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Characterization of Cabernet Sauvignon from Maipo Valley (Chile) Using Fluorescence Measurement*

Caracterización del Cabernet Sauvignon del Valle del Maipo (Chile) usando mediciones de uorescencia

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Abstract

Virus infection in *Vitis vinifera* negatively impacts vineyard production and grape quality. Examples are grapevine leafroll-associated viruses (GLRaVs) and grapevine *rupestris* stem pitting-associated viruses (GRSPaV). We investigated transient fluorescence of chlorophyll *a* in leaves of Cabernet Sauvignon, the primary red cultivar planted in Chile, using the OJIV test. First, we evaluated fluorescence in productive grapevines throughout their development, and then we analyzed the same parameters in plants infected with GLRaVs or GLRaVs+GRSPaV. We observed that throughout phenological development, as in the virus-affected clones, the kinetics of fluorescence emission decreased, particularly in values such as F_o and F_M, reflecting the limitation in electron donation by the oxygen-releasing complexes and changes in the PSII antenna complex. The parameter reflecting the most significant differences was the performance index (PI_{ABS}), whose value decreases throughout phenological development and is also lower in virus-affected plants compared to healthy plants. This decrease would indicate that PSII changes the conversion of light energy of chemical energy to heat dissipation, making PI_{ABS} an excellent parameter to evaluate the vitality of Cabernet Sauvignon vineyards.

Keywords: wine, photosynthesis, virus.

Resumen

Las infecciones virales en *Vitis vinifera* impactan negativamente en la producción de viñedos y en la calidad de la uva. Algunos ejemplos son los virus asociados al enrollamiento de la hoja de la vid (GLRaVs) y los asociados al picado del tallo en *rupestris* (GRSPaV). Analizamos la fluorescencia transitoria de clorofila *a* en Cabernet Sauvignon, principal cultivar tinto en Chile, utilizando la prueba OJIV. Primero evaluamos la fluorescencia en vides productivas a lo largo de su desarrollo, y luego la analizamos en plantas infectadas con GLRaVs o GLRaVs+GRSPaV. Observamos que, a través del desarrollo fenológico, al igual que en clones con virosis, la cinética de emisión de fluorescencia disminuyó, especialmente en valores como F_o y F_M, reflejando limitación en la donación de electrones por parte de los complejos liberadores de oxígeno y cambios en el complejo antena del PSII. El parámetro que reflejó las diferencias más significativas fue el índice de rendimiento PI_{ABS}, que disminuyó a lo largo del desarrollo fenológico y también en plantas con virosis. Dicha disminución indicaría que PSII cambia la conversión de la energía luminosa de química a disipación de calor, volviéndolo un excelente parámetro para evaluar la vitalidad de los viñedos de Cabernet Sauvignon.

Palabras clave: vino, fotosíntesis, virus.

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Introduction

Analysis of chlorophyll *a* fluorescence is a widely used technique for basic and ecophysiological studies on the plant's health status. Photosynthetic heterogeneity has been identified in many situations, giving valuable information about the extent of damage and the plant's photosynthetic ability to adapt against stress. The fast transient analysis of chl a fluorescence is a simple, sensitive, non-invasive, and highly reliable tool for rapid measurement of chloroplast functionality, particularly of PSII (Stirbet and Govindjee, 2011).

Virus-related diseases can affect the kinetic fluorescence of dark-adapted photosynthetic cells, showing several inflection points (O, J, I, and P) after exposure to actinic light with time, known as OJIP transients. These transients reflect the redox state of the photosystems and correspond to changes in the photosynthetic electron transport chain. The OJIP transients can be analyzed using the JIP-test (Strasser and Strasser, 1995), which conceives the PSII functionality by translating the fluorescence transient measurements into several phenomenological and biophysical expressions. These parameters interpret the essential indicators of PSII characteristics, such as energy trapping and electron transport kinetics (Stirbet and Govindjee, 2011).

