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Photosynthetic adjustments and proline concentration are probably linked to stress memory in soybean exposed to recurrent drought

Ajustes fotossintéticos e concentração de prolina provavelmente estão ligados à memória de estresse em soja exposta a seca recorrente

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ABSTRACT

Drought stress is the main abiotic factor limiting soybean yield. The memory of recurrent water stress can provide greater efficiency in minimizing the negative effects of drought. Thus, the aim of this work was to understand the temporal adjustments in photosynthesis presented by soybeans when exposed to recurrent drought at the beginning of the flowering and grain filling stages. The experiment was carried out in a randomized block design with five replications, consisting of four treatments: i) WS-R1 (moderate water deficit at the beginning of flowering), ii) WS-R5 (severe water deficit during grain filling), iii) WS-R1+R5 (moderate water deficit at early flowering and severe water deficit during grain filling), and iv) WW (well-watered condition). Severe stress caused reductions in gas exchange parameters and the relative water content, with increased initial fluorescence and water use efficiency. The plants from the WS-R5 and WS-R1+R5 treatments showed a reduction in the apparent rate of electron transport in photosystem II (PSII), photochemical quenching, and effective quantum yield of PSII, as well as increased nonphotochemical quenching values. Furthermore, the proline concentration in the leaves was higher in plants from the WS-R1+R5 treatment, contributing to the greater ability to maintain turgid cells compared to the WS-R5 plants. The photosynthetic adjustments related to faster isohydric responses and photoprotective mechanisms in soybean plants subjected to recurrent drought allowed the maintenance in the weight or number of grains compared to plants without water restriction, demonstrating the activation of efficient memory mechanisms of response to water stress.

Index terms: Gas exchange; *Glycine max*; chlorophyll fluorescence; photoprotection; water stress.

RESUMO

O estresse hídrico é o principal fator abiótico que limita a produtividade da soja. A memória ao estresse hídrico recorrente pode proporcionar maior eficiência na minimização dos efeitos negativos da seca. Assim, o objetivo deste trabalho foi compreender os ajustes temporais na fotossíntese apresentados pela soja quando exposta à ciclos recorrentes de seca, no início da floração e enchimento de grãos. O experimento foi conduzido em blocos casualizados com cinco repetições, composto por quatro tratamentos: i) WS-R1 (déficit hídrico moderado no início da floração), ii) WS-R5 (deficiência hídrica severa durante o enchimento de grãos), iii) WS-R1+R5 (déficit hídrico moderado no início da floração e déficit hídrico severo durante o enchimento de grãos) e iv) WW (condição bem irrigada ao longo de todo ciclo). O estresse severo causou reduções nos parâmetros de trocas gasosas e no teor relativo de água, aumento da fluorescência inicial e eficiência do uso da água. As plantas dos tratamentos WS-R5 e WS-R1+R5 apresentaram redução na taxa aparente de transporte de elétrons no fotossistema II (PSII), extinção fotoquímica e rendimento quântico efetivo de PSII, além de aumento nos valores de extinção não fotoquímica. Além disso, a concentração de prolina nas folhas foi maior nas plantas do tratamento WS-R1+R5, contribuindo para a maior capacidade de manter as células túrgidas em relação às plantas WS-R5. Os ajustes fotossintéticos relacionados a respostas isoídricas mais rápidas e mecanismos fotoprotetores em plantas de soja submetidas a seca recorrente permitiram a manutenção no peso ou número de grãos em comparação com plantas sem restrição hídrica, demonstrando a ativação de mecanismos eficientes de memória de resposta ao estresse hídrico.

Termos de indexação: Trocas gasosas; *Glycine max*; fluorescência da clorofila; fotoproteção; estresse hídrico.

INTRODUCTION

Soybean is an economically important crop worldwide, as it is the main source of oil and proteins in human and animal food (Mertz-henning et al., 2018). However, one-third of the world's population lives in regions with water shortages. With climate change, drought can become more frequent and severe, reducing crop yield. Soybean development is directly affected by water restriction, especially in the initial stages of growth and during flowering and grain filling. Soybeans have a high water requirement during the reproductive development stage, especially at the beginning of flowering (R1) and grain filling (R5). The drought stress imposed during stage R5 is as harmful as that imposed over the entire cycle of the soybean crop (Gava et al., 2016).

Plants have developed various strategies to cope with such drought conditions, exhibiting physiological, biochemical and molecular changes (Bruce et al., 2007; Galviz; Ribeiro; Souza, 2020). Under drought conditions, there is an increase in gene expression and production of stress-responsive proteins. In the recovery period (resumption of irrigation), the return to the basal levels can be observed. When a later stressful event occurs, the plant already has "machinery" that allows for an enhanced response when perceiving the second event. This ability to respond to stress cycles is linked to memory (Crisp et al., 2016; Galviz; Ribeiro; Souza, 2020).

It is well known that the exposition of soybean plants to non-lethal water deficit in different developmental stages affects the plant responses to subsequent drought (Kron; Souza; Ribeiro, 2008). The plant stage in which the first drought occurs is determinant to the recovery capacity after the second event of stress. If the first exposition to drought occurs in the R1 stage, the plant performance after the second exposition can be impaired (Kron; Souza; Ribeiro, 2008). However, despite the large number of studies focused on the water shortage effects on soybean plants (Burle; Rodrigues 1990; Cotrim et al., 2021), the temporal responses during drought and recovery after exposition to the stress at different stages of the soybean crop is poorly understood.

