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Article

Tolerance of Greek Indigenous Winegrape Cultivars to Heat Stress under Variable Light Conditions

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Abstract

This study investigates the effects of rising temperatures on photosynthetic efficiency and stress tolerance in major Greek grapevine cultivars (Savvatiano, Muscat, Assyrtiko, Mavrodafni, Moschofilero, and Agiorgitiko), using Sauvignon Blanc and Merlot as reference varieties. The primary aim was to identify heat tolerant genotypes suitable for cultivation in warm regions with high solar radiation. Another objective was to indicate shade-tolerant cultivars that could benefit from canopy shading techniques, contributing to broader climate adaptation strategies in viticulture. Muscat and Assyrtiko displayed the most heat-tolerant photosynthetic apparatus among white cultivars, while Mavrodafni was the most heat-tolerant among the red ones, by effectively managing excess light energy. Sauvignon Blanc although exhibiting heat susceptibility, it maintained high PSII functionality under heat stress by activating photoprotective mechanisms. Temperatures above 35 °C increased grapevine's susceptibility to strong light, thereby intensifying photooxidative stress. Savvatiano and Agiorgitiko were more vulnerable to photooxidative stress above 35°C, though Agiorgitiko maintained a functional photosynthetic apparatus even at 40°C by shifting to a more photoprotective strategy. In contrast, Merlot, despite its resistance to photooxidative stress, lacked photoprotective investment, resulting in suppressed PSII under heat stress. Moschofilero was the most susceptible cultivar to photooxidative stress. Leaf morphological traits contributed to heat and light stress tolerance, with smaller, thicker leaves facilitating thermoregulation. Taken together, Merlot, Mavrodafni, Sauvignon Blanc, and Assyrtiko are recommended for high-altitude cultivation due to their high tolerance to light. Conversely, Savvatiano, Moschofilero, and Agiorgitiko were better suited to lower light conditions, favoring higher latitudes and shading practices under heat stress. The present results provide important insights into cultivar-specific responses to heat and light stresses, presenting for the first time the respective tolerances of major Greek cultivars. This knowledge is essential for selecting heat-tolerant genotypes, optimizing vineyard site selection and management strategies, thereby supporting the sustainability and climate resilience of viticulture.

Keywords: climate change; grapevine; heat stress; tolerance; light; chlorophyll fluorescence; leaf traits; photooxidative stress; PSII; photoinhibition

1. Introduction

Climate change has led to more frequent, intense and prolonged heat events, with Mediterranean regions being at the center of this challenge by experiencing temperatures above the global average [1,2]. Except for quality degradation by producing wines with higher alcohol content, lower acidity and altered aromatic profiles, high temperatures also threaten the sustainability of several vineyard sites, by potentially making them unsuitable for viticulture [3]. Therefore,

implementing sustainable management strategies for the adaptation of viticulture to emerging stress conditions is essential for preserving its long-term sustainability [4]. Some of these strategies include the relocation of vineyards to cooler areas, the application of viticultural shading techniques, and the selection of genotypes with advanced heat-tolerance [5,6].

One of the most reliable indicators of heat tolerance is photosynthesis as it is considered the most heat-sensitive physiological process that can be inhibited before other heat-stress symptoms are detected [7,8]. The heat-tolerance of the photosynthetic apparatus is thought to be mainly dependent on the thermostability of PSII, one of the most heat-sensitive components of the electron transport chain [9,10,11,12]. This PSII vulnerability is primarily attributed to the increased fluidity of thylakoid membranes at temperatures above 35°C. Under these conditions, the major light-harvesting complex (LHCII) detaches from the PSII reaction center (PSII-RC), making it more susceptible to damage from excess light energy [13]. Therefore, disruptions in thylakoid membranes integrity may lead to damages in the electron transport system manifested by increases in the initial chlorophyll fluorescence emission which is a reliable indicator of PSII efficiency and photosynthetic apparatus stress tolerance [14,15].

However, high temperatures usually do not occur alone in nature but often coexist with high light intensities which synergistically intensify plant stress, making their evaluation essential [16,17]. Additionally, climate change with the upcoming scenarios of even higher temperatures, will potentially place in danger low latitude areas, by making them unsuitable for viticulture [18,19]. Therefore, the necessity for a sustainable viticulture will probably change the current geographical distribution of vineyards by pushing them to higher latitudes or altitudes [20]. Regarding this, we should consider that high-altitude regions are characterized by higher light intensities and therefore such locations are more suitable for cultivars with high-light tolerance [21,22,23,24,25]. In contrast, high-latitude regions are characterized by lower light intensities making them more suitable for cultivars better adapted to limited light conditions [26,27]. Therefore, the evaluation of cultivars' adaptability under low light intensities is equally important, considering also that viticultural practices such as artificial shading and proper canopy configuration (to protect grapes from sunburn), are widely used to mitigate the heating effects of intense solar radiation [5,28,29]. This evidence strongly indicates that light intensity is a decisive factor in determining suitable cultivation areas and appropriate practices for each variety, highlighting the importance of evaluating their performance under fluctuating light conditions.

The main goal of this study was to assess, compare and draw conclusions regarding the resistance of different grapevine cultivars under combined conditions of heat stress and fluctuating light intensities. Considering also that climate change will inevitably move viticulture at higher altitudes or latitudes, as well as the application of viticultural practices to mitigate heat-stress effects, this study offers a valuable perspective on a future climate adaptation scenario. Such knowledge will be crucial for identifying optimal cultivation sites and implementing appropriate viticultural practices for each variety, thereby supporting the wine sector to adapt to climate change implications.

2. Materials and Methods

2.1. Plant material and growth conditions

For the conduction of the experiments, two different groups of *Vitis vinifera* L. cultivars including white (Savvatio, Assyrtiko, Muscat, and Sauvignon Blanc) and red (Moschofilero, Agiorgitiko, Mavrodafni, and Merlot) ($n = 4$) were used. Six of them were Greek indigenous (Muscat refers to white Muscat of Samos), while two were international (Sauvignon Blanc and Merlot). All plants were heterografted onto Richter 110 rootstock and transplanted into 8L pots filled with a soil substrate mixture consisting of peat (60%), clay (30%), and zeolite (10%). After budbreak, one shoot per plant was maintained, and all of them were placed outdoors. All the pots were irrigated until runoff, and no symptoms of water stress detected, while balanced fertilization was performed at regular intervals to prevent nutrient deficiencies. Once the plants acquired 10-11 fully-expanded leaves, they were

transferred into a plant growth chamber (CMP-6050, Conviron, Winnipeg, MB, Canada) for acclimatization. The chamber was equipped with T5 fluorescent and halogen lamps providing a balanced light spectrum for plant growth and a heat exchange system for the temperature control. During the experiments, the lamps were operated at a constant $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, the day/night photoperiod cycle was set at 13/11 hours, and the relative humidity was maintained at 50-60%. All plants were exposed to three distinct temperature regimes: a control regime with day/night temperatures of $23^{\circ}\text{C}/15^{\circ}\text{C}$, a moderate heat stress regime at $35^{\circ}\text{C}/27^{\circ}\text{C}$, and a severe heat stress regime at $40^{\circ}\text{C}/32^{\circ}\text{C}$. All measurements were conducted on mature, fully-expanded leaves. Prior to the beginning of each group of measurements, plants were acclimatized for 4-5 days in each condition.

