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ORIGINAL ARTICLE

Severe drought stress strongly impacts physiological and morphoanatomical traits of soybean cultivars during vegetative stage

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Abstract

Several studies have investigated soybean response to drought, revealing significant changes in different physiological and anatomical traits. Investigating how plants respond to regulated deficits can help optimize water use efficiency and shed light on their tolerance and sensitivity to water stress at different phenological stages. Thus, this study aimed to assess the effect of water stress during the vegetative stages on the morphophysiological and water status characteristics of soybean cultivar. The experiment was conducted in a greenhouse at the State University of Maranhão. A completely randomized design with four irrigation levels: 100% field capacity (FC) (control), 75% FC, 50% FC, and 25% FC, and 20 replicates were used. Growth variables, physiological parameters, water-related parameters, and root, stem, and leaf anatomy were evaluated. Interaction was observed between water levels and assessment times for plant height and stem diameter growth rates. There was a significant reduction in gas exchange over time, with the greatest decline at 81 days after planting (DAP). The 25% FC treatment had the most pronounced effect on the photosynthetic CO_2 assimilation (A), stomatal conductance (g_s), transpiration (E), and carboxylation efficiency (CE), with reductions of 55, 72, 53, and 60%, respectively, compared to the control, at 80 DAP. Anatomical analysis revealed adaptations in the 25% FC treatment, characterized by increased stem sclerenchyma thickness and smaller root xylem vessel diameter. Plants in the 75% FC treatment showed similar responses to the control, demonstrating that this water regime did not compromise the growth and development of BRS Tracajá soybean plants.

Keywords Gas exchange · *Glycine max* · Water deficit · Plant anatomy

Introduction

Climate conditions play a pivotal role in determining favorable agricultural production. It is therefore essential to embrace innovative approaches to comprehend the impact of various climate scenarios on plant growth, encompassing physiological and phenological traits (Guo et al. 2022). Soybean (*Glycine max* (L.) Merr.) is an economically important crop worldwide (Santos et al. 2022). In the last century, breeding programs have developed highly productive soybean cultivars adapted to different environmental conditions. However, growing climate instability, which is predicted to affect rainfall and temperature, has raised concerns about the sustainability of soybean yields (Anderson et al. 2019). Several studies have investigated soybean response to drought, revealing significant changes

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in physiological and anatomical traits such as leaf area, gas exchange dynamics, chlorophyll fluorescence, and photosynthetic pigment levels (Chowdhury et al. 2016; Wang et al. 2018; Basal and Szabó 2018). Furthermore, at the anatomical level, differences in the continuity and thickness of the sclerenchyma layer and smaller xylem diameters have also been observed (Makbul et al. 2011; Prince et al. 2017).

The hydraulic efficiency of the xylem is closely linked to anatomical traits related to xylem vessel diameter and configurations (Lens et al. 2013). To ensure robust shoot growth, plants need an efficient root system to uptake water and essential nutrients. Root water uptake and its subsequent transport throughout the plant occur in a continuous flow, driven leaf evaporation. This establishes a hydraulic pathway known as the soil-plant-atmosphere *continuum*. In this context, high root hydraulic conductivity plays a pivotal role in supplying water to the aerial parts of plants (Figueiredo et al. 2014; Lopes et al. 2019).

Thus, investigating how plants respond to regulated deficit irrigation can help optimize water productivity (Fernández et al. 2020) and shed light on their tolerance and sensitivity to water deficit at different phenological stages (Coelho et al. 2020). This study aimed to assess the effects of drought stress during the vegetative stages on the growth, physiological, anatomical, and water status characteristics of soybean cultivar.

Materials and methods

Plant material

BRS Tracajá, a conventional soybean cultivar launched in 2003 by the Brazilian Agricultural Research Corporation (Embrapa) and the Research Support Foundation of the North Export Corridor (Fapcen), was used in this study. It is recommended for cultivation in the Brazilian North and Northeast regions. It has the following phenotypic characteristics: determinate growth, 108 to 120-day cycle, average nutritional requirements, lodging resistance, good production stability, and high physiological seed quality. It also exhibits a wide range of yields in response to genotype-environment interaction, performing differently according to variations in soil and climate conditions (El-Husny et al. 2003).

The seeds were inoculated with *Rhizobium* bacteria (Embrapa Agrobiology, RJ, Brazil) and sown in 8 L plastic pots. The substrate consisted of a soil, sand, and manure mixture 4:2:1. After germination, thinning was performed, leaving one plant per pot, representing one replicate. Irrigation was carried out daily, maintaining soil moisture at field capacity until the treatments began.

Experimental site, growing conditions, and treatments

The experiment was conducted in a greenhouse at the State University of Maranhão (2°35’30.5"S, 44°12’43.4"W) between May and November 2018. Climate variables (Fig. 1) such as temperature, relative humidity, and photosynthetically active radiation (PAR) were monitored using a set of data loggers (Spectrum Technologies, Illinois, USA) positioned above the crop canopy. Vapor pressure deficit (VPD) was calculated according to Jones (1992).

A completely randomized design with four treatments was used, consisting of irrigation at 100% field capacity (FC) (control), 75% FC, 50% FC, and 25% FC (Mwamlima et al. 2022), with 20 replicates. The treatments began when the plants reached stage V3 (15 days after planting, DAP). FC was estimated using five pot extra with soybean plants in addition to the main experiment. Trays were placed under each pot and the plants were irrigated with a known volume of water, sufficient to saturate the substrate. After complete drainage (approximately 30 min), the volume of drained water was measured, and the difference between the applied and drained volume represented the amount of water stored by the substrate, which was used for 100% FC. This procedure was performed daily in the late afternoon throughout the experimental period.

Growth variables

Plant height and stem diameter were assessed every three days after stress imposition. Height (cm) was measured using a measuring tape and diameter (mm) at the base of the stem with a stainless steel 150 mm digital pachymeter.

Specific leaf mass (SLM, g cm^{-2}) was determined at the end of the experiment by calculating the ratio of the area of fifteen 0.23 cm-wide leaf disks per plant to their dry mass (g), according to Matos et al. (2015). Total leaf area (LA, cm^2) was calculated by measuring the total area of the individual canopies in all the experimental units using a scanner and conveyor belt system (WinDIAS 3.2, Delta-T Devices).

Root volume (RV, cm^3) was measured via water displacement in a graduated cylinder. After washing, the roots were placed in a cylinder containing a known water volume. RV was calculated as the difference between water volume before root addition and water volume after root addition, applying the equivalence of units (1 mL = 1 cm^3). The number of nodules (NN) was determined by manual counting (Lopes et al. 2019; Nget et al. 2022). The plant parts were separated and dried in a forced-air oven at 65 °C until constant weight to determine leaf dry mass (LDM, g), stem dry mass (SDM, g), root dry mass (RDM, g), and nodule dry mass (NDM, g).