One of the first responses to the reduction in soil water potential is stomatal closure, which reduces water loss through transpiration but limits the photosynthetic capacity (Wang et al., 2018). The temporal dynamics of stomata closure during drought rely on the plant's capacity to set a threshold between the maintenance of carbon fixation and the regulation of the water supply capacity of the hydraulic systems avoiding embolism (Martin-StPaul; Delzon; Cochard, 2017; Volaire, 2018). However, the reduction in the internal concentration of CO_2 in the leaf mesophyll, can cause biochemical limitations due to the lower efficiency of instantaneous carboxylation (Chaves; Flexas; Pinheiro, 2009). The impairment of biochemical processes might be heightened due to a reduction in the electron transport chain, leading to an increase in the dissipation of excess energy by nonphotochemical quenching as a strategy to dissipate excess energy to prevent damage to the photosynthetic apparatus (Oya et al., 2004).

As stress severity increases, plants face drought through the accumulation of high intracellular levels of osmoprotectant compounds to protect cellular components and to restore the osmotic balance (Gurrieri et al., 2020). The accumulation of osmoregulatory compounds, such as proline, can aid in water absorption due to a decrease in the cell's osmotic potential. This decrease leads to the maintenance of water uptake and together with a reduction in transpirational flux, the turgor necessary for cell expansion and the photosynthetic integrity is maintained. In addition, proline stabilizes cytosolic proteins against degradation under dry conditions and reduces reactive oxygen species, minimizing cell damage (Mwenye et al., 2016).

Plant responses to recurrent water stress may vary according to hierarchical levels of observation, such as morphological, physiological, biochemical and molecular (Auler et al., 2021). Mantoan et al. (2020) observed an improvement in photosynthesis and in the antioxidant system and a reduction in dry mass in sorghum plants subjected to recurrent water stress. Walter et al. (2011) also found a reduction in photochemical parameters in grasses and justified it as an improved photoprotection under recurrent stress. According to Ribeiro et al. (2021), sugarcane plants exposed to three cycles of water deficit or propagules originating from stressed plants showed the highest resilience and/or lowest disturbance values when compared to well-irrigated plants or to propagules of well-watered plants.

Therefore, it is expected that soybean plants subjected to recurrent water stress in stages R1 and R5 will have faster responses when exposed to severe stress and will present greater efficiency in recovery and minimization of the effects of water deficit compared to plants subjected to stress only in the R5 stage. The aim of this work was to understand the temporal adjustments in photosynthesis of soybean plants in response to recurrent water stress in stages R1 and R5 that help maintain yield.

MATERIAL AND METHODS

Study area and plant species

The experiment was carried out in a greenhouse at the Federal University of Viçosa *Campus* Florestal (19°52'35.1"W 44°24'49.6"W), from February to May 2018. The soybean (*Glycine max* L.) cultivar used was M5917 IPRO (Monsanto®), which has indeterminate growth and early cycle. The seeds were treated with Vitavax Thiram® fungicide (Carboxanilide + Diamethyldithiocarbamate) 250ml/100 Kg of seed and inoculated with Adhere® 60g/100 Kg of seed. Then, 5 soybean seeds were sown in 10 L pots, with a substrate composed of soil, sand and tanned cattle manure, in a 3:1:1 (v:v) ratio, fertilized with K_20 and P_20_5 at a dosage of 40 kg/ ha (Ribeiro; Guimarães; Alvarez, 1999). When the plants reached the V1 stage (first node) (Fehr; Caviness, 1977) they were thinned, leaving one plant per pot. The experimental unit consisted of one plant per pot, totaling 20 pots.

Application of water stress treatments

When the plants reached the R1 development stage (Fehr; Caviness, 1977), treatments WS-R1 (moderate water deficit in stage R1) and WS-R1+R5 (moderate water deficit in stage R1 and severe water deficit in stage R5) were subjected to moderate water stress until the tension of water in the soil, evaluated by tensiometers in each pot, reached a pressure of 80 kPa. The period of moderate water stress lasted 4 days. Soon after, the soil was irrigated and maintained at field capacity. At the beginning of the grain filling stage (R5), the WS-R5 treatment (plants subjected to severe water deficit at the R5 stage) and the WS-R1+R5 treatment were subjected to water stress. The plants were not irrigated for 7 days until net photosynthesis reached values close to zero; this was characterized as severe stress. After this period, irrigation was resumed, and the recovery of photosynthetic metabolism was monitored for 6 days. There was no water restriction in the control treatment (WW) during the entire crop cycle. Soil moisture in each pot was monitored with tensiometers installed at 15-20 cm soil depth, maintaining the soil at field capacity.

Chlorophyll *a* fluorescence analysis

Measurements of chlorophyll *a* fluorescence were carried out with the aid of a Mini-PAM pulse-modulated fluorometer (Heinz Walz, Effeltrich, Germany). Evaluations were performed on the third fully expanded leaf from the apex of the central leaflet in each replicate. During the 13 days of exposure to water stress and recovery, measurements of the minimum fluorescence (F_0) and maximum fluorescence (F_m) were taken at predawn. The values obtained were used to determine the maximum quantum efficiency of photosystem II (PSII) ($F_v/F_m = (F_m - F_0)/F_m$).

After determination of F_v/F_m, the plant tissue was exposed to a photosynthetic photon flux density (PPFD) of 1000 µmol m^{-2} s^{-1} for 30 seconds, and then a saturating light pulse was applied. From this process, the following variables were determined: F, the fluorescence under *steady state* conditions before the saturating light pulse, and Fm’, the maximum fluorescence of the illuminated plant tissue. Light response curves were assessed on the 7th day of severe stress at noon. The following variables were calculated: effective quantum efficiency of photosystem II (ϕ_{PSII}) (Genty; Briantais; Baker, 1989), nonphotochemical quenching (NPQ) (Bilger; Bjrkman, 1990) and photochemical quenching of PSII (q_L) (Kramer et al., 2004). The minimal fluorescence values for the state acclimated to light (F_0’) were obtained according to Oxborough and Baker (1997). The apparent rate of electron transport (ETR) was calculated as ETR = $\phi_{PSII} \times \text{PPFD} \times \alpha$, where PPFD = photosynthetic photon flux density, α= the product of the leaf absorptivity coefficient and the fraction of excitation energy absorbed between PSII and PSI, defined as 0.47 for soybean (Gallé et al., 2013).

