DOI: 10.1007/s11099-015-0156-8 PHOTOSYNTHETICA 54 (1): 47-55, 2016

Kaolin-based, foliar reflective film protects photosystem II structure and function in grapevine leaves exposed to heat and high solar radiation

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Abstract

Extreme conditions, such as drought, high temperature, and solar irradiance intensity, are major factors limiting growth and productivity of grapevines. In a field experiment, kaolin particle film application on grapevine leaves was examined during two different summer conditions (in 2012 and 2013) with the aim to evaluate benefits of this practice against stressful conditions hindering photochemical processes. We used chlorophyll *a* fluorescence to investigate attached leaves. Two months after the application, during the hottest midday, the kaolin-treated plants showed by the JIP test significantly higher quantum yield of PSII photochemistry, flux ratios, maximum trapped excitation flux of PSI, absorption flux, electron transport flux, maximum trapped energy flux per cross section, and performance index than plants under control conditions in the warmer year. On the contrary, the treated plants showed a lower initial slope of relative variable fluorescence and a decrease in the absorption and electron transport per cross section. The JIP test showed higher efficiency of PSII in the plants treated with kaolin mainly in 2013 (higher temperature and drought). Our results supported the hypothesis that the accumulation of active PSII reaction centres was associated with decreased susceptibility to photoinhibition in the kaolin-treated plants and with more efficient photochemical quenching. Grapevines in the Douro Region seems to profit from the kaolin application.

Additional key words: chlorophyll *a* fluorescence transient; energy flux; *Vitis vinifera*.

Introduction

Winemaking has a large social, environmental, and economic relevance in Europe and it is responsible for nearly 60% of *Vitis vinifera* L. area under cultivation worldwide (OIV 2006). Grapevine production in Mediterranean

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Received 7 January 2014, *accepted* 15 May 2015, *published as online-first* 24 June 2015.

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Abbreviations: ABS/CS$_0$ – absorption flux per cross section; ABS/RC – average absorbed photon flux per PSII reaction centre; Car – carotenoids; Chl – chlorophyll; DF$_{ABS}$ or log (PI$_{ABS}$) – driving force on absorption basis; DI$_0$/RC – dissipated energy flux at time zero per PSII; ET$_0$/ABS – relative yield of electron transport; ET$_0$/CS$_0$ – electron transport flux per cross section; ET$_0$/RC – electron transport flux at time zero per PSII; F$_v$/F$_m$ – quantum yield of PSII photochemistry; M$_0$ – the relative Q$_A$ reduction; PI$_{ABS}$ – performance index; RCs – Q_A^- reducing PSII reaction centres; S$_m$ – normalized area above the OJIP transient; TR$_0$/CS$_0$ – maximum trapped energy flux per cross section; TR$_0$/RC – maximum trapped excitation flux at time zero per PSI; φ_{E0} – quantum yield of the electron transport (at t = 0) flux from Q$_A$ to Q$_B$; φ_{P0} – maximum quantum yield of primary PSII photochemistry (at t = 0); ψ_0 – efficiency/probability (at t = 0) with which a PSII trapped electron is transferred from Q$_A$ to Q$_B$.

Acknowledgements: The study was undertaken under the PTDC/AGR-ALI/110877/2009 and European Union Funds (Enoexcel – Norte – 07-0124-FEDER-000032) financed by 'Fundação para a Ciência e a Tecnologia' (FCT, Portugal). The postdoctoral fellowship (SFRH/BPD/84676/2012) awarded to the first author is appreciated. We would also like to thank "Quinta Do Vallado" for the collaboration and efforts in making the vineyard's facilities available for the research and particularly to Engineer António Pinto. G. Pinto is hired under the programme Ciência 2008 (FCT, Portugal), co-funded by the Human Potential Operational Programme (National Strategic Reference Framework 2007-2013) and European Social Fund (EU).

regions, especially in the Douro Demarcated Region (DDR, northeast Portugal), is subject to hot and dry summer climate conditions, which may irreversibly impair some physiological processes (Berry and Björkman 1980) and lead to poor grape yields and quality (Moutinho-Pereira *et al.* 2004, Ferreira *et al.* 2012). As it occurs in other species, *Vitis vinifera* photosynthetic activity declines during typical summer days due to stomatal and nonstomatal limitations, including disturbances in biochemical and photochemical processes (Chaves *et al.* 1987, Moutinho-Pereira *et al.* 2010). Many inorganic and organic products have been exogenously applied in order to mitigate negative effects caused by high irradiance and extreme temperature in grapevine (Song *et al.* 2012). One of the most commonly used compounds is kaolin, a white, inert clay mineral used as a base to develop a particle film (Pf) for foliar application to mitigate the damaging effects of heat and light stress on plant physiology, productivity, and quality of production (Ou *et al.* 2010). This treatment reduces heat and light stress in plants by reflecting infrared (IR) and ultraviolet radiation from the foliar surface and it improves water conditions (Glenn and Puterka 2005, Glenn *et al.* 2010). However, such a reduction does not impair leaf gas exchange because a leaf is able to intercept enough photosynthetically active radiation (Glenn 2009). Midday leaf water potential had no influence on grapevine, however, a decrease of supraoptimal air temperature results in a net diurnal increase in stomatal conductance (Shellie and Glenn 2008).

