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Effects of Short-term Drought Stress on Chlorophyll Fluorescence and Proline Content of *Ficus annulata*

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Abstract

Drought is still one of the key factors that directly affects the rate of photosynthesis and the reduction of plant growth and yield. This study was carried out to investigate the effect of drought and re-watering trials on the contents of chlorophyll, carotenoids, proline, CO_2 fluxes, and the photosynthetic efficiency of *Ficus annulata*. Treatments included control (no drought) and drought-stressed plants exposed to 21 days of drought followed by re-watering, with four replications conducted over 56 days. The results showed that drought stress greatly reduced the amounts of chlorophyll and carotenoids, with the highest reduction in relative water content (RWC) observed at 76–79% ($p \leq 0.05$). Conversely, proline content significantly increased during drought stress, exhibiting the highest value of 108.16 µg/g FW before re-watering ($p \leq 0.05$). A 21-day short-term drought had a statistically significant effect on changes in chlorophyll fluorescence parameters and CO_2 flux ($p \leq 0.05$). However, the overall plant response after re-watering showed no significant difference compared with the control ($p > 0.05$), suggesting recovery of physiological efficiency. Our findings indicated that *F. annulata* has the capacity to mitigate carbon dioxide emissions. These physiological responses enhance the plant's suitability for drought resistance, and re-watering supports effective survival under drought stress.

Keywords: *banyan tree; carbon dioxide; chlorophyll fluorescence; drought re-watering; photosynthesis; proline; short-term drought; water deficit*

1. Introduction

Ficus annulata (Moraceae), commonly known as the banyan tree, is an economically important plant species in Thailand. *F. annulata* Blume was previously recorded in Myanmar, Indo-China, Yunnan, Thailand, Sumatra, the Malay Peninsula, Banka, Java, Borneo, Sulawesi, the southwestern Philippines (Rasingam & Upadhyay, 2013). This plant species is found in India (Andaman and Nicobar Islands), Myanmar, Indo-China, Yunnan, Thailand, Sumatra, Malay Peninsula, Banka, Java, Borneo, Sulawesi, and southwestern Philippines. It is uncommon along the streams of semi-evergreen and littoral forests from sea level to 100 m altitude. It is very sparsely distributed inside inland semi-evergreen forests, with an estimated density of 5–7 individuals per 10,000 square meters (Rasingam & Upadhyay, 2013). It is an ornamental plant with a variety of shapes and dense, multilayered leaves, and is well known for absorbing dust and blocking sunlight. It is commonly planted in landscaped plots and gardens. Nonetheless, due to the fact that water is an important factor for the growth of plants, *F. annulata* is likely to wilt, lose leaves, and even die when exposed to drought stress. Generally, drought increases osmotic pressure, which decreases turgor pressure in plant cells, reducing carbon fixation and photosynthetic rate, causing cell damage and stomatal limitation (Kaewthongrach et al., 2019). The closure of stomata during drought stress diminishes the influx of carbon dioxide (CO_2) and limits CO_2 absorption, leading to significantly decreased photosynthetic activity. Furthermore, it affects various physiological and biochemical processes, including a decrease in leaf water content, gas exchange, ion exchange between the root and shoot, and growth restriction (Oguz et al., 2022). A significant decrease in net photosynthetic rate was observed in wheat (*Triticum aestivum*) plants exposed to drought in a growth chamber, due to reduced stomatal conductance, transpiration rates, and intercellular CO_2 concentration (Farooq et al., 2009). The chlorophyll concentration is a crucial factor influenced by drought stress and varies among plant species. The decline in chlorophyll concentration in leaves may be attributed to the degradation of chlorophyll caused by dryness. Furthermore, the decline in chlorophyll content during drought stress is ascribed to the inactivation of crucial chlorophyll synthesis enzymes (Rai & Rai, 2020). Numerous studies have sufficiently demonstrated that the oxidative damage of chloroplast lipids and the resulting loss of chlorophyll occur in tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), and canola plants (*Brassica napus*) (Oguz et al., 2022; Rai & Rai, 2020). As an osmotic regulatory substance in response to drought or plant cells being subjected to osmotic stress, proline is preferentially stored in plant vacuoles. Cell structure may be protected by proline because of its potent capacity to preserve the protoplasm of the cell and the external osmotic balancing environment. It mitigates oxygen damage induced by stress by chelating singlet oxygen and hydroxyl radicals (Yang et al., 2021).

Changing global climate patterns have caused irregular rainfall and drought stress. Thailand has encountered drought almost every year, and extreme droughts occur frequently during the summer season. Drought affects these regions by causing water shortages for consumption, daily use, and agriculture. The lack of water will affect plants, causing physiological, anatomical, and morphological changes. Drought stress reduces plant growth, making osmotic adjustment critical and leading to a reduction in the biomass of plants (Ozturk et al., 2021). Global climate change, meanwhile, primarily due to increased atmospheric CO_2, has complex effects on plant CO_2 absorption and proline content. On the other hand, photosynthesis is widely expected to rise due to increased atmospheric CO_2. Higher levels of CO_2 in the atmosphere also have had a positive effect on photosynthesis and water retention. Previous studies have reported the CO_2 absorption capacity of species in the *Ficus* genus (such as *F. religiosa*), approximately 72.42 kg/tree/year (Kliangsaard et al., 2020; Sevik et al., 2017). Planting trees is a promising option to reduce atmospheric CO_2 and alleviate global warming through plant photosynthesis and respiration. Carbon sequestration in plants will positively play a role in decreasing atmospheric CO_2 concentration and will lessen drought stress. However, the effect of higher temperatures and less soil moisture on photosynthesis and other plant physiological processes is not always positive. Thus, the net balance may be positive, neutral, or negative, depending on several factors.

For this reason, investigating photosynthetic efficiency under drought conditions has become an important research focus worldwide. However, there are no reports on the basic physiological response of *F. annulata*, including the ability to deal with drought when exposed to short-term drought stress and CO_2 absorption. Addressing this point is essential for developing strategies to improve drought tolerance in plants. Enhancing drought resilience not only benefits agricultural productivity but also supports sustainable practices in the face of climate change. Furthermore, understanding the adaptive responses of *F. annulata* to transient water deficits will provide valuable insights into plant resilience mechanisms, informing future efforts to optimize crop performance and ensure food security under changing environmental conditions.