Gas exchange and chlorophyll assessments

Gas exchange evaluations were performed daily in the morning, on the third fully expanded leaf for 13 days, from the imposition of severe water deficit in stage R5 and rehydration. Measurements were performed using an infrared gas analyzer, model LI-6400xt (Li-Cor Inc., Lincoln, Nebraska, USA), with a PPFD of 1200 µmol m^{-2} s^{-1}, a CO_2 concentration of 400 µmol mol^{-1}, an air temperature of 28 °C and air relative humidity of 44%. The following variables were obtained: net photosynthetic rate (*A*); stomatal conductance (g_s); transpiration (*E*), ratio between ambient and internal CO_2 concentrations in chloroplasts (Ci/Ca), instantaneous water use efficiency (WUE; *A*/*E*) and instantaneous carboxylation efficiency (*A*/Ci).

Evaluations of chlorophyll indices (total chlorophyll, chlorophyll *a*, chlorophyll *b* and the chlorophyll *a/b* ratio) were using a portable ClorofiLOG meter (Falker, Brazil). The indices represent the average of three measurements performed on the central leaflet of the third fully expanded leaf from the base apex.

Relative water content and proline determination

To evaluate the relative water content (RWC), a fourth date of the central leaflet of the fully expanded leaf with an area of 14 cm^2 was used, collected on the day of maximum water deficit (7 days after water withhold). Immediately after collection, the samples were weighed to determine the fresh mass and then placed for 24 h in Petri dishes with distilled water at a temperature close to 6 °C, and then the turgid mass was determined. Afterward, the samples were dried in an oven at 65 °C for 72 h, and the dry mass was determined (Turner, 1981). The RWC was obtained from the following Equation 1 :

$$RWC = \frac{\left(Fresh\ mass - dry\ mass\right)x100}{Saturated\ mass - dry\ mass} \quad (1)$$

The proline concentration was determined according to Bates, Waklren and Teare (1973) using 100 mg of leaf dry mass collected on the day of maximum water deficit. The sample was ground in 3% sulfo-salicylic acid (5 ml) in a chilled pestle and mortar. The homogenate was filtered, and 1 ml was taken in a test tube in which 1 ml acid ninhydrin solution (1.25 g ninhydrin+20 ml of 6M orthophosphric acid+30 ml glacial acetic acid) and 1 ml glacial acetic acid were added and heated in a boiling water bath for 1 h at 100°C, and the reaction was terminated by cooling in an ice bath. Two milliliters of toluene were added to the test tube, vortexed for 15–20 s, and allowed to stand, and then, the upper layer (chromophore) was taken. Absorbance was recorded at 520 nm using a spectrophotometer, and the concentration of proline was determined with the help of a standard curve (Bates;Waklren; Teare, 1973).

Morphological variables and production

At stage R8 (Fehr; Caviness, 1977), the length of the root and stem (cm) and the diameter of the stem were determined with a manual caliper (mm). Then, the plants were sectioned into roots, grains, pods, leaves and stems and dried in a forced-air circulation oven for 72 h at 65 °C to determine the dry biomass. The number of grains was also determined in stage R8.

Statistical analysis

The experiment was carried out in completely randomized blocks with five replications consisting of four treatments, namely, i) WS-R1 (moderate water deficit at the beginning of flowering), ii) WS-R5 (severe water deficit during grain filling), iii) WS -R1+R5 (moderate water deficit at the beginning of flowering and severe water deficit during grain filling), and iv) WW (optimal irrigation conditions during the entire cycle).

Data were submitted to analysis of variance (ANOVA) and a means test (Tukey p<0.05) using the statistical program R, version R i386 3.5, and the ExpDes.pt package. Total chlorophyll data were transformed (log *x*) to meet the ANOVA normal distribution assumption. The graphs were created using the SigmaPlot 14.0 program.

RESULTS AND DISCUSSION

Adjustments in gas exchange allowed a faster response to drought in soybean

Plants exposed to moderate water deficit only in stage R1 (WS-R1) did not show a significant difference ($p > 0.05$) in gas exchange compared to the control treatment (WW). After 3 to 4 days of the suspension of irrigation, plants subjected to recurrent water stress in stage R5 (WS-R1+R5) showed a significant reduction in net photosynthesis (A) (Figure 1a). The same occurred with stomatal conductance (g_s) (Figure 1b), transpiration (E) (Figure 1c), the ratio of the internal and external concentrations of CO_2 (Ci/Ca) (Figure 1d) and the instantaneous carboxylation efficiency (A/Ci) (Figure 1e). The maximum point of water stress, when the A of soybean plants exposed to severe stress reached values close to zero, occurred after seven days of stress (Figure 1). At this point, the Ci/Ca ratio of plants under severe stress increased; therefore, there was no significant difference ($p > 0.05$) between treatments (Figure 1d). Five days after the irrigation resumption the recovery of the g_s, Ci/Ca and A/Ci values was observed, with no significant differences compared to treatments WW and WS-R1 (Figure 1b, d, e). In contrast, the A and E of the plants in treatments subjected to severe stress in stage R5 recovered after six days of rehydration (Figure 1a, c).

The decrease in the stomatal conductance in soybean plants under drought, was an efficient mechanism to reduce transpiration and conservation of water, mainly in the WS-R1+R5 treatment, in which the plants presented a better isohydric response. The faster stomatal closure in isohydric plants is related to their higher drought resistance when compared to the anisohydric plants, which keep their stomata open at lower water potential, maintaining photosynthesis, but with higher susceptibility to hydraulic failure (Martin- StPaul; Delzon; Cochard, 2017). The memory of recurrent water stress in plants from WS-R1+R5 treatment allowed a faster isohydric response linked to a conservative strategy when exposed to a severe drought.