Fluorescence emission gives valuable information about infection with bacteria (Berger *et al*., 2007) and fungi (Swarbrick *et al*., 2006). Concerning virus-infected plants, there is considerable disagreement regarding the alterations in chlorophyll fluorescence parameters. It is being reported that Fv/Fm value did not change in *Eupatorium makinoi* infected by geminivirus (Funayama *et al*., 1997) but was significantly lower in *Nicotiana tabacum* (Ryšlavà *et al*., 2003) and Oncidium (Chia and He, 1999) after virus infection.

Virus-related diseases can affect grapevine physiological performance, vigor, yield, grape and wine composition, and quality (Alabi *et al*., 2016, Endeshaw *et al*., 2014). Changes in grapevine physiology resulting from virus infections are mainly related to photosynthesis and chlorophyll *a* fluorescence; both processes are directly or indirectly related to the ability of the grapevine to maintain vegetative and reproductive growth and ripen crops.

Grapevine leafroll disease (GLD) is one of the significant diseases of the grapevine and possibly the most important virus disease from an economic perspective. Grapevine leafroll-associated viruses 3 (GLRaV-3) is a member of the genus *Ampelovirus* and is the primary agent associated with GLD (Burger *et al*., 2017). Several studies have shown that GLRaV-3 affects different events related to photosynthesis in red-berried cultivars under field conditions (Bertamini and Nedunchezhian, 2002; Gutha *et al*., 2012; Moutinho-Pereira *et al*., 2012). A reduction in photosynthetic pigments, net photosynthesis, and chlorophyll a fluorescence was observed in symptomatic leaves during the pre-veraison stage but not in asymptomatic leaves, compared with data from corresponding healthy leaves. This dysfunctional photosynthesis physiology was attributed, at least in part, to the altered structural and functional integrity of PSII, especially the donor site that makes up the oxygen-evolving complex of the photosynthesis apparatus (Bertamini and Nedunchezhian, 2002).

Another frequent virus disease in the grapevine is rupestris stem pitting (RSP). This graft-transmissible disease induces a strip of pits below the inoculation site on the woody cylinder of the indicator *Vitis rupestris* St. George after graft inoculation. Grapevine rupestris stem pitting-associated virus (GRSPaV), a member of Fobeavirus, was discovered in 1998 to be a likely causal agent of RSP (Meng *et al*., 1999). Infected scions grafted onto virus-free rootstocks showed a three to fivefold reduction in photosynthetic potential and an increased dark respiration rate compared with healthy scion/rootstock combinations (Fajardo *et al*., 2004). In Italy, a reduced photosynthetic rate and chlorophyll content of GRSPaV-infected Bosco vines were also reported (Gambino *et al*., 2012).

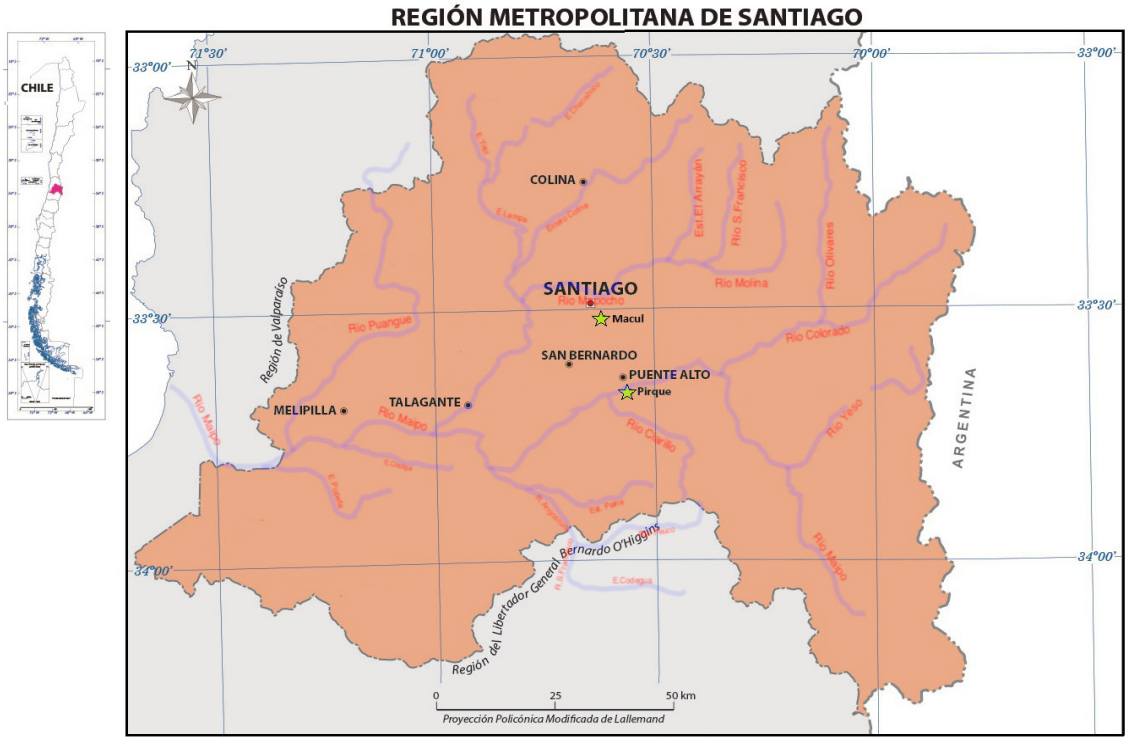
Photosynthesis and chlorophyll fluorescence were lower in GLRaV-3-infected Cabernet Franc grapevines in a Michigan (USA) vineyard than in healthy vines after veraison (Endeshaw *et al*., 2014). Following veraison, sugar accumulates in grape berries, and reduced leaf photosynthesis would explain the lower sugar accumulation in the GLRaV-3-infected berries (Alabi *et al*., 2016).

