

# Assessment of photosynthetic pigment quantification methods and physiological indicators in *Capsicum chinense* under prolonged water stress in arid conditions

Evaluación de métodos de cuantificación de pigmentos fotosintéticos e indicadores fisiológicos en *Capsicum chinense* bajo estrés hídrico prolongado en condiciones áridas

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## ABSTRACT

Habanero pepper (*Capsicum chinense* Jacq.) is a high-value crop in Mexico, whose production is limited by water deficit and salinity, particularly in arid regions such as the Mexicali Valley. The objective of this study was to compare methods for quantifying photosynthetic pigments and to evaluate the physiological response of *C. chinense* to prolonged water stress. Two extraction methods for chlorophylls and carotenoids were evaluated using UV-Vis spectroscopy, along with measurements of relative water content (RWC) and the normalized difference vegetation index (NDVI) with GreenSeeker™, under a completely randomized design with two irrigation treatments. Results showed that, from day 21 onward, water stress significantly reduced chlorophyll *a* and NDVI, while carotenoids remained stable. RWC remained unchanged for three weeks, reflecting mechanisms of osmotic adjustment and water retention. The leaf disc-based method reduced extraction time by 80 % and exhibited lower data dispersion. It is concluded that *C. chinense* displays tolerance to water deficit for up to 28 d under saline conditions, and that the integration of destructive and non-destructive methods constitutes a robust strategy to assess the impact of water stress on economically important crops.

**Keywords:** Chlorophyll, Relative water content, NDVI, Osmotic Fit, Soil salinity.

## RESUMEN

El chile habanero (*Capsicum chinense* Jacq.) es un cultivo de alto valor en México cuya producción se ve limitada por el déficit hídrico y la salinidad, particularmente en regiones áridas como el Valle de Mexicali. El objetivo de este estudio fue comparar métodos de cuantificación de pigmentos fotosintéticos y evaluar la respuesta fisiológica de *C. chinense* al estrés hídrico prolongado. Se evaluaron dos métodos de extracción de clorofilas y carotenoides mediante espectroscopía UV-Vis, junto con lecturas de contenido relativo de agua (CRA) y del índice de vegetación de diferencia normalizada (NDVI) con GreenSeeker™, bajo un diseño completamente al azar con dos tratamientos de riego. Los resultados mostraron que, a

partir del día 21, el estrés hídrico redujo significativamente la clorofila *a* y el NDVI, mientras que los carotenoides permanecieron estables. El CRA se mantuvo sin cambios durante tres semanas, reflejando mecanismos de ajuste osmótico y retención hídrica. El método basado en discos foliares redujo en un 80 % el tiempo de extracción y presentó una menor dispersión en los datos. Se concluye que *C. chinense* presenta tolerancia al déficit hídrico de hasta 28 días en condiciones salinas y que la integración de métodos destructivos y no destructivos constituye una estrategia robusta para evaluar el impacto del estrés hídrico en cultivos de importancia económica.

**Keywords:** Clorofila, contenido relativo de agua, NDVI, ajuste osmótico, salinidad edáfica.

## INTRODUCTION

Habanero pepper (*Capsicum chinense* Jacq.) is one of the most economically, culturally, and gastronomically valuable species in Mexico, particularly in the Yucatán Peninsula, where it holds a designation of origin and is extensively cultivated for domestic consumption and export (Valladares *et al.*, 2004; Ahmad *et al.*, 2022). Its growing demand has driven the expansion of cultivation areas into different regions of the country, including arid and semi-arid zones such as the Mexicali Valley, Baja California, where extreme climatic conditions and limited water availability pose a constant challenge to agricultural production (Mukarram *et al.*, 2021; CONAGUA, 2025). In this context, water stress is recognized as one of the most limiting factors for the yield and quality of habanero pepper crops, as it affects various physiological, biochemical, and morphological processes in the plant (Farooq *et al.*, 2009; Gupta *et al.*, 2020).

Water deficit directly alters photosynthetic efficiency by modifying the content of essential pigments such as chlorophyll *a*, chlorophyll *b*, and carotenoids. These compounds not only participate in the capture of light energy but also play a role in photoprotection under stress conditions (Szabados and Saviouré, 2010; Ghosh *et al.*, 2022). The reduction in photosynthetic pigment concentration under water

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scarcity has been documented in several *Capsicum* species, affecting growth, yield, and ultimately fruit quality (Molla *et al.*, 2021). Thus, the evaluation of photosynthetic pigments has become a key tool for understanding plant physiological responses to water stress and for establishing management strategies in production systems.

Several methods have been employed to quantify chlorophyll content and to assess the physiological status of plants subjected to water stress. Conventional UV-Vis spectroscopy, applied through pigment extraction and measurements based on fresh weight, is a widely used method in plant physiology due to its accuracy, although it involves a destructive and labor-intensive process. Moreover, in water stress evaluations, variations in leaf water content can introduce biases in chlorophyll quantification, since differences in fresh or dry biomass may yield apparently higher pigment readings in stressed plants, even when their actual concentration is not greater (Rodríguez *et al.*, 2019). More recently, innovative approaches such as the use of response surface methodologies applied to absorbance data have enabled the development of more robust and reliable models for pigment estimation. Additionally, chlorophyll fluorescence-based tools, such as the GreenSeeker optical sensor, offer the possibility of obtaining optical indices related to photosynthetic activity and crop vigor, with relevant applications in precision agriculture (Mendoza *et al.*, 2024).

