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ESTIMATING THE TOLERANCE OF THREE TABLE GRAPE VARIETIES TO WATER STRESS BY CHLOROPHYLL FLUORESCENCE ANALYSIS

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Abstract

Chlorophyll fluorescence analysis is one of the modern techniques used to study the effect of stress on the photosynthetic process. In our research, we monitored through periodic determinations the index of chlorophyll, the content of the main parameters of chlorophyll fluorescence and photosynthetic potential, in order to determine the thermal resistance threshold at three varieties of table grapes grown in the Stefanesti, Arges County area. All plants analyzed were subjected to three temperature thresholds: 3.3-7.1ºC, 19.4-21.6ºC and 36.5-43.5ºC (increased heat stress). The 'Argessis' variety proved to be the most resistant, followed by 'Victoria', and the 'Augusta' variety was the most affected by high temperatures, with highly significant positive correlations. Very significant positive correlations were found between OJIP indicators and Phi Do indicator, Pearson correlation values ranging from 0.749 to 0.701. These methods are supportive valuable indicators for establishing the tolerance of table grape varieties to water stress, for tailored irrigation management and for an appropriate choose of resilient varieties in the climate change context

***Key words**: Chlorophyll fluorescence water deficit, Vitis vinifera L, environmental drought stress, photosystem II efficiency.*

INTRODUCTION

Abiotic stresses are often interdependent, either individually or combined, lead to morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity and ultimately yield. Heat, drought, cold and salinity are the major abiotic stresses that induce severe cell damage in plant species.

Determination of drought tolerance crop plants can be expensive requiring time-consuming and labor-intensive techniques, field tests, etc. A promising approach is the use of chlorophyll fluorescence, a technique that can provide large amounts of data with minimal time and without harming plants. Chlorophyll fluorescence it works on the principle that photosynthesis is one of the basic functions in plant physiology. In the last decade, chlorophyll fluorescence has been widely used to document the physiological effects of plant stress; plants subjected to almost any stress, including heat stress, will show changes in fluorescence (Knight and Ackerly, 2002; Yamada et al., 1996). Baker and Rosenqvist, (2004), Gamon and Pearcy, (1989), state that, under stress conditions, Fv/Fm (where Fv is variable fluorescence and Fm is maximum fluorescence) decreases due to an increase of minimum chlorophyll fluorescence (Fo). In the last decade, chlorophyll fluorescence has been widely used to document the physiological effects of plant stress. Plants subjected to almost any stress, including heat stress, will show changes in fluorescence Fv/Fm ratio (Fv is variable fluorescence and Fm is maximum fluorescence) which decreases due to an increase in of minimum chlorophyll fluorescence (Fo) (Belkhodja et al., 1994;

Bukhov and Carpentier, 2004; Maxwell and Johnson, 2000).The OJIP test was first proposed by Strasser and Strasser (1995), being used to translate the original measurements of transient fluorescence in various phenomenological and biophysical expressions quantifying PSII function (Tóth et al., 2007).
According to the Manual of Opti-Sciences (http://www.optisci.com/cf.htm - Stress Testing), the OJIP test is a rapid, dark-adapted test that uses a high capture rate of signal for analyzing their fluorescence, with an emphasis on the kinetics of the rapid increase in initial fluorescence, using strong actinic light. In the initial phase of the fluorescence increase, the resulting curve was found to show intermediate inflections before reaching FM or P. These intermediate peaks or steps (levels) are designated as J, I and P, starting from O, this being the value of the signal of the initial fluorescence measured after 20 μsec (Strasser, 2004). In addition to the JIP steps, an additional step called K occurs during specific types of stress (Strasser, 2004). According to data from the specialized literature, thermal stress triggers the destruction of the manganese complex of the oxygen-producing complex (Yamane et al., 1998, cited by Tóth et al., 2006), which has the role of splitting water, the source of electrons for the transport chain of electrons. The most used parameter in the OJIP test is the PI performance index, an indicator with three main attributes that determine the potential of photosynthetic activity, the density of reaction centers, the probability that an absorbed photon to be used for charge separation (initial electron transfer) before electron transfer. Thermal stress causes significant changes in the fluorescence, including an increase in the initial fluorescence (F0) and a decrease in the maximum fluorescence values (FM). An additional peak at approximately 0.3 ms can also be observed, which is called band K (Oukarroum et al., 2012 Weng and Lai, 2005).
The objective of this study was to evaluate the effects of different temperatures on the photosynthetic activity of 3 varieties of table grapes in order to determine the resistance threshold to drought. The chlorophyll index, changes in chlorophyll content that can occur as a result of plant exposure to environmental stress, was also assessed.