Thus, few existing studies evaluate the health status of grapevine by measuring parameters derived from chlorophyll-a fluorescence. In addition to the studies mentioned above on viral infections, this technique has also been used to characterize and detect the stress response to esca disease, a wood disease of fungal origin (Christen *et al*., 2007). However, applications of this type in Chile have not been carried out to date.

Chilean viticulture is characterized by its strong French influence, which began with the importation during the second half of the XIX century of the most outstanding French varieties, such as Cabernet Sauvignon, Merlot, Sauvignon Blanc, and Chardonnay, among others, which extensive vineyards and wineries were established, some of which survive to this day. Of all these varieties, the most cultivated red cultivar to date is Cabernet Sauvignon, with an area of 40,053 ha as of 2020, according to the National Vineyard Register of the Servicio Agrícola y Ganadero (SAG). At the same time, some valleys in Chile stand out internationally in the wines produced with Cabernet Sauvignon, such is the case of the Maipo Valley. This wine-growing region stands out for its Mediterranean-type climate with well-defined seasons, low risk of rain during the harvest, and stony alluvial soil, which results in privileged conditions for growing grapes.

The objective of this work will be to study how the measurement of photosystem II fluorescence emission is helpful in the characterization of phenological development and phytosanitary status of Cabernet Sauvignon vines grown in the Maipo Valley. For this purpose, first, the fluorescence parameters along the productive development of field-grown plants in Pirque will be characterized. Second, the differences in these parameters will be studied between plants infected with GLRaV-3 and GRSPaV virus, versus sanitized plants, in greenhouse crops, in Macul. Both sectors belong to the Maipo Valley (Figure 1).

Figure 1. Map of Maipo valley. The vines evaluated for phenological development were planted in a vineyard situated in the town of Pirque, south of the Maipo River. The grapevines evaluated for viruses were in a greenhouse in Macul, north of the Maipo River
Figura 1. Mapa del Valle del Maipo. Las vides evaluadas según desarrollo fenológico se encontraban plantadas en un viñedo ubicado en la localidad de Pirque, al sur del río Maipo. Las vides evaluadas por virosis se encontraban en un invernadero ubicado en Macul, al norte del río Maipo



Source: adapted from Instituto Geográfico Militar, Chile.
Fuente: adaptado desde Instituto Geográfico Militar, Chile.

Materials and methods

Fluorescence measurement

The transient fluorescence of chlorophyll a from dark-adapted grape leaves was measured using a Handy-PEA® chlorophyll fluorometer (Handy-Plant Efficiency Analyzer, Hansatech Instruments, King's Lynn, Norfolk, UK). The transient was induced by illumination for 1 s with an array of six diodes emitting a maximum light intensity of 3000 µmol (photons) $m^{-2} \cdot s^{-1}$ and homogeneous irradiation in an area of about 4 mm in diameter. Rapid fluorescence kinetics (F_o s F_M) was recorded from 10 µs to 1 s. The fluorescence intensity at 50 µs was considered F_o (Strasser and Strasser, 1995).