Although each of these methodologies presents advantages and limitations, there is little comparative information available to determine which is the most suitable for evaluating the physiological responses of *Capsicum chinense* under water deficit conditions. Contrasting optical methods is essential not only to validate the applicability of emerging technologies in greenhouse conditions, but also to generate standardized protocols that facilitate decision-making in irrigation management (Mukarram *et al.*, 2021). The integration of portable, spectrophotometric, and remote sensing measurements may represent an effective alternative to understand crop adaptation mechanisms to stress, and to contribute to the development of mitigation strategies under water scarcity scenarios (Gupta *et al.*, 2020).

In this context, the objective of the present study was to evaluate different approaches for photosynthetic pigment quantification based on biomass and leaf surface area, and to validate their applicability using selected physiological and optical indicators in *C. chinense* subjected to prolonged water stress under arid conditions.

## MATERIAL AND METHODS

### Plant material and growing conditions

The experiment was conducted with *Capsicum chinense* Jacq. 'Megalodon' plants transplanted directly into soil, with a planting density ( $1.39 \text{ plants m}^{-2}$ ) of 0.4 m between plants and 1.8 m between rows, established in the Mexicali Valley under protected cultivation conditions. Plant material consisted of approximately nine-month-old plants at the beginning of the evaluation. Agronomic management prac-

tices included irrigation and fertilization every third day, with irrigation sessions lasting 2 h each (at a flow rate of  $1 \text{ L h}^{-1}$ ). Fertilization was based on the nutritional requirements per hectare for habanero pepper, consisting of 125-100-160 units of nitrogen, phosphorus, and potassium, respectively. The total fertilizer was distributed across four phenological stages: transplant stage lasting 20 d, vegetative growth stage lasting 30 d, flowering and fruiting stage lasting 30 d, and harvest stage lasting 60 d (Soria *et al.*, 2002). For the *Bemisia tabaci* (whitefly) control, Movento® ( $1 \text{ mL L}^{-1}$  of water) and Sivanto® ( $2.5 \text{ mL L}^{-1}$  of water) were applied. Weed control was carried out manually.

### Quantification of photosynthetic pigments

The quantification of photosynthetic pigments was carried out by adapting the methodologies reported by Inskeep and Bloom (1985), and Biehler *et al.* (2010). Two extraction methods were evaluated: in Method 1, 50 mg of fresh leaf tissue were chopped into small fragments, while in Method 2, the leaf area equivalent to 50 mg of fresh biomass was determined through preliminary tests using leaf discs of different diameters. It was found that an 11 mm diameter disc corresponded to an average biomass of  $12.5 \pm 1.5 \text{ mg}$ . Based on this value, it was established that four leaf discs represent approximately 50 mg of fresh biomass, the amount used for subsequent analyses (Figure 1). Samples were placed in Eppendorf tubes, and 1.5 mL of 80 % (v/v) acetone were added for extraction. Maceration was performed in darkness at  $5^\circ\text{C}$  for 24 h. Subsequently, solids were separated by centrifugation ( $10,000 \text{ rpm}$  for 5 min; alternatively,  $4,000 \text{ rpm}$  for 10 min), and the supernatant was used for spectrophotometric readings. Absorbance was recorded in triplicate using a UV-Vis spectrophotometer (model DR6000™ UV-Vis, Hach, Loveland, Colorado, USA) in quartz cuvettes with a 1 cm optical path, at wavelengths of 664 nm (chlorophyll a), 647 nm (chlorophyll b), and 450 nm (carotenoids). Pigment contents were calculated using the following equations:

$$\text{Chlorophyll a (Ca): } \text{Ca } (\mu\text{g/mL}) = 12.25 \times A_{664} - 2.79 \times A_{647} \quad (\text{Eq. 1})$$

$$\text{Chlorophyll b (Cb): } \text{Cb } (\mu\text{g/mL}) = 21.50 \times A_{647} - 5.10 \times A_{664} \quad (\text{Eq. 2})$$

$$\text{Chlorophyll total (Ct): } \text{Ct} = \text{Ca} + \text{Cb} \quad (\text{Eq. 3})$$

$$\text{Carotenoids (Cx+c): } \text{Cx+c } (\mu\text{g/mL}) = (1000 \times A_{450} - 1.82 \times \text{Ca} - 85.02 \times \text{Cb}) / 198 \quad (\text{Eq. 4})$$

### Pigment extraction time

The extraction time was recorded to compare the operational efficiency of both methods (fragments vs. discs). For this purpose, a stopwatch was activated at the moment of cutting or disc punching and stopped once the final volume of 80 % acetone was added to the Eppendorf tube, including the grinding/maceration steps required for each method. Times were expressed in seconds, and the processing order of the samples was documented to avoid bias due to temperature or air exposure.

