PHYSIOLOGY OF PROLONGED WATERLOGGING STRESS IN SEEDLINGS FROM WILD *Carica papaya* L. PLANTS COLLECTED AT YUCATAN, MEXICO †

[FISIOLOGÍA DEL ESTRÉS POR ANEGAMIENTO PROLONGADO EN PLÁNTULAS DE *Carica papaya* L. SILVESTRES COLECTADAS EN YUCATÁN, MÉXICO]

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SUMMARY

Background: Papaya (*C. papaya* L.) is one of the most economically significant fruit crops in Mexico, as it is the leading global exporter. Although Yucatán is not among the country's top producing states, it holds great importance as the center of origin and distribution for this crop. This explains the current presence of wild papaya populations in unperturbed remote sites within the Yucatan State, which can serve as a genetic source for genetic improvement of current cultivated varieties, as they exhibit a higher level of tolerance to stress factors such as waterlogging caused by climate change. Therefore, it is essential to characterize the physiological response of these wild genotypes to abiotic stress factors. **Objective:** To characterize the physiological response to prolonged waterlogging stress of wild *C. papaya* genotypes. **Methodology**: A system was designed to induce waterlogging in three-months-old *C. papaya* plants. Plantlets were subjected to waterlogging (100% of roots submerged) for seven days. During the waterlogging stress, the following variables were measured every 24 hours: PSII efficiency (Fv/Fm), photosynthesis, transpiration, stomatal conductance, leaf loss, and petiole binding angle. Data collected from each parameter were analyzed using student t test analysis. **Results:** Seedlings from wild papaya populations exhibited a rapid response to waterlogging stress of some photosynthetic parameters, and with prolonged exposure, the plants eventually lose several leaves but papaya wild seedlings survive after 7 days of waterlogging (pw). Transpiration and stomatal conductance variables dropped after 24 hours pw and remained low throughout the experiment. Leaf loss began to be noticeable at around 72 hours pw. Additionally, leaf inclination angle exhibited variations starting from 24 hours pw, probably related to epinasty caused by ethylene accumulation. However, Fv/Fm remained high even after 7 d of being waterlogged. **Implications:** The results suggest that waterlogging stress has an impact on the physiology of wild papaya plants, however, this rapid response of closing stomata and maintaining photosynthesis could be a mechanism to tolerate this stress. The fact that wild papaya plants were able to maintain high values of Fv/Fm even after 7d of waterlogging, might be associated to waterlogging tolerance, as there are reports that in commercial papaya cultivars, this parameter rapidly decline during the first 24h to values much lower than those found here for wild papaya plants. **Conclusions:** It is necessary to perform further comparative studies between wild papaya genotypes vs commercial papaya cultivars and assess the performance of wild papaya plantlets during recovery periods once the stress is released.

Key words: abiotic stress; climate change; physiological damage; waterlogging; wild papaya.

