STUDY OF THE MORPHOLOGY AND PHYSIOLOGY OF *Arabidopsis thaliana* COLUMBIA ECOTYPE DURING INDIVIDUAL AND COMBINED STRESS: DROUGHT AND HEAT †

[ESTUDIO DE LA MORFOLOGÍA Y FISIOLOGÍA DE *Arabidopsis thaliana* ECOTIPO COLUMBIA DURANTE EL ESTRÉS INDIVIDUAL Y COMBINADO: SEQUÍA Y CALOR]

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SUMMARY

Background: *Arabidopsis thaliana* is the most widely used plant organism as a study model, due to its short life cycle and small genome. Among the most used accessions, the Columbia (Col-0) ecotype is widely used for molecular and genetic research, however the physiological response of this model plant to abiotic factors is relatively unknown. **Objective:** Given its relevance in studying gene functionality, it is essential to understand its physiology and morphology under individual stress conditions: water deficit stress (WDS), thermal shock (50 °C for 2 hours), and under the combined effect of both stress types (WDS + 50 °C for 2 hours). **Methodology:** 75-day-old *A. thaliana* Col-0 plants were used for the 3 stress treatments: 14d of WDS, 50 °C/2 h, and 14d WDS + 50 °C/2 h. The survival percentage, water potential, electrolyte leakage, PSII status, and gas exchange were evaluated. **Results:** *A. thaliana* plants exhibited susceptibility to prolonged levels of stress, demonstrating different physiological mechanisms to cope with individual and combined stresses. The analysis of the photochemical state of PSII indicated that *Arabidopsis* is more vulnerable to the 50 °C/2 h stress and to the combined WDS + 50 °C/2 h stress, than to water deficit stress. The WDS + 50 °C/2 h treatment caused greater membrane damage, more negative water potential, and lower gas exchange compared to the individual stress. **Implications:** This system is proposed for future molecular analyses involving the overexpression of cloned transcription factor genes from tolerant species, with the aim of extrapolating these findings to commercially relevant crops. **Conclusion:** The differential response observed under different types of stress in this model plant, may facilitate the elucidation of underlying molecular mechanisms, which should be a central focus in future research aiming to increase resilience to climate change factors in commercially important agricultural crops.

Key words: *Arabidopsis thaliana*; Drought stress; Heat shock stress; Plant physiology; PSII.

RESUMEN

Antecedentes: *Arabidopsis thaliana* es el organismo vegetal más utilizado como modelo de estudio, gracias a su corto ciclo de vida y pequeño genoma. Entre las accesiones más empleadas para investigaciones moleculares y genéticas se encuentra el ecotipo Columbia (Col-0), sin embargo, poco sabemos sobre su respuesta fisiológica al estrés abiótico. **Objetivo:** Dada su relevancia en el estudio de la funcionalidad genética, es fundamental comprender su fisiología y morfología bajo condiciones de estrés individual: estrés hídrico (WDS), choque térmico (50 °C durante 2 horas) y el efecto combinado de ambos (WDS + 50 °C durante 2 horas). **Metodología:** Se utilizaron plantas de *A. thaliana* Col-0 de 75 días para los tratamientos de estrés WDS, 50 °C/2 h y WDS + 50 °C/2 h. Se evaluaron el porcentaje de supervivencia, el potencial hídrico, la fuga de electrolitos, el estado del PSII y el intercambio de gases. **Resultados:** Las plantas de *A. thaliana* mostraron susceptibilidad a niveles prolongados de estrés, presentando diferentes mecanismos fisiológicos para manejar el estrés individual y

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combinado. El análisis del estado fotoquímico del PSII indicó que *Arabidopsis* es más vulnerable al estrés de 50 °C/2 h y a WDS + 50 °C/2 h, que al estrés hídrico. El tratamiento WDS + 50 °C/2 h causó un mayor daño en las membranas, una disminución del potencial hídrico (más negativo) y un menor intercambio de gases en comparación con el estrés individual. **Implicaciones:** Se propone utilizar este sistema para futuros análisis moleculares que impliquen la sobreexpresión de genes de factores de transcripción clonados de especies tolerantes, con el objetivo de extrapolar estos hallazgos a cultivos de interés comercial. **Conclusión:** La respuesta diferencial observada ante distintos tipos de estrés en esta planta modelo puede facilitar la elucidación de los mecanismos subyacentes, lo que debería ser un enfoque central en futuros trabajos de investigación destinados a programas de mejoramiento genético de cultivos comerciales para lograr mayor resiliencia a factores del cambio climático.

Palabras clave: *Arabidopsis thaliana*; Estrés hídrico; Estrés térmico; Fisiología; PSII.