In both cases, the procedure consisted of recording with a stopwatch the elapsed time (in seconds) from the moment of sample preparation (cutting or disc punching) to

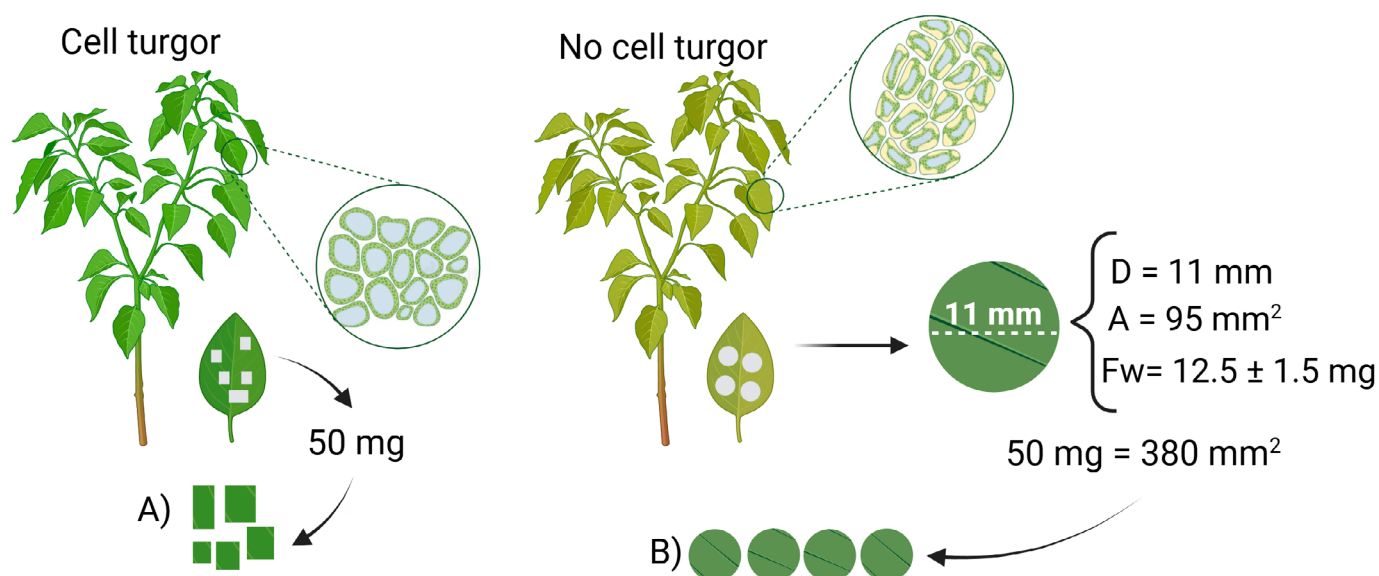


**Figure 1.** Quantification of photosynthetic pigments using two extraction methods. A) Method 1: leaf fragments; B) Method 2: 11-mm-diameter leaf discs (equivalent to 50 mg ± 4.5 mg).

**Figura 1.** Cuantificación de pigmentos fotosintéticos mediante dos métodos de extracción. A) Método 1: fragmentos de hojas; B) Método 2: discos foliares de 11 mm de diámetro (equivalentes a 50 mg ± 4.5 mg).

the grinding and maceration in Eppendorf tubes, including the addition of the final volume of 80 % acetone used as the extraction solvent. This allowed for a comparison of the time efficiency between both methods of leaf tissue processing.

Chlorophyll fluorescence measurements were performed to evaluate photosystem II activity using a portable fluorometer FluorPen FP 110<sup>®</sup> (Photon Systems Instruments, Czech Republic). Prior to measurements, leaves were dark-adapted for 15 min using specialized clips.

Measurements were taken at 12:00 h, with three readings per experimental unit, on fully expanded leaves corresponding to the third plagiotropic growth of the plants. Evaluations were conducted on the different sampling days of the experiment (0 and 21 d after the onset of water stress).

From the fluorescence induction kinetics (OJIP), the parameters of photochemical efficiency of photosystem II (ΦPSII) and non-photochemical quenching of energy dissipation (NPQ) were determined and used to analyze the functional status and photoprotective mechanisms of the photosynthetic apparatus under water stress conditions.