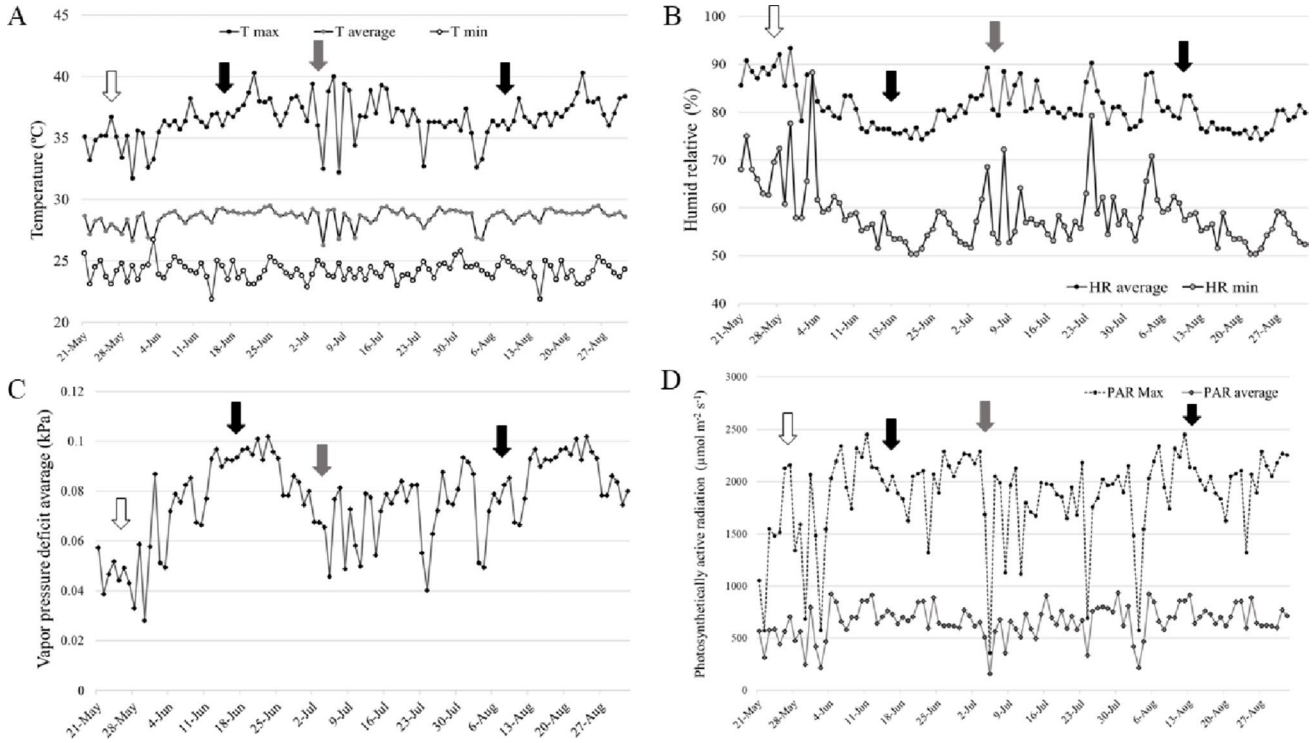

Fig. 1 Data climate collected during the experimental period (may – nov/2018, in a greenhouse, São Luís, Maranhão, Brazil. A – Temperature average, maximum and minimum, B – humid relative (HR), C – Vapor pressure deficit (VPD), and D – photosynthetically active radiation. White arrow: start of application of treatments; black arrows: physiological assessments; gray arrow: assessment of leaf water potential

Physiological parameters

Gas exchange, chlorophyll fluorescence, and greenness intensity were measured between 8:00 and 10:00 am on the fully expanded central leaflet of the trifoliate in the upper third of the plants at 38, 44, and 82 DAP, which corresponds to the transition from stages V4 to V5, transition from stages V5 to V6, and the end of the vegetative cycle and the beginning of the reproductive stages, respectively (Kumudini 2010). The photosynthetic CO_2 assimilation (A, µmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), and internal CO_2 concentration (Ci, µmol m^{-2} s^{-1}) were determined with an LI-6400 infrared gas analyzer (IRGA, Li-Cor), calibrated for 1500 µmol m^{-2} s^{-1} of photosynthetically active radiation and 400 ppm of CO_2. Water use efficiency (WUE, µmol mol^{-1}) and carboxylation efficiency (CE, [(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$]) were calculated via the A/E and A/Ci ratios, respectively. The Chlorophyll Index was estimated using a portable chlorophyll meter (SPAD-502), performing ten readings on one leaf per plant. Chlorophyll fluorescence variables, established based on variations in maximum efficiency of PSII (Fv/Fm) and photosynthetic index (PI), were measured using a Pocket-PEA fluorometer (Hansatech).

Estimation of water-related parameters

Leaf water potential (LWP, MPa) was measured predawn and at midday in leaves from the middle third of the canopy, at 44 DAP, using a Scholander pressure chamber (Soil Moisture, PMS Instrument Company) with N_2 gas.

Root hydraulic conductivity (RHC, m^3 H_2O cm^3 $root^{-1}$ MPa^{-1} s^{-1}) was determined at 60 DAP with a Scholander pressure chamber (Soil Moisture, PMS Instrument Company). By the methodology of Tyree and Zimmerman (2003), adapted by Figueiredo et al. (2014), plant stems were cut 7 cm from the base. The root system was then placed in a water container inside a pressure chamber. Increasing pressures of 0.1, 0.2, 0.3, and 0.4 MPa were applied for 4 min, and all the exuded water was collected and weighed between the 3rd and 4th minute on a precision analytical balance. The collection time was established based on a previous study that indicated flow stabilization between the 2nd and 5th minute within a 10-minute interval for all the treatments and pressures tested, and the collection interval was set between the 3rd and 4th min.

Anatomical analyzes

For leaf samples, a trifoliate was taken from the middle third of the plant, while for stem and root samples, 4 cm sections were removed 6 cm from the stem and root tips. After removal, the samples were immediately immersed in 50% FAA fixative solution (50% alcohol, formaldehyde, and acetic acid at a ratio of 90:5:5) for 24 h and then permanently stored in containers with 50% alcohol. Semi-permanent slides of root, stem, and leaf samples were manually sectioned according to the methodology of Jorge et al. (2006). The sections were then cleared in a 50% hypochlorite solution and stained with basic fuchsin, which highlights secondary tissues such as the xylem and sclerenchyma. The sections were mounted in 50% glycerin solution, sealed with acrylic varnish, and then selected and photographed using a Leica® optical microscope equipped with a camera and Laz Ez 3.4.0 software. The images were analyzed using Image J software, with the thickness (µm) and area (µm^2) of the following tissues measured on the leaf midrib and stem laminae: cortex (comprising epidermis, collenchyma, and cortical parenchyma), phloem sclerenchyma (strip below the cortex), phloem, and xylem.

