ORIVALDO BENEDITO DA SILVA

MORPHOPHYSIOLOGICAL CHARACTERISTICS OF SUNFLOWER GENOTYPES UNDER WATER DEFICIT

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Prof. Dr. Evaristo Mauro de Castro
Orientador
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CARACTERÍSTICAS MORFOFISIOLÓGICAS DE GENÓTIPOS DE GIRASSOL SOB DÉFICIT HÍDRICO

Tese Apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Botânica Aplicada, área de concentração em Botânica Aplicada para a obtenção do título de Doutor.

APROVADA em 15 setembro de 2023.
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LAVRAS-MG
2023

Aos meus pais, Antonio Manoel Benedito dos Santos e Maria Eremita da Silva

Meus maiores exemplos de vida.

Dedico

In memoriam

Ao Professor Dr. Arno Rieder

Professor da Universidade do Estado de Mato Grosso – UNEMAT

Profissional e amigo, importante na minha trajetória

Estará sempre em nossos corações.

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RESUMO

O girassol (*Helianthus annuus* L., Asteraceae) é uma planta classificada como tolerante à seca. Entretanto, a sua produtividade é afetada em condições de déficit hídrico, dependendo do estágio reprodutivo e tempo de duração da limitação hídrica. Portanto, é fundamental a investigação de características que atribuem tolerância ou susceptibilidade do girassol ao déficit hídrico controlado, contribuindo com programas de melhoramento genético da cultura. Neste sentido, o objetivo da pesquisa foi avaliar um conjunto de características morfológicas, anatômicas, fisiológicas e bioquímicas em quatro genótipos de girassol sob déficit hídrico controlado. O experimento foi desenvolvido em esquema fatorial 2×4 (duas condições hídricas e quatro genótipos de girassol), distribuídas em seis repetições, sendo planta por vaso do tipo rizotron, totalizando 48 plantas. As condições hídricas foram: plantas bem irrigadas (capacidade de campo) e déficit hídrico (40% da capacidade de campo). Os genótipos de girassol testados foram: OLISUN03, AGUARÁ06, BRS323 e HÉLIO250. O déficit hídrico promoveu redução no desenvolvimento, bem como massas secas da parte aérea e raízes dos genótipos de girassol. Na condição de déficit hídrico, ocorreram mudanças na morfologia e densidade estomática, refletindo na redução da condutância estomática, transpiração e concentração intercelular de CO_2. Nessas condições, ocorreram uma melhor eficiente no uso da água, eficiência instantânea de carboxilação e incremento de conteúdo de prolina foliar; aumento na área de espaços intercelulares do parênquima paliçádico e da câmara subestomática (AGUARÁ06 e BRS323) e na área do colênquima (OLISUN03, BRS323 e HELIO250) nas folhas. Em adição, os genótipos OLISUN03 e BRS323, sob déficit hídrico, possuem a arquitetura do sistema radicular estreita e profunda. Além disso, os genótipos OLISUN03 e AGUARÁ06, sob déficit hídrico, reduziram a espessura da endoderme e área do cilindro vascular, nas raízes. Os quatro genótipos de girassol possuem estratégias de absorção da água disponível no solo, evidenciadas nas características morfoanatômicas radiculares, modificações anatômicas foliares, contribuindo a eficiência fisiológica. Sendo assim, há um conjunto de características que atribuem tolerância ao déficit hídrico do girassol, contribuindo com programas de melhoramento genético da cultura.

Palavras-chave: *Helianthus annuus* L. Anatomia foliar. Anatomia radicular. Sistema radicular. Arquitetura radicular. Espaços intercelulares. Densidade estomática. Trocas gasosas.

ABSTRACT

Sunflower (*Helianthus annuus* L., Asteraceae) is a plant classified as drought tolerant. However, its productivity is affected under water deficit conditions, depending on the reproductive stage and duration of water limitation. Therefore, it is essential to investigate characteristics that attribute sunflower tolerance or susceptibility to controlled water deficit, contributing to crop genetic improvement programs. This study evaluated morphological, anatomical, physiological, and biochemical characteristics in four sunflower genotypes under controlled water deficit. The experiment was developed in a 2×4 factorial scheme (two water conditions and four sunflower genotypes), distributed in six replicates, with one plant per rhizotron-type pot, totaling 48 plants. The water conditions were well-irrigated plants (field capacity) and water deficit (40% of field capacity). The sunflower genotypes tested were OLISUN03, AGUARÁ06, BRS323, and HÉLIO250. Water deficit reduced development and dry masses of the aerial part and roots of sunflower genotypes. Changes in stomatal morphology and density occurred in the water deficit condition, reflecting a reduction in stomatal conductance, transpiration, and intercellular CO_2 concentration. Under these conditions, there was better water use and instant carboxylation efficiency and an increase in leaf proline content, intercellular spaces of the palisade parenchyma and substomatal chamber (AGUARÁ06 and BRS323), and collenchyma area (OLISUN03, BRS323 and, HELIO250) in the leaves. The OLISUN03 and BRS323 genotypes also have a narrow and deep root system architecture under water deficit. Furthermore, under water deficit, the OLISUN03 and AGUARÁ06 genotypes reduced the endodermis's thickness and the area of the vascular cylinder in the roots. The four sunflower genotypes have strategies for absorbing water available in the soil, evidenced in root morphoanatomical characteristics and leaf anatomical modifications, contributing to physiological efficiency. Therefore, a set of characteristics attribute tolerance to sunflowers' water deficit, contributing to the crops' genetic improvement programs.

Keywords: *Helianthus annuus* L. leaf anatomy. Root anatomy. Root system. Root architecture. Intercellular spaces. Stomatal density. Gas exchange.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

O girassol (*Helianthus annuus* L., Asteraceae) é uma cultura agrícola de importância na economia mundial, no qual dos frutos (aquênios) são extraídos óleo, utilizado no consumo humano, composição de cosméticos e indústria de biodiesel. Em adição, as sementes são utilizadas na composição de ração animal, alimentação de aves e a inflorescência, destacando-se como ornamental e fonte de recursos para abelhas, sendo, alternativa para a produção apícola. A cultura é classificada como tolerante à seca, quanto ao seu desenvolvimento, por isso, é utilizada na sucessão ou rotação de culturas, na segunda safra (CARVALHO et al., 2015; DALCHIAVON et al., 2016). Entretanto, o déficit hídrico pode afetar principalmente a fase inicial e intermediária da floração, afetando a produtividade. Em contraste, são aceitáveis níveis limitados de irrigação durante a formação dos frutos e sementes, resultados da diminuição da fotossíntese, com senescência das folhas (KEIPP et al., 2020).

O déficit hídrico é um fator limitante no rendimento de culturas em regiões tropicais e subtropicais. O grau de severidade causado pelo déficit hídrico na planta depende do tempo de duração e estágio de desenvolvimento, tornando-se mais severo para a produção no período antes e durante a floração das plantas (ZIA et al., 2013). Sob déficit hídrico, ocorrem adaptações anatômicas, morfológicas e fisiológicas nas plantas. Nessas condições, abertura estomática, fotossíntese e funções metabólicas são limitadas, regulados por sinais físicos e químicos, restringindo o crescimento e produtividade da planta (XU; ZHOU; SHIMIZU, 2010). Considerando-se esses fatores, são necessários estudos que busquem identificar materiais biológicos tolerantes e susceptíveis ao déficit hídrico. Assim, possibilitando evidenciar um conjunto de características que podem contribuir com programas de melhoramento genético das plantas, dentre elas, o girassol.

Atualmente, são realizadas pesquisas com espécies de interesses agrícola, as quais possuem materiais biológicos com características morfoanatômicos e fisiológicas de tolerância e susceptibilidade ao déficit hídrico, refletindo na produtividade, como em genótipos de milho (*Zea mays* L., Poaceae) (PIRES et al., 2020). Em adição, contribuem com características de plasticidade fenotípica de tolerância ao déficit hídrico. Por isso, estudam-se também plantas estabelecidas como tolerantes ao déficit hídrico, evidenciando-se um conjunto de características anatômicas, atribuídas à eficiência fotossintética, como o caso das plantas de sorgo [*Sorghum bicolor* (L.) Moench], destacando como planta modelo (OLIVEIRA et al., 2021).

Nesse contexto, pesquisas com genótipos de girassol, avaliados em condições controladas de déficit hídrico e estes, amplamente cultivadas em regiões semiáridas do Brasil, contribuem para caracterização morfológicas, anatômicas, fisiológicas e bioquímicas de tolerância da cultura ao déficit hídrico. Contribuindo com programas de melhoramento genético, expansão da cultura e sobretudo, maiores produtividades. Neste sentido, o objetivo da pesquisa foi avaliar um conjunto de características morfológicos, anatômicos, fisiológicos e bioquímico de quatro genótipos de girassol sob déficit hídrico controlado.

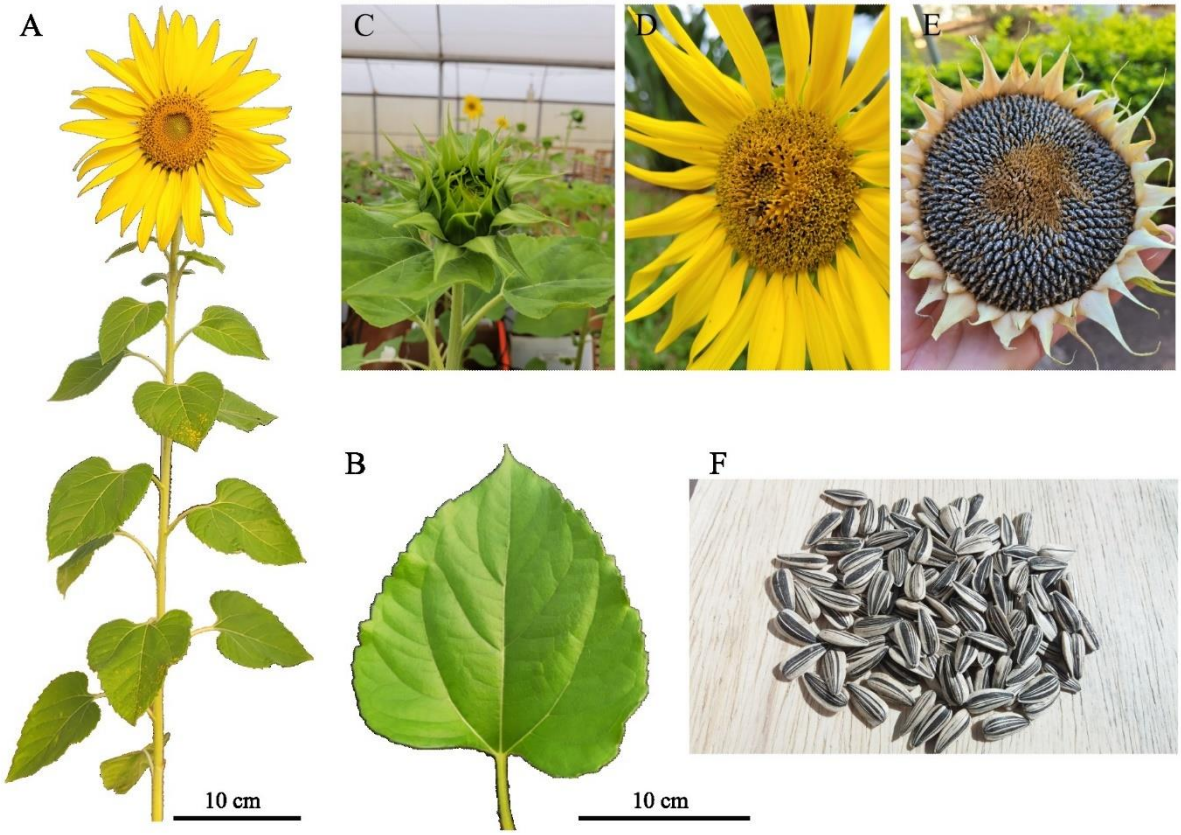
2 REFERENCIAL TEÓRICO

2.1 Aspectos gerais da cultura do girassol

O girassol (*Helianthus annuus* L., Asteraceae) é uma planta herbácea, podendo alcançar até 4 m altura, dependendo da cultivar (FIGURA 1). Possui folhas simples e organizadas em filotaxia oposta, correspondente a fase vegetativa (V4 a V8) e posteriormente, filotaxia alterna, marcando a passagem da fase vegetativa para a fase reprodutiva. As folhas (FIGURA 1B) são pecioladas, cordiformes e com grandes quantidades de tricomas, principalmente na face abaxial (CASTRO; FARIAS, 2005).

O sistema radicular do girassol é pivotante com grande quantidade de raízes secundárias e podendo alcançar até dois metros de profundidade, dependendo das características físicas do solo (CASTRO; FARIAS, 2005). É uma característica que possibilita alcançar água e nutrientes na camada mais profunda do solo e ancoragem da planta (CASTRO; FARIAS, 2005). Em adição, as raízes secundárias possibilitam a sustentação lateral da planta e absorção de água e nutrientes da camada superficial do solo (CASTRO; FARIAS, 2005).

Figura 1- Plantas de girassol em período reprodutivo.



Legenda: Planta de girassol com capítulo (A), folha (B), fase R1 (C), fase R5 (D), fase R9 (E), aquênios (F). Fonte: Do autor (2021).

O girassol é uma oleaginosa, originária da América do Norte e juntamente com o dendê (*Elaeis guineensis* Jacq.), a soja [*Glycine max* (L.) Merr.] e a canola (*Brassica napus* L.) desempenham um importante papel na economia mundial (CASTRO; LEITE, 2018). É uma espécie, amplamente cultivada em diversos continentes, e introduzida no Sul do Brasil por colonos Europeus no final século XIX (CASTRO; LEITE, 2018). O ciclo vegetativo varia entre 90 e 130 dias, dependendo da cultivar, época de semeadura e das condições edafoclimáticas da região de cultivo (OLIVEIRA et al., 2017).

Por se tratar de uma oleaginosa com alta adaptabilidade climática, bons rendimentos de frutos e alta qualidade de óleo, a sua produção tem crescido nos últimos anos em diversos países. No último levantamento mundial, o girassol alcançou uma produção de 58,1 milhões de toneladas (FAOSTAT, 2021). No Brasil, Segundo a Companhia Nacional de Abastecimento (CONAB, 2023) a produção brasileira de girassol em 2023, obteve uma estimativa de 85,2 mil toneladas, alcançando 56,1 mil hectares de área plantada e uma produtividade de 1.520 kg/ha.

A região Centro-Oeste é detentora da maior produção da oleaginosa do Brasil, sendo o Estado de Mato Grosso responsável por maior produção brasileira.

O girassol é utilizado na sucessão ou rotação de culturas, na segunda safra (CARVALHO et al., 2015; DALCHIAVON et al., 2016). Isso porque o girassol possui alta adaptabilidade edafoclimáticas, bem como tolerância a seca, ao frio e ao calor, quando comparado com outras oleaginosas cultivadas no Brasil (DALCHIAVON et a., 2016). A cultura de girassol é beneficiada pela polinização cruzada de abelhas (*Apis mellifera* L.), um eficiente polinizador de diversas culturas, proporcionando benefícios na reprodução e consequentemente na formação dos aquênios. Além disso, a polinização efetiva proporciona produtos secundários como produção de mel, tornando uma alternativa de renda para pequenos produtores (SILVA et al., 2010; TOLEDO et al., 2011; MARTIN; FARINA, 2016) e pode ser cultivado com outras culturas, como milho e feijão (CARVALHO et al., 2017; OLIVEIRA; MELO; SANTOS, 2017).

