Evaluation of intravarietal genetic variability of agronomic traits and stress tolerance in the Portuguese grapevine variety Arinto

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Summary

The valorization of genetic variability through the identification of the most suitable genotypes seems to be an effective strategy for tackling ongoing climate change. Heat and drought affect grapevine physiology in numerous aspects but still little information is available on intravarietal variability regarding responses to these stress. The objective of this work is to study the intravarietal genetic variability of the Portuguese variety *Vitis vinifera* L. CV Arinto in terms of yield and tolerance to abiotic stress, through indirect, rapid and non-destructive measurements carried out in the field. The experiment took place in PORVID's experimental vineyard in Pegões (Setubal, Portugal). The traits analyzed were Surface Leaf Temperature (SLT), yield, pruning wood weight, NDVI (Normalized Differences Vegetation Index), PRI (Photochemical Reflectance Index) and chlorophyll content through SPAD index. Linear mixed models were fitted to the data of the several traits evaluated, and the empirical best linear unbiased predictors (EBLUPs) of genotypic effects were obtained as well as the coefficient of genotypic variation (CVG) and broad sense heritability. The trait with the highest CVG was found to be yield, followed by those based on PRI and SPAD indices. Some hypotheses for polyclonal selection were explored: the highest predicted genetic gains were obtained with the selection based on the yield and on PRI and SPAD indices. In general, weak correlations between predicted genotypic values were found between the evaluated traits. The results obtained confirmed the effectiveness of the phenotyping methods and the experimental design used.

Keywords

Grapevine intravarietal variability – Polyclonal selection – Phenotyping – heat stress – drought stress

Resumo

A valorização da variabilidade genética através da identificação dos genótipos superiores relativamente a várias características de interesse cultural e económico parece ser uma estratégia eficaz para fazer face às alterações climáticas. Calor e seca afetam a fisiologia da videira em vários aspectos, mas ainda há poucas informações disponíveis sobre a variabilidade intravarietal em relação às respostas a esse stresse. O objetivo deste trabalho foi analisar a variabilidade genética intravarietal da variedade portuguesa Arinto em termos de rendimento e tolerância ao stresse abiótico por meio de medidas indiretas, rápidas e não destrutivas realizadas em campo. As avaliações foram realizadas no ensaio instalado no Pólo Experimental de Conservação e Diversidade da Videira da Associação Portuguesa para a Diversidade da Videira (PORVID), em Pegões (Setúbal, Portugal). As características analisadas foram: temperatura superficial das folhas (SLT), rendimento, peso da lenha de poda, NDVI (índice de vegetação por diferença normalizada), PRI (índice de reflectância fotoquimica) e teor de clorofila pelo índice SPAD. Foram ajustados aos dados das diferentes características modelos linear mistos com o objectivo de prever os melhores preditores lineares não enviesados empíricos (EBLUPs) dos efeitos genotípicos, calcular o coeficiente de variação genotípico (CVG) e a heritabilidade em sentido lato. Verificou-se que a característica com maior CVG foi o rendimento, seguida pelos índices PRI e SPAD. Foram exploradas algumas hipóteses de seleção policlonal, sendo que os maiores ganhos genéticos foram obtidos para o rendimento e para as seleções baseadas nos índices PRI e SPAD. A correlação entre os valores genotípicos previstos das diferentes características analisadas foi, em geral, fraca. Os resultados obtidos confirmaram a eficácia dos métodos de fenotipagem e do delineamento experimental adotado.

Palavras-chave

Variabilidade intravarietal da videira - Seleção policlonal - Fenotipagem – stresse por calor - stresse por seca

Resumo alargado

A videira é uma das espécies cultivadas economicamente mais importantes a nível mundial. Portugal tem mais de trezentas variedades oficialmente descritas na lista oficial de castas. É um país extremamente rico em termos de diversidade, tendo em conta também que para cada variedade existe uma ampla diversidade intravarietal (Almadanim et al., 2007).

Esta riqueza de biodiversidade constitui uma importante reserva de variabilidade potencialmente útil para lidar com as temperaturas médias mais elevadas e uma maior frequência de ondas de calor e secas que irão afectar o ambiente mediterrânico e a sua viticultura no futuro (Fraga et al., 2013). A resposta à seca, em particular o controle estomático, tem-se mostrado dependente do genótipo (Chaves et al., 2010), mas o comportamento das variedades em termos de eficiência no uso da água ainda é pouco conhecido. Os fatores que contribuem para a tolerância incluem aqueles relacionados à morfologia, anatomia e bioquímica foliar e algumas diferenças nessas características foram já encontradas (Shultz, 1996; Moutinho-Pereira et al., 2007). Quanto às diferenças intravarietais, existem poucos estudos disponíveis na literatura. Um resultado promissor vem da comparação feita entre a variabilidade de uma coleção de clones de Tempranillo e a de uma coleção de variedades, verificando-se que suas gamas de variação são semelhantes quanto ao uso da água e condutância estomática (Tortosa et al., 2016).

O objetivo deste trabalho é caracterizar a variabilidade genética intravarietal da variedade portuguesa Arinto, em termos de tolerância ao stresse abiótico, como o calor e a seca.

O trabalho experimental foi realizado num ensaio com 166 clones de Arinto, instalado de acordo com um delineamento experimental linha-coluna resolúvel, com 6 repetições e 3 plantas por unidade experimental. Este ensaio pertence ao primeiro ciclo de seleção da metodologia desenvolvida em Portugal (Gonçalves e Martins., 2012), recomendada pela Organização Internacional da Vinha e do Vinho (OIV) desde 2019, e está localizado no Pólo de Conservação da Diversidade da Videira da PORVID, em Pegões.

As características avaliadas foram escolhidas com base na possibilidade da sua medição expedita e na sua relação com alguns dos processos fisiológicos ligados à utilização de água e aos mecanismos de tolerância ao stresse térmico: SPAD, teor relativo de clorofilas; PRI, índice de reflectância fotoquimica, um estimador da eficiência do ciclo das xantofilas; NDVI, índice de vegetação por diferença normalizada, um estimador do crescimento vegetativo e da tolerância ao stresse; SLT, temperatura da superfície foliar, relacionada com a regulação estomática e o estado da água da planta. Para além destas características foram ainda avaliados o rendimento (kg/planta) e o peso da lenha de poda (kg/planta).

Para a análise dos dados foram ajustados modelos lineares mistos. Nestes modelos foram incluídos o factor genótipo e todos os factores associados ao delineamento experimental. Em todos os casos, admitiram-se esses factores como de efeitos aleatórios. As componentes de variância foram estimados pelo método de máxima verossimilhança restrita (REML; Patterson e Thompson, 1971). A partir das equações do modelo misto, foram obtidos os melhores estimadores lineares não enviesados empíricos (EBLUEs) dos efeitos fixos e os melhores preditores lineares não enviesados empíricos (EBLUPs) dos efeitos aleatórios (Henderson, 1975). A componente de variância genotípica foi testada através de um teste de razão de verossimilhanças restritas. Para a caracterização da variabilidade intravarietal das características avaliadas e para avaliação do sucesso da seleção policlonal foram calculados os seguintes indicadores: 1) coeficiente de variação genotípico; 2) heritabilidade em sentido

lato; 3) ganho genético previsto (média dos EBLUPs dos clones selecionados para cada característica).

Para uma melhor compreensão da relação existente entre o rendimento e as características avaliadas para analisar a tolerância ao stress abiótico, foram estudadas as diferenças de rendimento médio entre quatro classes de SLT, PRI e NDVI, definidas de acordo com os quartis das distribuições empíricas das respetivas características. Para cada característica, foi realizada uma análise de variância a um factor de efeitos de efeitos fixos. Para os casos em que foi encontrado efeitos significativo da classe sobre o rendimento, foi realizado o teste de Tukey para obter uma informação mais detalhada sobre as diferenças entre as médias de rendimento entre classes.

O software usado para análise de dados foi o R (R Core Team 2017, The R Foundation for Statistical Computing Platform). Para o ajustamento dos modelos lineares mistos foi usado o pacote ASREML-R (Butler et al., 2017).

Quanto aos resultados, encontrou-se variabilidade genotípica significativa para o NDVI, PRI, SPAD e rendimento (p-value <0,001). Comparando os coeficientes de variação genotípicos entre características, a que revelou maior variabilidade intravarietal foi o rendimento, seguida pelo PRI e SPAD. Quanto aos valores de heritabilidade em sentido lato, estes variaram entre 0,56, para NDVI, e 0,67, para o SPAD, revelando um satisfatório controlo da variabilidade ambiental. Estes resultados revelaram-se promissores para fins de seleção. No contexto da seleção policlonal, ao selecionar os 15 genótipos superiores para cada uma dessas características o maior ganho genético previsto foi observado para o rendimento (32,1%), seguido pelo PRI (13,7%) e SPAD (12,3%). Por outro lado, selecionando os 15 clones com menor temperatura foliar, observou-se uma redução da temperatura foliar prevista de 5,4% em relação à media da população, transportando este grupo um ganho genético previsto de cerca de 4,5% para o SPAD, 3,7% para o PRI e 14,1% para o rendimento. Foram conduzidos vários exercícios de seleção policlonal, todos eles com ganhos genéticos previstos associados.

Relativamente à correlação entre características, foram encontradas correlações positivas entre NDVI e PRI, SPAD e NDVI, e entre SPAD e PRI. Entre SLT e NDVI, PRI, SPAD e rendimento não foram encontradas correlações, tendo o mesmo sido verificado quanto ao rendimento. Quanto à relação existente entre o rendimento e PRI, a classe de genótipos com menores valores de PRI revelou um rendimento médio significativamente diferente das classes com maiores valores de PRI, tendo-se verificado o mesmo tipo de resultado para o NDVI.

Em síntese, este trabalho demonstrou a existência de diversidade intravarietal da casta Arinto quanto a características potencialmente atribuíveis a uma maior tolerância ao stress abiótico. É consensual que para fazer face às mudanças climáticas não existe uma solução única, mas sim um conjunto de esforços em diferentes frentes, principalmente ambientais, tecnológicas e genéticas. Com este trabalho tornou-se claro que a preservação da variabilidade intravarietal das castas autóctones e a seleção policlonal são ferramentas importantes para enfrentar o problema, com a vantagem de valorizar as castas autóctones, marca histórica e cultural da viticultura.

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ABA: Abscisic Acid

AQP: Aquaporins

CF: Cloropyhyll Fluorescence

CFI: Chlorophyll Fluorescence Imaging

CFIN: Chlorophyll Fluorescence Induction

CV%: Coefficient of variation

CV_G: Coefficient of genotypic variation

CWSI: Crop Water Stress Index

EBLUPs: Empirical best lienar umbiased predictors

ET_0: Reference evapotraspiration

ETc: Crop evaporation

ETR: Electron Transport Rate

Fs: Steady state fluorescence

$g_{L:}$ Leaf conductance

gm: Mesophyll conductance

gs: Stomatal conductance

H^2: Broad-sense heritability

HS: Heat Stress

IPCC: Intergovernmental Panel on Climate Change

Kc: Crop coefficient

Ks Hydraulic conductance

LA: Leaf Area

LAI: Leaf Area Index

LS: High Light Stress

NDVI: Normalized Difference Vegetation Index

NPQ: Non-Photochemical Quenching

PAR: Photosynthetic Active Radiation

PEV_i: Predicted Error Variance

PGV: Predicted Genotypic Value

Pn: Net photosynthetic rate

PRI: Photochemical Reflactance Index

PSII: photosystem II

R: Predicted genetic gain

R%: Predicted genetic gain in percentage of the mean

REML: Residual Maximum Likelihood

REMLRT: Residual Maximum Likelihood Ratio Test

S: Selection Differential

SLT: Surface Leaf Temperature

SPAD: Soil Plant Analysis Development

T_{leaf}: Leaf temperature

TN: Touriga Nacional

TR: Trincadeira

TrS: Transpiration rate

VLAI: Vine Leaf Area Index

WS: Water Stress

WUE: Water Use Efficiency

ψ_{pd}: Pre-dawn leaf water potential

ψ_{stem}: Stem water potential

1. Introduction

Grapevine is one of the most economically important crop species in Portugal. Its main product, grapes, feeds the wine sector, highly significant for the national economy and a major export. In 2017 Portugal was the fourth European country in terms of vineyard area (194,000 ha), the fifth wine producer in the EU (6.6 million hl), and the eleventh worldwide (OIV statistical report 2018). Portugal is also extremely rich in grapevine biodiversity with more than 300 officially described varieties ("castas"), consisting of hundreds of clones (Almandanim et al., 2007). These different genotypes are themselves the natural reservoir of the genetic and phenotypic variability of the varieties and behave differently regarding the most important economic traits (quality and productivity). The fact that climate change is underway is now widely confirmed. Higher average temperatures and more frequent climate extremes (such as events of drought and heat) will affect Mediterranean viticulture (Fraga et al., 2012). On the long term one of the most effective strategies to overcome these changes is the use of more suitable genotypes.

The objective of this work is to characterize the intravarietal genetic variability regarding tolerance to abiotic stress in the variety Arinto, based on a set of fast and non-destructive phenotyping techniques to be used in field conditions. Another aim is to identify the best methodology to obtain robust and reproducible data to help to assess diversity regarding abiotic stress tolerance. To achieve these goals it is first necessary to understand the main genetic traits characterizing *Vitis vinifera* as a crop species, as well as the domestication and breeding history which led to the current varieties.

Efficient breeding programs for crop species are necessarily based on knowledge of genetic and phenotypic characteristics which distinguish the species/genotypes in analysis. Because of the specific purposes of this work, an overview of the physiological effects of abiotic stress and the tolerance mechanisms that plants have developed is also a necessary premise. Some peculiarities of the *Vitis vinifera* species, such as the long history of asexual propagation, the high heterozygosity, the susceptibility to inbreeding depression, and also limitations imposed by the market and by the wine culture and history, affect the choice of the most suitable techniques. The clonal selection of ancient varieties is a potentially efficient method for selection regarding quantitative traits.The improved method developed in Portugal, and recommended by the International Organisation of Vine and Wine (OIV) since 2019 (OIV, 2019), overcome some limitations found in the classical protocol (Gonçalves and Martins, 2012).

One of the most challenging aspects of breeding programs is the phenotyping work. This is due to the high number of samples needed (several replicates of hundreds of genotypes) and the extensive layout required for an effective statistical analysis, which makes the measurement of several important physiological parameters impractical and sometimes even unfeasible. Therefore, the development of more adequate and feasible measurements for field phenotyping is paramount.

This experimental work was undertaken on the variety Arinto in a field trial corresponding to the first experimental cycle of a selection program following the methodology developed in Portugal. The field is located in the Experimental Center of PORVID, in Pegões and laid out in a row-column design (168 genotypes X 3 plants per plot X 6 resolvable replicates).

The traits evaluated were Normalized Difference Vegetation Index (NVDI), Photochemical Reflectance Index (PRI), Surface Leaf Temperature (SLT), Chlorophyll content (SPAD), yield and pruning weight. The measurements were performed during the period most affected by heat and drought stresses (June to September). The analyses made were chosen considering their correlation with some of the most important physiological parameters involved in stress response and tolerance and the feasibility of being carried out on the experimental design layout.

1.1. Grapevine breeding

Plant breeding is a branch of agriculture that aims to develop new or improved plant types by manipulation of the genetic material (Acquaah, 2012). To reach this goal several techniques are available, and the choice is often conditioned by the variety considered or by the specific breeding purpose. The oldest breeding activity dates back to the early days of agriculture (Zohary and Hopf, 2000), when the first farmers instinctively chose the best plants for propagation. Since then the history of agriculture has been and still is closely related to the genetic improvement of cultivated species.

Grapevine (*Vitis vinifera*) belongs to the Vitacea family, together with *circa* 60 wild species, most of the more important varieties for table and wine grape production are classified within the subsp. *vinifera* or *sativa* of *Vitis vinifera* L., derived from the wild *Vitis vinifera* L. subsp. S*ylvestris* (Terral et al., 2010). The origin of cultivated grapevines remains unclear. A recent research found traces of vinification 8000 years old in a site located 50 km south of Tbilisi, Georgia (Mc Govern et al., 2017). Although this cannot be considered a proof of domestication/cultivation it is an indication of an ancient connection between man and wine. According to several studies the domestication could be dated to between the 7^{th} and 4^{th} millennia BC (Terral et al., 2010). Parallel to the hypothesis of a single domestication center from which *Vitis vinifera* was then spread throughout Europe, there are elements that suggest that domestication has occurred more than once, in different places and times. In fact, a study based on chloroplast DNA polymorphism analysis pointed out the existence of at least two possible main centers of domestication, one in eastern Europe and another in the western Mediterranean region (Arroyo-Garcia et al., 2006). After domestication, and just like other perennial crops, vines were mainly propagated by vegetative means (cuttings, grafting) for two main reasons: the strong segregation during sexual reproduction caused by the high heterozygosity, that leads to the loss of traits previously selected, and the long time needed for the progeny to reach sexual maturity. Data from different studies suggest that European cultivars originated from importation, introgression and *de novo* domestication, but except for recent cultivars the origin of most varieties is unknown (Jackson, 2014).

During millennia of viticulture a great amount of variability has been accumulated thanks to accidental crossing, somatic mutation and in the last century, by intentional crossings. The current ampelographic landscape consists in hundreds of local varieties in the main European and Caucasian countries dedicated to viticulture. The number of grape varieties in the world is estimated at 6000 to 8000 (Maul et al., 2018) and other authors report a total of 10000 (OIV, 2017). The “Vitis International Variety Catalogue”, comprising accessions belonging to the genus *“Vitis”,* has 22422 registered accessions from 86 countries including breeding lines and Vitis species existing in grapevine repositories and/or described in bibliography (Maul et al., 2019), According to the catalogue, in 2011 a total number of 1902 varieties (for fruit production and rootstock) are officially authorized for cultivation at least in one state of the EU, of which 1246 are registered in one single state (Lacombe et al., 2011) and should be considered local varieties.

Table 1. Grape varieties included in the national catalogues (Lacombe et al., 2011).

	Country	Number of grape varieties in the national catalogue	Year of the national catalogue update
Members States of the European Union (* partners in GrapeGen06 program)	Austria (AUT)*	105	2008
	Belgium (BEL)	34	2000
	Bulgaria (BRG)	166	2009
	Cyprus (CYP)*	109	2000
	Czech Republic (CZE)*	89	2007
	Germany (DEU)*	163	2010
	Denmark (DNK)	38	2000
	Spain (ESP)*	222	2010
	France (FRA)*	338	2010
	United Kingdom (GBR)	45	2000
	Greece (GRC)*	197	2007
	Hungary (HUN)*	146	2010
	Italy (ITA)*	548	2010
	Luxembourg (LUX)	16	2000
	Malta (MLT)	56	2000
	Nederland (NLD)	50	2000
	Portugal (PRT)*	511	2010
	Romania (ROU)	124	2009
	Slovakia (SVK)*	37	2000
	Slovenia (SVN)	54	2000
	Sweden (SWE)	10	2000
Third Countries partners in GrapeGen06 program)	Switzerland (CHE)	113	2007
	Georgia (GEO)	58	1998
	Croatia (HRV)	231	2009
	Moldova (MDA)	86	2009
	Turkey (TUR)	78	2009

The first proof of intentional plant breeding goes back to the late 1600, when hybrid hyacinths were developed in Holland (Zohary and Hopf, 2000). However, selection of accidental improved strains of food crops began with agriculture itself.

Until about a century ago, deliberate operations to develop new grapevine cultivars were limited. Some of the earliest attempts involve crossings between *V. vinifera and V. labrusca, V. rupestris, V. riparia and V. aestivalis.* in order to obtain cultivars resistant to Phylloxera and with the oenological attributes of *V. vinifera* (Jackson, 2014). They were successively abandoned because of their different aromatic profiles. Nowadays there are several molecular techniques for fingerprinting that allow to discover the relationship between different varieties and how they were originated but it is not possible to ascertain whether the crossing was intentional or not (Jackson, 2014).

The goals of most grape breeding programs (e.g. improve tolerance to biotic and abiotic stress and increase yield), are almost the same since the early works of breeders (Jackson, 2014). The major changes occurred in the analytical techniques available and in the deeper understanding of genetic sequences resulting from the use of those tools. Currently, genetic maps can show the position of some genes of interest and adjacent SSR and SNP-based markers (Di Gaspero and Cattonaro, 2010). These maps could permit marker-assisted breeding and support traditional breeding techniques, identifying the desirable trait in parents for crossing or in the progenies without having to evaluate the phenotypic expression (Di Gaspero and Cattonaro, 2010). Nevertheless, this is mainly possible for traits associated with one or few dominant genes. Other new methods of genetic engineering involve the insertion or deletion of specific genes by gene editing (Pretorius and Høi, 2005).

The high heterozygosity of most desirable traits and inbreeding depression, combined with the fact that most of the interesting traits are quantitative are the main reasons why grapevine breeding is challenging. Other problems are the time necessary to reach the wine evaluation phase and the strict legislation regarding the varieties allowed in specific appellations. Also, the consumers' preferences are often addressed to "traditional" varieties. For these reasons classic breeding, specifically clonal and mass selection, still finds ample space in viticulture.

1.2. Clonal selection, an improved method

Clonal selection is the main method by which it is possible to breed grapevine for specific quantitative traits, and without modifying the variety's distinctive characteristics and consequently maintaining the same name. The starting point for any improvement is the existing genetic variability within the variety. Such variability is due to somatic mutations at bud level (somatic cells of the L1 layer) and the successive fixation by asexual propagation (Franks et al., 2002). Some mutations are visually recognizable, like the ones affecting grape pigments or vine morphology but most of them are more difficult to detect, such as the ones related to enzymes involved in aromatic compounds profile, yield or biotic and abiotic stress resistances. Mutations tend to accumulate slowly, for this reason they are present at higher rates in old varieties (Jackson, 2014).

According to the resolution "OIV-VITI-564A-2017" the clonal selection process must follow three main steps before the clone registration:

1. Selection of initial material

The selection of initial material should be done preferably in vineyards planted without selected clones and before the beginning of selection programs.

Varietal identity confirmation carried out by ampelographic and/or genetic investigation.

Elimination of individuals affected by transmissible diseases. Usually about 1000 plants in more than 20 vineyards are evaluated and this step ends with the selection of approximately 100 plants.

2. Observation and conservation of the vegetative progeny of selected individuals.

Individuals selected in the previous phase are propagated and planted in a comparable trial, preferably in two different environments, the trial should include one or more existing standard clones as reference. Each candidate clone should be planted at least with 5 vines replicated at least three times. Evaluation for 3-5 years with the aim to select 20-30 superior clones.

3. Full study of individuals selected in step 2.

Investigation of the candidates in several locations and on several rootstocks, with enough plants per clone in order to have enough grapes for microvinification. This evaluation should be carried out for at least two years. Experimental design must be done with a minimum of three repetitions per clone candidate and sanitary diagnosis is required.