2. Objectives

This study aimed to investigate the effects of short-term drought stress on the photosynthetic efficiency of *F. annulata* in a pot experiment. The effects of drought on chlorophyll content, proline accumulation, carbon dioxide absorption capacity, and relative water content (RWC) were also determined.

3. Materials and Methods

3.1 Plant Preparation and Treatments

The present study was conducted in a greenhouse at the Department of Biology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand (latitude 13°44´N, longitude 100°33´E). For plant preparation, seedlings cultured for 3 months and approximately 30 cm in height were selected and transplanted into plastic pots containing either 100% PC or 37.5% PC soil. The field capacity of the pot (PC) was determined by saturating the soil with water. The water content at PC was calculated as the difference between the soil weight after drainage and the soil dry weight. At 100% PC and 37.5% PC, 15,000 mL (53.95% VWC) and 4,000 mL (20.23% VWC) of water, respectively, were added to the pots. After 3 months of plant culture, the seedlings were transferred into plastic pots, and the water level in the pots was maintained by measuring soil water content using a reflectometer on day 0. Plants were subjected to two treatments: (1) 100% PC and (2) 37.5% PC, with four replicates and five plants per replicate for each treatment. The physiological responses and photosynthesis efficiency were measured every 7 days after treatments. To evaluate CO_2 flux, plastic pots with soil and *F. annulata*, as well as pots with soil at 100% and 37.5% PC, were prepared and also measured every 7 days after treatments.

3.2 Relative Water Content

The relative water content (RWC) was determined by the following equation of Schonfeld et al. (1988). RWC = (FW − DW)/(TW − DW) x 100, where FW is the fresh weight of leaves, and then the leaves were soaked in distilled water at room temperature for 24 h to determine the turgid weight (TW), and DW is dry weight, which was determined after drying at 60°C for 48 h.

3.3 Chlorophyll and Carotenoids

0.02 g of leaf sample was extracted with dimethyl sulfoxide (DMSO) under dark conditions overnight. The extract was then boiled at 65°C for 20 min and allowed to cool. The photosynthetic pigments were determined by spectrophotometer and the absorbance at 480, 649, and 665 nm were measured. The chlorophyll (Chl) *a*, Chl *b*, carotenoids, and total chl were calculated using the formula proposed by Sumanta et al. (2014).

$$\text{Chl } a\ (C_a) = (12.47A_{645} - 3.62A_{649})\ (V/1000W)$$

$$\text{Chl } b\ (C_b) = (25.06A_{649} - 6.50A_{665})\ (V/1000W)$$

$$C_{X+C} = ((1000A_{480} - 1.29C_a - 53.78C_b)/220)\ (V/1000W)$$

$$\text{Total chl} = C_a + C_b$$

3.4 Photosynthesis Efficiency

The OJIP chlorophyll a fluorescence parameters; Fv/Fm (the maximum quantum efficiency of PSII, which is correlated with the quantum yield of net photosynthesis), Pi (performance index for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors), ABS/RC (the absorption flux per reaction center), DIo/RC (dissipated energy flux per reaction center), TRo/RC (trapped energy flux per reaction center), and ETo/RC (electron transport flux per reaction center) indicate the maximum quantum efficiency of PSII were measured in the fully expanded leaves. The leaves were dark-adapted for 30 min. The Fv/Fm and Pi were measured by using a chlorophyll fluorometer (Pocket PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK).

3.5 Proline Content

0.1 g of leaf sample was extracted in 1 mL of 3% sulfosalicylic acid and centrifuged at 10,000 x g for 20 min. 0.5 mL of supernatant was made to react with 0.5 mL of acid ninhydrin in a water bath at 100°C for 30 min. Then extracted with 1 mL of toluene, cooled to room temperature, and the absorbance was measured at 520 nm (Bates et al., 1973).

3.6 CO_2 Fluxes

Soil respiration rate (CO_2 fluxes) was measured by a closed chamber method (Hanpattanakit et al., 2017). The system consisted of a chamber system and a data storing unit. The chamber consists of 2 parts; the cover and the base. The cover is made from acrylic of 35 x 35 x 70 cm (width x length x height) and the base is made from stainless steel with 35 x 35 x 10 cm (width x length x height) and inserted into the soil. The soil temperature was measured at a depth of 5 cm by thermocouple probes (TCAV, Campbell Scientific, Inc. USA). The air temperature was measured in a chamber by a thermometer. The soil water content was measured at a depth of 5 cm by soil water content reflectometers (CS615, Campbell Scientific, Inc., USA). The CO_2 concentration was determined by a non-dispersive infrared (NDIR) detector in a Licor 820 analyzer (LI-COR Corporation, Lincoln, Nebraska, USA) every 7 days. The data of CO_2 concentration was collected by a data logger (CR10X, Campbell Scientific, Logan, Utah, USA) every 15 seconds for 10 min. CO_2 fluxes were calculated using the following formula;

$$F = \frac{dC_i}{dt} \text{ x } \frac{1}{A} \text{ x } \frac{M_iPV}{RT} \text{ x } t_i$$

F = CO_2 fluxes (mg m^{-2} h^{-1})

$\frac{dC_i}{dt}$ = the increase/decrease rates of CO_2 concentrations (ppb/m)

M_i = the mass number (44 x 10^3) (mg·mol^{-1})

A = the area of the chamber (width x length) (m^2)

P = the atmospheric pressure (1 atm)

V = the chamber volume (width x length x height) (m^3)

R = the gas constant (0.82058 × 10^{-3} m^3·atm·mol^{-1}·K^{-1})

T = the air temperature inside the chamber (K)

t_i = time (60 min)

3.7 Statistical Analysis

The experiments consisted of 2 treatments. All the data were analyzed using a *t*-test with the IBM SPSS Statistics software package (SPSS Inc., 2016). Differences among treatments were considered significant at the 0.05 probability level.