Entre as aplicações da matéria prima do girassol encontra-se óleo extraído dos frutos (aquênios). Possui alto teor de óleo, variando entre 38% e 50%, de alta qualidade (LACHANCE; GRANGE, 2014). Sendo assim, adequado para fins comestíveis por ser rico em ácido linoleico e alta fonte de proteína (RAI; MOHANTY; BHARGAVA, 2016). Segundo Khan et al. (2015) o óleo de girassol possui cerca de 69% de ácido linoleico, 20% de ácido oleico e 11% de ácidos graxos saturados. Tais valores são próximos ou superiores aos encontrados no óleo de soja [*Glycine max* (L.) Merr.], que possui 53,2% de ácido linoleico e 23,4% de ácido oleico (WANG, 2011). O óleo de girassol é aplicado na indústria de produção de biodiesel (GAMA; GIL; LACHTER, 2010; SAYDUT et al., 2016) e os resíduos resultantes desse processo, são usados na formulação de ração de aves, oferecendo uma nutrição rica em fibras e proteínas brutas; energia e minerais, proporcionando qualidade na carcaça de frango de corte e qualidades dos ovos (ALAGAWANY et al., 2015). Em adição, utilizado na formulação de ração de bovinos, podendo substituir farelo de soja sem prejuízos nos limites nutricionais dos animais (MESACASA et al., 2015).

2.2 Déficit hídrico no crescimento e desenvolvimento de plantas

Em plantas em condições de déficit hídrico, ocorrem modificações morfológicas, anatômicas, fisiológicas e moleculares. Nas folhas são observadas modificações no ângulo foliar, diminuição do número de folhas, área foliar, bem como fechamento de estômatos para limitar a perda de água e com consequente redução na aquisição de CO_2, afetando diretamente

a fotossíntese (SCALON; MUSSURY, 2020). Nos estômatos são observadas modificações em tamanho, densidade e fechamento dos estômatos e estes, associados com mecanismo para evitar a perda de água para atmosfera pela transpiração foliar, sendo essas que definem a eficiências da condutância estomática (LAWSON; BLATT, 2014). Nos vasos condutores ocorrem alterações na relação xilema/floema, redução dos vasos do xilema; alteração na espessura das células do parênquima e aumento no tecido vascular e na espessura da parede celular (GONÇALVES et al., 2017).

Nas raízes, são observadas modificações com relação as barreiras apoplásticas, espessura das células corticais e cilindro vascular. Em adição, modificações na arquitetura do sistema radicular. Além disso, observa-se interação entre raiz/parte aérea para evitar a dessecação. Esse conjunto de modificações e interações permitem acessos à água com uso mínimo de carbono, contribuindo substancialmente ao desempenho da cultura, bem como aumento da produtividade, como observados em genótipos de sorgo e milho resistente à seca (HUND; RUTA; LIEDGENS, 2009).

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1: LEAF MORPHOANATOMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SUNFLOWER GENOTYPES UNDER WATER DEFICIT

ARTIGO A SER SUBMETIDO NA JOURNAL OF EXPERIMENTAL BOTANY

Leaf morphoanatomical and physiological characteristics of sunflower genotypes under water deficit

Morphoanatomical and physiological characteristics of sunflower

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Highlight

Leaf anatomical characteristics, such as an increase in the area of collenchyma, intercellular spaces and substomatal chamber, contribute to efficient photosynthesis under water deficit.

Abstract

Sunflower (*Helianthus annuus* L., Asteraceae) is a drought-tolerant crop whose yield is compromised when there is water scarcity during the flowering period. The objective of the present study was to evaluate, under controlled conditions, the morphoanatomical and physiological aspects of four sunflower genotypes grown under water deficit conditions. The experiment was conducted using a completely randomized design in a 2×4 factorial scheme (two water conditions and four genotypes), with six replicates of one plant per rhizotron pot, totalling 48 plants. The water conditions evaluated included plants under well-watered conditions (field capacity) and plants under water deficit conditions (40% field capacity). The studied sunflower genotypes included OLISUN03, AGUARÁ06, BRS323, and HELIO250. Water deficit induced developmental delay, reduced shoot and root dry mass, and changed stomatal morphology and density, which were reflected by reduced stomatal conductance, transpiration, and internal CO_2 concentrations. Changes in the areas of intercellular spaces and substomatal chambers, increased collenchyma area and proline leaf content were also observed. It was concluded that the four sunflower genotypes had reduced growth under water deficit, in addition to morphoanatomical changes.

Keywords: Collenchyma, Gas exchange, *Helianthus annus*, intercellular spaces, leaf morphoanatomical characteristic, physiological characteristics, substomatal chambers, sunflower, water deficit.

Introduction

Sunflower (*Helianthus annuus* L., Asteraceae) is one of the four most important oilseeds in the world (FAO, 2022). Oil extracted from the achenes is present in high proportions (38% to 50%) and is used for human consumption, the pharmaceutical industry (Castro and Leite 2018), and the biodiesel industry (Abubakar *et al*., 2020; Khattak *et al*., 2021). In addition, sunflower is among the ingredients used in silage and grain mixtures for bird feed (Castro and Leite 2018). It is a crop that has greater drought tolerance than most oilseed crops grown in Brazil (Carvalho *et al*., 2015; Ibrahim *et al*., 2016; Vilvert *et al*., 2018) and may be a good alternative for crop succession.

As a secondary crop, sunflower has been cultivated after corn and has become an alternative crop when the probability of productivity losses of other species is high due to water deficit. Its good water stress and heat tolerance has also enabled it to expand into the semiarid Northeast region which is characterized by low rainfall amounts, irregular rainfall distribution, and high temperatures and radiation (Lacerda *et al*., 2015; Mutti *et al*., 2019). Despite sunflower's tolerance to water stress, water deficit occurring at the beginning of flowering and achene filling can considerably decrease achene production and oil content (Hussain *et al*., 2018). The development of cultivars in breeding programs with higher achene and oil yields under water deficit conditions is essential for establishing crops in production systems facing a water deficit.

Several agricultural crops exposed to water deficit have developed morphoanatomical structures, such as the substomatic cavity (Oliveira *et al*., 2022), and mechanisms, such as the accumulation of osmocompatible solutes (such as proline) and osmotic adjustment, which favour tolerance to water deficit (Barros *et al*., 2019). Other anatomical parameters affecting tolerance are stomatal density and index; cuticle, epidermis, and mesophyll thickness; and adjustments in the water conduction systems, including the diameter of the xylem vessels (Nazaré *et al*., 2012; Gonçalves *et al*., 2017; Pires *et al*., 2020). Therefore, there is a set of characteristics involved in plant responses to water deficit that consequently provide varying levels of tolerance.

Identifying the morphoanatomical and physiological characteristics present in productive sunflower genotypes under water deficit conditions may contribute to the selection of more adaptable genotypes in breeding programs. Thus, the objective of the present study was to evaluate the leaf morphoanatomical and physiological characteristics of four sunflower genotypes grown under water deficit conditions in a controlled environment.

Materials and methods

Plant materials, cultivation conditions, and experimental design

In this study, four commercial hybrid sunflower genotypes were obtained from different breeding practices. They were evaluated in a greenhouse of the Botany sector of the Department of Biology, Federal University of Lavras, Lavras, MG, Brazil: AGUARÁ06 (Atlântica Seeds, Curitiba, PR, Brazil), BRS323 (Brazilian Agricultural Research Corporation - Embrapa, Brasília, DF, Brazil), HELIO250 (Heliagro Agricultura e Pecuária Ltda., Araguari, MG, Brazil), and OLISUN03 (Advanta Comércio de Sementes Ltda., Campinas, SP, Brazil). The four genotypes presented, in previous studies, high achene productivity in the semiarid conditions of northeastern Brazil (Carvalho *et al*., 2018; Souza *et al*., 2019; Carvalho *et al*., 2020). The sunflower plants were grown in a germination chamber at 25 °C with 12 h light and 12 h dark from seeds germinated on filter paper. When the rootlets of the seedlings were approximately 2 cm long (total time of four days), they were transferred to rhizotron pots (size: 42.5×29.5×3.5 cm) filled with 2.8 L of washed sand and the commercial substrate Tropstrato (Vida Verde®, Brazil) at a ratio of 1:1. The properties of the substrate were as follows: electrical conductivity: 1.5 mS cm^{-1}; dry basis density: 190 kg m^{-3}; wet basis density: 500 kg m^{-3}; moisture: 60% of the total substrate weight. The chemical attributes were as follows: pH $CaCl_2$: 5.75; P: 65.70 mg dm^{-3}; K: 1.60 $cmol_c$ dm^{-3}; Ca: 23.80 $cmol_c$ dm^{-3}; Mg: 12.40 $cmol_c$ dm^{-3}; Al: 0.0 $cmol_c$ dm^{-3}; H + Al: 4.20 $cmol_c$ dm^{-3}; sum of bases: 39.80 $cmol_c$ dm^{-3}; cation exchange capacity: 42.10 $cmol_c$ dm^{-3}; and base saturation (V%): 64.80.

The experiment was conducted in a 2×4 factorial scheme (two water conditions and four sunflower genotypes) using a completely randomized design, with six replicates and one plant per pot in the experimental unit, for a total of 48 plants. The water conditions evaluated included well-irrigated plants (WW), which correspond to field capacity, and plants under water deficit (WD). In the latter case, the field capacity progressively decreased from 15 to 30 days after transplanting (DAT) up to 40%, and was maintained at this point until 51 DAT, i.e., the beginning of the reproductive stage, which allowed the morphological, anatomical, and physiological effects of water deficit to be measured for the period that covered the beginning of flowering and achene filling.

Compost moisture was monitored using two resistive moisture sensors, one for each treatment, installed on the upper edge of the rhizotron pots and repositioned between the plants every 24 h, keeping all plants in the same conditions of water availability throughout the experiment. The resistive humidity sensors were connected to the voltage comparator module (LM393) and microcontroller (Arduino Mega 2560), programmed for each field capacity,

according to the resistance of the compound. In addition, the irrigation system consisted of an irrigation pump for each treatment, distribution hoses and two dripping stakes (15 cm), positioned at the top of each rhizotron pot, and the system was automatically activated. Irrigation of all plants was performed with Hoagland and Arnon (1950) nutrient solution at 40% ionic strength. The plants were kept in a greenhouse at a controlled temperature of 26 ± 2 °C, relative humidity ranging between 50% and 70%, average maximum photosynthetic photon flux density of 652 µmol m^{-2} s^{-1} (measured in the plant canopy) and a photoperiod of 12 h light and 12 h dark.

Analysis of plant growth

From 15 to 51 DAT, plant height and stem diameter were measured every two days between the surface of the substrate and the highest point of vegetative growth and subsequently reproductive growth with the aid of a ruler. The stem diameter was measured 3 cm from the substrate with a digital calliper.

At 51 DAT, the plants were harvested, and the leaves were scanned on an A3 Scanner (1200S, Mustek, China). The leaf areas of all leaves were determined by image analysis with ImageJ software. Subsequently, the leaves, stems, and roots were placed in a forced-air oven at 60 °C until reaching constant dry mass, which was determined on an analytical balance (AY220, Shimadzu, São Paulo, Brazil). With these data, the specific leaf area (SLA), leaf area ratio (LAR), leaf mass ratio (LMR) and root/shoot ratio (RSR) were calculated (Equations 1, 2, 3 and 4).

$$\mathrm{SLA} = \frac{La}{Dlm} \quad (1)$$

$$\mathrm{LAR} = \frac{La}{Tdm} \quad (2)$$

$$\mathrm{LMR} = \frac{Ldm}{Tdm} \quad (3)$$

$$\mathrm{RSR} = \frac{Rdm}{Apdm} \quad (4)$$

Were LA is leaf area; DLM is dry leaf mass; TDM is total dry mass; LDM is leaf dry mass; RDM is root dry mass; APDM is aerial part dry mass.

Leaf water potential (Ψw)

The leaf water potential was determined using a portable Scholander pressure pump (Model 1.000; PSM Instrument Company, Corvallis, Oregon, USA) with N_2 gas; the pressure necessary to bring sap to the cut in the midrib was applied, and the water potential was recorded. The evaluations were performed at 49 DAT, using the fourth and fifth leaves, fully expanded, from the top of the stem, between 4:00 h and 5:30 h, when the leaf water potential was maximum, and between 11:00 h and 12:30 h, i.e., when leaf water potential was minimum, as previously described.

Analysis of gas exchange

At 50 DAT, gas exchange was evaluated with an infrared gas analyser (IRGA) model LI-6400XT (Li-COR Biosciences, Lincoln, Nebraska, USA) equipped with a 6 cm^2 chamber and a red/blue LED light source (LI6400-02B, LI-COR, Lincoln, Nebraska, USA). The readings were performed between 8:00 am and 11:00 am on the third fully expanded leaf. The photosynthetic photon flux density (PPFD) was standardized at 1000 μmol m^{-2} s^{-1} in an equipment cuvette. In addition, the mixer function used carbon dioxide capsules to provide 400 ppm of CO_2 during the analyses. During the measurements, the leaf temperature was maintained at 28.5 °C. The net assimilation rate (A_N), stomatal conductance to water vapour (g_{sw}), transpiration rate (E), and CO_2 concentration at the substomatal cavity (Ci) were evaluated. The internal and external carbon (Ci/Ca) ratio, instantaneous water-use efficiency (A_N/E), and carboxylation efficiency (A_N/Ci) were calculated.

Determination of proline

At 49 DAT, the proline content was determined according to the methodology described by Bates *et al*. (1973). For the extraction of proline from the samples, fully expanded leaves, i.e., the fourth and fifth leaves from the top of the stem, were collected and used to evaluate the leaf water potential. The leaves were dried at 60 °C, ground in a mill, weighed to 200 mg, and transferred to test tubes, and 3% sulfosalicylic acid was added. Proline was quantified upon reaction with ninhydrin (ninhydrin, glacial acetic acid, and phosphoric acid) and incubated at 100 °C for 60 min in a water bath. The absorbance reading was measured in a spectrophotometer at 520 nm, and the values were expressed in μmol g^{-1} of dry mass.

Leaf anatomical analyses

At the end of the experiment, at 51 DAT, the third most apical leaf was collected and fully expanded for the study of leaf anatomy. The leaf samples were fixed in 70% FAA solution (formaldehyde, glacial acetic acid, and 70% ethanol, 1:1:18) for 72 h and transferred to 70% ethanol solution for analysis (Johansen, 1940). Samples for cross-sections, obtained in the median region of the leaf, were dehydrated in increasing concentrations of ethanol (70, 80, 90, and 100%) at intervals of 2 h at room temperature, infiltrated for 24 h in historesin (Leica Microsystems, Heidelberg, Germany), cross-sectioned (7 µm thickness) with a semiautomatic rotary microtome, stained with 0.05% toluidine blue (w/v) (Feder & O'Brien, 1968), and mounted on permanent slides with Entellan (Merck, Darmstadt, Germany).