INTRODUCTION

The native species *A. thaliana* is widely distributed across Europe, continental Asia, and northern Africa (Fulgione and Hancock, 2018). This relatively limited geographic distribution suggests a preference for temperate climates with low precipitation, reflecting its adaptation to specific environmental conditions (Krämer, 2015). Since its discovery, early ecotypes have emerged through natural mechanisms, leading to remarkable genetic diversity. Currently, over 750 natural ecotypes have been collected worldwide, available at two major seed storage centers: The Arabidopsis Biological Resource Center (ABRC) and the Nottingham Arabidopsis Storage Center (NASC). This extensive collection not only facilitates research but also enables comparisons of genetic and phenotypic characteristics among different ecotypes. The Columbia ecotype (Col-0) was selected as the natural accession for the sequencing and annotation of the complete Arabidopsis genome, highlighting its importance in genetic and functional studies, (Arabidopsis Genome Initiative, 2000).

Climate change poses one of the greatest challenges to modern agriculture, as phenomena such as drought and high temperatures often occur simultaneously, limiting crop growth and productivity. It is projected that the global average temperature will rise by 2 °C by 2100 and by 4.2 °C by 2400, with an expectation that factors such as salinity, drought, and cold stress will become more frequent, thus compromising food security in many regions of the world (Malhi *et al.*, 2021). This situation demands a proactive approach to agricultural research.

A fundamental strategy to support food security involves conducting genetic and molecular studies using model organisms like Arabidopsis Col-0, given its short life cycle and small genome. To achieve this, it is essential to investigate where and when genes are expressed, how they integrate into signaling pathways, and to establish techniques and methods for measuring tolerance at morphological and physiological levels (Østergaard and Yanofsky, 2004). This knowledge will not only enrich our basic understanding of plant responses towards water deficit stress and heat but also it will have practical applications in crop improvement.

It has been recognized that under conditions of water deficit, stomatal closure is the primary cause of reduced photosynthesis, (Kijne, 2006). On the other hand, high-temperature stress alters photosynthetic activity by modulating enzyme activity rates and suppressing electron transport (Lloyd, 2008). Other relevant parameters include water relations, water potential, and relative water content, as well as membrane integrity, assessed through electrolyte leakage (Bajji *et al.*, 2002). Moreover, chlorophyll fluorescence is a useful indicator of PSII integrity and it has been employed to predict plant tolerance (Pleban *et al.*, 2020). *A. thaliana* is a model plant for physiological analyses assessing tolerance to individual and combined abiotic stress factors. This research may allow the development of future programs aimed at enhancing crop tolerance to adverse environmental conditions.

Therefore, the objective of the present study was to assess the physiological response of *A. thaliana* Col-0 ecotype when exposed to water deficit stress, to heat stress and when exposed to the combined effect of both stress types. This research will not only contribute to understanding the physiological responses of this model plant to abiotic stress factors, but it will also provide a reference point to advance genetic improvement programs aiming to increase drought and heat tolerance in commercial agricultural crops, thereby ensuring greater resilience in the face of future climate challenges.

MATERIALS AND METHODS

Plant material and growth conditions

A. thaliana ecotype Col-0 seeds were treated with 20% sodium hypochlorite for disinfection and subsequent germination in 1X MS medium, sucrose and 5 g L^{-1} agar. Seedlings were transplanted once they presented their first true leaves to individual polystyrene containers (12 Oz) with Peat Moss Sunshine Mix ® substrate, Agrolite and Vermiculite (3:1:1). An irrigation program was established with 20 mL of distilled water every second day and fertilized every week with Root Plant 500. The plants were maintained with a long photoperiod of 16 hours of light and 8 hours of darkness, with an average temperature of 25±2 °C, 70% relative humidity and a photosynthetic photon flux density (PPFD) of 90-100 µmol $m^{-2}s^{-1}$.

Conditions for individual and combined stress treatments

75 d old plants were subjected to 3 different types of stress. 1) Heat stress; for high temperature stress, a thermal chamber was set up at a temperature of 50 °C where plants were placed at 50 ±2 °C for 2 h. 2) Water deficit stress (WDS); it was imposed by suspending irrigation for 14 days reaching a soil potential of –2.5 MPa, with a controlled temperature of 25±2 °C. 3) Combined stress; the third treatment consisted of maintaining plants without irrigation for 14 days and then placing them at 50 ±2 °C for 2 h.

In addition, a group of plants was assigned to each of the three stress treatments where after being exposed to their respective stress treatment, plants were returned to the irrigation and temperature conditions of control plants, for further 168 h (Recovery). Three replicates of 5 plants each were allocated for each stress treatment (that is, 15 plants per treatment). Control plants for each group were maintained under conditions of temperature 25±2 °C and adequate irrigation (soil Ψ of -0.8 MPa).

Water potential

To determine the magnitude of stress in the plant, the change in the water status of the plants (Ψ_L; MPa) was evaluated as follows: 3 samples of the intermediate leaves of the rosette were selected and cut into 3 cm diameter discs. Discs were placed in a humid chamber at a temperature of 24±2 °C for 40 minutes to acclimatize. After this time, the reading was taken in the sealed chamber of a WP4C Dewpoint potentiaMeter© equipment (Decagon Devices, Inc).

