Relationships between sensitivity to high temperature, stomatal conductance and vegetative architecture in a set of grapevine varieties

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Introduction

High temperatures influence plant development and induce a large set of physiological responses at the leaf scale. Stomatal closure is one of the most observed responses to high temperatures. This response is commonly considered as an adaptive strategy to reduce water loss and embolism in the vascular system caused by the high evaporative demand (Jones and Sutherland., 1991). Nevertheless, this response negatively impacts plant functioning, as it decreases photosynthesis and raises the leaf temperature (Tuzet et al., 2003). This increase in temperature is due to a decrease in energy loss by evaporative cooling. In extreme cases, this increase can induce leaf burning symptoms and lead to leaf or entire plant mortality (Webb et al., 2010).

In the context of global warming, the occurrence of extreme heatwaves events is expected to increase in almost all the vineyard areas. These events can cause major risks for the perennity of this cropping system. In this context there is a need to develop new varieties more adapted to high temperatures. For instance in the south of France in June 2019 a major heatwave was observed with air temperature higher than 45°C. Previous analyses made during this period, showed high genotypic variability in the sensitivity to this leaf burn symptoms in a core collection of varieties that was grown in Montpellier (South of France).

To apprehend the physiological determinants explaining these genotypic differences, it is necessary to understand the factors that affect leaf temperature. Leaf temperature results from the leaf energy balance. This energy balance depends on the amount of solar radiation intercepted by the canopy and on the ability of the leaf to transfer this energy through evapotranspiration. In that context, there exist two leverages that limit this increase in leaf temperature. First, reducing the amount of light intercepted and secondly maintaining stomatal aperture even under high temperature. Previous studies in grapevine showed high genotypic variability in stomatal behavior under water deficit in grapevine (Coupel-Ledru et al., 2014). Conversely, the studies on the response to temperature are more scarce. Regarding the amount of light intercepted, plant architecture plays a major role in light capture (Louarn et al., 2008). From the multitude of architectural traits: leaf shape and size, petiole length, and leaf 3D orientation significantly influence the efficiency of radiation interception (Falster and Westoby, 2003; Valladares and Brites, 2004).

A large genotypic variability in architectural traits was also observed in many plants (Segura et al., 2007 in apple). However, no study investigated the genotypic variation in architectural traits in grapevines and their potential impact on leaf functioning. In grapevine, a previous study showed intra-plant variability in leaf angles with respect to the training systems (Mabrouk et Carbonneau, 1997). However, this study did not consider any genotypic variability. Consequently, the definition of new architectural and functional ideotypes to face hatewaves in vineyards is a particularly relevant research topic.

Research Objectives

In the present study we investigated in a set of 30 genotypes displaying contrasted architectural traits and different sensitivity to leaf burning symptom observed in 2019, 1) the genotypic variability in the leaf temperature and stomatal conductance under a wide range of climatic conditions, 2) the existing genotypic variability in some architectural traits and 3) the relationships between architectural traits, stomatal conductance and the previously observed leaf burning symptoms. The 30 genotypes we studied were selected from a panel of 279 genotypes optimized to represent the genetic diversity of Vitis vinifera L (Nicolas et al., 2016).

Material and methods

Plant material and experimental site

Among the 30 genotypes we studied, 16 genotypes were identified in the experimental vineyard of the Institut Agro - Montpellier in June 2019 during the heatwave period. They were selected on the basis of the intensity of burning symptoms. Phenotyping of the burning symptoms was conducted based on the proportion of leaves that were burned (0-100%) and the burning intensity on individual leaves (qualitatively noted from 0 to 5). Among the 16 genotypes 8 were sensitive to high temperatures (called ‘sensitive’ genotype) and 8 low or not sensitive (called ‘resistant’ genotype). The 16 other genotypes were selected to maximize the variability in architectural traits such as leaf orientation, leaf shape and internode length. Measurements were conducted on two-year-old plants in 2021 and on one-year-old cuttings in 2022. The plants were grown in pots and fert-irrigated to avoid any water deficit or nitrogen deficiency. The experiments were carried out at the campus of the Institut Agro - Montpellier from the 10th to 19th of August in 2021 and from 19th of July to the 8th of August in 2022. The rows in this vineyard had an east-west orientation. Only one annual shoot with all the secondary axes removed was kept on each plant. The plants belonging to the same genotypes were placed with two pots facing each other in the row to represent self-shading conditions that could be observed in the vineyard. As a consequence half of the individuals of a given genotype were oriented in western direction and the other half in eastern direction.