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RESUMEN

Antecedentes: La papaya (*C. papaya* L.) es uno de los frutales de mayor importancia económica para México, debido a que es el principal exportador a nivel mundial. Aunque Yucatán no figura entre los principales estados productores del país, tiene una gran importancia debido a que es parte del centro de origen y distribución del cultivo. Lo anterior explica la presencia actual de poblaciones silvestres de papaya en lugares remotos y poco perturbados del Estado, las cuales pueden ser fuente de mejoramiento genético para variedades cultivadas, ya que presentan un mayor nivel de tolerancia a factores de estrés causado por el cambio climático. Por tanto, es esencial caracterizar la respuesta fisiológica de estos genotipos silvestres a factores del cambio climático como el anegamiento. **Objetivo:** Caracterizar la respuesta fisiológica de plantas de genotipo Silvestre de *Carica papaya* ante el estrés por anegamiento prolongado. **Metodología:** Se diseñó un sistema para inducir el anegamiento en plantas de *C. papaya* de tres meses de edad. Se sometieron a anegamiento (100 % de raíces sumergidas) durante siete días. Cada 24 h posteriores al anegamiento (paa) se tomaron las siguientes variables: eficiencia del PSII (Fv/Fm), fotosíntesis, transpiración, conductancia estomática, pérdida de hojas y ángulo de inclinación de los pecíolos. Los datos obtenidos de cada día se analizaron mediante prueba t de Student. **Resultados:** El anegamiento prolongado generó una disminución de fotosíntesis, a las 24 h paa, sin embargo, su caída a valores cercanos a 0 se dió hasta las 120 h paa. Las variables de transpiración y conductancia estomática cayeron a las 24 h paa y se mantuvieron así durante todo el experimento. La pérdida de hojas comienza a observarse a partir de las 72 h paa. Por otro lado, los peciolos mostraron una variación en el ángulo de inclinación desde las 24 h paa, tentativamente debido a epinastia generado por acumulación de etileno. Sin embargo, Fv/Fm presentó solo un ligero descenso en relación a las plantas control. **Implicaciones:** Los resultados sugieren que el estrés por anegamiento genera un cambio en la fisiología de plantas silvestres de papaya, sin embargo, la rápida respuesta de cerrar estomas y mantener la eficiencia fotosintética (Fv/Fm) podrían ser un mecanismo para tratar de tolerar dicho estrés. El hecho de que las plantas silvestres de papaya mostraran altos niveles de Fv/Fm aun después de 7 días de anegamiento pudiera sugerir que estos genotipos pueden ser más tolerantes a este estrés, particularmente cuando existen reportes de que Fv/Fm decae drásticamente en genotipos de papaya comercial. **Conclusiones:** Las plántulas derivadas de poblaciones silvestres de papaya tiene una respuesta rápida al estrés por anegamiento, sin embargo es posible que su capacidad para mantener altos valores de Fv/FM aun después de 7 d de inundación, le permitan tener una adecuada recuperación posterior. Por supuesto que se requieren más estudios del comportamiento de estas plantas de papaya silvestres en comparación con cultivares comerciales durante periodos de estrés por anegamiento y durante periodos de recuperación, toda vez que hayan sido removidas del anegamiento.

Palabras clave: Estrés abiótico; cambio climático; daño fisiológico; anegamiento; papaya silvestre.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important fruit crops in Mexico, as the country is a leading global exporter. While the state of Yucatán is not among the main producers of this tropical crop, contributing only around 1% of the national production (SIAP, 2023), its significance lies in being part of the center of origin and distribution of this species (Chávez-Pesqueira and Nuñez-Farfán, 2017). Therefore, various wild populations of *C. papaya* have been identified in non-perturbed remote sites of Yucatán (Fuentes and Santamaría, 2013). This source of genetic reservoir is highly valuable when considering the improvement of cultivated varieties, whether through traditional breeding or biotechnology (Dhekney *et al*., 2016). For instance, previous studies have demonstrated that wild papaya populations identified in Yucatán are an important source of genetic material for drought tolerance (Estrella-Maldonado *et al*., 2021; Girón-Ramírez *et al*., 2021).

However, drought is not the only environmental challenge facing papaya production in the country and the state of Yucatán. Recent reports suggest that global climate change is causing atypical extreme rainfall in the state (Bautista-Zuñiga and Aguilar-Duarte, 2021), posing the risk of waterlogging in production areas. There are already reports suggesting that these changes in precipitation patterns could affect yields in other crops in the Yucatán Peninsula (Mardero *et al*., 2018).

Previous reports have demonstrated that the Maradol papaya cultivar is highly susceptible to flooding, experiencing irreparable damage to the plants after only 24 hours of waterlogging (Thani *et al*., 2016). However, it has been observed in other species that genotypes exhibiting drought tolerance may also show tolerance to flooding (Chen *et al*., 2023).

Therefore, the aim of the present study was to characterize the physiological response to prolonged waterlogging stress of seedlings derived from wild papayas collected at Yucatán, México.

MATERIALS AND METHODS

Plant material

Seeds were obtained from fruits collected from a wild papaya population located on the Federal Highway Cancun-Merida, kilometer 75 (20.9° N, 89.2° W). Fruits were collected in July 2022. Subsequently, the fruits were placed in dark chambers with absorbent paper to favor ripening, for a period of 5 to 8 days, depending on the individual needs of each fruit. Furthermore, measurements of the equatorial and longitudinal diameter were taken using a digital caliper, and the weight of both the fruit and seeds was measured using an analytical balance. The seeds underwent a pre-germination treatment, which was achieved with a lab´s protocol. Once germinated, the seeds were placed in a germination tray with sterile substrate until the seedlings reached 60 days of age. After reaching this stage, they were transplanted into black polyethylene bags filled with sterile substrate (peat moss + agrolite: 1:1).