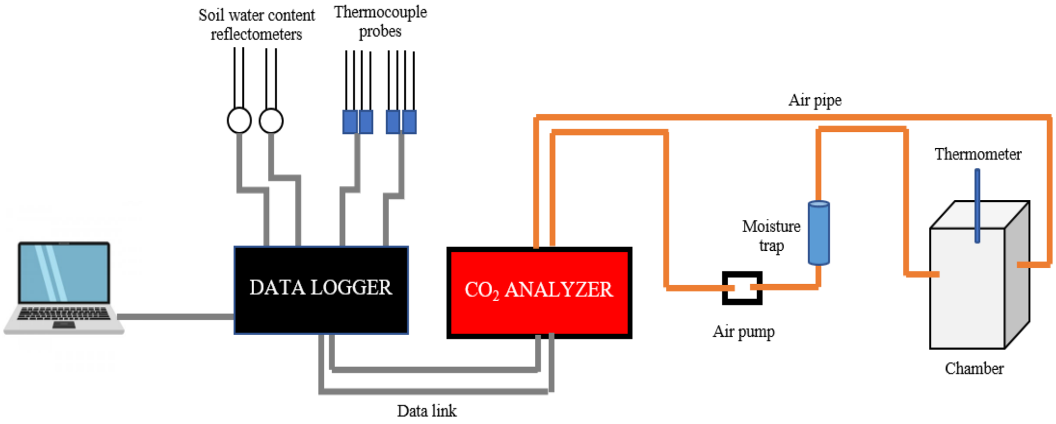

Figure 1 The installation of equipment for studying carbon dioxide levels.

Figure 2 The installation of equipment for measuring carbon dioxide absorption in plants.

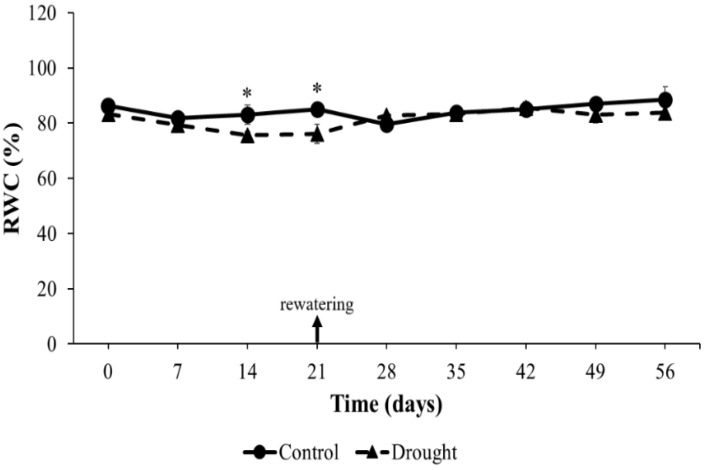

Figure 3 Effect of short-term drought stress on relative water content (RWC) of *F. annulata* under control, stress and recovery water conditions during the 56-day experiment (means of 4 replicates ± S.E). The asterisk indicates significance at $p \leq 0.05$ according to the Student's *t*-test.

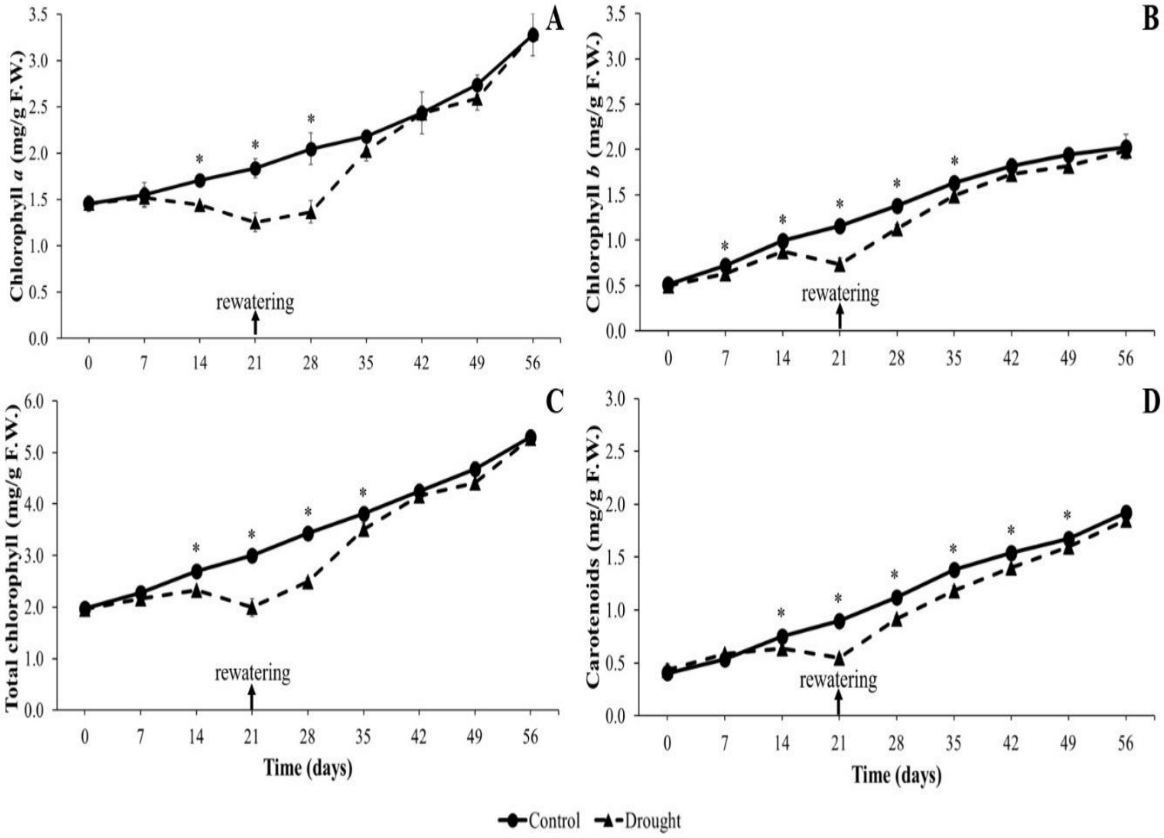

Figure 4 Illustrates the impact of short-term drought stress on chl *a* (A), chl *b* (B), total chl (C), and carotenoids (D) in *F. annulata* under control, drought stress, and recovery water conditions throughout the 56-day experiment (mean values of 4 repetitions ± S.E). The asterisk denotes significance at $p < 0.05$ as determined by the Student's *t*-test.