2.2. Photosynthetic light response curves (LRCs)

Photosynthetic light response curves (LRCs) were undertaken with the steady-state method using the autoprogram function of Li-COR 6400XT (LI-COR Environmental, Lincoln, Nebraska USA) under all the temperature regimes (23°C , 35°C and 40°C).

The measurements were performed with decreasing light intensity from 2000 to $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ in ten steps (2000 , 1500 , 1000 , 500 , 250 , 120 , 60 , 30 , 15 and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$) with an average waiting time of 120-200 seconds at each step. The A_n/I data collected from the LRCs were then modeled by fitting them to a Michaelis-Menten based model [30], using non-linear regression techniques in SPSS 29 to determine the parameters of light-saturated photosynthesis (A_{sat}), dark respiration rate (R_{dark}), light saturation point (LSP) and light compensation point (LCP). The particular model was based on a rectangular hyperbola function which is described as follows:

$$A_n = \frac{\phi_{(I_0)} \times I \times A_{g \max}}{\phi_{(I_0)} \times I + A_{g \max}} - R_{\text{Dark}} \quad (1)$$

where A_n is the net photosynthetic rate [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$], $\phi(I_0)$ is the quantum yield at $I = 0$ [$\mu\text{mol}(\text{CO}_2) \mu\text{mol}(\text{photon})^{-1}$], I is the photosynthetic photon flux density [$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$], $A_{g \max}$ is the estimate of the maximum gross photosynthetic rate [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$] and R_{dark} is the dark respiration rate.

2.3. Temperature dependence model

Temperature responses of light-saturated photosynthesis (A_{sat}) were modeled by fitting the measured values into a modified Gaussian growth function model [31], which provides bell-shaped curves with great flexibility and accurate fit to the measured data and it is described as follows:

$$A(T) = A_{\text{opt}} * \exp\left(-\frac{(T - T_{\text{opt}})^2}{\sigma^2}\right) \quad (2)$$

where A_{opt} is the maximum photosynthetic rate, T_{opt} is the temperature where A_{opt} occurs and σ represents the spread of the Gaussian curve, defining how broadly or narrowly the curve is distributed around T_{opt} . The optimal temperature for photosynthesis (T_{opt}) was determined as the temperature corresponding to the maximum of the fitted curve.

2.4. Chlorophyll content measurements

Leaf chlorophyll content (LCC) was estimated by a non-destructive method which is based on measuring the leaf absorbances in the red and near-infrared regions with the Minolta SPAD-502 chlorophyll meter (Konica Minolta Inc., Osaka, Japan). Using these two absorbances, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf. In order to convert the SPAD data (unitless) to absolute values of chlorophyll content ($\mu\text{g}/\text{cm}^2$), we adopted the empirical statistical model of [32].

$$\text{LCC} = 1.034 + 0.308 * \text{SV} + 0.11 * \text{SV}^2 \quad (3)$$

Where LCC is leaf chlorophyll content and SV is the Spad Value

2.5. Chlorophyll fluorescence quenching analysis

Chlorophyll *a* fluorescence measurements were performed using the LI-600 Fluorometer (LI-COR Biosciences, Inc., Lincoln, NE). Light-adapted state measurements were first performed with the light intensity inside the growth chamber to be set at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The steady-state fluorescence (F_s) was first determined and thereafter a 800ms saturating light of 7500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was applied to measure the maximum fluorescence in the light-adapted state (F_m') which were then used for the estimation of the actual quantum efficiency of PSII (ΦPSII):

$$\Phi\text{PSII} = \frac{F_m' - F_s}{F_m'} \quad (4)$$

Subsequently, the lights of the chamber were closed and all the desired leaves were dark-acclimated for 30 min before the start of the measurements in the dark-adapted state. The parameters of maximum fluorescence (F_m) and minimum fluorescence (F_o) were then determined providing a 800ms saturating pulse of continuous red light at 6000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ which were then used for the calculation of the maximum quantum efficiency of PSII using the following equation:

$$\frac{F_v}{F_m} = \frac{F_m - F_o}{F_m} \quad (5)$$

The parameters of non-photochemical quenching (NPQ) and photochemical quenching (qP) were also determined following Shin et al. [33] with the below equations:

$$\text{NPQ} = \frac{F_m - F_m'}{F_m'} \quad (6)$$

$$\text{qP} = \frac{F_m' - F_s}{F_m' - F_o'} \quad (7)$$

where F_o' is the minimum chlorophyll *a* fluorescence in the light-adapted leaf, calculated using the formula of Oxborough and Baker [34]:

$$F_o' = \frac{F_o}{\frac{F_v}{F_m} + \frac{F_o}{F_m'}} \quad (8)$$

2.6. Leaf transpirational cooling and thermoregulation

Leaf temperature (T_{leaf}) measurements were conducted using a non-contact infrared thermometer (IRT) integrated into the LI-600 Porometer (LI-COR Biosciences, Inc., Lincoln, NE). Transpiration rate was also computed from the difference in H_2O in an air-stream flowing through a leaf cuvette:

$$E = \left(- \frac{\text{Mr}(\text{W}_s - \text{W}_r)}{S(1 - \text{W}_s)} \right) \quad (9)$$

where E is apparent transpiration ($\text{mol m}^{-2} \text{s}^{-1}$), S is leaf area (m^2), Mr (mol s^{-1}) is the molar flow rate into the leaf cuvette, and W_r with W_s are water vapor mole fractions into and out of the leaf cuvette respectively ($\text{mol H}_2\text{O mol air}^{-1}$).

2.7. Leaf morphology

For the estimation of leaf size, we used a destruction method following the instructions of Martin et al. [35]. After collecting the plants, the leaves were removed and placed on a sheet of A4 paper bearing a completely black rectangle of 3 x 15 cm (45 cm^2) used for software calibration. All the leaves

were then photographed using a digital camera and the photos uploaded to the ImageJ software for the estimation of leaf area. The average distance between the camera and the leaf was 50 cm. For estimation of mesophyll thickness, we followed the instructions of Luo et al. [36] by performing semi-thin leaf cross-sections which were then photographed at 400× magnification using the digital microscope camera Leica DFC450 (Leica Microsystems, Milton Keynes, UK). All the images were then uploaded to the ImageJ software for the estimation of mesophyll thickness.