Electrolyte leakage

The increase in electrolyte leakage was evaluated as an indicator of membrane stability. To do this, a 1 cm diameter punch was used to make circular cuts. For each plant, 3 discs were taken from the same leaf, which were placed in 15 mL of distilled water. They were incubated and shaken for 3 h, and the initial electrical conductivity (C1) was measured. Subsequently, those discs were placed in an autoclave for 40 min to achieve cell damage. Once the sample cooled down to room temperature, the final electrical conductivity (C2) was measured in an Orion conductivity meter, model 162 (USA). The percentage of ion leakage was calculated according to the following equation: Electrolyte leakage = (C1/C2) *100.

Photochemical state of PSII: Fv/Fm, PIabs and OJIP inflection curves

Two parameters, Fv/Fm and PIabs, were considered to determine the level of stress to which the plants are exposed in each treatment. These measurements were performed using an M-PEA chlorophyll fluorometer (FMS 2 Hansatech lnstruments Ltd, Norfolk, UK). It consisted of adapting leaves to darkness for 30 min for complete oxidation of the reaction centers. After this time, they were then exposed to a pulse of saturating red light (650 nm, 3000 µmol m^{-2} s^{-1}) for 1 second. The biophysical parameters established by the OJIP test were calculated based on all fluorescence transients on logarithmic time scale provided by the M-PEA software.

Gas exchange: Photosynthesis (Pn), Stomatal Conductance (gs), Intercellular CO_2 (Ci) and Transpiration (E)

The photosynthetic rate of leaves of *Arabidopsis thaliana* was determined using a portable gas analysis system in the infrared spectrum (IRGA, LICOR LI 6400, Lincoln, Nebraska, USA) with a chamber size adjusted to 4 cm^2 with a ratio of stomata between the adaxial/abaxial of 0.1. Measurements were carried out under a constant air flow of 350 µmol s^{-1} CO_2 and 100 µmol PPFD m^{-2} s^{-1}.

Data analysis

Data are presented as means ± standard deviation (SD) and analyzed using analysis of variance (ANOVA) with IBM SPSS Statistics V21.0 software (IBM Corporation, Armonk, New York). Mean comparisons were performed using Tukey's test, with a significance level of $p < 0.05$. Each experiment was conducted with three biological replicates, and graphs were created using SigmaPlot 11.0 software.

RESULTS AND DISCUSSION

Morphological response of *Arabidopsis thaliana* in response to abiotic stress

In general, *Arabidopsis* exhibited noticeable damage across all stress treatments, including reduced turgor, chlorosis, and cell death. Its limited geographic distribution suggests a preference for temperate climates with low precipitation, indicating that the survival of *A. thaliana* may be influenced by temperature, water availability, and day length (Lasky *et al.*, 2012). Control plants showed 100% survival and exhibited no signs of chlorosis, leaf abscission, or any damage indicative of biotic or abiotic stress (Figure 1A, B, and C). Exposure of plants to 50 °C for 2 hours, resulted in 30% mortality due to turgor loss caused by the increased temperature and water loss (Figure 1A). After 7 days of recovery, the old leaves showed a marked reduction in chlorophyll, the vital pigment responsible for capturing light energy during photosynthesis; this reduction in chlorophyll levels could lead to decreased photosynthetic rates.

Regarding the water stress treatment imposed by withholding irrigation for 14 days, a 50% mortality rate was observed. Most leaves displayed a purplish coloration (Figure 1B). This purplish hue has been attributed to the accumulation of anthocyanins, which appear to help control water loss and act as antioxidants (Cirillo *et al.*, 2021). In *Arabidopsis*, which is more susceptible to environmental changes, the accumulation of anthocyanins serves as an indicator of plant stress; their presence is beneficial for enhancing production as a protective response. This accumulation helps to mitigate the damage caused by reactive oxygen species (ROS) accumulation and improves the plant's ability to cope with oxidative stress (Sperdouli and Moustakas, 2012).