In addition, free-form paradermal sections from the adaxial and abaxial surfaces were obtained using a steel blade, clarified with sodium hypochlorite (50%), washed in distilled water, stained with 1% safranin, and mounted on semipermanent slides with 50% glycerol (Johansen, 1940). The slides were photographed with a camera coupled to a microscope (Eclipse E100-LED; Nikon, Tokyo, Japan). The quantitative anatomical data were obtained using ImageJ software.

In the midrib region of the leaf cross-sections, the areas occupied by the midrib, collenchyma and vascular bundles were estimated, and the diameter of the xylem vessels was determined. The areas of collenchyma (CO) and vascular bundles (VB) were estimated following equations 5 and 6.

$$\mathrm{CO} = \frac{Ac}{Tam}\, x\ 100 \tag{5}$$

$$\mathrm{VB} = \frac{Avb}{Tam}\, x\ 100 \tag{6}$$

Were AC is area of the collenchyma; TAM is total area of the midrib; AVB is area of the vascular bundles.

In the internerval region of the leaf, the thickness of the adaxial and abaxial epidermis, mesophyll, palisade parenchyma, and spongy parenchyma (= lacunose) and the distance between the vascular bundles were quantified. In addition, the percentages of area occupied by the substomatal chamber and intercellular space (ASC and IS) in the palisade parenchyma and spongy parenchyma were determined (Equation 7).

$$\mathrm{ASC\ and\ IS} = \frac{Sca + Isa}{Tap}\, x\ 100 \tag{7}$$

Were SCA is substomatal chamber area; ISA is intercellular space area; TAP is total area of the palisade parenchyma or spongy parenchyma.

In the paradermal sections, the density of stomata (DS) and stomatal index (SI) was determined (Equations 8 and 9). The size of the stomata was quantified from measurements of the polar and equatorial diameters of the stomata and the area of the stomatal pore.

$$DS = \frac{Ne \times 10^6}{Sa} \quad (8)$$

$$SI = \frac{Ne}{Ne + Nce} \times 100 \quad (9)$$

Were NE is number of stomata; SA is section area (103,867.38 μm^2); NCE is number of regular epidermal cells.

Statistical analysis

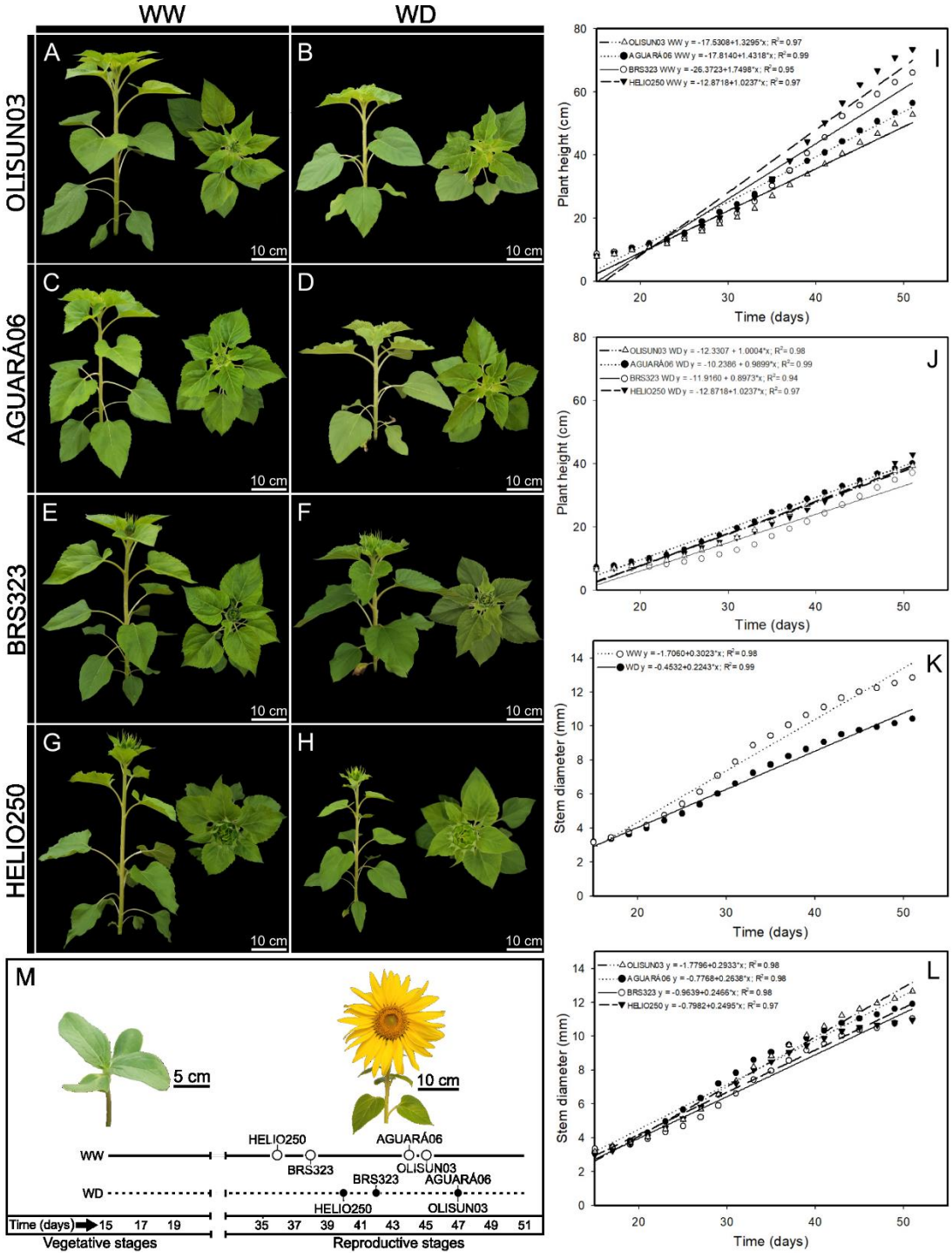
The data were tested for normality using the Shapiro–Wilk test. The means were subjected to analysis of variance (ANOVA), followed by the Scott–Knott test. The data obtained over time (stem height and diameter) were subjected to regression analysis, all at 5% significance. All analyses were performed using the software Sisvar 5.0 (Ferreira, 2011).

Results

Plant growth

The plant heights of the four sunflower genotypes, evaluated between the onset of water stress (15 DAT) and the end of the experiment (51 DAT), showed increases under both water conditions (Fig. 1I and J), with the increments being significantly higher in the irrigated plants. Under WW conditions, the highest plant height was observed in HELIO250 (69.97 cm) and BRS323 (62.87 cm), and the lowest was observed in OLISUN03 (50.28 cm; Fig. 1I). Under WD conditions, the highest plant height was observed in AGUARÁ06 (40.25 cm), while the lowest plant height was observed in BRS323 (33.85 cm; Fig. 1J). Regarding stem diameter, there was no significant interaction between the water condition factors and genotypes. The largest stem diameter (13.71 mm) occurred in the WW condition, while the smallest stem diameter (10.99 mm) was observed under the WD condition (Fig. 1K). Regarding the sunflower genotypes, the largest stem diameter (13.18 mm) occurred in OLISUN03, while the smallest stem diameter (11.61 mm) occurred in BRS323 (Fig. 1L). Initial flowering, that is, when the first ligulated flowers (stage R4) appeared, and full flowering (stage R5; Fig. 1M) occurred first in plants under the WW condition, particularly in the HELIO250 genotypes (at 36 DAT),

269 followed by BRS323, AGUARÁ06, and OLISUN03 (at 38, 44 and 45 DAT, respectively). A
270 delay in the reproductive stage was observed under WD conditions, which was greater for the
271 BRS323 and HELIO250 genotypes than for the other genotypes (Fig. 1M).



272

273 **Fig. 1.** Growth characteristics of four sunflower genotypes grown in rhizotron pots under well-
274 watered (WW) and water deficit plant conditions (WD). (A-H) Morphological aspects of
275 sunflower genotypes in well-watered condition (WW) and water deficit (WD), (I) Height of
276 plants in well-watered condition (WW), (J) Height of plants in water deficit (WD), (K) Stem

277 diameter in conditions of well-irrigated (WW) plants and water deficit (WD), (L) plant stem
278 diameter, independent of water conditions, and (M) reproductive stages of four sunflower
279 genotypes grown in pots under well-watered and water deficit plant conditions. Symbols
280 represent the mean values. Asterisks indicate the significance (Scott–Knott test, *P*-value <0.05).
281
282 Regarding biomass accumulation, there was a significant interaction between water
283 conditions and sunflower genotypes for total dry mass, root dry mass, leaf dry mass, and total
284 leaf area. For the four genotypes, there was a significant reduction in leaf area and leaf biomass
285 accumulation due to WD (Table 1). In AGUARÁ06, BRS323, and OLISUN03, there was also
286 a reduction in root dry weight and total plant dry weight. However, under WD conditions, no
287 difference was observed between the genotypes regarding the accumulation of dry mass in
288 leaves and roots and the total shoot and leaf area. Regarding the dry mass accumulated in the
289 stems, there was no significant interaction between water conditions and sunflower genotypes
290 (data not shown).
291 For the four estimated growth indices, a significant interaction was observed between
292 water conditions and sunflower genotypes (Table 2). The specific leaf area (SLA) and
293 root/shoot ratio (RSR) of the four genotypes were reduced under WD conditions, whereas the
294 leaf area ratio (LAR) and mean leaf ratio (MRL) increased. Under WD conditions, the
295 genotypes did not differ significantly in terms of any of the four indices. In contrast, for plants
296 under the WW condition (maintained at field capacity), there was a difference between the
297 genotypes. Under this condition, higher SLA, LAR, and leaf mass were observed in HELIO250;
298 the BRS323 genotype, in turn, stood out for a higher root/shoot (RSR) ratio in the WW plants
299 than in the WD plants.
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Table 1. Plant growth characteristics of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions.

	Dry mass							
	LDM (g plant^{-1})		RDM (g plant^{-1})		TDM (g plant^{-1})		TLA (cm^2 plant^{-1})	
Genotypes	WW	WD	WW	WD	WW	WD	WW	WD
OLISUN03	6.63 ± 0.33 Ba	3.83 ± 0.70 Ab	61.03 ± 12.51 Ba	20.50 ± 7.21 Ab	76.93 ± 14.68 Ba	30.88 ± 8.21 Ab	1329.60 ± 122.94 Ba	676,72 ± 109.66 Ab
AGUARÁ06	7.88 ± 0.32 Aa	3.69 ± 0.47 Ab	105.08 ± 9.12 Aa	18.98 ± 4.42 Ab	124.86 ± 9.66 Aa	30.07 ± 4.88 Ab	1687.54 ± 185.93 Aa	630.98 ± 132.40 Ab
BRS323	5.36 ± 0.89 Ca	3.51±0.49 Ab	66.00 ± 22.59 Ba	14.63 ± 4.63 Ab	81.06 ± 23.45 Ba	23.56 ± 5.28 Ab	1283.61 ± 67.93 Ba	654.51 ± 78.05 Ab
HELIO250	4.94 ± 0.54 Ca	3.89±0.59 Ab	28.50 ± 7.89 Ca	16.84 ± 3.30 Aa	42.43 ± 7.80 Ca	27.18 ± 4.50 Aa	1213.99 ± 42.94 Ba	676.72 ± 251.72 Ab

LDM, leaf dry mass; RDM, root dry mass; TDM, total shoot; LA, total leaf area. Data are means ± SD. The means followed by the same lowercase letter in the rows (comparing humidity conditions) and uppercase letters in the columns did (comparing genotypes) not differ according to the Scott–Knott test (P<0.05).

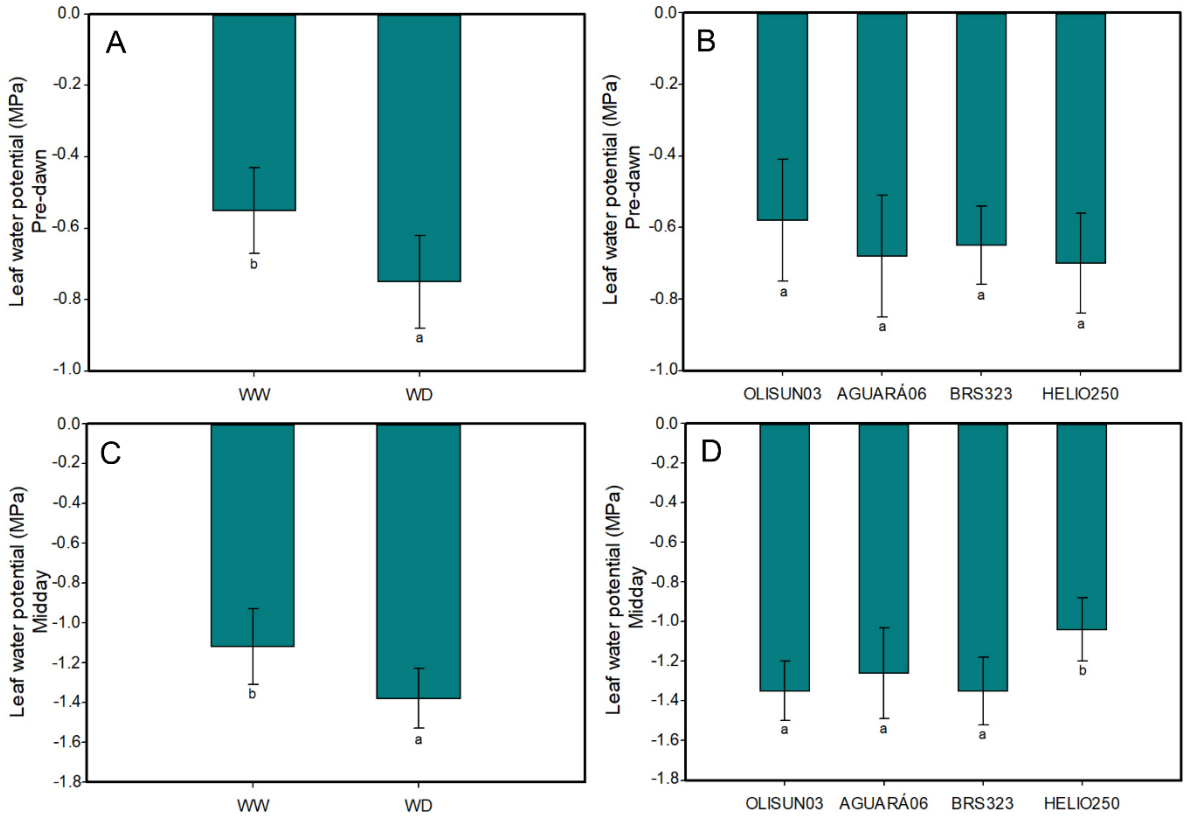
Table 2. Physiological growth indexes of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions.