4. Results

4.1 Effect of Drought Stress on Relative Water Content

The plants were sufficiently irrigated on day 21 (re-watering), and there was no significant difference in mean RWC among all treatments at the end of the trial ($p > 0.05$) (Figure 3). However, the RWC of *F. annulata* at 37.5% PC was found to be affected by short-term drought and showed a significantly lower RWC (76–79%) than the control (≥90%) before re-watering *(*$p \leq 0.05$).

4.2 Effect of Drought Stress on Chlorophyll and Carotenoids Content

The content of Chl *a*, Chl *b*, total Chl, and carotenoids of plants under drought stress and recovery water conditions during the 56-day experiment are depicted in Figure 4. At the end of the experiment, drought stress had not markedly decreased the content of chlorophyll and carotenoids in the plant ($p > 0.05$). As can be seen in Figure 4A–C, *F. annulata* subjected to drought stress exhibited significantly lower amounts of Chl *a*, Chl *b*, and total Chl compared to the control between 14 and 35 days of trial ($p \leq 0.05$), indicating that a short period of drought has a positive temporal effect on the reduction of the concentration of chlorophyll in the plant. Nevertheless, those content were increased after a 21-day re-watering and showed no significant difference when compared with the control after a 35-day trial ($p > 0.05$). Although the highest reduction of carotenoids content in drought-stressed plants was exhibited at 21 days of treatment (Figure 4D). After re-watering till the end of the trial, the carotenoids content in the drought-stressed plants was not significantly different compared to the control ($p > 0.05$).

4.3 Photosynthesis Efficiency

Chlorophyll fluorescence response is commonly referred to as photosynthetic efficiency of photosystem (PS) II in plants. The chlorophyll fluorescence parameters Fv/Fm, Pi, ABS/RC, DIo/RC, TRo/RC, and ETo/RC measure the change in energy flow per reaction center. Before the 21-day re-watering period, *F. annulata* was influenced by drought and exhibited chlorophyll fluorescence (Fv/Fm) values ranging from 0.75 to 0.65, compared to the control (0.82–0.81) (Figure 5A). From re-watering to the end of the experiment, however, the Fv/Fm value of plants under drought stress showed no significant mean difference when compared to the control ($p > 0.05$). Similar results were observed in the value of the performance index (Pi); there was no significant difference in Pi value compared to the control at the end of the experiment ($p > 0.05$) (Figure 5B). Meanwhile, the values of the ABS/RC (light energy absorbed per reaction center), DIo/RC (the light energy dissipated as heat), TRo/RC (the changes in light energy captured in PSII per reaction center), and ETo/RC (the changes in energy transferred to the electron transport system) showed a significantly the highest increase at 21 days of treatment compared with the control ($p \leq 0.05$) (Figure 5C–F). Evidently, our investigation revealed that short-term drought stress prior to a 21-day re-watering significantly impacted the photosynthetic efficiency of *F. annulata*.

4.4 Proline Content

The proline content in drought-stressed plants (108.16 µg/g FW) was higher than that in the control during the 21-day short-term drought stress period, and the difference was statistically significant ($p \leq 0.05$) (Figure 6). A slight but non-significant reduction ($p > 0.05$) in the proline content was observed in *F. annulata* after a 21-day re-watering when compared to the control. It can be stated that the proline content of *F. annulata* was unaffected by the end of the trial but strongly affected only by a 21-day short-term drought.

4.5 CO_2 Fluxes

In the present study, it is clear from Figure 7A–C that the treatment of soil with *F. annulata* exhibited overall lower CO_2 fluxes compared to soil without planting under normal conditions (100% PC) in the clear chamber. The cumulative CO_2 fluxes were observed at 59.07 g CO_2/m^2 over 56 days, or 8.12%, indicating the efficiency of CO_2 release from the soil-plant system. Under a 21-day short-term drought, the soil + *F. annulata* was treated with 37.5% PC, resulting in higher CO_2 fluxes than those in the soil without plants. The results indicate that the period from drought re-watering to the end of the experiment led to a significant decrease in CO_2 fluxes, with the lowest reduction observed at day 49 (Figure 8A–C). This suggests that changes in CO_2 flux values are influenced by plants grown in soil experiencing drought stress.