The analysis of fast phase OJIP and the equations proposed for the quantification of the behavior of PSII (Bolhàr-Nordenkampf and Öquist, 1993), the following information can be obtained (Table 1): (a) the specific energy flows for absorption (ABS), trapping (TR_o), electron transport (ET_o), and dissipation (DI_o) by reaction center or by the active area of the leaf; (b) quantum efficiency or flux ratio as the quantum yield of primary photochemistry (ϕP_o), the efficiency with which a trapped exciton can move an electron

in the electron transport chain beyond Q_A^- (ψ_o), and the yield quantum electron transport (ϕE_o); (c) the number of active reaction centers per emission area; performance index (PI_{ABS}), which combines structural and functional criteria of the PSII; (d) the number of very slow reopening reaction centers or reaction centers that do not bind Q_B^-; (e) estimation of the number of oxygen releasing centers (OEC); (f) the number of times that Q_A^- is reduced from the time zero (t_o) to the time in which the maximum fluorescence t_{Fmax} (N) is reached, and (g) the average fraction of reaction centers opens during the time necessary to reduce all reaction centers.

Table 1. OJIP analysis using data obtained in the fast phase of fluorescence emission
Tabla 1. Análisis OJIP usando datos obtenidos en la fase rápida de la emisión de fluorescencia

Technical parameters			
Fluorescence at 50 µs	F_o		
Maximum fluorescence	F_M		
Variable fluorescence to 2 ms	F_v	=	F_M-F_o
Slope from the origin of fluorescence	dV/dt_o	=	$(F_{300\mu s}$-$F_o)/(F_M$-$F_o)$
Variable fluorescence relative to 2 ms	V_j	=	$(F_{2ms}$-$F_o)/(F_M$-$F_o)$
Specific fluxes expressed by reaction centers (RC)			
RC absorption	ABS/RC	=	$(M_o/V_j)/(1$-$F_o/F_M)$
Trapping at time 0 by RC	tRo/RC	=	$M_o/Vj = (ABS/RC)\phi_{Po}$
Dissipation at time 0 per RC	Dio/RC	=	(ABS/RC)-(TR_o/RC)
Electronic transport on time 0 by RC	eTo/RC	=	$(TR_o/RC)\psi_o$
Specific flows expressed by area (Cross-section) (CS)			
CS absorption	ABS/CS	=	F_o o F_M
Trapping at time 0 by CS	tRo/CS	=	$(TR_o/ABS)/(ABS/CS)$
Dissipation at time 0 per CS	Dio/CS	=	(ABS/CS)-(TR_o/CS)
Electronic transport on time 0 by CS	eTo/CS	=	$(ET_o/RC)(RC/ABS)$
Density of RC por CS	RC/CS	=	(ABS/CS)(RC/ABS)
Quantum efficiencies (or flow relationships)			
Maximum quantum yield of primary photochemistry	ϕ_{Po}	=	$TR_o/ABS = (F_M$-$F_o)/F_M = (1$-$(F_o/F_M) = F_v/F_M$
Maximum quantum yield of non-photochemical deexcitation	ϕ_{Do}	=	$DI_o/ABS = 1$- $\phi_{Po} = F_o/F_M$
Probability that a trapped exciton moves an e- further Q_A^-	ψ_o	=	$ET_o/TR_o = 1 - V_j$
Probability that an absorbed exciton moves an e- further Q_A^-	ϕ_{Eo}	=	ϕ_{Po} –- $\psi_o = (TR_o/ABS)/(ET_o/TR_o) = ET_o/ABS = (1$-$F_o/F_M)(1$-$V_j)$
Vitality Index			
Performance Index	PI_{ABS}	=	$[RC/ABS][\phi_{Po}/(1$-$\phi_{Po})][\psi_o/1$-$\psi_o]$
Driving force of photosynthesis	DF_{ABS}	=	Log $[PI_{ABS}]$

Source/fuente: González *et al*. (2008).