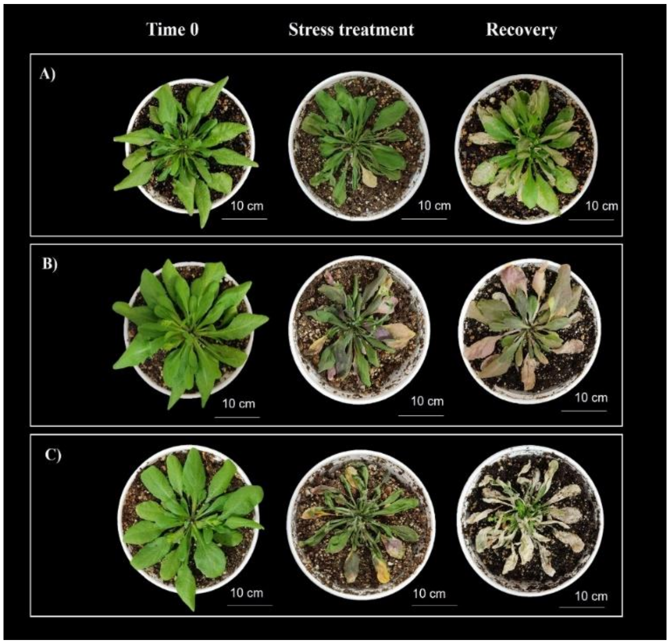

Figure 1. Morphological responses of *A. thaliana* plants under three different conditions: non stressing conditions (Time 0 plants were well irrigated and grown at 25 ± 2 °C); or under A) **thermal stress** (50 ± 2 °C for 2 hours); B) **water deficit stress** for 14 day and C) stress **combination** (plants were exposed to 50 ± 2 °C for 2h, after being exposed to 14 day of WDS. **Recovery** in the 3 cases, consisted of stress-release in A, by returning plants to 25 °C for further 7days. In the case of B, by re-watering plants for further 7 days and in C, by returning plants to 25°C and rewatering them for further 7 days. The experiment was performed with 75-day old plants.

Finally, the combined stress treatment (14 days without irrigation followed by exposure to thermal shock at 50 °C for 2 hours) resulted in a higher mortality rate (80%), and plants exhibited significant damage in terms of turgor loss and decreased chlorophyll content (Figure 1C). When plants were returned to non-stressful conditions for another 7 days, it was observed that 96% of the leaves showed chlorosis, and the plants did not recover. Equivalent results were obtained in the experiment by Rizhsky *et al.* (2004), where they studied the combined effect of drought and heat stresses by subjecting *Arabidopsis* plants grown with 70% to 75% water content to thermal stress at 38 °C for 6 hours.

Water potential in *A. thaliana* leaves in response to abiotic stress

Plants at time 0 exhibited average leaf water potentials of -0.48 ± 0.16 MPa. These values indicate that the plants at time 0 were in adequate hydration conditions; our results are very similar to those reported (Ψ of -0.4 MPa) by Christmann *et al.* (2007) for *A. thaliana* Col-0.

During thermal stress, the effects on water potential were not evident, and there were no statistically significant differences (P = 0.22) compared to the plants at time 0 (Figure 2). In other words, 50 °C for 2 hours was not sufficient to cause plasmolysis or a significant reduction in turgor in *A. thaliana*. This is further demonstrated in the recovery treatment, where, after the thermal shock, the plants maintained their water potentials at values close to -0.4 ± 0.13 MPa (Figure 2).

In contrast, the suspension of irrigation for 14 days significantly affected the water potential, reaching average Ψ values of -3.1 ± 0.76 MPa recorded during the treatment. Following rehydration, *Arabidopsis* showed partial recovery capacity, with water potential values around -2.1 ± 0.20 MPa (Figure 2). However, these values did not approach those at time 0, indicating that the permanent wilting point had not been reached. This suggests the activation of osmotic adjustment mechanisms that help cells cope with the stress episode. Some authors have reported that the permanent wilting point is generally found in a range of water potentials around -3.0 MPa; however, these studies do not consider post-stress conditions (De Ollas *et al.*, 2019).

Similar but more severe effects were observed in the combined stress treatment (14 days under WDS + 50ºC for 2 hours), where plants reached water potentials of -6 ± 0.58 MPa. During the recovery phase, the water potential only increased to values of -5.1 ± 0.46 MPa. The results suggest that the combined effect of both factors (water stress plus high temperature conditions) may have caused some degree of plasmolysis in the cells, (Figure 2). However, it would be worthwhile to include studies of osmotic and turgor potentials in future research, as noted by Juenger *et al.* (2023).

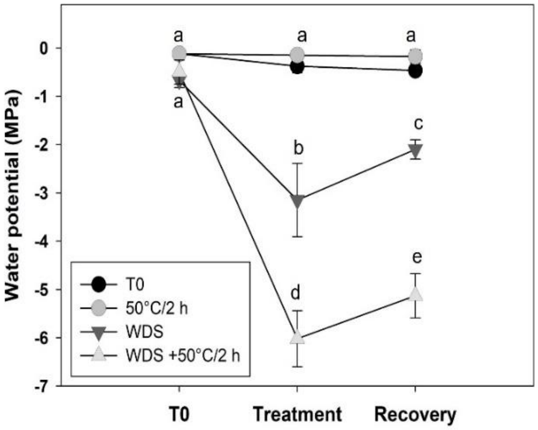

Figure 2. Water potential measured in leaves of *A. thaliana* plants. T0 plants under control conditions (well irrigated and grown at 25ºC), and plants exposed to three different stress treatments, and during the recovery period from the corresponding stress condition: Plants exposed to thermal stress of 50 °C for 2h; plants subjected to water deficit stress (WDS) for 14 days; plants exposed to a combination of WDS followed by thermal stress (50ºC for 2 hours); followed by a recovery period under well-watered conditions at 25 °C for an additional 168 hours. The data are means ± SD from 3 replicates. Different letters indicate significant differences ($p < 0.05$) compared to time 0.