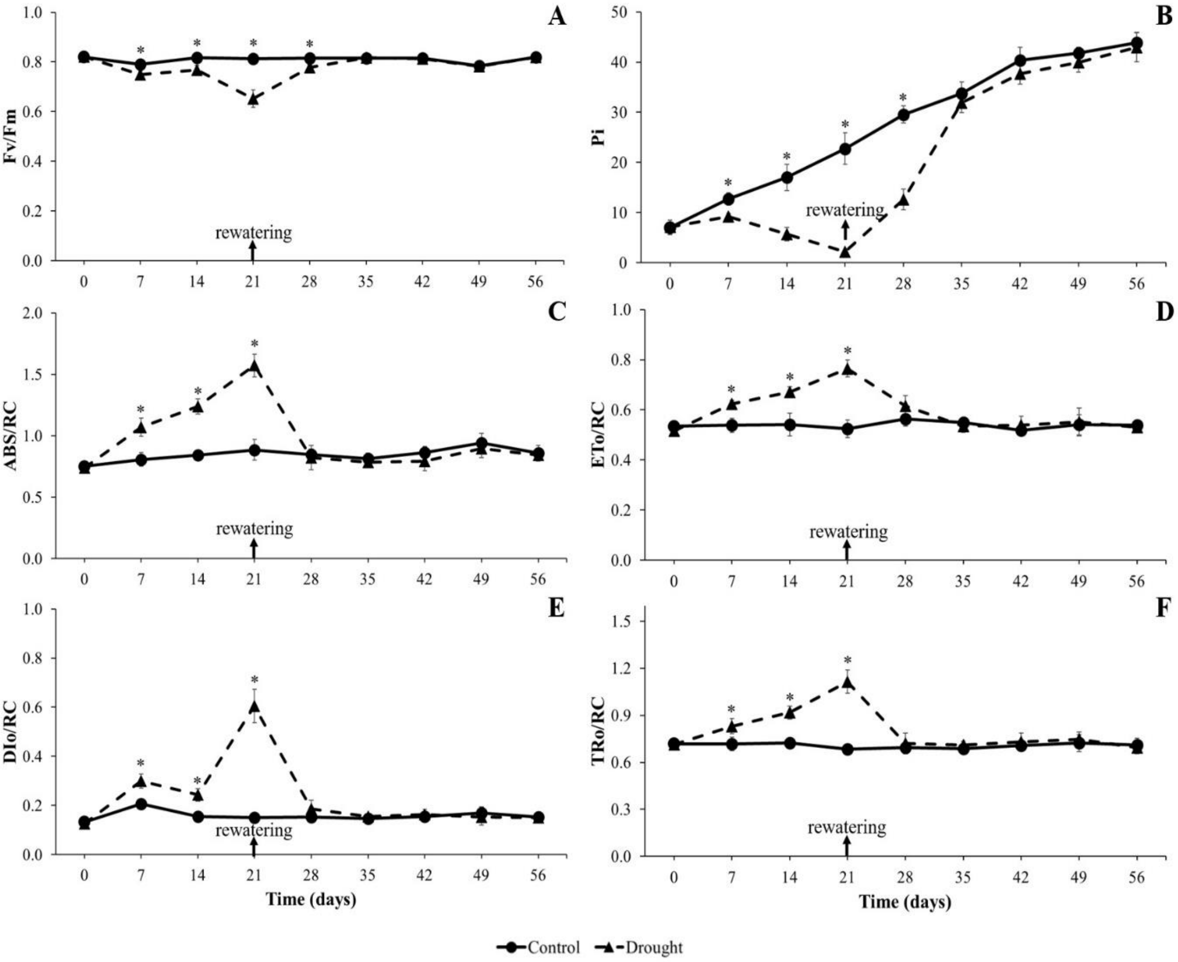

Figure 5 Effect of short-term drought stress on the chlorophyll fluorescence; Fv/Fm (A); performance index; Pi (B); absorption per active reaction center; ABS/RC (C); electron transport flux per active reaction center; ETo/RC (D); dissipation energy per active reaction center; DIo/RC (E); and trapped energy flux per active reaction center; TRo/RC (F) in *F. annulata* under control, drought stress and recovery water conditions during the 56-day experiment (means of 4 replicates ± S.E). The asterisk indicates significance at $p \leq 0.05$ according to the Student's *t*-test.

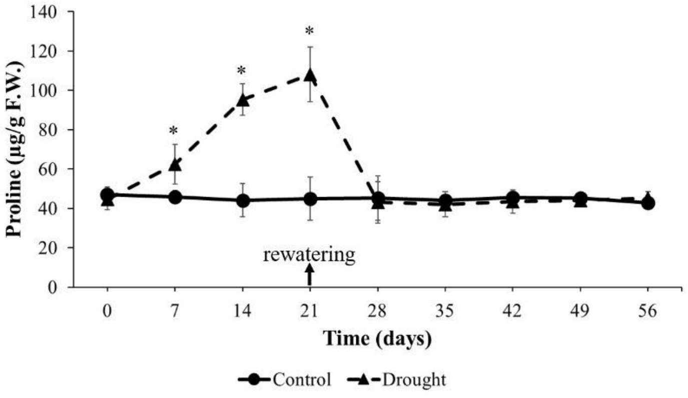

Figure 6 Effect of short-term drought stress on the proline content in *F. annulata* under control, drought stress and recovery water conditions during the 56-day experiment (means of 4 replicates ± S.E). The asterisk indicates significance at $p \leq 0.05$ according to the Student's *t*-test.