With the exception of the advices regarding the vineyards suitable for the first selection, this methodology is almost the same that has been used from the beginning of clonal selection programs and has been followed during the first selection studies in Portugal and many other countries. However, results obtained after 1984 in experimental clone collections in Portugal

revealed some weaknesses in this method (Martins and Gonçalves, 2015), especially regarding the inefficacy of the initial phase of individual phenotypic selection in vineyards. According to these authors, the first problem was identified observing the large variation in yield between plants of the same genotype in an experimental field trial, corresponding to the second phase of the process. The variation due to the environment was much larger than the one caused by the genotypes, making it impossible to identify genotypic differences through visual analysis of phenotypes. This suggests that the initial visual selection of superior clones is ineffective. Another fact that supports this idea is the lack of correlation between the yield of mother plants and the yield of the related clones in experimental conditions, observed in a collection of 52 clones of Antão Vaz selected in four vineyards. The third observation that prompted the researchers to develop a new method was the very low heritability of yield calculated in large computer-simulated collections, applying the expression of genetic gain R (Falconer and Mackay, 1996), where R is the genetic gain, S the selection differential (the difference between the mean of the selected genotypes and the overall mean) and h^2 is broad-sense heritability.

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

The run of 10000 simulations obtained an estimation of broad-sense heritability between 0 and 0.25.

The premises that clonal selection can be effective only if there is enough initial variability and the weaknesses found in the classical method led to some changes in the methodology applied in Portugal, the main one concerns the first phases of the protocol. As it was proven that that the visual selection of ‘apparently’ superior clones is ineffective, a new method of sampling the intravarietal genetic variability was developed. The importance of having a good representation of the variability is inferable from Eqn 1, where it is possible to see that the genetic gain (R) depends on the selection differential (S) and this depends on the diversity within the population. To obtain a representative sample it is first necessary to define the minimum number of mother plants; this number has been determined through simulation experiments (Martins et al., 1990; Gonçalves and Martins, 2012) and corresponds to 50 - 70 genotypes per each region where the variety is cultivated. A minimum number of 70 genotypes is thus recommended where there aren’t optimal trial conditions. Other advices to obtain representative samples are given in order to minimize the probability of collecting plants with the same genotype: 1) for each region the samples should be taken from old vineyards, planted before homogeneous material was available from the nurseries, 2) few plants should be chosen in each vineyard (from two to five plants in more than 20 vineyards), belonging to different owners and if possible geographically distant; 3) during sampling a visual and enzymatic diagnosis must be performed to exclude virus-infected plants. The variety identification is usually obtained with ampelographic analysis and in case of doubts, with genetic identification. The genotypes collected should be grafted onto the same rootstock in an experimental field with homogeneous soil and an adequate experimental design that allows to manage the spatial variability. In fact, a trial with so many genotypes requires an area between 0.75 and 1.5 ha (Gonçalves et al., 2010), and this surface is large enough to cause large environmental variation. To overcome this, it was established, after many years of experiments, that the more suitable designs to manage dozens or hundreds of different genotypes are alpha designs and row-column designs (Gonçalves et al., 2010; Gonçalves and Martins, 2012). If the genotypes are more than 400 and the main objective is to study genetic variability it is possible to use also unreplicated designs (Gonçalves et al., 2013). The new methodology obtained, recommended by the OIV since 2019 (OIV, 2019) is briefly schematized in Figure 1.

The most important aspects of this method are the possibility to quantify the intravarietal genotypic diversity of the varieties studied and to perform the selection of a superior group of genotypes (mass genotypic selection or polyclonal selection) and to predict the corresponding genetic gain of selection during the second step of the process. The setting up of the first field trial is also very useful in terms of conservation against genetic erosion (Martins and Gonçalves, 2015). The genetic variability for any quantitative trait can be analyzed with various metrics, among these the coefficient of genotypic variation turned out to be particularly adequate (Gonçalves and Martins, 2012). Polyclonal selection is based on the principles of quantitative genetics and allows prediction of genetic gains by using Eqn 1. It could be performed several times concerning different quantitative traits, allowing to follow the needs of the producer, which often change over time. It is also possible to obtain a first fast result useful for producers, during the long clonal selection procedure.

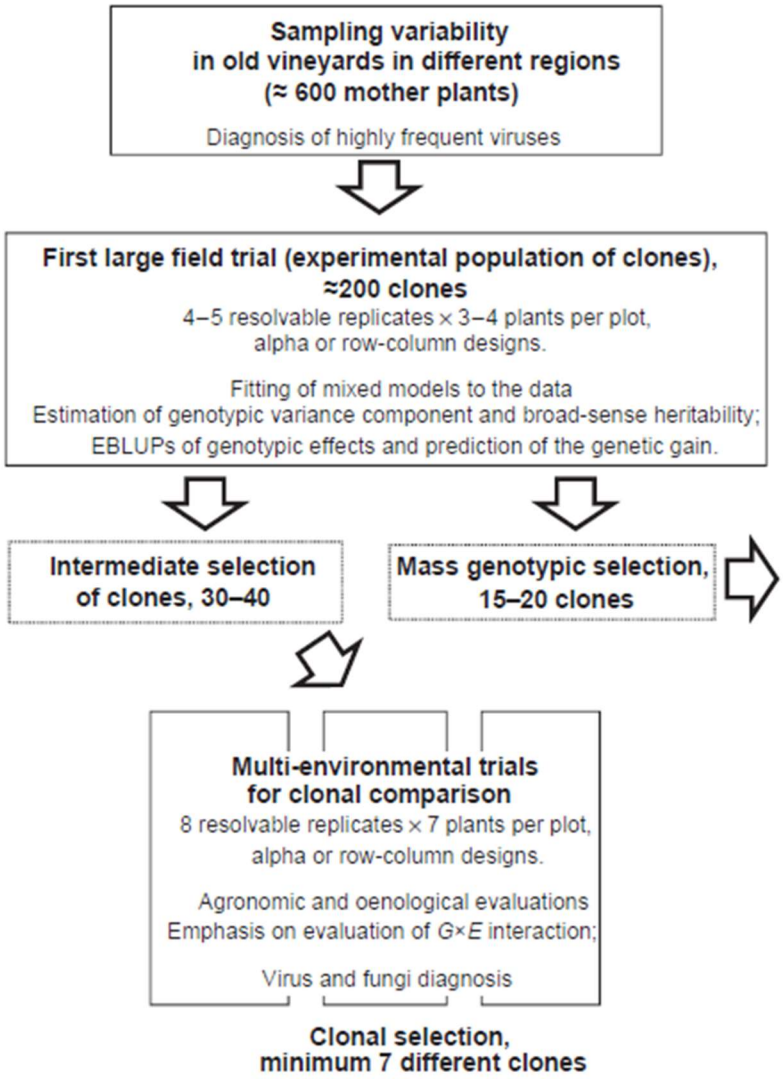


Figure 1. Methodology of grapevine selection developed in Portugal (Martins and Gonçalves, 2015) and recognized by the OIV since 2019 (OIV,2019).

Polyclonal selection (mass genotypic selection) was tested in two Portuguese varieties, Arinto and Vital, considering yields and °brix data collected in several years (Gonçalves and Martins, 2012). The trials were laid out according to a randomized complete block design with 247 genotypes for Arinto and 232 for Vital. Linear mixed models were fitted to yield and brix data and the empirical best linear unbiased predictors (EBLUPs) of genotypic effects for these traits were obtained. After finding that the genotypic variance component was statistically significant (p-value

<0.0001), the EBLUPs of genotypic effects were used to select two groups of 30 superior clones per each variety. The performance of the group of clones selected has been compared to the performance of individual clones during different years and the result was that the predicted genetic gain in the group is more stable than the behavior of individual clones. This experiment highlights another important advantage of polyclonal selection that is to minimize genotype X environment interaction. This methodology of polyclonal selection was expressly recognized in 2019 by the International Organization of Vine and Wine, through the "Resolution OIV-VITI 564B-2019: OIV Process for the recovery and conservation of the intra-varietal diversity and the polyclonal selection in grape varieties with wide genetic variability".

1.3. Importance of water and temperature on grapevine physiology in a warming world

1.3.1. High temperature scenarios

According to the Intergovernmental Panel on Climate Change (IPPC), the average global temperature has risen about 0.85 °C between 1880 and 2012 (IPPC, 2013) and many regions worldwide have experienced even greater warming. The trend in soil temperature recorded at the Nanchang Weather Station also shows a significant increase from 1960 to 2018 with an even more rapid increase from 1990 and it also shows a reduction in the difference between air and soil temperature at different depths (Zhan et al., 2019). The projection shows that the increase of global surface temperature is likely to be from 0.3 to 1.7 °C in the lowest emissions scenario and between 2.6 and 4.8 °C under the highest emissions scenario (IPCC, 2014). More frequent high temperature extremes are also predicted, together with more frequent heat waves and changes in precipitation intensity and frequency (IPCC, 2014).

Due to the risks posed to agriculture, it is undoubtedly necessary to reverse this trend. Although grapevine and wine production are not crucial for survival of humanity viticulture is an important socio-economic activity in several countries, and wine belongs to the tradition and culture in many countries as well (Estreicher, 2006; Dougherty, 2012). For these reasons, it is crucial that the wine sector finds solutions for higher sustainability under adverse climate conditions.

Grapevine is considered susceptible to short duration climate variability and to long-term climate changes (Jones and Webb, 2010). Literature also points out changes in the geographic distribution of wine production (Lallanilla, 2013), mainly with a shift to higher latitudes and/or uphill. An increased frequency of heat wave events has already been observed in the most susceptible regions (Perkins et al., 2012). Projections for future climatic conditions in Portugal indicate a warming and drying trend, with a potential impact on grapevine phenology and growth and on wine characteristics. Also, the suitability of some regions for viticulture will be affected, suggesting a reshaping of Portugal's wine geography (Fraga et al., 2017).

Despite the possibility of more or less efficient agronomic interventions against drought and heat stress, in the long term the strategies can only be aimed at the choice of less exposed sites and at the use of suitable genotypes, through the use of less sensitive varieties and possibly, considering the historical value of some varieties, less sensitive clones within those varieties.

1.3.2. Temperature and water: influences on grapevine physiology

The importance of temperature and water on vine's growth and berry ripening have long been known. The main aspects relating to them are summarized by Keller (2015). The most relevant physiological processes influenced by temperature and water are reported below.

Temperature, together with water, is one of the main factors responsible for the changes in berry quality between seasons. The optimal temperature for grapevine metabolism for growth and ripening is below 30 °C (Keller, 2015), heat stress is usually defined when temperature exceeds 5 °C above the optimal temperature for grapevine metabolism. Higher temperature accelerates plant growth and development, speeding up phenology. Acceleration of phenological stages under global warming conditions may be particularly relevant because earlier *veraison* anticipates ripening to the warmer period of the growing season. This shift has been already observed in France (Duchêne and Schneider, 2005), and led to higher sugar content and lower acidity (Drappier et al., 2019). In some cases this could be correlated with higher wine quality, but this is not a general rule. Moreover, the gap between technological and phenolic ripeness increases.

More than 5 °C above the optimum causes a marked decline in photosynthetic mechanism efficiency (Salvucci and Crafts-Brandner, 2004). In hot regions the temperatures could exceed the photosynthetic optimum during the day and increase the proportion of carbon lost through respiration during the night, directly affecting the sugar available to the clusters; also, prolonged periods above 40 °C inhibit the long-distance transport of assimilates through the phloem and can limit berry size and fruit ripening. The decrease of photosynthesis and the shift of carbon partition in favor of vegetative growth can lead to a suppression of fruit growth and ripening (Greer and Weston, 2010; Sepulveda et al., 1986), especially when it happens in post-*veraison*. Anthocyanin content is also affected by temperature, the synthesis increases until 30 °C and is inhibited above 35 °C (Kliewer, 1977). Excessive temperature may also cause oxidative stress, which could lead to anthocyanin degradation (Mori et al., 2007). Also, tannins and amino acids increase with higher temperature. Heat stress could also interfere with pollen maturation and viability (De Storme and Geelen, 2014; Zinn et al., 2010).

Even brief periods of leaf temperature above 45 °C can cause irreversible decline in stomatal conductance and photosynthesis, and longer periods can cause death (Gamon and Pearcy, 1989; 1990). High temperatures are often associated with high vapor pressure deficit and stomata close in response to this condition. Heat damage usually depends on the irreversible disintegration of membranes caused by lipid and protein denaturation. As in other stresses, the recovery capacity depends on intensity, duration and on the phenological stage at which it occurs. There is also a variation in terms of sensitivity among *Vitis* species and cultivars (Keller, 2015). An example reported by Keller (2015) concerns the different behavior between Sangiovese and Montepulciano observed during an hot summer in Italy in which Sangiovese vines lost most of their basal leaves, in particular on the west side of the canopy, whereas Montepulciano vines didn't lose any leaves (Palliotti et al., 2009). Heat stress tends to have similar symptoms to drought stress because it affects plant water relations.

Leaves are able to regulate their temperature and thus increase heat tolerance through evaporative cooling due to transpiration, whereas grape berries are designed to minimize water loss by transpiration, which is particularly true after *veraison* and which makes berries quite susceptible to overheating (Keller, 2015). This can lead to inhibition or denaturation of berries' proteins (Keller, 2015), despite the synthesis of heat shock proteins, which help other proteins keep their native structure (Iba, 2002). The accumulation of calcium ions in the cytosol of heat-stressed cells seems to help reduce membrane permeability and stimulate plant's antioxidant system to protect the photosynthetic apparatus against oxidative damage (Keller, 2015). Isoprene, monoterpenes and other volatile isoprenoid emissions help leaves to survive brief episodes of high temperature (>40 °C) by stabilizing the thylakoid membranes and removing reactive oxygen species (Loreto and Shnitzler, 2010; Pichersky and Gershenzon, 2002).

Water is crucial for plant growth and survival. It is also very important for yield, fruit composition, and ripening (Keller, 2015). *V. vinifera* is quite tolerant to drought stress and can grow in areas with less than 300 mm annual precipitation but with very limited yield (Huglin and Schneider, 1998). Most of the world's vineyard acreage is located under Mediterranean type climate

conditions, characterized by dry and warm summers, with high evaporative demand and low soil moisture. In warm climates, vineyard water use ranges approximately from 450 to 800 mm during the growing season (Williams and Baeza, 2007), water availability depends on rainfall quantity but also on evaporation, soil holding capacity, depth, texture and organic matter content (Keller, 2015). The water potential at field capacity varies between -0.01 to -0.03 MPa, and under arid conditions it can decline to -3.0 MPa. We must bear in mind that the lowest limit for grapevine's suction activity (permanent wilting point) is -1.5 MPa (Keller, 2015).

A high soil water content stimulates vigor, which can lead to a dense canopy and cluster shading, and also requires more water for transpiration, making the plant more susceptible to drought (Keller, 2015). Conversely, severe water deficit may cause leaves abscission by early senescence and consequent exposure of the fruits (Romero et al., 2010). Shoot growth and tendril development are highly sensible to water stress, shoot growth is even more sensitive than photosynthesis (Hsiao and Xu, 2000; Stevens et al., 1995). In a non-stressed vine the uppermost tendrils extend beyond the top of the shoot, in water stress conditions tendrils remain small. In severe stress conditions (leaf water potential below -1.5 MPa and stomatal conductance reduced below 20% compared to well-watered conditions) growth can slow drastically or even stop and photosynthetic metabolism is damaged, initially by a decreasing of electron transport and ATP synthesis which leads to the inhibition of the Calvin cycle and in severe conditions by inhibition of light harvesting in photosystem II, accompanied by a small decrease in respiration (Keller, 2015). This metabolic limitation of photosynthesis, in contrast to stomatal limitation, is irreversible (Escalona et al., 1999; Lawlor and Cornic, 2002). In water stress conditions, an increasing proportion of CO_2 derived from stored carbohydrates is lost from the leaves (Mc Dowell, 2011). When leaves lose turgor, they wilt and reduce light absorption because the surface area decreases, avoiding a potential "energy overload" that could damage the photosynthetic system (Flexas et al., 1999; Lawlor and Cornic, 2002).

Usually reduction of vegetative growth due to water stress is more severe than reduction of fruit growth (Williams, 1996). However, water deficit also affects yield, especially when deficits occur early in the growing season, and even moderate and short water stress periods during male meiosis can interfere with pollen development because of the inhibition of sugar transport (De Storme and Geelen, 2014), with negative consequences for pollination and fruit set. After fruit set vines usually maintain fruit development rather than the vegetative growth and carbohydrate storage (Eibach and Alleweldt, 1985) and this could have long term implications in budbreak. During berry growth, the most sensitive phase to water stress is cell expansion (Ojeda et al, 2001). Other authors consider that yield reduction can be severe even after fruit setting but they regard it insensitive to changes in water status after *veraison* (Hardie and Considine, 1976; Hofacker et al 1976, McCarthy, 1997; Williams and Matthews, 1990). The effect of early drought stress on shoot elongation and yield was studied on Merlot (Korkutal et al., 2011) under water deficit. The authors report yellowing of leaves and partial leaf fall, smaller shoots and a 50% yield reduction.

Water can also affect berry sugar content, acidity, pH and color. Indirect improvement of berry quality could be achieved because of lower yield and smaller berry size but in case of severe stress, especially during ripening, the decrease in photosynthesis and sugar export can reduce sugar accumulation (Keller, 2015). Mild water deficit could have positive effects on grapes aroma potential, increasing the amount of thiol precursors (Peyrot et al., 2005) and accelerate the post-*veraison* breakdown of methoxypyrazine (Sala et al., 2005).

One universal mechanism adopted by plants that have to face cell dehydration caused by drought is the accumulation of solutes inside the cells (Bohnert et al., 1995) to support water uptake and prevent water loss. Thanks to stomata regulation plants are also able to avoid xylem cavitation caused by water deficit (Brodribb and Holbrook, 2003; Jones, 1998). Decreasing stomata transpiration and keeping the leaf water potential constant in low soil water potential conditions is

a mechanism to avoid cavitation. Different behavior in response to drought and vapor pressure deficit could be attributed to aquaporins activity (Sade et al, 2009; Vandeleur et al., 2009). Twenty-eight different aquaporins (AQP) have been identified in *Vitis* (Fouquet et al., 2008). There is a relationship between leaf hydraulic conductance and AQP gene expression (Cochard et al., 2007). They could also influence shoot recovery from embolism (Lovisolo and Shubert, 2006). A study regarding isohydric and anisohydric vines behavior suggests the major role of physiological and anatomical differences in water transport in roots (Vandeleur et al., 2009) and their relationship with gene expression for some major AQPs at the membrane level. The role of AQP in the hydraulic behavior has also been demonstrated for tomatoes (Sade et al., 2009). Another important factor is the mesophyll conductance (*gm*) (Flexas et al., 2002; 2006). If genetic differences in stomatal conductance are confirmed it could be a way to improve WUE (Flexas et al., 2006). Also night time transpiration and water use could affect WUE but there is still little information about these processes in grapevine (Shultz and Stall 2010; Coupel-Ledru et al., 2014). In many horticultural crop species an incomplete stomatal closure during the night has been observed (Caird et al., 2007) which can lead to water loss through transpiration. The quantity of water transpired depends on the cuticular conductance and on the vapor pressure deficit and can be between 5 and 15% or even more compared to the daytime rates (Shultz and Stoll, 2010).

1.4. Genetic differences in drought and heat stress tolerance

1.4.1. Differences among varieties

The response to drought stress, in particular stomatal control, is mostly genotype-related (Escalona et al., 1999) but the behavior concerning WUE is still unknown for most genotypes. Stomata regulation allows to avoid xylem embolism maintaining the water flow within safe limits (Sperry et al., 2002) and grapevine is generally considered efficient in this mechanism. Other factors involved in drought resistance could be related to leaves morphology, anatomy and biochemistry and differences in these characteristics have been found among varieties (Schultz et al., 1996; Moutinho-Pereira et al., 2007). Varieties can be classified as isohydric, when they reduce the transpiration rate by closing the stomata in drought conditions, or anisohydric when they maintain the transpiration rates almost constant and decrease leaf water potential (Schultz et al., 2003; Soar et al., 2006). In the case of Grenache, a nearly isohydric variety, the stomata behavior has been correlated with the higher concentration of ABA in the xylem and higher expression of key genes involved in ABA biosynthesis in leaves, compared with Syrah, an anisohydric variety (Soar et al., 2006). However, a partition of varieties in two strict categories may be inadequate because stomatal regulation is also dependent on rootstock, climatic conditions and intensity and duration of water deficit as well as on growing conditions (Chaves et al., 2010). Several varieties subjected to moderate water deficit had different transpirational behavior and WUE, Cabernet Sauvignon, Syrah, and Touriga Nacional behaved as anisohydric (Chaves et al., 2010; Costa et al., 2012) while Aragonez can be isohydric, near-isohydric or even anisohydic (Chaves et al., 2010). Another study on 23 varieties in field conditions (Bota et al., 2015) pointed out that a strict classification into iso- or anisohydric varieties is inappropriate, despite confirming significant genotypic variations in stomatal behavior and WUE among the varieties.

Thermography can be used to assess plant temperature and plant water status (Jones et al., 2002; Moller et al., 2007; Costa et al., 2012). A close correlation between leaf temperature (T_{leaf}), stomatal conductance (g_s) and leaf water potential (ψ_{pd}) was found. The comparison of thermal imaging and leaf-gas exchange between five varieties showed that for very similar leaf water potential, leaf temperature changes with the variety, because of different stomata regulation, suggesting different adaptations to warmer climate, with Touriga Nacional identified as the more

able to adopt evaporative cooling strategies under conditions of water availability. Conversely Syrah seems to be more efficient in water use but less in heat dissipation which can be problematic for heat stress. The results found regarding Syrah contrast with previous reports which identify the variety as isohydric.

Also Carvalho et al. (2016) studied the varieties Touriga Nacional (TN) and Trincadeira (TR), in order to better understand the origin of such different behavior in relation to water stress (WS), heat stress (HS) and high light stress (LS), individually and combined. The authors report that during stress conditions involving heat stress the thermal dissipation in light-harvesting complexes tended to be higher than in other stress conditions, in particular when water and heat stresses were combined there were significantly higher values of intrinsic water use efficiency because of the very low *gs*. TN had higher rates of water use and higher water use efficiency. The authors conclude that each genotype has specific responses to the different stresses and TN seems to be more tolerant to abiotic stress, keeping the stomata open and allowing evaporative cooling and photosynthesis, except when water stress was involved, while TR could be considered as more “stress sensitive”.

Couple-Ledru et al. (2014) hypothesized that the hydric behavior can be due to differences in stomatal sensitivity to water deficit, which affect the more or less efficient maintenance of leaf water potential during the daytime (Buckley., 2005). The mechanism of stomatal closure involves abscisic acid production in response to drought (Tardieu and Davies, 1992; Borel et al., 1997; Tardieu and Simonneau, 1998) and plant hydraulic conductance plays a role on leaf water potential (Franks et al, 2007; Pantin et al., 2013); differences in hydraulic conductivity in roots (Vandeleur et al., 2009) and petioles (Shultz, 2003) have also been related to isohydric or anisohydirc behavior. The study was conducted on a pseudo-F_1 progeny obtained by a reciprocal cross between Syrah and Grenache. A QTL approach in the genomic region associated with the trait was used to analyze leaf water potential variability, in parallel with transpiration rates (TrS) and hydraulic conductance (KS) measurements. The authors found a highly significant genotypic effect on leaf water potential under water deficit. Also, KS and TrS have been found to be highly variable in the progeny. A high genotypic correlation was also found for leaf area, which was negatively correlated with TrS and also with leaf water potential. The leaf area was the trait which showed the highest broad-sense heritability (H) and with an amplification of variability in drought conditions. Thus, the variability in leaf water potential in water deficit conditions is largely determined by the genotype and four QTL, each one explaining 11% of the variability, were detected. The role of hydraulic conductance was also analyzed, high heritability and many QTLs were found but only one of these co-localized with a QTL for leaf water potential, suggesting that genetic variability in hydraulic conductance cannot fully explain hydric behavior. A physiological explanation can be found in the water transport capacity mediated by aquaporin expression in roots (Vendeleur et al., 2009) or leaves (Pou, et al., 2013).