Physiological variables

Measurements of stomatal conductance and leaf temperatures were performed using a fluo-porometer (Li-600, Licor, Lincoln, NE, USA). These measurements were made on four leaves per genotype in each year (two leaves on two plants in 2021, one leaf on four plants in 2022). Measurements were made on sun-exposed leaves during 6 days in 2021 and 11 days in 2022. The measurement days were most of the time divided into measurement sessions with one session in the morning and another one in the afternoon (10 sessions in 2021 and 21 sessions in 2022). The climatic conditions during these different measurement periods were thus contrasted. The choice to perform measurements under contrasted climatic conditions allowed us to assess the repeatability of the observed results. Additional measurements were made on leaves. We measured photosynthesis at saturating light and ambient CO_2 concentration (Amax) and photosynthesis assimilation at saturating light and saturation CO_2 concentration (Asat), water potential (stem and leaf potential), non structural carbohydrate contents and chlorophyll concentration. These complementary measurements are not presented in this abstract.

Architecture description

Two plants per genotype on the western side of the row were digitized in 2021 and 2022. Digitizing was done with a Polhemus electromagnetic digitizer (3Space Fastrak; Polhemus Inc., Colchester, VT, USA). Seven 3D coordinates on each metamer along the primary axis were recorded: the insertion point of the petiole on the stem, the insertion of the blade on the petiole and the five extremities of the veins of the leaf blades. Based on these 3D coordinates, we computed various architectural traits among them the internode length, the individual leaf area and the leaf elevation, azimuth and roll angles (azimuth and roll angles are not presented in this abstract). In our study the elevation angles varied between 0 and 180°. An elevation angle equal to 90° represents horizontal leaves, 180° erected leaves in upward direction and 0° bending leaves in downward direction.

Statistical analysis

All statistical tests and plots were made with R software. For architectural traits, we used a mixed model with the genotype as a random effect and the year as a fixed effect to extract the Best Linear Unbiased Predictors (BLUPs) of genetic values. For functional traits we also considered the genotype as a random effect but we used as a fixed effect a variable called afterward ‘year_period’. This variable combines the year, the day and the period of measurements (morning or afternoon conditions). Variance estimates of the models were used to estimate the broad-sense heritability (H^2) as :

$$H^2 = \frac{\sigma^2{}_G}{(\sigma^2{}_G + \frac{\sigma^2{}_R}{n})}$$

where σ^2G is the genetic variance, σ^2R the residual variance, and n the number of replicates per genotype. The heritability for all the traits was computed for the year 2021 and 2022 considered separately and for both years together (2021 & 2022).

We performed two ways ANOVA to analyze the possible relationship between the group of sensitivity to burning symptoms and architectural and functional traits. Two-way ANOVAs including the group of genotypes (‘sensitive’ or ‘resistant’ based on burning symptoms) and the year or the year_period effect was included in these ANOVAs.To characterize the existing variability in climatic conditions during the different measurement periods, we used a clustering approach (Ward method). The variables included were the air temperature (T, °C), relative humidity, wind speed ($m.s^{-1}$), vapor pressure deficit (VPD, kPa) and the incident solar radiation (Rs, W m^{-2}).

Results

Climatic variability between the measurement periods

The clustering performed on the climatic variables showed that there were four main types of measurement periods (the result of the clustering is not presented in this abstract). The first type corresponded to the warmest and driest periods with an average T= 35.8°C, Rs= 833.3 W/m² and VPD= 4.1 Kpa. 12 measurement sessions belonged to this group. The second type corresponded to warm and dry periods with average T= 31.5°C and VPD= 3.2 Kpa (11 measurement sessions). The third type corresponded to warm (T= 32°C) but more humid periods (VPD= 2.4 Kpa) (7 measurement sessions). Finally, the last type corresponds to the coldest periods with an average T= 27°C, Rs= 563.2 W/m² and VPD= 1.8 Kpa (3 measurement sessions).