The JIP test is a tool to analyse the polyphasic rise of the Chl *a* fluorescence transient and corresponds to the redox states of PSII and PSI and to the efficacies of electron transfer through the intersystem chain to the end electron acceptors at the PSI acceptor side (Strasser *et al.* 2004). The polyphasic fluorescence rise is defined by O, J, I, and P steps and is observed after the illumination of dark-adapted leaves (Rasineni *et al.* 2011); it gives information on the relationship between function and structure of PSII reaction centre (RC) and core complexes (Papageorgiou and Govindjee 2004). The goal of the study was to investigate main effects of kaolin foliar application on photochemistry in Touriga Nacional.

Materials and methods

Plant material: The grapevine (*Vitis vinifera* L.), variety Touriga Nacional (grafted on the rootstock 110 R) was used to study the effect of foliar kaolin application on photochemistry of photosynthetic apparatus. The experiment was undertaken in 2012 and 2013 at a commercial vineyard of the "Quinta do Vallado" located at Peso da Régua in the Douro Demarcated Region (DDR), northern Portugal. The Mediterranean type weather is known by warm temperate climate and dry and hot summer (Kottek *et al.* 2006) characterized by most rainfall falling during the winter months and very little during the summer. The soil is a typical schistous and the plants were managed without irrigation and grown using standard cultural decisions as applied by commercial farmers.

Values of monthly rainfall (R, from April to October in 2012 and 2013), total solar irradiation (SR), and mean air temperature (T) from May to October in 2012 and 2013 are shown in Fig. 1. Rainfall values were higher in 2012 than that in 2013, except those in October. The amount of rainfall (May–October) was 9.0 and 6.2 mm, in 2012 and 2013, respectively. Temperature values were higher in 2013, except those in May, and solar irradiation was also higher in 2013 except in May and June. Thus, 2013 was the warmer and drier year than 2012.

Two groups of 60 plants each (three lines with 20 plants per line) were studied: one group received no treatment (control, C), while the plants of another group (kaolin-treated, KT) were sprayed, soon after veraison (11 July 2012 and 17 July 2013), with 5% kaolin (w/v; 2,250 g of kaolin were suspended in 45 l of water without any surfactant) (*Surround WP*, *Engelhard Corp*., Iselin, NJ). The amount of kaolin was sufficient to be well distributed and the plants showed a homogeneous appearance.

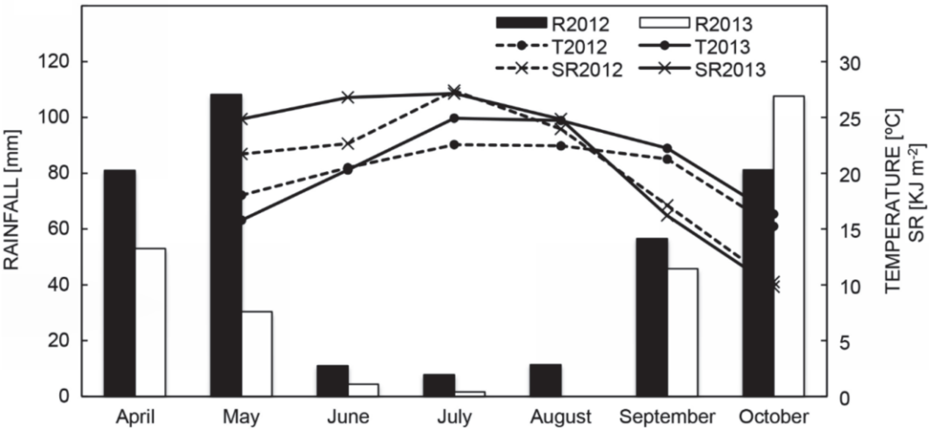

Fig. 1. Monthly rainfall (R), total solar irradiance (SR), and mean air temperature (T) obtained during 2012 and 2013.

All plants were grown under the same edaphoclimatic conditions and the vineyards were located on a steep hill with rows N-S orientated. The six-year-old vines were trained to unilateral cordon and the spurs were pruned to two nodes each with 10–12 nodes per vine. In 2012, after foliar kaolin application, there was a heavy rain that caused a partial removal of the kaolin particles. The second application was done to compensate this effect on 30 July 2012. The first measurements were performed two weeks after pulverization.