Fluorescence study in phenological development

Healthy and adult *Vitis vinifera* clones were studied in production (7 years) of the cultivar Cabernet Sauvignon (clone 56, Foundation Plant Materials Service [PMS], University of California, Davis, USA) trained in lyre trellis system and located in Pirque, Santiago (33°38′07″S, 70°34′22″W, Figure 1).

Four repetitions of three plants were made, measuring two leaves on each plant using four clips, giving 96 measurements by date. Measurements were made according to the stipulations of the fluorometer program. The phenological stage was determined according to the Modified Eichhorn-Lorentz System (Coombe, 1995).

Fluorescence study in clones affected by viruses

Fluorescence measurements were made in plants of Cabernet Sauvignon clone 060114, grown in greenhouses in the San Joaquín Campus of Pontificia Universidad Católica de Chile, located in Macul, Santiago (33°30′00″S, 70°34′00″O, Figure 1).

Fluorescence measurements were done on January 15 and January 27, 2016. Three groups of plants were evaluated. A first group was infected with GLRaV-3, a second group was infected with GLRaV-3 and GRSPaV, and a third group was certified as healthy plants.

Four repetitions of three plants were made for each group and date, measuring two leaves on each plant using four clips, giving 96 measurements. Measurements were made according to the stipulations of the fluorometer program.

All the evaluated plants were analyzed by RT-PCR, performing a total nucleic acid extraction through the Silica Capture method. Reverse transcription was performed using Randon Primers (Promega) and MMLV enzyme (Invitrogen). 5 µl of the nucleic acid extract was mixed with 1 µl of random primer (0.5 µg/µl) and 24 µl of nuclease-free water. Denaturation was performed at 95 °C for 5 min, then the tubes were placed on ice for 5 min, and then a mixture containing 5 µl of 5X Buffer, 2.5 µl of the dNTPs mix at a concentration of 10 mM, 2.4 µl of DTT, 1 µl of M-MLV 200 U/µl enzyme and 4.1 µl of water was added to each tube. Incubation was performed at 42 °C for 1 hour to obtain the cDNA. The PCR step was performed using LC1/LC2 partitions for GLRV-3, amplifying a 546 bp fragment, C3310/H2999 for GFLV, amplifying a 312 bp fragment, H28/B/Bo sedown for GVB yielding a 155 bp fragment and 48d/49d for RSPaV, with an expected fragment of 330 bp. The reaction was performed by making a mixture consisting of 5 µl of 10X buffer, 1 µl of 2.5 mM dNTPs, 2 µl of 25 mM MgCl2, 1 µl of each partitioner, 0.2 µl of Invitrogen 5 U/µl Taq polymerase, and 35 µl of water, to which 5 µl of cDNA was added. Alignment cycles and temperatures were according to those indicated for each primer, and an MJ Research thermocycler was used for this purpose. PCR products were observed by 1.5% agarose gel electrophoresis.

Results

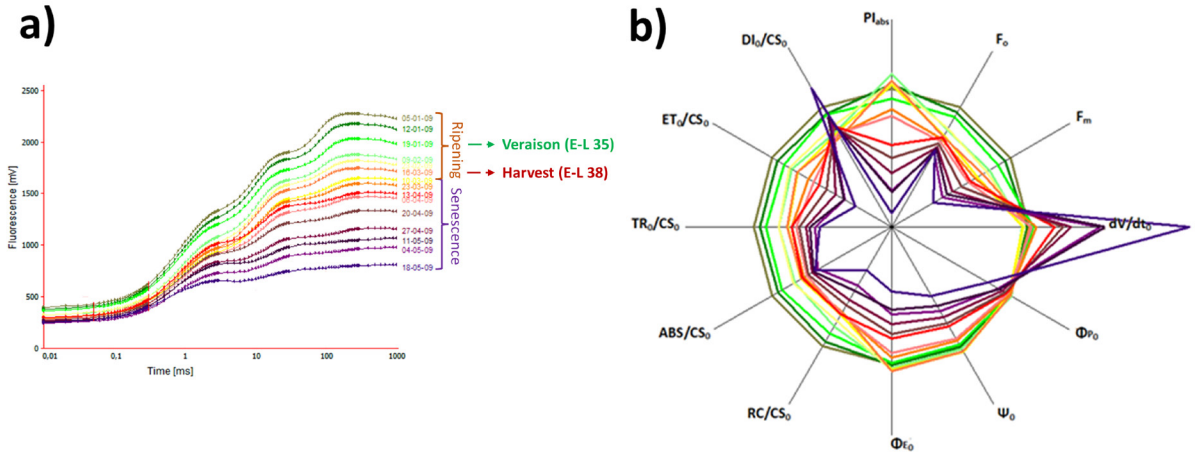
Fluorescence behavior during phenological development