Membrane integrity indicator under abiotic stress conditions

Electrolyte leakage is used as a parameter to evaluate the tolerance of plant tissues; the higher the percentage of electrolyte leakage, the greater the membrane damage (Demidchik *et al.*, 2014). This parameter has been employed to compare tolerance between wild-type (WT) and transformed lines of *Arabidopsis*, where WT lines show a leakage percentage of 24% (Li *et al.*, 2024), similar to that found at T0 of our system, which averages 25%. In contrast, thermal stress treatment (50 ± 2 °C for 2 hours) caused an increase in electrolyte leakage, reaching up to 60%, that continued to rise during the recovery phase, possibly due to membrane damage. This damage could include denaturation of channels in the lipid bilayer, affecting the maintenance of ion flow in response to stress (Figure 3).

Regarding the water deficit stress treatment, the damage observed was not as evident as in the previous case; under recovery conditions, *A. thaliana* plants approached the values of the T0 treatment (Figure 3). This suggests that thermal stress and water deficit stress affect plasma membranes in different ways, with thermal stress having a more direct impact on membrane structure and function. Although water deficit stress also causes damage, this is primarily due to dehydration and solute concentration (Opoku *et al.*, 2024).

Finally, in the combined treatment (drought + heat), we found values of up to 81% ion leakage, reflecting significant cellular damage that could not be repaired even after 7 days of recovery (Figure 3).

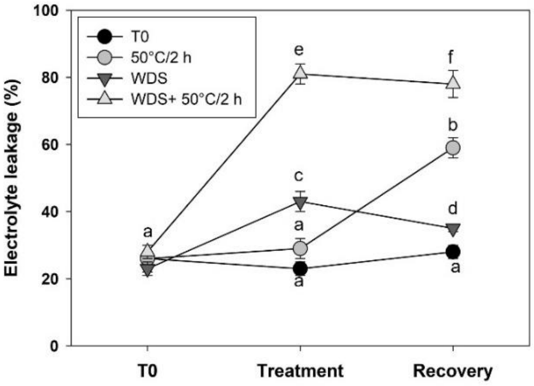

Figure 3. Electrolyte leakage from the leaves of time 0 (T0) plants of *A. thaliana* and those subjected to three stress treatments: thermal shock stress (50 °C for 2 hours), drought stress (WDS), or combined thermal shock and drought stress (WDS + 50 °C for 2 hours), followed by a recovery period under well-watered conditions at 25 °C for an additional 168 hours. The data are expressed as means ± standard error from three replicates. Different letters indicate significant differences ($p < 0.05$) compared to time 0.

Photochemical status of PSII in response to abiotic stress

Chlorophyll fluorescence is a non-destructive parameter that indicates whether the excitation energy efficiency in the photochemical process is adequate. When photosystem II (PSII) is intact, leaves exhibit values close to 0.8. Conversely, if plants experience any degree of damage, fluorescence efficiency decreases. Another parameter, PIabs, reflects the concentration of reaction centers, electron transport, and the dissipation of excess excitation energy, determining the photochemical performance of PSII. In non-stressed plants, this parameter typically reaches values between 6 and 8 in species such as *Carica papaya* (Estrella *et al.*, 2021).

In *A. thaliana*, the values observed in non-stressed plants indicated that the PSII photochemical process is functioning well, with an Fv/Fm of 0.83 ± 0.0015 and a PIabs of 6 ± 0.73. These results are consistent with those reported by Rico *et al.* (2023) in *Arabidopsis* Col-0 under optimal watering conditions, where values of 0.77 were recorded. However, under thermal stress conditions, the efficiency of the photochemical reactions decreased to approximately 0.44 ± 0.05. Plants in recovery conditions still exhibited low photochemical reaction rates of 0.35 ± 0.18 (Figure 4A), suggesting significant damage to PSII. In fact, Fv/Fm values lower than 0.44 resulted in high mortality among the plants (Figure 1A).

Regarding membrane damage, this was not as evident in the case of water stress, as no significant differences were found in the drought treatment, where values of 0.79 ± 0.01 were recorded. During the recovery phase, values were 0.76 ± 0.002 compared to the T0 treatment, which had a value of 0.83 ± 0.0035. This may be attributed to the adaptation of most plants to stress during the 14 days without watering. However, we did find differences with the PIabs parameter, which proved to be a sensitive indicator of stress response, allowing for the identification of damage comparable to that from other stress factors, with values of 1.09 ± 0.22 at the end of the recovery phase (Figure 4B). Other studies have also identified that the performance index PIabs is a very sensitive parameter across different crops and in most environmental stress situations (Živčák *et al.*, 2008).

On the other hand, in the combined stress treatment, chlorophyll fluorescence was compromised, with values of 0.30 ± 0.16 for Fv/Fm and it showed limited recovery capacity, reaching only 0.50 ± 0.08 for Fv/Fm. Regarding the PIabs parameter, values of 0.15 ± 0.20 were recorded, indicating that this type of stress was severe for the species.