In conclusion, there are significant differences between varieties in the response to drought stress, and this supports the need for carrying out selection of more resistant genotypes to face climate change conditions. However, differences seem to depend on a series of distinct complex physiological factors, whose complete understanding still needs to be clarified.

1.4.2. Intravarietal differences

In the last years much research was undertaken in order to understand the different behavior in terms of heat and drought stress tolerance between different varieties. It is known that the intravarietal differences regarding traits such as yield, especially in ancient varieties, are huge, but very few studies tackled with intravarietal genetic variability related to abiotic stress. One of them was performed by Spanish researchers to evaluate the variability in water use efficiency in a clone collection of 30 accessions of Tempranillo, compared with a collection of 23 varieties

(Tortosa et al., 2016). The accessions were analyzed for water status using several physiological parameters and three water status categories: plants under non-stress conditions, moderate water stress and severe water stress. The results show a wide range of variation for cultivars in the same water availability conditions, proving the different behavior of the varieties. Tempranillo clones show a range of variation similar to those found among cultivars and it was possible to identify six clones with higher WUE and three with lower WUE. The coefficient of variance, calculated as an estimator of the variability, was lower between clones than between cultivars for WUE and g_s, achieving about 70-80% of the cultivars' variance. The authors conclude that the different behavior between clones and the genetic variability found were enough to consider clonal selection an effective method to improve cultivar water use efficiency.

1.5. The challenge of phenotyping

Plant phenotyping consists in the quantification of plant physiological, agronomical and qualitative traits. Its main goals are to study plant physiology or to evaluate and select superior genotypes in breeding programs. The work necessary is often long and problematic because of the large number of accessions and the high spatial variability required, especially in field conditions and because of the impracticality of some longsome physiological measurements. As much as the urgency to obtain improved crop species to overcome climate change, the need for fast and efficient methodologies to perform phenotypic analysis is universally recognized. This topic has been discussed and studied by several researchers especially for herbaceous plants and cereals; in the last years good progress has been made in remote and proximal sensing tools that could meet the need for phenotyping technologies. These technologies could be helpful especially for greenhouse trials on annual crop species, where it is now possible to use automated multi-sensor phenotyping machines. For perennial crops with complex canopies, such as grapevine, the use of these devices is still difficult, especially in field conditions, and there is less experimentation in this area. However, the principle of using indirect measurements to quantify physiological traits is suitable for grapevine phenotyping. This approach is getting more attention in parallel with the increasing availability of the necessary technologies. The main analyses useful for phenotyping with a proximal sensing approach, especially for "stress-tolerance" purposes are Visible RGB imaging, canopy temperature and thermo-imaging, chlorophyll fluorescence and Hyperspectral imaging. Their applications are briefly described below. In the next years the major challenges for crops phenotyping will be the development and validation of new experimental methods based on the technology available nowadays in terms of sensors and data collection devices, allowing to fully explore the potentials of these new technologies.

1.5.1. Visible RGB imaging

Shoot growth, leaf area and yield are important agronomical and morphological parameters related to plant vigor. The classical measurement methods consist in weighing or measuring shoot elongation and yield and/or weight or measure leaf area. Despite the ease of these measurements they are very time-consuming and inadequate for large scale experiments.

RGB imaging analysis has shown to be an efficient method to assess leaf area and also yield in grapevine (Mabrouk and Sinoquet, 1998; Nuske et al., 2011; Arnò et al., 2013; Diago et al., 2012; Dogan et al., 2018). Image analysis performed automatically after selection of representative pixels for each category such as "leaves" "wood" or "grapes", in comparison with destructive methods showed high values of R^2 for leaves and for yield (Diago et al., 2012). RGB also proved to be a feasible strategy to estimate yield. Berry detection was based not only on the color but also on berries geometry, specifically the radial symmetry, to distinguish them from the background even when green (Nuske et al., 2011). More recently Munitz et al. (2019) established

a relationship between Leaf Area Index (LAI) and the crop coefficient (Kc), obtained as the ratio between ET_c, the actual crop evaporation and ET_0, the reference evapotranspiration. They measured LAI with a canopy analysis system based on 64 quantum sensors sensitive to Photosynthetic active Radiation (PAR), and compared the results with a destructive measurement using an area meter. They found a high correlation between the two methods and also between LAI and K_c.

RGB images have also been used to estimate the whole plant leaf area (LA) and fresh biomass (Couple-Ledru et al., 2014). The use of RGB images has also proved to be effective as a simple and inexpensive method of calculating single leaf area, which consists of counting the pixels using a Photoshop program tool and then apply a proportion between the pixels value and the pixels relative to figures with a known area (Doğan et al., 2018). LAI was also successfully estimated with a laser sensor (Arnò et al., 2013), the authors obtained good correlations between LAI and canopy volume, as well as between LAI and tree area index.

1.5.2. Canopy and leaf temperature

Infrared thermography or canopy temperature also shows potential for plant/crop phenotyping in both greenhouse and field crops and assess their response to drought (Jones, 2002; Maes and Steppe, 2012; Costa et al., 2013). Stomatal behavior is correlated with the plant water status and is also responsible for plant evaporative cooling, because when stomata are closed plant temperature increases. According to these principles there have been attempts to use thermal images instead of the time-consuming leaf gas exchange measurements. The temperature analysis can be performed using cameras recording electromagnetic radiation in the infrared region (Humplik et al., 2015), where most of the heat electromagnetic spectrum is located. Even if thermal imaging has proven to be adequate for different plant species and in different conditions, factors responsible of environmental variability such as wind speed, light intensity, humidity etc. could affect the representativeness of the imaging compared to the actual plant status (Costa et al., 2013). This can be minimized with the use of so-called thermal indexes.

In 2005 in a vineyard in Israel thermal and visible images were used for grapevine water status estimation (Möller et al., 2006). The devices used were a FLIR thermal imaging system and a digital camera, aluminum crosses were used to match the two types of images and an artificial wet surface was used as reference wet temperature to calculate the crop water stress index (CWSI). The spatial variability of leaf temperature, another parameter previously identified as related to water stress, was calculated (Jones, 1999; Jones et al., 2002), but in this experiment the correlation between canopy temperature variability and g_L was very weak and without statistical significance. For LAI calculation, the Gap Fraction Inversion method, based on visible image analysis was used (Cohen et al., 1997; 2000). To test the efficacy of the method several physiological parameters were also measured. The CWSI (Jackson et al., I988) index is calculated as the difference between canopy temperature and a well-watered crop temperature ($CWSI = T_{canopy} – T_{wet} / T_{dry} – T_{wet}$) and has proved to be robust in arid and semi-arid conditions but is sensitive to environmental factors such as wind and radiation (Jackson et al., 1988; Jones, 1999) and the identification of the non-water-stressed crop temperature could be problematic. A development of this method (Clawson et al., 1989) is based on the use of wet and dry reference surface temperatures. T_{dry} can be calculated as T_{air}+5 °C (Irmak et al., 2000). A high and stable correlation between CWSI and g_L, was found especially when CWSI was calculated using the temperature at the center of the canopy or its sunlight fraction. T_{wet} was calculated from the Penman-Monteith-FAO equation or by using an artificially wet canopy, with high correlation, while when T_{dry} was computed from climate data the correlation decreased during the season. A high positive correlation between g_L and ψ_{steam} during the season was also found.

In turn, Matese et al. (2015) compared the CWSI obtained from proximal and remote thermal sensing with physiological parameters in the varieties Vermentino, Caberent and Cagnulari. The authors found a high correlation between CWSI and Pn and Fv'/Fm' in all the varieties. Other reports emphasize the fact that canopy size and architecture and leaves orientation can be responsible for temperature variations with the same stomatal conductance (Grant et al., 2006). A very important development is the use of low cost equipment (e.g. thermal camera connected to a smartphone) to calculate several water status indices, including CWSI and the conductance index (Petrie et al., 2019). The same approach was used in a research in South Australia, with promising results for the development of smartphone-based sensors methodology to evaluate grapevine water status (Skewes et al., 2018). A comparison between a low cost thermal camera connected with a smartphone and a conventional one has also been successfully performed on almond (García-Tejero et al., 2018).

1.5.3. Chlorophyll fluorescence

The emission of chlorophyll fluorescence is a process through which plants protect the photosynthetic apparatus by dissipating energy that exceeds the photosynthetic demands. The antennae system of photosystem II is the first CF emission source (Dobrowski et al., 2005).The intensity of CF is variable over time depending on the photosynthetic activity and has been used to estimate plant stress, PSII efficiency, quantum yield and electron transport rate. At leaf level it is measured with a class of instruments called pulse amplitude modulating fluorometers. The measurement consists in exposing the leaves to light at low wavelength in the visible range and the successive registration of the re-emitted light at long wavelengths after applying a light pulse for a short time. It is a widespread analysis in plant physiology with which it is possible to determine parameters related to photosynthesis activity, also it is useful to biotic and abiotic stress susceptibility evaluation (Walter et al., 2012).

The photosynthetic light-harvesting apparatus is often damaged by cold, heat and UV stress and this makes chlorophyll fluorescence analysis a useful tool for screening for stress tolerance (Furbank and Tester, 2011). Cold and heat stress affect the photosynthetic apparatus and membrane properties and thus can rapidly cause changes in chlorophyll fluorescence (Berry and Biorkman, 1980). Because of its easiness of measurement, the most common parameter used in studies of stress resistance is dark-adapted Fv/Fm, which represents the maximum intrinsic photochemical efficiency of light harvesting in photosystem II. Other parameters which can be obtained from chlorophyll fluorescence analysis are the electron transport rate (ETR) and the non-photochemical quenching (NPQ), which is related with the dissipation of energy as heat and is a valid indicator of the photoprotection mechanism in the photosynthetic apparatus; it has been frequently used as a stress indicator in model plants and also crop species (Baker, 2008). Chlorophyll fluorescence induction (CFIN) is a method widely used in physiology research related to photosynthesis because it provides several information and is cheap and non-destructive at the same time (Humplik et al., 2015).

CF can be measured at canopy or plant level by fluorescence imaging obtained with laser or flash lamp to induce CF, the small spatial extension of the laser pulse and the difficulties to control spatial variability still don't allow an application on large spatial scales (Dobrowski et al., 2005). A parameter related to CF is the steady-state fluorescence (Fs), the fluorescence emitted in the range between 600 nm and 800 nm under constant illumination without saturating flashes. Currently, researchers are exploring the link between Fs and plant photosynthetic status; there are evidences of a strong positive correlation between Fs and water and irradiance-related parameters such as stomatal conductance and CO_2 assimilation (Flexas et al., 1999; 2000; 2002).

The use of chlorophyll fluorescence imaging (CFI) allows the study of spatial and temporal heterogeneities in fluorescence emission patterns at the level of cells, leaves or whole plants with a potential use in stress detection and genotype screening for breeding programs (Gorbe and Calatayud, 2012). CFI is useful to asses stomatal patchiness and photosynthetic activity heterogeneity (Omasa and Takayama, 2003), overcoming the problems of punctual measurements due to the high variability at leaves level (Ehlert and Hincha, 2008). A review for the use of CFI in horticulture was proposed by Gorbe and Calatayud (2012) in order to explore the usefulness of CFI for stress detection in greenhouse and field conditions, postharvest fruit and flower quality evaluation and phenotyping for stress tolerance. There are several reports of successful uses of fluorescence indexes for water stress detection in tobacco leaves (Lang et al., 1996), bean leaves (Lichtenthaler et al., 2000; 2005) and roses (Calatayud et al., 2006) and also of correlations between fluorescence and heat and light stress effects on the photosynthetic apparatus.

1.5.4. Hyperspectral imaging

Some important photosynthesis-related parameters can be investigated through the spectral composition of the light reflected by the plant canopy. The principle is that color differences are related to chlorophyll, carotenoids and or/nitrogen content, vigor, water status and other parameters (Walter et al., 2012), in particular the reflectance analyzed in the visual, near-infrared, and short wavelength infrared spectrum (SWIR) reflectance is used for the estimation of plant's water status. These measurements initially used for remote sensing analysis result to be very suitable for phenotyping (Humplink et al., 2015), the main limitation, as for the canopy temperature analysis, is the spatial variability to which the plants are subjected during the measurement. SWIR measurements are at the basis of indices like the normalized difference vegetation index (NDVI), an estimator of the chlorophyll content, and the proportion of chl *a* in relation to chl *b*, and the photochemical reflectance index (PRI) which allows to estimate the photosynthetic efficiency by measuring the redox status of carotenoids (Humplik et al., 2015) that is part of the non-photochemical de-excitation pathway (Demmig-Adams and Adams, 1992), and in turn is correlated with photosynthetic light use efficiency (Gamon et al.,1992; Peñuelas et al., 1995).

A portable apparatus for NDVI ground-based measurement was tested for estimation of vine vigor by vine leaf area index (VLAI; Drissi et al., 2009), and the authors report that the sensor is adequate to estimate vine vigor as VLAI and canopy gap but only until the canopy growth doesn't saturate the response.

An experiment has been carried out to verify if a simple reflectance index, calculated in the red-edge spectral region could be adequate to track changes in steady-state chlorophyll fluorescence due to heat and water stress (Dobrowsky et al., 2005). In the experiment the link between reflectance and physiological parameters was evaluated. During the experiment, after imposing water and heat stress the authors measured simultaneously leaf gas exchange parameters and Fs. They calculated two indices based on reflectance measurements: R690/R600 and R740/R800, where R690 and R740 are the chlorophyll fluorescence emission peaks and R600 and R800 are bands not affected by chlorophyll fluorescence. Both indices had a strong positive curvilinear relation with Fs.

Both proximal and remote sensing technologies have undergone great developments in recent years, the attention of the scientific and production communities towards them is high due to their potential in the analysis and subsequent management of variability in the field. These technologies are constantly being developed and improved. Although their main application, especially in viticulture, is in the agronomic management of the vineyard, they are also potentially very promising for phenotyping work. For this purpose, it will be necessary to deepen and clarify

the link between the indirect measurements obtained by the sensors and the physiological parameters under examination, which sometimes is still doubtful. The following step would be to develop specific protocols for the use of these technologies for grapevine phenotyping in greenhouse and in field conditions regarding traits closely related to physiologically complex phenomena such as that of resistance to abiotic stress.

1.6. Objectives of the work

The objectives of the experimental work are the following:

- to verify the existence of genetic variability within Arinto variety and to quantify its consistency for the analyzed traits, including agronomic traits such as yield and pruning weight and traits related to tolerance to water and heat stress;

- to identify the clones or the group of clones with the most suitable characteristics to tolerate abiotic stress conditions;

- to investigate the existence of correlations between the traits, to validate the experimental approach, namely the use of indirect measurements;

- to identify the traits and indices more adequate to set up a selection process aiming at increasing tolerance to abiotic stress.

2. Material and methods

2.1. Field location and set up

The field trial is located in the experimental center of PORVID in Pegões(38°38'54.9"N 8°38'38.2"W; Setúbal district, Southern Portugal), and is laid out in a resolvable row-column design consisting of six resolvable replicates (Figure 2), with incomplete blocks in row and column direction within each resolvable replicate (complete block). The climate of the region is Mediterranean with hot and dry summers and mild winters. The average annual rainfall is 550 mm, 400 of which usually fall during autumn and winter. The soil is mostly sandy with some clay-rich spots and is derived from podzols (Santos et al., 2007). The rows have a Northeast – Southwest orientation.

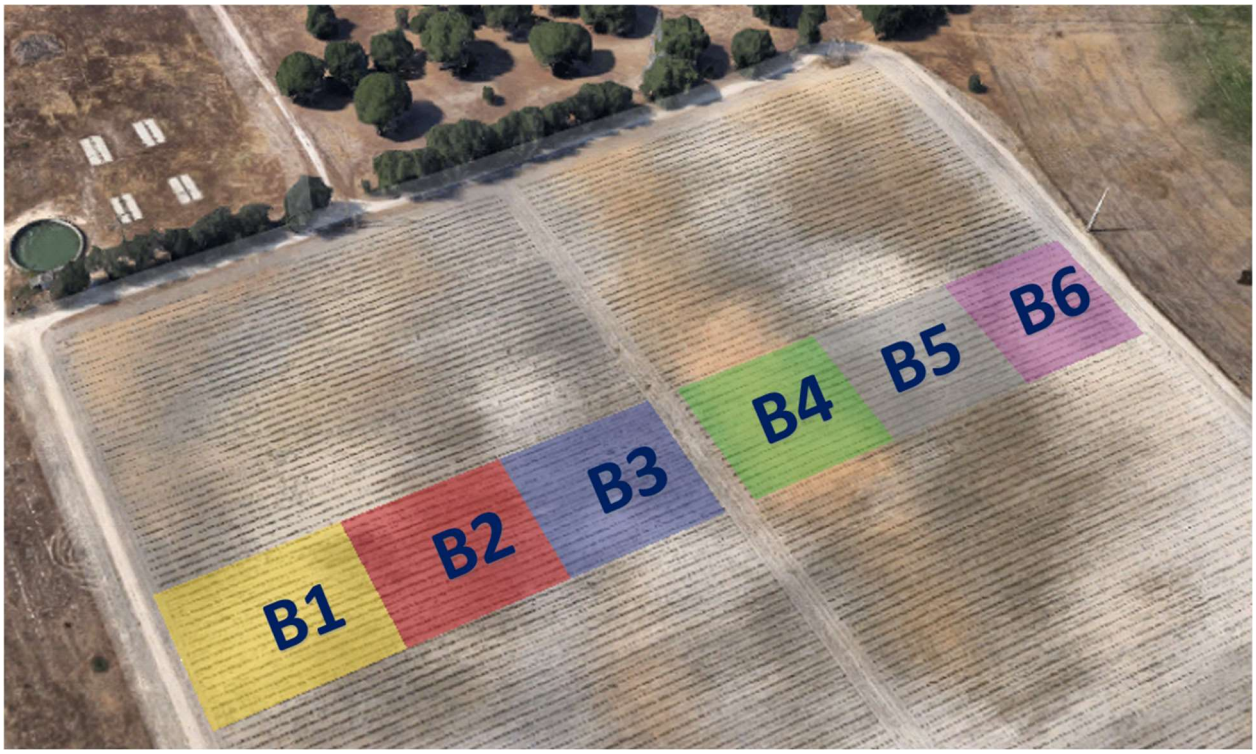


Figure 2. PORVID experimental field with a representation of the six resolvable replicates.

2.2. Climatic data

Meteorological data were recorded from the nearest weather station, which is located in Faias, about 15 km from Pegões experimental field (Figure 3). The annual rainfall for 2019 was 401.00 mm which is in line with the average annual rainfall of the region. Most of the precipitation was recorded from October to December while only about 5 mm of rain fell from the beginning of June to the end of August. The highest maximum and medium average temperatures were recorded in August with a maximum temperature of 31.03 °C and a medium temperature of 22.53 °C, followed by September and July with maximum average temperatures of 29.67 °C and 29.20 °C respectively, while the mean temperature was 22.08 °C in July and 21.27 °C in September. In July there were eleven days with maximum temperature above 30 °C. The two hottest days of the season were July 10th and July 11th with a Tmax of 35.6 °C and 38.0 °C respectively. In August, 17 days had a Tmax above 30 °C and five days above 35 °C. The coldest month was January with an average Tmin of 3.29 °C.

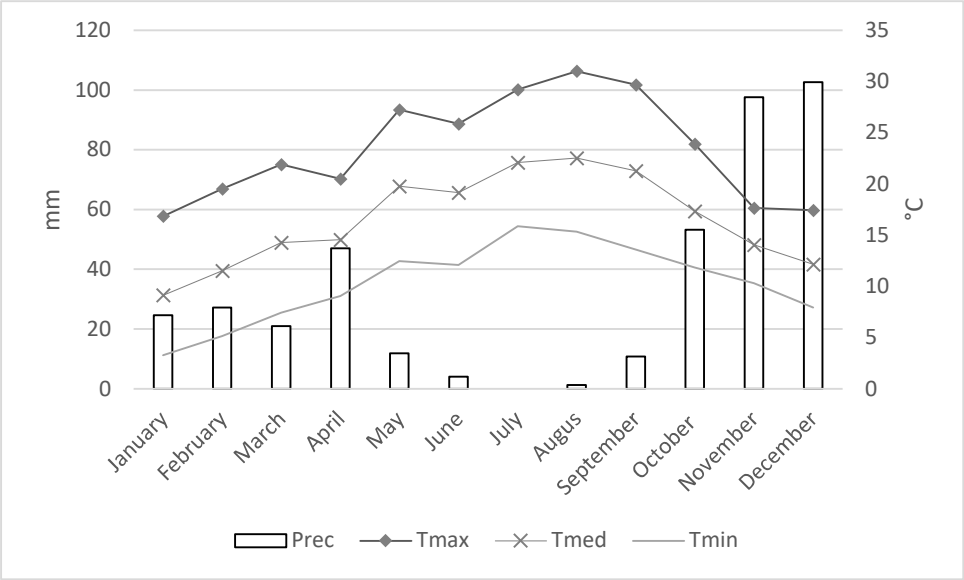


Figure 3. Monthly total rainfall and average maximum, minimum and medium day temperatures (source: weather station of Faias, IPMA).

2.3. Plant material

In this work 168 different genotypes/clones of Arinto were evaluated. For each clone three consecutive plants are present in each plot of each complete block. The genotypes belong to the first stage of selection (random sampling of the intravarietal variability) - explained in section 3. This field trial belongs to the second stage of the selection methodology (Figure 1). All the plants are the same age and are grafted onto 1103 Paulsen rootstock, trained as bilateral cordon with vertical shoots position and pruned and managed with standard procedure and without irrigation. The non-completely formed plants and the ones visually recognizable as affected by diseases were not included in the experiment.

2.4. Data collection

2.4.1. Physiological parameters measured in the field

The measurements related to the physiological condition of the plants, namely indicators of possible stress conditions were chlorophyll content (through SPAD), PRI index, NDVI index and surface leaf temperature (SLT).

All the measurements were carried out during the months of July and August 2019 and were performed on all the repetitions in all the clones in the shortest time possible, and proceeding incomplete block by incomplete block within each complete block to reduce environmental variability. All the leaf parameters were measured on leaves chosen using the same criteria: one fully developed and fully exposed leaf per plant, in the central region of the canopy. The measurements were performed in the hottest hours of the day, between 11 AM and 16 PM, in days with clear sky, intense solar irradiation and absent or weak wind. Chlorophyll content, PRI

and NDVI were measured at the same time for each leaf and the results were exported to a spreadsheet for analysis.