Fluorescence measurements were made in the cultivar Cabernet Sauvignon in 2009, during the ripening and senescence period (Figure 2). Veraison (E-L 35) occurred around the January 19 measurement, while harvesting (E-L 38) took place on March 16. As phenological development progressed, we observed that the fluorescence emission decreased, with a fluorescence range between 2500 and 500 mV (Figure 2a). We see a clear decreasing trend in the technical parameters such as F_o and F_M throughout development, consistent with the overview obtained by viewing the OJIV curves (Figure 2b). Similarly, it appears that the slope at the origin of the increase in normalized fluorescence (dV/dt_o) increases with time, which gives an idea of the increase in the relative rate of reduction of Q_A. Concerning the quantum efficiency parameters, we observed a marked decrease in the maximum primary photochemical yield (ϕP_o) and the probability of electron movement beyond Q_A^- per trapped (ψ_o) and absorbed exciton (ϕE_o).

Regarding the specific flows by area, we also see a strong tendency to decrease their values as the phenological state advances. There is a decrease in the density of reaction centers per area (RC/CS), absorption (ABS/CS), trapping at time zero (TR_o/CS), and electronic transport at time zero (ET_o/CS). In the case of dissipation at time zero (DI_o/CS), on the contrary, we see a substantial increase as the phenological state advances. Finally, the vital index of functioning (PI_{ABS}) shows a slight increase as soon as the phenological state progresses, until it decreases significantly towards the last dates.

Figure 2. OJIV fluorescence emission curves (a) and derived parameters (b) of Cabernet Sauvignon leaves from the town of Pirque, where fluorescence is evaluated during phenological development. Each curve represents the average of 96 measurements

Figura 2. Curvas OJIV de emisión de fluorescencia (a) y parámetros derivados (b) de hojas de Cabernet Sauvignon de la localidad de Pirque, donde se evaluó la fluorescencia durante el desarrollo fenológico. Cada curva representa el promedio de 96 mediciones