### Relative Water Content (RWC)

RWC was determined through leaf sampling carried out on four evaluation dates (weekly, up to day 28), all conducted at 12:00 h in order to reduce variability due to diurnal changes in water status. On each time, the third plagiotropic growth was used as reference, ensuring uniformity in the position and physiological condition of the leaf tissue. Collected leaves were immediately weighed to obtain fresh biomass and subsequently dried in an oven at 40 °C per 72 h (constant weight). Based on this information, RWC was calculated using Equation (5) proposed by Jothimani and Arulbalachandran (2020):

$$\text{RWC (\%)} = \frac{\text{Fresh leaf mass} - \text{dry leaf mass}}{\text{sat leaf mass} - \text{dry leaf mass}} \times 100 \quad (\text{Eq. 5})$$

### NDVI Readings

Canopy vigor and condition were quantified using a portable optical sensor GreenSeeker™ (Trimble Inc., USA), which estimates the Normalized Difference Vegetation Index (NDVI) based on reflectance in the red and near-infrared bands. Measurements were taken in triplicate at the middle section of the plants, maintaining a uniform distance between the sensor and the foliage, as well as homogeneous lighting conditions to minimize variability. This methodology allows for obtaining non-destructive and comparable data on crop growth and productivity and has proven effective in different agricultural systems (Singh and Singh, 2022).

### Experimental design and statistical analysis

The study was established under a completely randomized design with two irrigation treatments. The first treatment corresponded to the non-stress condition, in which irrigation was applied three times per week by wetting the planting beds using drip irrigation tape with emitters spaced at 20 cm intervals and a flow rate of 1 L per hour per emitter. Irrigation events were applied every other day for 2 h, resulting in an approximate water supply of 10 L per day per linear meter of bed. The second treatment corresponded to the water stress condition, in which plants were irrigated only at the beginning of the experiment and subsequently maintained under water deficit until day 28.

Each treatment included four replicas, consisting of individual plants. Evaluations were carried out in four weekly samplings starting from the onset of flowering in autumn, with the last sampling corresponding to day 28. At each sampling, leaves were collected at 12:00 h, using the third

plagiotropic growth as reference, and simultaneously RWC, photosynthetic pigments, and NDVI were quantified.

The statistical analysis considered irrigation treatments and sampling times as factors. To identify differences among weeks, a one-way ANOVA followed by Tukey's test ( $p < 0.05$ ) was applied, while Student's *t*-test was used to compare treatments at each date. Data processing was performed using Statgraphics Centurion XVI software (Statgraphics Technologies, 2009).

## RESULTS AND DISCUSSION

Figure 2 shows the behavior of photosynthetic pigments in *C. chinense* under water stress conditions, comparing the two extraction methods evaluated. For chlorophyll a content, no statistically significant differences were detected between methods during the first three weeks (Figure 2A and 2B). However, water stress caused a reduction of 30 % with Method 1 and 26 % with Method 2 by day 21 (Figure 2C). By day 28 (Figure 2D), stressed plants showed a significant decrease of 34 % in Method 1 and 14% in Method 2 compared to non-stressed plants, with no differences between methods.

For chlorophyll b, a higher content was observed in stressed plants at day 7 when Method 1 was used. Subsequently,

between days 14 and 21 (Figure 2 A and B), values remained similar across methods and treatments, with a general trend toward decreasing in stressed plants. By day 28 (Figure 2 D), non-stressed plants showed 39 % more chlorophyll b content with Method 1, whereas in Method 2 the difference compared to stressed plants was 10 % and not significant.

Regarding total chlorophyll, no significant differences were recorded between methods in most samplings. Only at day 21 (Figure 2 C) an increase was observed in non-stressed plants to 30 % with Method 1 and 27 % with Method 2 compared to stressed plants. By day 28, the trend of higher total chlorophyll content in non-stressed plants continued, particularly with Method 1, although no statistical differences were detected between methods.

For carotenoid content, no significant differences were detected either between extraction methods or irrigation conditions throughout the entire evaluation period. It is worth noting that Method 2 showed lower dispersion and closer means between stressed and non-stressed treatments, indicating greater homogeneity in pigment quantification. Overall, up to day 14, plants did not show significant changes in any of the evaluated pigments, evidencing that the photosynthetic machinery remained stable during the first two weeks of water deficit.