To minimize differences in the thickness and total area of the tissues identified in the stem and leaf midrib slides, the measurements were expressed as percentages to allow comparison of thickness and area for all the tissues. In the root sections, the average diameter of xylem vessels and the proportion of vessels with diameters up to 20 µm, >20 to 30 µm, >30 to 40 µm, >40 to 50 µm, and >50 µm were evaluated. In addition, the flow-conducting area of the root xylem was compared between treatments, calculated by the ratio between the sum of the total diameter of the vessels (µm^2) and the total area of the xylem tissue in the section (µm^2).

Statistical analysis

Data on the effect of treatments on plant height, stem diameter, gas exchange, SPAD index, and chlorophyll fluorescence parameters were submitted to analysis of variance (ANOVA) in a split-plot design, with interaction between treatments and assessment times. The remaining data were analyzed by one-way ANOVA. Data normality was assessed by the Shapiro-Wilk test and means were compared using Tukey's test at 5% significance. Principal component analysis was performed between treatments and variables that showed significant differences to understand the influence of water deficit on the variables studied. All statistical analyses were performed using R software version 4.3.1 with the "ExpDes.pt", "ggplot2" and "factoextra" packages.

Results

Shoot growth responses of soybean plants under water deficit

The height and stem diameter growth in all treatments were significant and positively correlated with the days after the imposition of water restriction treatments. Differences were also observed among the treatments ($p<0.001$; Fig. 2A and B).

Regarding dry matter, the treatment that most affected this parameter was 25% FC, with a 73% decrease in leaf dry mass (Fig. 3A) and 80% in stem dry mass (Fig. 3B) about the control (100% FC) ($p<0.05$). There was no difference between 75% FC and the control for leaf area, whereas the 25% FC treatment negatively affected leaf area, with an 84% decline in leaf area concerning the control (Fig. 3C). Specific leaf mass increased by 13% at 25% FC level when compared to the control (Fig. 3D) ($p<0.05$).

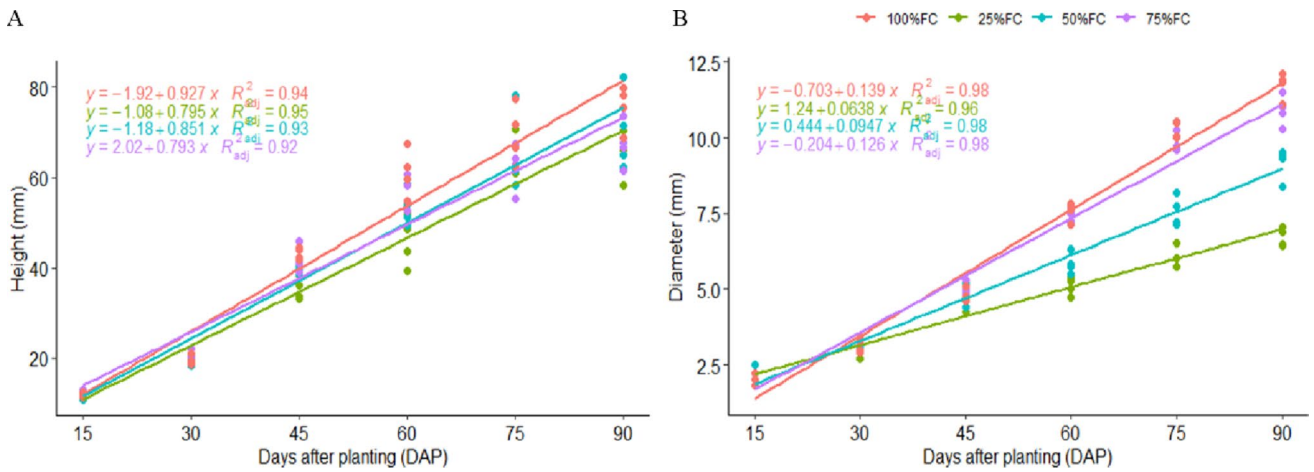

Fig. 2 Growth rate of soybean plants height and diameter under different field capacity (FC) levels and assessment times ($n=4$)

