

Chlorophyll fluorescence and other physiological parameters as indicators of waterlogging and shadow stress in lulo (*Solanum quitoense* var. *septentrionale*) seedlings

Parámetros de fluorescencia de la clorofila y otros parámetros fisiológicos como indicadores del estrés por anegamiento y sombrío en plántulas de lulo (*Solanum quitoense* var. *septentrionale*)



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Experiment with lulo plants in greenhouse under shadow net.

Photo: G. Fischer

ABSTRACT

Climate change has resulted in an increasing frequency of the phenomenon “La Niña,” generating prolonged periods of waterlogging and low light. The objective of the present study was to evaluate the effects of two abiotic stresses: shading (65%) and waterlogging, and their interaction on fluorescence parameters of chlorophyll *a* in lulo (*Solanum quitoense* var. *septentrionale*) seedlings. A completely randomized design with a factorial arrangement was implemented. The first factor consisted of two levels of light (with and without shading). The second factor were four levels of duration of the waterlogging period (0, 3, 6 and 9 days), for a total of 8 treatments with three replicates. The response variables were recorded at 6, 12 and 18 days after the application of the waterlogging treatments began. Measurements of relative water content (RWC), electrolyte leakage, chlorophyll content and chlorophyll *a* fluorescence were recorded. The lulo plants appeared to be more susceptible to waterlogging than to shading, with a lower RWC when waterlogged 6 and 9 days,

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presenting damage at the level of photosystem II from day 3, causing a decrease in the chlorophyll content. The plants flooded under shading had a greater tolerance to this factor than those cultivated in full light. The techniques of quantification of the chlorophyll *a* fluorescence, especially the maximum quantum efficiency of the PSII, the effective photochemical quantum yield of PS II and the photochemical quenching were useful tools that characterized the lulo seedlings under stress conditions.

Additional keywords: oxidative stress; hypoxia; light energy dissipation; electrolyte leakage.

RESUMEN

El cambio climático trae como consecuencia el aumento en la frecuencia de fenómenos como los eventos “La Niña”, generando periodos prolongados de anegamiento y sombrío. El objetivo del presente estudio fue evaluar los efectos de dos estreses abióticos sombrío (65%) y anegamiento y su interacción sobre parámetros de fluorescencia de la clorofila *a* en plántulas de lulo (*Solanum quitoense* var. *septentrionale*). Se implementó un diseño completamente al azar con un arreglo factorial. El primer factor consistió en dos niveles de sombrío (sin y con). El segundo factor fueron cuatro niveles (0, 3, 6 y 9 days) de duración del periodo de anegamiento, para un total de 8 tratamientos con tres repeticiones. Las variables de respuesta se registraron a los 6, 12 y 18 días después de iniciada la aplicación de los tratamientos de anegamiento. Se registraron medidas de contenido relativo de agua (CRA), fuga de electrolitos, contenido de clorofilas y fluorescencia de la clorofila *a*. Se encontró que las plantas de lulo son más susceptibles al anegamiento que al sombrío, evidenciado en una reducción del CRA en condiciones de anegamiento durante 6 y 9 días, presentando daño a nivel de fotosistema II a partir del día 3 y causando disminución en el contenido de clorofilas. Las plantas bajo sombrío presentaron mayor tolerancia al anegamiento en comparación a aquellas sin sombrío. Las técnicas de cuantificación de la fluorescencia de la clorofila *a*, especialmente la eficiencia máxima del PSII, la eficiencia real del PSII y el *quenching* fotoquímico, fueron una herramienta útil que permitió la caracterización de plántulas de lulo a condiciones de estrés.

Palabras clave adicionales: estrés oxidativo; hipoxia, disipación de energía lumínica, fuga de electrolitos.

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INTRODUCTION

The lulo plant (*Solanum quitoense* Lam.; Solanaceae) is a tropical shrub from the inter-Andean areas (Huertas *et al.* 2011), whose fruit is desired for its organoleptic characteristics, such as soft and exotic aroma, taste, color and bright appearance in the pulp (Ardila *et al.*, 2015). In addition, it has antioxidant properties and a high nutritional potential with its important source of vitamins and minerals (Gancel *et al.*, 2008). It is widely consumed in Colombia, desired mainly for its high potential in agroindustry with uses for its pulp, nectars and juices (González *et al.*, 2014). It belongs to the group of exotic Solanaceae that enjoys demand in the international market, especially in Europe (Álvarez-Herrera *et al.*, 2015; Cruz *et al.*, 2007; Orjuela-Castro *et al.*, 2017).