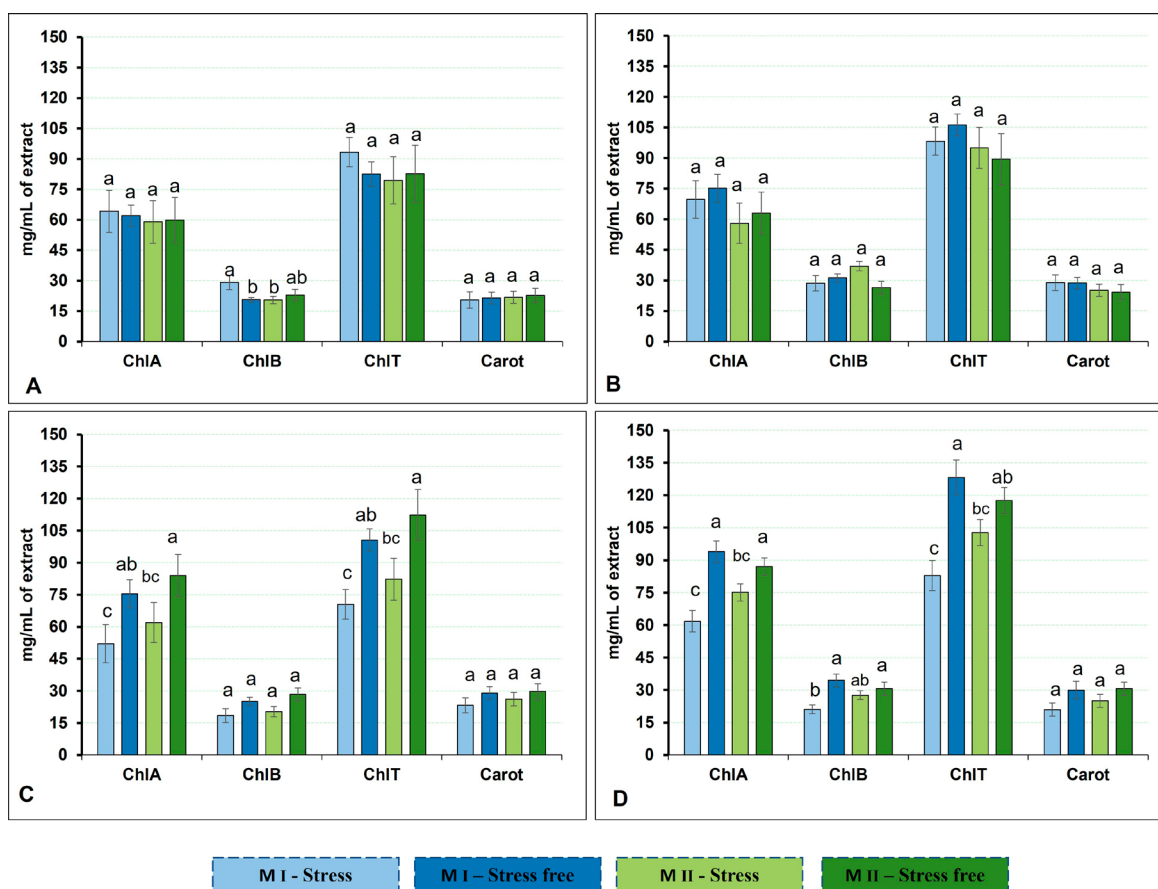


**Figure 2.** Dynamics of photosynthetic pigment content in habanero pepper plants under water deficit during flower-to-fruit development. A: 7 days; B: 14 days; C: 21 days; D: 28 days. M I: Method 1; M II: Method 2.

**Figura 2.** Dinámica del contenido de pigmentos fotosintéticos en plantas de chile habanero bajo déficit hídrico durante el desarrollo de flor a fruto. A: 7 días; B: 14 días; C: 21 días; D: 28 días. M I: Método 1; M II: Método 2.

Water stress causes adverse effects in plants, affecting the synthesis and stability of photosynthetic pigments, mainly reducing chlorophyll a, as observed in *C. chinense* from day 21 onward. This decrease, more pronounced than that of chlorophyll b, is consistent with reports in other solanaceous species, where photoinhibition and damage to photosystem II accelerate chlorophyll a degradation (Peralta *et al.*, 2021; Campos *et al.*, 2024).

The stability of pigments up to day 14 suggests initial tolerance to water deficit, probably due to osmotic adjustments and solute accumulation (Florido and Bao, 2014). Carotenoids remained constant, reinforcing their photoprotective role against the generation of reactive oxygen species (Amari and Abdelly, 2021).

Table 1 summarizes the values of photosystem II photochemical efficiency ( $\Phi$ PSII) and non-photochemical energy dissipation (NPQ) in *C. chinense* plants under water stress and non-stress conditions. No statistically significant differences were observed for  $\Phi$ PSII between treatments or among evaluation days, with similar values maintained in both stressed and non-stressed plants. Likewise, NPQ values did not show statistically significant differences between treatments in most cases. However, on day 21, a statistically significant difference was detected, in which water-stressed plants exhibited lower NPQ values compared to non-stressed plants, indicating a transient modulation of photoprotective responses under water deficit conditions.

The stability observed in  $\Phi$ PSII suggests that photosystem II in *C. chinense* maintained its functionality under the evaluated water stress conditions, reflecting the preservation of electron transport efficiency even under water deficit (Banks, 2018). This behavior has been reported in drought-tolerant species, where  $\Phi$ PSII stability is associated with physiological adjustment mechanisms rather than photochemical damage (Sperdouli and Moustakas, 2012; Hu *et al.*, 2023).

The general absence of significant differences in NPQ indicates that thermal energy dissipation remained regulated throughout the stress period. Although the reduction observed on day 21 was not sustained over time, it suggests

**Table 1.** Photosystem II photochemical efficiency ( $\Phi$ PSII) and non-photochemical energy dissipation (NPQ) in *Capsicum chinense* plants under water stress and non-stress conditions.