MATERIALS AND METHODS

The study has been carried out in 2022, in a table grape vineyard, located at the National Research and Development for Biotechnology in Horticulture Ștefanești, Argeș County (NRDIBH Stefanesti). The region is characterized by a humid temperate-continental climate, with a mean annual temperature (T. mean) of 9.6°C and a precipitation amount of 671.8 mm for the 1979-2020 period not uniformly distributed across the year. The large amplitude of meteorological conditions occurring during the vegetation period from 2022 at NRDIBH Stefanesti, has the specificity of an excessively continental climate, this year's vegetation season being a very dry one (only 530 mm compared to the multi-year average of 671.8 mm). The driest months were May, June and October, with precipitation of only 16.2 mm, and 3.6 mm, respectively 7.2, the number of rainy days being 4-9. In the air, the last minimum temperature was recorded in April (-2.8ºC) and the maximum temperature in July 36.08ºC.

Determination of the chlorophyll index (CCI)

In our study, in August, we monitored the influence of the 3 varieties of table grapes (‘Augusta’, ‘Victoria’, and ‘Argessis’) and the position of the plants along the watering tubes through 3 periodic determinations of the chlorophyll content index with CCM-200 OPTI-SCIENCES device. The measurements were performed on young leaves, in the phenophase of plant’s growth (Filimon et al., 2014). For each experimental variant, 30 determinations were performed from a bifactorial scheme, as follows:

- factor A, table grape variety with 3 gradations:
- a_1 - ‘Augusta’,
- a_2 - ‘Victoria’,
- a_3 - ‘Argessis’;
- factor B - the position of the plants along the watering tube:
- b_1 - I-Third location - the analyzed plant in the first third of the watering tube, where it is assumed that the supply of water is richer;

- b2. - II third location - in the middle of the watering tube and
- b_3 - III- third location - in the last third of the watering tube.

Determination of the OJIP chlorophyll fluorescence indicator

Three leaves from each variety ('Augusta', 'Victoria' and 'Argessis') were detached on August 12th, quickly placed with the base in water in Erlenmayer dishes and adapted to the dark and passed through three times successively through three temperature thresholds: 3.3-7.1ºC, 19.4-21.6ºC and 36.5-43.5ºC (accentuated thermal stress).

Using the OJIP test, determined with the FP110 fluorometer, 25 indicators were calculated at each temperature change (14 determinations), as follows:

Fo = F50 µs; chlorophyll fluorescence intensity at 50 µs (initial);

Fk = fluorescence intensity at level k (at 300 µs);

Fj = fluorescence intensity at level j (at 2 ms);

Fi = fluorescence intensity at level i (at 60 ms);

Fm = maximum fluorescence intensity;

Fv = Fm - Fo - maximum fluorescence amplitude;

Vj = (Fj - Fo)/(Fm - Fo) - the relative fluorescence amplitude at level j, for non-connected FS II units;

Vi = (Fi - Fo)/(Fm - Fo) - the relative fluorescence amplitude at level i;

Fm/Fo = ratio of maximum to minimum (initial) fluorescence;

Fv/Fo = capture probability performance ΦPo (PTR) [ΦPo/(1 - ΦPo)], contribution to the performance index (PI) of light-driven reactions for primary photochemical centers. The contribution of light-produced reactions to primary photochemistry is estimated according to the JIP test as [ΦPo/(1 - ΦPo)] = Fv/Fo;

Fv/Fm = quantum potential yield (efficiency) of photosystem II (FS II), in a dark-adapted leaf. It is an indicator of the FS II integrity of the plant. A healthy land plant will almost always have an Fv/Fm value close to 0.8. A decrease in the ratio value will indicate the presence of stress conditions and a fluorescence quenching mechanism;

Mo or (dV/dt)0 = TRo / RC - ETo / RC = 4 (F300 - Fo) / (Fm - Fo) – initial approximate slope (in ms-1) of transient fluorescence V = f(t); FS II net closing rate: (dV/dt) or Mo = 4 (F300µs – Fo)/(Fm – Fo).