The stomatal closure in response to water stress resulted in a decrease in the availability of carbon dioxide in the leaf mesophyll, as evidenced by the values of Ci/Ca and A/Ci (Chaves; Flexas; Pinheiro, 2009). However, in the period of maximum stress, the increase in CO_2 in the leaf mesophyll under severe stress indicated a reduction in carboxylation efficiency in the Calvin-Benson cycle, as shown by the A/Ci result and a significant decrease in F_v/F_m (Maxwell; Johnson, 2000).

The water use efficiency (*WUE*) demonstrates the amount of CO_2 assimilated in relation to the use of water in the transpiration process. The WUE of plants of WS-R5 and WS-R1+R5 increased significantly ($p < 0.05$) at 5 and 6 days after the suspension of irrigation. However, in the period of maximum stress (7 days), the plants subjected to the WS-R5 treatment showed a significant reduction in WUE compared to those in the other treatments, except for WS-R1+R5. After one day of plant rehydration under severe stress, the *WUE* values between treatments were equivalent ($p < 0.05$) (Figure 1f).

With the imposition of water stress, the earlier increase in WUE observed in drought-stressed plants helped them to maintain the relative water content on the 7th day of stress to avoid critical values that could harm the development of the plants (Lawlor; Cornic, 2002). The decrease in WUE occurred on the day of maximum stress because net photosynthesis approached zero. The results of this work agree with those presented by Buezo et al. (2019), which showed that the increase in WUE coupled with photoprotective mechanisms under drought is a key aspect of high-yielding varieties of soybean.

Chlorophyll *a* fluorescence and chlorophyll assessments

The chlorophyll content did not differ statistically between treatments ($p>0.05$) during the period when plants were exposed water stress (Figure 2). However, during rehydration at 8 and 13 days, WS-R1+R5 plants showed a significant decline in chlorophyll *a* compared to WS-R1 and WW treatments (Figure 2a). The levels of total chlorophyll and chlorophyll *b* showed significant differences at 8, 9 and 10 days between the WS-R1+R5 and WS-R5 treatments and the WW and WS-R1 treatments. For these variables, at 12 days, there was also a significant difference between treatments WS-R1+R5 and WW and WS-R1 (Figure 2b, c). During this same period, between 8 to 10 and 12 days, the chlorophyll *a/b* ratio increased significantly in plants from treatments WS-R1+R5 and WS-R5 compared to those from treatments WW and WS-R1 (Figure 2d). The chlorophyll indices of plants subjected to severe stress in stage R5 fully recovered ($p < 0.05$) at 13 days compared to those of treatments without severe stress.

The maintenance of the chlorophyll indices in the period of severe stress in plants of treatments WS-R5 and WS-R1+R5 was possibly due to the insufficient duration and intensity of stress for the degradation of pigments. Reductions in chlorophyll *a* and *b* levels in these plants occurred after rehydration, resulting in an efficient protective mechanism, as they reduced light absorption by the antenna complex and the amount of energy present in PSII. This reduces the possibility of the formation of reactive oxygen species (ROS) by water-restricted plants and provides faster recovery of F_v/F_m. ROS can oxidize cellular components, such as membrane lipids and proteins, and can cause other cell damage, leading to plant cell death (Fang; Xiong, 2015).

There was a significant reduction ($p < 0.05$) in the maximum quantum efficiency of PSII (F_v/F_m) of plants under water stress (WS-R5 and WS-R1+R5) only on the day of maximum stress (7 days). Plants from treatment WS-R1+R5 recovered faster (8 days) than plants exposed solely to severe stress during stage R5 (WS-R5) (Figure 3a). Plants subjected to treatment WS-R1+R5 showed a significant increase ($P < 0.05$) in the values of initial fluorescence (F_0) on the 6th day compared to plants in treatment WS-R5. In the period of maximum stress, the plants of both treatments under severe water stress (WS-R5 and WS-R1+R5) presented significantly higher values of F_0 ($p<0.05$) than plants without a water shortage (WW and WS-R1). This increase in F_0 along with the reduction in F_v/F_m in the period of maximum stress is indicative of the decline in energy transfer to PSII in plants under water stress. The early increase in F_0 (on the 6th day) along with the maintenance of F_v/F_m in the plants in the WS-R1+R5 treatment demonstrates the activation of photoprotection mechanisms more efficiently than in the plants subjected to the WS-R5 treatment (Maxwell; Johnson, 2000). Consequently, the decrease in F_v/F_m in plants under severe stress on the 7th day is indicative of nonphotochemical adjustments in plants exposed to water stress, without permanent damage to the photosynthetic apparatus.

These adjustments allowed the recovery of F_v/F_m values once irrigation resumed, especially in plants exposed to recurrent water stress (WS-R1+R5). On the first day of rehydration, plants from treatments WS-R5 and WS-R1+R5 matched those from treatments WS-R1 and WS-R5 (Figure 3b). This faster recovery of plants in the WS-R1+R5 treatment compared to those in WS-R5 demonstrates the presence of efficient mechanisms in response to water stress that may have helped to maintain yield at the end of the cycle.

At light intensities lower than 400 µmol photons $m^{-2} s^{-1}$, the plants showed similar ETR responses. However, with increasing irradiance, plants under severe stress (WS-R5, WS-R1+R5) showed significant reductions in ETR compared to those of the other treatments (Figure 4a). The effective quantum yield of PSII (ϕ_{PSII}) decreased with increasing irradiance in all treatments (Figure 4b), with significantly lower values in plants under severe stress.