Measurements of Chl *a* fluorescence were performed on 31 July and 4 September in 2012, and 30 July, 20 August, and 11 September in 2013, and six sunlit leaves were used for each grapevine group (18 per treatment). All leaves were washed before measurements to ensure that the readings were taken only from leaves and not from kaolin particles. Midday measurements (13:00 h, local time) were done in both years, but the morning measurements (8:00 h) were obtained only in 2013. A portable chlorophyll fluorometer *OS-30p* (*Opti-Sciences Inc*., USA) was used for the measurements, which were performed on sun-exposed, healthy, and mature leaves (usually between 8th and 11th node on the shoot axis). The leaves were dark-adapted in clips for 30 min prior to measurements and later Chl *a* fluorescence transients of dark-adapted leaves were measured. The transients were induced by 1 s illumination providing a maximum light intensity of 3,000 μmol(photon) m^{-2} s^{-1} and a homogenous irradiation over a 4 mm diameter of leaf area. The fast fluorescence kinetics (F_0 to F_m) was recorded from 10 μs to 1 s. The fluorescence intensity at 50 μs was considered as F_0 (Strasser *et al.* 2000).

Analysis of the fluorescence transients using the JIP test: A conversion of the measured parameters into the JIP test parameters provided structural and functional information and enabled us to evaluate the PSII behaviour after different treatments. The data from the original measurements were described in Table 1. The biophysical parameters derived from the OJIP transients were calculated according to the JIP test equations (Strasser and Tsimilli-Michael 2001, Strasser *et al.* 2010, Stirbet and Govindjee 2011). The detail formulae and terms used by the JIP test for analysis of the fluorescence transient OJIP were given in Table 1 (Strasser and Tsimilli-Michael 2001).

Photosynthetic pigments concentration: The photosynthetic pigments were obtained from six leaves per treatment, collected during two different periods (July and September in 2012 and 2013). In the field, each leaf was separated into two parts: one was immediately frozen in liquid N_2; another one was taken to the laboratory for area, and fresh and dry mass determinations. These data were used for pigment expression on an area basis. For pigment quantification, each frozen leaf was ground with liquid N_2 and stored at –80°C in eppendorfs to use for future analysis. For determination of Chl and carotenoids (Car), samples (10 mg of ground leaf) were homogenised with 80% acetone. Chl *a*, Chl *b*, total chlorophyll Chl(*a*+*b*), the ratio of Chl *a*/*b* (Šesták *et al.* 1971), and total Car concentration (Lichtenthaler 1987) were determined spectrophotometrically (*CARY 100 Bio*, *Varian*, Australia).

Statistical analysis: Results were represented as mean ± SD. The significance of the difference between mean values of control and kaolin application was determined using *SPSS 20.0* program (*SPSS Inc*., Chicago, IL, USA). Results were considered statistically significant at $p<0.05$.

Results

Chl *a* fluorescence: F_v/F_m showed significantly higher values in the midday period in 2013 in KT grapevines (July: 4.1% higher, September: 24.4% higher). The log function of the relative performance index log (PI_{ABS}) was linearly correlated with the relative electron transport activity (φ_{E0} = ET/ABS) in the morning and midday period (R^2 = 0.6536 and R^2 = 0.6517, respectively) for both years (Fig. 2). In addition, these variables presented higher variability and low values in the midday than those in the morning. Taking into account that the statistical significance of the previous variables was only observed in the midday period, the following data only represented this period. The JIP test showed a higher efficiency of PSII in the KT plants, especially in 2013. In fact, the slope at the origin of the normalized Chl *a* fluorescence (relative rate of Q_A reduction) was 28.7 and 17.8% lower in August and September 2013, respectively, in the KT grapevine (Fig. 3). Density of RCs (Q_A^- reducing PSII reaction centres) was significantly lower in the KT plants in 2012 and in August 2013 (Fig. 4).

Radar plot of JIP test parameters: The relative values (the lower value in each variable means relative change against the maximum value, set as 100%) of the JIP test parameters are shown as a multiparametric radar plot (Fig. 5). As no significant differences were obtained in the morning period, Fig. 5 only represents the midday period. During 2012, the multiple turnover of Q_A reduction events (S_m) and the maximal yield of primary photochemistry (φ_{P0}) were statistically insignificant both in the C and KT plants. From July (kaolin application, Fig. 5*A*) to September (grape maturation, Fig. 5*B*), ABS per RC

Table 1. Terms and formulae used by the JIP test for the analysis of the fluorescence transient OJIP (Strasser and Tsimilli-Michael 2001).