**Tabla 1.** Eficiencia fotoquímica del fotosistema II ( $\Phi$ PSII) y disipación de energía no fotoquímica (NPQ) en plantas de *Capsicum chinense* en condiciones de estrés hídrico y sin estrés.

| Treatment | Fluorescence      |                   |                   |                   |
|-----------|-------------------|-------------------|-------------------|-------------------|
|           | $\Phi$ PSII       |                   | NPQ               |                   |
|           | Stress            | Stress-free       | Stress            | Stress-free       |
| Day 0     | 0.74<br>± 0.02 aA | 0.78<br>± 0.03 aA | 1.76<br>± 0.02 aA | 1.64<br>± 0.18 aA |
| Day 21    | 0.68<br>± 0.08 aA | 0.62<br>± 0.10 aA | 1.47<br>± 0.01 aB | 1.53<br>± 0.11 aA |
| HSD       | 0.14              | 0.17              | 0.04              | 0.34              |

Means ± standard deviation. Lowercase letters indicate a statistical difference between treatments (Test t,  $p < 0.05$ ). HSD: Honestly Significant Difference.

a short-term adjustment in photoprotective mechanisms, possibly related to changes in energy demand or in the utilization of absorbed energy (Fracheboud and Leipner, 2003; Zhuang *et al.*, 2020).

Table 2 shows the differences in extraction time between the two evaluated methods. Method 2 presented a processing time per sample approximately 80 % shorter than Method 1, a statistically significant difference. This reduction implies lower acetone volatilization during the procedure and, consequently, less chlorophyll degradation as the number of cuts increases, in addition to representing a substantial saving in the total sample processing time.

These methodological results are relevant because chlorophyll preservation directly influences the accuracy of vegetation indices. In this regard, Table 3 shows that, starting from day 14, the NDVI index exhibits a decline that continues until day 28. The temporal dynamics indicate that non-stressed plants progressively increased this index as the days advanced, whereas water-stressed plants displayed the opposite trend. The consistency observed between photosynthetic pigment dynamics and NDVI behavior supports the use of non-destructive optical indices as complementary tools to validate spectrophotometric pigment quantification methods under water stress conditions.

The progressive decrease of the NDVI index in water-stressed plants from day 14 onwards, indicates a reduction in photosynthetic activity and chlorophyll content, consistent with the trend observed in pigments (Florido and Bao, 2014). In contrast, the sustained increase in well-irrigated plants reflects normal leaf development and greater efficiency in light use (Santamaría *et al.*, 2023). This pattern suggests that the NDVI index is a sensitive indicator for detecting early al-

**Table 2.** Average pigment extraction time in *Capsicum chinense* under two quantification methods.

**Tabla 2.** Tiempo promedio de extracción de pigmentos en *Capsicum chinense* bajo dos métodos de cuantificación.

| Treatment       | Extraction time (s) |
|-----------------|---------------------|
| M1: Leaf pieces | 202.4 ± 9.37 a      |
| M2: Leaf discs  | 117.0 ± 5.74 b      |
| HSD             | 11.33               |

Means ± standard deviation. Lowercase letters indicate statistical difference between treatments (Test t,  $p < 0.05$ ). HSD: Honestly Significant Difference.

**Table 3.** Dynamics of the NDVI optical index measured with GreenSeeker in *Capsicum chinense* plants under water deficit and irrigation conditions.

**Tabla 3.** Dinámica del índice óptico NDVI medido con GreenSeeker en plantas de *Capsicum chinense* bajo déficit hídrico y condiciones de riego.

| Day | Treatments      |                 |      |
|-----|-----------------|-----------------|------|
|     | Stress          | Stress-free     | HSD  |
| 7   | 0.72 ± 0.03 aA  | 0.73 ± 0.01 aB  | 0.02 |
| 14  | 0.69 ± 0.04 bAB | 0.73 ± 0.02 aB  | 0.03 |
| 21  | 0.65 ± 0.05 bBC | 0.74 ± 0.01 aAB | 0.04 |
| 28  | 0.63 ± 0.02 bD  | 0.76 ± 0.02 aA  | 0.02 |
| HSD | 0.05            | 0.02            |      |

Lowercase letters indicate statistical difference between treatments (t-test,  $p < 0.05$ ). Capital letters indicate statistical difference between days (Tukey,  $p < 0.05$ ). HSD: Honestly Significant Difference.

terations in water status and in the photosynthetic capacity of the crop (Mendoza-Pérez *et al.*, 2024).

Regarding relative water content (RWC), plants maintained stable values during the first 21 d, with no significant differences between treatments. It was not until day 28 that stressed plants showed a decrease in this parameter (Table 4). Significant differences between treatments began to be detected on day 21, with well-watered plants maintaining an RWC similar to that recorded in the initial measurements of the trial. Notably, under the edaphic conditions of the Mexicali Valley (characterized by clay soils, high pH, and elevated salinity), *C. chinense* plants maintained comparable RWC values in both stressed and control plants. Under these saline conditions, stressed plants also showed modifications in leaf size and an increase in solute accumulation, which was reflected in greater leaf weight.

In this study, RWC was incorporated not as a detailed hydraulic descriptor, but as a supporting physiological indicator aimed at contextualizing pigment responses and minimizing potential biases associated with tissue water content during pigment quantification. This approach allowed a more accurate interpretation of pigment dynamics under water deficit conditions.