Heritability of functional and architectural traits

The broad sense heritability of functional traits (Tleaf, gs) was high (gs = 0.67, Tleaf = 0.76) if both years were considered separately except for Tleaf in 2022 for which H^2 = 0.31, only. Finally, the functional traits were less heritable if we consider both years but remained non negligible above all for stomatal conductance. Such differences between the two years could be due to the fact that (i) plants were measured at different periods on older leaves in 2021 than in 2022 and (ii) plants did not have the same age in both years (1 year old in 2022 and 2 year old in 2021). The architectural traits (individual leaf area, elevation angle and internode length) are highly heritable for all cases. As for functional traits, H^2 remained higher if we considered the years separately. The leaf elevation angle was the most heritable trait (H^2 = 0.89) closely followed by the individual leaf area (H^2=0.75) and internode length (H^2= 0.71).

Impact of leaf architecture, and functional traits on observed burning symptoms.

We tried to find out if we could find any differences on functional and physiological traits which could be associated with the intensity of the leaf burning symptoms observed during the 2019 heatwave. First, a significantly higher stomatal conductance was observed for the ‘sensitive’ genotypes ($P<0.001$). Consistently with this higher stomatal conductance a lower temperature was observed. This result could be considered as unintuitive but it should be noted that the temperature during the measurements in 2021 and 2022 were lower than the ones observed during the heatwave in 2019. A first conclusion could be that the ‘sensitive’ genotypes had a different regulation of stomatal conductance than the ‘resistant’ genotypes. Their strategy could be to keep their stomata open even at very high temperatures. This behavior would eventually cause some hydraulic failure in the vascular system under high temperature with high evaporative demand.

Then, we observed the architectural traits depending on the sensitivity to the heatwave. First, the ‘resistant’ genotypes had shorter internodes than the ‘sensitive’ genotypes. We assume that this shorter internodes leads to higher self-shading within the canopy and could reduce the amount of light intercepted by each individual leaf. There were also differences in individual leaf areas between the two groups with greater values for the ‘sensitive’ genotypes but these differences were not significant. Finally, no significant differences in leaf elevation angle between the ‘resistant’ and 'sensitive’ groups was observed. 3D mockups of one ‘resistant’ and one ‘sensitive’ genotype are represented in Fig. 2f. The ‘resistant’ plants had short internodes and small leaves, whereas the ‘sensitive’ one had longer internodes and larger individual leaf area.

Conclusion

This study was based on a large set of data collected over a wide range of climatic conditions. It showed that a part of the variability in the sensitivity to leaf burning symptoms could be associated with architectural traits and stomatal behavior. We are also investigating other traits that could explain these differences in leaf sensitivity such as leaf and stem water potential as well as the impact of leaf temperature on leaf photosynthesis. Finally we are also making use of 3D modeling approaches to estimate the potential impact of architectural traits while keeping in mind that leaf temperature is probably not completely associated with the observed leaf burning symptoms. Other physiological adjustments such as the accumulation of heat shock proteins could explain the observed genotypic differences. Such kinds of mechanisms can lead to contrasted symptoms between genotypes for a similar leaf temperature.