In Colombia, two botanical varieties predominate: a) septentrionale, with thorns on the stem and leaves and higher acidity in the fruits; and b) quitoense, without thorns and with sweeter fruits (Bonnet and Cárdenas, 2012). The lulo exhibits its best development in sites with temperatures of 15 to 24°C, with an optimum of 20°C, requires slightly acidic soils, with pH between 5.5 and 6.0, with good moisture retention capacity and deep and good drainage (Gómez *et al.*, 2014). It is a plant native to humid forests, adapted to fresh and shaded zones, but, in the course of its domestication, commercial crops have been adapted to areas free from light exposure (Ardila *et al.*, 2015; Huertas *et al.*, 2011).

Fischer *et al.* (2018) stated that “the environmental conditions of the site (climate and soil) are crucial for the process of crop quality formation” when plants are exposed to multiple factors of abiotic and biotic stress (Visser *et al.*, 2015). The Intergovernmental Panel on Climate Change (IPCC) reported that the increase in rainfall as a result of climate change might have a greater impact on tropical regions as a result of the increased world hydrological cycle (Bailey-Serres and Voesebeck, 2008), generating waterlogging conditions as a result of heavy rains and poor drainage and gloomy weather because of the greater presence of clouds. This situation is aggravated on mixed plantations and can negatively affect photosynthesis and the performance of crops (Soleh *et al.*, 2018). The lulo was classified as a species susceptible to waterlogging (Flórez-Velazco *et al.*, 2015) and moderately susceptible to shaded conditions (Cardona *et al.*, 2016).

In general, plants are often limited by a number of stressful factors that occur simultaneously, which makes it difficult to predict their geographical distribution based on physiological responses of an individual factor (Mittler, 2006; Fischer *et al.*, 2016; Fischer and Melgarejo, 2020); thus, the effect of a combined stress on plant physiology is likely to be key to understanding the mechanisms of susceptibility to stress under natural field conditions (Casierra-Posada and Cutler, 2017). However, the potential effects of the stress combination may vary depending on the level of each of the individual stresses combined (e.g. severe vs. low) and the type of plant or pathogen involved (Mittler, 2006; Villarreal-Navarette *et al.*, 2017; Jiménez *et al.*, 2015).

Some physiological responses reported about the effects of stress from waterlogging and cloudy conditions suggest that both factors interact in such a way that the adverse effect of the shade is amplified in flooded soils (Laan *et al.*, 1990). Waterlogging and shading can affect the growth of plants independently or interact in such a way that a factor reduces or increases the impact of additional factors (Urbas and Zobel, 2000).

The effects of abiotic stress caused by waterlogging and shading, mainly on growth and development factors, have been studied in Solanaceae species, such as the cape gooseberry (*Physalis peruviana* L.) by Aldana *et al.* (2014), tomatoes (*Solanum lycopersicum* L.) by Ezin *et al.* (2010) and Baracaldo *et al.* (2014) and lulos (*Solanum quitoense*) by Flórez-Velasco *et al.* (2015).

It has been shown that the decrease in chlorophyll content under stressful conditions can be considered a typical symptom of oxidative stress as a result of photooxidation of pigments and degradation of photosynthetic pigments (Anjum *et al.*, 2011). Chlorophyll fluorescence, which indicates the photosynthetic efficiency of photosystem II (PSII) (Bansal *et al.*, 2019), is a highly informative parameter of plant traits under adverse environmental conditions. Nowadays, chlorophyll fluorescence measurements are nearly universally used in ecophysiological plant studies, where, especially under natural and managed growth conditions, plant reactions can be easily understood when factors are changing (Hanelt, 2018).

Generally, environmental stress can be detected early through the quantification of chlorophyll fluorescence since this methodology uses information on the photochemical activity of plants (Marques *et al.*, 2017). This is possible because the chlorophyll molecule is fluorescent and, through the dissipation of photons, changes in electron transfer at the level of chloroplast membranes can be detected (Do Nascimento and Marques, 2018). However, studies on the effects of the interaction of two abiotic stresses on the fluorescence in lulo plants are scarce.