2.8. Statistical data analysis

Analysis of variance (ANOVA) was conducted to assess the effects of variety and temperature on all the dependent variables, followed by Tukey's HSD post-hoc test at $p \leq 0.05$. Prior to performing ANOVA, the assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilk and Levene's tests, respectively, at a significance level of $p \leq 0.05$. Linear and non-linear regression analyses were also performed for curve normalization, fitting data to 3rd and 4th degree polynomial growth functions. All statistical analyses were conducted using SPSS Statistics v.29.

3. Results

3.1. Temperature dependence of light response curves (LRCs) parameters

LRCs revealed different photosynthetic responses under fluctuating light intensities with the data being well fitted to the rectangular hyperbola model ($R^2 > 0.992$). Light-saturated photosynthesis (A_{sat}) showed thermal optima (T_{opt}) at 36.4-40.1°C for the white cultivars with significant differences between treatments and genotypes ($P < 0.001$). A_{sat} increased by 36.2-56.2% up to 35°C whereas different responses recorded at 40°C, with either decreases, minimal changes, or increases. Sauvignon Blanc exhibited the highest A_{sat} whereas Muscat significantly the lowest across treatments ($P < 0.01$) (Figure 1A). Light-saturation point (LSP) was also non-linear correlated with temperature with increases of 55.4-78.8% up to 35°C and fluctuating responses at 40°C depending on cultivar. We did not observe significant differences between genotypes at 23°C ($P = 0.458$), in contrast to 40°C where Sauvignon Blanc and Muscat recorded the highest LSP and Savvatiano significantly the lowest ($P = 0.003$) (Figure 1C).

A_{sat} and LSP also significantly differed among red cultivars and treatments ($P < 0.001$). A_{sat} increased by 15.3-32.7% up to 35°C and declined by 33.5-43.5% at 40°C revealing different thermal optima ($T_{opt} = 32.3-34.2^\circ\text{C}$). Mavrodafni exhibited the highest rates across treatments ($P < 0.001$), whereas no significant differences were observed among the rest cultivars ($P > 0.340$) (Figure 1B). LSP increased by 21.5% up to 35°C and declined by 10-23.9% at 40°C where Mavrodafni presented the highest and Agiorgitiko the lowest values (Figure 1D).

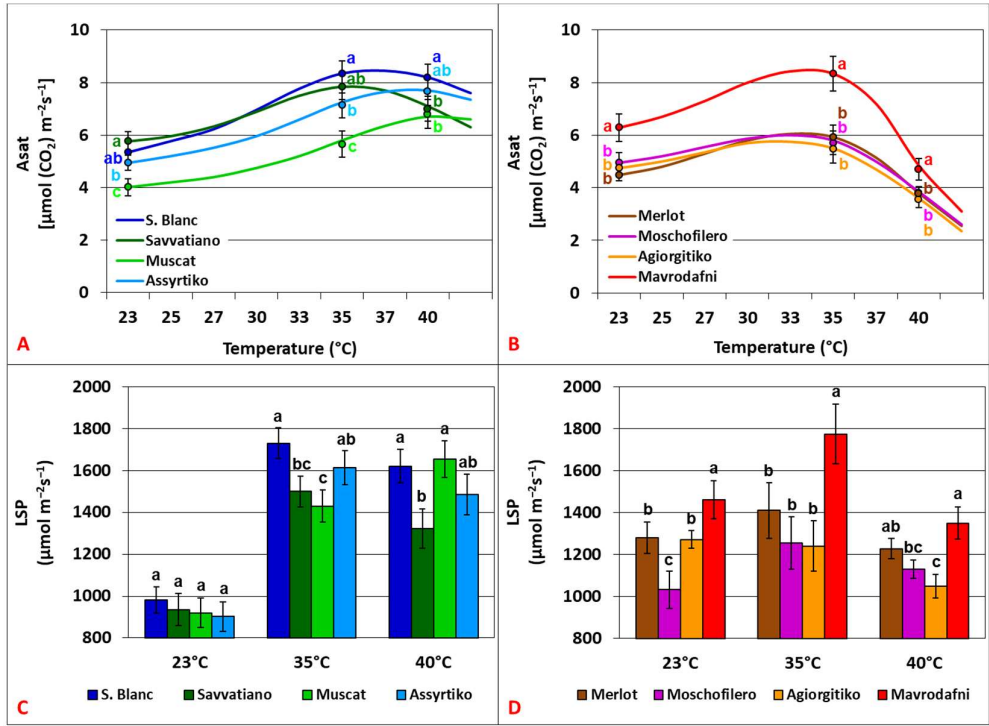


Figure 1. Temperature responses of light-saturated photosynthesis (Asat) (A, B) and light saturation point (LSP) (C, D) in two grapevine varietal groups. Data points with error bars represent mean $\pm$ standard deviation (SD). Different lowercase letters denote statistically significant differences between cultivars ($P<0.05$). A and C refer to white cultivars, while B and D to red ones ($n=4$).

Dark respiration rates (R_{dark}) and light compensation point (LCP) increased with rising temperature, more exponentially above 35°C, fitting well to quadratic functions ($R^2=0.982-0.994$). No significant differences were observed in R_{dark} or LCP between genotypes at 23°C ($P>0.062$) in contrast to 35°C ($P<0.003$) and 40°C ($P<0.001$) in both varietal groups. Sauvignon Blanc exhibited the highest R_{dark} and LCP values, whereas Savvatiano the lowest at 40°C (Figure 2A,C). Among the red cultivars Merlot demonstrated the highest R_{dark} and LCP values while Mavrodafni the lowest 40°C (Figure 2B,D).