Waterlogging treatments

60-day-old plants were placed in black bags and left under the waterlogging treatment, bags containing the plants were placed inside larger 10 L plastic pots without drainage holes, allowing water to completely inundate the root system. These pots were filled with 6 L of purified water, ensuring that the entire root system (100%) was submerged. The treatments consisted in subjecting the papaya plants to 1, 2, 3, 4, 5, 6 and 7 days under waterlogging conditions. Physiological parameters were measured each day.

Evaluation of morphological variables

A photographic record was taken every 24 hours to visualize any morphological changes caused by waterlogging. Additionally, leaf loss and petioles binding were monitored. The number of leaves remaining after subjecting plants to waterlogging stress were counted daily during the 7d waterlogging period. Similarly, the angle formed between the petioles and the main stem was measured using an angle protractor daily during the 7d waterlogging period.

Evaluation of physiological variables

Measurements were taken for the following photosynthetic variables (Pn, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), and transpiration (E, mmol H_2O m^{-2} s^{-1}). These measurements were conducted on the third fully expanded leaf of each plant using a portable infrared gas analyzer (IRGA) from Li-COR, model LI6400 (LiCor Inc., Lincoln, Nebraska, USA), using a 6 cm^2 chamber. Before measurements, the following settings were established: 0.1 as stomatal ratio between the adaxial and abaxial sides, photon flux density of 1000 μmol s^{-1} and an airflow of 400 μmol s^{-1} at 25 °C. Once the parameters were stabilized, one leaf per plant per treatment was placed in the center of the chamber, and 5 measurements of each parameter were taken with a time difference between them of 1 to 3 s. Additionally, the photosynthetic efficiency was determined using the variable chlorophyll fluorescence ratio (Fv/Fm) of papaya leaves. For this purpose, a chlorophyll fluorometer of the Multi-function Plant Efficiency Analyzer type (MPEA) (FMS 2 Hansatech Instruments Ltd, Norfolk, UK) with its respective optical sensor was used. Before placing the equipment, the area of the leaf where the sample was taken was left in darkness for 20 minutes, using a leaf clip. After that time, the optical sensor emitted a pulse of saturating red light (650 nm, 3000 μmol m^{-2} s^{-1}) for 1 s, and the chlorophyll fluorescence reading was taken.

Experimental design

A completely randomized experimental design with two treatments (waterlogging and control) and three replicates was employed. Measurements were taken at 0, 1, 2, 3, 4, 5, 6, and 7 d following the waterlogging. The data were analyzed using a two-tailed Student's t-test ($p < 0.05$) performed with R statistical software version 2.12.2. SigmaPlot 11 software was used to generate the graphs.

RESULTS AND DISCUSSION

Effect of waterlogging on plant Morphology

Wild papaya plants exhibited progressive morphological changes over the seven days of waterlogging. During the initial three d of waterlogging, no significant signs of turgor loss or changes in petiole angles were observed (Fig. 1A-D). However, starting from the fourth day, the morphological changes became more evident (Fig. 1E). After 6 days under waterlogging treatment, petiole binding and leaf loss became more evident and after 7 days of waterlogging, only the upper leaves remained attached to the plant. This response differs from cultivated papaya genotypes, as there are reports that cultivated papayas begin to show severe structural damage after only 24 h of being waterlogged (Rodriguez *et al*., 2014).

Leaf loss data indicates that defoliation begins after 3d of waterlogging, remaining relatively stable until the fifth day, however, wild papaya plants still maintain two leaves even after being waterlogged for 7 days (Fig. 1 and Fig. 2A). These leaves might be the support to generate more leaves during a recovery period.

In terms of petiole binding (epinasty), plantlets showed variation in petiole angles after 24 hours of being waterlogged (Fig. 2B, C). The lower leaves (leaves A, B and C in Fig. 2D) showed decreased angles over the waterlogging treatment, only after seven days under this treatment. However, in upper leaves (D and E leaves), the angle increased as waterlogging stress progressed (Fig. 2C). This variation in petiole angle has been associated with a rapid accumulation of intracellular ethylene (Iqbal *et al*., 2017), which, in turn, could be linked to a plant response to hypoxia stress caused by waterlogging (Khan *et al*., 2020).