Area = the area between the fluorescence curve and Fm (subtract the starting level).

Fix Area = The total area above transient OJIP fluorescence - between F40µ and F1s (subtract the starting level);

Sm = Area/Fm - Fo ("turn-over" multiple);

Ss = the smallest Sm turn-over (a single "turn-over" - inflection of the transient curve);

N = Sm . Mo . (1/Vj) – number "turn-over" QA (QA reduction through FS II activity).

Production or Quantum efficiency or electron flux ratios:

Phi_Po (ΦPo) = 1- (Fo/Fm) (or Fv/Fm) = the maximum quantum yield of primary photochemical reactions at t=0.

Psi_o (Ψo or ETo/TRo) = 1 - Vj = the probability (at time 0) that an excited donor transfers an electron in the electron transport chain beyond QA (the primary acceptor);

Phi_Eo (ΦEo) = (1 - Fo/Fm)). Psi_o - quantum yield for electron transport at t = 0;

Phi_Do (ΦDo) = 1 - Phi_Po - (Fo/Fm) - the quantum yield at t = 0 for energy dissipation;

Phi_Pav (ΦPav) = Phi_Po - (Sm/tFm); (tFm) = rise time at Fm (ms);

Pi_Abs = absorption-based performance index (PI).

Specific flows or activities expressed per reaction center (RC):

ABS/RC = Mo . (1/VJ) . (1/Phi_Po);

TRo/RC = Mo . (1/Vj) - energy flow captured on the reaction center (RC) la t=0;

ETo/RC = Mo . (1/Vj) . Phi_o) - the flow of electrons carried on the RC at t=0.

Relations between chlorophyll content and photosynthesis variables were tested, with linear regression analysis, and Pearson correlation coefficients. Analysis of variation (ANOVA) and Duncan's test were performed to test the mean differences between experimental factors.

RESULTS AND DISCUSSIONS

Maximum fluorescence (FM) showed significant decreases starting from 36.4°C, where the lowest values were recorded. (Table 1, Figure 1). Temperature thresholds significantly affected Fo and Fm, which resulted in significantly different values of Fv/Fm ratio. This was true for all three varieties studied. The Fv/Fm measurements had values between 0.80 and 0.61, after reaching the threshold of 36.4ºC. A healthy land plant will almost all the time have an Fv/Fm value close to 0.8 (Tóth et. al., 2007). A decrease in the ratio value will indicate the presence of stress conditions and a fluorescence quenching mechanism (Tóth et. al., 2007). Analyzing this indicator, at the temperature threshold 36.5-43.5ºC, from table 2 it can be seen that the highest values were recorded in the 'Augusta' variety, and the lowest in the 'Argessis' variety, the latter suggesting a greater tolerance to water stress. The 'Victoria' variety recorded intermediate values of FO (421), at the same temperature threshold analyzed. Bussotti et al. (2011) in their research have affirmed that an F0 increase can be interpreted as indicative of irreversible damage to PSII caused by uncontrolled heat dissipation that produces an excess of excitation energy. Fo values can increase when there is a slowdown in excitation energy transfer from the light collection system to the reaction center (Baker and Rosenqvist, 2004), or when there is some type of damage in the PSII reaction centers themselves (Vieira et al., 2010). This parameter represents the number of open reaction centers, or, rather, the first electron acceptor of PSII, QA, in its oxidised state. (Bussotti et al., 2011; Strasser et al., 1995). However, the higher values of the Fv/Fm ratio in the 'Argessis' variety, closely followed by the 'Victoria' variety, measured at a temperature of 43.5ºC (0.64 and 0.63, respectively), compared to only 0.58 recorded in the 'Augusta' variety, indicates greater tolerance of plants to higher temperatures (increased heat stress) (Figure 1). The flux of absorption and trapping per reaction center (RC) of PSII, defined as ABS/RC, (RC) of PSII, ABS/RC, and TR_0/RC, respectively, were significantly higher in leaf discs incubated above 36.5ºC (Table 1). The specific fluxes expressed per reaction centers (ABS/RC; TR_O/RC; DI_O/RC; ET_O/RC) were derived from Sironval's theory of energy flow through biomembranes and were calculated using the OJIP test (Strasser et. al., 2001).