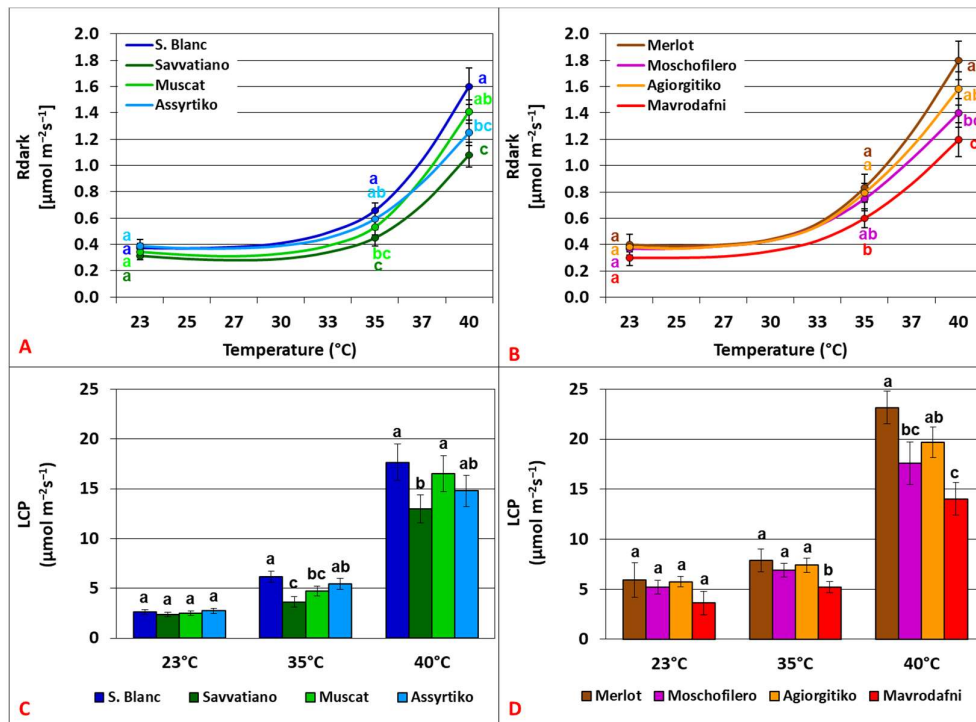


Figure 2. Temperature responses of dark respiration rate (R_{dark}) (A, B) and light compensation point (LCP) (C, D) in two grapevine varietal groups. Data points with error bars represent mean $\pm$ standard deviation (SD). Different lowercase letters denote statistically significant differences between cultivars ($P < 0.05$). A and C refer to white cultivars, while B and D to red ones ($n=4$).

3.2. Temperature dependence of chlorophyll fluorescence parameters

The F_v/F_m parameter remained almost stable with rising temperature up to 35°C in most cases, in contrast to 40°C where most genotypes recorded significant reductions. We observed significant variations among genotypes across all treatments in both varietal groups ($P < 0.001$). Sauvignon Blanc, Assyrtiko and Muscat remained F_v/F_m above 0.75 at 40°C whereas Savvatiano displayed 3.9-5.1% lower values ($P < 0.001$) (Figure 3A). $\Phi PSII$ demonstrated a stable or slightly decreasing trend up to 40°C with significant differences among genotypes across all treatments in both varietal groups ($P < 0.001$). Sauvignon Blanc, Savvatiano and Muscat exhibited the highest performance, whereas Assyrtiko consistently the lowest with reduced $\Phi PSII$ by 7.2-11.1% at 40°C ($P < 0.001$) (Figure 3C).

Among the red genotypes Mavrodafni exhibited the highest F_v/F_m across treatments, Agiorgitiko 1.71% lower ($P < 0.001$) and Moschofilero with Merlot 4.1-4.4% lower at 40°C ($P < 0.001$). Mavrodafni exhibited also the highest $\Phi PSII$, Agiorgitiko with Merlot significantly lower at 35°C and 40°C (5.8-7% and 10.4-13% respectively) whereas Moschofilero consistently the lowest across treatments ($P < 0.001$) (Figure 3D).

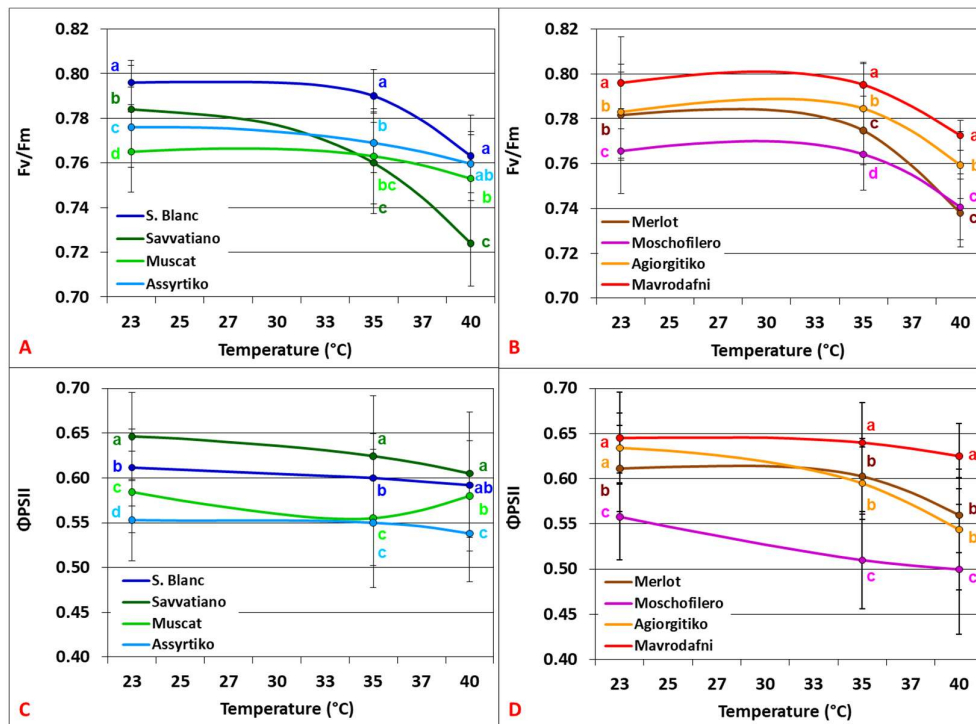


Figure 3. Temperature responses of maximum efficiency of PSII (Fv/Fm) (A, B) and effective efficiency of PSII (ΦPSII) (C,D) in two grapevine varietal groups. Data points with error bars represent mean ± standard deviation (SD). Different lowercase letters denote statistically significant differences between cultivars (P<0.05). A and C refer to white cultivars, while B and D to red ones (n=4).

NPQ varied greatly among cultivars across all treatments in both varietal groups (P<0.001). Sauvignon Blanc, Muscat and Assyrtiko exhibited high NPQ across treatments, whereas Savvatiano consistently the lowest with reduced NPQ by 46-52% at 40°C (P<0.001) (Figure 4A). qP demonstrated a constant or slightly decreasing trend with rising temperature up to 40°C in most cases. Significant variations among cultivars across all treatments were observed in both varietal groups (P<0.001). Sauvignon Blanc, Savvatiano and Muscat demonstrated the highest performance, whereas Assyrtiko consistently the lowest with reduced qP by 4.5-6.95% at 40°C (P<0.001) (Figure 4C).

Among the red genotypes Moschofilero presented the highest NPQ, Agiorgitiko 15.5% lower, Merlot 26% even lower, and Mavrodafni the lowest at 40°C (P<0.011) (Figure 4B). Mavrodafni displayed the highest qP, Merlot and Agiorgitiko 3.6% and 7.8% lower respectively at 40°C and Moschofilero consistently the lowest across treatments (P<0.001)(Figure 4D).