Selected information obtained from the fast OJIP fluorescence	
$F_0 = F_{50\mu s}$	Initial value of fluorescence intensity
$F_{300\mu s}$	Fluorescence value at 300 µs
$F_J = F_{2ms}$	Fluorescence value at 2 ms (J level)
$F_I = F_{30ms}$	Fluorescence value at 30 ms (I level)
$F_m = F_P$	Maximal fluorescence intensity – peak of OJIP curve
t_{Fmax}	Time to reach the maximum fluorescence value F_m
Area	Area between OJIP curve and the line $F = F_m$
Technical fluorescence parameters	
$F_V = F_M - F_0$	Maximum variable fluorescence
$V_J = (F_J - F_0) - (F_m - F_0)$	Relative variable Chl fluorescence at the J-step
$M_0 = (dV/dt)_0 = 4\,(F_{300\mu s} - F_0)/(F_m - F_0)$	Approximated initial slope of relative variable Chl fluorescence curve (= the relative Q_A reduction)
$S_m = Area/F_V$	Normalized area above the OJIP transient (reflecting the number of reduction and oxidation of one Q_A^- molecule during the fast OJIP transient and therefor related to the electron carriers per electron transport chain)
Specific energy fluxes expressed per active PSII reaction centre (RC)	
$ABS/RC = (M_0/V_J)/(1 - F_0/F_m)$	Average absorbed photon flux per PSII RC
$TR_0/RC = M_0/V_J$	Maximum trapped excitation flux at time zero per PSII(leading to Q_A reduction)
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Dissipated energy flux at time zero per PSII
$ET_0/RC = \psi_0\,(TR_0/RC)$	Electron transport flux at time zero per PSII
Phenomenological energy fluxes expressed per excited cross section (CS)	
$ABS/CS_0 = F_0$	Absorption flux (apparent PSII antenna size) per cross section
$TR_0/CS_0 = F_0\,(TR_0/ABS)$	Maximum trapped energy flux per cross section
$ET_0/CS_0 = \phi_{E0}\,(ABS/CS_0)$	Electron transport flux per cross section
$DI_0/CS_0 = (ABS/CS_0) - (TR_0/CS_0)$	Dissipated energy flux per cross section
$RC/CS_0 = \varphi_{P0}\,F_0\,(V_J/M_0)$	Density of RCs (Q_A reducing PSII reduction centres) per cross section
Quantum yields and efficiencies/probabilities	
$\varphi_{P0} = TR_0/ABS = 1 - F_0/F_m$	Maximum quantum yield of primary PSII photochemistry (at t = 0)
$\varphi_{D0} = DI_0/ABS = 1 - \varphi_{P0} = F_0/F_m$	
$\psi_0 = ET_0/TR_0 = 1 - V_J$	Efficiency/probability (at t = 0) with which a PSII trapped electron is transferred from Q_A to Q_B
$\varphi_{E0} = ET_0/ABS = (1 - F_0/F_M)\,(1 - V_J) = \varphi_{P0}\,(1 - V_J)$	Quantum yield of the electron transport (at t = 0) flux from Q_A to Q_B
"Vitality" indexes (combination of parameters)	
$PI_{ABS} = (RC/ABS)\,(\varphi_{P0}/(1 - \varphi_{P0}))\,(\psi_0/(1 - \psi_0))$	Performance index of energy conservation from photons absorbed by PSII antenna to the reduction of Q_B
$RC/ABS = (V_J/M_0)\,(F_v/F_m)$	Density of RCs per chlorophyll
Total driving forces (for photochemical activity)	
$DF_{ABS} = \log\,(PI_{ABS})$	Driving force on absorption basis

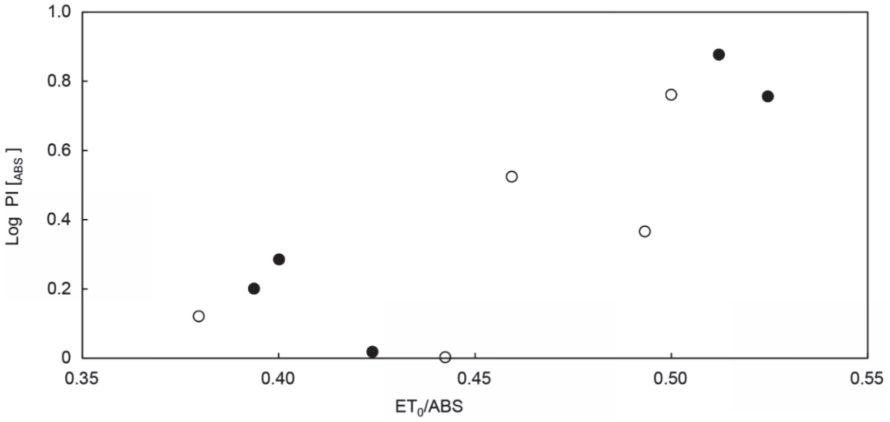

Fig. 2. Correlation between the log function of the relative performance index [(log (PI_{ABS})] and the relative yield of electron transport (ET_0/ABS, probability that an absorbed photon moves an electron further than Q_A^-; φE_0). Values correspond to the average of five measurements in the midday period (July and September in 2012, and July, August, and September in 2013 in control and kaolin-treated plants; $y = 4.9219x - 1.8377$, $R^2 = 0.6517$) (•) and in the morning period (July, August, and September 2013 in control and kaolin-treated plants; $y = 2.3468x - 0.4153$, $R^2 = 0.65355$) (°).