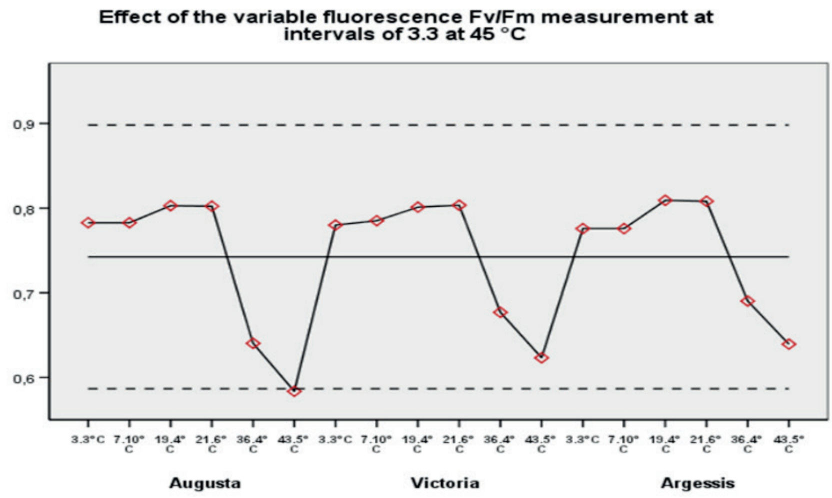

Figure 1. Variation of the OJIP - Fv/Fm indicator according to the combinations of gradations of the 2 experimental factors (Variety and Temperature, NRDIBH Stefanesti, 2022)

In our study, the ABS/RC indicator recorded the highest values in the 'Augusta' variety (7.53) after exposing the plants to the threshold of temperature by 36.5-43.5ºC, compared to only 6.40, respectively 6.45 that were recorded in the 'Argessis' and 'Victoria' varieties, at the same temperature threshold analyzed. The lowest values of ABS/RC were evident at the temperature threshold of 3.3-7.1ºC, in all the varieties analyzed, with values between 1.47

and 1.49 (Table 1). The term absorption (ABS) refers to the absorption of photons by chlorophyll molecules in antenna-like complexes. The electron transport flux per RC of PSII defined as ET_0/RC started to increase at the temperature of 36.5°C. It was not possible to estimate the reduction in terminal electron acceptor flux (RE_0/RC) for temperatures above 36.5°C due to the change in the shape of the kinetics of the fluorescence emission curve (Table 1). These statements are also supported by Chen et al. (2008) in selling research. Therefore, the intervals 3.3-7.1ºC were chosen; 19.6-21.4ºC, respectively 36.5-43.5ºC, between these thresholds there are no differences between the measurements (Table1).

Table 1. Chlorophyll fluorescence intensity of the OJIP assay parameters from the fluorescence transient at three table grapes subjected to different temperatures

Variety	T (ºC)	Intensity chlorophyll a fluorescence				
		To	**Tm**	**Fm/Fv**	**Fv/Fo**	**Fi**
'Augusta'	3.3-7.1ºC	315^{b}	1450^{ab}	0.78^{a}	487^{b}	3.60^{ab}
	19.6-21.4⁰C	336^{b}	1700^{a}	0.80^{a}	641^{a}	3.90^{a}
	36.5- 43.5ºC	454^{a}	$1175,5^{b}$	0.61^{b}	571^{a}	1.59^{b}
'Victoria'	3.3-7.1ºC	332^{b}	1525^{ab}	0.78^{a}	431^{b}	$3.6a^{b}$
	19.6-21.4⁰C	339^{b}	1715^{a}	0.80^{a}	641^{a}	4.06^{a}
	36.5- 43.5ºC	421^{a}	1210^{b}	0.65^{b}	645^{a}	1.87^{b}
'Argessis'	3.3-7.1ºC	325^{b}	1450^{ab}	0.78^{a}	495b	3.46^{ab}
	19.6-21.4⁰C	330^{b}	1725^{a}	0.80^{a}	641a	4.22^{a}
	36.5-43.5ºC	415^{a}	1245^{c}	0.66^{b}	654a	2.00^{b}