	Physiological growth indexes							
	SLA (cm^2 g^{-1})		LAR (cm^2 g^{-1})		LMR (g g^{-1})		RSR (g g^{-1})	
Genotypes	WW	WD	WW	WD	WW	WD	WW	WD
OLISUN03	199.87 ± 8.32 Ca	176.91 ± 7.24 Ab	17.92 ± 2.72 Bb	22.84 ± 2.76 Aa	0.09 ± 0.01 Bb	0.13 ± 0.01 Aa	3.79 ± 0.40 Ca	1.93 ± 0.49 Ab
AGUARÁ06	223.97 ± 9.46 Ba	182.61 ± 5.80 Ab	14.56 ± 1.64 Bb	22.87 ± 2.43 Aa	0.06 ± 0.01 Cb	0.12 ± 0.01 Aa	5.32 ± 0.55 Ba	1.70 ± 0.34 Ab
BRS323	222.36 ± 17.00 Ba	179.66 ± 5.22 Ab	13.03 ± 2.12 Bb	26.16 ± 3.17 Aa	0.06 ± 0.01 Cb	0.15 ± 0.02 Aa	6.29 ± 1.08 Aa	1.77 ± 0.59 Ab
HELIO250	248.80 ± 11.26 Aa	178.62 ± 15.51 Ab	27.08 ± 2.52 Ab	19.58 ± 2.88 Aa	0.13 ± 0.02 Aa	0.13 ± 0.01 Aa	2.08 ± 0.73 Da	1.63 ± 0.21 Aa

SLA, specific leaf area; LAR, leaf area ratio; LMR, leaf mass ratio; RSR, root/shoot ratio. Data are means ± SD. The means followed by the same lowercase letter in the rows (comparing humidity conditions) and uppercase letters in the columns did (comparing genotypes) not differ according to the Scott–Knott test (P<0.05).

316 *Leaf water potential*

317 There was no significant interaction between the factors water condition and genotype, but
318 interactions occurred within each factor. At 49 DAT, between dawn and noon on the same day,
319 there was a reduction in the water potential of the plants (Fig. 2). Regarding the water
320 conditions, WD induced a reduction in water potential in both periods evaluated, i.e., dawn and
321 noon (Fig. 2A and C). Water potential at dawn did not differ among sunflower genotypes (Fig.
322 2B). At noon, however, the OLISUN03, AGUARÁ06, and BRS323 genotypes showed a
323 reduction in water potential, while HELIO250 showed no change in water potential (Fig. 2D).



324

325 **Fig. 2.** Leaf water potential of four sunflower genotypes grown in rhizotron pots under well-
326 watered (WW) and water deficit (WD) plant conditions. (A and B) Leaf water potential
327 recorded at dawn and (C and D) close to noon. Histograms represent the mean value ± SD.
328 Means followed by the same letters in water conditions and genotypes did not differ by the
329 Scott–Knott test (P<0.05).

330

331 *Physiological characteristics*

332 Regarding gas exchange, there was no significant interaction between the factors water
333 condition and genotype, and only the individual effects of each occurred (Table 3).

Table 3. Means of gas exchange of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions.

Water condition	A_N (µmol CO_2 m^{-2} s^{-1})	g_{sw} (mol H_2O m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Ci (mmol m^{-2} s^{-1})	Ci/Ca	A_N/E (µmolCO_2 mmol H_2O^{-1})	A_N/Ci (µmol m^{-2} s^{-1} Pa^{-1})	Proline (µmol g-1 DW)
WW	19.80 ± 2.19 a	0.69 ± 0.03 a	9.00 ± 0.51 a	307.16 ± 7.83 a	0.77 ± 0.02 a	2.20 ± 0.30 b	0.065 ± 0.009 b	1.98 ± 0.18 b
WD	21.14 ± 2.82 a	0.59 ± 0.09 b	7.63 ± 0.74 b	290.07 ± 16.87 b	0.72 ± 0.04 b	2.83 ± 0.45 a	0.073 ± 0.012 a	4.77 ± 0.33 a
Genotypes								
OLISUN03	22.07 ± 1.97 a	0.61 ± 0.08 a	8.16 ± 0.80 a	289.31 ± 12.46 a	0.72 ± 0.03 a	2.76 ± 0.41 a	0.077 ± 0.010 a	3.71 ± 0.40 a
AGUARÁ06	19.57 ± 2.23 a	0.64 ± 0.11 a	8.21 ± 1.23 a	296.59 ± 18.17 a	0.74 ± 0.04 a	2.54 ± 0.56 a	0.065 ± 0.008 a	4.00 ± 0.37 a
BRS323	20.00 ± 2.74 a	0.67 ± 0.04 a	8.35 ± 0.34 a	305.32 ± 9.81 a	0.76 ± 0.02 a	2.39 ± 0.28 a	0.065 ± 0.011 a	2.69 ± 0.46 b
HELIO250	20.22 ± 3.15 a	0.66 ± 0.05 a	8.56 ± 0.66 a	303.24 ± 11.29 a	0.76 ± 0.02 a	2.38 ± 0.38 a	0.068 ± 0.014 a	3.10 ± 0.32 b

A_N, net assimilation rate; g_{sw}, stomatal conductance for water vapour; E, transpiration rate; Ci, CO_2 concentration at the substomatal cavity; Ci/Ca, internal carbon and external carbon ratio; A_N/E, instantaneous water-use efficiency; A_N/Ci, instantaneous carboxylation efficiency. Data are means ± SD. Means followed by the same letters in water conditions and genotypes did not differ by the Scott–Knott test (P<0.05).

The net assimilation rate (A_N) was not altered as a function of water condition or genotype. However, there was a reduction in the stomatal conductance rate for water vapour (g_{sw}), transpiration rate (E), CO_2 concentration at the substomatal cavity (Ci), and internal and external carbon (Ci/Ca) in plants under WD conditions. The instantaneous water-use efficiency (A_N/E) and carboxylation efficiency (A_N/Ci) were higher in plants under WD conditions than in those under WW conditions. For the genotypes, no significant differences were found. The WD condition induced an increase of 140.91% in proline accumulation in sunflower genotypes, with higher levels observed in AGUARÁ06 and OLISUN03 than in the other genotypes.

Anatomical characteristics of the leaf

No significant differences were observed in mesophyll thickness (Fig. 4C), palisade parenchyma thickness (Fig. 4D), spongy parenchyma thickness (Fig. 4E), or lower epidermis thickness (Fig. 5D). However, there was a change in the area of the intercellular spaces and substomatic chambers of these tissues (Fig. 3).

In the palisade parenchyma, WD induced an increase in the intercellular space and substomatal chamber areas in AGUARA06 and BRS323 (28.15 and 103.63%, respectively) (Fig. 3E). In the spongy parenchyma, WD induced an increase in the intercellular space and substomatal chamber areas in AGUARÁ06 (41.46%) and BRS323 (39.62%) and a reduction in HELIO250 (43.15%; Fig. 3F). On the other hand, when the plants were maintained at field capacity (WW condition), larger areas of the intercellular space and substomatal chamber of the palisade parenchyma under the WW condition were observed in AGUARÁ06 (22.31%) and OLISUN03 (22.08%). Regarding the spongy parenchyma, the genotypes did not differ regarding the area of the intercellular space and the substomatal chamber of the WW plants (Fig. 3F).

There was no significant interaction between the studied factors and the distance between the vascular bundles in the mesophyll and the thickness of the upper epidermis. Under WD conditions, a 13.74% reduction in the distance between the vascular bundles was observed (Fig. 4G). There were no significant differences in the distance between the vascular bundles among the genotypes (Fig. 4H). The upper epidermis was thicker (22.04 µm) in the genotypes under the WW condition than in those under the WD condition (20.20 µm) (Fig. 4A). Among the genotypes, OLISUN03 and AGUARÁ06 (22.31 and 21.88 µm, respectively) were thicker, and HELIO250 and BRS323 (20.72 and 19.56 µm, respectively) were thinner (Fig. 4B).

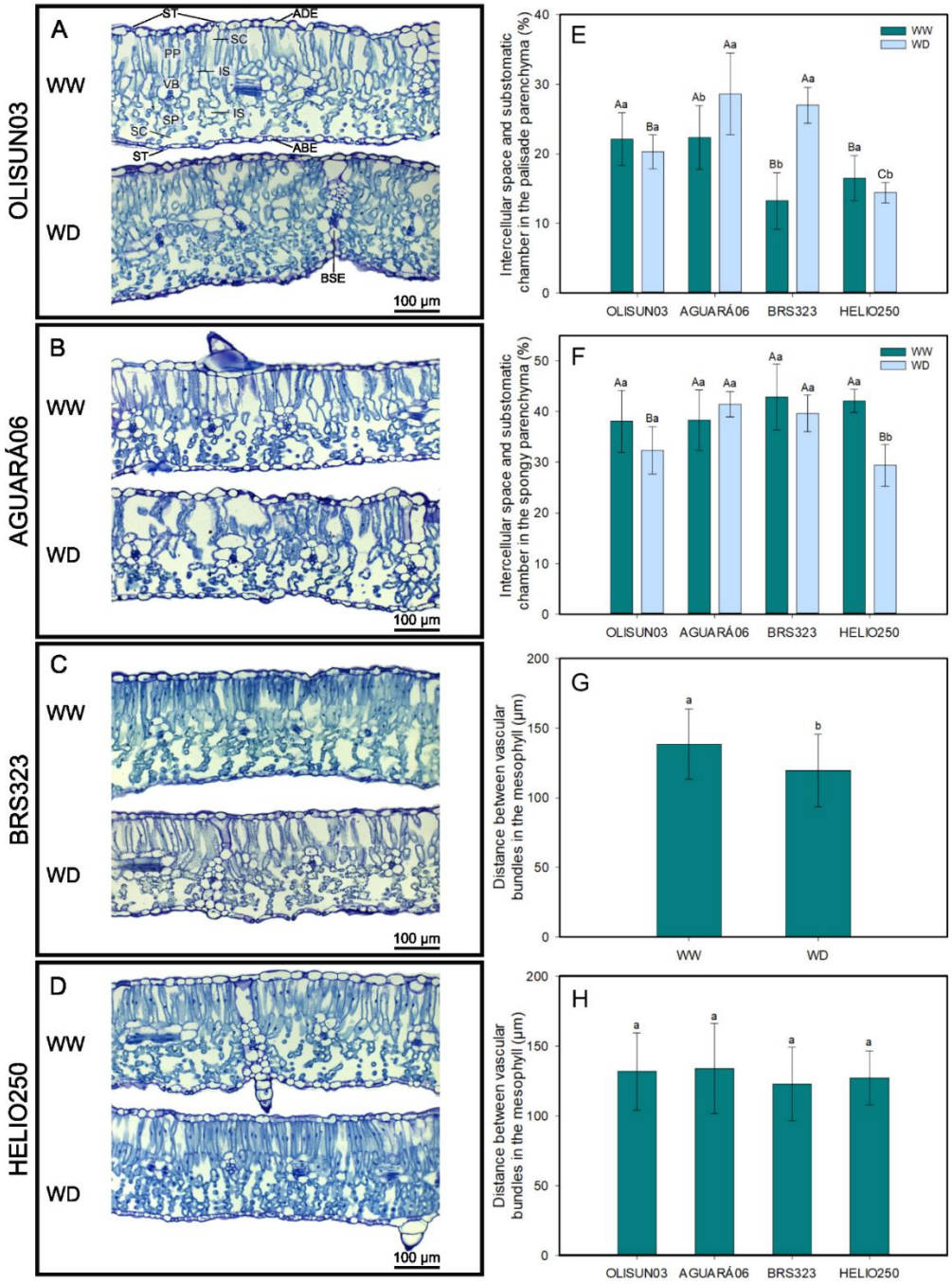


Fig. 3. Transverse sections of the leaf blade and leaf anatomical characteristics of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. BSE, bundle sheath extension; ABE, abaxial epidermis; ADE, adaxial epidermis; IS, intercellular space; PP, palisade parenchyma; SC, substomatal chamber; SP, spongy parenchyma; ST, stomata; VB, vascular bundle. Histograms represent the mean value ± SD. Means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors); means followed by equal letters in water conditions and genotypes (isolated factors) do not differ by the Scott–Knott test (P<0.05).

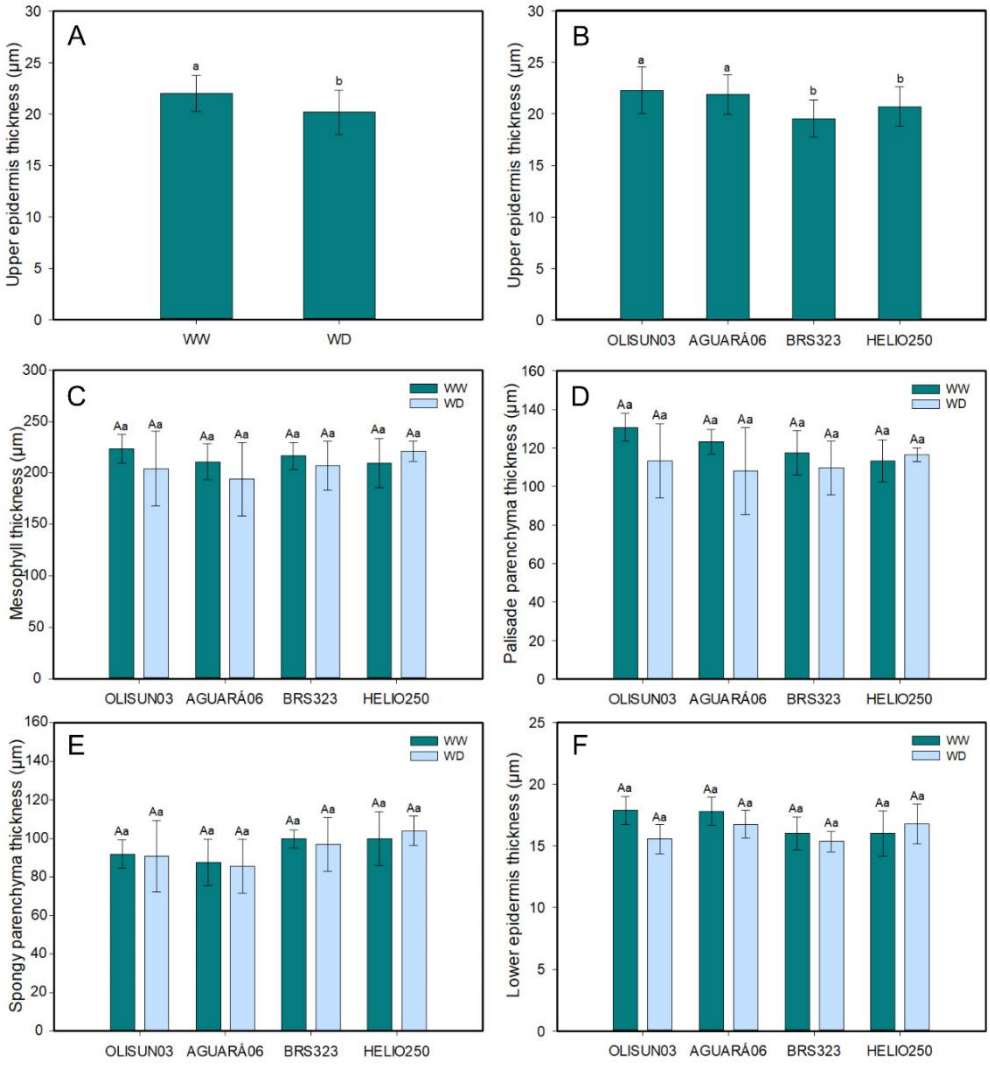


Fig. 4. Leaf anatomical characteristics of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. Histograms represent the mean value ± SD. Means followed by equal letters in water conditions and genotypes (isolated factors) means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors) do not differ by the Scott–Knott test (P<0.05).