Therefore, the objective was to evaluate the effects of two abiotic stresses (shading and waterlogging) and their interaction on physiological parameters such as fluorescence of chlorophyll, relative water content and electrolyte leakage in seedlings of lulo (*Solanum quitoense*) var. *septentrionale*.

MATERIALS AND METHODS

Plant material and experiment conditions

This study was conducted in a plastic greenhouse at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogota, located at 4°35'56" N and 74°04'51" W and at 2,556 m a.s.l.

The plant material was lulo seedlings (*Solanum quitoense* var. *septentrionale*) obtained from seeds, with an age of 10 weeks. The seedlings were transplanted into 5 L pots, which contained a substrate composed of quartzite sand and sieved soil at a ratio of 1:1 v/v. The substrate had the following physical and chemical properties: pH 5.2; organic carbon 6.33%; N 0.55%; Ca 6.21 meq/100 g; K 1.7 meq/100 g; Mg

1.42 meq/100 g; Na 0.29 meq/100 g; P 37.2 mg kg⁻¹; Cu 0.20 mg kg⁻¹; Fe 47.0 mg kg⁻¹; Mn 4.57 mg kg⁻¹; Zn 1.45 mg kg⁻¹; B 0.34 mg kg⁻¹; Al 0.23 meq/100 g; CEC 9.94 meq/100 g; and a sandy loam texture (sand 72%, lime 17% and clay 11%).

Experiment design and treatments

A completely randomized design was carried out with a factorial arrangement, where the first factor consisted of the two levels of light conditions: a) without shading (WS); with an average noon radiation of 66,694±10,981 lux; b) shading (SH); with an average shading percentage of 65±4% (radiation of 29,935±3,428 lux), which was done through the installation of a black polyshade, 1.5 m high, covering the seedlings on all sides. The second factor corresponded to the four levels of waterlogging (WA) period (0, 3, 6 and 9 d). To do this, the drainage holes of the materials were closed hermetically, and, through irrigation, the water level was established at 3 cm above the substrate during the experiment. At the end of each period of waterlogging, the drainage holes of the pots were reopened to begin the recovery period. The experiment had a total of 8 treatments with three replicates for each one. Additionally, the response variables were recorded at 6, 12 and 18 d after the application of the waterlogging treatments began.

The mean air temperature for the two light conditions, with and without shading, was 25.3 and 25.6°C, respectively, while the mean relative humidity in the polyshade chamber was higher than that of without shading, with 61.2 and 25.7%, respectively.

Relative water content of leaves

The relative water content (RWC) was determined by extracting 1 cm diameter discs, taken from the middle area of the leaf blade of the second fully expanded leaf. The following equation was used to calculate the RWC (1)

$$RWC = [(FW-DW) / (WT-DW)] \times 100 \quad (1)$$

where, *FW* is the fresh weight, *WT* is the weight at turgidity, measured after 24 h of saturation in distilled water at 4°C in darkness and *DW* is the dry weight determined after 48 h of drying in an oven at 80°C.

Electrolyte leakage

The methodology described by Jiang and Zhang (2001) was followed to determine the electrolyte leakage. Ten discs, with a 0.5 mm diameter, were extracted from the middle area of the leaf blade from the second fully expanded leaf (counted from the apical part of the plant) in each treatment replicate. The discs were lightly washed with deionized water and placed in a test tube with 30 mL of deionized water. The tubes were subsequently incubated in a water bath (model B-480; Büchi Waterbath, Flawil, Switzerland) at 30°C for 2 h. The initial electrical conductivity (EC_1) was measured with a conductimeter (Bench Meter, model P700; Oakton Instruments, Vernon Hills, IL). Subsequently, they were incubated again in the water bath, for 15 min at a temperature of 100°C, in order to extract all released electrolytes, and, with a conductivity meter, the final electrical conductivity (EC_2) was measured. The percentage of electrolytes was calculated with the following equation (2)

$$\text{Electrolyte leakage (\%)} = [(CE_1/CE_2)] \times 100 \quad (2)$$

Chlorophyll content

The chlorophyll content was estimated using a chlorophyllometer (atLEAF, FT Green, Wilmington, DE). The readings were taken on the second fully expanded leaf, taking three data per replicate.