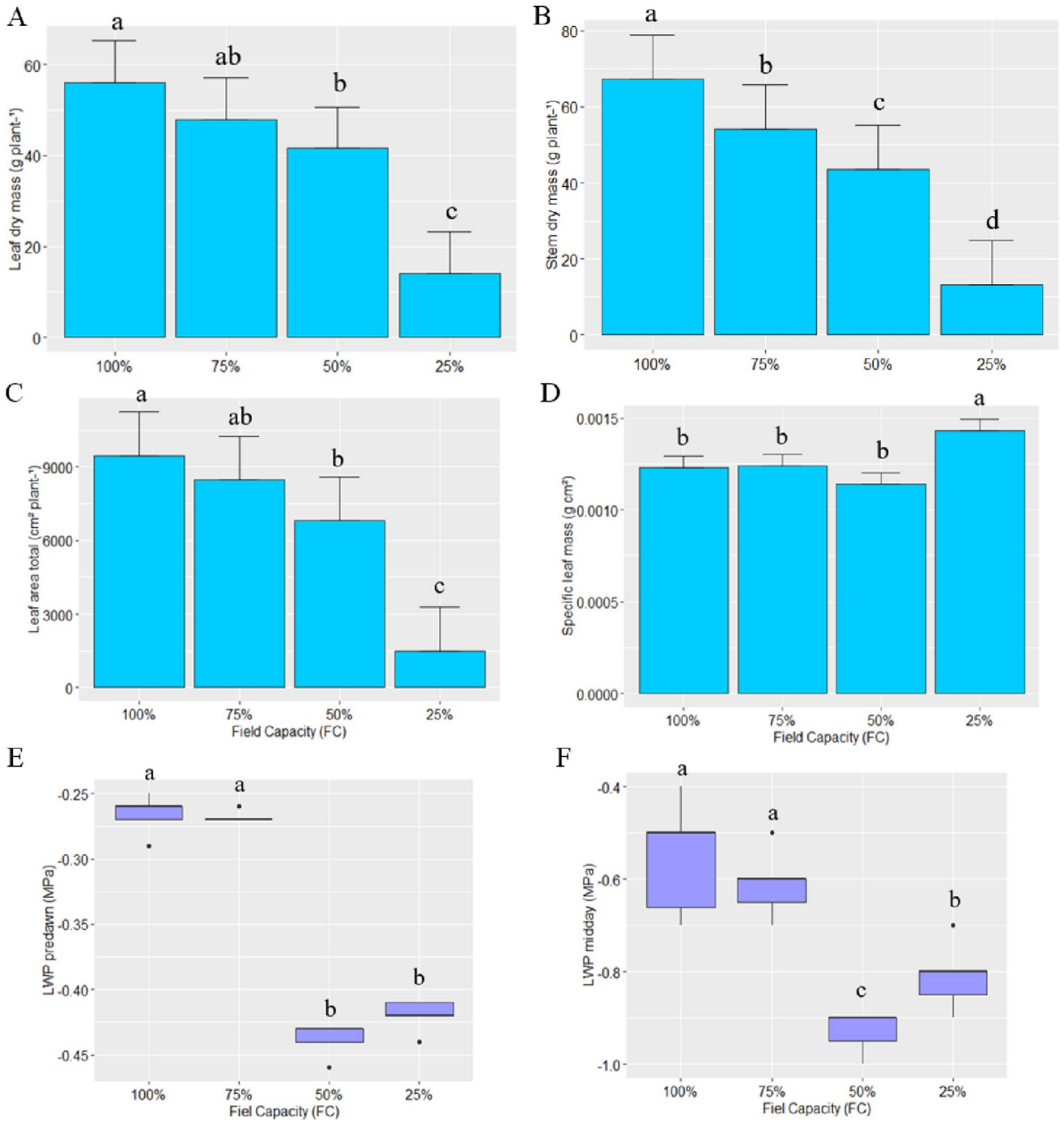

Fig. 3 Leaf dry mass (**A**) and stem dry mass (**B**), total leaf area (**C**), specific leaf mass (**D**) and predawn leaf water potential (**E**) and midday leaf water potential (**F**) of soybean plants under different field capacity (FC) levels ($n=4$). Means followed by the same letter do not differ according to Tukey's test ($p<0.05$). The bar graphs represent means ± standard error (A-D). The boxplots represent the replicates ($n=4$; E-F). The analyses and collections were conducted 90 days after planting

The mean comparison showed no difference between the control and 75% FC treatments for predawn and midday LWP (Fig. 3E and F). At 25 and 50% FC, predawn LWP declined by 81 and 93%, respectively, about the control (Fig. 3E). The lowest midday LWP was obtained at 50% FC, with a value 96% lower than that observed in control ($p<0.001$) (Fig. 3F).

In the leaf midrib, the most significant cortical thickness and proportion concerning other vessels occurred in the water-restricted treatments. At the same time, the phloem scleraemic exhibited an equivalent total area across all the

treatments. Among the conducting tissues, the most significant xylem thickness and area were observed at 25%, 75%, and 100% FC and the lowest at 25%. At the same time, the phloem displayed proportional thicknesses and areas across all the treatments, i.e., no difference between the means (Table 1).

In the stem, the thickness, area, and proportion of cortical and phloem tissues and the relationship between conducting tissues remained unchanged ($p<0.05$). Phloem sclerenchyma showed equivalent areas in all the treatments, and the xylem exhibited proportional thickness across treatments, with a larger area at 75% FC compared to 25% FC (Table 1). Vessel anatomy in the cortex, sclerenchyma, phloem, and xylem showed the same organizational pattern in all the treatments. Additionally, comparing stem and leaf sections from the different treatments indicated larger xylem vessels at 75% FC (Fig. 4).

Physiological responses of soybean plants under water restriction

There was a significant reduction in gas exchange over time ($p<0.001$, Table 2), with the most significant decrease observed at 81 DAP (Fig. 5). The 25% FC treatment had the most pronounced effect on *A*, *gs*, *E*, and CE, which declined by 55, 72, 53, and 60%, respectively, about the control at 81 DAP. The highest WUE was observed at 25% FC and 44 DAP, exhibiting an increase of 33% compared to the control. There was no interaction between treatments and assessment times for the SPAD index and Fv/Fm ($p>0.05$). At 81 DAP, PI differed between treatments, with the most significant reduction recorded at a 50% level (32% lower than the control) (Fig. 5).

Root responses of soybean plants under water deficit

There was no difference between the 75% FC and control treatments for nodule number (NN) and root volume (RV) ($p<0.05$). The 25% FC level negatively affected NN and RV, which declined by 85% and 81%, respectively, when compared to the control. Root dry mass (RDM) was most affected at 25% FC with reductions of 81% about the control (100% FC). There was a difference ($p<0.05$) among the means for RHC, with the highest average value (5.53 m^3 H_2O cm^3 $root^{-1}$ MPa^{-1} s^{-1}) recorded at 100% FC. The 75% FC treatment showed a similar response to the control (5.09 m^3 H_2O cm^3 $root^{-1}$ MPa^{-1} s^{-1}), while average RHC declined by 50.5% at 50 and 25% FC compared to 100% FC (Fig. 6).

Root anatomy demonstrated a decrease ($p<0.05$) in average vessel diameter at 25% FC, whereby a most significant percentage of vessels had diameters up to 20 µm and a lower percentage above 40 µm. Vessels with diameters between 20 and 40 µm showed no statistical difference among the

Table 1 Thickness and area of leaf midrib and stem tissue in soybean plants at 25, 50, 75, and 100% field capacity (FC) ($n=4$)

Anatomical traits			Treatments			
			100%FC	75%FC	50%FC	25%FC
Leaf midrib		Cortex	33.4 b	34.8 ab	37.9 a	35.1 ab
		Phloem scleraemic	13.7 a	12.0 b	13.0 ab	12.3 ab
	Thickness (%)	Phloem	12.2 a	12.7 a	12.2 a	11.8 a
		Xylem	40.7 a	40.4 ab	37.7 b	40.8 a
		Phloem/xylem	0.30 a	0.32 a	0.33 a	0.29 a
		Cortex	44.3 b	45.4 ab	49.2 a	45.6 ab
		Phloem scleraemic	16.5 a	15.2 a	14.3 a	15.3 a
	Area (%)	Phloem	11.6 a	12.9 a	11.0 a	11.2 a
		Xylem	27.4 a	26.3 ab	25.3 b	27.7 a
		Phloem/xylem	0.42 ab	0.49 a	0.43 ab	0.40 b
Stem		Cortex	17.6 a	16.6 a	19.7 a	16.0 a
		Phloem scleraemic	7.40 b	7.70 ab	9.90 a	7.70 ab
	Thickness (%)	Phloem	13.5 a	17.9 a	15.0 a	16.3 a
		Xylem	61.5 a	57.8 a	55.5 a	60.0 a
		Phloem/xylem	0.23 a	0.32 a	0.27 a	0.28 a
		Cortex	18.8 a	21.3 a	25.0 a	20.4 a
		Phloem scleraemic	9.00 a	9.60 a	10.6 a	8.70 a
	Area (%)	Phloem	15.9 a	21.7 a	18.3 a	18.8 a
		Xylem	56.3 a	47.3 ab	46.0 b	52.0 ab
		Phloem/xylem	0.30 a	0.46 a	0.40 a	0.38 a

Means followed by the same letter do not differ according to Tukey's test ($p<0.05$)