There was an interaction between the studied factors and the total area of the midrib and area of the collenchyma. Under WD conditions, the total area of the midrib was reduced in the sunflower genotypes (Fig. 5A). However, the greatest reductions in the midrib area in genotypes under the WD condition occurred in AGUARÁ06 (51.52%) and BRS323 (39.40%), which had the smallest areas (Fig. 5B). In three of the studied genotypes, there was an increase in the collenchyma area under WD conditions (Fig. 5C), and among the genotypes, HELIO250 had the greatest collenchyma area (25.01%). No differences were observed among the genotypes

for the area of the midrib vascular bundles (Fig. 5D). For vessel diameter, a reduction of 11.72% was observed between the genotypes under the WD condition in comparison to those under the WW condition (Fig. 5E). Among the genotypes, BRS323 and AGUARÁ06 (51.91 and 49.65 µm, respectively) had the largest vessel diameters, while OLISUN03 and HELIO250 (46.87 and 46.35 µm, respectively) had the smallest vessel diameters (Fig. 5F).

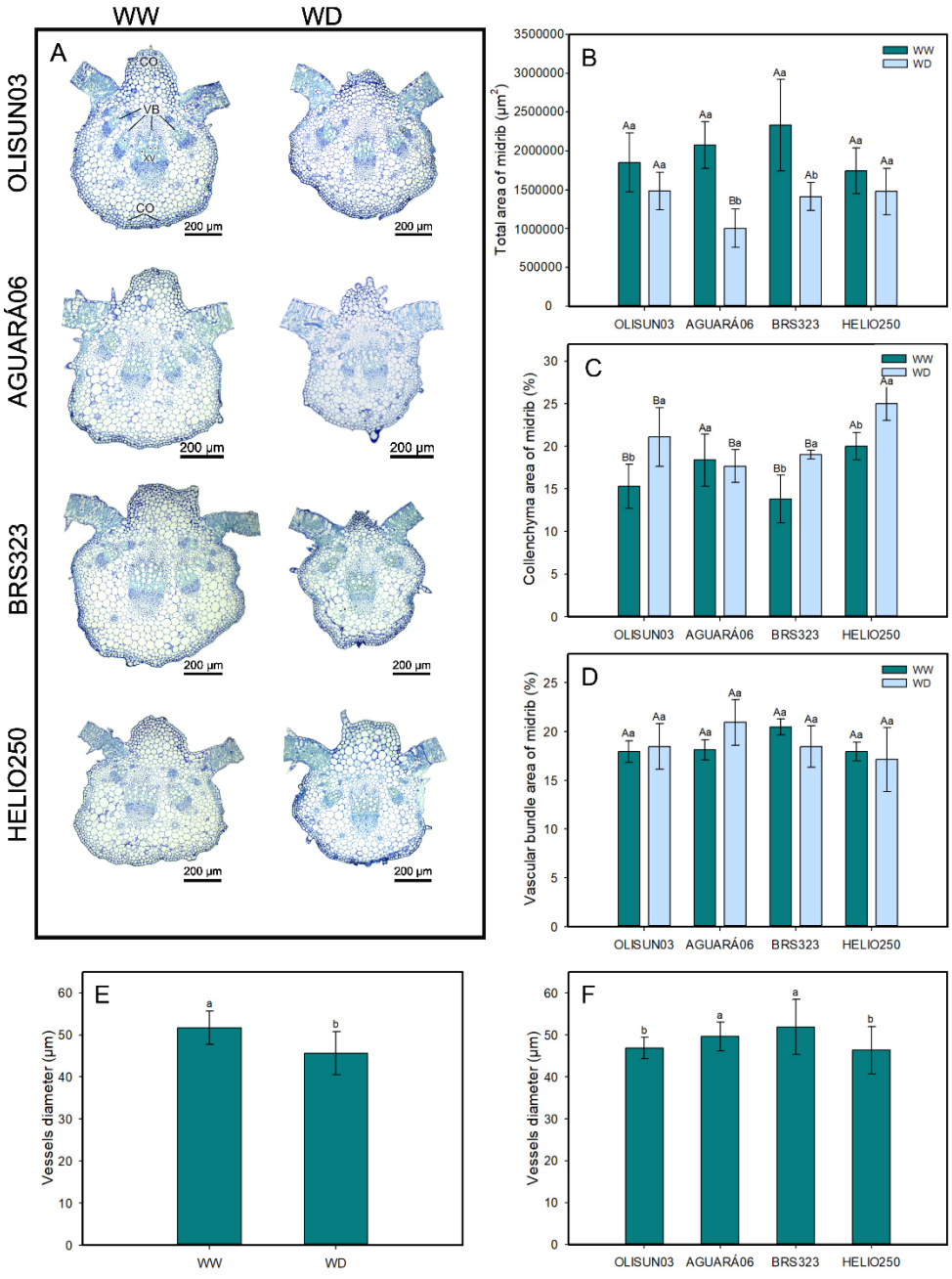


Fig. 5. Anatomical sections and leaf anatomical characteristics of the midrib of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. CO, collenchyma; VB, vascular bundle; XV, xylem vessels. Histograms represent the mean value ± SD. Means followed by equal letters, uppercase for genotypes and lowercase

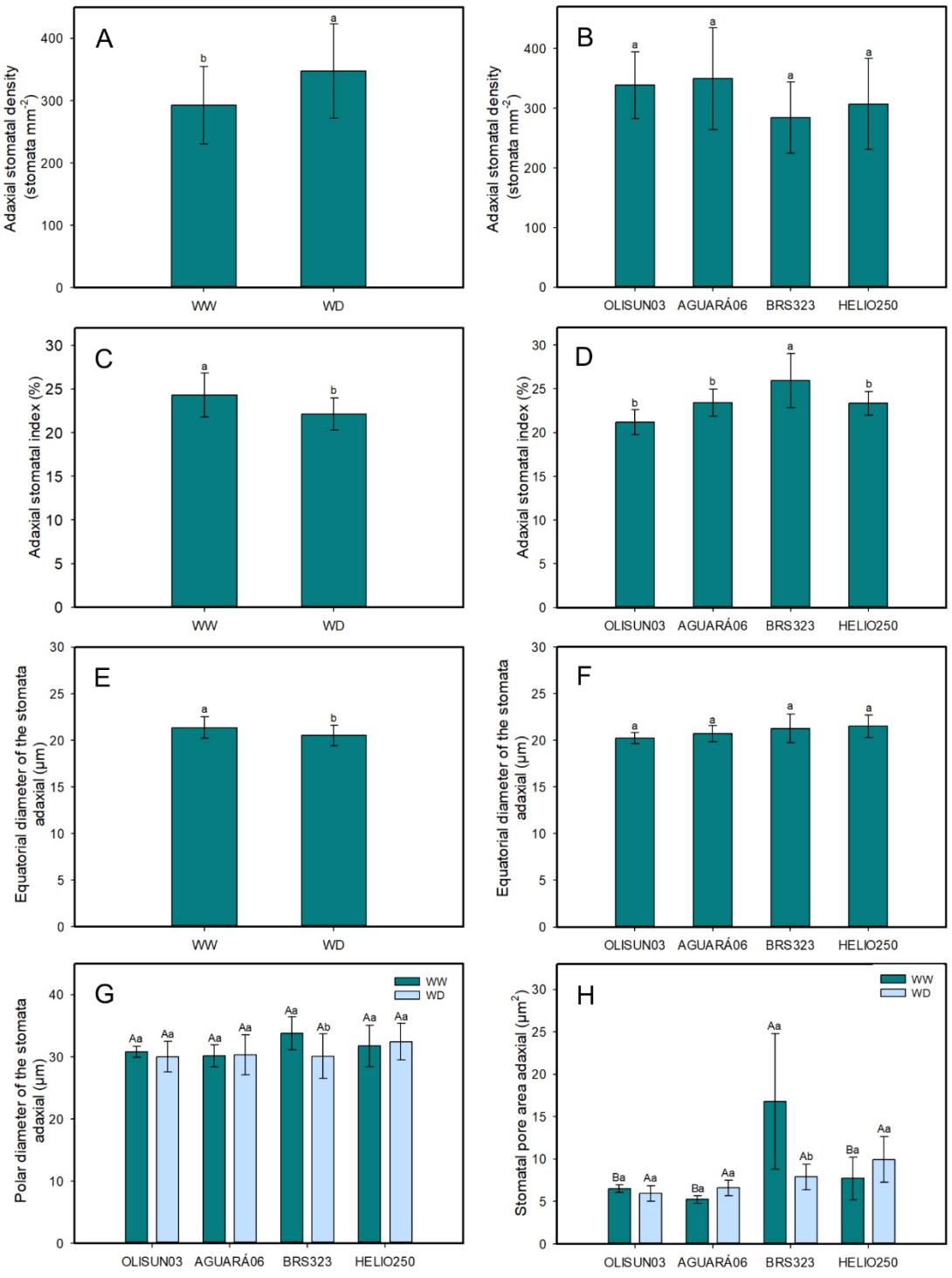
for water conditions (interaction between factors); means followed by equal letters in water conditions and genotypes (isolated factors) do not differ by the Scott–Knott test ($P<0.05$).

In the adaxial surfaces of the leaves, an increase of 18.70% in the stomatal density of the genotypes was observed under the WD condition compared to that under the WW condition (Fig. 6A). Among the genotypes, no differences were observed (Fig. 6B). In contrast, for genotypes under the WD condition, a reduction of 8.97% in the SI on the adaxial face was observed (Fig. 6C). Among the genotypes, BRS323 had the highest SI (25.92%), while AGAURÁ06, HELIO250 and OLISUN03 had SIs of 23.42, 23.33 and 21.17%, respectively (Fig. 6D). There was also a significant reduction in the adaxial equatorial diameter of 3.98% among the genotypes under the WD condition compared to the genotypes under the WW condition (Fig. 6E). There were no differences in the equatorial diameter among the genotypes (Fig. 6F). In addition, no differences in polar diameter were observed between water conditions or between genotypes (Fig. 6G).

There was an interaction between water conditions and genotypes in the opening of the adaxial stomatal pore. Adaxial stomatal pore opening only occurred in genotypes under WD conditions, with no difference among the genotypes. The same trend was observed for genotypes under the WW condition, except for BRS323, which obtained a higher adaxial stomatal pore opening compared to genotypes under the same condition and an increase of 112.55% in relation to the BRS323 genotype under the WD condition (Fig. 6H).

The abaxial anatomical characteristics (except for equatorial diameter) showed interactions between water conditions and genotypes. Compared to the WW condition, under the WD condition, an increase in stomatal density was observed in the BRS323 and HELIO250 genotypes (77.17 and 64.15%, respectively) (Fig. 7A). Under WD conditions, the highest stomatal density (520 stomata mm^{-2}) occurred in BRS323, while under WW conditions, the highest stomatal density (546 stomata mm^{-2}) occurred in AGUARÁ06 (Fig. 7A). SI was not statistically different among genotypes under the WD condition, however, under the WW condition, SI increased by 23.47 and 12.69% in BRS323 and OLISUN03, respectively (Fig. 7B). The area of the abaxial stomatal pore opening in OLISUN03, AGUARÁ06, and HELIO250 was smaller under WD conditions. Between the two conditions, the greatest reduction in the abaxial stomatal pore area (76.40%) occurred in HELIO250 (Fig. 7C). There was a significant reduction (12.76 and 8.00%) in the polar diameter of the abaxial stomata in HELIO250 and BRS323, respectively, under the WW condition and the WD condition (Fig.

450 7D). Under WW conditions, the largest polar diameters (35.99 and 34.65 µm) occurred in
451 HELIO250 and BRS323, respectively, differing from those of OLISUN03 and AGUARÁ06
452 (32.73 and 30.18 µm, respectively). No differences were observed among the genotypes under
453 the WD condition (Fig. 7D). In comparison to the WW condition, the WD condition promoted
454 a significant reduction of 5.31% in the equatorial diameter of the abaxial stomata (Fig. 7E).
455 Among the genotypes, HELIO250 had the largest equatorial diameter of abaxial stomata (23.04
456 µm), differing from those of BRS323, AGUARÁ06, and OLISUN03 (22.13, 21.77 and 21.75,
457 respectively) (Fig. 7F).



458

459 **Fig. 6.** Characteristics of stomata of the adaxial face of four sunflower genotypes grown in
460 rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. Histograms
461 represent the mean value ± SD. Means followed by equal letters in water conditions and
462 genotypes (isolated factors) means followed by equal letters, uppercase for genotypes and
463 lowercase for water conditions (interaction between factors) do not differ by the Scott–Knott
464 test (P<0.05).