Fluorescence of chlorophyll *a*

The maximum quantum efficiency of photosystem II (PSII) (F_v/F_m), the effective photochemical quantum yield of PSII (Y_{II}), the photochemical quenching (qP) and the non-photochemical quenching (NPQ) were determined at 6, 12 and 18 DAOW, using a Modulated fluorescence chlorophyll meter (MINI-PAM, Walz, Effeltrich, Germany). The leaves were adapted to darkness with clips for 15 min. The maximal fluorescence (F_m) was estimated with a 0.8 s long saturating light pulse (2,600 $\mu\text{mol m}^{-2} \text{s}^{-1}$), with 20,000 Hz frequency. The variable fluorescence (F_v) was estimated with the difference between F_0 and F_m . The potential maximal PSII quantum yield (F_v/F_m ratio) was calculated from the F_v and F_m . The photochemical and non-photochemical quenching were assessed as qP =

$(F_m' - F)/(F_m' - F_0)$, and $NPQ = (F_m - F_m')/F_m'$ according to Schreiber *et al.* (1994).

Statistical analysis

The data were analyzed using Statistix program v. 9.0 (analytical software; Informer Technologies, Tallahassee, FL). When significant differences were obtained in the ANAVA, the Tukey mean comparative test at $P \leq 0.05$ was used.

RESULTS AND DISCUSSION

Relative water content (RWC) of leaves

The RWC expresses the percentage of water content and is relative to the turgidity or total saturation in a given tissue (Melgarejo, 2010). Significant differences were found in the waterlogging factor at the three

evaluation moments (Tab. 1). The waterlogging period of 6 d and longer negatively affected the RWC (Tab. 2). Although at 12 and 18 d after the onset of waterlogging (DAOW) the plants in the 6 and 9 d waterlogging treatments had a recovery time (6 and 3 d at the first evaluation point; 12 and 9 d at the second evaluation point, respectively), the values were close to 55 and 65% of the RWC at the two evaluation points, respectively, and were significantly lower than the control treatment (about 80% RWC).

The reduction of the RWC after 6 d of waterlogging was possibly caused by the low absorption and transfer of water to the leaves, negatively affecting cellular turgor (Moreno and Fischer, 2014) as caused by anoxia conditions in the root system (Moreno *et al.*, 2019), a situation that can damage numerous metabolism processes in plants (Yan *et al.*, 2018). Similarly, six tree species in Bogota constantly decreased their RWC with an increase in waterlogged time between 0 and 28 d (Moreno *et al.*, 2019).

Table 1. Summary of analysis of variance of the effect of shading (SH) and waterlogging (WA) treatments on physiological variables of lulo seedlings at 6, 12 and 18 d after the onset of waterlogging (DAOW).

Variable	Abbreviation	6 DAOW			12 DAOW			18 DAOW		
		SH	WA	SH × WA	SH	WA	SH × WA	SH	WA	SH × WA
Relative water content	RWC	NS	*	NS	NS	***	NS	NS	**	NS
Electrolyte leakage		NS	NS	***	***	**	NS	***	NS	*
Chlorophyll content	At-LEAF	*	NS	NS	*	***	**	NS	***	**
Real efficiency of PSII	$Y_{(II)}$	NS	*	NS	NS	***	NS	***	***	NS
Photochemical quenching	qP	***	*	***	NS	**	NS	*	***	NS
Non-photochemical quenching	NPQ	NS	***	**	**	NS	**	***	**	**
Maximum efficiency of PSII	F_v/F_m	**	NS	NS	**	***	*	**	**	NS

NS: non-significant; *significance level $P \leq 0.05$, **significance level $P \leq 0.01$, *** significance level $P \leq 0.001$.

Table 2. Relative water content (RWC) of the lulo seedling leaf under conditions of 0, 3, 6 and 9 d of waterlogging and subsequent recovery of 6, 12 and 18 d (DAOW).

Treatment	RWC (%)		
	6 DAOW	12 DAOW	18 DAOW
Waterlogging (d)			
0	81.45 ± 2.66 a	79.75 ± 2.26 a	79.45 ± 2.97 a
3	75.27 ± 4.27 ab	83.42 ± 2.73 a	74.70 ± 2.51 a
6	70.22 ± 4.31 b	56.73 ± 8.25 b	68.52 ± 3.58 ab
9	71.68 ± 3.85 ab	52.62 ± 2.48 b	60.48 ± 8.17 b
CV (%)	8.68	9.91	10.73

Means with different letters indicate a significant statistical differences according to Tukey test ($P \leq 0.05$) ($n=3 \pm$ standard error); CV, coefficient of variation.