The collections were conducted 90 days after planting

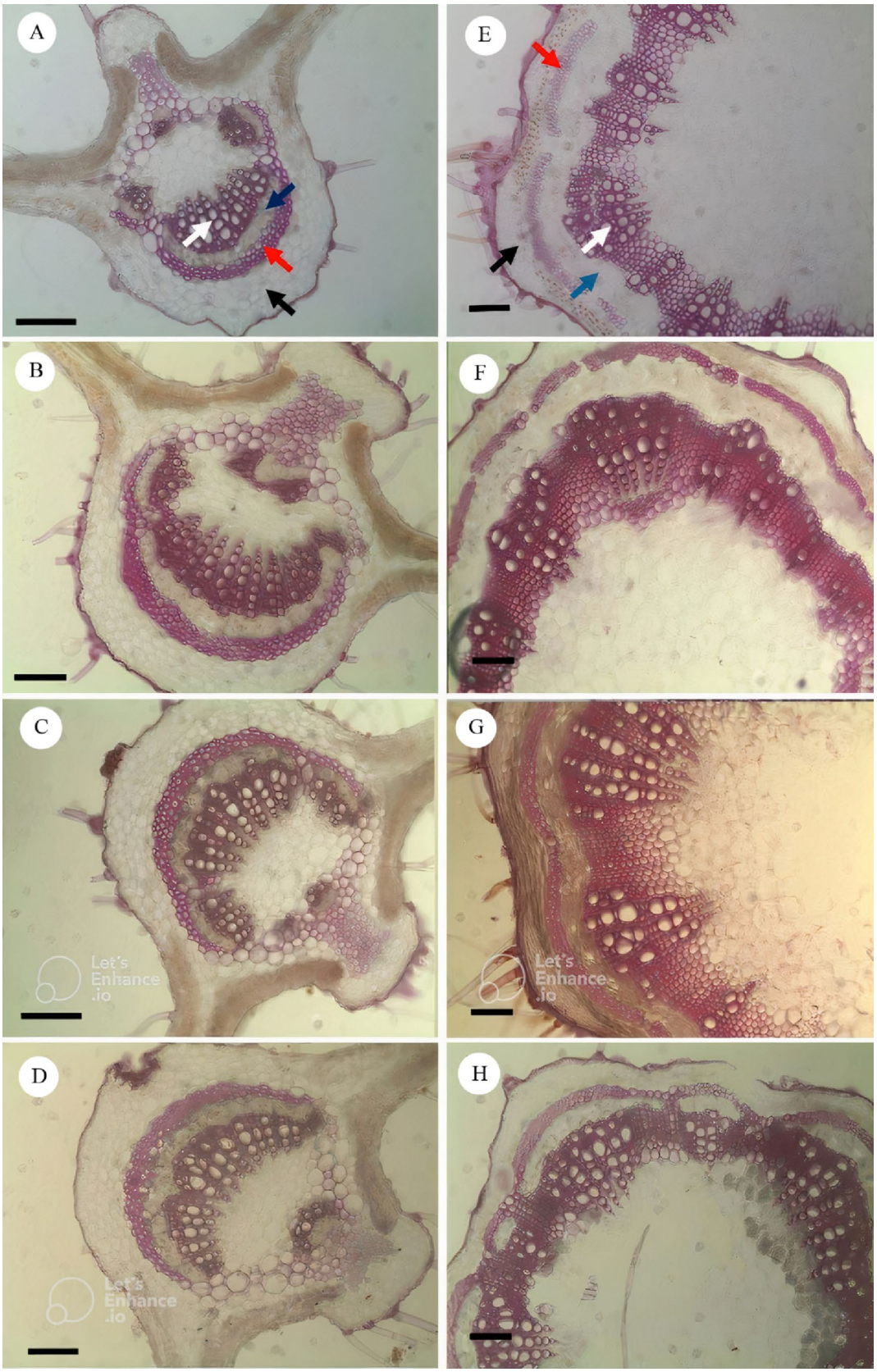

Fig. 4 Cross-sections of the leaf midrib (A – 25% FC; B – 50% FC; C – 75% FC; D – 100% FC) and stem (E – 25% FC; F – 50% FC; G – 75% FC; H – 100% FC). Arrows indicate the cortex (black), phloem sclerenchyma (red), phloem (blue), and xylem (white). Scale bar = 100 µm

Table 2 Root thickness (µm) and area (µm^2) in soybean plants at 25, 50, 75, and 100% field capacity (FC)

	Treatments	100%FC	75%FC	50%FC	25%FC
Root	Average vessels diameter (µm)	42.2 a	42.0 a	39.7 a	29.9 b
	Up to 20 µm (%)	1.90 b	3.80 b	2.70 b	25.1 a
	>20 to 30 µm (%)	21.2 a	19.2 a	21.5 a	30.2 a
	>30 to 40 µm (%)	26.0 a	27.4 a	30.8 a	23.6 a
	>40 to 50 µm (%)	23.0 ab	21.4 ab	27.0 a	13.7 b
	>50 µm (%)	28.0 a	28.1 a	18.0 ab	7.50 b
	Vessels/xylem (µm^2/µm^2)	0.18 ab	0.23 a	0.12 b	0.19 ab

Means followed by the same letter do not differ according to Tukey's test ($p<0.05$)

The collections were conducted 90 days after planting

treatments, while those larger than 50 µm had a higher percentage at 50, 75, and 100% FC (Table 2).

The root xylem was equally stained for all the treatments and contained xylem parenchyma with spherical to oval vessel elements, with no differences identified between treatments (Fig. 7).

Principal component analysis

In principal component analysis (PCA), the first component (PC1) explained 47.5% of the variance, and the second (PC2) 21.4%, corresponding to 68.9% of total variance (Fig. 8). The PC1 and PC2 provide sufficient information to support the results obtained. Gas exchange and response variables in the shoot and root system contributed to the highest data variance, with only *Ci* exhibiting a negative correlation with the other variables.

Most of the variance observed is explained by PC1. The similarity between shoot and root variables in the 75 and 100% FC treatments indicates a correlation, with the water regime influencing these variables. A similar response was observed for gas exchange at 50% FC. The 25% FC treatment negatively correlated with the other treatments and variables.

Discussion

In the present study, severe water stress (25% FC) had a significant adverse effect on the biometric, physiological, anatomical, and water status characteristics of the soybean cultivar during the vegetative stages. Understanding the impact of drought stress on soybeans is the key to improving agricultural production in an environment characterized by constant changes. The data from this study enhance our understanding of how water deficit affects soybeans during the vegetative stages, thereby improving soybean tolerance and yield potential in environments with low water availability. Soil moisture stress is a crucial limiting factor during the vegetative phase of soybean growth and establishment, affecting cell elongation, leaf expansion, and development. Among different growth and developmental traits, node number, plant height, internode length, and leaf area expansion have been suggested as indicators of tolerance to soil moisture deficit in soybeans (Khan et al. 2014; Wijewardana et al. 2019). According to Staniak et al. (2023), understanding how soybean plants respond to stress factors and react to stress is essential for innovation in agronomic practices that benefit soybean production in the face of climate change.

The growth rate, physiological responses, and morphology of above-ground and root components were affected by water deficit. Among the water regimes studied, the 25% FC treatment compromised the most significantly variables (height, diameter, gas exchanges, biomass, leaf area, and number of nodules). Crop growth and yields are severely impacted by inadequate water supply, reducing carbon assimilate contents. According to Hasanuzzaman et al. (2013), plant drought stress in can alter gene expression and cellular metabolism, reduce mitotic cell division in mesophyll tissues and other organs, and decrease stomatal conductance.