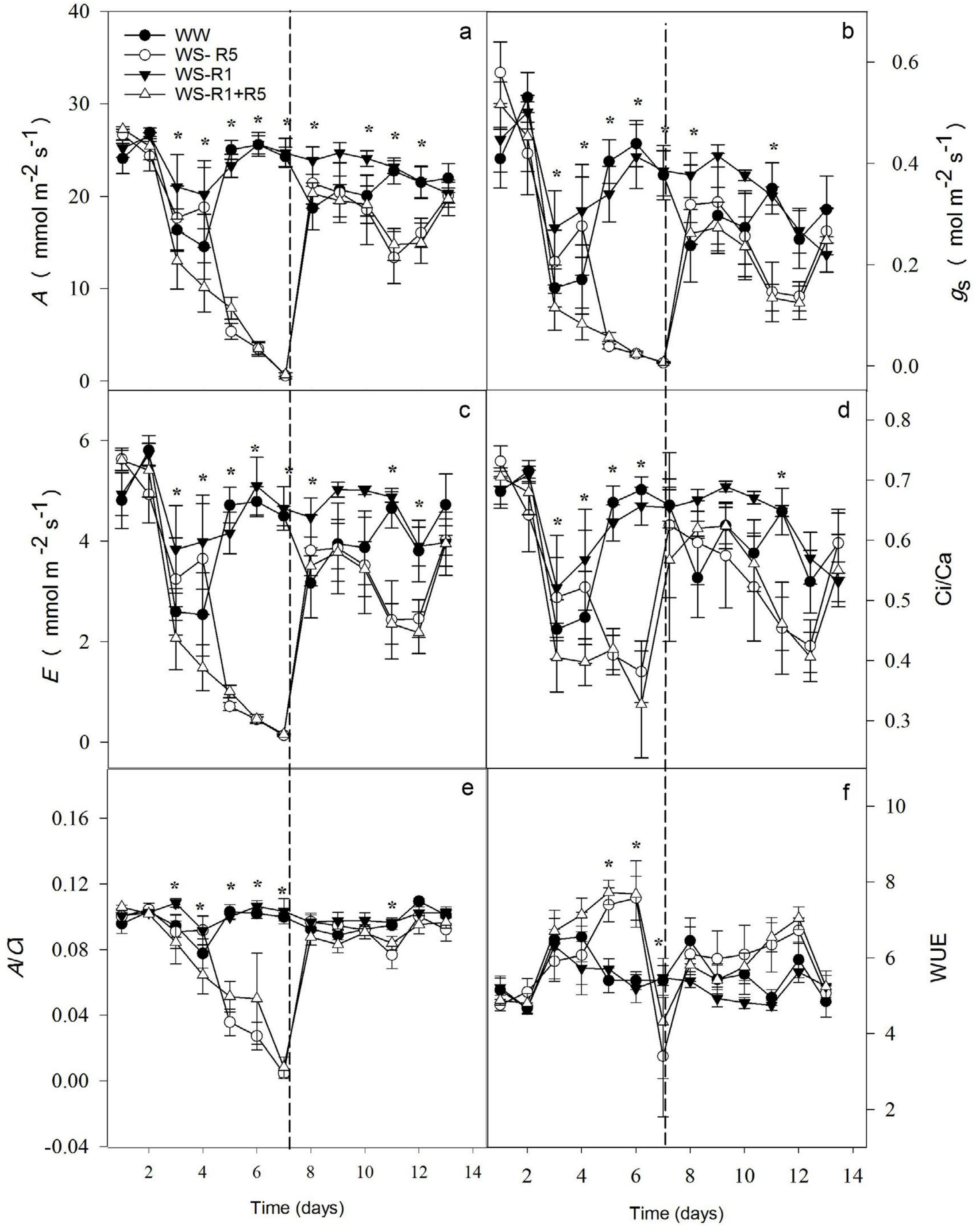

Figure 1: Net photosynthesis (*A*; a), stomatal conductance (g_s; b), transpiration (*E*; c), ratio between ambient and internal CO_2 concentrations in chloroplasts (Ci/Ca; d), instantaneous carboxylation efficiency (*A*/Ci; e) and instantaneous water use efficiency (WUE; f) of soybean plants exposed to different treatments with daily water replacement (WW), moderate stress in stage R1 (WS-R1), severe stress in stage R5 (WS-R5) and moderate stress in stage R1 and severe stress in R5 (WS-R1+R5). The bars indicate the standard error of the mean of five repetitions, the dotted line represents rehydration, and the asterisk indicates a significant difference between at least two treatments based on the Tukey test (p<0.05).