Variety	T (ºC)	Flux per reaction centers		
		ABS/RC	**TR_0 /RC**	**ET_0 /RC**
'Augusta'	3.3-7.1ºC	1.48^{b}	1.17^{b}	0.38^{b}
	19.6-21.4⁰C	1.62^{b}	1.20^{b}	0.61^{a}
	36.5-43.5ºC	7.53^{a}	3.28^{a}	-
'Victoria'	3.3-7.1ºC	1.47^{b}	1.20^{b}	0.31^{b}
	19.6-21.4⁰C	1.56^{b}	1.21^{b}	0.58^{a}
	36.5-43.5ºC	6.43^{a}	3.15^{a}	-
'Argessis'	3.3-7.1ºC	1.49^{b}	1.18^{b}	0.29^{b}
	19.6-21.4⁰C	1.58^{b}	1.20^{b}	0.58^{a}
	36.5-43.5ºC	6.40^{a}	3.08^{a}	-

*Values in the same column followed by different letters as the exponent are significantly different at $p < 0.05$.

In order to test the effect of thermal thresholds on the 26 OJIP indicators measured with the FP 110 fluorometer, the intensity of linear correlations was established using the simple Pearson correlation coefficient (r), and their statistical significance was established (Sig.), after which the OJIP indicators were ordered in ascending order by "r" values (Table 2). It can be seen that the OJIP indicators are most affected by the increase in air temperature (lower and lower values with the increase in air temperature in the range of 3.3-43.5ºC), having the lowest values of the simple correlation coefficient (in the conditions where the significance correlation statistic was highly significant), were in order: Ss (lowest Sm - transient curve inflection) with r = -0.704ºººº, Fv/Fm (quantum potential yield of photosystem II - FS II) whose optimal value is 0.8) and Phi_Po (maximum quantum yield of primary photochemical reactions at t = 0. Thus, the correlation coefficient for the maximum quantum yield of primary photochemical reactions (Phi_Po) has a value of r = -0.692ººº, and for Fm/Fo (ratio between maximum and minimum fluorescence) together with Fv/Fo (contribution to the performance index). (PI) of the reactions carried out in the light) a correlation coefficient was found with r = -0.628ººº, respectively r= -0.626 ººº. At the opposite pole, with highly significant positive correlations (the increase of temperature increased the values of the respective indicators), the OJIP indicators of energy dissipation: the specific fluxes or activities expressed on the reaction centers (ETo/RC, TRo/RC, DIo/RC and ABS/ RC), and Phi_Do which represents the quantum yield at t = 0 for energy dissipation. Pearson correlation coefficient values ranged from 0.749 to 0.640

(Figure 2). The lower values of PI_ABS recorded at high temperatures to which the plants were subjected, may be caused by the absorption of energy by inactive reaction centers, which results in lower values of a maximum quantum yield of primary photochemical reactions at t = 0 (Phi_Po) and a reduction in yield of Phi_Eo electron transport (Table 2). Similarly, Zushi et al., 2012, found that specific fluxes per RC increase with temperature in most horticultural species. The increase in ABS/RC indicators, TRo/RC and DIo/RC on active RC was observed due to the inactivation of more RCs, which also suggests an increase of the total dissipation ratio to active RCs due to the high dissipation caused by the RCs inactive.

Table 2. The intensity of the correlation between air temperature and OJIP indicators of chlorophyll fluorescence of the three table grapes varieties

Chlorophyll fluorescence indicator	Simple Pearson correlation indicator	Sig. (2-tailed)	Symbol of statistical significance
Ss	-0,704	0,000	ooo
Fv/Fm	-0,692	0,000	ooo
Phi_Po	-0,691	0,000	ooo
Fm/Fo	-0,628	0,000	ooo
Fv/Fo	-0,626	0,000	ooo
Pi_Abs	-0,462	0,000	ooo
Fv	-0,458	0,000	ooo
Area	-0,422	0,000	ooo
Fm	-0,389	0,000	ooo
Fi	-0,344	0,000	ooo
Fix Area	-0,337	0,000	ooo
Vi	-0,287	0,000	ooo
Phi_Eo	-0,265	0,001	oo
Fj	-0,240	0,003	oo
Vj	-0,201	0,020	o
Sm	-0,161	0,058	
Psi_o	0,191	0,018	*
N	0,261	0.001	**
Fo	0,328	0,000	***
Phi_Pav	0,447	0,000	***
Mo	0,431	0,000	***
Phi_Do	0,64	0,000	***
ETo/Rc	0,658	0,000	***
TRo/RC	0,72	0,000	***
Dio/RC	0,736	0,000	***
ABS/RC	0,744	0,000	***

Analyzing Figure 2, we observe both the main effects of the 2 experimental factors, as well as the interactions A (variety) x B (plant position on the watering tube row) generated by them on the chlorophyll index values.