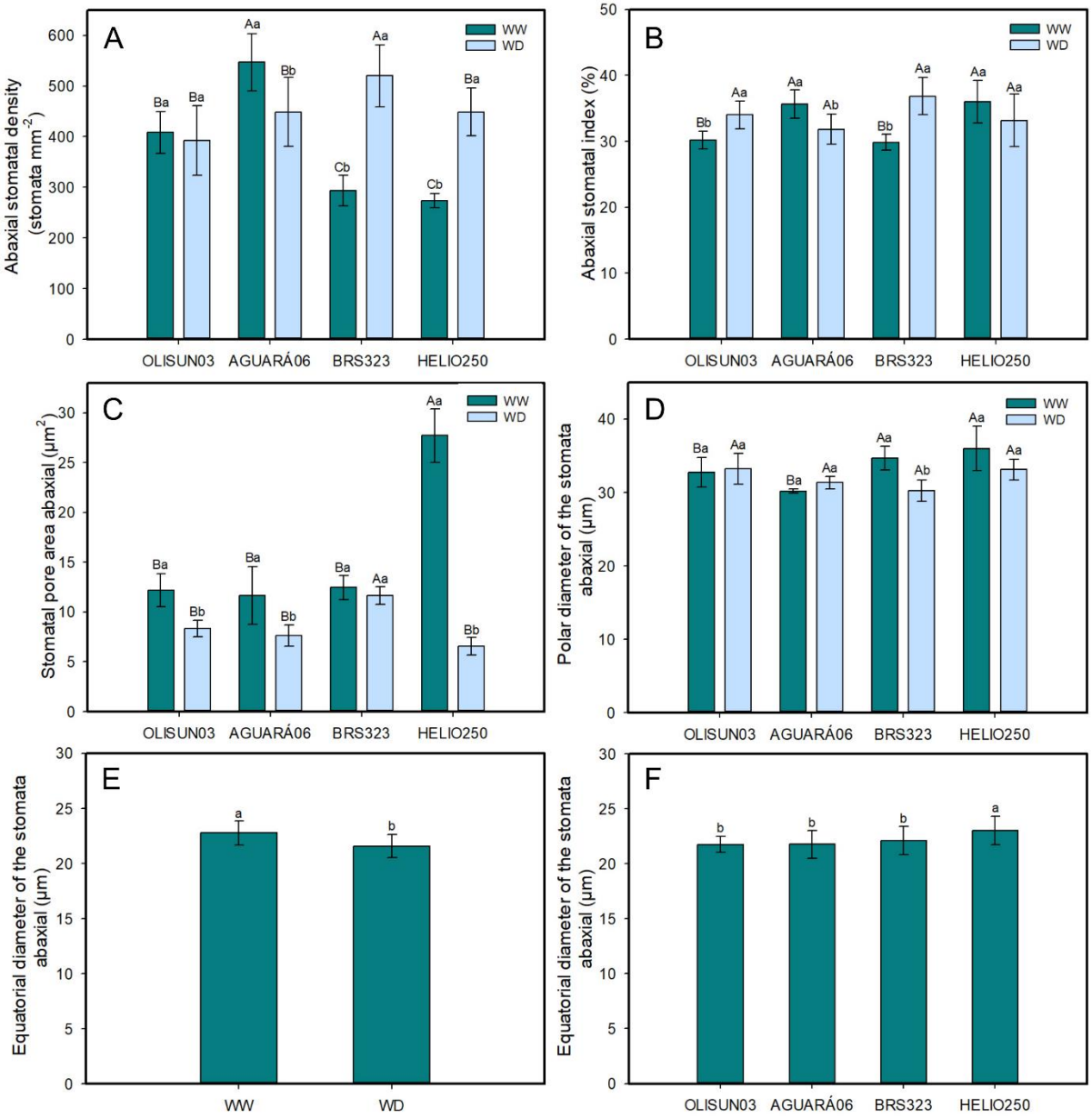


Fig. 7. Characteristics of stomata of the abaxial face of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. Means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors); means followed by equal letters in water conditions and genotypes (isolated factors) do not differ by the Scott–Knott test (P<0.05).

Discussion

This study monitored a set of morphological, anatomical and physiological characteristics in sunflower genotypes under controlled water deficit conditions. Responses to water deficit vary, enabling tolerance in sunflowers. The four commercial sunflower plant genotypes evaluated, when grown in pots at 40% field capacity, i.e., under severe water restriction, showed delayed reproductive development, as indicated by the later emergence of flowers. A greater delay was observed in BRS323 and HELIO250 than in the other genotypes. Growth in terms of height, although it lasted longer, particularly in these genotypes, was reduced under WD conditions in all genotypes.

Except the HELIO250 genotype, changes in growth due to WD also included changes in SLA, LMR, and root/shoot ratio. According to these indices, HELIO250 plants also stood out because they did not show a reduction in root dry mass accumulation in response to water deficit. However, the genotypes did not differ in terms of biomass accumulation in the roots, leaves or leaf area. This explains the absence of a significant difference between the genotypes regarding gas exchange, particularly the rate of photosynthetic CO_2 assimilation.

For all four genotypes, WD conditions induced a reduction in leaf area and leaf biomass accumulation, although the rates of A_N and quantum yield were not significantly modified. It is suggested that this scenario developed from the morphoanatomical and physiological changes observed in these plants. In plants under WD conditions, reductions in stomatal conductance, leaf transpiration rates and leaf carbon concentration were observed in this study, similar to those observed in other crops subjected to WD conditions (Pires *et al*., 2020; Langner *et al*., 2021; Becker *et al*., 2021), contributing to changes in the mechanism of internal input and diffusion of CO_2 and, consequently, in the A_N/E (Bertolino *et al*., 2019).

In sunflower, stomata are present on both sides of the epidermis. The changes in the CO_2 input mechanisms involved a reduction in the number and density of stomata in the adaxial epidermis, a change in morphology (flattening), and a reduction in the pore area. All these modifications have been cited as drought tolerance strategies (Ozkur *et al*., 2009). In this study, an increase of more than 100% in leaf proline levels was also determined, similar to the results of Carvalho *et al*. (2018) for the same northeastern semiarid genotypes and those of Barros *et al*. (2019) in a controlled environment. The increase in proline accumulation in response to WD suggests osmotic adjustment, a mechanism that allows plants to decrease water potential and thus favour water absorption and increased turgor (Rauf, 2008).

The rate of A_N was not altered by water conditions. However, the stomatal conductance and transpiration rates were altered under WD conditions. It has been suggested that the ability of plants to adjust photosynthesis in response to water deficit may be correlated with the leaf anatomy of the sunflower genotypes, involving mechanisms of internal input and diffusion of CO_2, such as the density index, as well as space for the diffusion of CO_2 in tissues, such as intercellular spaces and substomatal chambers of the mesophyll. Sunflower plants have stomata on both sides of the leaves, and under water deficit conditions, the stomatal density and SI data, in addition to the polar and equatorial diameters and opening of the stomatal pore, were adjusted, suggesting that they contributed to the net assimilation rate (Figs. 7 and 8). Greater stomatal closure and changes in stomatal morphology are mechanisms (or strategies) to prevent the impact of drought (Ozkur *et al*., 2009). These changes favour the optimization of CO_2 absorption for photosynthesis and minimize water loss, which is related to A_N/E (Bertolino *et al*., 2019). These changes were reflected in the reduction in g_{sw}, E, Ci and the Ci/Ca ratio for genotypes under WD conditions.

Under WD conditions, the HELIO250 genotype stood out from the other genotypes because there was no reduction in dry mass accumulation in the roots, SLA, leaf mass ratio or root/shoot ratio. Although the number of stomatal openings in the adaxial epidermis did not reduce in response to WD, this genotype saw a decrease in the area of its stomatal pore, which minimizes water loss by transpiration. In addition, near the adaxial epidermis, there was no change in the area of the intercellular spaces and the substomatic chamber of the palisade parenchyma or reductions in the total area of the main vein, which are characteristics that support the CO_2 diffusion necessary to maintain photosynthetic activity and biomass accumulation. In comparison to the other genotypes, this genotype also had a higher minimum leaf water potential, regardless of water conditions.

On the other hand, under WD conditions, the genotypes OLISUN03, BRS323, and HELIO250 invested in increasing the area of the collenchyma (Fig. 5C), a dynamic tissue that plays a role in increasing structural support and maintaining water balance (Dos Anjos et al., 2015). Under this condition, the genotypes AGUARÁ06 and BRS323 showed an increase in the area of intercellular space and the substomatal chamber in the palisade parenchyma. This change favours the internal storage of CO_2 and greater efficiency in the diffusion of CO_2 and water use, partially compensating for losses due to stomatal restrictions and consequently contributing to the efficiency of photosynthesis. According to Oliveira et al. (2022), in plants with drought tolerance, the relationship between increased intercellular spaces and lower water

content is important to modulate the movement of CO_2 and water vapour within the leaf. In the present study, this was observed and was reflected in the increase in A_N/E for photosynthetic CO_2 assimilation and in the instant carboxylation efficiency of Rubisco, which were higher under the WD condition than under the WW condition (Table 3).

Another adjustment, observed in the OLISUN03 and HELIO250 genotypes, which is notable is the reduction in the diameter of the xylem vessels and the distance between the vascular bundles in the main vein of the leaves. This scenario favours the maintenance of the water balance and makes it possible to reduce damage to the leaf water conduction systems, as well as improve water distribution along the entire leaf extension. In addition, the sunflower genotypes differed regarding the accumulation of proline in the leaves in response to WD, and proline accumulation was higher in the OLISUN03 and AGUARÁ06 genotypes than in the other genotypes (Table 3).

In this study, it was verified that in sunflower cultivars, some characteristics favoured greater tolerance to water deficit. Among these, it is worth highlighting the increase in the area of the intercellular space and the substomatic chamber of the palisade parenchyma (greater in AGUARÁ06 and BRS323), the area of the intercellular space and the substomatic chamber of the spongy parenchyma (greater in HELIO250) and the area of the collenchyma in the midrib (larger in OLISUN03, BRS323 and HELIO250). The increase in air spaces, as already discussed, favoured the efficiency of photosynthesis, which was reflected in the development of the sunflower. In addition, it is necessary to emphasize the characteristics related to the stomata, which showed the importance of the stomatal mechanism for sunflower cultivars in the face of water restrictions: increase in stomatal density (higher in BRS323 and HELIO250), in the stomatal index (higher in OLISUN03 and BRS323) and in the polar diameter of the stomata (larger in BRS323) on the abaxial surface, reduction of the stomatal pore opening on the abaxial surface (larger on OLISUN03, AGUARÁ06 and HELIO250), and reduction of the stomatal pore opening on the adaxial surface (larger on BRS323). The study developed under controlled conditions of water deficit allowed us to identify attributes related to the drought tolerance of sunflower cultivars for use as morphophysiological descriptors. These leaf anatomical characteristics can contribute to the selection of new sunflower genotypes for the Sunflower Genetic Improvement Program for cultivation in regions with water restrictions.

Conclusions

Water deficit affected the development of sunflower genotypes, which was observed in growth characteristics and mass accumulation. We found that anatomical characteristics, such as the intercellular space area and substomatal chamber, as well as changes in stomatal density and morphology, xylem diameter, and proline content, modulated and allowed efficient gas exchange for all genotypes.

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Author Contributions

EMC, MPP: Conceptualization; EMC, CGPC and LMC: Resources; EMC, OBS and MPP: Methodology; OBS, MPP, IAB and ELC: Investigation. All authors contributed to writing and review the manuscript.

Conflict of Interest

No conflict of interest declared.

Data Availability

Data are available upon request to the corresponding author.

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Table 1. Plant growth characteristics of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. LDM, leaf dry mass; RDM, root dry mass; TDM, total shoot; LA, total leaf area. Data are means ± SD. The means followed by the same lowercase letter in the rows (comparing humidity conditions) and uppercase letters in the columns did (comparing genotypes) not differ according to the Scott–Knott test (P<0.05).

Table 2. Physiological growth indexes of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. SLA, specific leaf area; LAR, leaf area ratio; LMR, leaf mass ratio; RSR, root/shoot ratio. Data are means ± SD. The means followed by the same lowercase letter in the rows (comparing humidity conditions) and uppercase letters in the columns did (comparing genotypes) not differ according to the Scott–Knott test (P<0.05).

Table 3. Means of gas exchange of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. AN, net assimilation rate; g_{sw}, stomatal conductance for water vapour; E, transpiration rate; Ci, CO_2 concentration at the substomatal cavity; Ci/Ca, internal carbon and external carbon ratio; AN/E, instantaneous water-use efficiency; AN/Ci, instantaneous carboxylation efficiency. Data are means ± SD. Means followed by the same letters in water conditions and genotypes did not differ by the Scott–Knott test (P<0.05).

Fig. 1. Growth characteristics of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit plant conditions (WD). (A-H) Morphological aspects of sunflower genotypes in well-watered condition (WW) and water deficit (WD), (I) Height of plants in well-watered condition (WW), (J) Height of plants in water deficit (WD), (K) Stem diameter in conditions of well-irrigated (WW) plants and water deficit (WD), (L) plant stem diameter, independent of water conditions, and (M) reproductive stages of four sunflower genotypes grown in pots under well-watered and water deficit plant conditions. Symbols represent the mean values. Asterisks indicate the significance (Scott–Knott test, P-value <0.05).

Fig. 2. Leaf water potential of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. (A and B) Leaf water potential recorded at dawn and (C and D) close to noon. Histograms represent the mean value ± SD. Means followed by the same letters in water conditions and genotypes did not differ by the Scott–Knott test (P<0.05).

Fig. 3. Transverse sections of the leaf blade and leaf anatomical characteristics of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. BSE, bundle sheath extension; ABE, abaxial epidermis; ADE, adaxial epidermis; IS, intercellular space; PP, palisade parenchyma; SC, substomatal chamber; SP, spongy parenchyma; ST, stomata; VB, vascular bundle. Histograms represent the mean value ± SD. Means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors); means followed by equal letters in water conditions and genotypes (isolated factors) do not differ by the Scott–Knott test (P<0.05).

Fig. 4. Leaf anatomical characteristics of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. Histograms represent the mean value ± SD. Means followed by equal letters in water conditions and genotypes (isolated factors) means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors) do not differ by the Scott–Knott test ($P<0.05$).

Fig. 5. Anatomical sections and leaf anatomical characteristics of the midrib of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. CO, collenchyma; VB, vascular bundle; XV, xylem vessels. Histograms represent the mean value ± SD. Means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors); means followed by equal letters in water conditions and genotypes (isolated factors) do not differ by the Scott–Knott test ($P<0.05$).

Fig. 6. Characteristics of stomata of the adaxial face of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. Histograms represent the mean value ± SD. Means followed by equal letters in water conditions and genotypes (isolated factors) means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors) do not differ by the Scott–Knott test ($P<0.05$).

Fig. 7. Characteristics of stomata of the abaxial face of four sunflower genotypes grown in rhizotron pots under well-watered (WW) and water deficit (WD) plant conditions. Means followed by equal letters, uppercase for genotypes and lowercase for water conditions (interaction between factors); means followed by equal letters in water conditions and genotypes (isolated factors) do not differ by the Scott–Knott test ($P<0.05$).

ARTIGO 2: **ROOT SYSTEM MORPHOANATOMY OF SUNFLOWER GENOTYPES UNDER WATER DEFICIT**

ARTIGO A SER SUBMETIDO NA PLANT BIOLOGY

Root system morphoanatomy of sunflower genotypes under water deficit

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ABSTRACT

- Sunflower is classified as a drought tolerant crop. However, its productivity is affected under water deficit when it reaches the flowering period. Information about the morphoanatomical characteristics of the root system and trichome densities in leaves, tested under controlled water deficit conditions, can contribute to sunflower breeding programs.
- The objective of this study was to identify a set of root morphoanatomical characteristics and trichome density on leaves of four sunflower genotypes subjected to controlled water deficit. We tested four commercial sunflower genotypes (OLISUN03, AGUARÁ06, HELIO250 and BRS323) under well-irrigated (field capacity) and water restriction (40% of field capacity) conditions.
- Under water deficit, the genotypes OLISUN03 and BRS323 have a narrow and deep root system architecture (RSA), contributing to the survival of the plants under limited soil water conditions. In this condition, tissue differentiation occurred first near the root apex. Under

water deficit, the genotypes OLISUN03 and AGUARÁ06 had reduced endoderm thickness and vascular cylinder area.

- The four sunflower genotypes tested have water absorption strategies and morphoanatomical modifications. Characterization of this set of traits contributes to sunflower breeding programs.

Keywords: *Helianthus annuus* L.; root development; root system architecture; anatomical changes.

Introduction

Sunflower (*Helianthus annuus* L., Asteraceae) is an oilseed crop with a high oil content (40 to 60%) and protein content (17 to 20%) and with global potential for the production of edible oil and animal feed (Hussain et al., 2018). It is grown in regions with water restriction or supplemental irrigation (Hussain et al., 2018) and under drought conditions, especially relative to other crops such as maize and wheat (Ibrahim et al., 2016). However, water deficit in the vegetative, flowering and/or achene filling phase promotes significant reductions in yield and oil percentage (Ibrahim et al., 2016). Therefore, identifying root characteristics that may contribute to drought tolerance and ensure yield is essential for crop breeding programs.