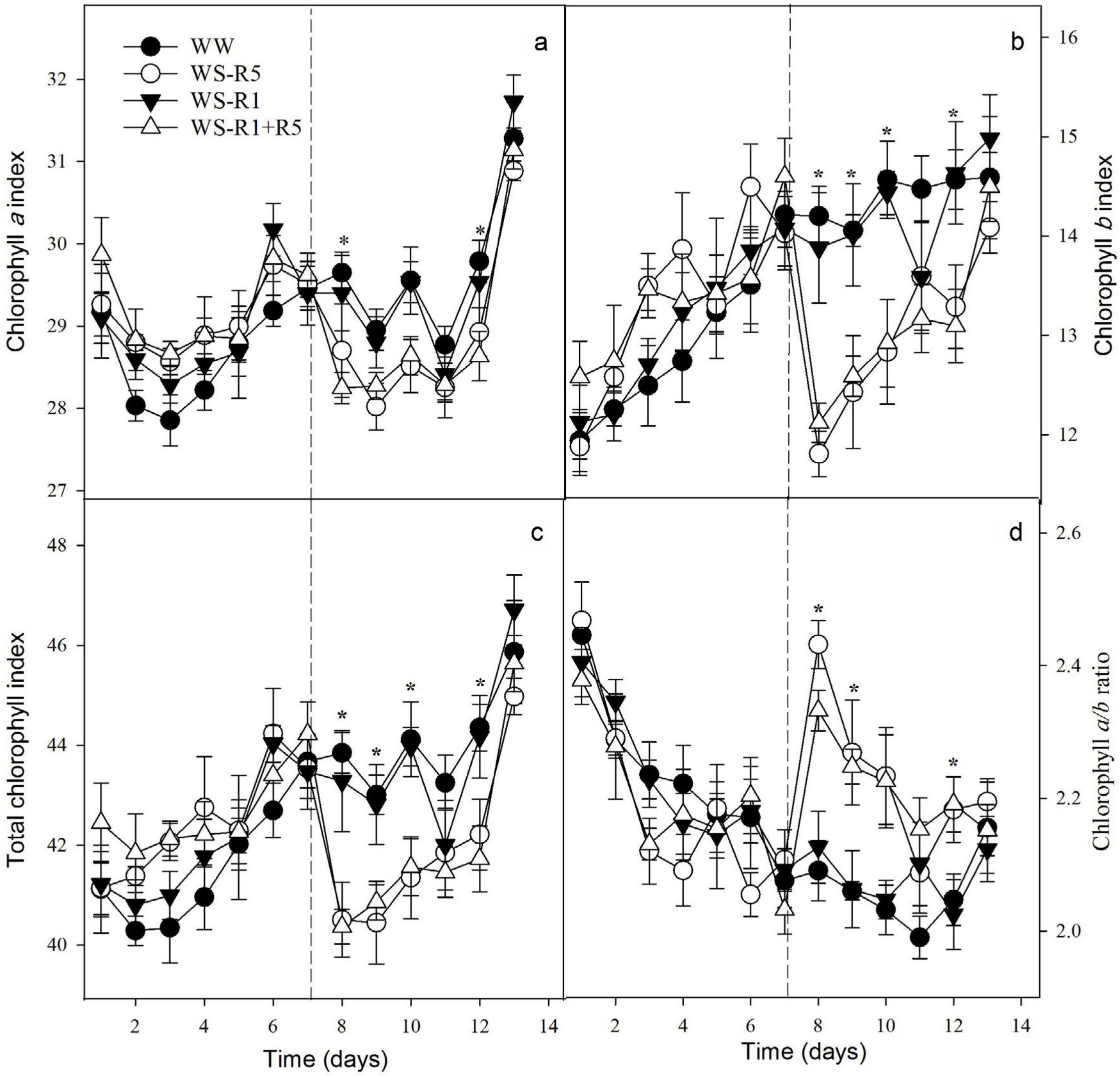

Figure 2: Indices of chlorophyll *a* (a), chlorophyll *b* (b), total chlorophyll (c) and the chlorophyll *a/b* ratio (d) in soybean plants exposed to different treatments with daily water replacement (WW), moderate stress in stage R1 (WS-R1), severe stress in stage R5 (WS-R5) and moderate stress in stage R1 and severe stress in R5 (WS-R1+R5). The bars indicate the standard error of the mean of five repetitions, the dotted line represents rehydration, and the asterisk indicates a significant difference between at least two treatments based on the Tukey test (p<0.05).

Only at high irradiances (above 900 μmol photons $m^{-2} s^{-1}$) did that plants from treatments WW and WS-R1 present q_L values significantly higher than those of the other treatments (Figure 4c). The lower values of photochemical quenching (q_L), the effective quantum yield of PSII and the apparent electron transport rate (ETR) in the maximum stress period contributed to nonstomatic limitations of photosynthesis in plants exposed to drought in the R5 stage, thus reducing the production of NADPH and ATP but increasing nonphotochemical quenching (NPQ) (Flexas; Medrano, 2002).

At all levels of irradiance, plants from treatments WS-R5 and WS-R1+R5 showed higher values ($p<0.05$) of nonphotochemical dissipation than those from treatments WW and WS-R1 (Figure 4d). The increase in NPQ in plants under severe stress demonstrates a mechanism for dissipating excess energy as heat, thus protecting the photosynthetic apparatus against oxidative damage to PSII due to excess energy (Baker, 2008; Pereira et al., 2013). This mechanism also prevents the formation of ROS, thus protecting against oxidative damage in the thylakoid membrane (Demmig-Adams et al., 2017; Murchie; Ruban, 2020).

Proline accumulation is a key component of soybean responses to recurrent drought

The adjustments in gas exchange variables and photoprotection during the temporal responses to drought in soybean, required adjustments in a later stage, with the accumulation of osmotically active compounds, such as proline. The leaf proline concentration was significantly higher ($p < 0.05$) in plants from the WS-R1+R5 treatment compared with other treatments (Figure 5a). The increase in the concentration of proline in plants subjected to recurrent water deficit (WS-R1+R5) is an alternative to minimize the effect of water deficit since this reduces the water potential, helping to reduce water loss from plant cells (Taylor, 1996; Szabados; Savouré, 2010).

Soybean plants exposed to severe stress (WS-R5, WS-R1+R5) showed a significant reduction in the relative leaf water content (RWC) compared to plants without severe water stress (WW, WS-R1) (Figure 5b). The combined responses of initial stomatal closure and solute accumulation in plants from WS-R1+R5 treatment, allows the retention of water under very high tension (Martin-StPaul; Delzon; Cochard, 2017) and allowed a better recover during rehydration with the maintenance of yield. In addition, proline also contributes to carbon and nitrogen storage and can be used for protein synthesis in response to water deficit and in the TCA cycle during stress recovery (Hare; Cress, 1997).