Drought stress compromises dry matter accumulation, leaf area index, and photosynthetic efficiency (Li et al. 2019). The degree of soybean sensitivity to water is closely related to the growth period, with short-term and moderate water deficit during vegetative growth generally not reducing soybean yield significantly (Xiong et al. 2021). Under drought stress, plants may partially or fully close stomata to reduce water loss; the resulting decline in the photosynthetic electron transport chain forces excitation energy to dissipate through nonphotochemical quenching, producing reactive oxygen species that damage the photosynthetic apparatus (Xiong et al. 2021). As observed in Fig. 4B, there was a reduction in stomatal conductance over the exposure period to stress in the treatment with 25% FC.

The decline in *A* is mainly attributed to stomatal closure, as observed in Fig. 4, which is in line with the findings of Wijewardana et al. (2019). The stomatal limitation is likely the primary cause of reduced net photosynthesis in water-stressed soybeans. Typically, the response of leaf stomata is regulated to minimize water loss or maximize carbon gain, depending on the plant's water status and photosynthetic CO_2 assimilation. Some studies have reported reduced *A* and gs in response to soil moisture stress (Makbul et al. 2011; Ku et al. 2013).

Soil moisture stress decreases leaf water potential (LWP), which reduces turgor pressure and, subsequently, stomatal closure. Midday LWP is a reliable measure to assess plant water status, given its relationship with leaf gas exchange

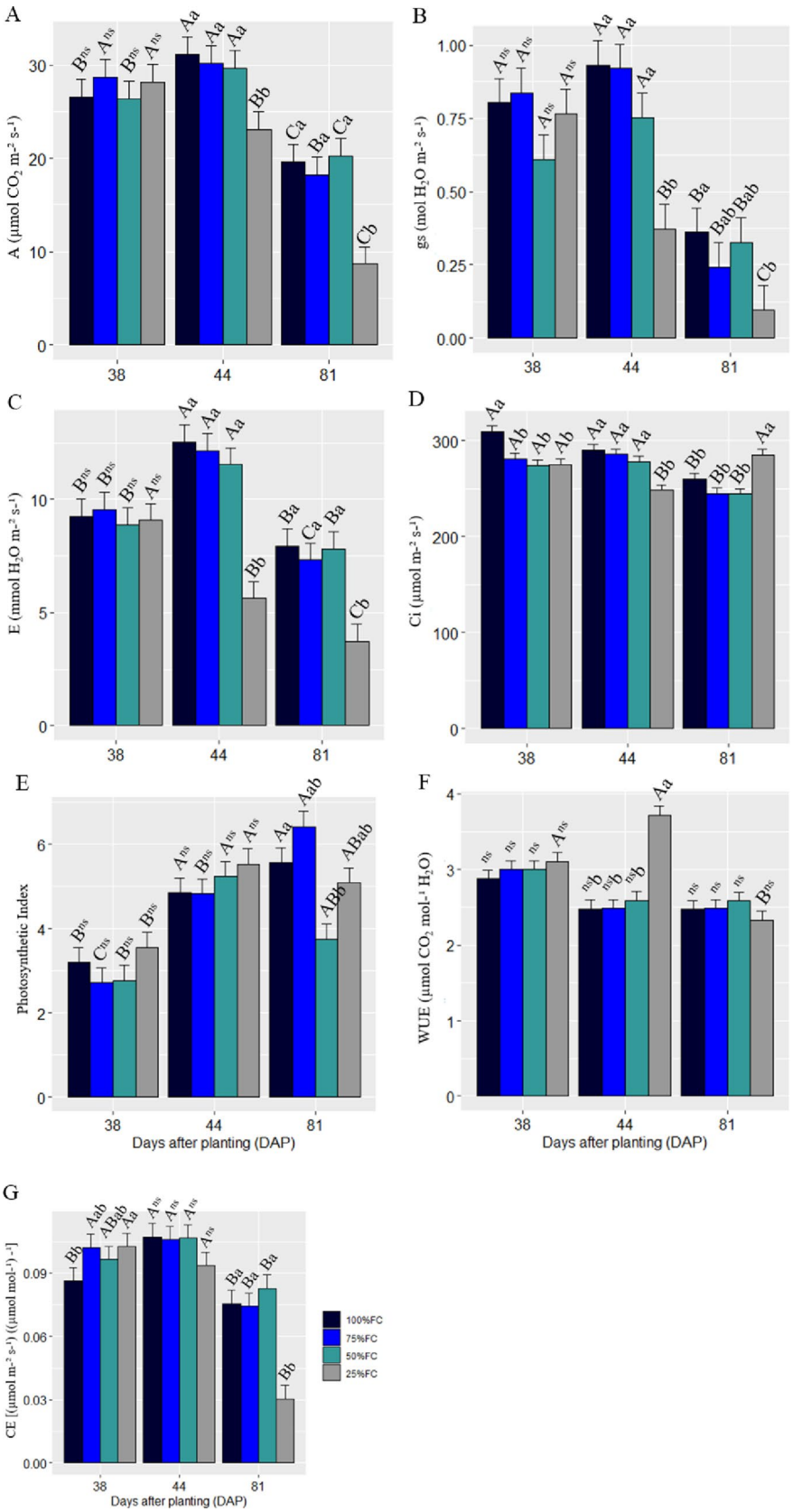

Fig. 5 Physiological responses of soybean plants under different field capacity (FC) levels and assessment times ($n=4$). Photosynthetic CO_2 assimilation (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), photosynthetic index (PI), water use efficiency (WUE), and carboxylation efficiency (CE) of soybean plants under different field capacity (FC) levels and assessment times ($n=4$). The bar graphs represent means ± standard error