The plants with up to 3 d of waterlogging (DWA) without any influence on the RWC or recovery time may have activated mechanisms that diminished the water potential in the plant cells through the synthesis of compatible osmolytes, such as proline, soluble sugars, or glycine betaine in order to maintain a water potential gradient that favored water intake (Oh and Komatsu, 2015). On the other hand, and according to Cardona *et al.* (2016), lulo plants waterlogged for 3 d do not suffer damage to their root system, so the absorption and transport of water in this organ is much greater and is related to the high RWC values compared to the 6 and 9 d waterlogged plants, which presented more serious damage, especially through the reduced diameter of the root neck.

Electrolyte leakage

For the loss of electrolytes, significant differences were found at the level of the shading×waterlogging interaction at the three sampling points (Fig. 1).

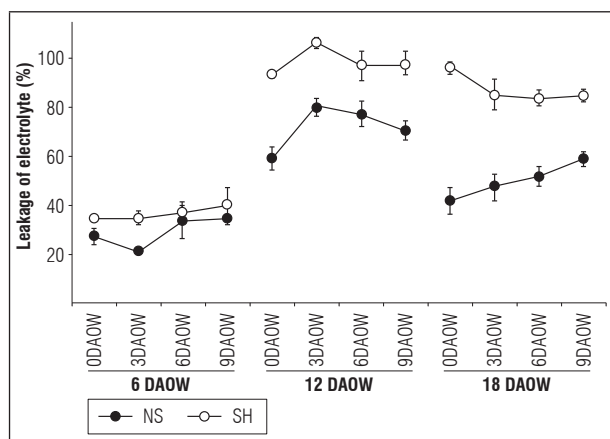


Figure 1. Electrolyte leakage in lulo seedlings under non-shading (NS) and shading (SH) conditions and at 0, 3, 6 and 9 d of waterlogging (DWA) and subsequent recovery of 6, 12 and 18 d after onset of waterlogging (DAOW). The vertical bars indicate \pm standard error.

In general, it was observed that the treatments that did not have the additional shade (SH) presented the lowest values for electrolyte leakage. At 6 DAOW, the loss of electrolytes from the seedlings with shading maintained values close to 40%, while the seedlings without shading had values that were on average 35%. At 12 DAOW, the leakage of electrolytes from the seedlings with shadowy conditions

was higher than 90%. Also, for the seedlings without shading with 0 d of waterlogging, the electrolyte leakage values were close to 60%, probably because of a mild dry air stress during this evaluation period (Sánchez-Reinoso *et al.*, 2019). For the plants with some period of waterlogging stress, these values were close to 80%. At 18 DAOW, the percentage of electrolyte leakage from the plants of the different SH treatments was close to 85%. However, the highest values found in the seedlings of the different WS treatments were observed in those that had 9 d of waterlogging, with about 60% electrolyte leakage, and the lowest values were evidenced in the seedlings with 0 d of waterlogging (about 40% electrolyte leakage). However, the treatment of the lulo seedlings with 9 d of waterlogging reached values of approximately 60% loss of electrolytes.

Obviously, the flooding stress was increased by the conditions of shading, increasing the leakage of electrolytes, which is related to oxidative stress (Moreno *et al.*, 2019). Similar results were found by Bansal *et al.* (2019) in black beans, in which the membrane stability decreased as a result of the greater loss of electrolytes generated by the conditions of the lack of oxygen in the radical system (waterlogging for 10 d starting at 30 d after sowing), which was also manifested by cell membrane damage by ROS-induced peroxidation of membrane lipids (Andrade *et al.*, 2018).

Chlorophyll content

For the chlorophyll content, significant differences were obtained in the shading×waterlogging interaction at the three sampling points (Fig. 2; Tab. 1). Particularly, the plants in the different shading treatments had lower chlorophyll contents than the shaded plants. Additionally, the chlorophyll values at 6 DAOW were, on average, \sim 50 at-LEAF units. In general, it was evident that, with the increase in the period of waterlogging, the reduction of chlorophyll content was greater. This reduction was more pronounced in the treatments with 6 and 9 d in the last two evaluation points when the plants were unshaded, which reached values close to 30 at-LEAF units. However, the SH plants with 9 d of waterlogging reached the lowest values at 18 DAOW (about \sim 35 at-LEAF units), followed by SH plants with 6 d of waterlogging (\sim 40 at-LEAF units) at the same evaluation point.