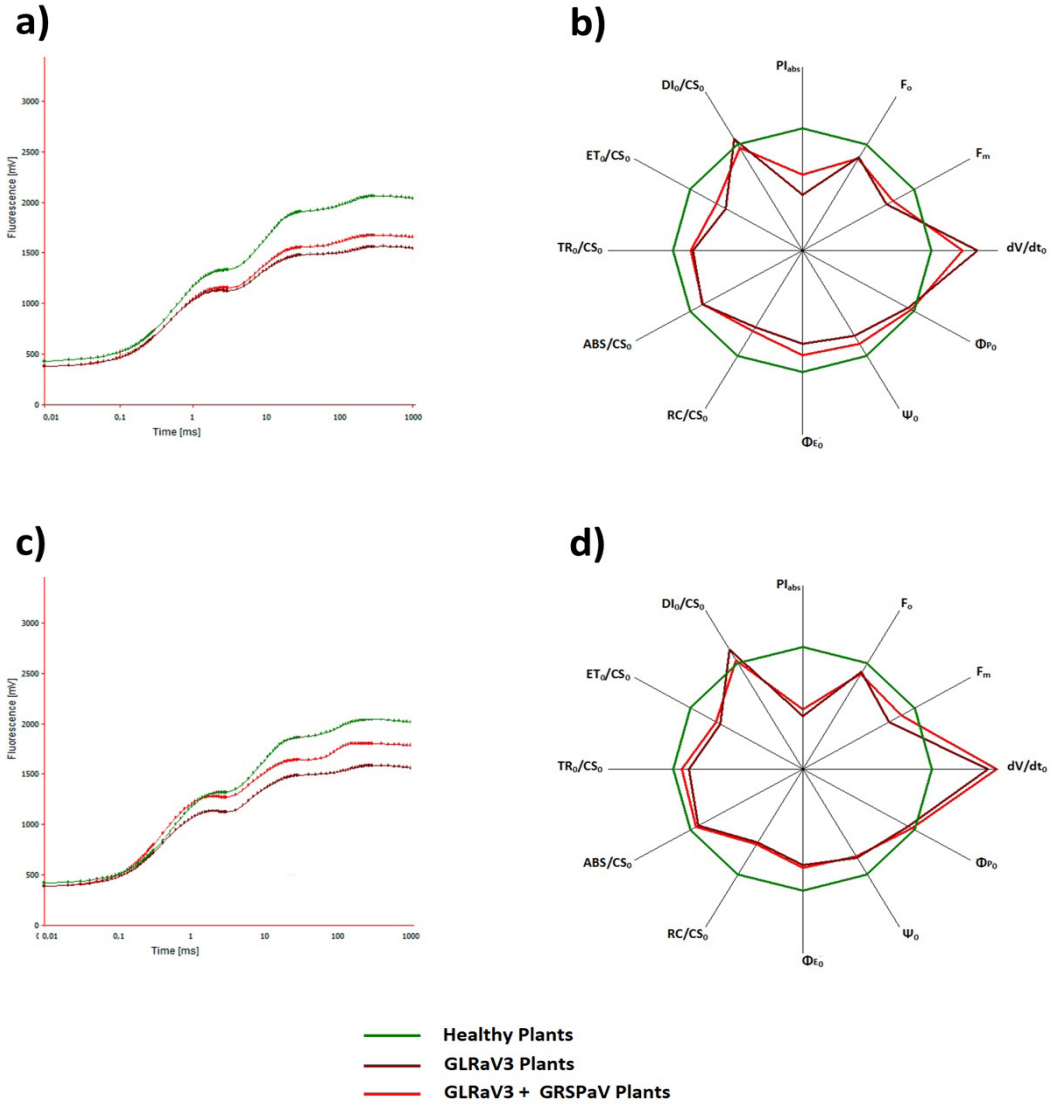


Source: own elaboration. Fuente: elaboración propia.

The behavior of fluorescence in vines affected by viruses

Fluorescence emission decreased in plants affected by viruses (around 1500 mV) compared to healthy plants (around 2000 mV) on the two dates evaluated (January 15, Figure 3a; and January 27, Figure 3c). In technical parameters (Figure 3b and d), we see a clear decrease in Fo and FM when plants are infected, according to what we observed in the OJIV curves. Similarly, the slope at the origin of the increase in normalized fluorescence (dV/dt_o) increases in plants with virus infections. About the quantum efficiency parameters, a slight decrease is observed in the maximum primary photochemical yield (ϕP_o) and a marked reduction in the probability of electron movement beyond Q_A^- per exciton trapped (ψ_o) and absorbed (ϕE_o) in plants with viruses.

Figure 3. OJIV fluorescence emission curves (a and c) and derived parameters (b and d) of greenhouse Cabernet Sauvignon leaves. Green curves correspond to healthy plants; red curves correspond to diseased plants. Figures a and b correspond to January 15, 2016, and c and d correspond to January 27
Figura 3. Curvas OJIV de emisión de fluorescencia (a y c), y parámetros derivados (b y d) de hojas de Cabernet Sauvignon provenientes de invernadero. Las curvas verdes corresponden a plantas sanas; curvas rojas corresponden a plantas enfermas. Figuras a y b corresponden al 15 de enero del 2016, c y d corresponden al 27 de enero



Source: own elaboration. Fuente: elaboración propia.

We also see a strong tendency to decrease their plants' values with virus infections regarding the specific flows by area. There is a decrease in the density of reaction centers per area (RC/CS), absorption (ABS/CS), entrapment at time 0 (TR_o/CS), and electronic transport at time zero (ETo/CS). On the contrary, in dissipation at time zero (DI_o/CS), we see similar values and even slightly higher concerning healthy plants. Finally, the vital performance index (PI_{ABS}) shows a significant decrease in plants with virus infections.