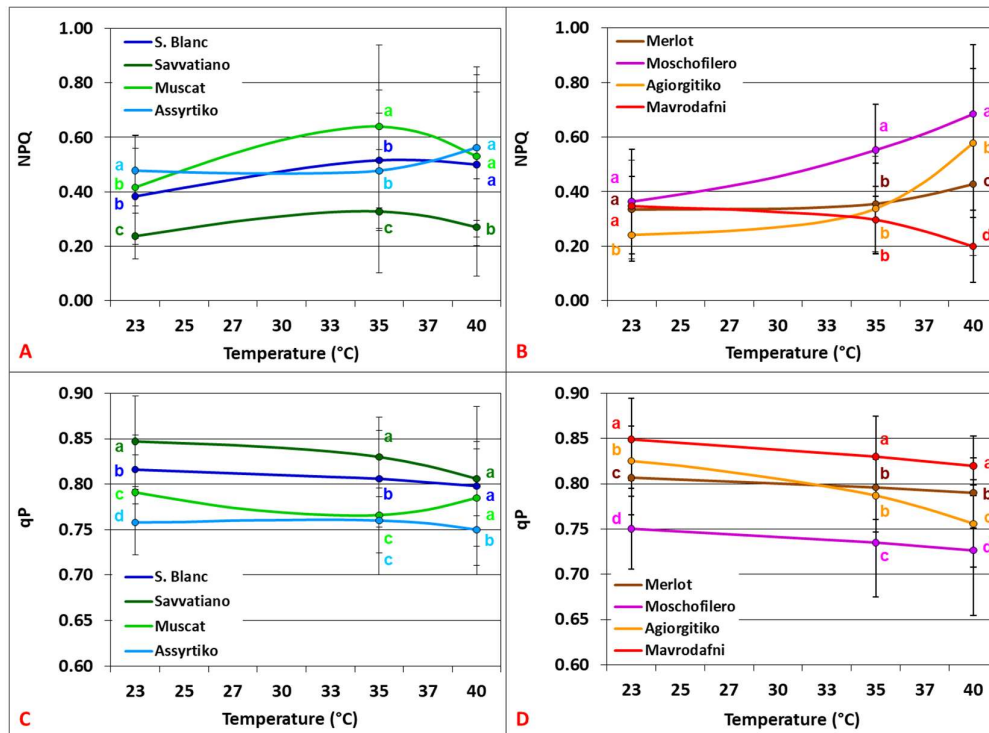


Figure 4. Temperature responses of non-photochemical quenching (NPQ) (A, B) and photochemical quenching (qP) (C, D) in two grapevine varietal groups. Data points with error bars represent mean $\pm$ standard deviation (SD). Different lowercase letters denote statistically significant differences between cultivars ($P<0.05$). A and C and refer to white cultivars, while B and D to red ones ($n=4$).

3.3. Temperature-induced changes in chlorophyll content

Chlorophyll content varied greatly among cultivars across all treatments in both varietal groups ($P<0.001$). White cultivars did not change their chlorophyll concentration from 23°C to 35°C ($P=0.981$), while reductions of 2.7-11.6% were recorded at 40°C ($P<0.001$). Sauvignon Blanc presented the highest chlorophyll content at 40°C, Muscat and Assyrtiko reduced concentrations by 7.1 and 12.5% respectively, whereas Savvatiano significantly the lowest ($P<0.001$) (Figure 5A).

Red cultivars increased their chlorophyll content by 16.5-33.1% from 23°C to 35°C but decreased it by 10.1-31.5% at 40°C. Mavrodafni exhibited the highest chlorophyll content across treatments, Merlot and Moschofilero reduced concentrations by 20.8-28.5% whereas Agiorgitiko presented significantly the lowest at 40°C ($P<0.001$) (Figure 5B).

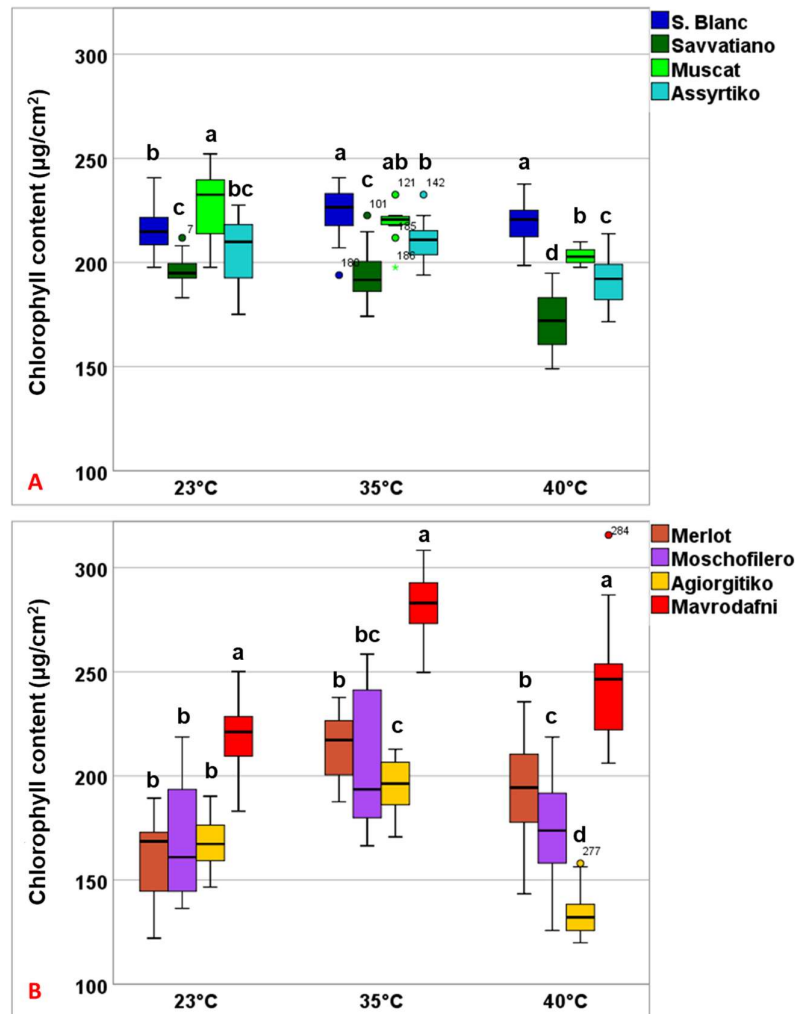


Figure 5. Box plots presenting the median values of chlorophyll content with upper and lower quartiles. The whiskers represent the range of the variabilities outside the quartiles, and the outliers are plotted as individual points. Different lowercase letters denote statistically significant differences between cultivars ($P<0.05$). A refers to white cultivars, while B to red ones ($n=4$).

3.4. Leaf transpirational cooling and thermoregulation

Transpiration (Tr) exponential increased with rising temperature up to 35°C, followed by a slowdown or stabilization of rates at 40°C in most cases. Significant variations among treatments and genotypes were recorded in both varietal groups ($P<0.001$). Assyrtiko exhibited the highest rates whereas Savvatiano the lowest at 40°C. We observed a better Tr retention in Assyrtiko and Muscat above 35°C suggesting a higher T_{opt} whereas Savvatiano exhibited the sharpest decrease from 35°C to 40°C (-13.2%)(Figure 6A). Leaf temperature (T_{leaf}) showed small deviations from growth temperature (T_{growth}) at 23°C which gradually increased as the temperature risen up to 40°C. Significant variations among treatments and genotypes were recorded in both varietal groups ($P<0.011$). Savvatiano exhibited the highest T_{leaf} at 40°C whereas Assyrtiko significantly the lowest ($P<0.001$) (Figure 6C).