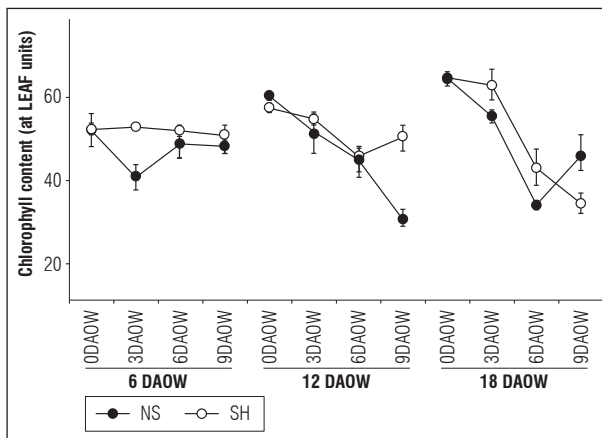


Figure 2. Chlorophyll content in at-LEAF units in lulo seedlings under non-shading (NS) and shading (SH) conditions and at 0, 3, 6 and 9 d of waterlogging (DWA) and subsequent recovery of 6, 12 and 18 d after onset of waterlogging (DAOW). The vertical bars indicate \pm standard error.

A decrease in chlorophyll content with the duration of waterlogging stress has been observed in many species, such as the cape gooseberry (Aldana *et al.*, 2014), tomatoes (Ezin *et al.*, 2010; Baracaldo *et al.*, 2014), black beans (Bansal *et al.*, 2019), and cabbage (Casierra-Posada and Cutler, 2017), among others, which is manifested by foliar yellowing, followed by wilting, affecting photosynthesis (Wu *et al.*, 2015) and consequently reducing the biomass of the lulo plants, such as with a decrease in leaf area and root volume (Cardona *et al.*, 2016).

The lower reduction of chlorophyll content in the lulo plants under additional shade as waterlogging continued (Fig. 2) coincided with that observed by Mielke and Schaffer (2009) in the Solanaceae pitanga, taking into account that shaded leaves generally have a higher concentration of chlorophyll and more pigment molecules per electron transport chain than leaves in full light (Kadereit *et al.*, 2014). However, Visser *et al.* (2015) found the opposite in *Solanum dulcamara*, which means that the flooded and shaded plants synthesized a smaller amount of chlorophyll than those waterlogged and in full light.

Chlorophyll fluorescence parameters

The results of the maximum quantum efficiency of PSII (F_v/F_m), effective photochemical quantum yield of PSII (Y_{II}), photochemical quenching (qP), and

non-photochemical quenching (NPQ) were affected by the waterlogging (Fig. 3). In general, regardless of the duration of waterlogging or the shading condition, there was a tendency towards a reduction of the parameters F_v/F_m , Y_{II} , and qP. Additionally, the reduction was higher in the seedlings that were not shaded (NS), being lower in those that had 9 d of waterlogging (Fig. 3A, B and C). However, the NPQ tended to increase its values up to 40% with respect to the waterlogging condition, being greater when the lulo plants were exposed to a greater number of days (especially 6 and 9 d of waterlogging). However, at 6 DAOW, the highest values were found in the plants in shading conditions, but, as time progressed, the shaded plants had higher values (Fig. 3D).

For the reduction of the NPQ values at 12 and 18 DAOW, it is supposed that the lulo plants under shady conditions did not have stress conditions, with no need to dissipate energy in the form of heat since lulo plants are native to undergrowth areas (Bonnet and Cárdenas, 2012). This may be related mainly to the fact that the light compensation point may be lower than in other species grown with free exposure (Taiz *et al.*, 2018).

A reduction in the maximum quantum efficiency of PSII, when the condition of waterlogging stress is increased, is common in many different plant species (e.g. black beans, Bansal *et al.*, 2019; pitanga, Mielke and Schaffer, 2010; and several tree species, Moreno *et al.*, 2019). Most likely, the decrease in F_v/F_m in the flooded lulo indicated that it adversely affected the photochemistry of photosynthesis in these stressed plants (Mielke and Schaffer, 2010), while Janowiak *et al.* (2002) observed tomato damage in the light-harvesting complex of PSII in waterlogged plants.