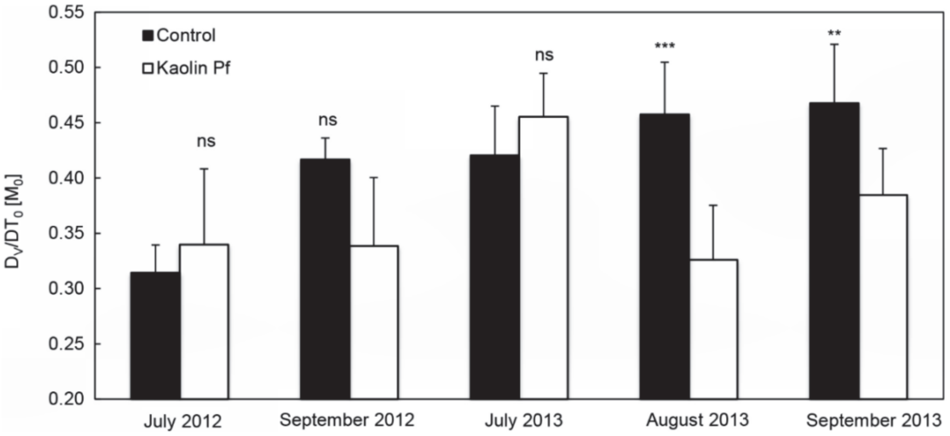

Fig. 3. Slope at the origin of the normalized Chl *a* fluorescence rise D_V/DT_0, which corresponds to the maximal relative rate of Q_A reduction ($D_V/DT_0 = M_0$) of grapevines with (*white columns*) and without (*black columns*) exogenous kaolin-particle film (Pf). Columns indicate mean values ($n = 6$) of leaves and error bars indicate standard deviation. Effects of Pf were tested by *Tukey*'s test. *** – significant at $p<0.001$, ** – significant at $p<0.01$, ns – not significant.

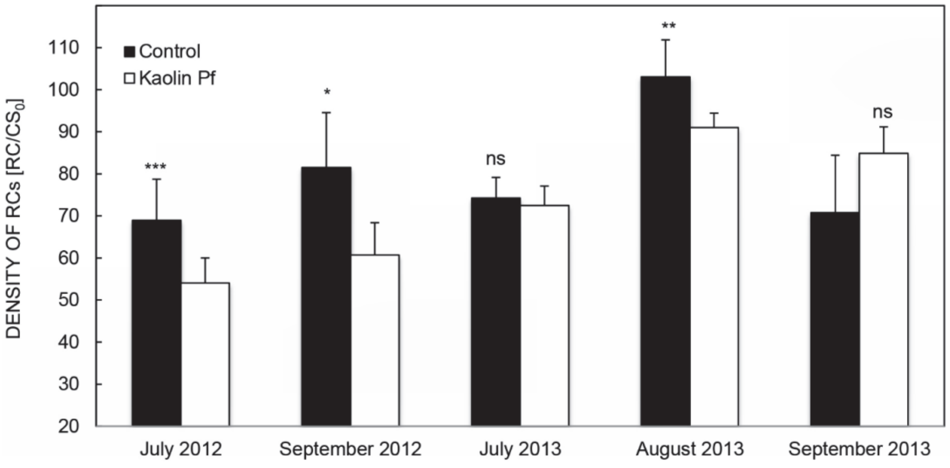

Fig. 4. Changes in the density of the RCs (Q_A^- reducing PSII reaction centres) of vines with (*white columns*) and without (*black columns*) exogenous kaolin particle film (Pf) in both years. Effects of Pf were tested by *Tukey*'s test. *** – significant at $p<0.001$, ** – significant at $p<0.01$, * – significant at $p<0.05$, ns – not significant.

and TR_0 per RC decreased in the KT plants. A decrease in the absorption and trapping flux per cross section occurred in the leaves of KT compared with the C ones. The quantum yield for electron transport (φ_{E0}) and the efficiency of energy conservation in the electron transport (ψ_0) were slightly lower in the KT grapevine leaves. Ψ_0 was 7.5% lower in the KT leaves. In September, Ψ_0 and φ_{E0} KT leaves were 7.5% and 11.5% lower, respectivelly. Similar tendency appeared in the electron transport per cross section (ET_0/CS) and, with greater relevance, in the performance index (PI_{ABS}), with lower values in the KT leaves.

The excitation energy per RC and per CS was lower by 76.1% and 43.9%, respectivelly, in the KT plants than in C plants.