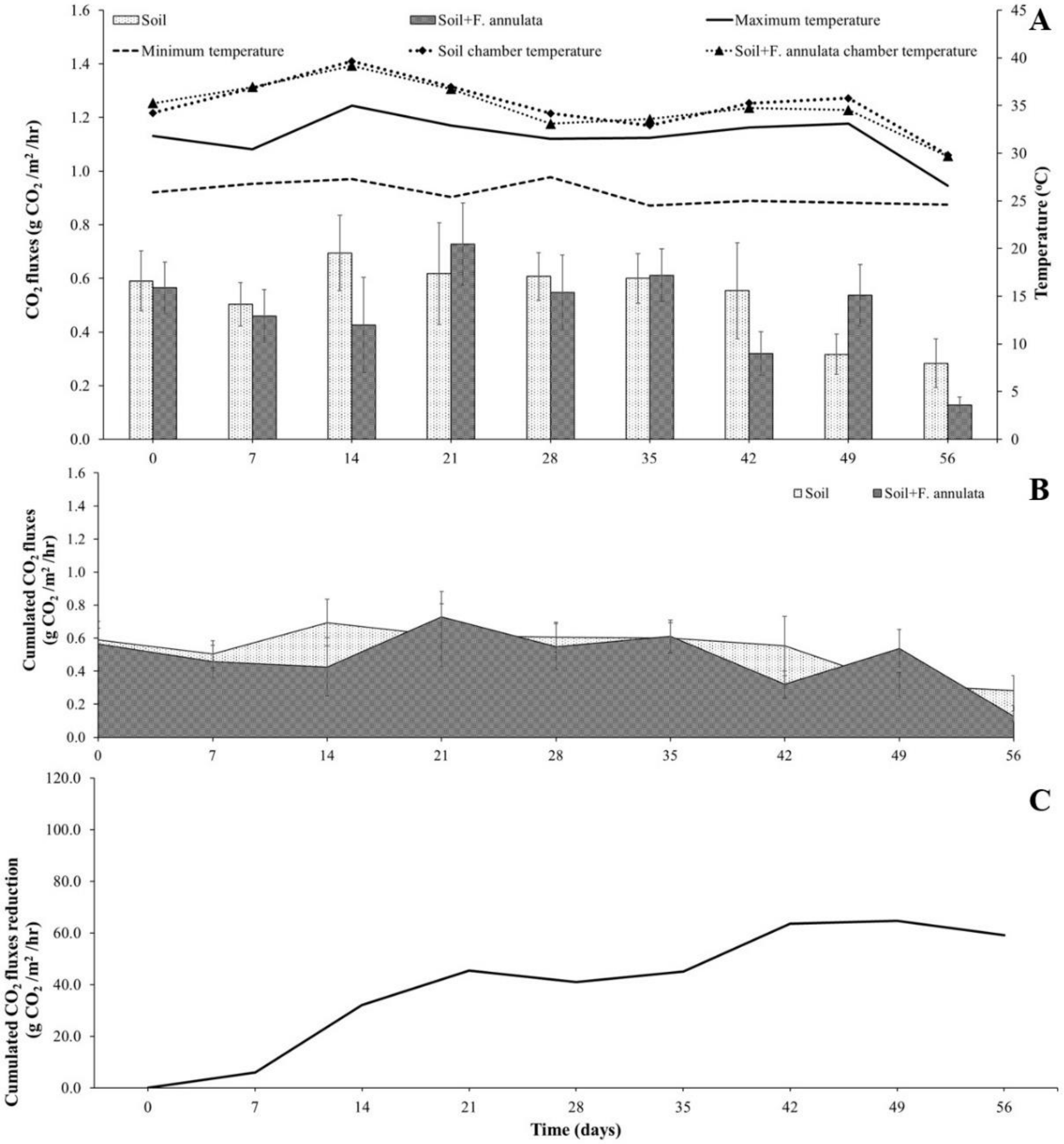

Figure 7 The CO_2 fluxes (g CO_2/m²) (A); cumulated CO_2 fluxes (g CO_2/m²) (B); and cumulated CO_2 fluxes reduction (g CO_2/m²) (C) of *F. annulata* under control conditions during the 56-day experiment (means of 4 replicates ± S.E) according to the Student's *t*-test.

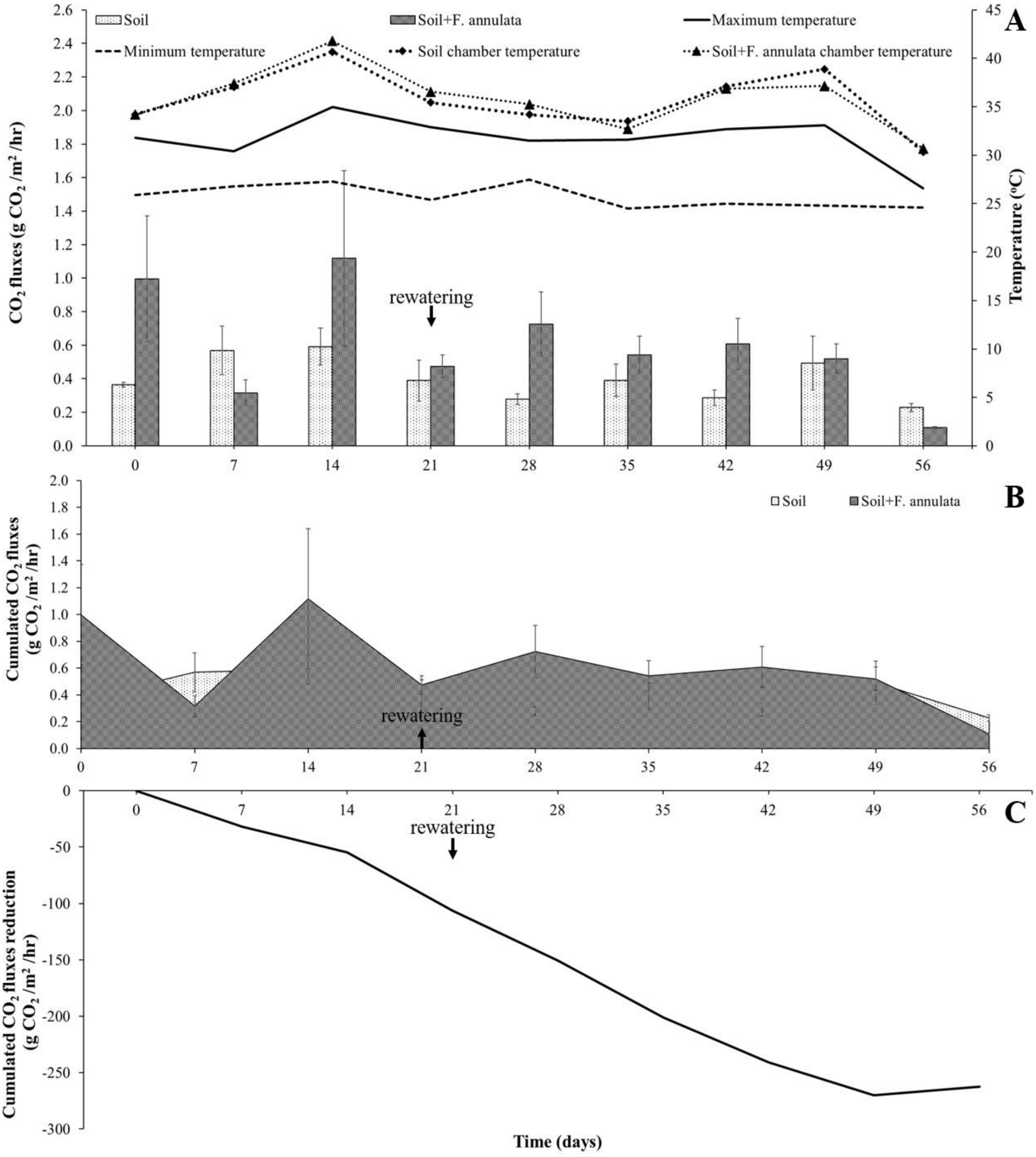

Figure 8 The CO_2 fluxes (g CO_2/m^2) (A); cumulated CO_2 fluxes (g CO_2/m^2) (B); and cumulated CO_2 fluxes reduction (g CO_2/m^2) (C) of *F. annulata* under short-term drought stress and re-watering conditions during the 56-day experiment (means of 4 replicates ± S.E) according to the Student's *t*-test.