Acknowledgements

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Tables and Figures

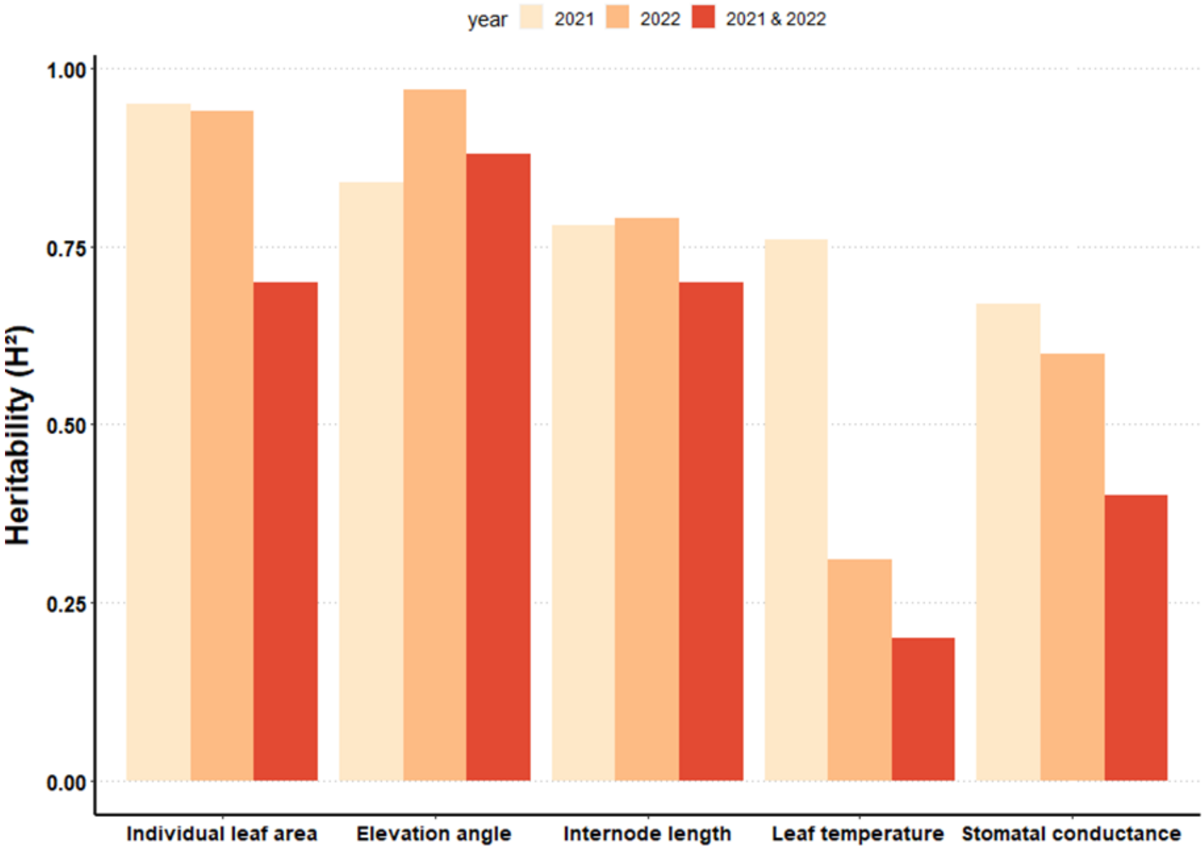

Figure 1. Broad sense heritabilities of architectural (individual leaf area, leaf elevation angle and internode length) and functional traits (leaf temperature and stomatal conductance) computed for the two years of measurement (2021, 2022) or for both years together.