2.4.1.1. Chlorophyll content

Chlorophyll content was indirectly measured at leaf level with the portable Chlorophyll content meter CL-01 (Hansatech Instruments Ltd, Pentney, King's Lynn, Norfolk, UK). The device is equipped with 2 LED light sources at 620 nm and 940 nm, the relative chlorophyll content range varies between 0 and 2000 units. Calibration and temperature compensation are automatic. Chlorophyll content is expressed in logarithmic scale through the SPAD index.

2.4.1.2. PRI

The photochemical reflectance index was measured at leaf level using a Plant Pen PRI 200 (Photon System Instrument, Drásov, Czech Republic). The device has an internal dual wavelength light source and measures in two narrow wavelength bands, centered close to 531 nm and 570 nm with a +/- 5 nm tolerance. The PRI index is defined as:

$$PRI = \frac{(p531 - p570)}{(p531 + p570)},$$

where p531 is the reflectance at 531nm and p570 is the reflectance at 570nm. A calibration program is also included in the device and the need for calibration is indicated by the instrument itself.

2.4.1.3. NDVI

Normalized Difference Vegetative Index was measured by a device similar to the one used for PRI, the Plant Pen NDVI 300 (Photon System Instrument, Drásov, Czech Republic), this instrument has an internal dual wavelength light source, one in the visible (VIS: 625 nm – 645 nm) and one in the near infra-red (NIR: 750 nm – 760 nm), the tolerance is +/- 5 nm. NVDI is calculated from individual measurement as:

$$NVDI = \frac{(NIR - VIS)}{(NIR + VIS)}.$$

Calibration modality and frequency are indicated by the instrument itself.

2.4.1.4. Surface Leaf temperature

Individual surface leaf temperature (SLT) was measured using an infrared thermometer SCANTEMP 440 (Dostmann Electronic, Wertheim-Reich-olzheim, Germany), a non-contact infrared thermometer. For each leaf, selected as previously explained, ten consecutive measurements were performed to overcome the instrumental error. Temperature measurements were performed under the same conditions as the previous parameters, measuring incomplete block by incomplete block to manage environmental variability. Data were also exported to a spreadsheet for analysis.

2.4.2. Yield evaluation

Harvest was performed on the 6th of September, in parallel with yield measurement. The harvest date was chosen according to Brix and sensorial analysis. All the bunches from every plot,

consisting of three plants per plot, were harvested in individual boxes and then weighted, so that the yield of each clone was not an estimation but the actual yield. During the harvest the actual number of plants harvested was registered, as sometimes a plant is missing or malformed. In the data analysis that followed the yield per plot was divided by the actual number of plants present in each plot to obtain the mean yield per plant.

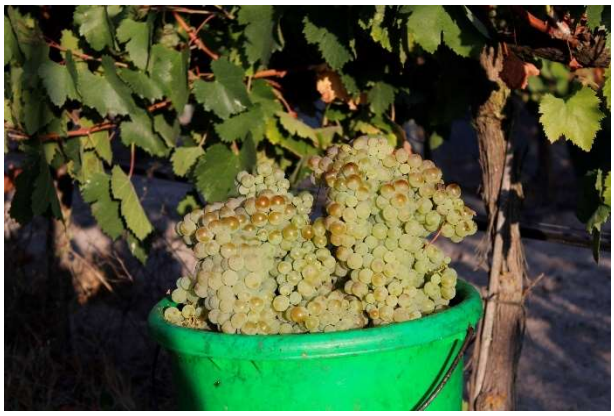



Figure 4 and 5. Yield evaluation in the field trial of Arinto located in the experimental center of PORVID in Pegões.

2.4.3. Pruning weight evaluation

Pruning was performed on January 22nd 2020. The plants are trained in a double permanent cordon, for each vegetative point two buds were left for the next year production, the remaining part of the canopy, consisting of shoots and lateral shoots was pruned and weighted. Shoots from plants in the same plot, corresponding to the same clone were weighted together and the final weight was then divided by the number of plants. Pruning weight was measured in three repetitions.


Figure 6 and 7. Pruning weight evaluation in the field trial of Arinto located in the experimental center of PORVID in Pegões.

2.5. Data analysis

2.5.1. Statistical model

According to the available literature, linear mixed models are the most suitable for data analysis from initial trials for selection within ancient grapevine varieties (Gonçalves and Martins, 2012). In matrix notation, the general expression for a linear mixed model is:

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{e}$$

where $\mathbf{y}$ is the $\mathrm{n} \times 1$ vector of observations; $\boldsymbol{X}$ is the $\mathrm{n} \times \mathrm{p}$ design matrix of fixed effects; $\boldsymbol{Z}$ is the n×q design matrix of random effects; $\boldsymbol{u}$ is the q×1 vector of random effects and $\boldsymbol{e}$ is the n×1 vector of random errors. Vectors $\boldsymbol{u}$ and $\boldsymbol{e}$ are assumed to be mutually independent with multivariate normal distribution, with mean zero and variance-covariance matrices $\boldsymbol{G}$ and $\boldsymbol{R}$, respectively. As a consequence, vector $\boldsymbol{y}$ has multivariate normal distribution, with mean $\boldsymbol{X\beta}$ and variance covariance matrix $\boldsymbol{V} = \boldsymbol{ZGZ^T} + \boldsymbol{R}$ ($\mathbf{Z}^{\mathrm{T}}$ is the transposition of $\boldsymbol{Z}$).

In this study, the linear mixed models fitted to the collected data followed the experimental design of the field trial, a resolvable row-column design, which can be described by the general equation:

$$y_{ijlm} = \mu + u_{gi} + u_{rj} + u_{col(r)ji} + u_{row(r)jm} + e_{ijlm} \quad (2)$$

for $i = 1, \cdots, g,\ j = 1, \cdots, r,\ l = 1, \cdots, s,\ m = 1, \cdots, k.$ y_{ijlm} represent the observations, μ the population mean, u_{gi} the genotypic effects (a representative sample of Arinto variety), u_{rj} the resolvable replicate effects, $u_{col(r)ji}$ the column effects within replicates, $u_{row(r)jm}$ the row effects within replicates, and e_{ijlm} the random errors associated with observations y_{ijlm}.

For yield data, the mean yield of the plot was used, therefore the fitted model was the exact model (2). For physiological traits (NDVI, PRI, SPAD and SLT) individual measurements on different leaves were made, as a result in the fitted models the effect of the plot, u_{plot}, was added. In all cases, model effects (with the exception of μ) were assumed independent and identically distributed normal variables with zero mean and respective variances σ_g^2, σ_r^2, $\sigma_{col(r)}^2$, $\sigma_{row(r)}^2$ σ_{plot}^2 and σ_e^2. All random effects were assumed mutually independent. As a consequence of this model, in the same random effect the observations are correlated (Searle et al., 1992; McCulloch et al., 2008).

2.5.2. Genetic parameters estimation

Residual maximum likelihood (REML) estimation method was used for covariance parameter estimation (Patterson and Thompson, 1971). Empirical Best Linear Unbiased Estimators (EBLUE) of fixed effects and Empirical Best Linear Unbiased Predictors (EBLUP) of random effects were obtained from mixed model equations (Henderson, 1975). The EBLUPs of the genotypic effects obtained for each evaluated traits were ranked and used to perform selection.

The genotypic variance component was tested (hypotheses: $H_0: \sigma_g^2 = 0\ vs\ H_1: \sigma_g^2 > 0$) using a residual maximum likelihood ratio test. This test compares two models: model without genotypic effects (H_0) and model considering genotypic effects (H_1). Because the null hypothesis was on the boundary of the parameter space, the p-value of the test was assumed to be half of the reported p-value from the Chi-squared distribution with one degree of freedom (Self and Liang, 1987; Stram and Lee, 1994).

Additionally, other genetic indicators were computed: (1) genotypic coefficient of variation ($\mathrm{CV_G}$) and (2) a generalized measure of broad-sense heritability ($\mathrm{H^2}$).

The $\mathrm{CV_G}$ is the ratio between the estimate for the genotypic standard deviation ($\hat{\sigma}_g$) and the overall mean. It is a relative measure of variability, therefore it is useful to compare genetic variability among traits,

$$CV_G = \frac{\hat{\sigma}_g}{\text{Overall Mean}} \times 100.$$

Broad sense heritability *(H^2)* is necessary to evaluate how much phenotypic variability is due to genetic causes, and consequently, to evaluate the potential success of genetic selection (as it reflects the relationship between true and predicted genotypic effects). The classical formula for broad sense heritability is

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{e/r}^2}$$

where σ_g^2 is the genotypic variance and $\sigma_g^2 + \sigma_{e/r}^2$ is the phenotypic variance at the level of the mean of the genotypes. However, this formula is only applicable for balanced data and models with one random effects factor with diagonal variance-covariance matrix. As such conditions were not satisfied in our analysis, a generalized measure of broad-sense heritability (H^2) (Oakey et al., 2006; Gonçalves et al., 2013) was considered,

$$H^2 = \frac{\sum_{i=1}^{q_1}\left(1 - \frac{PEV_i}{\hat{\sigma}_g^2}\right)}{t}$$

where PEV_i is the predicted error variance of the genotypic effect for genotype i ($i = 1, \ldots., q_1$), $\hat{\sigma}_g^2$ is the genotypic variance component estimate, and $t = q_1 - 1$.

For each trait, the predicted genetic gain of polyclonal selection was computed as the mean of the EBLUPs of the top-ranked 15 selected clones.

2.5.3. Relation between the traits studied

2.5.3.1. Correlation between traits

The Pearson correlation coefficient (r) was used to study the linear correlation between the predicted genotypic values of two traits.

2.5.3.2. Differences in yield among four classes of SLT, PRI and NDVI

For SLT, PRI and NDVI, the clones were divided into four classes corresponding to the quartiles of the distributions, and for each one the mean yield value was calculated (yield data of four different classes of SLT, PRI, and NDVI). In this approach, the response variable is the yield and the factor is the class of the trait (with four levels). For each trait, one-way fixed effects analysis of variance (ANOVA) was conducted. When statistically significant differences between class yield means were found, the Tukey's honestly significant difference (HSD) test was performed to obtain a more detailed information about the differences between the class yield means.

2.5.4. Software

Data analysis was carried out with R (R Core Team, 2017, The R Foundation for Statistical Computing Platform). Linear mixed models were fitted using ASREML-R software (Butler et al., 2017). To perform ANOVA and Tukey's HSD test the functions *aov* and *TukeyHSD* were used, respectively.

3. Results and discussion

3.1. Intravarietal genetic variability for the studied traits

The results regarding intravarietal genetic variability for all the studied traits are shown in Table 2. The first results obtained are the estimates of the variance components and the related standard errors. For NDVI, PRI, SPAD and yield the highest variance value is associated with the error, and the lowest with the row within replicate; for SLT the highest variance is the replicate's variance, and the lowest is associated to the error. The genotypic variance is the second highest for yield, SPAD and PRI, while it is the 4th and the 5th for SLT and NDVI respectively. In general, these results reflect the experimental design adopted. For example, the higher replicate's variance for SLT reflects the differences in air temperatures among the days of the measurements. Also, there is a strong relationship between the genotypic variance estimates and the values of broad-sense heritability.

Considering the genotypic variance component for NDVI, PRI, SPAD and yield, the null hypothesis was rejected (p-value<0.001), stating that there is genotypic variability relative to these traits, while for pruning weight no genotypic variability was observed.

Once the significance of the genotypic variability had been ascertained, the following step was to make it comparable among the various traits. For this purpose, the genotypic coefficient of variation (CV_G) was calculated. Comparing the obtained results, it emerges that the trait with the greatest genotypic variability, with the highest CV_G, corresponds to yield. This is consonant with bibliographic sources that previously had identified yield as a trait with high variability among clones (Martins and Goncalves, 2015).

Regarding a clonal selection focused on lower susceptibility to heat and water stresses no literature is available for grapevine, while regarding other crops, four cotton genotypes analyzed for drought resistance had coefficient of variation (*CV%*) values of *circa* 6.6 for chlorophyll content under moderate and severe drought (de Brito et al., 2011). In lima bean subjected to water and salt stress a coefficient of variation of 7.69 was reported for SPAD under drought and of 12.19 under salinity (Pereira-Filho et al., 2019). A significant reduction of SPAD values in drought stress was also found in wild Tibet barley genotypes (Zhang et al., 2009).In our results, among the indexes related to physiological traits and susceptible to stress conditions, the higher CV_G is associated with PRI, immediately followed by SPAD. The parameters with the lowest genotypic variation coefficients are SLT and NDVI.

In the perspective of a genetic selection process, a higher coefficient of variability, together with a good heritability widens the selection possibilities for specific performances that meet specific needs of the users of the selected material. In fact, genetic variability is the "raw material" on which selection is applied, the higher the variability, the larger the breeding opportunities. Broad sense heritability provides an indication of the component of a given character due to genetic causes, it is a value that varies between zero and one. In the present work the trait associated with the highest heritability value is SPAD, followed by Yield, PRI, SLT, and NDVI (Table 2). All the values of H^2 are higher than 0.5, except for pruning weight but, as previously explained, this trait didn't show a significant genotypic variance. All the other values obtained for H^2, ranging from 0.57 to 0.67, indicate a genetic control on the traits. The obtained result for yield was expected and is in line with other previous works (Martins and Gonçalves, 2015). For physiological traits, the results constitute a new knowledge and are of fundamental importance in the perspective of an effective genetic selection. The analysis of heritability of traits related to drought stress on 140

Table 2. Variance components estimates and respective standard errors, residual maximum likelihood ratio test for genotypic variance component and associated p-value, overall mean, coefficient of genotypic variation and broad sense heritability for NDVI, PRI, SPAD, SLT (Surface leaf temperature), yield and pruning weight.

Trait	Variance component	Variance estimate	Standard error	REMLRT (p-value)	Overall mean	CV_G	H^2
NDVI	σ^2_g	0.0000535	1.094E-05	47.478 (<0.001)	0.679	1.077	0.565
	σ^2_r	0.0001291	8.797E-05				
	$\sigma^2_{row(r)}$	0.0000113	5.167E-06				
	$\sigma^2_{col(r)}$	0.0001135	2.101E-05				
	σ^2_{plot}	0.0000800	1.235E-05				
	σ^2_e	0.0003635	1.206E-05				
PRI	σ^2_g	0.0000063	1.216E-06	60.300 (<0.001)	0.0187	13.485	0.597
	σ^2_r	0.0000025	1.928E-06				
	$\sigma^2_{row(r)}$	0.0000004	4.042E-07				
	$\sigma^2_{col(r)}$	0.0000048	1.124E-06				
	σ^2_{plot}	0.0000042	1.368E-06				
	σ^2_e	0.0000523	1.736E-06				
SPAD	σ^2_g	1.7424131	2.919E-01	96.496 (<0.001)	14.275	9.247	0.674
	σ^2_r	1.3564718	9.476E-01				
	$\sigma^2_{row(r)}$	0.2236592	1.077E-01				
	$\sigma^2_{col(r)}$	1.3925592	2.891E-01				
	σ^2_{plot}	1.7185961	2.539E-01				
	σ^2_e	7.3183833	2.429E-01				
SLT	σ^2_g	1.4884611	2.851E-01	69.770 (<0.001)	31.157 °C	3.916	0.583
	σ^2_r	15.2055702	1.559E+01				
	$\sigma^2_{row(r)}$	6.0712044	1.393E+00				
	$\sigma^2_{col(r)}$	0.4127452	1.664E-01				
	σ^2_{plot}	7.2444456	3.035E-01				
	σ^2_e	0.2649085	3.351E-03				
Yield	σ^2_g	0.994924	1.750E-01	84.420 (<0.001)	4.586 kg/plant	21.752	0.636
	σ^2_r	0.461406	3.354E-01				
	$\sigma^2_{row(r)}$	0.193722	7.934E-02				
	$\sigma^2_{col(r)}$	0.483789	1.248E-01				
	σ^2_e	2.784582	1.610E-01				
Pruning weight	σ^2_g	0.0056617	0.0047111	1.5524 (0.106)	0.698 kg/plant	10.777	0.132
	σ^2_r	0.0010321	0.0025368				
	$\sigma^2_{row(r)}$	0.0049569	0.0029034				
	$\sigma^2_{col(r)}$	0.0076062	0.0035492				
	σ^2_e	0.0845698	0.0072733				

σ^2_r - replicate variance; $\sigma^2_{row(r)}$ - row within replicate variance; $\sigma^2_{col(r)}$ - column within replicate variance; σ^2_g - genotypic variance; σ^2_{plot} - plot variance; σ^2_e - error variance. REMLRT – value of Residual Maximum Likelihood Ratio Test statistic; CV_G - Coefficient of genotypic variation; H^2 - Broad sense heritability.

genotypes of *Arachis hypogea* L. reports a broad sense heritability of 0.27 for chlorophyll content measured through SPAD (Painawadee et al., 2009). The authors also found that most of the drought resistance traits were not correlated with yield. Among the authors' conclusions it emerges that the heritability was considered low to moderate, implying potentially poor results in terms of breeding for stress resistance.

According to these first results the parameters that appear to be more suitable for a selection process are yield, PRI and SPAD. PRI and SPAD results seem to be the most promising for drought and heat stress resistance selection.
Nowadays the most important objectives in grapevine breeding are related to resistance to biotic and abiotic stresses. For the type of abiotic stress we have focused on, namely resistance to heat and drought, it could also be interesting to study intravarietal variability in terms of phenology. However, obtaining the necessary data is complicated by the breadth of the experimental design and the number of clones used.

3.2. Predicted genotypic values

The following step in processing the results was to calculate the empirical best linear unbiased predictors (EBLUP) of the genotypic effects for each trait. These values allow the understanding of how much of the calculated genotypic variability actually affects the traits of each clone (the deviations from the overall mean explained by genetic causes). With the EBLUPs of genotypic effects, the predicted genotypic values were obtained. For a given clone and trait, the predicted genotypic value (PGV) is the predicted value of the trait due to genotypic causes, that is, the predicted value of the trait in the absence of environmental deviations. These values allow us to make a comparison between clones regarding their actual genotypic potential for each trait. The complete lists of clones with the respective EBLUPs of genotypic effects and PGVs for NVDI, PRI, SPAD, SLT and yield are shown in annex 1.

Table 3 shows a classification of the first and the last fifteen clones in terms of predicted genotypic values for all the parameters analyzed. On the basis of these rankings it is possible to set a selection process, for example choosing the group of clones with the highest or lowest yield, or according to their performances in terms of photosynthetic activity. From a quick visual comparison of the classifications, a similar behavior of the clones for the photosynthesis-related indexes is recognizable. In fact, at the first 15 positions there are several recurring clones for NDVI, PRI and SPAD. The relationship between the various parameters is further analyzed.

Considering yield and SLT, the first thing that emerges is the result associated to clone AR2601: it revealed the lowest SLT and the highest yield. Additionally, the clone AR2651 is in sixth position for SLT and third for Yield, while the clone AR40509 is in tenth position for both SLT and Yield, and AR2572 is in the fourteenth position for SLT and second for yield. But several other clones are among the first fifteen for different traits. For example, the clone AR4101 appears in the top 15 positions for NDVI, PRI, SPAD, and yield. The first 4 clones for SLT and Yield are among the top 15 at least for one of the other traits studied. Altogether 22 clones are present among the first 15 positions in at least two of the traits analyzed.

Table 3. Ranking of the first and last 15 clones for each trait according to their performance based on the predicted genotypic value (PGV).

Rank	NDVI		PRI		SPAD		SLT		YIELD	
	clone	PGV	clone	PGV	clone	PGV	clone	PGV	clone	PGV
1	AR41205	0.6899	AR11203	0.0218	AR2672	16.594	AR2601	28.531	AR2601	7.268
2	AR40507	0.6880	AR0498	0.0218	AR3204	16.351	AR40105	28.759	AR2572	6.310
3	AR2521	0.6875	AR41206	0.0216	AR41203	16.263	AR2612	28.958	AR2651	6.280
4	AR41203	0.6874	AR4104	0.0215	AR6118	16.232	AR41206	29.006	AR2521	6.115
5	AR3203	0.6870	AR1632	0.0214	AR40507	16.172	AR2671	29.262	AR1501	6.102
6	AR2019	0.6866	AR11313	0.0214	AR12601	16.153	AR2651	29.322	AR11201	6.088
7	AR2424	0.6864	AR4101	0.0213	AR3504	16.058	AR41201	29.476	AR3020	5.943
8	AR4104	0.6861	AR2410	0.0211	AR3112	15.987	AR3402	29.633	AR12401	5.920
9	AR11306	0.6860	AR12609	0.0211	AR4101	15.966	AR10505	29.672	AR2424	5.904
10	AR2403	0.6859	AR10702	0.0211	AR10702	15.941	AR40509	29.811	AR40509	5.902
11	AR3204	0.6857	AR0459	0.0210	AR2692	15.818	AR2662	29.830	AR4101	5.885
12	AR11201	0.6854	AR12601	0.0209	AR11202	15.774	AR3701	29.930	AR3501	5.879
13	AR4101	0.6852	AR3112	0.0209	AR2612	15.736	AR11202	29.935	AR12502	5.849
14	AR2410	0.6849	AR3502	0.0208	AR40105	15.702	AR2572	30.007	AR2642	5.712
15	AR3502	0.6846	AR2671	0.0207	AR2641	15.684	AR11205	30.013	AR41305	5.704
...										
151	AR3808	0.6719	AR12502	0.0165	AR3808	12.739	AR41207	32.460	AR3105	3.482
152	AR41303	0.6718	AR3701	0.0164	AR3402	12.731	AR2664	32.555	AR3807	3.358
153	AR3305	0.6717	AR3305	0.0161	AR10501	12.729	AR8204	32.591	AR3812	3.353
154	AR3810	0.6717	AR10502	0.0161	AR12604	12.706	AR41202	32.613	AR2693	3.335
155	AR0503	0.6716	AR0503	0.0160	AR6116	12.457	AR2511	32.751	AR10502	3.331
156	AR3103	0.6715	AR11502	0.0160	AR1510	12.385	AR2574	32.759	AR10504	3.305
157	AR10207	0.6714	AR3810	0.0160	AR10207	12.382	AR3807	32.787	AR10503	3.219
158	AR3901	0.6714	AR10501	0.0157	AR10503	12.226	AR11603	32.838	AR11310	3.217
159	AR6116	0.6671	AR3808	0.0155	AR12607	11.906	AR10312	32.903	AR11501	3.166
160	AR10503	0.6661	AR11308	0.0149	AR11502	11.799	AR3605	32.960	AR2671	3.154
161	AR11304	0.6660	AR11304	0.0148	AR3305	11.712	AR2425	32.973	AR40507	2.950
162	AR11502	0.6625	AR10503	0.0132	AR10804	11.596	AR1117	33.224	AR10312	2.923
163	AR10804	0.6622	AR10504	0.0126	AR11304	11.533	AR3105	33.381	AR6118	2.877
164	AR10504	0.6581	AR10804	0.0106	AR10504	11.091	AR3601	33.500	AR10804	2.861
165	AR10312	0.6489	AR10312	0.0064	AR10312	10.990	AR12607	33.571	AR2701	2.814

3.3. Empirical density functions of the empirical best linear unbiased predictors of the genotypic effects for each trait

Figure 8 shows a representation of the empirical distribution of the empirical best linear unbiased predictors (EBLUPs) of the genotypic effects for each trait. Except for the presence of a few outliers, it is clear that for all the traits these predictors are distributed according to a curve similar to a normal one and rather symmetrical, and with mean zero.