Among the red cultivars Mavrodafni exhibited the highest Tr and Agiorgitiko significantly the lowest at 40°C ($P<0.001$) (Figure 6B). Contrastingly, Agiorgitiko recorded the highest T_{leaf} and Mavrodafni significantly the lowest at 40°C ($P<0.001$) (Figure 6D).

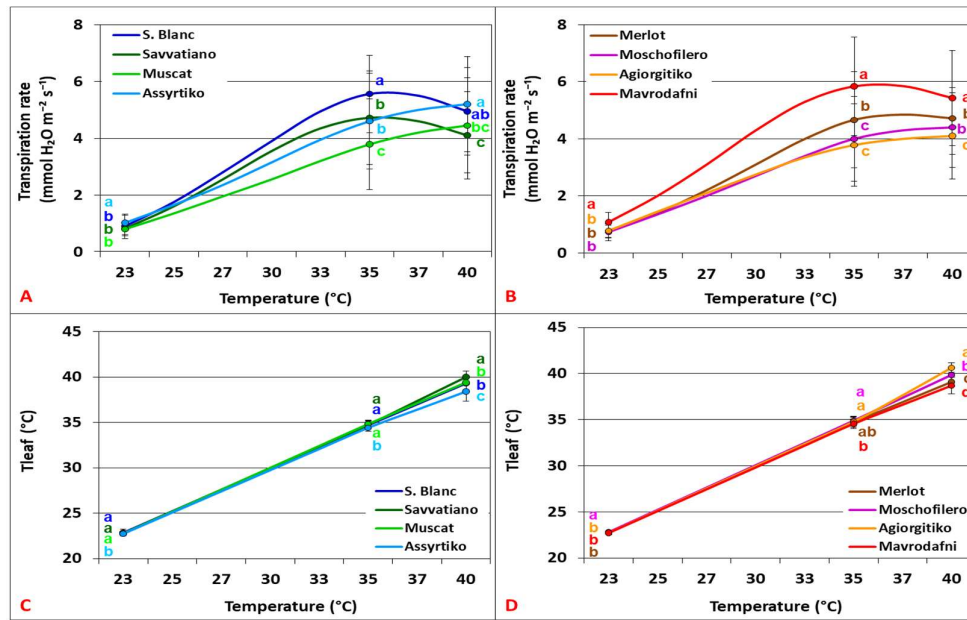


Figure 6. Temperature dependence of transpiration rate (A, B) and leaf temperature (Tleaf) (C, D) in two grapevine varietal groups. Data points with error bars represent mean $\pm$ standard deviation (SD). Different lowercase letters denote statistically significant differences between cultivars ($P < 0.05$). A and C and refer to white cultivars, while B and D to red ones ($n=4$).

3.5. Leaf traits contributing to thermotolerance

Leaf morphological analysis revealed significant differences in leaf size ($P < 0.001$) and mesophyll thickness ($P < 0.017$) among genotypes in both varietal groups. Savvatiano and Muscat displayed the largest leaves among white cultivars whereas Sauvignon Blanc and Assyrtiko 23.6-30.4% smaller ($P < 0.001$) (Figure 7A). Similarly, Assyrtiko with Muscat displayed the thickest mesophylls, whereas Sauvignon Blanc and Savvatiano thinner by 16.8-18.2% ($P < 0.001$) (Figure 7C).

In the case of red cultivars, Moschofilero displayed the largest leaf, Agiorgitiko 45% smaller and Mavrodafni with Merlot 51.5% and 53.6% smaller respectively ($P < 0.001$) (Figure 7B). Mavrodafni displayed the thickest mesophyll, Moschofilero with Agiorgitiko thinner by 6.6-7.1% and Merlot the thinnest mesophyll (Figure 7D).

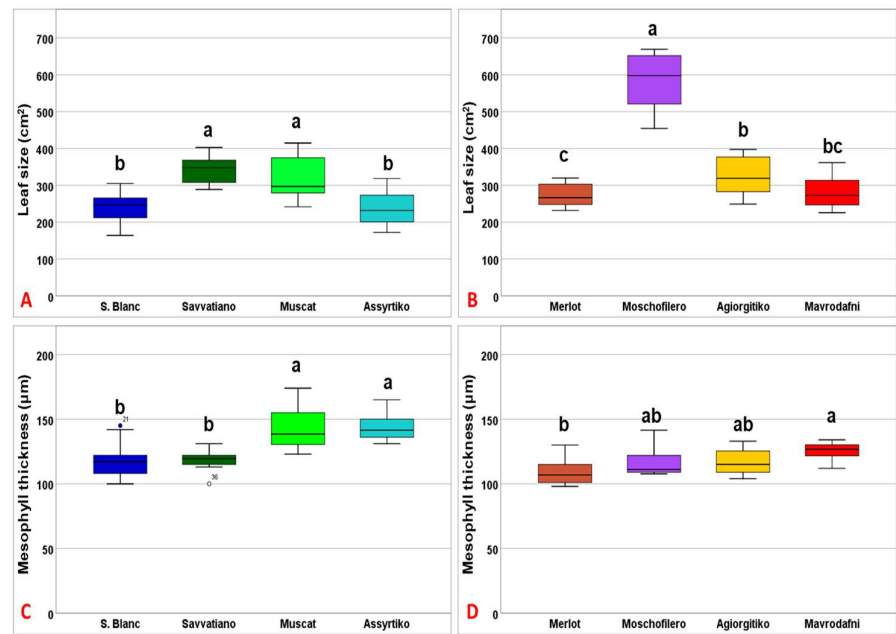


Figure 7. Box plots presenting the median values of leaf size (A, B) and mesophyll thickness (C, D) with upper and lower quartiles. The whiskers represent the range of the variabilities outside the quartiles, and the outliers are plotted as individual points. Different lowercase letters denote statistically significant differences between cultivars ($P<0.05$). A and C and refer to white cultivars, while B and D to red ones ($n=4$).