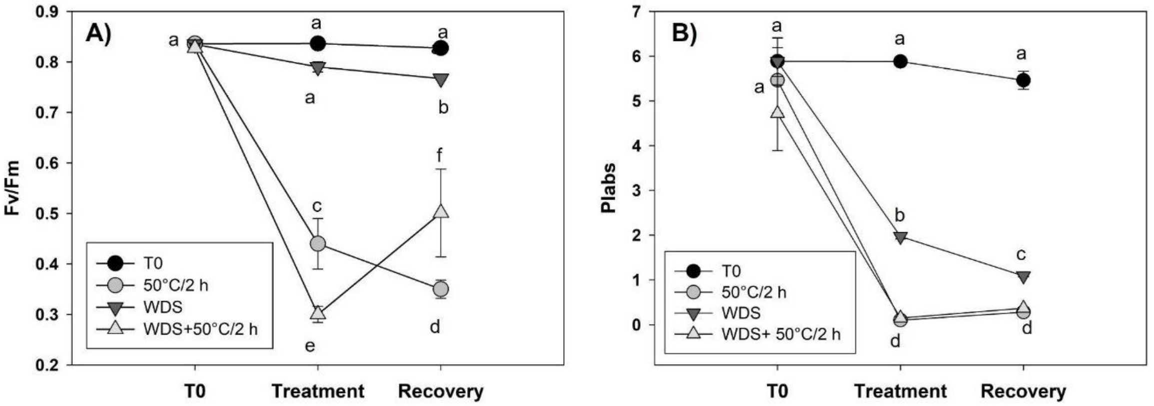

Figure 4. Photochemical state of PSII in *A. thaliana* in response to thermal shock stress (50 °C/2 h), drought (WDS), and combined thermal shock plus drought (WDS + 50 °C/2 h). A) Chlorophyll fluorescence (Fv/Fm). B) Performance index (PIabs). Data means ± SE from 3 replicates. Different letters indicate significant differences ($p < 0.05$) compared to time 0.

Electron transport in the photochemical phase of PSII, (OJIP analysis)

The OJIP curves represent chlorophyll fluorescence over time, reflecting the efficiency of electron transport (González *et al.*, 2008). In these curves, the points O, J, I, and P correspond to different stages of the process: O (F0): minimum fluorescence, where energy is absorbed but electron transport has not yet occurred. J: Represents an increase in fluorescence as electron transport begins. I: maximum fluorescence indicating that the system is operating at full capacity. P (Fm): maximum fluorescence, showing that all electrons have been efficiently transported (Kalaji *et al.*, 2016).

In *Arabidopsis* plants at time 0 (T0), O (F0) values ranged between 4822 and 6993 relative fluorescence units (mV). The subsequent steps exhibited a characteristic polyphasic curve, indicating proper electron transport and capture in the photosynthetic apparatus, facilitating the production of NADPH, ATP, and ultimately the assimilation of carbon dioxide (Figure 5).

In response to thermal stress, PSII is considered one of the most sensitive components of the photosynthetic apparatus due to increased fluidity of the thylakoid membranes. The light-harvesting complex of PSII can easily detach from the membrane, compromising its integrity and negatively affecting photosynthetic electron transport (Mathur *et al.*, 2010). In *A. thaliana*, exposure to 50 °C for 2 hours inhibited electron transport (J phase), and during the repair phase, irreversible damage was observed, potentially impacting the photosynthetic carbon assimilation process (Figure 5A).

In contrast to thermal shock stress, water deficit in *A. thaliana* plants did not cause significant damage to the oxygen-evolving complex. This allowed for similar values in the stress treatment and recovery, albeit with lower efficiency compared to control conditions. The results suggest a reduction in electron transfer efficiency; however, no significant damage to PSII was observed (Figure 5B).

In response to the combined stress, the typical polyphasic curve was significantly altered. During WDS + 50ºC for 2 h, similar values were observed starting from the J phase, with 5804; and 4128 relative fluorescence units for recovery, indicating a drastic reduction in electron transport and damage to PSII (Figure 5C). According to our results, this type of analysis might serve as a valuable tool for monitoring and quantifying the behavior and performance of the PSII apparatus in *A. thaliana*.