Results from July 2013 are shown in Fig. 5*C*. S_m was lower and TR_0/RC was higher in the KT leaves, while the other variables were insensitive to kaolin. Two months after foliar kaolin application (September), almost all variables showed sensibility to kaolin application. Flux

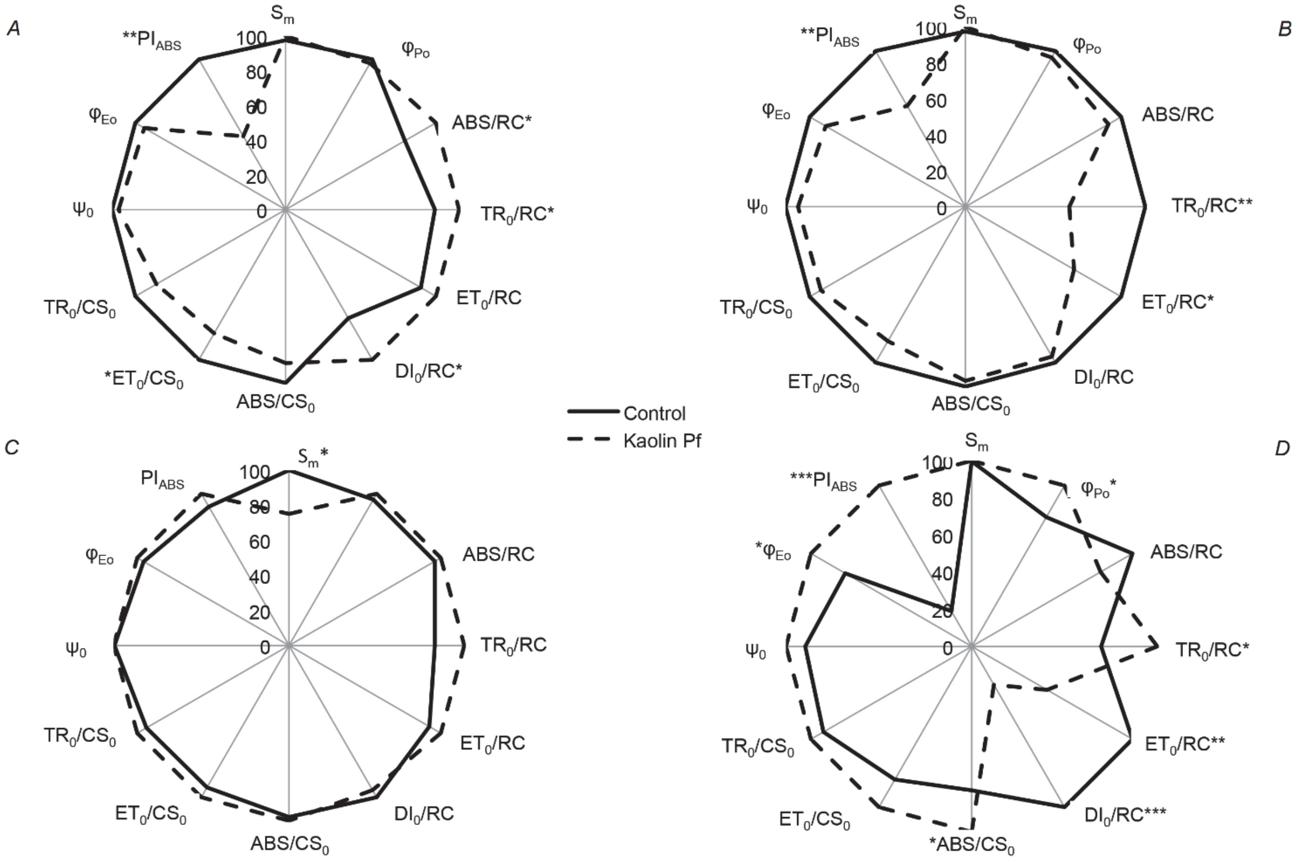

Fig. 5. Spider plot of JIP parameters (the lower value in each variable means relative change against the maximum value, set as 100%) deduced from chlorophyll *a* fluorescence OJIP transient curves in control and kaolin particle film (Pf)-treated plants from midday in July and September in 2012 (*A* and *B*, respectively) and in July and September in 2013 (*C* and *D*, respectively). Effects of Pf were tested by *Tukey*'s test. *** – significant at $p<0.001$, ** – significant at $p<0.01$, * – significant at $p<0.05$, ns – not significant.

ratios, TR_0/RC, ABS/CS_0, ET_0/CS_0, TR_0/CS_0, and PI_{ABS} were higher in the KT plants, while ABS/RC, ET_0/RC, and DI_0/RC were higher in the C plants (Fig. 5*D*).

Photosynthetic pigment concentrations: No significant differences between the C and KT leaves were detected in July of either year. In September of both years, the total Chl and total Car concentration per unit of leaf area were higher in the KT leaves. The particle film had no detectable influence on the ratios of Chl *a*/*b* and Chl/Car (Table 2).