The maintenance of relative water content (RWC) during the first 21 d in both treatments indicates an osmotic adjustment capacity in *C. chinense*, which allowed the water status to be sustained despite the deficit (Rodríguez Ledesma *et al.*, 2019). The decrease observed on day 28 in stressed plants reflects the limitation of this mechanism as water deficit is prolonged, consistent with reports in other solanaceous species (Peralta *et al.*, 2021; Campos *et al.*, 2024). The edaphic conditions of the Mexicali Valley, likely favored additional adaptive responses such as leaf size reduction and accumulation of compatible solutes, which contribute both to water retention and to increased leaf weight (Nieto-Garibay *et al.*, 2009). These results suggest that the evaluated *C. chinense* variety exhibits a physiological capacity to withstand water deficit for up to 28 d without irrigation, under the saline and high-pH conditions characteristic of the Mexicali Valley. The initial stability of RWC, together with the reduction in leaf size and the accumulation of compatible solutes, reinforces the hypothesis that osmotic adjustment mecha-

nisms buffer the effects of water stress during early stages. In scenarios of agriculture in arid environments, this interval may be considered a reasonable margin of resistance before the physiological vigor of the crop is compromised. Future studies should focus on determining the permanent wilting point and evaluating the impact of controlled reductions in irrigation on fruit yield and quality, in order to establish more efficient water management strategies.

## CONCLUSIONS

Prolonged water stress in *Capsicum chinense* caused a significant reduction in photosynthetic pigment content and in the NDVI index, indicating a progressive decline in photosynthetic activity. However, relative water content (RWC) remained stable throughout the first three weeks, suggesting the presence of osmotic adjustment and water retention mechanisms that help maintain plant physiological status under moderate deficit conditions. Chlorophyll fluorescence parameters ( $\Phi$ PSII and NPQ) remained largely stable throughout the evaluation period, indicating the preservation of photosystem II functionality and supporting their use as complementary physiological indicators for interpreting plant responses to water stress. The edaphic conditions of the Mexicali Valley, promoted additional morphophysiological responses, such as reduced leaf size and the accumulation of compatible solutes. From a methodological perspective, although both pigment extraction methods yielded comparable results, the method based on leaf discs of known area stood out due to its greater operational efficiency and lower data dispersion, making it a suitable alternative for chlorophyll and carotenoid studies. Overall, these findings highlight the value of integrating destructive and non-destructive techniques to achieve a more accurate physiological assessment of *C. chinense* under water stress conditions in arid environments.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest

## REFERENCES

- Ahmad, A., Aslam, Z., Javed, T., Hussain, S., Raza, A., Shabbir, R. and Tauseef, M. 2022. Screening of wheat (*Triticum aestivum* L.) genotypes for drought tolerance through agronomic and physiological response. *Agronomy*. 12(2): 287.
- Amari, T. and Abdelly, C. 2021. Responses of *Digitaria commutata* and *Cenchrus ciliaris* to water stress: antioxidative reactions, proline and soluble sugars. *BIOAGRO*. 33(3): 171–181.
- Banks, J.M. 2018. Chlorophyll fluorescence as a tool to identify drought stress in *Acer* genotypes. *Environmental and Experimental Botany*. 155: 118–127.
- Campos, H., Mancera, A.V., Trejo, C., Quintana, F.U., García, T.C., García-Díaz, S.E., Hernández-García, F.A., Cruz-Aviña, J.R., Pérez-Mendoza, N. and Parada, J.R.B. 2024. Gas exchange and its relationship with chlorophyll fluorescence in bell pepper (*Capsicum annuum* L.) plants under water stress. *Acta Agrícola y Pecuaria*. 10(1): 1.

**Table 4.** Relative water content in *Capsicum chinense* plants under water-stressed and non-stressed conditions.

**Tabla 4.** Contenido relativo de agua en plantas de *Capsicum chinense* bajo condiciones de estrés hídrico y sin estrés.

| Day        | Treatments       |                  | HSD  |
|------------|------------------|------------------|------|
|            | Stress           | Stress-free      |      |
| 7          | 78.03 ± 3.80 aA  | 78.31 ± 3.15 aA  | 7.91 |
| 14         | 75.39 ± 1.66 aAB | 76.26 ± 2.65 aAB | 5.02 |
| 21         | 70.7 ± 1.85 aBC  | 69.65 ± 2.40 aB  | 4.86 |
| 28         | 67.59 ± 2.49 bC  | 72.29 ± 1.24 aB  | 4.46 |
| <b>HSD</b> | 7.29             | 5.85             |      |

Lowercase letters indicate statistical difference between treatments (t-test,  $p < 0.05$ ). Capital letters indicate statistical difference between days (Tukey,  $p < 0.05$ ). HSD: Significant Honest Difference.