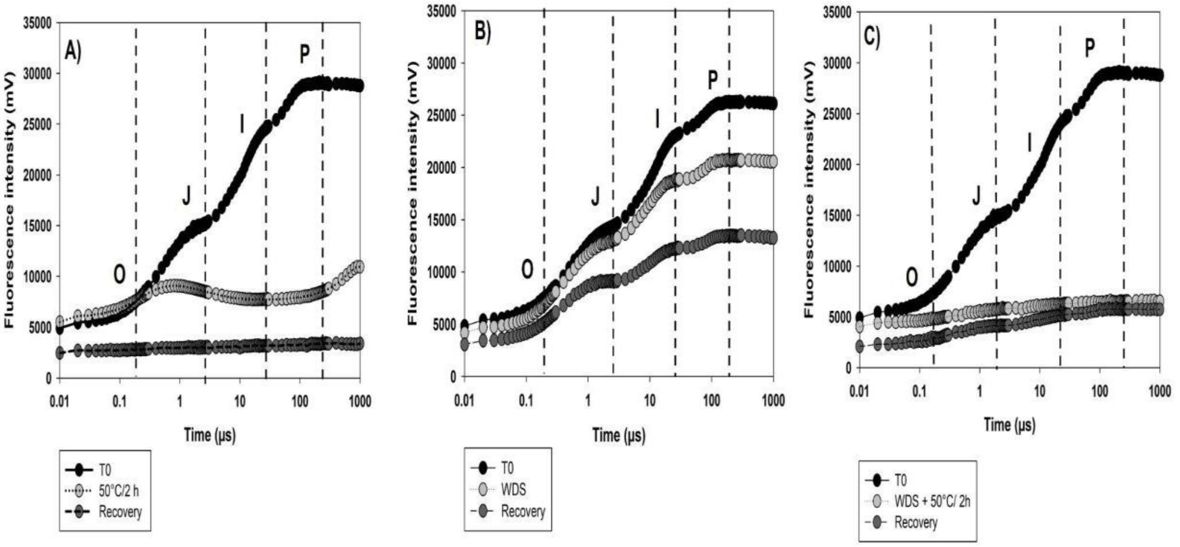

Figure 5. Kinetic representation of fluorescence transients (OJIP) of *A. thaliana* leaves in response to abiotic stress. A) Response to heat shock stress at 50 °C for 2 hours. B) Response to water deficit stress for 14 days (WDS). C) Plants exposed to the combined effect of both stress types (drought plus thermal shock) (14 days without irrigation + 50 °C for 2 hours). Time 0: well irrigated plants under controlled temperature conditions 25°C±2. Recovery refers to plants restored to initial conditions (well-watered at 25°C). In all cases, leaves were dark-adapted for 30 min. The signals are the fluorescence intensity O (at 50 µs); the fluorescence intensities J (at 2 ms), I (at 30 ms) and the highest peak P (at 500 ms) that represents the maximum fluorescence (Fm).

Gas exchange of *A. thaliana*: Photosynthesis, Stomatal Conductance, Intercellular CO_2 and Transpiration in response to abiotic stress

The gas exchange analysis indicated that under time 0 conditions, *A. thaliana* plants exhibited photosynthesis values of 6 µmol CO_2 m^{-2} s^{-1}, stomatal conductance of 0.18 mol H_2O m^{-2} s^{-1}, transpiration of 2.8 mmol H_2O m^{-2} s^{-1}, and intercellular CO_2 of 320 µmol CO_2 mol^{-1}, reflecting plants under no stress (Figure 6A-D).

During heat shock stress, the plants showed photosynthetic rates of -0.93 µmol CO_2 m^{-2} s^{-1}, suggesting that photorespiration occurred temporarily, limiting the reduction of net carbon fixed by the Calvin cycle, while maintaining intercellular CO_2 levels higher than the control (391 µmol CO_2 mol^{-1}) (Figure 6A). With Rubisco acting as an oxygenase, transpiration was measured at 7.07 mmol H_2O m^{-2} s^{-1} and stomatal conductance at 0.53 mol H_2O m^{-2} s^{-1} (Figure 6B). This phenomenon has also been attributed to the plants increasing stomatal opening to respire and possibly regulate leaf temperature, allowing CO_2 entry (Mittler, 2006). In the recovery stage (plants at 25 °C), our data indicate a recovery in photosynthetic processes to 1.72 µmol CO_2 m^{-2} s^{-1}, with stomatal conductance at 0.10 mol H_2O m^{-2} s^{-1}, transpiration at 1.81 mmol H_2O m^{-2} s^{-1}, and intercellular CO_2 at 359 µmol CO_2 mol^{-1}. These results are consistent with findings reported by Rizhsky *et al.* (2002) under thermal stress in *Arabidopsis* Columbia, where plants subjected to 38 °C for 6 hours showed increased conductance and higher respiration rates.

During drought stress, the plants exhibited a photosynthetic rate of 1.05 µmol CO_2 m^{-2} s^{-1}, with significantly lower stomatal conductance and transpiration values compared to time 0, measuring 0.06 mol H_2O m^{-2} s^{-1} and 0.78 mmol H_2O m^{-2} s^{-1}, respectively (Figure 6C). This stomatal closure mechanism is associated with preventing water loss through transpiration under water deficit conditions, which is a beneficial trade-off for plant survival (Pirasteh *et al.*, 2016). In the recovery phase, stomatal conductance, transpiration, and intercellular CO_2 values partially returned to time 0 conditions, except for photosynthesis.

In the assessment of plants that survived after combined stress (WDS+50°C/2 h), we observed that photosynthesis was lower than in the drought treatment, measuring 0.74 µmol CO_2 m^{-2} s^{-1}. Stomatal conductance, transpiration, and intercellular CO_2 values during the stress and recovery treatments were very close to the time 0 values, possibly due to the adaptation the plants underwent during the 14 days of water stress (Figure 6D).