It can be seen that the 2^{nd} and 3^{rd} positions on the watering tube provided a significant increase in CCI compared to the 1^{st} third (Figure 2).

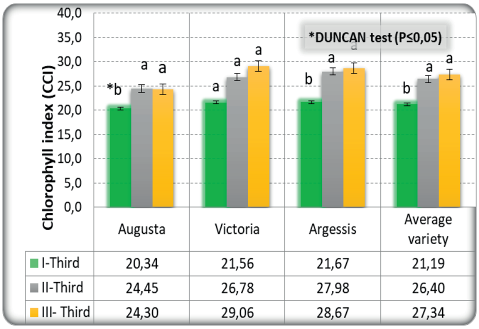

Figure 2. The influence of the position of the watering tube on the chlorophyll index of the three table grapes varieties

Table 3. The intensity of the correlation between the leaf chlorophyll index (CCI) and the OJIP indicators of the chlorophyll fluorescence of the three table grapes varieties (NRDIBH Stefanesti, 2022)

Chlorophyll fluorescence indicator	Simple Pearson correlation indicator	Sig. (2-tailed)	Symbol of statistical significance
Mo	-0,478	0,000	ooo
Vj	-0,457	0,000	ooo
Fo	-0,271	0,000	ooo
Phi_Do	-0,269	0.001	ooo
Fj	-0,256	0,001	ooo
DIo/RC	-0,201	0.020	oo
ABS/RC	-0,178	0,025	o
Vi	-0,177	0,026	o
TRo/RC	-0,156	0,048	o
N	-0,04	0,678	
Fi	-0,037	0,710	
Phi_Pav	0	1,000	
Phi_Eo	-0,265	0,001	
ETo/Rc	0,008	0,917	
Sm	0,051	0,572	
Fix Area	0,074	0,263	
Fm	0,105	0.221	
Area	0,114	0,185	
SS	0,209	0,018	*
Fv/Fm	0,256	0,001	***
Phi_Po	0,257	0,001	***
Fv/Fo	0,316	0,000	***
Psi_o	0,316	0,000	***
Phi_Eo	0,578	0,000	***
ABS/RC	0,585	0,000	***

The investigations carried out in 2022 aimed at verifying the studied varieties, their behavior under the excessive continental climate and irrigation efficiency measures applied especially to overcome water stress. This has been done in particular by analyzes of CCI chlorophyll index dynamics, chlorophyll fluorescence and photosynthetic potential, taking into account 26 OJIP parameters. The chlorophyll content index is highly positively correlated significantly with indicators of chlorophyll fluorescence - OJIP, of quantum production or efficiency, ABS/RC, Psi_Eo, Psi_o, Phi_Eo, Phi_Po, but also with Fv/Fm the quantum potential of IInd photosystem (Table 3).

CONCLUSIONS

From our research, it was found that there was a positive relationship between Fv/Fm ratio and temperatures. As the temperature increased, after 36.5°C, Fv/Fm ratio decreased greatly, indicating a decrease in the photochemical efficiency of PSII.

The Fv/Fm ratio and the valuable OJIP indicators can still be useful for determining the threshold between moderate and excessive water deficit in vines. The higher values of the Fv/Fo, Fv/Fm ratio and absorption flux per reaction center (RC) of PSII, defined as ABS/RC, ET_0/RC and TR_0/RC, respectively, in 'Argessis' and 'Victoria' varieties, measured at 36.5-43.5°C, indicate a greater tolerance of the plants to higher temperatures (increased thermal stress).

These varieties are recommended to be further studied by reducing the water supply in a controlled manner within the vineyard, being an important tool for a moderate level of water deficit that allows the plants to maintain the functions of the leaves and the entire vegetative system, as well as table grape quality indicators.

Highly significant positive correlations were found between temperature and OJIP indicators of energy dissipation (ETo/RC, TRo/RC, DIo/RC and ABS/RC), as well as Phi_Do. Thus, the increase of temperature led to increase the values of the OJIP indicators of energy dissipation and Phi_Do, the values of the Pearson correlation coefficients ranging between 0.749 and 0.640.

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