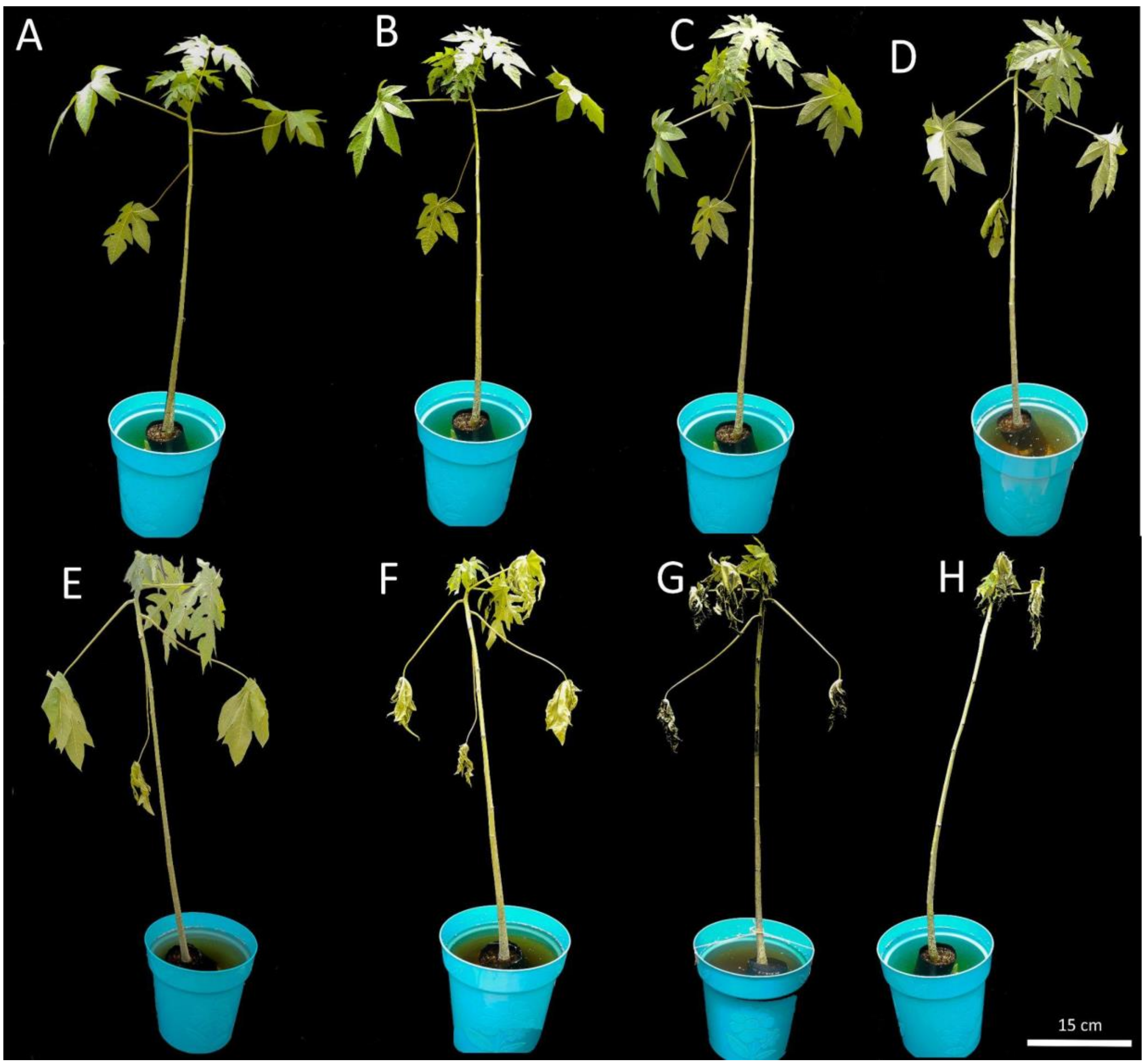

Figure 1. Temporal progression of morphological changes in wild papaya plants due to prolonged waterlogging: 0 days A), 1 day B), 2 days C), 3 days D), 4 days E), 5 days F), 6 days G) and 7 days H) under waterlogging treatment.