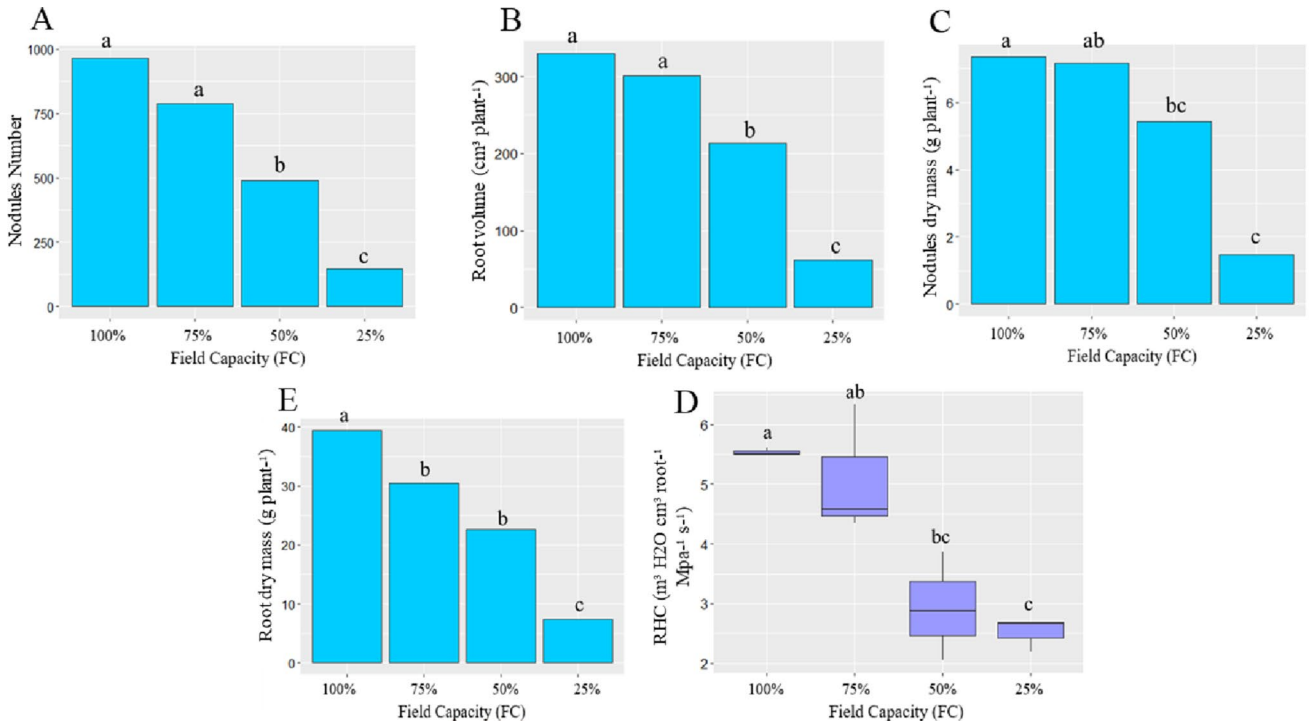

Fig. 6 Nodule number (**A**), root volume (**B**), nodule dry mass (**C**) root dry mass (**D**), and root hydraulic conductivity (**E**) of BRS Tracajá under different field capacity (FC) levels and assessment times ($n=4$). Means followed by the same letter do not differ according to Tukey's test ($p<0.05$). The bar graphs represent means ± standard error (A-D). The boxplots represent the replicates ($n=4$; E). The analyses and collections were conducted 90 days after planting

and other growth and development parameters (Wijewardana et al. 2019).

Water use efficiency is an essential trait in plants and a key factor linking water and carbon cycles. Understanding the physiological mechanisms involved in this trait and predicting their response in a constantly changing environment is a considerable challenge. Instantaneous water use efficiency (*A/E*) and intrinsic water use efficiency (*A/gs*) are obtained from the gas exchange and express how efficiently plants use water while simultaneously assimilating carbon. High WUE values are characteristic of plants tolerant to low water availability and work as a parameter that indicates physiological plasticity to abiotic factors (Costa et al. 2022).

Factors limiting photosynthesis are generally attributed to restricted CO_2 conductance from the atmosphere to the carboxylation site and limited CO_2 assimilation in the stroma of chloroplasts under saturated light conditions (Hu et al. 2019). Rubisco is a fundamental catalyst of carbon fixation, a process regulated primarily by chloroplast CO_2 concentration and the content and activity of the Rubisco enzyme. Photosynthesis depends on CO_2 diffusion and assimilation capacity and coordination, which are critical in achieving maximum photosynthetic capacity (Galmés et al. 2005).

Under prolonged water deficit, plants undergo morphological changes to cope with the adverse effects of stress (Wang et al. 2022). A complex network of anatomical adaptations, such as reduced vessel size with greater wall thickness, decreased formation of cortical and mesophyll parenchyma, and increased stomatal density, is needed to maintain water potential and energy storage under drought stress (Boughalleb et al. 2014). Confirming the authors mentioned earlier, in the present study, we found a reduction in the thickness and area of the cortex in the leaf midrib (Table 1). Additionally, an increase in root xylem vessels with a diameter of < 20 µm and a reduction in vessels > 50 µm was observed (Table 2).

Root vascular systems showed smaller xylem vessel diameters under water deficit (Table 2). A decrease in root xylem diameter in response to drought is a mechanism to prevent xylem embolism (Comas et al. 2013). Makbul et al. (2011) also reported a decline in stele and xylem diameter in soybean roots as a drought tolerance mechanism. In the present study, severe stress (25% FC) reduced root volume and xylem vessel diameter, leading to a decline in root hydraulic conductivity (RHC) (Fig. 6; Table 2). According to Suku et al. (2013), within a stable water potential gradient, RHC is altered via two pathways: increasing root surface area or enhancing intrinsic properties (transport volume).

Furthermore, water flow resistance in the xylem is partly determined by the diameter and length of conducting vessels responsible for water transport (Figueiredo et al. 2014). Kisman et al. (2022) reported that drought stress increased