- CONAGUA. 2025. Drought monitor in Mexico. National Meteorological Service. Retrieved from <https://smn.conagua.gob.mx/es/climatologia/monitor-de-sequia/monitor-de-sequia-en-mexico>
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M. 2009. Plant drought stress: effects, mechanisms and management. In: Lichtfouse, E., Navarrete, M., Debaeke, P., Véronique, S. and Alberola, C. (eds.), *Sustainable Agriculture*. Springer, Dordrecht. pp. 153–188.
- Florido Bacallao, M. and Bao Fundora, L. 2014. Tolerance to water deficit stress in tomatoes (*Solanum lycopersicum* L.). *Cultivos tropicales*. 35(3): 70–88.
- Fracheboud, Y. and Leipner, J. 2003. The application of chlorophyll fluorescence to study light, temperature, and drought stress. In *Practical applications of chlorophyll fluorescence in plant biology* (pp. 125–150). Boston, MA: Springer US.
- Ghosh, U.K., Islam, M.N., Siddiqui, M.N., Cao, X. and Khan, M.A.R. 2022. Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. *Plant Biology*. 24(2): 227–239.
- Gupta, A., Rico-Medina, A. and Caño-Delgado, A.I. 2020. The physiology of plant responses to drought. *Science*. 368(6488): 266–269.
- Hu, C., Elias, E., Nawrocki, W.J. and Croce, R. 2023. La sequía afecta a ambos fotosistemas en *Arabidopsis thaliana*. *New Phytologist*. 240(2): 663–675.
- Mendoza-Pérez, C., Valle-Gough, R.E., Samaniego-Gámez, B.Y., del Rosario Ruelas-Islas, J. and Núñez-Ramírez, F. 2024. Relationship Between NDVI Readings and Growth in Tomatillo, Cabbage, and Habanero Peppers. *Invurnus*. 19(1).
- Molla, M.R., Rohman, M.M., Monsur, M.B., Hasanuzzaman, M. and Hassan, L. 2021. Screening and assessment of selected chilli (*Capsicum annuum* L.) genotypes for drought tolerance at seedling stage. *Phyton*. 90(5): 1425–1439.
- Mukarram, M., Choudhary, S., Kurjak, D., Petek, A. and Khan, M.M.A. 2021. Drought: Sensing, signalling, effects and tolerance in higher plants. *Physiologia Plantarum*. 172(2): 1291–1300.
- Nieto-Garibay, A., Troyo-Diéguez, E., García-Hernández, J.L., Murillo-Amador, B., Ruiz-Espinoza, F.H., and Pimentabarri, E. 2009. Effect of edaphic water stress on seedling emergence and development on the species of chili pepper *Capsicum frutescens* L. And *Capsicum annuum* L. *Tropical and Subtropical Agroecosystems*. 10(3): 405–413.
- Peralta, R.M., Peralta, M.D.L.Á.M., García, A.R.R. and Vargas, D.S. 2021. Effect of water stress on native Mexican tomato populations. *Ecosystems and Agricultural Resources*. 8(2): 31.
- Rodríguez Ledesma, N.D., Torres Sevillano, C.N., Chaman Medina, M. E. and Hidalgo Rodríguez, J.E.M. 2019. Effect of salt stress on growth and relative water content in the IR-43 and Amazonian varieties of *Oryza sativa* “rice” (*Poaceae*). *Arnaldoa*. 26(3): 931–942.
- Santamaría, M.I.R., Cabrera, D.T., Santiago, A.I.L., Trejo, J.P., García, M.S. and Navarrete, A. . 2023. Impact of climate change on photosynthesis, photorespiration and respiration of C3 plants. *ICAP Agricultural Sciences Bulletin*: 9: 6–11.
- Seleiman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y. and Battaglia, M.L. 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants*. 10(2): 259.
- Sousaraei, N., Mashayekhi, K. and Mousavizadeh, S.J. 2021. Screening of local tomato varieties for drought tolerance based on growth analysis and chlorophyll fluorescence. *Horticulture, Environment, and Biotechnology*. 62: 521–535. <https://doi.org/10.1007/s13580-020-00328-5>
- Sperdoui, I. and Moustakas, M. 2012. Differential response of photosystem II photochemistry in young and mature leaves of *Arabidopsis thaliana* to the onset of drought stress. *Acta Physiologiae Plantarum*, 34(4): 1267–1276.
- Statgraphics Technologies. 2009. *Statgraphics Centurion XVI User’s Manual*. The Plains, VA, USA: Statgraphics Inc.
- Szabados, L. and Saviouré, A. 2010. Proline: a multifunctional amino acid. *Trends in Plant Science*. 15(2): 89–97.
- Valladares, F., Vilagrosa, A., Peñuelas, J., Ogaya, R., Camarero, J.J., Corcuera, L. and Gil-Pelegrín, E. 2004. Water stress: ecophysiology and drought scales. In: Valladares, F. (ed.), *Ecology of the Mediterranean Forest in a Changing World*. Ministry of Environment, EGRAF S.A., Madrid. pp. 163–190.
- Zhuang, J., Wang, Y., Chi, Y., Zhou, L., Chen, J., Zhou, W. and Ding, J. 2020. Drought stress strengthens the link between chlorophyll fluorescence parameters and photosynthetic traits. *PeerJ*. 8: 10046.