The root system comprises a set of characteristics associated with several functions involved in plant development. Among them are anchorage and water and nutrient uptake from soil (Li et al., 2021). Root system architecture (RSA) includes root positioning, length, angle, branching, surface area, coverage and diameter (Karlova et al., 2021). These are adaptive traits for capturing soil resources, such as water and nutrients, thus contributing to plant breeding programs (Alahmad et al., 2019; Zhan et al., 2019; Li et al., 2021).

Anatomical changes involved in the radial transport of water, such as apoplastic and symplastic pathways, occur in the root system (Díaz et al., 2018). The main changes are in the

cortical parenchyma cells and apoplastic barriers, epidermis and sometimes exodermis and endodermis (Klein et al., 2020). Other changes occur in the vascular cylinder, which is the tissue responsible for transporting water to the aerial part of the plant (Klein et al., 2020). In this case, the main changes occur in the xylem vessels, which are modulated according to water availability, either in the diameter and number of vessels or in the thickness of the cell wall (Klein et al., 2020). This set of modifications promotes better water absorption and efficiency, enhancing the productivity of crops such as soybean (Prince et al., 2017) and the reduction in xylem vessels, which prevents xylem embolisms, as evidenced in woody plants (Levionnois et al., 2021).

The characteristics of RSA include the spatial distribution of the root system and anatomical characteristics involved in water absorption and transport. This study highlights a set of characteristics of the root system that may contribute to sunflower breeding programs. Therefore, the hypotheses are as follows: 1) sunflower genotypes have a narrow root system that absorbs water in deep soil layers; 2) sunflowers under water deficit conditions promote root tissue differentiation near the root apex compared to those with adequate irrigation; and 3) in sunflower plants of different genotypes under water stress, there is an increase in apoplastic barriers and a reduction in the vascular cylinder and xylem diameters. The objective of the present study was to evaluate the root morphoanatomical characteristics of four sunflower genotypes subjected to controlled water deficit.

Material and Methods

Location and growth conditions

The experiment was conducted in a greenhouse located in the Botany Sector of the Department of Biology (DBI) of the Federal University of Lavras (UFLA), Lavras, MG, Brazil. The environmental temperature was maintained at 26 ± 2 °C, the relative humidity was between 50% and 70%, and the average photosynthetic photon flux density was 652 µmol m^{-2} s^{-1}, as measured in the plant canopy. A photoperiod of 12 hours in the light and 12 hours in the dark was used. The plants were kept in rhizotron pots (size: 42.5×29.5×3.5 cm) containing a transparent glass plate inclined at 43° towards the horizontal plane, promoting root development next to the plate and consequently facilitating the evaluation of the root system. The rhizotron vessels were filled with 2.8 L of compost, containing washed sand and the commercial substrate Tropstrato (vida verde®, Brazil) at a 1:1 ratio. The substrate had the following properties: pH $CaCl_2$: 5.75; P: 65.70 mg dm^{-3}; K: 1.60 cmolc dm^{-3}; Ca: 23.80 cmolc dm^{-3}; Mg: 12.40 cmolc dm^{-3}; Al: 0.0 cmolc dm^{-3}; H+Al: 4.20 cmolc dm^{-3}; sum of bases: 39.80 cmolc dm^{-3}; cation exchange capacity: 42.10 cmolc dm^{-3}; base saturation (V%): 64.80; electrical conductivity: 1.5 mS cm^{-1}; density on a dry basis: 190 kg m^{-3}; density on a wet basis: 500 kg m^{-3} ; moisture: 60% of the total weight of the substrate.

Plants and experimental design

Four sunflower genotypes were tested, including three commercial hybrids from different breeding programs: OLISUN03 (Advanta Comércio de Sementes Ltda., Campinas, SP, Brazil), AGUARÁ06 (Atlântica Sementes, Curitiba, PR, Brazil), HELIO250 (Heliagro Agricultura e Pecuária Ltda., Araguari, MG, Brazil) and BRS323, a hybrid developed by Embrapa (Empresa Brasileira de Pesquisa Agropecuária, Brasília, DF, Brazil). Sunflower plants were obtained from seeds germinated on Germitest® paper in a germination chamber at

25 °C under 12 h of light provided by lamps, reaching a photosynthetically active photon flux density of 96 µmol m^{-2} s^{-1}. The plants were transferred to the pot rhizotrons when the rootlets reached approximately 2 cm in length.

The experiment was conducted with a 2×4 factorial design (two water conditions and four sunflower genotypes), and a completely randomized design with six replicates was applied, with one plant per rhizotron pot, totalling 48 plants. The water conditions evaluated included well-irrigated plants (WW), corresponding to field capacity, and plants under water deficit (WD), in which the field capacity was progressively decreased from 15 to 30 days after transplanting (DAT), up to 40% and maintained at that point until 51 DAT, i.e., the beginning of the reproductive stage. At this stage, it is possible to determine architectural and anatomical parameters of the root system involved in the effects of water deficit in the period that includes the beginning of flowering and the filling of the achenes.

All plants were irrigated with Hoagland and Arnon (1950) nutrient solution at 40% ionic strength. The moisture content of the compost in the rhizotron vessels was monitored with soil resistive moisture sensors connected to the voltage comparator module (LM393) and regulated with a microcontroller (Arduino Mega2560). The irrigation system for each water condition was automatically activated when the moisture content of the compost reached the field capacity, which was determined for each water regime. In addition, the system consisted of an irrigation pump, distribution hoses and two drip pipes (length 15 cm) positioned at the upper edge of each rhizotron vessel.

Moisture content and root angle

At 51 DAT, the vessels were scanned using an A3 scanner (1200S, Mustek, China), and all analyses were performed using ImageJ software. To visualize the moisture content and distribution in the substrate, the images were stacked, obtaining the average grayscale intensity

values. The images were coloured with 16 colours (LUT), and the intensity and moisture distribution of the rhizotron vessel were visualized with a colour scale (Rellán-Álvarez et al., 2015). The angle of the root system was obtained between the beginning of the main root at the upper edge of the compost and the limit of the secondary roots on the side of the rhizotron vessel. A representation of the root system architecture was created from six stacked images and the configuration of a time-lapse colour coder (LUT-Spectrum).

Anatomical root analyses

At 51 DAT, the rhizotron vessels were disassembled, and roots approximately 20 cm long were collected from the apex and fixed in 70% FAA solution (formaldehyde, glacial acetic acid and 70% ethanol, 1:1:18) for 72 hours, after which they were transferred to 70% ethanol (Johansen, 1940). Subsequently, sections were obtained at 6, 12, 14 and 16 cm from the root tip towards the root base (Fig. 1) and dehydrated in increasing concentrations of ethanol (70, 80, 90 and 100%). At intervals of 2 hours at room temperature, the cells were infiltrated for 24 hours in historesin (Leica Microsystems, Heidelberg, Germany). The cross-sections (7 µm thickness) were obtained using a semiautomatic rotating microtome, stained with 0.05% (w/v) toluidine blue (Feder and O'Brien, 1968) and mounted on permanent slides with Entellan (Merck, Darmstadt, Germany). The slides were photographed with a camera attached to a microscope (Eclipse E100-LED; Nikon, Tokyo, Japan). Quantitative anatomical data were obtained using ImageJ software, and analyses in which all tissues were differentiated were performed in 12-cm sections from the root apex (Figure 1). The thicknesses of the epidermis, exodermis, cortex and endodermis were determined, as were the diameters of the metaxylem, vascular cylinder and cortex areas.

Statistical analyses

The data were tested for normality using the Shapiro–Wilk test. The means were subjected to analysis of variance (ANOVA) followed by the Scott–Knott test. All analyses were performed using Sisvar 5.0 software (Ferreira, 2011).

Results

Moisture content and root angle

The moisture mapping in the rhizotron vessels showed that in the OLISUN250 and HELIO250 genotypes, under the two water conditions, water uptake by the root system occurred in the middle and superficial regions of the rhizotron vessels (Fig. 2a, b, g, h). In the OLISUN250 genotype, under water deficit, a higher moisture content was observed in the upper side of the vessels (Fig. 2b), while in HELIO250, under both water conditions, higher moisture levels were observed along the sides and in the deep region of the rhizotron vessels (Fig. 2 g, h). In the AGUARÁ06 and BRS323 genotypes, under the two water conditions, water absorption was observed in the different regions of the rhizotron vessels (Fig. 2c, d, e, f). However, for BRS323 water absorption occurred under water deficit in the deep region of the rhizotron vessel (Fig. 2f).

The angle of the root system of the AGUARÁ06 genotype under water deficit was wider (Fig. 2d; Fig. 3) than that under field capacity (Fig. 2c; Fig. 3). On the other hand, the root systems of the OLISUN03 and BRS323 genotypes in the well-watered treatment reached greater angles than those in the water deficit treatment (Fig. 2a, and; Fig. 3). A comparison within the water deficit conditions showed that the root system of the genotype AGUARÁ06 had a greater angle than that of the other genotypes, while in the well-irrigated treatment, the root systems of the genotypes OLISUN03, BRS323 and AGUARÁ06 reached larger angles (Fig. 3).

Root anatomical characteristics

There was a difference in the differentiation of root tissues between the two irrigation conditions, as observed in the anatomical sections collected in different positions, starting from the root tip (Fig. 1). In the plants under water deficit, all the root tissues located 6 cm from the apex were differentiated, while in those under field capacity, several tissues were differentiated, especially those of the vascular cylinder (Fig. 1d).

The interaction between genotype and water condition had an effect on endoderm thickness and vascular cylinder area. The endoderm thickness was greater in OLISUN03 in the WW condition than in the other genotypes in the same condition; this was the only genotype for which a significant reduction was observed when the plants were subjected to the water deficit condition (Table 1).

Comparing the genotypes under water deficit, it was observed that BRS323 presented greater endodermis thickness (Table 1). Water deficit caused a reduction in the vascular cylinder area only in AGUARÁ06. Among the genotypes under the WW condition, the smallest areas of vascular casts occurred in BRS323 and HELIO250 (Table 1).

There was no interaction among the thicknesses of the epidermis, exodermis, cortex, and cortex area or metaxylem diameter. However, for all the genotypes, water deficit allowed for an increase in the thickness of the root epidermis and a reduction in the diameter of the metaxylem vessel, while the field capacity condition allowed for greater thickness and total cortex area (Table 2). Regardless of the water conditions, a reduction was observed in the thickness of the exodermis in OLISUN03 and in the diameter of the metaxylem in HELIO250.

Discussion

This study, conducted in rhizotron vessels under controlled water deficit conditions, allowed us to obtain parameters that reflect the development of sunflower under field conditions. Thus, the characteristics evaluated, such as RSA and root anatomy, which are involved in the mechanisms of water absorption, promoted structural and anatomical changes under controlled water deficit conditions. Therefore, we have evidenced a set of root system characteristics that may be related to sunflower tolerance to water deficit and may affect grain yield (achenes) in areas with prolonged summers (Carvalho et al. 2018; Souza et al. 2019; Carvalho 2020). In addition, the rhizotron vessel research model presented highly relevant, complex and interesting results, corroborating a previous study conducted with maize (*Z. mays* L.) genotypes (Pires et al. 2020).

The mapping of substrate moisture and the angle occupied by the root system showed strategic water uptake by the root systems of sunflower genotypes subjected to controlled water deficit (Fig. 2). The efficiency of water uptake by the root systems of sunflowers belonging to different genotypes is related to the smaller angle of the root system, characterized as narrow and deep, as evidenced in the genotypes BRS323 and OLISUN03 under water deficit (Fig. 2a and 2f).

The RSA is an important characteristic that determines the efficiency of soil water capture to prevent water stress in crops (Li et al., 2021). Crops under water deficit tend to develop roots with a narrower and deeper angle, thus allowing access to nutrients and water in deeper soil layers (Alahmad et al., 2019). This response was observed for the genotypes OLISUN03 and BRS323 under water deficit.

Depending on the water conditions, the sunflower genotypes showed variation in the time of differentiation of the root tissues, observed in anatomical sections from the root apex to the root base (Figure 1). In the plants under water deficit, there was total differentiation of

tissues near the root apex (6 cm), while in those under field water capacity, there was differentiation even further away from the root apex (12 cm). Therefore, this differentiation is a strategy for capturing available water in the deepest part of the soil, evidenced by the narrow, deep root system and water absorption in this region of the rhizotron vessel, as observed in the moisture mapping of genotypes BRS323 and OLISUN03 (Figure 2 B, F). Plants grown under abiotic stress promote anatomical structural changes in different regions of the roots; such an effect was observed for soybean under saline stress [*Glycine max* (L.) Merr.] (Silva et al., 2021) and for maize under high air temperature stress, drought stress or combinations of these two conditions (*Zea mays*) (Pei et al., 2023).

Plants under water deficit underwent root anatomical changes, contributing to water deficit tolerance (Fig. 4, Table 1). In this study, root anatomical characteristics involved in water absorption and conduction, such as endoderm thickness and vascular cylinder area, exhibited plasticity; that is, they responded to water deficit. In particular, a reduction in the vascular cylinder was observed in the AGUARÁ06 genotype and in the endodermis in OLISUN03 (Figure 1, Table 1). These adjustments in the endoderm allow a lower apoplastic barrier, contributing to the radial absorption of water. In addition, the reduction in the vascular cylinder is a protective mechanism and contributes to the movement of water into the shoots and the maintenance of ideal conditions for continued growth of the root system (Hazman & Brown, 2018).

Conclusion

The controlled water deficit induced morphoanatomical changes that were observed in the RSA, the mechanisms of radial water absorption and water transport to the aerial parts of the sunflower plants. This set of traits contributes to the tolerance of sunflower genotypes to water

deficit. In addition, the results shown here can contribute to sunflower genetic improvement programs.

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Table 1. Root anatomical characteristics of four sunflower genotypes (*Helianthus annuus* L.) grown in pots with full irrigation (field capacity; WW) and under controlled water deficit (40% of field capacity; WD).