4. Discussion

4.1. Photosynthetic dynamics under light-saturated conditions and heat stress

Rising light leads to increased photosynthetic rates up to a threshold, known as light saturation point (LSP), above which additional light does not lead to further increase but to a decrease in photosynthesis due to excessive light energy [37]. Light levels above LSP can induce photoinhibition by reducing the photosynthetic efficiency and damaging PSII proteins faster than they can be repaired [38]. Therefore, plants with higher LSP and light-saturated photosynthetic capacity (Asat) exhibit a greater tolerance and a better ability to sustain photosynthesis under strong light without experiencing photoinhibition [39]. Our results demonstrated a significant temperature effect on LSP, as photosynthetic saturation occurred with less light energy at 23°C ($LSP<1200\mu\text{mol m}^{-2}\text{s}^{-1}$), whereas at 35°C, 19-79% higher light intensities were required to achieve Asat. Asat also increased with rising temperature up to 35°C suggesting a positive effect on photosynthetic efficiency. However, at 40°C, the significant LSP and Asat reductions indicated that heat stress can enhance grapevine’s susceptibility to intense light and photoinhibition. Synergistic stress effects were also reported in tomato leaves [40], seagrass species [41,42] and other grapevine cultivars [43] where high irradiance intensified heat stress causing severe photoinhibition. Conversely, low light exposure mitigated heat stress impacts on grape leaves by protecting PSII from photooxidative damage [44,45]. Cultivars’ comparison revealed that Sauvignon Blanc exhibited high photosynthetic efficiency and strong tolerance to high light intensities. Assyrtiko demonstrated good tolerance and enhanced photosynthetic efficiency under light-heat stress, while Muscat also displayed improved light tolerance under high temperatures. Contrastingly, Savvatiano, demonstrated increased light susceptibility and reduced photosynthetic efficiency above 35°C. Mavrodafni exhibited also high photosynthetic efficiency and strong light tolerance under heat stress. Moschofilero consistently presented increased light susceptibility, whereas Agiorgitiko only above 35°C. Merlot finally, although more tolerant to high light intensities than Moschofilero and Agiorgitiko, was more susceptible than Mavrodafni under heat stress. Contradictory performances were also reported in

other grape cultivars such as 'Jing Hongbao' and 'Ruidu Cuixia' which showed better tolerance under combined light and heat stress, compared to 'Ruidu Zaohong', 'Ruidu Xiangyu', and 'Ruidu Wuheyi' [46].

4.2. PSII functionality and photoprotective responses under heat stress

The effects of heat stress and high light energy absorption on cultivars' photosynthetic machinery were reflected in chlorophyll fluorescence parameters which serve as sensitive indicators of heat tolerance and photoinhibition [47,48]. The Fv/Fm which represents the health of the photosynthetic apparatus [49,50], was downregulated in most cases above 35°C indicating PSII suppression under heat stress [51]. Sauvignon Blanc, despite a sharper decline in Fv/Fm above 35°C, maintained satisfactory values at 40°C suggesting a functional PSII under heat stress. This was associated to the effective activation of photoprotective mechanisms, such as NPQ, which enables the dissipation of excess energy as heat, thereby protecting PSII from photooxidative damage [52,53,54]. Assyrtiko showed minimal reductions in Fv/Fm without signs of photoinhibition even at 40°C, suggesting a heat tolerant PSII. This response was mainly attributed to the sufficient activation of NPQ and the consistently low values of Φ PSII and qP. qP represents the proportion of open PSII reaction centers (PSII-RCs) capable of photochemistry, while Φ PSII is the fraction of light energy converted into chemical [55]. Therefore, it was concluded that Assyrtiko adopted a photoprotective strategy involving a partial closure of PSII-RCs (low qP) to reduce the light energy supplied for photochemistry and alleviate further pressure on PSII [56]. Although this strategy sacrifices PSII photochemical efficiency (Φ PSII), it enhances excess energy dissipation (NPQ) and prevents photoinhibition. Muscat also maintained relatively stable Fv/Fm without signs of photoinhibition even at 40°C. This PSII thermotolerance was highly attributed to the strong activation of photoprotective mechanisms at 35°C, including high NPQ and partial closure of PSII-RCs. The same photoprotective strategy have been also reported in Vitis labrusca L. cv. Concord under light-heat stress exposure [57]. Contrastingly, Savvatiano showed significant reductions in Fv/Fm above 35°C indicating severe PSII photoinhibition. This heat susceptibility was associated to the inability of managing excessive energy due to insufficient activation of photoprotective mechanisms. However, the results showed no irreversible damage of PSII as Fv/Fm maintained at 0.72 which is above the risk threshold in grapevine [58]. Mavrodafni maintained the highest Fv/Fm among the red cultivars even at 40°C, suggesting a heat-tolerant PSII. It also exhibited high Φ PSII and low NPQ, indicating efficient management of the absorbed energy without the need for extra heat dissipation. Moschofilero, as the most susceptible to photooxidative stress, adopted a preventive photoprotective strategy characterized by high NPQ and low Φ PSII to avoid PSII overload. The pronounced increase in NPQ, and the sharp decline in Fv/Fm above 35°C, indicated a high demand for energy dissipation and serious photoinhibition under heat stress. These results are consistent with findings in Panax notoginseng, where low-light-adapted plants prioritized the protection of photosynthetic apparatus by downregulating Φ PSII and enhancing NPQ under strong light exposure [59]. Despite its susceptibility to photooxidative stress, Agiorgitiko maintained PSII integrity at 40°C through strong NPQ activation and partial closure of PSII-RCs above 35°C. This reflects a strategy that prioritizes photoprotection over photochemical efficiency to prevent PSII overload. In contrast Merlot, although more resistant to photooxidative stress, exhibited a sharp decline in Fv/Fm above 35°C, indicative of severe PSII photoinhibition. This heat-induced suppression was attributed to its limited NPQ capacity and the higher proportion of open PSII-RCs, reflecting a strategy that prioritizes maximum Φ PSII over photoprotection. Interestingly, this observation partly contrasts with Qiu et al. [54] who reported enhanced photoprotective mechanisms in Merlot.

4.3. Chlorophyll reduction as an adaptive response to light and heat stress

Cultivars' distinct energy management strategies are highly reflected in chlorophyll content (Chl), which is responsible for light absorption during photosynthesis [60,61,62]. Several studies on

grapevine cultivars such as Cabernet Sauvignon, Vitis davidii cv. Junzi [63], Assyrtiko [64], Trebbiano [65] Hongti [66], Merlot and Muscat Hamburg [54] have reported chlorophyll reduction under heat stress, the degree of which depends on the cultivar's heat tolerance [67]. Light intensity is also correlated with chlorophyll content as strong light can damage chlorophyll, leading to reduced photosynthetic efficiency [68,69]. Our results indicate that cultivars with increased light susceptibility such as Savvatiano, tend to maintain lower chlorophyll content under heat stress which is associated with damage to the thylakoid membranes and PSII photoinhibition [70,71]. However, chlorophyll reduction is not necessarily associated with photooxidative damage, but may also be part of an adaptive response for heat acclimatization which helps managing reactive oxygen species (ROS) and maintain PSII stability [72,73]. For example, in a Syrian barley landrace, chlorophyll reduction led to reduced light absorbance which in turn minimized the harmful heating effects of intense light. This response is considered a key mechanism contributing to heat tolerance [74]. Additionally, in perennial ryegrass, chlorophyll catabolism was proven to be critical for maintaining PSII stability during heat stress [75]. Considering the photoprotective strategy adopted by Agiorgitiko, we can assume that its chlorophyll reduction above 35°C, may be also part of a broader photoprotective response to reduce light absorption and maintain PSII stability under heat stress. In contrast, Moschofilero and Merlot, which maintained higher chlorophyll content at 40 °C, were unable to prevent excess light absorption, PSII overload, and consequent photoinhibition.