Discussion

There was the increase in the efficiency of PSII photochemistry in the KT plants as shown by the F_v/F_m values in 2013 (Fig. 6). Moreover, the results showed that the capacity of light protective mechanisms was exceeded, although it was reversible, because during the morning, the differences were not significant, certainly due to the overnight recovery (Souza *et al.* 2004). During the midday period, the significantly higher F_v/F_m values after the kaolin application indicated that plants sprayed with the particle film on adaxial surface had the higher photochemical efficiency of PSII. The relationship between the log function of performance index [log (PI_{ABS})] and the probability that an absorbed photon moves an electron further than Q_A^- ($\varphi_{E0} = ET_0/ABS$), in the morning and midday, revealed a great difficulty of grapevine plants to live under high radiance and temperature (the midday period) (Fig. 2). Thus, we could separate high- and low-performing plants, independently of the treatments, according to their performance index ranking. In addition, this correlation confirmed the susceptibility of plants to the stressful midday period. Similar results with the "vitality" ranking were obtained previously in grapevine (Christen *et al.* 2007).

The JIP test data shows the biochemical and biophysical performance of photosynthetic apparatus and performance index of PSII.

Table 2. Concentration of total chlorophyll (Chl), total carotenoids (Car), and Chl *a*/*b* ratio in 2012 and 2013 in leaves with and without (control) kaolin application. Values are means ± SD of six leaves per treatment ($n = 6$). The significance was determined according to *Tukey*'s test. ** – significant at $p<0.01$, * – significant at $p<0.05$, ns – not significant

Parameters	July Control	Kaoline	September Control	Kaoline
2012				
Total Chl [mg dm^{-2}]	3.80 ± 0.37	3.58 ± 0.39^{ns}	2.80 ± 0.65	3.95 ± 0.61**
Chl *a*/*b*	2.74 ± 0.21	2.55 ± 0.17^{ns}	2.36 ± 0.29	2.37 ± 0.19^{ns}
Total Car [mg dm^{-2}]	0.831 ± 0.098	0.787 ± 0.012^{ns}	0.640 ± 0.103	0.797 ± 0.066**
Chl/Car	4.59 ± 0.29	4.60 ± 0.56^{ns}	4.37 ± 0.58	4.95 ± 0.54^{ns}
2013				
Total Chl [mg dm^{-2}]	3.40 ± 0.43	3.17 ± 0.31^{ns}	2.64 ± 0.87	3.10 ± 0.46*
Chl *a*/*b*	3.13 ± 0.16	3.03 ± 0.11^{ns}	2.70 ± 0.64	2.73 ± 0.27^{ns}
Total Car [mg dm^{-2}]	0.883 ± 0.094	0.838 ± 0.053^{ns}	0.690 ± 0.134	0.789 ± 0.115*
Chl/Car	3.84 ± 0.13	3.77 ± 0.18^{ns}	3.82± 0.44	3.89 ± 0.80^{ns}

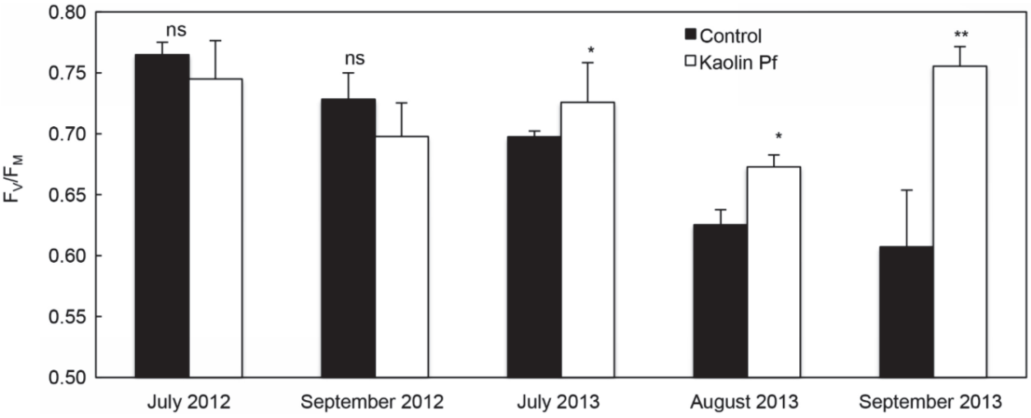

Fig. 6. Maximum potential quantum yield of PSII photochemistry (F_V/F_m) at midday period in July and September in 2012, and July, August, and September in 2013, in leaves of grapevines with (*white columns*) and without (*black columns*) exogenous kaolin-particle film (Pf). Bars indicate mean values ($n = 18$) of leaves and error bars indicate standard deviation. Effects of Pf were tested by *Tukey*'s test. ** – significant at $p<0.01$, * – significant at $p<0.05$, ns – not significant.