This finding indicates the validation of the model assumptions concerning genotypic effects and allows an in-depth analysis of the relationship between the different parameters.

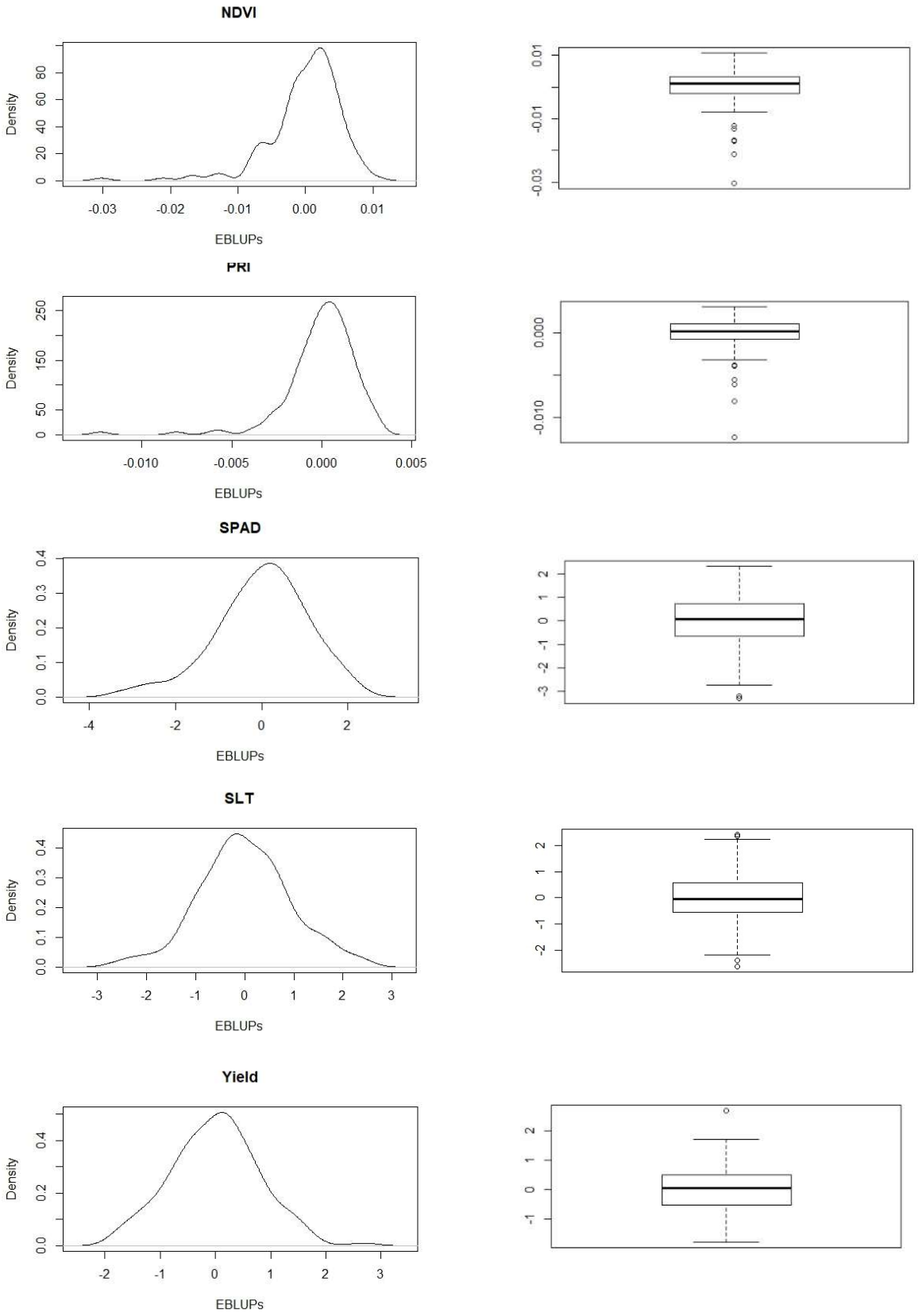


Figure 8. Empirical density function of EBLUPs of the genotypic effects for NDVI, PRI, SPAD, SLT and yield.

The analysis of the clones with EBLUPs of genotypic effects values external to the population's distributions revealed that for NDVI and PRI three of the four outliers are the same for the two traits (clones AR10312, AR10504 and AR10804). Among the latter clones are the two that also hold the position of outliers for SPAD (clones AR10312 and AR10504). The SLT distribution has four outliers, one of which (clone AR2601) is also an outlier of the distribution for yield. The other outliers not shared between the different distributions are AR11502 for NDVI, AR10503 for PRI, and AR40105, AR3601 and AR12607 for SLT.

The presence of these outliers, and the fact that some of them are the same for different traits, requires to formulate hypotheses about their nature, among which one possible, although in need of further confirmation, is that they do not belong to the Arinto variety, i.e., at the time of prospection they were considered as Arinto variety while instead they belong to other variety. This result recommends a new observation in the field focused on ampelography and, in the case of doubt, this hypothesis can be definitively confirmed or denied through molecular analysis. This operation will probably be carried out to clarify any doubt before future work, but given the lack of certainty during the execution of the current study, the correctness of the previous varietal identification has not been questioned and the aforementioned clones have been considered as part of the Arinto variety.

3.4. Genetic gains for polyclonal selection

The purpose of this work is to study the genetic variability of a population of clones for traits connected to stress tolerance and to give an overall description of the clones' performances. The ultimate goal of such a work is to perform a selection process allowing the use of genetic variability to benefit the needs of winemakers and viticulturists. Although the ranking of clones' performances allows the identification of clones with the highest and lowest genotypic values, the actual phenotypic performances of these clones are always susceptible to genotype×environment interaction and, as reported in the introduction, the stability of a group of clones rather than a single one is considerably greater. For this reason, the work focused mostly on studying the behavior of clone groups (polyclonal selection), rather than individual clones.

Table 4 shows the predicted genetic gains associated to the selection of the first 15 genotypes for the traits evaluated. The genetic gain for pruning weight was not calculated since no genotypic effects were found.

Table 4. Predicted genetic gains (R) obtained for each trait with the selection of the top 15 selected genotypes.

Trait	Overall mean	Predicted genetic Gain (*R*) Mean of the EBLUPs of the genotypic effects of the selected clones	Predicted genetic gain in percentage of the mean ***R(%)***
NVDI	0.679	0.007	1.1
PRI	0.019	0.003	13.7
SPAD	14.275	1.753	12.3
SLT (ºC)	31.157	-1.680	-5.4
Yield (kg/plant)	4.586	1.472	32.1

The highest genetic gain can be obtained through the selection of the best genotypes in terms of yield, followed by PRI and SPAD, while for SLT the value is modest, as well as for NDVI, with the lowest value. For temperature the negative value is due to the fact that the genotypes with the

lowest temperature values were chosen. These results are in accordance with the values obtained for heritability and CV_G, from which it emerges that the most promising parameters are yield, PRI, and SPAD. The most suitable parameters for a selection process seem to be once again yield, PRI and SPAD.

3.4.1. Polyclonal selection based on SLT

Table 5 shows the genetic gains for each trait that would be obtained through the selection of the first 15 clones in terms of SLT. The analysis carried out revealed that there is a significant genetic component for SLT, however, the genotypic coefficient of variation is low. Despite this value, and due to its physiological meaning, it was still decided to calculate the genetic gain relative to the other traits in the hypothesis of selecting the first 15 clones with the lower SLT. With a selection based on SLT it is possible to achieve a genetic gain of 3.6% for PRI, 4.5% for SPAD and 14.0% for yield. It therefore emerges that with this type of selection the performance of the clones can be increased for all of the most important parameters, not least the yield, which is also indicative of a better physiological adaptation of the plant and certainly a trait of interest for producers.

Table 5. Predicted genetic gains (R) for each trait with the selection of the top 15 selected genotypes for SLT.

Trait	**Overall mean**	Predicted genetic Gain (*R*) Mean of the EBLUPs of the genotypic effects of the selected clones	**Predicted genetic gain in percentage of the mean *R(%)***
NVDI	0.679	0.001	0.1
PRI	0.019	0.001	3.6
SPAD	14.275	0.648	4.5
SLT (ºC)	31.157	-1.680	-5.4
Yield((kg/plant)	4.586	0.640	14.0

3.4.2. Polyclonal selection based on PRI

When selecting the first 15 clones for PRI the genetic gain obtained is 5.0% for SPAD and 3.7% for yield (Table 6). The opportunity to use PRI to perform a selection in relation to water and heat stress resistance arises from the fact that in numerous works carried out on grapevine as well as on other crops, PRI was significantly correlated to the main photosynthetic parameters and sensitive to the different conditions of water availability. PRI is based on the xanthophyll cycle, a process by which plants dissipate energy exceeding the photosynthetic demand. A certain amount of light energy is always lost during the photosynthetic process in the form of heat and fluorescence, this process is in competition with photosynthetic electron transport so changes in the photosynthetic rate cause a complementary change in fluorescence and/or heat dissipation (Schreiber et al., 1995). For these reasons leaf pigment content evaluation can be a useful tool for leaf physiological performance estimation (Sims and Gamon, 2002). PRI has been evaluated as an indicator of efficiency of photosynthetic radiation use in seven species with C3, C4 and CAM photosynthesis and it has been found to be exponentially related to it in several species and field conditions (Penuelas et al., 1995).

To summarize the results regarding PRI already presented, a CV_G of 13.5% was found, the heritability was 0.597 and by selecting the first 15 clones the predicted genetic gain is 13.7%. Differences in PRI values between clones from a physiological point of view indicate differences

in terms of photosynthetic activity and photoprotection processes, what allows to hypothesize that the first ranked clones are better adapted to withstand stress conditions.

The use of PRI can provide non-destructive assessments of photosynthetic radiation use efficiency (Penueles et al., 1997) and it is one of the few spectral indices which has been shown to be able to highlight rapid changes in plant photosynthetic status (Gamon et al., 1992; Penuelas et al., 1997). Schreiber et al. (1995) reported variations of PRI during the day from 0.11 to 0.07 while in shaded leaves used as a control the PRI values was always 0.125, in several species.

Table 6. Predicted genetic gains (R) for each trait with the selection of the top 15 selected genotypes for PRI.

Trait	Overall mean	Predicted genetic Gain (*R*) Mean of the EBLUPs of the genotypic effects of the selected clones	Predicted genetic gain in percentage of the mean *R(%)*
NVDI	0.679	0.003	0.4
PRI	0.019	0.003	13.7
SPAD	14.275	0.710	5.0
SLT (ºC)	31.157	-0.286	-0.9
Yield (kg/plant)	4.586	0.171	3.7

Leaves from plants affected by stress have significantly higher reflectance in the visible spectrum and lower in the near infra-red compared to non-stressed plants; this is the principle that explains why NDVI index can identify stressed leaves, but PRI provides better physiological information (Penuelas et al., 1994). PRI increases until midday and then decreases until dusk and also correlates well with several physiological parameters (Penuelas et al., 1994; Gamon, 1997). However, Dobrowski, (2005) warned that that the relationship of PRI with steady state fluorescence (Fs) was masked by the high noise of the PRI values compared to other indexes (for example FRI fluorescence ratio index) in plants subjected to heat and water stress.

A selection performed on the basis of PRI finds support in the fact that PRI correlates well with important physiological mechanisms and responses to environmental factors related to temperature and water availability. From the results of the current work the genetic gain for a polyclonal selection based on this trait appears satisfactory. However, while selecting this group of clones, the genetic gain for the other traits, in particular SLT, appears modest. It is therefore a matter of deepening in physiological terms which of these two parameters should be given greater importance in terms of adaptation to environmental stress.

3.4.3. Polyclonal selection based on SPAD

The last trait considered as adequate for a polyclonal selection process is SPAD, this index is considered a quick tool to evaluate the photosynthetic performance of plants and is also reported as enabling the detection of water stress.

A reduction in chlorophyll concentration is common in plants subjected to water stress, indicating oxidative stress probably due to the photo-oxidation of pigments associated with the degradation of chlorophyll (Carlin et al., 2012), but few studies are already available regarding the accuracy of spectral indices to detect this type of stress (Lin et al., 2015).

In a recent work SPAD quantified in Sauvignon Blanc leaves had a positive correlation with chlorophyll content, allowing an efficient indirect and non-destructive estimation of the chlorophyll

content in grapevine leaves (Cantona et al., 2017). In the same study it was reported that SPAD varied from 12 to 42 (Cantona et al., 2017), confirming previous results on 13 grapevine cultivars that had set SPAD values between 11 and 41 (Fanizza et al., 1997). SPAD was found to be accurate in the estimation of leaf chlorophyll content when it is below 300 mg m^{-2} (SPAD around 30), above this level the sensitivity of SPAD to chlorophyll content decreases (Steele et al., 2008).

The Predicted genotypic SPAD values ranged from 10.99 in clone AR10312 to 16.59 in clone AR 2672, well within the values considered as well correlated with chlorophyll content (Steele et al., 2008). The mean of the first 15 clones was 16.02, while the mean of the last 15 was 12.06. The group formed by the last 15 clones had values close to the minimum values recorded for vine leaves in its vegetative cycle (Fanizza et al., 1997; Cantona et al., 2017). This finds its causes in the period in which the surveys were carried out and in the type of leaf that was chosen: the analysis was performed on leaves almost at the end of their vegetative cycle, which coincides with the period in which the vines are exposed to low water availability and heat stress. This allows to emphasize the differences between the more and the less resistant genotypes. However, with the choice of this type of leaves at this time of year there is a risk of "flattening" any genotypic differences in terms of chlorophyll content and maximum photosynthetic efficiency. The choice of method included a compromise between the goals of the study and the practicality of carrying out field surveys on a large scale and in the shortest time interval possible.

Even if is not related to the aim of this work, it is important to highlight that SPAD index is also a good predictor of leaf N concentration, and thus of vegetative vigor. SPAD values from berry set to maturity were found to be positively correlated with yield and wood production (Taskos et al., 2015).

Table 7 shows how the selection of the group of clones with best SPAD values affects the other traits, and it is possible to see that the genetic gain for NDVI is negligible, for PRI and SLT a slight increase is obtained in the group's performance compared to the population. Regarding yield, the selection of SPAD would lead to its slight decrease, compared to the entire population.

Table 7. Predicted genetic gains (R) for each trait with the selection of the top 15 selected genotypes for SPAD.

Trait	**Overall mean**	**Predicted genetic Gain (*R*) Mean of the EBLUPs of the genotypic effects of the selected clones**	**Predicted genetic gain in percentage of the mean *R(%)***
NVDI	0.679	0.003	0.4
PRI	0.019	0.001	5.7
SPAD	14.275	1.753	12.3
SLT (ºC)	31.157	-0.521	-1.7
Yield (kg/plant)	4.586	-0.072	-1.6

Looking for an index that could efficiently detect water stress in an experiment conducted on *Cinnamomum camphora* L., Lin et al. (2015) reported for the first time a significant correlation between SPAD and Chl content but they also found that SPAD is not adequate for analysis of wilting or highly water-stressed leaves. Regarding water stress on grapevine, leaf greenness evaluated through SPAD has been successfully used to analyze table grape genotypes in water stress conditions (Fanizza et al., 1991), with SPAD varying from 34 to 44 in expanded leaves of non-stressed genotypes and from 34 to 41 in stressed genotypes, while in the apical leaves values ranged from 14 to 17 in stressed plants and from 9 to 10 in non-stressed ones. The authors concluded that the most tolerant genotypes had the lowest SPAD values (Fanizza et al., 1991).

Similar results were observed in potato (*Solanum tuberosum* L.) (Rolando et al., 2015), where there was an initial increase of chlorophyll content after a drought stress, due to the decrease of leaf growth. SPAD has been tested together with temperature and other indexes as a potentially fast, easily assessed and non-destructive tool for screening drought-tolerant sugarcane plants (Silva et al., 2007). Significant decreases of SPAD were reported in drought stressed plants compared to well-watered ones, with sensitive genotypes showing the higher decreases. Leaf temperature followed a similar behavior and was *circa* 4 °C higher in drought-stressed plants than in well-watered ones, with higher values reported in susceptible genotypes. The authors conclude that the most promising traits for a rapid and non-destructive screening to distinguish between tolerant and susceptible genotypes seem to be chlorophyll fluorescence, SPAD index and leaf temperature.

It therefore seems that the SPAD index may be useful to identify different responses to stress and that the range of about 6 units between the genotype with the highest and the lowest value found in the present work is enough to distinguish tolerant genotypes from sensitive ones. The fact that by selecting for SPAD small increases in PRI and decreases in leaf temperature are obtained is undoubtedly positive, while the fact that the yield decreases slightly compared to the population can be considered positive or negative depending on the producer's demands.

3.5. Correlation between different traits

Figures 9 to 11 show the reciprocal relationships between the three reflectance indexes measured and their relationship with yield. The correlation between, SLT and yield is low and negative (Figure 9). There is a high positive correlation between PRI and NDVI, and a moderate positive correlation between SPAD and NDVI, and between SPAD and PRI (Figure 10). The correlation between these indexes and leaf temperature is less evident (Figure 11), in all the three cases negative and with coefficient of correlation (r) of about 0.20, confirming a low correlation.

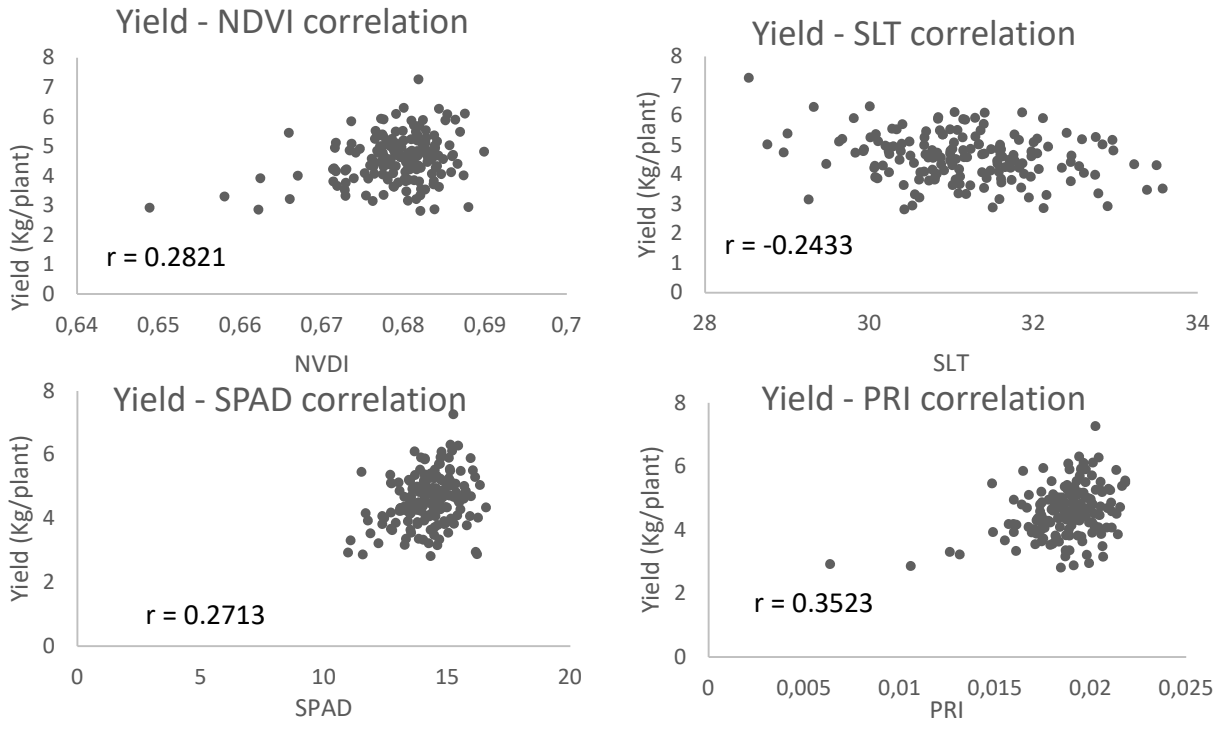
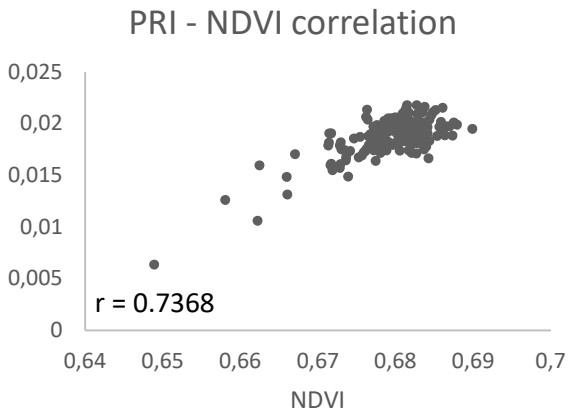
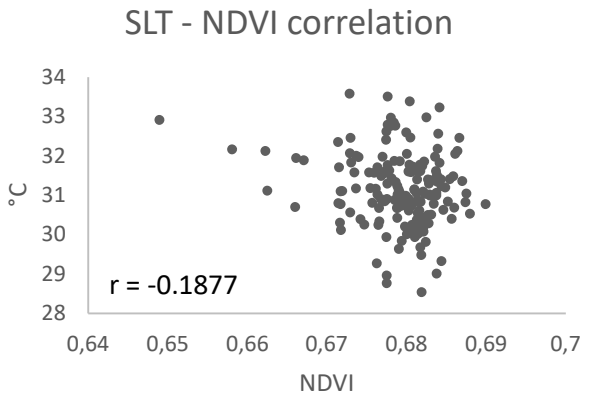
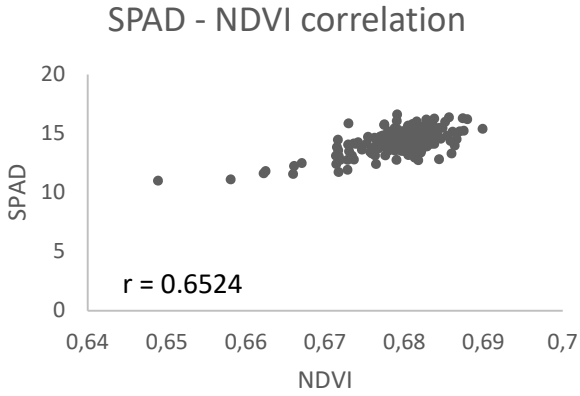
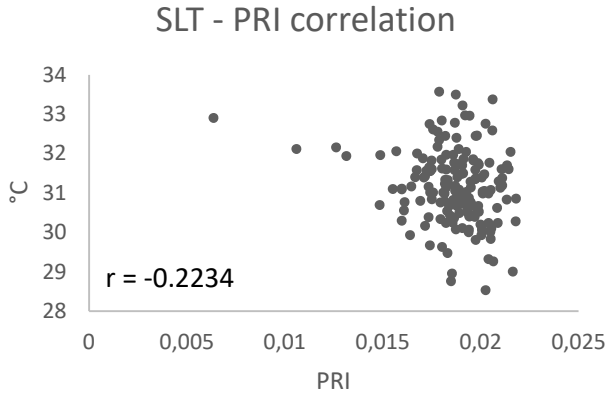


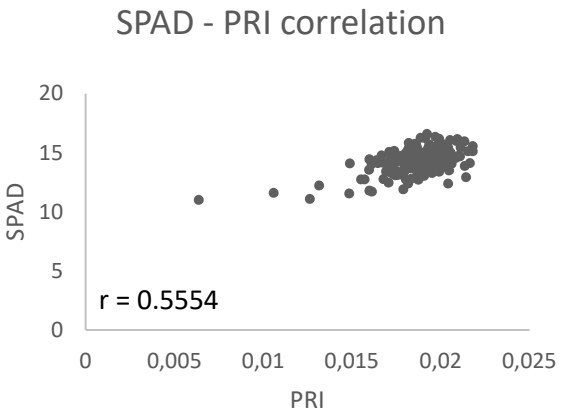
Figure 9. Correlation between the predicted genotypic values of NDVI, PRI, SPAD, SLT and Yield (kg/Plant)











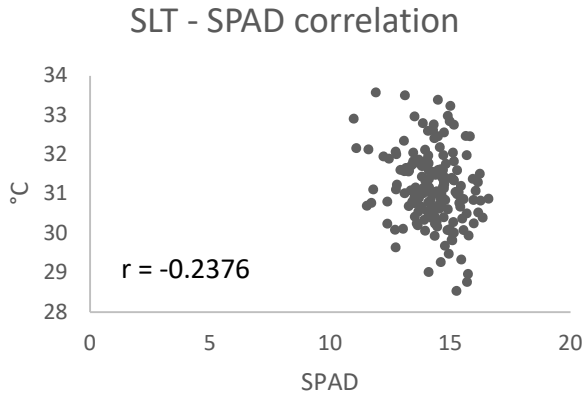


Figure 10. Correlation between the predicted genotypic values of SPAD, PRI and NDVI.