It is important to mention that this technique for evaluating the physiology and health status of the vine is relevant today due to two main factors. Firstly, it is a non-invasive technique and therefore does not affect the integrity of the plants. On the other hand, it is a portable measurement system, which over time has been miniaturized, making it much easier to use in the field. Thus, it is an analysis that remains valid, and that will be very useful in evaluating stressful conditions for plants in the current context. For example, the drought tolerance of different corn genotypes in Mozambique has been tested by determining the parameters associated with chlorophyll fluorescence (Chiango *et al.*, 2021).

Discussion

From the results obtained, we observed that the advance of the vines throughout the phenological stages, as well as the presence of viruses, produces, in general terms, a decrease in the fluorescence emission kinetics, which reflects a limitation of the donation of electrons by the oxygen-releasing complex and changes in the architecture of the PSII antenna complex, consequently altering the energy distribution. The decrease in the "J" point of the fluorescence kinetics is probably caused by an accumulation of the reduced pool of Q_A (Lazár and Ilík, 1997) due to a decrease in the transport of electrons beyond Q_A^-, which limits reoxidation reactions. Step 1, in the last part of the curve, can be assigned to the reduction of Q_B (Q_B^- and Q_B^{2-}) and the subsequent protonation, generating Q_BH_2. It has been observed that a transient limitation on the acceptor side of PSI leads to an obstruction of the traffic of transiently formed electrons in the electron transport chain and is responsible for the I-P transient. Thus, this limitation on the PSI side can be caused initially by ferredoxin-NADP+ oxidoreductase (FNR) and then by an inactive Calvin-Benson cycle (Schansker *et al.*, 2005).

In the radial graphs, we observed that the maximum quantum yield for PSII photochemistry (ϕ_{Po}) is one of the minor variable parameters throughout the vine's phenological development and against viruses.

Variations in photosynthetic efficiency, expressed by PIABS, were one of the parameters most sensitive to change throughout phenological development, through which it decreases, as well as its low value observed in plants with viruses, compared to healthy ones, is related to the variations of reaction centers by area (RC_o/CS) (Paoletti *et al.*, 2004). The reduced efficiency of the processes associated with the capture of energy caused by viruses can be interpreted as a strategy to lower the high excitation pressure in the mesophyll cells. The decrease in the fraction of fully active reaction centers (reduced centers Q_A and Q_B) in favor of an increase in the fraction of heat sink centers (non-reducing centers Q_A or silent centers) is considered a negative regulation

mechanism to dissipate excess absorbed light in a controlled way. PSII changes from being a light energy converter system in chemistry to a light energy converter system in heat dissipation. In this way, PIABS appears like an excellent parameter for evaluating the vines' vitality (Novak *et al*., 2007).

Conclusion

Photosynthesis is vital in photoautotrophic organisms' function. The analysis of its functioning is essential for practical situations in vineyard management, such as evaluating the vines' physiological state as their development progress and the operation of plants under different phytosanitary states. The quantitative determination of fluorescence emission of chlorophyll a is a valuable and rapid tool to obtain information at the molecular level of plants' physiology, particularly of the vine. In this study, it was observed that vine plants, as they progress through their phenological development, present a decrease in fluorescence kinetics, particularly in values such as F_o and F_M, reflecting changes in electrons' transport in the photosynthetic apparatus. Similar behavior can be observed in plants affected by viruses, allowing the plant's phytosanitary status to be predicted. A suitable parameter to evaluate the plant's vitality is PI_{ABS}, which proved to be one of the most sensitive to phenological changes and virus infection.

As can be deduced from this work, this type of study can be carried out in vineyards located in different parts of the country and the world, making it possible to characterize the physiological state of the vines. One of the particularities of this technique, which would be very useful for the wine industry, is that it would detect diseases in the vineyard before the plants show symptoms. In this sense, future work could investigate how the different parameters obtained from chlorophyll-*a* fluorescence are affected by biotic or abiotic stress factors, allowing a preventive differential diagnosis.

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