The strong decrease in the F_v/F_m ratio was especially evident in the plants with 9 d of waterlogging and 12 of recovery, with values below 0.7, which indicated severe stress that generated serious damage in PSII (Moreno *et al.*, 2019) and showed, according to Ashraf (2012), the inability of the plants to regenerate Rubisco under these stressful conditions. In the other treatments, with levels that exceeded 0.7, the functioning of PSII was not impaired (Bansal *et al.*, 2019); rather, there was a dynamic photoinhibition, without presenting real damage to the photosystems (Moreno *et al.*, 2019).

The waterlogged and shaded lulo showed a lower reduction in the maximum efficiency of PSII than the

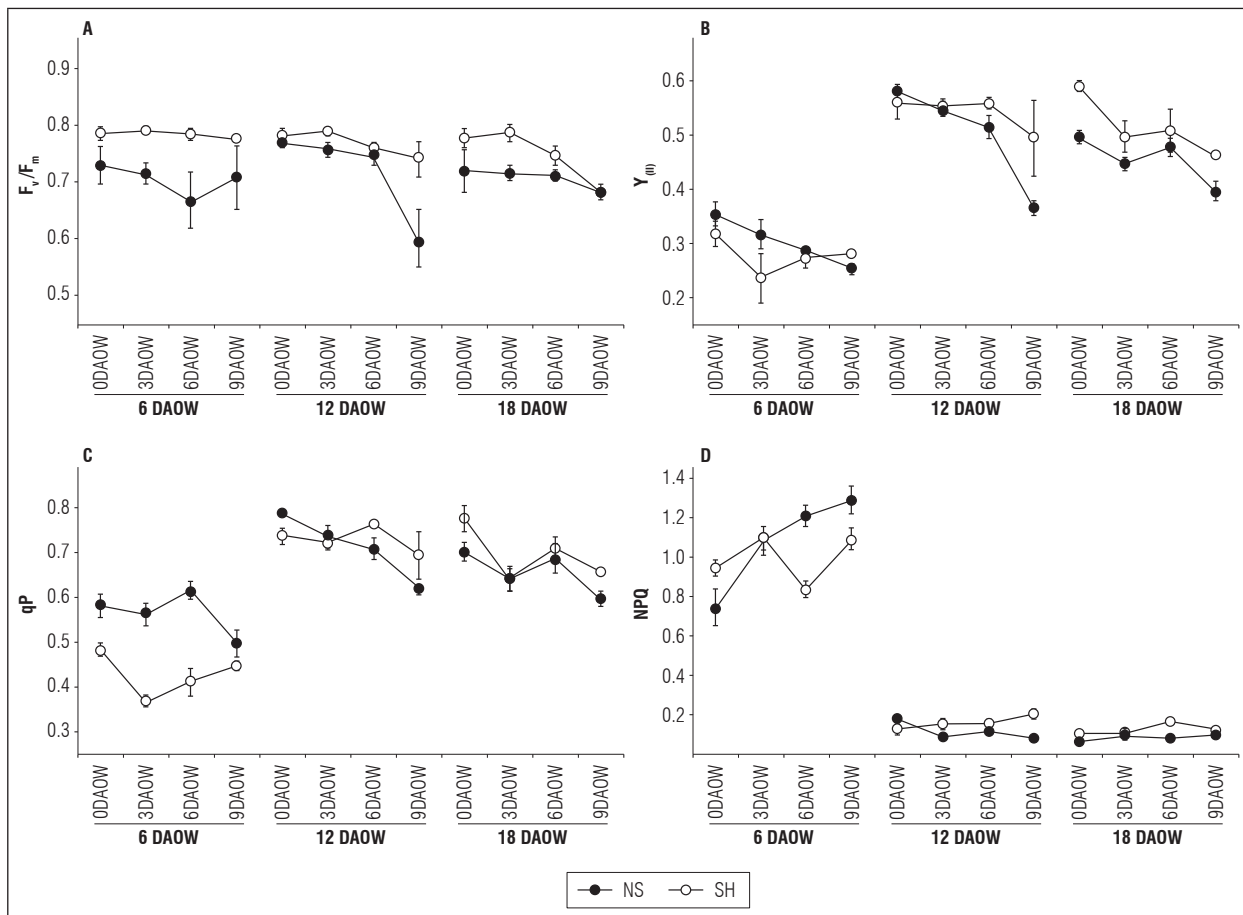


Figure 3. Fluorescence parameters of chlorophyll a in lulo seedlings under non-shading (NS) and shading (SH) conditions and at 0, 3, 6 and 9 d of waterlogging (DWA) and subsequent recovery of 6, 12 and 18 d after onset of waterlogging (DAOW). A) Maximum quantum efficiency of PSII (F_v/F_m); B) Effective photochemical quantum yield of PSII (Y_{II}); C) Photochemical quenching (qP); and D) Non-photochemical quenching (NPQ). The vertical bars indicate \pm standard error.

plants at full light, a result that Mielke and Schaffer (2010) also observed in pitanga. Supposedly, a higher concentration of chlorophyll, larger grana and a higher proportion of PSII compared to PSI in shaded leaves (Kadereit *et al.*, 2014) led to the increase in F_v/F_m in the lulo.

The effective photochemical quantum yield of PSII (Y_{II}) indicates the part of the energy absorbed by chlorophyll, which is associated with PSII and is used in the photochemical activity, evidencing the sum of transported electrons and, thus, becomes an indicator for photosynthesis (Lichtenthaler *et al.*, 2005; Jiménez-Suanca *et al.*, 2015). Overall, the Y_{II} of the waterlogged lulo plants decreased with the increased stress, as Else *et al.* (2009) observed in tomatoes. With the increase in recovery time (at 12 and 18 DAOW), this stress was reduced.

Photochemical quenching (qP) quantifies the photochemical capacity of PSII and refers to the proportion of excitation energy trapped by open PSII reaction centers (Lichtenthaler *et al.*, 2005; Hanelt, 2018). While non-photochemical quenching (NPQ) indicates the activation of non-photochemical processes that lead to the dissipation of non-radiant energy, such as changes in the transthylacoidal pH gradient, photoinhibition, interruption of light collection complexes, and formation of zeaxanthin, among others (Jiménez-Suanca *et al.*, 2015; Roháček, 2002).