Figure 11. Correlation between the predicted genotypic values of NDVI, SPAD, PRI and SLT.

Among the three most physiologically connected indices, namely SPAD, PRI and NDVI, as expected there was a positive correlation as visible in Figure 10. All the three indices are based on pigment (chlorophyll or carotenoids) content and/or photosynthetic efficiency, they are therefore intimately connected also from a physiological and genetic point of view.

On the other hand, the correlation between the three aforementioned indices and SLT was less predictable. A weak correlation ($r < 0.3$) was detected for all three indices with SLT. Maybe this occurred because NDVI, PRI, and SPAD respond to changes in water, heat and radiative state

of the plant in the medium-long term, while SLT provides an "instantaneous" data, with high levels of fluctuation during the same day, for this reason this parameter may be suitable make comparisons between different genotypes on short intervals of time, but not to express the overall response of the plant to heat and water conditions over the long term.

Figure 11 shows the correlation between yield and NDVI, PRI, SPAD and SLT. It is possible to say that there is no correlation between any of these parameters, even if the tendency agrees with the assumption that greater photosynthetic activity and lower SLT correspond to less stress and greater yield. In the case of SLT, the lack of correlation can be explained, with the aforementioned arguments. Regarding the other parameters, it could have been thought that greater photosynthetic activity corresponded to greater yield, this relationship is however mediated by very complex physiological mechanisms of regulation and vegetative-productive balance, in support of this hypothesis there is also the fact that the analysis of the data on the pruning wood weight, an expression of the vigor of the plant, did not reveal any significant genetic differences.

3.6. Differences in yield among classes of SLT, PRI and NDVI

The correlations analysis took into consideration the entire population but the division of the population into quartiles for one trait and the comparison between the means of these groups for another trait may reveal significant differences between groups of clones that were previously missed when analyzing the population as a whole. The population was thus divided, for each of the traits deemed most relevant, into four groups of about 40 clones each, corresponding to the four quartiles, and tested looking for significant differences in terms of yield.

3.6.1 SLT classes

One of the attempts made was to verify whether different classes of SLT corresponded to significant differences in terms of yield. Despite the low correlation between yield and SLT, dividing the clones into groups according to increasing temperature classes, the average yield for each class appears decreasing, with the highest average yield for clones with lower SLT and lower yield for clones with the highest SLT (Figure 12). However according to the low correlation

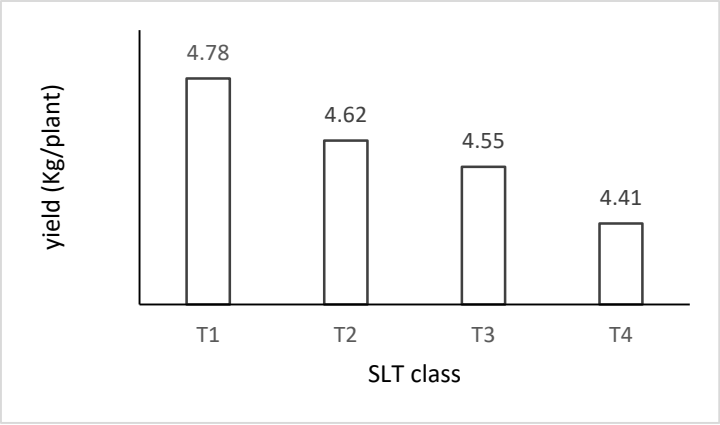


Figure 12. Average yield (kg/plant) at different SLT levels (result of ANOVA: Fcalc=1.54; p-value 0.2056). T1, from 28.531°C to 30.605°C; T2, from 30.605°C to 31.103°C; T3, from 31.103°C to 31.721°C; T4, from 31.721°C to 33.571°C.

observed, no significant difference emerges from the analysis of variance carried out with this approach (p-value 0.2056).

3.6.2. PRI classes

The same approach was adopted to investigate the possibility to create classes for the various indexes that corresponded to significant differences in terms of yield. With this purpose, the most interesting parameter should be PRI, because of the greater correlation with yield compared to the other indexes and because of the higher coefficient of genotypic variability. The ANOVA shows significant differences (p-value 0.00631) in terms of mean yield between the groups of clones formed according to their PRI values (Figure 13). A further investigation of these differences performed through Tukey test is reported in Table 8. It is possible to sustain that group 1, formed by the first 40 clones in ascending order by PRI values, has a significantly lower mean yield than groups 3 and 4**.** This result provides information that could be used to exclude a group of clones if a reduction in yield is not included among the selection goals.

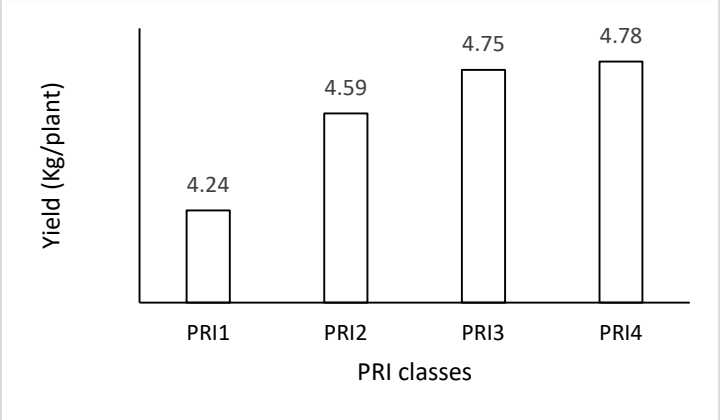


Figure 13. Average yield (kg/plant) at different PRI levels (result of ANOVA: Fcalc=4.26, p-value =0.00631). PRI1, from 0.006 to 0.018; PRI2, from 0.018 to 0.019; PRI3, from 0.019 to 0.020; PRI4, from 0.020 to 0.022.

Table 8. Tukey test results for mean yield differences at different PRI levels

Classes	Differences of means	p-value
PRI2-PRI1	0.3544	0.166
PRI3-PRI1	0.5128	0.016
PRI4-PRI1	0.5434	0.009
PRI3-PRI2	0.1584	0.790
PRI4-PRI2	0.1890	0.686
PRI4-PRI3	0.0306	0.998

3.6.3. NDVI classes

Differences in terms of yield between NDVI classes are also significant according to the ANOVA (p-value 0.01949). In Figure 14 it is possible to see that the first class of NDVI, i.e. the first forty clones with lower NDVI values, have a lower average yield than the other classes, while the highest yield value is associated with class 2, followed by classes 4 and 3, to confirm the absence of correlation and the non-linearity of the relationship between yield and NDVI.

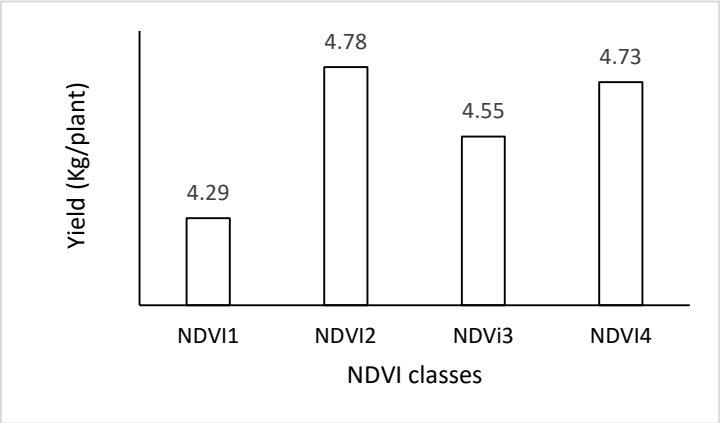


Figure 14. Average yield (Kg/plant) at different NVDI levels (result of ANOVA: Fcalc=3.39; p-value=0.01949). NDVI1, from 0.649 to 0.677; NDVI2, from 0.677 to 0.680; NDVI3, from 0.680 to 0.682; NDVI4, from 0.682 to 0.689.

Table 9 shows the results of the Tukey test for the average yield when considering NDVI levels and indicates that there are significant differences between group 1 and groups 2 and 4. As with regard to PRI, also in this case this result can be used in a selection process where yield improvement is one of the goals, to discard the clones in group 1, as they have the lowest yield.

Table 9. Tukey test results for mean yield differences at different NDVI levels

Classes	Differences of means	p-value
NDVI2-NDVI1	0.495	0.023
NDVI3-NDVI1	0.268	0.407
NDVI4-NDVI1	0.446	0.050
NDVI3-NDVI2	-0.228	0.550
NDVI4-NDVI2	-0.049	0.992
NDVI4-NDVI3	0.179	0.727

The combined analysis of the data of the whole population did not reveal any correlation between PRI or NDVI with yield. However, this analysis highlights significant differences in yield between

the group of clones with the lowest values for PRI and NDVI and the rest of the population. Furthermore, the fact that the clones with the lowest values, therefore the ones most affected by stress also have a lower yield, suggests that in conditions of more severe stress this trend could be even more marked and could affect the yield of the entire population, with different results also in terms of correlation between the traits.

4. Conclusions

The work carried out has led to the addition of several interesting details on the profile of the clones of Arinto. First of all, a significant genetic component was found for almost all the traits studied (Table 2), with the exception of the pruning wood weight. The coefficients of variability found were low for SLT and NDVI but consistent for yield, PRI and SPAD, heritability stands at values ranging from 0.57 to 0.67.

The calculation of the predicted genotypic values (Table 3) allowed to rank the clones in terms of performance according to each trait. The empirical probability density distributions (Figure 8) were similar to a normal curve, however, the presence of some outliers will advise that, in the future, a molecular analysis should be performed to confirm that these clones actually belong to the Arinto variety.

Considering a polyclonal selection process, one of the ultimate goals and practical application of this type of study, the predicted genetic gain obtainable with the selection of 15 clones was calculated on the basis of different selection hypotheses. In the first of these hypotheses (Table 6), to select the group of clones for each parameter that maximizes the genetic gain for the parameter itself, the results ranged from a low *circa* 1% for NDVI to 32% for yield. The second approach was to choose the index or traits more correlated to heat and drought tolerance and to select the best clones according to them without neglecting the genetic gain or the genetic lost for the other traits. With this in mind, the selection was based on SLT (Table 7), PRI (Table 8) and SPAD (Table 9).

Regarding the correlations between different traits, calculated with the predicted genotypic values, a moderate to high positive correlation was found between the three traits related to photosynthetic performance: NDVI, PRI and SPAD (Figure 10). Nevertheless, between yield and SLT and between these two traits and the others mentioned above (Figures 9 and 11), no correlation was identified.

Regarding NDVI, the genotypic variance was significant. However, a low level of genetic variability was detected (low coefficient of genotypic variation, 1.077), while the heritability was good. The predicted genetic gain obtained when selecting for NDVI was very low (1.1%), this is clearly due to the low coefficient of genotypic variation. As for the analysis of the literature, nothing has been found that supports the idea that this index can be used to discriminate between genotypes in terms of water and heat stress. On the basis of these elements it is possible to conclude that for the moment there are no elements supporting a selection based on this index.

PRI has a more consistent coefficient of variation, which leads to higher values of genetic gain (13.7%). The relationship between this index and water and heat stress conditions is widely treated in literature, several authors hypothesize the use of PRI to detect the presence or absence of stress. For these reasons, in this study it was considered as one of the most suitable for selection. When checking the behavior of the other traits when the first 15 best clones are selected for PRI, there is a decrease of 1% in SLT and a yield increase of 3.7%. Among the indexes used, PRI appears to be the one which better meets the goals of this work.

SPAD, whose genotypic component was also significant, had a coefficient of genotypic variation of 9.24, slightly lower than that of PRI but still high, and the predicted genetic gain obtained by selecting

the first 15 clones was 12.3%. Also in this case the literature consulted highlights a close relationship with physiological processes dependent on environmental factors such as temperature and water, thus supporting its use in identifying situations of tolerance and susceptibility. By selecting for SPAD, there is a 5.7% increase in PRI, a 1.7% reduction in SLT, and a 1.6% reduction in yield. Regarding this last item, it must be said that the decrease is minimal, but its importance is still relative depending on the objectives of the selection and the needs of the producers; if among the goals there was an increase in yield, a selection centered on this index would have to be excluded. It also remains to be clarified why by selecting the clones that should be less sensitive to stress, there is a decrease in yield, even if only slight. In conclusion, SPAD together with PRI are the most promising indices.

SLT is one of the most interesting traits because it is closely linked to leaves transpiration and the regulation of stomatal opening, thus also reflecting the water status of the plant. However, it showed a rather small coefficient of genetic variation, which in turn limits the genetic gain in a selection perspective, probably the problem lies within the variability of this trait when short periods of time are considered. Even if the analysis of variance for yield between the different classes of SLT revealed that higher temperatures correspond to lower yields, the differences found were not significant. On the basis of the data collected so far, the information is not enough to set a selection on this trait but still encourages further studies in this direction.

Yield is the trait that expressed the greatest genotypic variability and the largest range between the first and last clone in predicted genotypic values. The predicted genetic gain obtained from the selection of the first fifteen clones is 32.36%, the highest in this study, confirming that there is a great chance for genetic improvement by clonal selection for this trait. Some sources also suggest that a higher yield is itself indicative of a better adaptation to stress. In this work, however, there are no elements to confirm or deny the relationship between water and heat conditions and production.

Regarding the pruning wood weight, the genotypic variance component was not significant, for this reason there was no purpose in taking this trait further into consideration for analysis.

The experimental design was confirmed to be adequate for the collection of data and their statistical analysis, highlighting genotypic differences in a very large collection of clones in open field and therefore subject to high environmental variability. The phenotyping work has been effective but nevertheless it is certainly possible to optimize it in the future, with the help of faster and better performing technologies. The choice of the indices and traits analyzed did not always have the desired results. In the future, it will probably make sense for this type of work to focus on SPAD, PRI and yield and less on other indices, and to test different methods for SLT detection. Furthermore, the link between reflectance indices and the physiological processes involved in stress should be further explored.

We are still far from univocally defining the behavior of clones in terms of greater or lesser tolerance to water and heat stress, but it has been confirmed that there are genetic differences between clones potentially involved in tolerance traits, and some details were added to Arinto clones characterization. Certainly, the approach used, namely to perform indirect measurements and to work with an adequate statistical model, is effective and deserves to be improved. Furthermore, with this type of work, large *in situ* collections of plant material are maintained, which otherwise would not be economically sustainable.

There is still work to be done on phenotyping techniques, finding faster and more effective systems, for example testing devices based on the detection of images and their automated processing, for which new technologies are being developed at a fast rate.

It is well known that there is no single solution to the global warming conditions we are facing, but the approach must be to put together efforts on different fronts: environmental, technological and genetical. We also have the responsibility to preserve intra-varietal genetic variability, polyclonal and clonal selection are tools that allow us to keep old varieties as part of historical and cultural heritage, and to adapt them to conditions that are necessarily changing.

5. References

- Acquaah G., 2012. Principles of plant genetic and breeding.. John Wiley & Sons, Ltd, West Sussex, UK, 732 p
- Almandanim M.C., Baleiras-Couto M.M., Pereira H.S., Carneiro L.C., Fevereiro P., Eiras-Dias, J.E., Morais-Cecílio, L., Viegas, W., Veloso, M.M. 2007. Genetic diversity of the grapevine (*Vitis vinífera* L.) cultivars most utilized for wine production in Portugal. Vitis. 46, 116-119
- Arnò, J., Escolà, A., Vallès, J.M., Llorens, J., Sanz, R., Masip, J., Palacìn, J., Rosell-Polo, J.R., 2013. Leaf area index estimation in vineyards using a ground-based LiDAR scanner. Precision Agric. 14, 290–306.
- Arroyo-Garcia, R., Ruiz-García, L., Bolling, L., Ocete, R., López, M.A., Arnold, C., Ergul, A., Söylemezoğlu, G., Uzun, H.I., Cabello, F., Ibáñez, J. et al., 2006. Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. sativa) based on chloroplast DNA polymorphisms. Mol. Ecol. 15, 3707–3714.
- Baker N.R. 2008. Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. Annu Rev Plant Biol. 59, 89–113.
- Berry, J.A., Bjorkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Ann Rev Plant Physiol. 31, 491–543.
- Bohnert H.J., Nelson D.E., Jensen R.G. 1995. Adaptations to environmental stresses. Plant Cell. 7, 1099-1111
- Borel, C., Simonneau, T., This, D., Tardieu, F. 1997. Controls of stomatal conductance and ABA concentration in the xylem sap of barley lines of contrasting genetic origins. Aust J Plant Physiol. 24, 607–615
- Bota, J., Tomàs, M., Flexas, J., Medrano, H. 2016. Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. Agric Water Manag. 164, 91-99
- Brito, G.G., Sofiatti, V., de Andrade Lima, M.M., de Carvalho, L.P., da Silva Filho, J.L., 2011. Physiological traits for drought phenotyping in cotton. Acta Sci Agron. 33, 117-12.
- Brodribb T.J., Holbrook N.M. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiol. 132, 2166-2173.
- Buckley, T.N. 2005. The control of stomata by water balance. New Phytol. 168, 275–292
- Butler, D.G., Cullis, B.R., Gilmour, A. R., Geogel B, J., Thompson, R., 2017. ASReml-R reference manual, version 4. University of Wollongong, Wollongong.
- Cantona, M., Borghezanb, M., Silva, T.C., Welter, J.F., Villar, L., Rosa, D.J., Silva, A.L., Pescador, R., 2017. Chlorophyll evaluation on leaves of 'Sauvignon Blanc' during vegetative growth in São Joaquim, Santa Catarina, Brazil. Acta Hortic. 1188, 83 - 90
- Caird M.A., Richards J.H., Donovan L.A. 2007. Night time stomatal conductance and transpiration in C3 and C4 plants. Plant Physiol. 143, 4-10.
- Calatayud A., Roca D., Martínez P.F. 2006. Spatial-temporal variations in rose leaves under water stress conditions studied by chlorophyll fluorescence imaging. Plant Physiol Biochem 44, 564–573
- Carlin, S. D., Rhein, A.F.L., Santos, D.M.M., 2012. Efeito simultâneo da deficiência hídrica e do alumínio tóxico no solo na cultivar IAC91- 5155 de cana-de-açúcar. Semina: Ciências Agrárias, 33, 553-564.
- Carvalho, L.C., Coito, J.L,, Gonçalves, E.F., Chaves, M.M., Amâncio, S.. 2015. Differential physiological response of the grapevine varieties Touriga Nacional and Trincadeira to combined heat, drought and light stresses. Plant Biol. 18, 101–111.
- Chaves, M.M., Zarrouk O., Francisco R., Costa J.M., Santos, T., Regalado, A.P., Rodrigues, M.L, Lopes, C.M., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. Ann. Bot. 105, 661–676.
- Clawson, K.L., Jackson, R.D. Pinter Jr, P.J., 1989. Evaluating Plant Water Stress with Canopy Temperature Differences. Agronomy J. 81, 858-863.
- Cochard, H., Venisse, J.-S., Barigah, T.S., Brunel, N., Herbette, S., Guillot, A., Tyree, M.T., Sakr, S., 2007. Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. Plant Physiol. 143, 122–143.
- Cohen, S., Rao, R.S., Cohen, Y.. 1997. Canopy transmittance inversion using a line quantum probe for a row crop. Agr. Forest Meteorol. 86, 225–234.
- Cohen, S., Striem, M.J., Bruner, M., Klein, I.. 2000. Grapevine leaf area index evaluation by gap fraction inversion. Acta Horti. 537, 87–92.