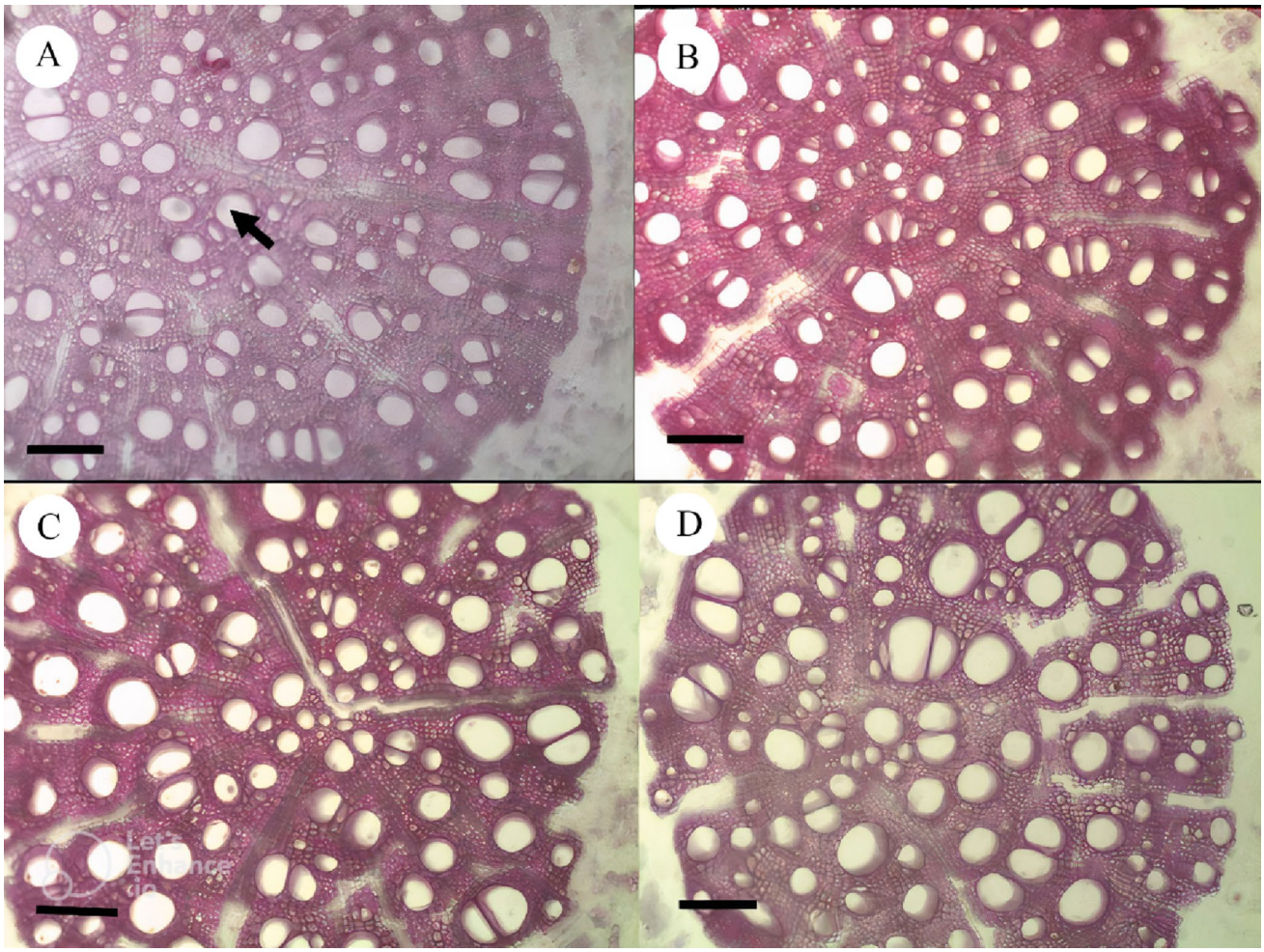

Fig. 7 Cross-section of root xylem (A − 25% FC; B − 50% FC; C − 75% FC; D − 100% FC). The arrow indicates the vessel element. Scale bar = 100 µm

epidermal and phloem thickness and decreased cortex, stele parenchyma, and xylem thickness in root anatomy. Similarly, in stem anatomy, epidermal, xylem, and phloem thickness decreased, but there were no differences in leaf anatomy. Similar responses were observed in our study (Table 1).

Efficient water and nutrient uptake by the roots significantly affect shoot development, governed by the balance between carbon assimilation through photosynthesis and carbon loss via respiration (Oliveira et al. 2019). Consequently, alterations that decrease hydraulic conductivity, particularly in the early stages of the pathway, such as the root, increase stress and may reduce leaf gas exchange, with a potentially adverse effect on growth and yield (Gilbert et al. 2011).

The 25% reduction in field capacity (75% FC treatment) did not compromise shoot and root responses, as shown in Fig. 8. This demonstrates the potential for water resource conservation and more effective water management.

Conclusions

The water regimes studied decreased the growth rate of plant height and stem diameter, especially from 30 to 60 days after planting. Severe drought stress (25% FC) drastically reduced the biomass of soybean plants. Gas exchange was also affected by water deficit, decreasing the photosynthetic CO_2 assimilation, stomatal conductance, transpiration, and carboxylation efficiency. Chlorophyll fluorescence and the SPAD index were not altered by the treatments applied. Anatomical analysis revealed adaptations at 25% FC, characterized by increased stem sclerenchyma thickness and a smaller average diameter of root xylem vessels. The 75% FC treatment showed similar responses to the control (100% FC), demonstrating that the former water regime did not compromise the growth and development of BRS Tracajá soybean plants.

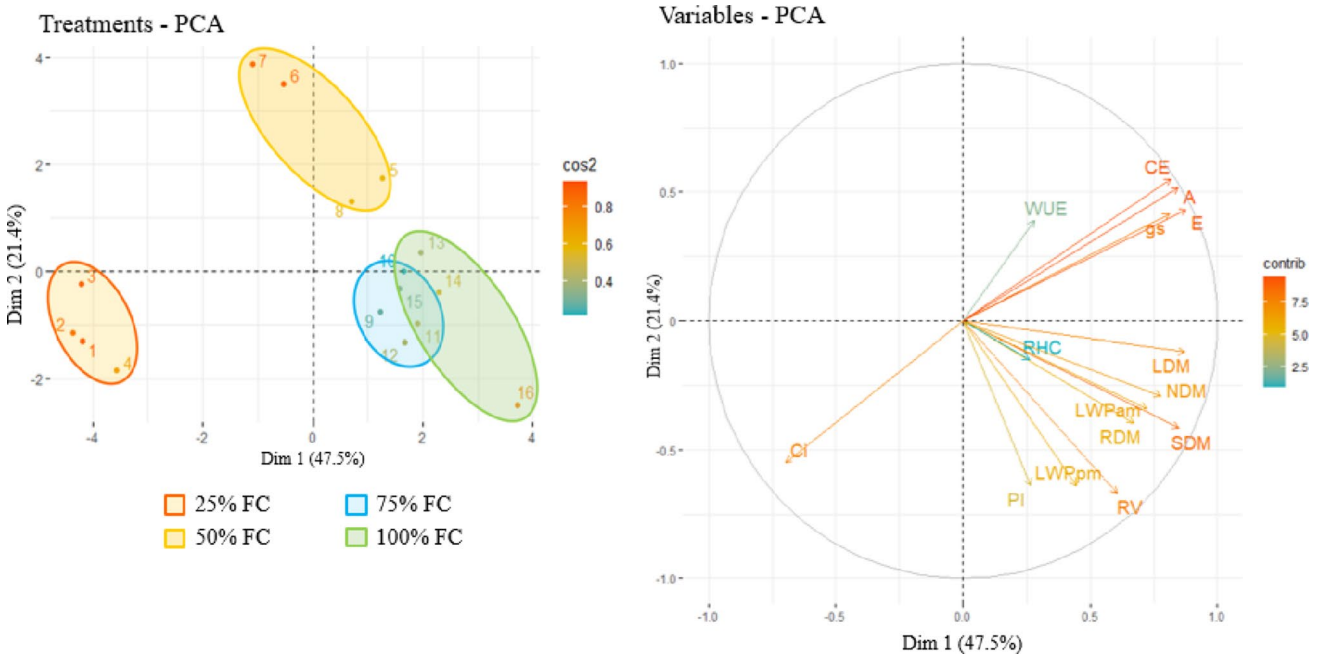

Fig. 8 Principal component analysis (PCA) between treatments (A) and variables (B). Variables: *A*, photosynthetic CO_2 assimilation; *gs*, stomatal conductance; *E*, transpiration; *Ci*, internal CO_2 concentration; PI, photosynthetic index; WUE, water use efficiency; CE, carboxylation efficiency; RHC, root hydraulic conductivity; LWP_{am}, predawn leaf water potential; LWP_{pm}, midday leaf water potential; RV, root volume; LDM, leaf dry mass; SDM, stem dry mass; RDM, root dry mass and NDM, nodule dry mass

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Declarations

Conflict of interest The authors have declared that no competing interests exist.

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