The adjustments to recurrent drought in soybean did not affect morphological variables, but resulted in the maintenance of production

Plants exposed to moderate water stress (WS-R1) did not show significant changes in the morphological variables evaluated in relation to the control (WW) (Table 1). Plants subjected to moderate and severe stress (WS-R1+R5) maintained grain production (mass and number) and stem length (Table 1), but the plants in the WS-R5 treatment showed a significant decline in total and stem dry masses (Table 1) compared to control plants (WW). There was no significant difference ($p > 0.05$) between treatments in terms of root dry mass, pods, leaves, root length or stem diameter (Table 1).

Soybean water stress tolerance should result in the ability to minimize negative effects related to yield (Hasanuzzaman et al., 2016). The maintenance of the mass and number of grains of plants under recurrent stress (WS-R1+R5) in relation to plants grown under optimal development conditions (WW) indicates possible adjustments in grain production related to water stress memory (Dolferus; Ji; Richards, 2011).

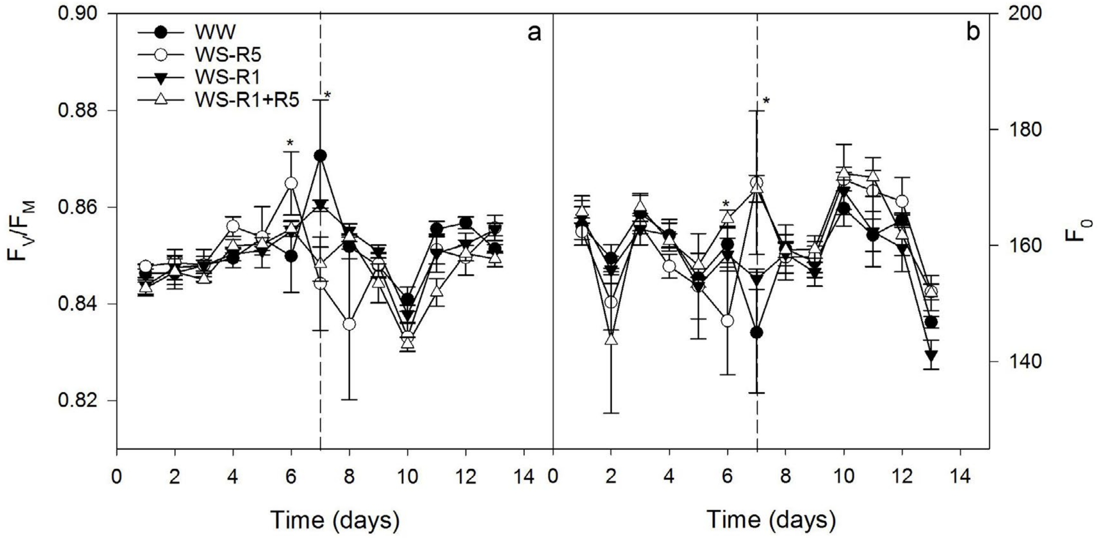

Figure 3: Maximum quantum efficiency of PSII (F_v/F_m; a) and initial fluorescence (F_0, b) of soybean plants exposed to different treatments with daily water replacement (WW), moderate stress in stage R1 (WS-R1), severe stress in stage R5 (WS-R5) and moderate stress in stage R1 and severe stress in stage R5 (WS-R1+R5). The bars indicate the standard error of the mean of five repetitions, the dotted line represents rehydration, and the asterisk indicates a significant difference between at least two treatments based on the Tukey test (p<0.05).

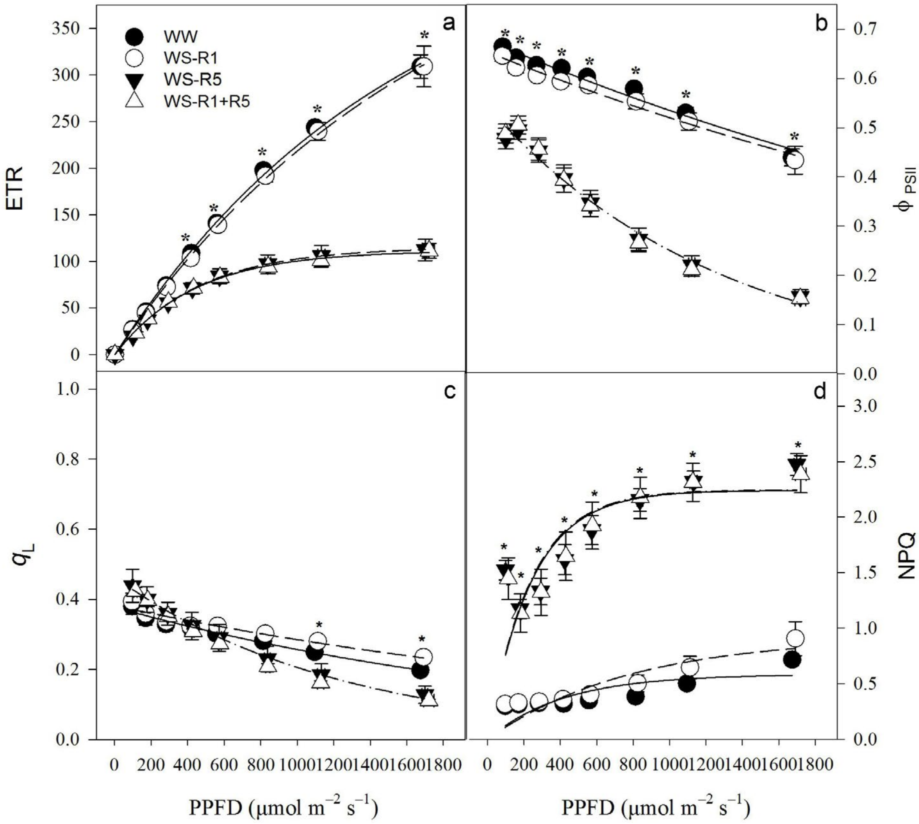

Figure 4: Light response curves of the PSII apparent electron transport rate (ETR, a), effective quantum yield of PSII (f_{PSII}, b), photochemical quenching (q_L, c) and nonphotochemical quenching (NPQ, d) in soybean plants exposed to different treatments with daily water replacement (WW), moderate stress in stage R1 (WS-R1), severe stress in stage R5 (WS-R5) and moderate stress in stage R1 and severe stress in stage R5 (WS-R1+R5). The assessment was carried out on the 7th day of severe stress at noon. The bars indicate the standard error of the mean of five repetitions, and the asterisk indicates a significant difference between at least two treatments based on Tukey's test (p<0.05).