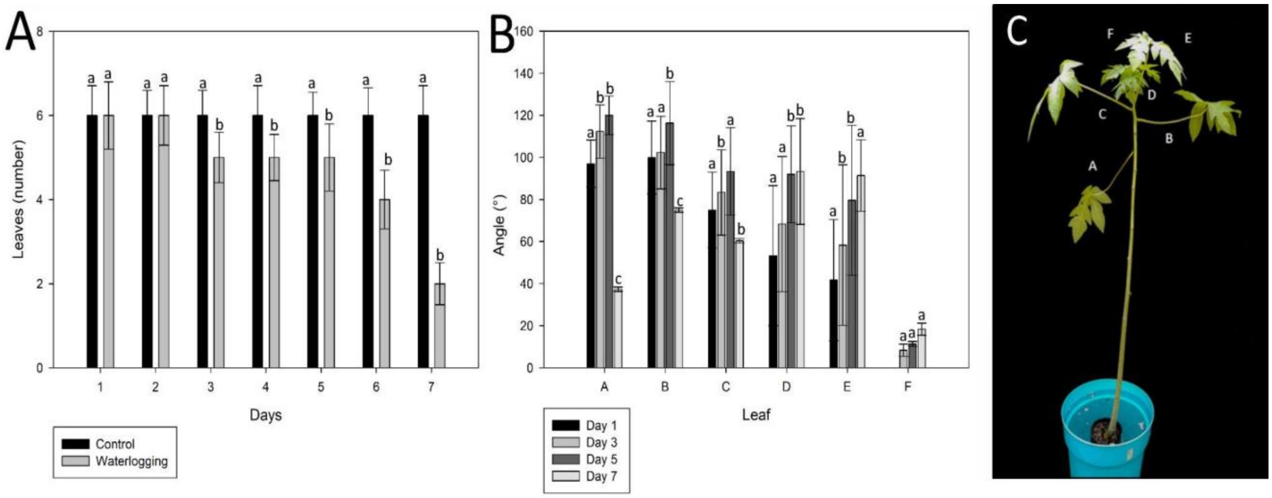

Figure 2. Number of leaves that remained on the plant A) and changes in the internal angle (of the petioles with the main stem) B), in wild papaya plants subjected to prolonged waterlogging stress. The leaf positions for analysis are depicted in C). Bars in each point are means ± SD. Different letters indicate statistically significant differences (p< 0.05).

Effect of waterlogging on gas exchange parameters

The analysis of physiological response of wild papaya plants revealed that most variables (except Fv/Fm) decreased in different levels between days one and two, persisting throughout the experiment. The decline in stomatal conductance (Fig. 3B) and transpiration (Fig. C) from day one of waterlogging may be attributed to the plant's tendency to close its stomata to accumulate more oxygen, explaining the decrease in transpiration as reported for other plant species under waterlogging stress (Yordanova *et al*., 2005; Khayrul *et al*., 2019), irrespective of their level of tolerance to this stress.