- Costa, J.M., Ortunõ, M.F., Lopes, C.M., Chaves, M.M., 2012. Grapevine varieties exhibiting differences in stomatal response to water deficit. Func. Plant Biol. 39, 179-189.
- Costa, J.M., Grant, O.M., Chaves, M.M., 2013. Thermography to explore plant–environment interactions. J. Exp. Bot. 64, 3937–3949.
- Coupel-Ledru, A., Lebon, E., Christophe, A., Dolingez, A., Cabrera-Bosquet, L., Péchier, P., Hamard, P., This, P., Simonneau, T., 2014. Genetic variation in a grapevine progeny (*Vitis vinifera* L. cvs Grenache×Syrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought. J. Exp. Bot. 65, 6205-6218.
- De Storme, N., Geelen, D., 2014. The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms. Plant Cell Environ. 37, 1-18.
- Demmig-Adams, B., Adams, W.W., 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol., 43, 599– 626.
- Di Gaspero, G., Cattonaro, F., 2010. Application of genomics to grapevine improvement. Aust. J. Grape Wine Res. 16, 122–130.
- Diago, M.P., Correa, C., Millàn, B., Barreiro, P., Constantino, V., Tardaguila, J., 2012. Grapevine Yield and Leaf Area Estimation Using Supervised Classification Methodology on RGB Images Taken under Field Conditions. Sensors, 12, 16988-17006.
- Dobrowski, S.Z., Pushnik, J.C., Zarco-Tejada, P.J., Ustin, S.L., 2005. Simple reflectance indices track heat and water stress-induced changes in steady-state chlorophyll fluorescence at the canopy scale. Remote Sens. Environ. 97, 403-414.
- Doğan, A., Uyak, C., Keskin, N., Akcay, A., Ilknur, R., Sensoy, R., Ercisli, S., 2018. Grapevine Leaf Area Measurements by Using Pixel Values. Compt. Rend. Acad. Bulg. Sci. 71, 6.
- Dougherty, P.H., 2012. The geography of wine. Springer Science, The Netherlands, 256p. ISBN: 978-94-007-0464-0.
- Drappier, J., Thibon, C., Rabot, A., Geny-Denis, L., 2019. Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming.) Crit. Rev. Food Sci. Nutr., 59, 14-30.
- Drissi, R., Goutouly, J.P., Forget, D., Gaudilliere, J.P., 2009. Nondestructive Measurement of Grapevine Leaf Area by Ground Normalized Difference Vegetation Index. Agron. J. 101, 226-231.
- Duchêne, E., Schneider, C., 2005. Grapevine and climatic changes: a glance at the situation in Alsace. Agron. Sustain. Dev. 25, 93-99.
- Ehlert, B., Hincha, D.K., 2008. Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in Arabidopsis leaves. Plant Methods. 27, 4-12.
- Eibach, R., Alleweldt, G., 1985. Einfluss der Wasserversorgung auf Wachstum, Gaswechsel und Substanzproduktion traubentragender Reben. III. Die Substanzproduktion. Vitis. 24, 183-198.
- Escalona, J.M., Flexas, J., Medrano, H., 1999. Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. Australian Journal of Plant Physiology 26, 421–433.
- Estreicher, S.K. 2006, Wine from Neolithic times to 21th century. New York, Algora Publishing
- Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics. 4th Edition, Addison Wesley Longman, Harlow.
- Fanizza, G., Della gatta, C., Bagnulo, C. 1991. A non-destructive determination of chloropyll in Vitis vinifera. Annals of applied Biology, 119, 203-205.
- Flexas, J., Escalona, J. M., Medrano, H., 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant Cell and Environment. 22, 39–48.
- Flexas, J., Briantais, J., Cerovic, Z. G., Medrano, H., Moya, I., 2000. Steady state and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: A new remote sensing system. Remote Sensing of Environment, 73, 283– 297.
- Flexas, J., Bota, J., Escalona, J.M., Sampol, B. Medrano, H., 2002. Effects of drought on photosynthesis in grapevines underfield conditions: an evaluation of stomatal and mesophyll limitations. Functional Plant Biology. 29, 461–471.
- Flexas, J., Escalona, J. M., Evain, S., Gulias, J., Moya, I., Osmond, C. B., Medrano, H., 2002. Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO2 assimilation and stomatal conductance during water-stress in C-3 plants. Physiologia Plantarum. 114(2), 231– 240.
- Flexas, J., Bota, J., Galmés, J., Medrano, H., Ribas-Carbó, M., 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiologia Plantarum, 127, 343–352.

- Flexas, J., Diaz-Espejo, A., Galmés, J., Kaldenhoff, R., Medrano, H. and Ribas-Carbó, M., 2007. Rapid variations of mesophyll conductance in response to changes in CO_2 concentrations around leaves. Plant, Cell and Environment. 30, 1284–1298.
- Flexas, J., Ribas-Carbó, M., Diaz-Espejo, A., Galmés, J., Medrano, H., 2008. Mesophyll conductance to CO_2: current knowledge and future prospects. Plant, Cell and Environment 31, 602–621.
- Fouquet, R., Léon, C., Ollat, N., Barrieu, F., 2008. Identification of grapevine aquaporins and expression analysis in developing berries. Plant Cell Reports, 27, 1541–1550.
- Fraga H., Malehiro, A. C., Moutinho Pereira, J., Santos, J.A. 2013. An overview of climate change impacts on European viticulture. Food Energy Secur, 1: 94-110.
- Frank, T., Botta, R., Thomas, M.R., Franks, J., 2002. Chimerism in grapevines: implication for cultivar identity, ancestry and genetic improvement. Theor Appl Genet.104(2-3), 192-199.
- Franks, P.J., Drake, P.L., Froend, R., 2007. Anisohydric but isohydrodynamic: seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance. Plant, Cell and Environment, 30, 19–30.
- Furbank, R.T., Tester, M., 2011. Phenomics – technologies to relieve the phenotyping bottleneck. Trends in Plant Science, 16(12), 635 – 644.
- Gamon, J.A., Pearcy, R.W., 1989. Leaf movement, stress avoidance and photosynthesis in *Vitis californica*. Oecologia 79, 475-481.
- Gamon, J.A., Pearcy, R.W., 1990. Photoinhibition in *Vitis californica*: interactive effects of sunlight, temperature and water status. Plant Cell Environ. 13, 267-275.
- Gamon J.A., Penuelas, J., Field, C.B. 1992. A Narrow-Waveband Spectral Index That Tracks Diurnal Changes in Photosynthetic Efficiency. Remote Sens. Environ. 41, 35-44.
- Gamon, J. A., Serrano, L., Surfus, J. S., 1997. The photochemical reflectance index: An optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. Oecologia, 112, 492– 501
- García-Tejero, I.F., Ortega-Arévalo, C.J., Iglesias-Contreras, M., Moreno, J.M., Souza, L., Tavira, S.C., Durán-Zuazo, V.H., 2008. Assessing the crop-water status in almond (*Prunus dulcis* mill.) trees via thermal imaging camera connected to smartphone. Sensors (Switzerland), 18 (4), 1050.
- Gonçalves, E., St Aubyn, A. Martins, A., 2010. Experimental designs for evaluation of genetic variability and selection of ancient grapevine varieties: a simulation study. Heredity 104, 552- 562.
- Gonçalves, E., Martins, A., 2012. Genetic variability evaluation and selection in ancient grapevine varieties. In Abdurakhmonov, I.Y. - Plant Breeding. InTech. ISBN 978-953-307-932-5. p. 333-352.
- Gonçalves, E., Carrasquinho, I., St. Aubyn, A., Martins, A., 2013. Broad-sense heritability in mixed models for grapevine initial selection trials. Euphytica, 189, 379-391.
- Gorbe, E., Calatayud, A., 2012. Applications of chlorophyll fluorescence imaging technique in horticultural research: A review. Sci Horti, 138, 24–35.
- Grant, O.M., Tronina, L., Jones, H.G., Chaves, M.M., 2006. Exploring thermal imaging variables for the detection grapevine. Journal of Experimental Botany, 58 (4), 827–838, special issue.
- Greer, D.H., Weston, C., 2010. Effects of fruiting on vegetative growth and development dynamics of grapevines (Vitis vinifera cv. Semillon) can be traced back to events at or before budbreak. Funct. Plant Biol. 37, 756-766.
- Hardie, W.J., Considine, J.A., 1976. Response of grapes to water-deficit stress in particular stages of development. Am. J. Enol. Vitic. 27, 55-61.
- Henderson, C.R, 1975. Best Linear Unbiased Estimation and Prediction under a Selection Model. Biometrics, 31, 423-447.
- Hofacker, W., Alleweldt, G., Khader, S., 1976. Einfluss der Umweltfaktoren auf Beerenwachstum und Mostqualitat bei der Rebe. Vitis 15, 96-112.
- Hsiao, T.C., Xu, L.K., 2000. Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. J. Exp. Bot. 51, 1595-1616.
- Huglin, P., Schneider, C., 1998. Biologie et Ecologie de la Vigne, second ed. Lavoisier, Paris.
- Humplìk, J.F., Lazàr, D., Husičková, A., Spìchal, L., 2015. Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses – a review. Plant Methods, 11, 29.
- Iba, K., 2002. Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annu. Rev. Plant Biol. 53, 225-245.
- IPCC 2013, Climate Change 2013
- IPCC 2014. Climate Change 2014

- Irmak, S., Haman, D., & Bastug, R., 2000. Determination of Crop Water Stress Index for Irrigation Timing and Yield Estimation of Corn. Agronomy J. 92. 1221–1227.
- Jackson, R.D., Kustas, W.P., Choudhury, B.J. 1988. A re-examination of the crop water stress index. Irrigation Science 9, 309–317.
- Jackson R., 2014; Wine science, principle and application; 4th edition. Academic Press. Elseiver
- Jones, G.V., Webb L.B., 2010. Climate Change, Viticulture, and Wine: Challenges and Opportunities. Journal of Wine Research, 21(2), 103 -106.
- Jones, H.G., 1998. Stomatal control of photosynthesis and transpiration. J Exp Bot. 49, 387-398.
- Jones, H.G. 1999. Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant Cell Environ. 22, 1043–1055.
- Jones, H.G., Stoll, M. Santos, T., De Sousa, C. Chaves, MM., Grant, O.M., 2002. Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. J Exp Bot., 53, 2240–2260.
- Keller, M., 2015. The Science of Grapevines, second edjtion. Academic Press.
- Kliewer, W.M., 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. Am. J. Enol. Vitic. 28, 96-103.
- Korkutal, I., Bahar, E., Carbonneau, A. 2011. Growth and yield responses of cv. Merlot (*Vitis vinifera* L.) to early water stress. Afr J Agric Res, 6(29), 6281-6288.
- Lacombe, T., Audeguin L., Boselli M., Bucchetti B., Cabello F., Chatelet P., Crespan M., D'onofrio C., Eiras Dias J. et al., 2011.Grapevine European Catalogue: towards a comprehensive list. Vitis, 50, 65–68.
- Lallanilla, M., 2013. Will Global Warming Crush the Wine Industry? Live Science. Available at: ⟨http://www.livescience.com/ 28577-wine-global-warming.html⟩.
- Lang, M., Lichtenthaler, H.K., Sowinska, M., Heisel, F., Miehé, J.A., 1996. Fluorescence imaging of water and temperature stress in plant leaves. J. Plant Physiol. 148, 613–621.
- Lawlor, D.W., Cornic, G., 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 25, 275-294.
- Lichtenthaler, H.K., Babani, F., 2000. Detection of photosynthetic activity and water stress by imaging the red chlorophyll fluorescence. Plant Physiol. Biochem. 38, 889–895
- Lichtenthaler, H.K., Langsdorf, G., Lenk, S., Buschmann, C., 2005. Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system. Photosynthetica, 43, 355-369.
- Lin, C., Popescu, S.C. Huang, S.C., Chang, P.T. Wen H.L., 2015. A novel reflectance-based model for evaluating chlorophyll concentration of fresh and water-stressed leaves. Biogeosciences, 12, 49–66.
- Loreto, F., Schnitzler, J.P., 2010. Abiotic stresses and induced BVOCs. Trends Plant Sci. 15, 154-166.
- Lovisolo, C. and Schubert, A., 2006. Mercury hinders recovery of shoot hydraulic conductivity during grapevine rehydration: evidence from a whole-plant approach. New Phytol. 172, 469–478.
- Mabrouk, H. and Sinoquet, H., 1998, Indices of light microclimate and canopy structure of grapevines determined by 3D digitising and image analysis, and their relationship to grape quality. Aust J Grape Wine Res, 4, 2-13.
- Maes, W.H., Steppe, K. 2012. Estimating evapotranspiration and drought stress with ground-based thermal remote sensing in agriculture: a review. J Exp Bot, 63, 4671–4712.
- Martins, A. Carneiro, L.C., Castro, R., 1990. Progress in mass and clonal selection of grapevine varieties in Portugal. Vitis 29, 485-489.
- Martins, A., Goncalves, E., 2015. Grapevine breeding programs in Portugal, Grapevine Breeding Programs for the wine industry. 8,159 - 182.
- Matese, A., Baraldi, R., Berton, A., Casaraccio, C., Di Gennaro, S.F., Duce, P., Facini, O., Mameli, M.G., Piga, A., Zaldei, A., 2018. Estimation of Water Stress in Grapevines Using Proximal and Remote Sensing Methods. Remote Sens. 10, 114.
- Maul, E., Carka, F., Cunha, J., Eiras Dias, J. E., Gardiman, M., Gazidova, A., Ivanisevic, D., Maletic, E., Maras, V., Organero, G. M. Nikolic, D. et al., 2018. On-farm inventory of minor grape varieties in the European Vitis Database. ECPGR Activity Grant Scheme – Third Call, 2016 Activity Report.
- Maul, E. et al., 2019: Vitis International Variety Catalogue. www.vivic.de – (accessed february 2019)
- McCarthy, M.G., 1997. The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). Aust. J. Grape Wine Res. 3, 102-108
- McCulloch, C. E., Searle, S.R., Neuhaus, J.M. 2008. Generalized, Linear, and Mixed Models, 2nd Edition.John Wiley and Sons, New York.

- McGovern, P., Jalabadze, M., Batiuk, S., Callahan, M.P., Smith, K.E., Hall, G.R., Kvavadze, E., Maghradze, D., Rusishvili, N., Bouby, L., et al., 2017. Early Neolithic wine of Georgia in the South Caucasus. PNAS 114, 10309-10318.
- Möller, M., Alchanatis, V., Cohen, Y., Meron, M., Tsipris, J., Naor, A., Ostrovsky, V., M. Sprintsin, M., Cohen S. 2006. Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. J Exp Bot. 58, 827-38
- Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K., 2007. Loss of anthocyanins in red-wine grape under high temperature. J. Exp. Bot. 58, 1935-1945.
- Moutinho-Pereira J, Magalhães N, Gonçalves B, Bacelar E, Brito M, Correia C. 2007. Gas exchange and water relations of three *Vitis vinifera* L. cultivars growing under Mediterranean climate. Photosynthetica 45: 202–207.
- Munitz, S., Schwartz, A., Netzer, Y., 2019. Water consumption, crop coefficient and leaf area relations of a *Vitis vinife*ra cv. 'Cabernet Sauvignon' vineyard. Agric. Water Manag, 219, 86–94.
- Nuske, S., Achar, S., Bates, T., Narisimahan, S., Singh, S., 2011. Yield Estimation in Vineyards by Visual Grape Detection. 2011 IEEE/RSJ International Conference on Intelligent Robots and Systems September 25-30, 2011. San Francisco, CA, USA
- Oakey, H., Verbyla, A., Pitchford, W., Cullis, B., Kuchel, H. 2006 Joint modeling of additive and non-additive genetic line effects in single field trials. TAG 113, 809-819.
- OIV (2017) Distribution of the world's grapevine variety
- OIV (2017) Resolution OIV-VITI 564A-2017
- OIV (2018) Statistical report on world vitiviniculture 2018
- OIV (2019) resolution OIV-VITI 564B-2019; OIV process for the recovery and conservation of the intravarietal diversity and The polyclonal selection of the vine in grape varieties with wide genetic variability
- Ojeda, H., Deloire, A., Carbonneau, A., 2001. Influence of water deficits on grape berry growth. Vitis 40, 141-145
- Omasa, K., Takayama, K., 2003. Simultaneous measurement of stomatal conductance, non-photochemical quenching, and photochemical yield of photosystem II in intact leaves by thermal and chlorophyll fluorescence imaging. Plant Cell Physiol. 44, 1290–1300.
- Painawade, M., Jogloy, S., Kesmala, T., Akkasaeng, C., Patanothai, A. 2009. Heritability and Correlation of Drought Resistance Traits and Agronomic Traits in Peanut. Asian J. Plant Sci, 8, 325-334.
- Palliotti, A., Silvestroni, O., Petoumenou, D., 2009. Photosynthetic and photoinhibition behavior of two fieldgrown grapevine cultivars under multiple summer stresses. Am. J. Enol. Vitic. 60, 189-198.
- Pantin, F., Monnet, F., Jannaud, D., Costa, J.M., Renaud, J., Muller, B., Simonneau, T., Genty, B. 2013. The dual effect of abscisic acid on stomata. New Phytol 197, 65–72.
- Patterson, H., Thompson, R., 1971. Recovery of Inter-Block Information when Block Sizes are Unequal. Biometrika, 58, 545-554.
- Penuales, J., Gamon, J.A., Fredeen, A.L., Merino, J., Field C.B. 1994. Reflectance Indices Associated with Physiological Changes in Nitrogen- and Water-Limited Sunflower Leaves. Remote Sens. Environ. 48, 135-146.
- Penuelas, J., Filella, I., Gamon, J.A., 1995. Assessment of photosynthetic radiation-use efficiency with spectral reflectance. New Phytol. 131, 291-296.
- Penuelas, J., Llusia, J., Pinol, J., Filella, I., 1997. Photochemical reflectance index and leaf photosynthetic radiation-use-efficiency assessment in Mediterranean trees. Int. J. Remote Sens 18, 2863– 2868.
- Pereira-Filho, J.V., de A. Viana, T.V., de Sousa, G.G., Chagas, K.L. de Azevedo, B.M., de S. Pereira, C.C.M. 2019. Physiological responses of lima bean subjected to salt and water stresses. R. Bras. Eng. Agríc. Ambiental, 23(, 959-965.
- Perkins, S. E., Alexander, L. V., Nairn, J. R., 2012. Increasing frequency, intensity and duration of observed global heatwaves and warm spells, Geophys. Res. Lett., 39, L20714
- Petrie, P.R., Wang, Y., Liu, S., Lam, S., Whitty, M. A., Skews, M.A., 2019. The accuracy and utility of a low-cost thermal camera and smartphone-based system to assess grapevine water status. Biosyst Eng, 179, 126 - 139.
- Peyrot des Gachons, C., Van Leeuwen, C., Tominaga, T., Soyer, J.P., Gaudille`re, J.P., Dubourdieu, D., 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L cv Sauvignon blanc in field conditions. J. Sci. Food Agric. 85, 73-85.
- Pichersky, E., Gershenzon, J., 2002. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr. Opin. Plant Biol. 5, 237-243.

- Pinto, C., Henriques, M.O., Figueiredo, J.P., David J.S., Abreu, F.G., Pereira, J.S., Correia I., David T.S., 2011. Phenology and growth dynamics in Mediterranean evergreen oaks: Effects of environmental conditions and water relations. For. Ecol. Manag. 262, 500–508.
- Pou, A., Medrano, H., Flexas, J., Tyerman, S.D. 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. Plant Cell Environ. 36, 828–843.
- Pretorius, I. S., HØJ, P. B., 2005. Grape and wine biotechnology: Challenges, opportunities and potential benefits. Aust J Grape Wine Res, 11,83-108.
- Rolando, J.L., Ramirez, D.A., Yactayo, W., Monneveux, P., Qiroz, R., 2015. Leaf greenness as a drought tolerance trait in potato. Environ Exp Bot, 110, 27-35.
- Romero, P., Fernandez J.I., Martinez-Cutillas, A., 2010. Physiological thresholds for efficient regulated deficit-irrigation management in winegrapes grown under semiarid conditions. Am. J. Enol. Vitic. 61,300-312.
- Ronald, J., 2014. Wine science, chapter 1,2. Academic Press.
- Sade, N., Vinocur, B.J., Dibes, A., Shatil, A., Ronen, G., Nissan, H., Wallach, R., Karchi, H. Moshelion, M., 2009. Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? New Phytol, 181, 651–661
- Sala, C., Busto, O., Guasch, J., Zamora, F., 2005. Contents of 3-alkyl-2-methoxypyrazines in musts and wines from *Vitis vinifera* variety Cabernet Sauvignon: influence of irrigation and plantation density. J. Sci. Food Agric. 85, 1131-1136.
- Salvucci, M.E., Crafts-Brandner, S.J., 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiol Plant 120:179–186.
- Santos T., Lopes C., Rodrigues M., Souza C., Ricardo-da-Silva J., Maroco J., Pereira J., Chaves M.. 2007. Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of Moscatel field-grown grapevines. Sci Horti. 112. 321-330.
- Schreiber, U., Bilger, W., Neubauer, C. 1995. Chlorophyll Fluorescence as a Nonintrusive Indicator for Rapid Assessment of In Vivo Photosynthesis. In: Schulze ED., Caldwell M.M. (eds) Ecophysiology of Photosynthesis. Springer Study Edition, vol 100. Springer, Berlin, Heidelberg
- Schultz, H.R. 1996. Water relations and photosynthetic responses of two grape vine cultivars of different geographical origin during water stress. Acta Horti. 427, 251–266.
- Schultz, H.R. 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behavior of two field-grown *Vitis vinifera* L. cultivars during drought. Plant Cell Environ. 26, 1393–1405.
- Schultz H.R., Stoll M., 2010. Some critical issue in environmental physiology of grapevine: future challenges and current limitation. Aust, J. of Grape and Wine Res., 16. 4-24.
- Searle, S. R., Casella, G., and McCulloch, C. E. 1992. Variance Components, Wiley,New York
- Self, S., Liang, K., 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio test under nonstandard conditions. J. Am. Stat. Assoc, 82, 605-610.
- Sepulveda, G., Kliewer, W.M., 1986. Effect of high temperature on grapevines (Vitis vinifera L.). II. Distribution of soluble sugars. Am. J. Enol. Vitic. 37, 20-25.
- Silva, A.M., Jifon, J.L., Da Silva, J.A.G., Sharma, V., 2007. Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. Braz. J. Plant Physiol. 19: 193-201.
- Sims, D., Gamon, J., 2002. Relationships Between Leaf Pigment Content and Spectral Reflectance Across a Wide Range of Species, Leaf Structures and Developmental Stages. Remote Sens Environ, 81 (2-3), 337 – 354.
- Skewes, M., Petrie, P.R., Liu, S., Whitty, M., 2008. Smartphone tools for measuring vine water status. Acta Horticulturae, 1197, 53-58.
- Soar, C.J., Speirs, J., Maffei, S.M., Penrose, A.B., McCarthy, M.G., Loveys, B.R. 2006. Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue. Aust J Grape Wine Res 12, 2–12
- Solie, J.B., M.L. Stone, W.R. Raun, G.V. Johnson, K. Freeman, R. Mullen, D.E. Needham, S. Reed, C.N. Washmon. 2002. Real-time sensing and N fertilization with a field scale greenseeker TM applicator. In P.C. Robert (ed.) Proc. Int. Conf. Precision Agriculture and Other Precision Resources Management,6th, Minneapolis MN.14–17.
- Sperry, J.S., Hacke, U.G., Comstock, J.P., Oren, R. 2002. Water deficits and hydraulic limits to leaf water supply. Plant Cell Environ. 25: 251–264.
- Stevens, R.M., Harvey, G., Aspinall, D., 1995. Grapevine growth of shoots and fruit linearly correlate with water stress indices based on root-weighted soil matric potential. Aust. J. Grape Wine Res. 1,58-66.