4.4. The thermodynamic role of leaf structure and transpirational cooling to light and heat stress management

Leaf morphological traits have also been reported to affect grapevine's abiotic stress tolerance, by regulating leaf energy balance, through variations in light absorption, thermal insulation, and heat dissipation [76]. Mesophyll thickness plays an important role in heat tolerance by regulating thermal insulation. Thicker leaves are characterized by higher thermal mass, greater water storage and reduced water loss during heat stress, contributing to increased thermal stability [77,78,79,80]. In grapevine, Razegui and Muscat Italia cultivars exhibited thicker leaf blades and therefore thicker mesophyll as an adaptive response to prolonged heat stress exposure [81]. Leaf size plays also crucial thermodynamic role via the thickness of the boundary layer, which serves as an insulator, regulating heat transfer to the environment [82]. Large leaves are characterized by a thicker boundary layer which limits heat convection, leading to higher leaf temperatures (T_{leaf}) and therefore reduced stress tolerance [78,83,84]. An illustrative example is that Grenache noir leaves exhibit a smaller total surface area than Syrah, which is regarded as a cultivar specific adaptation correlated with abiotic stress tolerance [85]. Similar findings were also reported in Fagus sylvatica [86], Pinus koraiensis [82] and 74 representative species of the eastern Australian flora [87], where small-leaved plants demonstrated an advantage under hot, high-light conditions, while large-leaved plants thrived under cool, low-light environments. Indeed, our results showed that cultivars with small, thick leaves (e.g. Assyrtiko and Mavrodafni), tend to exhibit better light-heat stress tolerance compared to those with larger, thinner leaves (e.g. Savvatiano and Moschofilero). This may explain why, at higher altitudes with more intense sunlight, plants with smaller leaves are more commonly found, as they have developed greater tolerance to high light conditions. [88,89,90]. Therefore, cultivars such as Merlot, Mavrodafni, Sauvignon Blanc and Assyrtiko are highly recommended for cultivation at higher altitudes where solar radiation is more intense. The degree of this tolerance is highly reflected in T_{leaf} , which indicates the heat load experienced by the leaf, where light and heat tolerant genotypes exhibit a better thermoregulation and a lower T_{leaf} [91,92]. However, T_{leaf} depends not only on the exposed leaf area and its thermal insulation properties but also on the rate of transpiration [93,94,95,96]. Higher transpiration rates lead to more effective leaf cooling, lower T_{leaf} and thereby improved heat and light stress tolerance [97,98]. All these data suggest that the increased susceptibility of Savvatiano and Agiorgitiko above 35°C, is partly attributed to their lower transpiration cooling capacity indicating that these two cultivars follow a more conservative, water-saving strategy under heat stress. This

aligns with previous findings on Savvatiano, which demonstrated an isohydric stomatal closure response at temperatures above 35 °C [99].

4.5. The cultivar-specific performances under limited light conditions

Several studies have highlighted the important role of dark respiration (R_{dark}) and light compensation point (LCP) in determining the carbon balance at low light conditions [100]. Shade-tolerant species typically exhibit lower R_{dark} and LCP values [101], enabling them to photosynthesize more than shade-susceptible under low light intensities [102]. Among the white cultivars, Sauvignon Blanc exhibited the most shade-susceptible performance, Assyrtiko and Muscat showed intermediate responses whereas Savvatiano was the most shade-tolerant. In addition to its high light tolerance, Mavrodafni performed equally well under low light conditions, showing characteristics of a versatile cultivar, capable of thriving in a wide range of environmental conditions. On the contrary, Merlot did not respond well under limited light conditions, whereas Moschofilero and Agiorgitiko exhibited a more shade-tolerant behavior. These findings suggest that light-susceptible cultivars under heat stress (e.g. Savvatiano, Moschofilero and Agiorgitiko), may reduce light exposure by forming canopies that intercept a greater fraction of the total incoming radiation [103]. For example in Syrah, differences in shoot architecture accounted for up to 25% of the variations in light interception among cultivars or trellis systems [104] whereas in Cabernet Franc maintaining dense canopies under light-heat stress regimes improved photosynthesis [105]. Moreover, artificial shading is highly recommended for light and heat susceptible cultivars, given reports of reduced canopy temperatures up to 7 °C, and lower incident radiation by 26%–46% [106]. Shading applications in Nero d'Avola [103], Thompson Seedless [107], Sangiovese [108], Cabernet Sauvignon [5], Nebbiolo [109] and Shiraz [110] confirmed that lower light exposure leads to significant reductions in microclimate temperature, mitigating heat stress impacts. Finally, cultivars such as Moschofilero, Savvatiano and Agiorgitiko are also more recommended for cultivation at cooler high-latitude regions where lower light intensities prevail.

5. Conclusions

The present study revealed cultivar-specific resistance under heat stress and fluctuating light intensities. Muscat, Assyrtiko and Mavrodafni demonstrated the most heat tolerant and Savvatiano with Moschofilero the most heat susceptible photosynthetic apparatus. Sauvignon Blanc exhibited heat susceptibility but maintained PSII functionality under heat stress. Findings indicate that climate change requires a more specialized approach for vineyard management, including site selection and the design of targeted viticultural practices. Canopy management should be adapted to the specificities of each cultivar using shading techniques for the light and heat susceptible genotypes and more open canopies for the resistant ones. Microclimate mapping and the assessment of cultivar tolerance to various stressors could provide useful models for predicting vineyard establishment. Therefore, the physiological profile of each cultivar could be matched to local climate forecasts, ensuring production stability and wines' typicality. Further research, including the study of other promising grapevine varieties, is needed to reveal new untapped genetic potential for adaptation to climate change.

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Abbreviations

The following abbreviations are used in this manuscript:

PSII	Photosystem II
LHCII	Light-harvesting complex
PSII-RCs	PSII reaction centers
LRCs	Light response curves
Asat	Light-saturated photosynthesis
Rdark	Dark respiration rate
LSP	Light saturation point
LCP	Light compensation point
An	Net photosynthetic rate
$\phi(I_0)$	Quantum yield at $I = 0$ [$\mu\text{mol}(\text{CO}_2) \mu\text{mol}(\text{photon})^{-1}$]
I	Photosynthetic photon flux density [$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]
Agmax	Estimate of the maximum gross photosynthetic rate
Topt	Optimal temperature for photosynthesis
LCC	Leaf chlorophyll content
SV	Spad value
Fs	Steady-state fluorescence
Fm'	Fluorescence in the light-adapted state
ΦPSII	Actual quantum efficiency of PSII
Fm	Maximum fluorescence
Fo	Minimum fluorescence
Fv/Fm	Maximum quantum efficiency of PSII
NPQ	Non-photochemical quenching
qP	Photochemical quenching
Fo'	Minimum chlorophyll fluorescence in light-adapted leaf
Tleaf	Leaf temperature
E	Apparent transpiration
ROS	Reactive oxygen species.

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