Variables, such as Ψ_0 and S_m, reveal information on photosynthetic capacity and they are proportional to the amount of PSII centres capable of moving electrons from Q_A to PQ pool *via* Q_B (Antal *et al.* 2009). These variables reached their highest values when electron transport between Q_A and Q_B was enhanced by kaolin application, mainly in 2013, as we could see by the increase between July and September. Moreover, the similar trend in F_v/F_m, Ψ_0, and S_m in grapevines with kaolin particles (Figs. 2; 6*C*,*D*) suggested a close association between the amount of photochemically competent PSII (F_v/F_m) and capability to transfer electrons between Q_A and PQs (Ψ_0 and S_m). Furthermore, between July (few days after foliar kaolin application) and September 2013, the energy fluxes φ_{E0}, Ψ_0, and φ_{P0} of PSII decreased in control plants, certainly due to heat stress (Fig. 6*C*,*D*). On the other hand, this trend did not occur in 2012, probably due to the removal of kaolin caused by a storm rain, which occurred after the kaolin application. However, the results (in 2012) were in accordance with other study done in tomato leaves and fruits (Zushi *et al.* 2012).

Concerning to phenomenological fluxes per CS_0, ABS/CS_0, TR/CS_0, and ET_0/CS_0, no significant differences were detected in 2012 (Fig. 6*A*,*B*), whereas there was a reduction in the values of the control plants in 2013 (Fig. 6*C*,*D*). The decrease in ABS/CS_0 reflects an increased density of inactive reaction centres. Thus, reduced TR/CS_0 and ET_0/CS_0 indicates that active RCs are converted into inactive RCs, reducing the efficiency of trapping and a decline in PSII activity (Zushi *et al.* 2012). On the contrary, relating to the specific fluxes per RC, an increase occurred in ABS/RC, TR_0/RC, ET_0/RC, and DI_0/RC in 2012, between July and September (Fig. 6*A*,*B*), and the increase in ABS/RC, ET_0/RC, and DI_0/RC was observed in the same period in 2013 (Fig. 6*C*,*D*) in the C plants and a decrease in the KT grapevines. These results suggest that average absorption (ABS/RC) and trapping (TR_0/RC) per active RC increased in the C plants due to the inactivation of some RCs, and that the ratio of total dissipation to the amount of active RCs (DI_0/RC)

increased because of high dissipation of the inactive RCs (Zushi *et al.* 2012). The effective antenna size of an active RC is represented by ABS/RC and it is calculated as a total number of photons absorbed by Chl molecules of all RCs divided by total number of active RCs (Mehta *et al.* 2010). Consequently, it is affected by the ratio of active/inactive RCs. In our study, the decrease in ABS/RC values was found in the KT plants between July and September in both years. However, the increase of the ABS/RC values in the C plants indicated that the summer stress decreased the antenna size of active RCs, as it occurred in passion fruit plants (Gomes *et al.* 2012).

The stability of light absorption capacity of the photosystems could be dependent on the alterations in the total Chl concentration (Kitajima and Hogan 2003). Usually, in Douro Demarcated Region, leaves developed in typically moderate to severe abiotic stresses, showed the lowest concentrations of photosynthetic pigments (Moutinho-Pereira *et al.* 2007) similarly as they occurred in the C plants in September 2012 and 2013 (Table 2). Apparently, the calculated average effective antenna size (Chl/RC) (active) slightly augmented in the KT grapevines. We believe that effective antenna size should have been increased if RCs were converted into heat sinks or because of the effective electron transport (Rasineni *et al.* 2011). The decline in total Chl of the C leaves found in September compared with the KT leaves (Table 2) could be a strategy to avoid a photooxidative or senescence effect (Yoo *et al.* 2003). Thus, we could see the particle film also as a protector against photodamage, namely in the midday period, when sunlight intensity was frequently supraoptimal. Meanwhile, the absence of significant difference in the Chl *a*/*b* ratio was a good indicator of the fact that the presence of kaolin did not lead to an acclimation to lower light intensity. Furthermore, it is known that Car are involved in the protection of the photosynthetic apparatus against photoinhibitory damage by singlet oxygen, which is produced by the excited triplet state of Chl (Bacelar *et al.* 2006). The KT leaves showed the higher Car content as well, which is important to prevent Chl degradation. In the present study, the susceptibility of the control plants was demonstrated by the lowest values of total Chl and Car when compared with the plants sprayed with kaolin. As reported by Smirnoff (1993), the low Chl concentration is a characteristic sign of oxidative stress and could be a consequence of higher Chl degradation or of Chl synthesis reduction with changes in thylakoid membrane composition (Brito *et al.* 2003). In conclusion, our results showed that the application of kaolin was a short-term measure that could mitigate the summer stress effects on grapevine leaves. However, the kaolin application showed functionality in hot and dry years such as 2013. Further studies should be done to optimize the adhesion of kaolin to leaves to improve their benefit.

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