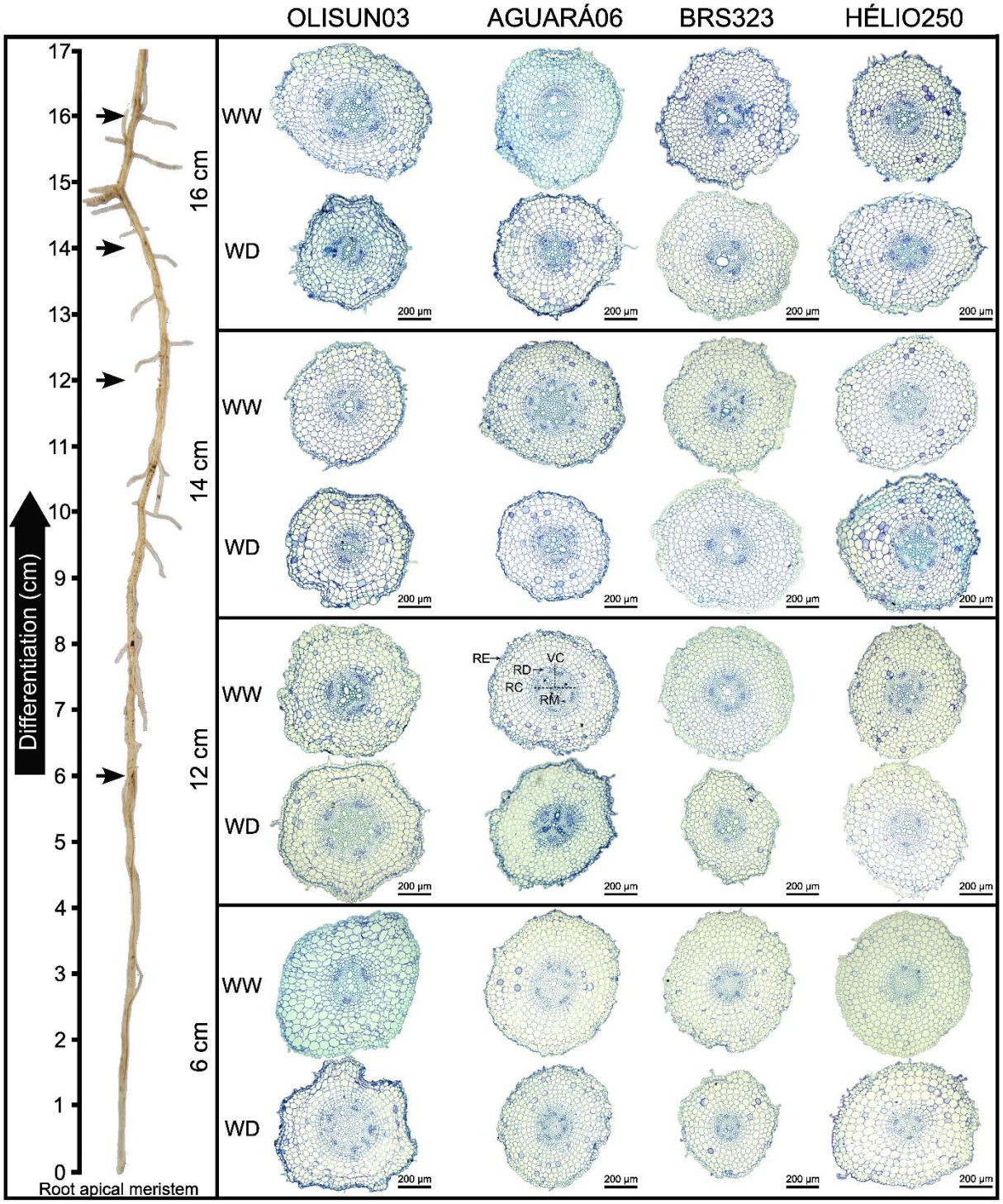
	RDT (µm)		VCA (µm^2)	
Genotypes	WW	WD	WW	WD
OLISUN03	19.96 ± 1.35 Aa	16.43 ± 0.83 Bb	83162 ± 24747 Aa	70013 ± 20407 Aa
AGUARÁ06	16.11 ± 1.31 Ba	15.54 ± 0.97 Ba	104417 ± 30343 Aa	61395 ± 19379 Ab
BRS323	16.80 ± 0.69 Ba	18.67 ± 1.00 Aa	58187 ± 4041 Ba	63374 ± 8770 Aa
HELIO250	16.78 ± 1.82 Ba	15.97 ± 1.64 Ba	45811 ± 9726 Ba	55576 ± 18327 Aa

The means followed by the same uppercase letter in the columns and lowercase letters in the rows did not differ from each other within 5% using the Scott Knott test. Means ± SD. RDT = Root endodermis thickness; VCA = Vascular cylinder area.

Table 2. Root anatomical characteristics of four sunflower genotypes (*Helianthus annuus* L.) grown in pots with full irrigation (field capacity; WW) and under controlled water deficit (40% of field capacity; WD).

Water condition	RET (µm)	REXT (µm)	RCT (µm)	RCA (µm^2)	RMD (µm)
WW	23.1 ± 2.2 b	21.8 ± 2.4 a	285.7 ± 23.7 a	473726 ± 90383 a	33.5 ± 5.1 a
WD	25.1 ± 2.9 a	20.0 ± 3.4 a	249.1 ± 22.1 b	382047 ± 80502 b	30.5 ± 4.4 b
Genotypes					
OLISUN03	22.8 ± 1.3 a	18.5 ± 2.4 b	267.0 ± 29.0 a	433224 ± 67955 a	34.4 ± 5.2 a
AGUARÁ06	24.9 ± 3.0 a	21.0 ± 3.8 a	267.5 ± 30.1 a	474036 ± 141039 a	32.7 ± 3.5 a
BRS323	23.2 ± 1.7 a	21.1 ± 2.7 a	274.3 ± 27.5 a	439883 ± 65669 a	35.6 ± 2.9 a
HELIO250	25.3 ± 3.8 a	22.9 ± 2.2 a	260.8 ± 19.5 a	364403 ± 54537 a	25.4 ± 3.0 b

Means followed by equal letters in water conditions and genotypes, respectively, do not differ from each other using the Scott-Knott test at 5% probability. Means ± SD. RET = Root epidermis thickness; REXT = Root exodermis thickness; RCT = Root cortex thickness; RCA = Root cortex area; RMD = Root metaxylem diameter.



411

412 **Fig. 1.** Transverse sections in different root regions of four sunflower genotypes (*Helianthus*
413 *annuus* L.) grown in pots with full irrigation (field capacity; WW) and under water deficit (40%
414 of field capacity; WD). RE = Root epidermis; REX = Root exodermis; RC = Root córtex; VC =
415 Vascular cylinder; RD = Root endodermis; RM = Root metaxylem.

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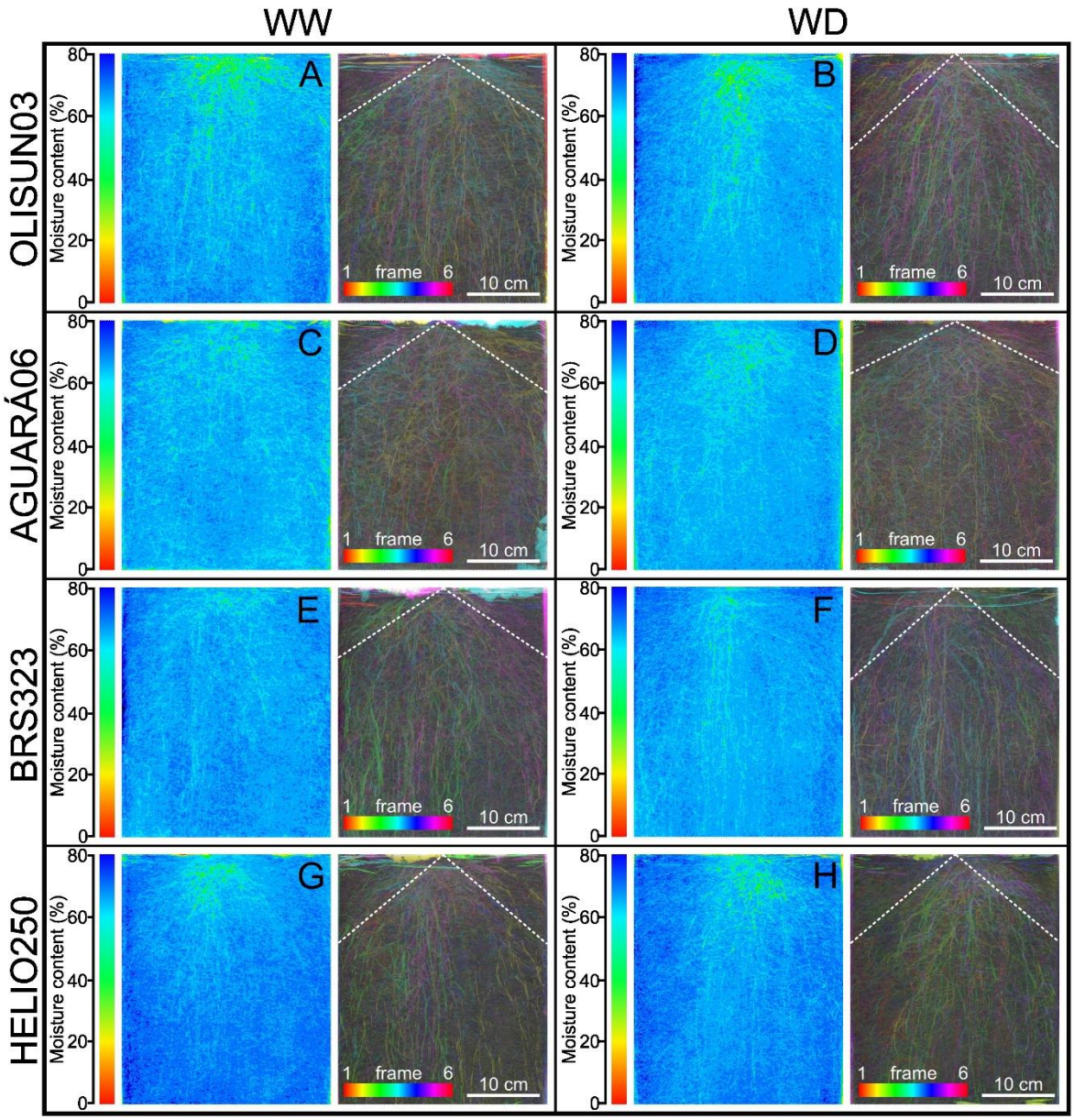

417

418 **Fig. 2.** Moisture content and root system angle of four sunflower genotypes (*Helianthus annuus*

419 L.) grown in pots with full irrigation (field capacity; WW) and under water deficit (40% of field

420 capacity; WD).

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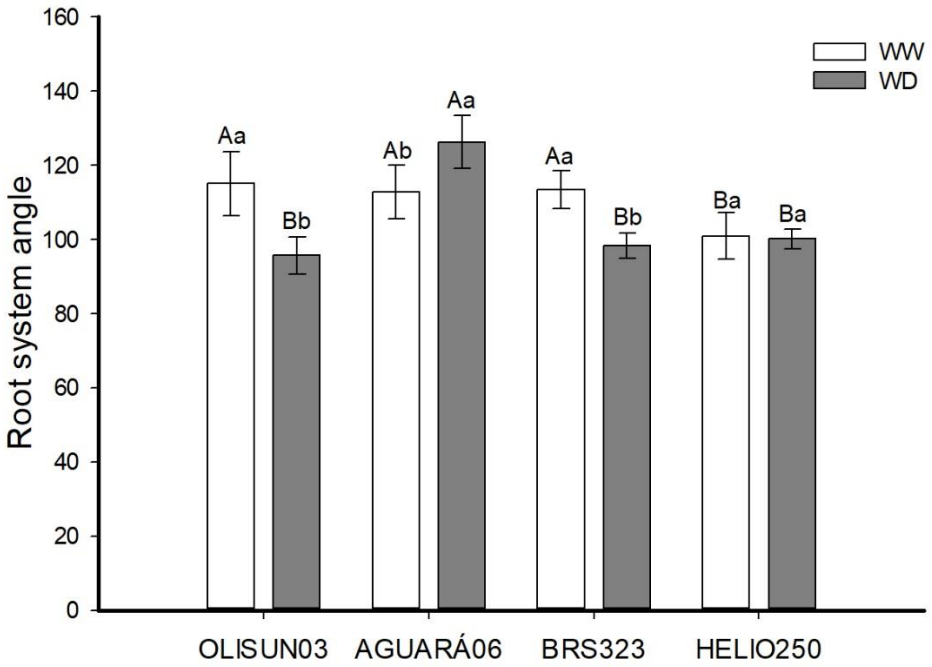

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427 **Fig. 3.** Root system angle averages of four sunflower genotypes (*Helianthus annuus* L.) grown
428 in pots with full irrigation (field capacity; WW) and under controlled water deficit (40% of field
429 capacity; WD).

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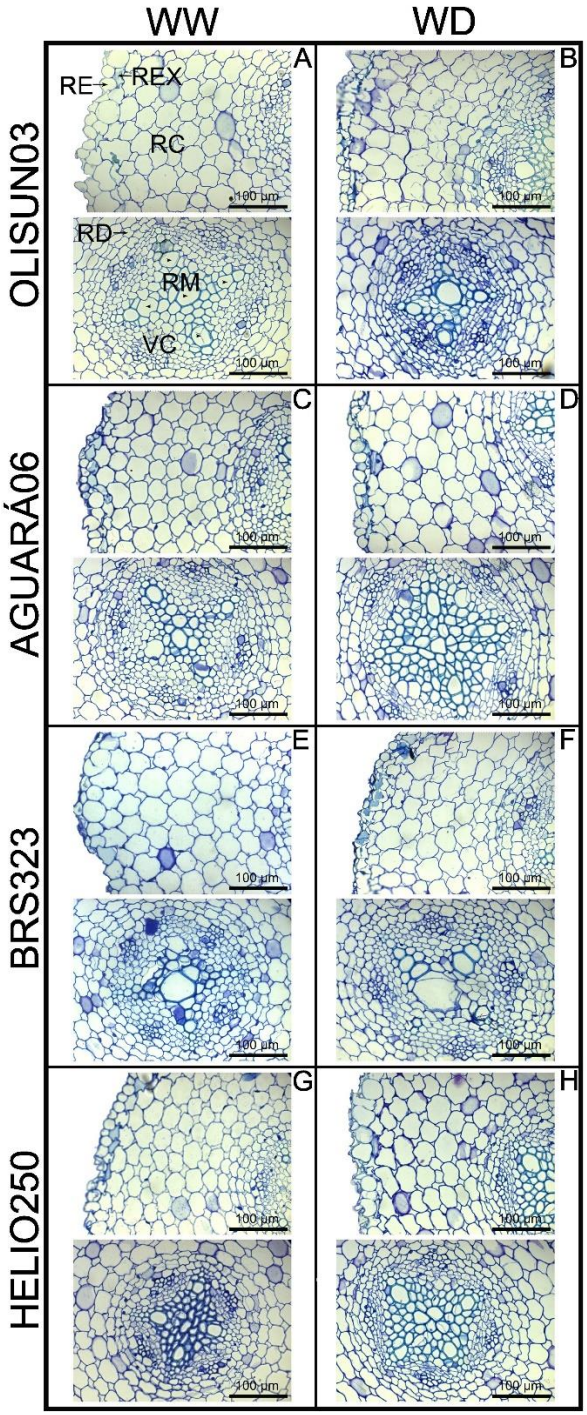

443

444 **Fig. 4.** Transverse sections in roots of four sunflower cultivars (*Helianthus annuus* L.) grown

445 under water conditions of well-irrigated plants - A (field capacity; WW) and under water deficit

446 - B (40% of field capacity; WD). RE = Root epidermis; REX = Root exodermis; RC = Root

447 córtex; VC = Vascular cylinder; RD = Root endodermis; RM = Root metaxylem.