5. Discussion

Our results show that drought can cause many negative effects on *F. annulata*. Short-term droughts typically last from a few days to a few weeks and may cause temporary stress. However, if adequate water becomes available again, many plants can recover without lasting damage, and the impacts are generally reversible. In the present study, leaf relative water content (RWC) and chlorophyll content can quickly recover after re-watering and remain at a higher level for a longer period of 56 days. Although a decrease in water content causes turgor loss, which disrupts metabolic processes, plants can quickly recover after re-watering at a certain RWC. Probably because it is attributed to adjustment in osmotic balance resulting from elevated proline content. Unlike the observed reduction in RWC due to drought without re-watering, various plant species, including *F. carica*, *T. aestivum*, and *Lycopersicon esculentum*, have been reported (Del Rosario Jacobo-Salcedo et al., 2024; Sousaraei et al., 2021; Ulfat et al., 2021). Exposure of plants to drought results in significant water loss, which subsequently induces heightened generation of reactive oxygen species (ROS) (Farooq et al., 2019; Hasanuzzaman et al., 2018). Nevertheless, plants subjected to drought and subsequently re-watered often demonstrate an enhanced capacity to scavenge and reduce ROS levels. Modifications in antioxidant enzyme metabolism can influence drought tolerance in plants, such as ROS scavenging mechanisms. This adaptive response helps mitigate oxidative stress caused by drought conditions, protecting cellular components from damage and supporting plant recovery and survival (Mahmood et al., 2019). In addition, various functional genes and genetic networks control complex agricultural traits in cotton to enhance the plant's drought tolerance recovery (Mahmood et al., 2019). In the case of *F. annulata*, re-watering appears to alleviate drought-induced oxidative stress, possibly leading to decreased ROS levels and consequently an increase in RWC, indicating improved water status and recovery capacity.

The present study indicates that a 21-day short-term drought directly influenced the content of chlorophyll and carotenoids, with the lowest reduction observed. According to earlier research, the depletion of chlorophyll under drought stress is caused by either the oxidative damage to chloroplast lipids, which causes chlorophyll degradation, or the inactivation of key enzymes involved in chlorophyll biosynthesis (Rai & Rai, 2020; Oguz et al., 2022). A reduction in chlorophyll content in plants under drought stress without re-watering was confirmed by previous studies in various plants, i.e., watermelon (*Citrullus lanatus*), tomato (*L. esculentum*), lettuce (*Lactuca sativa*), and Arabidopsis (Banks, 2018; Yao et al., 2018; Malambane et al., 2021; Shin et al., 2021). In contrast, the drought re-watering trials showed an increase in Chl *a*, Chl *b*, total Chl, and carotenoids content. These findings indicate that *F. annulata* is capable of either regenerating or restoring its chlorophyll content after drought following re-watering. Consequently, the levels of chlorophyll and carotenoids continued to increase throughout the duration of the trials, highlighting the recovery potential of *F. annulata* under drought stress. The increase in chlorophyll content acts as a protective mechanism, aiding in the preservation of photosynthesis efficiency and protecting the plant's photosynthetic system from damage (Challabathula et al., 2016). Chl *a* plays a necessary role in the electron transfer process during photosynthesis and must remain resilient to sustain photosynthetic activity under drought stress (Muhammad et al., 2021). Alternatively, it has been viewed as an adaptive trait that minimizes the risk of additional damage to the efficiency of photosynthesis caused by the formation of ROS (Mahmood et al., 2019). The present finding confirmed that the increase of RWC in drought re-watering trials was associated with a higher significance in proline content before a 21-day re-watering. This suggests that proline may play a crucial role in osmotic adjustment, accumulating at high concentrations within the cytosol as drought conditions become more severe. Such higher accumulation likely helps stabilize cellular membranes and preserve protein structures, thereby contributing to the plant's resilience under drought stress (Reddy et al., 2004). Similarly, Binukumar & Lovely (2024) demonstrated the accumulated proline of green gram (*Vigna radiata*), likely contributed to osmotic adjustment, and played a crucial role in maintaining turgor pressure under fluctuating soil water potential.

To evaluate the effect of short-drought stress, chlorophyll fluorescence parameters were measured. Interestingly, the photochemical efficiency of PSII (e.g., the Fv/Fm ratio and Pi values) tends to decrease during the initial 3 weeks of drought, indicating impaired photosynthetic performance. This decrease aligns temporally with reductions in chlorophyll content, which can be caused by degradation of chlorophyll molecules or inhibited synthesis under short-term drought before re-watering. Drought stress diminished the electron transport capacity and primary photochemical activity of PSII, along with the negative impact of excessive accumulation of excitation energy on photosynthesis (Demmig-Adams & Adams, 2018). The observed decrease in Fv/Fm and Pi values agrees with the findings of Xu et al. (2022), who reported that drought stress in maize (*Zea mays*) leads to reductions in these photosynthetic parameters. In the case of the ABS/RC, DIo/RC, TRo/RC, and ETo/RC values, there were negative effects of short-term drought stress on these parameters in *F. annulata*, indicating a decline in the efficiency of photosynthetic processes. These changes suggest that drought stress impairs the light-harvesting complex and subsequent energy transfer within the photosystems, possibly leading to reduced utilization of absorbed light energy. This disruption can cause an accumulation of energy that cannot be effectively used for photochemistry, potentially increasing the risk of photoinhibition and oxidative damage (Shanker et al., 2022). Generally, under drought stress conditions, the energy fluxes for absorption, trapping, and dissipation were higher than those in normal conditions. The increase in ABS/RC, TRo/RC and ETo/RC in *F. annulata* indicated that some reaction centers had become partially inactive leading to an increase in efficiency per active reaction center. The ABS/RC reflects the total photons absorbed by chlorophyll molecules in all reaction centers, divided by the total number of active reaction centers. It is affected by the ratio of active to inactive centers, so as the number of inactive centers rises, the ABS/RC ratio also increases (Mathur et al., 2011). TRo/RC indicates the maximum rate at which the photon is captured by the reaction center, leading to the reduction of QA. An increase in this ratio suggests that all QA has been reduced, but it cannot be oxidized back due to drought stress. Additionally, ETo/RC represents only active centers; the increased ratio suggests a rise in inactive centers, preventing QA from efficiently transferring electrons to QB. As a result, the ratio has increased while electron efficiency has declined. However, the increase in DIo/RC suggested that the reaction centers activated a protective mechanism by dissipating excess excitation energy as heat, thereby minimizing potential damage to the plant from excessive light energy, similar to the heat-stressed leaves of *Prunus persica* (Martinazzo et al., 2012).