The reduction of the qP value in all treatments, especially between 0 and 3 d of waterlogging and also in the comparison between the 6 and 9 d waterlogged plants, indicated that the plants were under stress. Casierra-Posada and Cutler (2017) and Wu *et al.* (2015) observed a reduction of photochemical dissipation in

Triticum aestivum as the result of a reduction in the efficiency of the excited energy collected in the open photosystem II centers. In the lulo, it was observed that the plants tried to tolerate waterlogging in a specific way when they were stressed for 6 d, as seen in the increases in the qP and YII values, mainly in the shading treatments (Fig. 3B and C), but, in the end, they could not resist, as the results showed at 9 DWA, at recovery times of 12 and 18 DAOW. The increase in the non-photochemical dissipation with the increase in the stress duration was observed in the lulo only when the recovery of the plants occurred for 6 d (Fig. 3D), an effect that occurred in tomatoes (Else *et al.*, 2009) and manifested mainly in plants under full light (Waldhoff *et al.*, 2002); this situation did not occur in the plants with recovery periods of 12 and 18 d.

Interestingly, the qP values were near 0.8 at 0 DWA on 12DAOW; presumably, the low radiation accompanied by a mild dry air stress condition that occurred in the evaluation period resulted in this value (Sánchez-Reinoso *et al.*, 2019).

CONCLUSIONS

In the lulo seedlings, the effect of waterlogging and shading was especially observed in the treatments evaluated at 12 and 18 d after the onset of the treatments, in which a lower light intensity (shade) favored the chlorophyll content and the maximum and real efficiency of photosystem II, but also increased the electrolyte leakage.

The lack of water absorption and translocation that resulted from the waterlogging of the root system reduced the relative water content in the leaves, especially in the plants with a longer waterlogging time (6 and 9 d) and observation (12 and 18 d).

For the chlorophyll *a* fluorescence, the measurements of the maximum quantum efficiency of PSII (F_v/F_m), the effective photochemical quantum yield of PSII (Y_{II}), and the photochemical quenching (qP) proved to be a good indicator of the effects of this double stress on the lulo seedlings.

Conflict of interest: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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