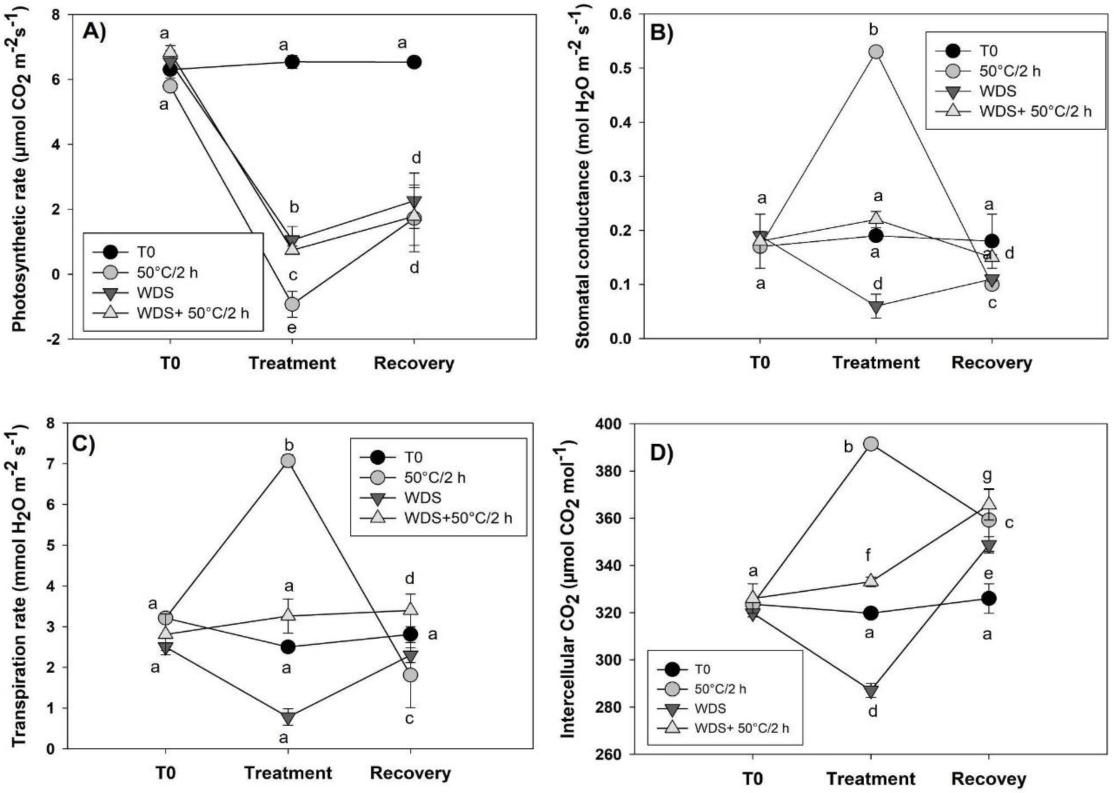

Figure 6. Gas exchange data A) Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}). B) Stomatal conductance (mol H_2O m^{-2} s^{-1}). C) Transpiration rate (mmol H_2O m^{-2} s^{-1}). D) Intercellular CO_2 (µmol CO_2 mol^{-1}). At time 0 (black circles) or in response to heat shock stress (50 °C/2 h; gray circles), drought (WDS; gray inverted triangle) and drought plus thermal shock (WDS +50 °C/2 h; gray triangle). Plants returned to non-stressing initial conditions (Recovery) for 168 h. Data points are means ± SE from 3 replicates. Different letters indicate significant differences ($p < 0.05$) compared to time 0.

CONCLUSIONS

In conclusion, we explored the physiological response of the model plant *A. thaliana* to conditions of water deficit, thermal stress, and their combination. The evaluated parameters water potential, chlorophyll fluorescence (OJIP curves), electrolyte leakage, and gas exchange prove to be suitable tools for assessing the tolerance level of this species, which was found to be relatively susceptible to both heat and drought, as well as to the combination of these stresses. Our study revealed that the main physiological processes affected under thermal and combined stress conditions include membrane damage and electron transport efficiency in PSII. A differential physiological response was observed to the individual stresses: during thermal stress, increases in respiration, stomatal opening, and internal CO_2 were recorded, leading to greater membrane damage. In contrast, water deficit stress resulted in decreased respiration, stomatal opening, and CO_2 uptake, causing less membrane damage. However, under combined stress conditions, the severity of the stress was so high that plant survival was lower compared to their individual stress treatments.

This study provides not only valuable insights into the morphological and physiological response of the Columbia ecotype of *A. thaliana* towards water deficit stress, heat stress and the combination of both stress types, but also it might set the basis to advance genetic improvement programs aimed to increase drought and heat tolerance in agricultural commercial crops, thereby ensuring greater resilience in the face of future climate challenges.

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