Therefore, the results of this experiment demonstrated that reduced photochemical efficiency of PSII reaction centers (Fv/Fm) and electron transport rate (ETo/RC) together with increased energy dissipation (DIo/RC) are the causes of the fall in Pi during drought stress (Meng et al., 2016; Oukarroum et al., 2009). Our results indicate that following a 21-day re-watering period after drought stress, however, the chlorophyll fluorescence parameters-namely Fv/Fm, Pi, ABS/RC, DIo/RC, TRo/RC, and ETo/RC rapidly returned to control levels. This recovery was accompanied by an enhancement in the photosynthetic efficiency of *F. annulata*. This suggests that chlorophyll fluorescence, which reflects the efficiency of light capture and energy transfer during photosynthesis, becomes highly effective post-rewatering. Such improvements facilitate sustained photosynthetic activity, agreeing with findings by Farooq et al. (2009), who reported that efficient chlorophyll fluorescence indicates optimal light utilization and energy transfer in re-watered plants.

Under short-term drought stress, *F. annulata* released higher CO_2 compared to normal conditions. It may be possible to explain that when *F. annulata* is exposed to drought or a water deficit, closing their stomata helps minimize water loss via transpiration. However, this adaptive response reduces the entry of CO_2 into the leaves, which can consequently limit photosynthetic activity and affect overall carbon assimilation. Once water availability improves, stomata generally reopen, allowing normal gas exchange and photosynthesis to resume. This dynamic regulation of stomatal conductance is crucial for balancing water conservation with carbon gain. Additionally, earlier research has suggested that higher levels of CO_2 in the air might reduce the harmful effects of drought, but the results can vary depending on the type of plant and how severe the treatment is (Holopainen et al., 2018). For instance, Lupitu et al. (2023) observed varying responses in cauliflower (*Brassica oleracea* var. botrytis) and green cabbage (*B. oleracea* var. capitata). Our findings indicate that *F. annulata* utilises CO_2 for photosynthesis to sustain growth during drought stress, despite a reduction in overall photosynthetic efficiency. On the contrary, when water becomes available again, a marked increase in soil CO_2 fluxes is observed. This possible rebound shows an increase in the activity of microbes and plant breathing, caused by better moisture levels that help their metabolic processes, leading to more CO_2 being released. These findings enhance our understanding of the physiological responses of *F. annulata* to short-term drought stress, offering guidance for its cultivation management under drought conditions and for its potential use as a carbon dioxide mitigation crop. Moreover, plants capable of recovering from drought conditions can sustain yields despite water shortages, reducing economic losses and enhancing food security. The recovery of long-term drought tolerance in crops may reduce the necessity for irrigation and water management under extreme climate change.

6. Conclusion

This study demonstrates that *Ficus annulata* possesses robust physiological mechanisms to withstand and recover from short-term drought stress. For 21 days, a short-term drought at 37.5% pot capacity temporarily reduced leaf relative water content, Chl *a*, Chl *b*, total Chl, and PSII performance (Fv/Fm, PIABS). In contrast, it also slightly increased ABS/RC, TRo/RC, ETo/RC, and DIo/RC and proline content. After rewatering, *F. annulata* exhibited recovery in measured physiological parameters, with pigments, relative water content, and chlorophyll fluorescence parameters returning to values comparable to the control. These findings indicate that, although this plant species is sensitive to water deficits, it demonstrates strong short-term drought resilience. Therefore, *F. annulata* is suitable for cultivation in drought-affected environments and for short-term cultivation in regions with variable rainfall.

7. Abbreviations

Abbreviation	**Full Term**
ABS/RC	Absorption flux per reaction center
Chl	Chlorophyll
DIo/RC	Dissipated energy flux per reaction center
DMSO	Dimethyl sulfoxide
ETo/RC	Electron transport flux per reaction center
Fv/Fm	Maximum quantum efficiency of PSII
NDIR	Non-dispersive infrared
OJIP	Chlorophyll a fluorescence induction curve (O–J–I–P)
PC	Pot capacity (field capacity of the pot)
Pi (PIABS)	Performance index for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
PSII	Photosystem II
QA, QB	Primary and secondary quinone electron acceptors in PSII
RC	Reaction center
RWC	Relative water content
TRo/RC	Trapped energy flux per reaction center

8. CRediT Statement

Sirilak Nimnuan: Investigation, data curation, writing original draft, writing review & editing.
Anan Piriyaphattarakit: Conceptualization, methodology, formal analysis, funding acquisition.
Phongthep Hanpattanakit: Investigations, resources, data curation, validation, visualization, supervision.
Kongkeat Jampasri: Data curation, methodology, validation, formal analysis, writing review & editing.
Sukhumaporn Saeng-ngam: Conceptualization, methodology, supervision, writing review & editing, funding acquisition, and project administration.

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