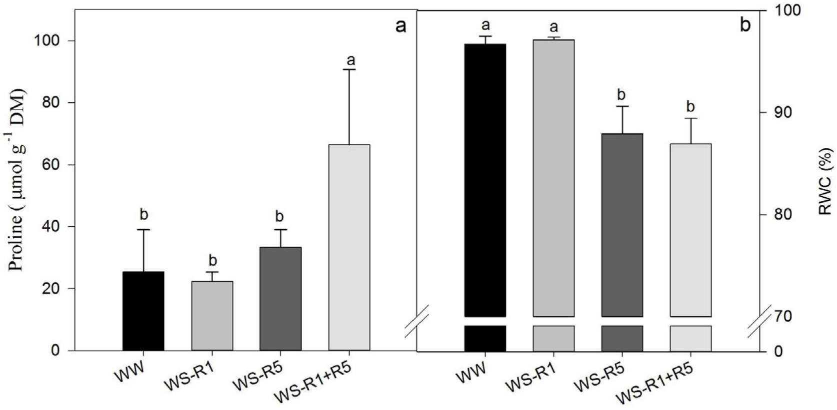

Figure 5: Proline concentration (a) and the relative leaf water content (RWC, b) in soybean plants exposed to different treatments with daily water replacement (WW), moderate stress in stage R1 (WS-R1), severe stress in stage R5 (WS-R5) and moderate stress in stage R1 and severe stress in stage R5 (WS-R1+R5). The bars indicate the standard error of the mean of five repetitions, and the different letters indicate a significant difference based on the Tukey test (p<0.05).

Table 1: Dry mass of grains, pods, leaves, stem, root, total dry mass, stem and root length, stem diameter and number of grains of soybean plants exposed to different treatments with daily water replacement (WW), moderate stress in stage R1 (WS-R1), severe stress in stage R5 (WS-R5) and moderate stress in stage R1 and severe stress in stage R5 (WS-R1+R5). Evaluations were carried out in stage R8. Means followed by different letters show differences between the morphological variables evaluated according to the Tukey test (p<0.05). Means ± standard error of five repetitions.

	Dry mass (g)						Length (cm)		Diameter of stem (mm)	Number of grains
Treatments	grains	Pods	leaves	stem	root	total	stem	root		
WW	16.10±1.00a	11.24±0.65a	3.13±1.03a	5.27±0.34a	2.97±0.44a	38.92±1.43a	46.4±1.50a	47.2±4.09a	7.9±0.49a	122.8±7.14ab
WS-R5	13.53±0.29b	8.62±1.16a	1.89±0.79a	3.54±0.26b	2.15±0.19a	29.90±1.31c	38.2±1.56b	50.4±4.80a	7.76±0.66a	89±2.34c
WS-R1	16.02±1.10a	10.62±0.46a	2.93±0.36a	4.65±0.2ab	2.68±0.14a	36.10±1.75ab	43.6±1.20ab	47±2.09a	7.22±0.22a	124.8±5.20a
WS-R1+R5	15.30±0.34ab	8.47±0.43a	2.88±1.04a	4.06±0.36b	2.24±0.26a	33.02±1.91bc	43.6±1.39 ab	45.6±4.22a	6.62±0.26a	103.2±5.40bc

Under water stress soybean can increase the root/shoot ratio by partitioning carbon to the root system, which results in increased root length and dry mass, culminating in greater exploration of deeper soil layers in search of nutrients and water (Hasibeder et al., 2015). However, in this work, even when using vessels measuring 10L, root growth may have been limited, with no significant differences, as observed by other works (Chavarria et al., 2015; Kron; Souza; Ribeiro, 2008).

The accumulation of total dry mass is important for soybean productivity; however, it is affected by water stress. The decrease in plant biomass under severe stress is related to the effects on stomatal closure and decreased net photosynthesis (Silva et al., 2020). However, the nonsignificant difference in grain dry mass between WW and WS-R1+R5 demonstrates memory to minimize the effect of stress, maintaining the production of photoassimilates, partitioning them, and allocating them more efficiently, resulting in the maintenance of grain production.

CONCLUSIONS

Photosynthetic adjustments related to faster isohydric responses and photoprotective mechanisms beyond proline accumulation, demonstrate the memory development in response to water stress in soybean plants subjected to moderate stress before severe stress (WS-R1 + R5). Plants subjected to recurrent water stress recovered the effective quantum yield earlier than WS-R5 plants when irrigation was resumed. The WS-R1+R5 plants showed that the weight and number of soybeans were statistically equivalent to those of the plants without water restriction (WW).

AUTHOR CONTRIBUTION

Conceptual idea: Pereira, E.G. ; Methodology design: Pereira, E.G.; Medina, I.R. ; Rocha, G.H. ; Data collection: Medina, I.R. ; Rocha, G.H. ; Data analysis and interpretation: Pereira, E.G. ; Medina, I.R. ; Rocha, G.H. ; Writing and editing: Pereira, E.G.; Medina, I.R.

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