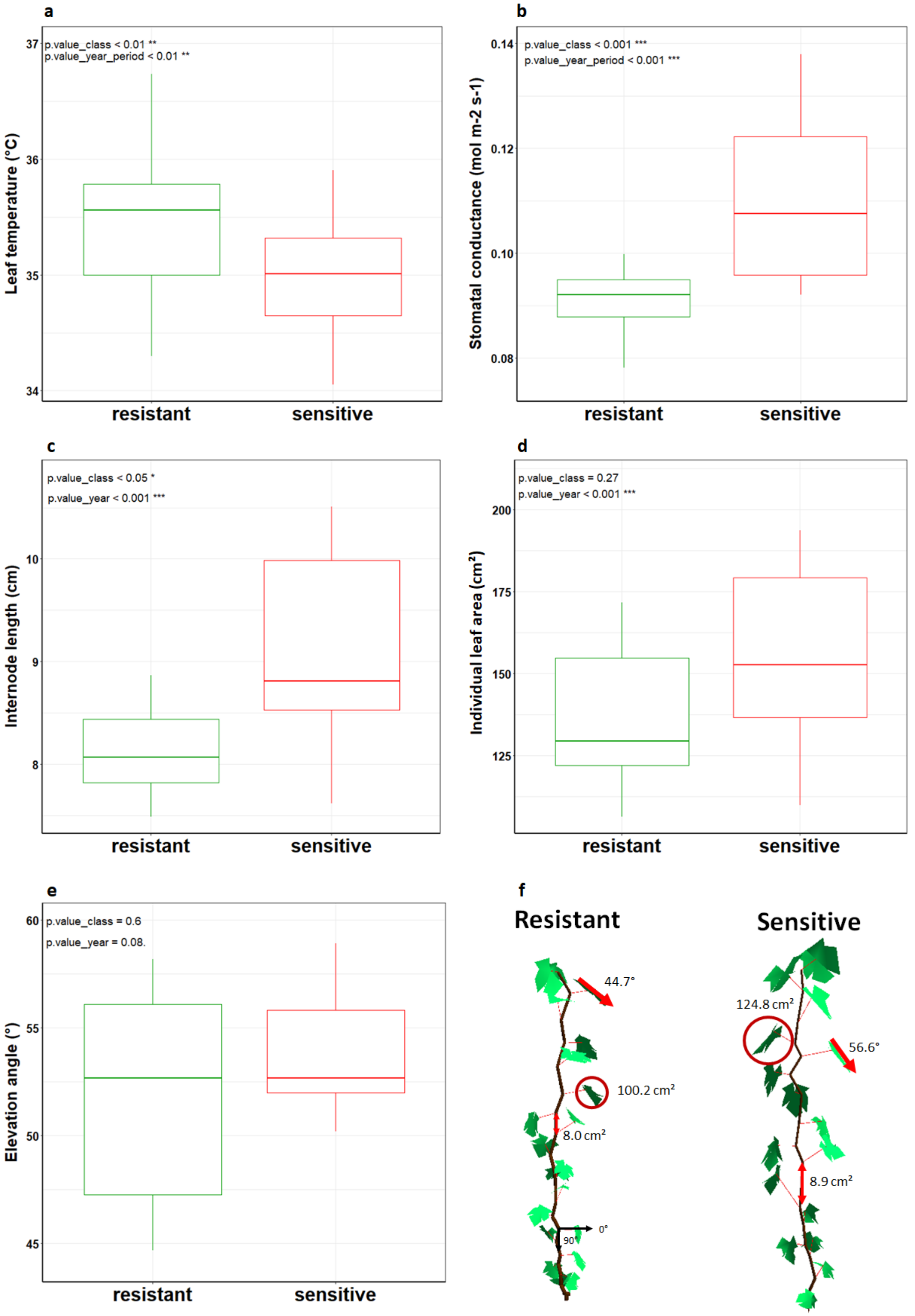
a
Leaf temperature (°C)
p.value_class < 0.01 **
p.value_year_period < 0.01 **
37
36
35
34
resistant
sensitive
b
Stomatal conductance (mol m-2 s-1)
p.value_class < 0.001 ***
p.value_year_period < 0.001 ***
0.14
0.12
0.10
0.08
resistant
sensitive
c
Internode length (cm)
p.value_class < 0.05 *
p.value_year < 0.001 ***
10
9
8
resistant
sensitive
d
Individual leaf area (cm²)
p.value_class = 0.27
p.value_year < 0.001 ***
200
175
150
125
resistant
sensitive
e
Elevation angle (°)
p.value_class = 0.6
p.value_year = 0.08.
60
55
50
45
resistant
sensitive
f
Resistant
Sensitive
44.7°
100.2 cm²
8.0 cm²
0°
90°
124.8 cm²
56.6°
8.9 cm²

Figure 2. Box plot representation of the physiological and architectural traits depending on the genotype sensitivity based on the burning symptoms observed in June 2019. For physiological traits, a two-way ANOVA with the genotype class (‘resistant’ vs ‘sensitive’) and the year-period factor effects was performed. For architectural traits, a two-way ANOVA with the genotype class and year effects was performed. The significance of each effect is represented on the top leaf corner of each sub-figure. The sub-figure on the bottom right side represents two 3D mockups of two genotypes belonging to the two classes of genotypes. On this sub-figure, the internode length, individual leaf area and elevation angle are represented.