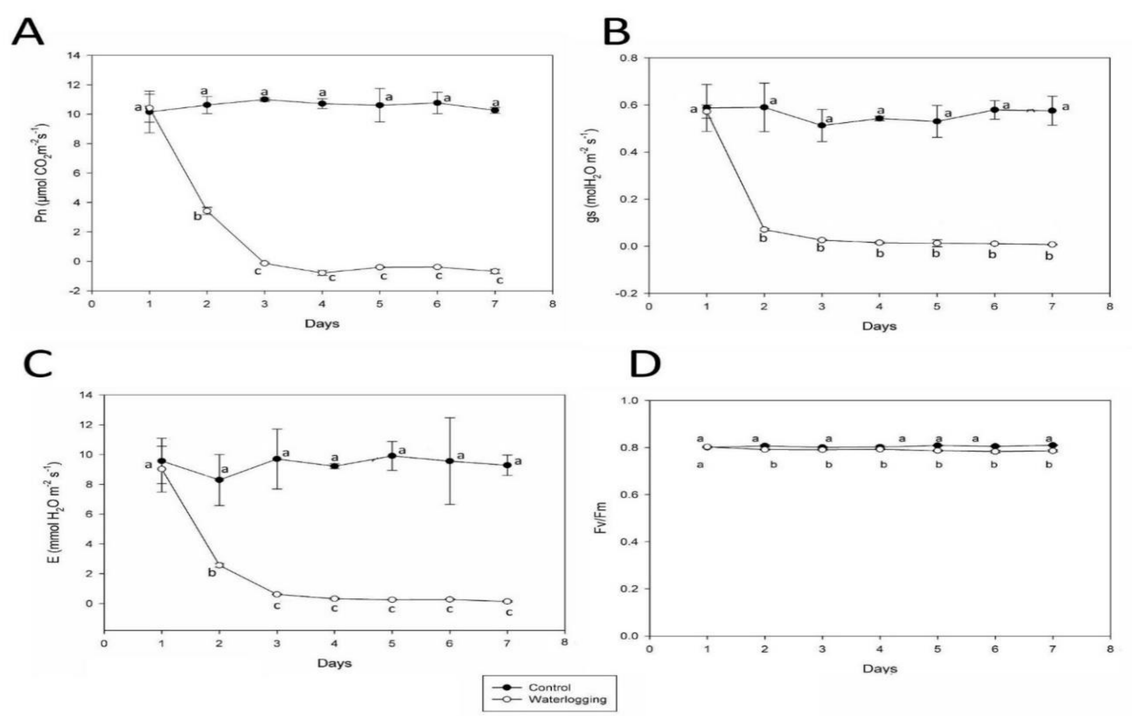

Figure 3. Kinetics of Photosynthesis (Pn) A), Stomatal conductance (gs) B), Transpiration C) and Photosynthetic efficiency (Fv/Fm) D), in wild papaya plants during prolonged waterlogging stress. Bars in each point are means ± SD. Different letters indicate statistically significant differences (p< 0.05).

The decrease in Pn (Fig. 3A) and transpiration (Fig. 3C) was less drastic than that occurring in stomatal conductance (Fig 3A), as it was after three days that Pn and transpiration reached 0 values. Thus, stomatal conductance was more sensitive to waterlogging as gs values reached 0 after only two days of being exposed to waterlogging stress. In fact, the reduction in photosynthesis has been suggested as being a common feature in plants under waterlogging stress, associated with stomatal closure and damage to PSII, due to photoinhibition (Sharma *et al*., 2022).

Effect of waterlogging on the Chlorophyll a fluorescence parameter Fv/Fm

On the contrary, photosynthetic efficiency (Fv/Fm) only decreased from values around 0.8 at day 0 in unstressed wild papaya plants, to values of 0.78 even after 7 days of waterlogging stress (Fig. 3D). This is interesting as there are reports from commercial papaya cultivars such as Red Lady, where Fv/Fm values reached values as low as 0.4 after 24h of waterlogging (even when they have removed plants from waterlogging, that is during the “recovery” stage) (Thani et al., 2016). Other stress factors such as heat may also decrease Fv/Fm to values as low as 0.4 in other species like beans (González-Moreno *et al*., 2008).

In the present study, Fv/Fm values from wild papaya plantlets did not decline below 0.78 even after 7d of being waterlogged, suggesting that the damage to the photosynthetic mechanism might be reversible, given appropriate conditions for recovery as suggested by Sharma *et al*., (2022). In fact, recently, Fv/Fm has been suggested as a sound parameter to compare drought tolerance among plant genotypes (Rico-Cambron *et al*., 2023), and although clearly more research is needed, our results might suggest that Fv/Fm might also be used as an estimate of waterlogging tolerance.

CONCLUSIONS

Wild papaya seedlings exhibited morphological changes (petiole binding and leaf loss) under waterlogging stress. However, those morphological changes became apparent only after three days of being waterlogged. The petioles binding (epinasty) could be associated with ethylene accumulation. Waterlogged wild papaya seedlings suffered defoliation after being waterlogged for more than 3 days, but they still retained leaves even after 7 days of waterlogging. Other physiological parameters such as photosynthesis, conductance and transpiration decreased during the first 24 h after being waterlogged.

On the other hand, the chlorophyll fluorescence parameter Fv/Fm, showed only a slight reduction after 24 hours of being waterlogged, and even after 7 days of being subjected to waterlogging stress, wild papayas were capable of still maintaining high photosynthetic efficiency (Fv/Fm) values, that suggests that photosynthetic apparatus might not have been severely damage and it may recover once the waterlogging stress may be reliesed.

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Compliance with ethical standards. Does not apply.

Author contribution statement (CRediT). N.A. González-Oviedo- Data curation, Formal Analysis, Methodology, Writing-original draft, **G. Fuentes-Ortíz-** Analysis**,** Writing, Editing. **J. M. Santamaría-** Conceptualization, Investigation, Methodology, Resources, Supervision, Writing - review and editing.

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