- Steele, M.R., Gitelson, A.A., Rundquist, D. C. 2008. A comparison of Two Techniques for Nondestructive Measurement of Chlorophyll Content in Grapevine Leaves. Agronomy Journal, 100(3).
- Stram, D., Lee, J., 1994. Variance components testing in the longitudinal mixed effects model. Biometrics, 50(4),1171 – 1177.
- Tardieu, F., Davies, W.J. 1992. Stomatal response to ABA is a function of current plant water status. Plant Physiol. 98, 540–545.
- Tardieu, F., Simonneau, T. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behavior s. Journal Exp Bot 49, 419–432.
- Taskos, D. G., Koundouras, S., Stamatiadis, S., Zioziou E., Nikolaou, N., Karakioulakis, K., Theodorou, N. 2015. Using active canopy sensors and chlorophyll meters to estimate grapevine nitrogen status and productivity. Precis Agric, 16,77-98.
- Terral, J.F., Tabard, E., Bouby, L., Ivorra, S., Pastor, T., Figueiral, I., Picq, S., Chevance, J.B., Jung, C., Fabre, L., Tardy, C., Compan, M., Bacilieri, R., Lacombe, T., This, P., 2010. Evolution and history of grapevine (Vitis vinifera) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient european cultivars. Ann. Bot. 105, 443–455.
- Tortosa I., Escalona, J.M., Bota, J., Tomás, M., Hernández, E., Escudero E.G., Medrano H. 2016. Exploring the genetic variability in water use efficiency: Evaluation of inter and intra cultivar genetic diversity in grapevines. Plant Sci. 251, 35-43.
- Vandeleur, R.K., Mayo, G., Shelden, M.C., Gilliham, M., Kaiser B.N. Tyerman, S.D. 2009. The role of PIP aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. Plant Physiol. 149, 445–460.
- Walter, A, Scharr, H., Gilmer, F., Zierer, R., Nagel, K.A., Ernst, M,. 2007. Dynamics of seedling growth acclimation towards altered light conditions can be quantified via GROWSCREEN: a setup and procedure designed for rapid optical phenotyping of different plant species. New Phytol. 174, 447–455.
- Walter, A., Studer, B., Kolliker, R. 2012. Advanced phenotyping offers opportunities for improved breeding of forage and turf species. Ann. Bot. 110, 1271–1279.
- Williams, L.E., 1996. Grape. In: Zamski, E., Schaffer, A. (Eds.), Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships. Dekker, New York, NY, pp. 851-881.
- Williams, L.E., Baeza, P., 2007. Relationships among ambient temperature and vapor pressure deficit and leaf and stem water potentials of fully irrigated, field-grown grapevines. Am. J. Enol. Vitic. 58, 173-181.
- Williams, L.E., Dokoozlian, N.K., Wample, R., 1994. Grape. In: Schaffer, B., Andersen, P.C. (Eds.), Handbook of Environmental Physiology of Fruit Crops, vol. I. Temperate Crops. CRC Press, Boca Raton,FL, pp. 85-133.
- Williams, L.E., Matthews, M.A., 1990. Grapevine. In: Stewart, B.A., Nielson, N.R. (Eds.), Irrigation of Agricultural Crops, Agronomy Monograph No. 30. ASA-CSSA-SSSA, Madison, WI, pp. 1019-1055.
- Zhang, M., Jin, Z., Zhang G., Wu F., 2015.Physiological and biochemical responses to drought stress in cultivated and Tibetan wild barley. Plant Growth Regul 75, 567–574.
- Zhan M., Xia L., Zhan L., Wang Y., 2019. Recognition of Changes in Air and Soil Temperatures at a StationTypical of China's Subtropical Monsoon Region (1961–2018). Advances in Meteorology.
- Zinn, K.E., Tunc-Ozdemir, M., Harper, J.F., 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. J. Exp. Bot. 61, 1959-1968.
- Zohary, D, Hopf, M., 2000. Domestication of plants in the Old World. 3rd edn. New York: Oxford University Press

Annex 1. Empirical Best Linear Unbiased Predictor (EBLUP) of the genotypic effect and predicted genotypic value (PGV) for all clones and traits evaluated.

	NDVI		PRI		SPAD		SLT		YIELD	
clone	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV
AR0223	-0.00199	0.67716	0.001	0.020	-0.166	14.109	0.435	31.591	0.421	5.006
AR0459	0.00181	0.68096	0.002	0.021	0.463	14.738	-0.020	31.137	0.665	5.250
AR0498	0.00360	0.68276	0.003	0.022	0.850	15.125	-0.877	30.279	0.966	5.552
AR0503	-0.00752	0.67164	-0.003	0.016	0.160	14.436	-0.863	30.294	0.364	4.949
AR0508	0.00497	0.68413	0.001	0.020	0.876	15.151	0.665	31.822	0.581	5.167
AR0527	0.00292	0.68208	-0.001	0.017	0.193	14.468	-0.992	30.165	-0.289	4.297
AR10207	-0.00773	0.67143	-0.001	0.018	-1.893	12.382	-0.361	30.796	-0.765	3.820
AR10305	0.00381	0.68297	0.001	0.020	-0.166	14.110	0.218	31.375	0.091	4.676
AR10312	-0.03023	0.64893	-0.012	0.006	-3.284	10.990	1.747	32.903	-1.662	2.923
AR10501	-0.00630	0.67286	-0.003	0.016	-1.547	12.729	0.906	32.062	-0.406	4.180
AR10502	-0.00625	0.67291	-0.003	0.016	-0.228	14.048	-0.600	30.557	-1.255	3.331
AR10503	-0.01306	0.66609	-0.006	0.013	-2.049	12.226	0.786	31.943	-1.366	3.219
AR10504	-0.02108	0.65808	-0.006	0.013	-3.184	11.091	1.001	32.157	-1.280	3.305
AR10505	0.00255	0.68171	-0.001	0.017	0.508	14.784	-1.484	29.672	0.613	5.199
AR10702	0.00255	0.68171	0.002	0.021	0.420	14.695	0.448	31.605	0.265	4.851
AR10804	-0.01692	0.66224	-0.008	0.011	-2.679	11.596	0.964	32.121	-1.725	2.861
AR11002	0.00087	0.68003	0.001	0.019	-0.815	13.460	0.886	32.043	0.621	5.207
AR1116	-0.00038	0.67877	0.000	0.018	1.146	15.422	-0.488	30.668	-0.492	4.093
AR1117	0.00497	0.68413	0.000	0.019	0.733	15.009	2.068	33.224	-0.245	4.340
AR11201	0.00624	0.68540	0.000	0.019	0.507	14.782	0.248	31.405	1.502	6.088
AR11202	0.00188	0.68104	0.001	0.020	1.498	15.774	-1.222	29.935	0.272	4.857
AR11203	0.00237	0.68152	0.003	0.022	1.285	15.561	-0.297	30.860	0.906	5.491
AR11205	0.00228	0.68144	0.002	0.021	0.803	15.079	-1.144	30.013	0.674	5.260
AR11301	0.00511	0.68427	-0.002	0.017	0.484	14.759	0.245	31.401	0.113	4.699
AR11303	-0.00107	0.67808	-0.001	0.017	-0.459	13.816	0.274	31.430	-0.705	3.881
AR11304	-0.01317	0.66599	-0.004	0.015	-2.742	11.533	-0.464	30.693	0.873	5.458
AR11305	-0.00192	0.67723	-0.001	0.017	0.083	14.359	0.399	31.556	-0.124	4.462
AR11306	0.00683	0.68599	0.001	0.020	-1.000	13.275	-0.474	30.683	0.092	4.677

	NDVI		PRI		SPAD		SLT		YIELD	
clone	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV
AR11308	-0.00522	0.67394	-0.004	0.015	-0.193	14.083	0.810	31.967	-0.661	3.925
AR11309	-0.00029	0.67887	-0.001	0.017	0.065	14.340	-0.143	31.014	-0.947	3.638
AR11310	0.00244	0.68160	0.001	0.020	-0.009	14.266	-0.548	30.609	-1.369	3.217
AR11313	-0.00282	0.67634	0.003	0.021	-0.407	13.868	0.549	31.706	-0.086	4.500
AR11314	0.00378	0.68294	0.002	0.020	0.360	14.635	-0.171	30.986	-0.105	4.480
AR11501	0.00140	0.68055	0.000	0.019	-0.982	13.293	0.428	31.584	-1.420	3.166
AR11502	-0.01666	0.66250	-0.003	0.016	-2.476	11.799	-0.050	31.106	-0.656	3.929
AR11601	-0.00498	0.67418	-0.001	0.017	-0.048	14.227	-0.772	30.385	0.206	4.791
AR11602	-0.00264	0.67651	0.000	0.019	0.207	14.483	-0.484	30.672	0.672	5.257
AR11603	-0.00077	0.67839	-0.001	0.018	0.700	14.976	1.682	32.838	0.427	5.013
AR1170	-0.00237	0.67679	0.000	0.019	0.141	14.416	0.326	31.482	0.271	4.857
AR11902	-0.00285	0.67631	-0.001	0.018	-1.167	13.108	-0.146	31.011	-0.270	4.316
AR11905	0.00457	0.68373	0.000	0.019	1.018	15.293	-0.107	31.050	-0.758	3.827
AR12304	-0.00218	0.67698	0.000	0.018	0.441	14.716	0.817	31.973	0.194	4.780
AR12401	-0.00157	0.67759	0.001	0.019	0.467	14.742	0.133	31.290	1.334	5.920
AR12501	-0.00550	0.67365	-0.002	0.017	-1.522	12.753	0.844	32.000	0.511	5.097
AR12502	-0.00553	0.67363	-0.002	0.016	-0.160	14.115	0.010	31.166	1.263	5.849
AR12601	0.00364	0.68280	0.002	0.021	1.878	16.153	0.136	31.293	0.707	5.292
AR12604	0.00263	0.68179	0.000	0.019	-1.569	12.706	-1.076	30.080	0.781	5.366
AR12605	0.00117	0.68033	0.002	0.020	-0.786	13.490	0.615	31.772	-0.119	4.467
AR12607	-0.00633	0.67283	-0.001	0.018	-2.369	11.906	2.414	33.571	-1.062	3.523
AR12608	0.00372	0.68288	-0.001	0.018	-0.397	13.878	-0.146	31.011	0.277	4.862
AR12609	0.00436	0.68351	0.002	0.021	1.665	15.941	0.219	31.376	-0.513	4.073
AR12610	0.00278	0.68194	0.000	0.019	-0.465	13.811	0.578	31.734	-0.767	3.819
AR1410	0.00354	0.68269	-0.002	0.017	0.780	15.056	0.241	31.397	-1.040	3.545
AR1501	-0.00003	0.67913	0.001	0.020	-0.587	13.688	0.703	31.860	1.516	6.102
AR1510	-0.00271	0.67645	0.002	0.020	-1.890	12.385	-0.918	30.239	-0.511	4.075
AR1515	0.00522	0.68438	0.000	0.018	-1.488	12.788	0.071	31.228	-0.957	3.628
AR1550	0.00223	0.68139	0.000	0.018	-0.598	13.677	-0.614	30.543	-0.201	4.384

	NDVI		PRI		SPAD		SLT		YIELD	
clone	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV
AR1632	0.00216	0.68132	0.003	0.021	-1.343	12.932	0.447	31.604	-0.724	3.862
AR2019	0.00746	0.68662	0.001	0.020	0.214	14.490	1.296	32.453	-0.169	4.416
AR2027	-0.00135	0.67781	-0.001	0.018	0.331	14.606	0.464	31.620	0.017	4.602
AR2403	0.00671	0.68587	0.002	0.020	0.070	14.346	0.320	31.477	-0.456	4.129
AR2404	0.00151	0.68067	0.000	0.018	0.086	14.362	-0.919	30.237	0.208	4.794
AR2410	0.00571	0.68486	0.002	0.021	1.177	15.453	0.035	31.192	-0.006	4.580
AR2423	0.00442	0.68357	0.000	0.019	1.407	15.683	0.817	31.974	0.032	4.617
AR2424	0.00722	0.68638	0.000	0.019	-0.321	13.954	0.960	32.116	1.318	5.904
AR2425	0.00332	0.68248	0.001	0.019	0.625	14.900	1.816	32.973	0.224	4.809
AR2511	-0.00118	0.67798	-0.001	0.017	0.883	15.159	1.595	32.751	-0.598	3.988
AR2512	0.00150	0.68065	0.002	0.021	0.703	14.979	-1.056	30.101	-0.715	3.870
AR2521	0.00837	0.68752	0.001	0.020	0.939	15.215	-0.119	31.037	1.530	6.115
AR2531	0.00247	0.68163	0.001	0.020	-0.325	13.950	-1.096	30.061	-0.357	4.228
AR2561	0.00190	0.68105	0.000	0.019	-0.090	14.186	-0.328	30.829	0.154	4.740
AR2562	0.00468	0.68384	0.000	0.018	0.876	15.151	0.177	31.334	0.440	5.026
AR2571	-0.00302	0.67614	-0.001	0.017	-0.724	13.551	0.000	31.157	0.000	4.586
AR2572	0.00089	0.68004	0.001	0.019	0.880	15.155	-1.150	30.007	1.724	6.310
AR2573	0.00163	0.68079	0.001	0.020	-0.876	13.399	0.565	31.721	-0.357	4.229
AR2574	-0.00058	0.67858	0.002	0.020	0.039	14.315	1.602	32.759	0.677	5.263
AR2581	-0.00573	0.67342	-0.001	0.017	-1.186	13.090	0.416	31.573	0.279	4.865
AR2601	0.00270	0.68186	0.002	0.020	0.991	15.266	-2.625	28.531	2.683	7.268
AR2602	0.00047	0.67963	-0.001	0.018	-0.778	13.498	-0.399	30.757	0.058	4.643
AR2603	0.00112	0.68028	0.001	0.019	0.621	14.897	-0.552	30.605	-0.764	3.821
AR2611	-0.00258	0.67658	-0.001	0.018	-0.366	13.909	-0.823	30.334	0.942	5.527
AR2612	-0.00168	0.67748	0.000	0.019	1.460	15.736	-2.199	28.958	0.160	4.745
AR2613	-0.00055	0.67860	0.000	0.018	-0.302	13.973	0.173	31.330	0.429	5.015
AR2621	-0.00162	0.67753	0.000	0.019	0.305	14.580	-0.261	30.895	0.763	5.349
AR2631	0.00024	0.67940	0.001	0.020	0.356	14.631	-0.429	30.728	-0.798	3.787
AR2641	0.00392	0.68307	0.001	0.019	1.409	15.684	-0.657	30.500	0.053	4.639

	NDVI		PRI		SPAD		SLT		YIELD	
clone	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV
AR2642	0.00216	0.68132	0.001	0.020	0.410	14.686	0.235	31.392	1.126	5.712
AR2651	0.00523	0.68438	0.002	0.020	1.183	15.459	-1.835	29.322	1.695	6.280
AR2652	0.00101	0.68017	0.001	0.020	0.619	14.895	-1.086	30.071	-0.679	3.906
AR2661	0.00318	0.68233	0.001	0.019	1.085	15.361	-0.125	31.032	0.497	5.083
AR2662	0.00023	0.67939	0.002	0.021	0.786	15.061	-1.327	29.830	0.151	4.737
AR2664	0.00481	0.68396	-0.001	0.018	0.462	14.737	1.399	32.555	-0.308	4.277
AR2671	-0.00291	0.67624	0.002	0.021	0.335	14.610	-1.894	29.262	-1.432	3.154
AR2672	-0.00007	0.67909	0.001	0.019	2.318	16.594	-0.282	30.875	-0.246	4.340
AR2681	0.00425	0.68341	0.000	0.019	-0.189	14.086	-0.380	30.777	-0.018	4.567
AR2682	-0.00153	0.67762	0.001	0.020	0.548	14.824	0.601	31.758	0.071	4.657
AR2691	0.00212	0.68128	0.000	0.019	1.224	15.500	-0.790	30.367	-0.086	4.499
AR2692	-0.00619	0.67296	0.000	0.018	1.542	15.818	1.297	32.453	-0.811	3.775
AR2693	-0.00373	0.67543	0.000	0.019	0.425	14.700	0.022	31.179	-1.251	3.335
AR2701	0.00292	0.68208	0.000	0.018	0.064	14.339	-0.728	30.429	-1.772	2.814
AR3001	0.00105	0.68021	0.000	0.018	1.007	15.283	0.438	31.595	0.086	4.671
AR3016	-0.00239	0.67677	0.000	0.019	-0.573	13.702	-0.261	30.896	0.769	5.354
AR3020	-0.00189	0.67727	-0.001	0.018	0.524	14.799	-0.319	30.838	1.358	5.943
AR3103	-0.00766	0.67149	0.000	0.019	-0.458	13.818	0.544	31.700	-0.321	4.264
AR3105	0.00122	0.68038	0.002	0.021	0.209	14.485	2.225	33.381	-1.103	3.482
AR3112	0.00243	0.68159	0.002	0.021	1.711	15.987	-0.913	30.243	0.103	4.688
AR3203	0.00781	0.68697	0.001	0.020	0.863	15.138	0.196	31.353	0.913	5.498
AR3204	0.00650	0.68565	0.001	0.020	2.075	16.351	-0.763	30.394	0.458	5.043
AR3205	0.00145	0.68060	0.000	0.019	0.248	14.523	-0.439	30.718	-0.011	4.575
AR3305	-0.00744	0.67172	-0.003	0.016	-2.563	11.712	-0.388	30.768	-0.425	4.161
AR3402	-0.00012	0.67904	-0.001	0.018	-1.545	12.731	-1.523	29.633	0.534	5.119
AR3404	0.00062	0.67978	0.001	0.020	-0.629	13.646	-0.960	30.197	0.384	4.969
AR3501	0.00183	0.68099	0.001	0.020	-0.162	14.114	-0.013	31.144	1.293	5.879
AR3502	0.00546	0.68462	0.002	0.021	0.265	14.541	-0.535	30.622	-0.517	4.069
AR3504	-0.00008	0.67908	0.002	0.021	1.782	16.058	-0.081	31.076	0.923	5.509

	NDVI		PRI		SPAD		SLT		YIELD	
clone	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV
AR3601	-0.00157	0.67759	0.000	0.019	-1.158	13.117	2.343	33.500	-0.2681	4.31739
AR3605	-0.00113	0.67803	0.001	0.019	-0.757	13.518	1.804	32.960	0.5810	5.16653
AR3701	-0.00169	0.67747	-0.002	0.016	0.086	14.362	-1.227	29.930	0.2071	4.7926
AR3702	0.00107	0.68023	0.000	0.019	-0.761	13.515	-0.247	30.910	-0.4437	4.14184
AR3803	0.00302	0.68218	-0.001	0.018	-0.718	13.557	0.692	31.849	-1.0327	3.55276
AR3804	-0.00346	0.67570	-0.002	0.017	-0.849	13.426	-0.355	30.801	-0.6717	3.91382
AR3806	-0.00034	0.67881	0.001	0.020	-0.746	13.529	-0.739	30.417	-0.9482	3.63726
AR3807	-0.00156	0.67760	0.000	0.019	-0.412	13.863	1.631	32.787	-1.2277	3.35775
AR3808	-0.00726	0.67190	-0.003	0.016	-1.536	12.739	-0.054	31.103	-0.9166	3.66887
AR3809	-0.00612	0.67303	-0.001	0.018	-0.813	13.462	0.668	31.825	-0.3611	4.22444
AR3810	-0.00747	0.67169	-0.003	0.016	-0.724	13.551	-0.062	31.094	-0.4022	4.18329
AR3812	0.00294	0.68209	0.000	0.019	-0.926	13.350	-0.073	31.084	-1.2324	3.35309
AR3901	-0.00779	0.67137	-0.001	0.018	-1.195	13.080	1.189	32.346	-0.3562	4.22931
AR3902	0.00253	0.68169	0.000	0.019	-0.170	14.106	0.613	31.769	0.7712	5.35669
AR3903	0.00354	0.68270	0.000	0.019	-0.144	14.132	-0.667	30.489	0.5340	5.11959
AR3905	-0.00071	0.67845	0.000	0.019	-0.241	14.035	0.709	31.866	-0.2082	4.37736
AR3910	-0.00449	0.67467	0.000	0.019	-0.666	13.610	-0.911	30.246	0.3354	4.92089
AR40105	-0.00168	0.67748	0.000	0.019	1.427	15.702	-2.398	28.759	0.4288	5.01436
AR40502	-0.00067	0.67849	0.001	0.019	0.446	14.721	-0.284	30.873	-0.0161	4.56943
AR40503	0.00208	0.68124	0.002	0.020	0.161	14.437	-0.101	31.056	-0.0683	4.51724
AR40506	-0.00323	0.67593	-0.001	0.017	-1.007	13.269	0.406	31.562	-0.2239	4.36164
AR40507	0.00880	0.68795	0.001	0.020	1.896	16.172	-0.632	30.524	-1.6356	2.94991
AR40509	0.00324	0.68240	0.001	0.020	0.809	15.085	-1.346	29.811	1.3168	5.90231
AR40510	-0.00174	0.67741	0.000	0.019	0.069	14.344	1.248	32.404	0.8214	5.40693
AR40709	0.00455	0.68371	0.001	0.020	-0.039	14.236	-0.169	30.987	0.2109	4.79642
AR40802	0.00076	0.67991	0.001	0.019	0.235	14.511	-0.174	30.983	0.9462	5.53179
AR4101	0.00601	0.68516	0.003	0.021	1.690	15.966	-0.320	30.836	1.2991	5.88468
AR4103	0.00138	0.68054	-0.001	0.017	-1.125	13.150	0.498	31.654	-0.3432	4.24228
AR4104	0.00692	0.68608	0.003	0.022	0.835	15.111	0.887	32.044	0.1308	4.7163

	NDVI		PRI		SPAD		SLT		YIELD	
clone	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV	EBLUPs	PGV
AR4105	-0.00387	0.67528	-0.002	0.017	-0.142	14.134	0.421	31.578	-0.493	4.09207
AR4107	0.00007	0.67923	-0.001	0.018	1.008	15.284	-0.171	30.986	0.128	4.71371
AR4110	0.00474	0.68390	-0.001	0.018	0.294	14.570	1.019	32.176	0.356	4.94116
AR41201	0.00266	0.68182	0.000	0.018	0.664	14.940	-1.681	29.476	-0.229	4.35656
AR41202	-0.00171	0.67745	-0.001	0.018	0.012	14.288	1.456	32.613	-0.528	4.0575
AR41203	0.00822	0.68738	0.000	0.019	1.987	16.263	-0.338	30.819	-0.565	4.02052
AR41204	0.00453	0.68369	-0.001	0.018	0.226	14.502	0.449	31.606	-0.862	3.72331
AR41205	0.01075	0.68990	0.001	0.019	1.103	15.379	-0.390	30.767	0.233	4.81859
AR41206	0.00462	0.68378	0.003	0.022	-0.169	14.107	-2.151	29.006	0.796	5.3811
AR41207	0.00132	0.68048	0.001	0.020	1.368	15.643	1.303	32.460	0.054	4.63941
AR41303	-0.00741	0.67175	0.000	0.019	-1.236	13.039	-1.050	30.107	0.537	5.12275
AR41304	-0.00019	0.67897	0.000	0.018	-0.386	13.890	-0.013	31.144	0.236	4.82179
AR41305	0.00280	0.68196	0.001	0.019	0.446	14.722	-0.755	30.402	1.118	5.70394
AR6116	-0.01208	0.66707	-0.002	0.017	-1.819	12.457	0.729	31.886	-0.571	4.01469
AR6118	0.00464	0.68380	0.000	0.019	1.956	16.232	0.345	31.501	-1.709	2.8768
AR6701	-0.00031	0.67885	0.001	0.020	-0.473	13.802	-0.487	30.669	0.408	4.99382
AR7503	-0.00034	0.67882	0.000	0.018	0.485	14.760	0.079	31.236	0.283	4.86847
AR8202	0.00261	0.68177	0.001	0.019	-0.724	13.552	-0.331	30.825	0.107	4.69223
AR8204	0.00080	0.67995	0.002	0.021	-0.199	14.077	1.435	32.591	0.602	5.18801
AR8807	0.00296	0.68212	0.001	0.019	1.275	15.550	-1.087	30.070	-0.299	4.28617