



BETHÂNIA SILVA MORAIS DE FREITAS PEDRINI

**STRUCTURAL AND PHYSIOLOGICAL TRAITS OF
SOYBEAN AND CORN GENOTYPES UNDER WATER
DEFICIT**

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

Prof. Dr. Evaristo Mauro de Castro
Orientador

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BETHÂNIA SILVA MORAIS DE FREITAS PEDRINI

**STRUCTURAL AND PHYSIOLOGICAL TRAITS OF SOYBEAN AND CORN
GENOTYPES UNDER WATER DEFICIT**

**CARACTERÍSTICAS ESTRUTURAIS E FISIOLÓGICAS DE GENÓTIPOS DE
SOJA E MILHO SOB DÉFICIT HÍDRICO**

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

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*A minha irmã gêmea Bheatriz, a minha mãe Doraci e ao meu pai Geraldo que são a minha
fortaleza.*

Ao meu amado Esposo Evandro por todo o amor e companheirismo.

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RESUMO

A soja e o milho são plantas amplamente cultivadas de grande importância econômica para o Brasil e para o mundo. O desenvolvimento das culturas agrícolas desde a sua domesticação foi conduzida pela seleção de características reconhecidas a nível fenotípico. No entanto, a seleção direta para o rendimento de grãos sob condições de stress hídrico foi prejudicada por questões de baixa herdabilidade. A produção agrícola mundial depende em grande parte da capacidade de uma cultura tolerar e aclimatizar-se a determinados fatores ambientais. Atualmente, existem muitos trabalhos relatando o papel do aerênquima e de características do sistema radicular na tolerância à seca de plantas cultivadas. Características como arquitetura radicular, absorção de nutrientes e fixação de nitrogênio são apontadas como promissoras para uma nova e necessária revolução verde. O Brasil destaca-se como grande produtor mundial de milho e soja. Dessa forma, o estudo com variedades de milho e soja, amplamente cultivadas no Brasil, oferece o potencial para a criação de variedades mais tolerantes às nossas condições climáticas, visto que existe uma grande variação nas respostas adaptativas das plantas à seca. Nesse trabalho estudamos a relação das características estruturais e fisiológicas do milho e da soja com a resistência a seca. Em adição foi avaliado os mecanismos moleculares e hormonais envolvidos na formação de aerênquima em raízes de milho.

Palavras-chave: Anatomia da raiz. Déficit hídrico. *Zea mays* L. *Glycine max* (L.) Merrill. Tolerância a seca.

ABSTRACT

Soybean and maize are widely cultivated plants of great economic importance for Brazil and the world. The development of agricultural crops since their domestication has been driven by the selection of traits recognized at the phenotypic level. However, direct selection for grain yield under water stress conditions was hampered by low heritability issues. World agricultural production depends to a large extent on the ability of a crop to tolerate and acclimatize to certain environmental stresses. Currently, there are many studies reporting the role of aerenchyma and characteristics of the root system in drought tolerance of cultivated plants. Traits such as root architecture, nutrient absorption and nitrogen fixation are promising for a new and necessary green revolution. Brazil stands out as a major world producer of maize and soybeans. Thus, the study with varieties of maize and soybeans, widely cultivated in Brazil, offers the potential for creation of varieties tolerant to our climatic conditions, since there is a many variations in the adaptive responses of plants to drought. In this work we study the structural and physiological characteristics of corn and soybeans tolerant to drought. In addition, the molecular and hormonal mechanisms involved in aerenchyma formation in maize roots were evaluated.

Keywords: Root anatomy. Water deficit. *Zea mays* L. *Glycine max* (L.) Merrill. Drought tolerance.

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A seca é uma das principais limitações para a produção agrícola mundial, devido à redução na fotossíntese pelas plantas em função da necessidade de preservar água. A produção agrícola depende em grande parte da capacidade de uma cultura tolerar a determinados estresses ambientais (GAMBETTA, 2016). Dessa forma, as plantas desenvolveram uma série de mecanismos que podem atuar em níveis morfológico, fisiológico, bioquímico, celular e molecular (FANG; XIONG, 2015).

Quando as plantas são expostas ao déficit hídrico, diversas respostas podem ocorrer integrando mecanismos adaptativos. No entanto, diferentes espécies ou genótipos de plantas dentro de uma mesma espécie geralmente possuem grandes variações na utilização desses mecanismos (FANG; XIONG, 2015). Entender como as plantas percebem sinais de estresse e se adaptam aos diversos ambientes são questões biológicas fundamentais (ZHU, 2016). A escassez de água e nutrientes no solo tem causado preocupação e atraído cada vez mais a atenção de pesquisadores acerca da resistência à seca de plantas cultivadas e de características específicas da raiz para o desenvolvimento de culturas resistentes (LYNCH, 2007; FANG; XIONG, 2015).

O milho e a soja, são duas importantes espécies cultivadas no Brasil. A soja é cultivada em 18 estados brasileiros, sendo o Mato Grosso o maior estado produtor, responsável por 26% da produção nacional (CONAB, 2021). O milho é cultivado praticamente em todo o Brasil, e a maior parte da produção nacional do milho concentra-se nas regiões Sul, Centro-Oeste e Sudeste, correspondendo a 90% do total produzido no País (CONAB, 2021). Assim como diversas outras culturas, o milho e a soja são sensíveis ao déficit hídrico. A necessidade de água para a cultura da soja é relativamente elevada e a planta tem baixa eficiência de uso da água (YANG et al., 2003). Quando essas culturas encontram-se em situações de estresse hídrico o primeiro efeito fisiológico é a redução da fotossíntese (BRUCE; EDMENDES; BARKER, 2002).

A complexidade dos mecanismos de tolerância à seca explica o lento progresso na melhoria da produtividade de plantas sob essas condições (CATTIVELLI et al., 2008). Embora as técnicas de seleção tenham sido direcionadas para selecionar características de alto rendimento, a necessidade de selecionar simultaneamente características que atribuam resistência ao estresse tem sido a força motriz na seleção de híbridos (DUVICK, 2005).

Alterações fisiológicas, morfológicas e bioquímicas podem permitir a aclimatização de plantas à seca, constituindo nas estratégias de escape, de evitação e de tolerância (SALEHI-LISAR; BAKHSHAYESHAN-AGDAM, 2016). Através dessas estratégias as plantas podem apresentar uma resposta característica ou uma resposta plástica hereditária que será desencadeada pelos sinais ambientais como o déficit hídrico no solo. No entanto, a sobrevivência da planta sob seca se contradiz com uma elevada produtividade pois há necessidade de alocação de recursos tanto para aclimatação ao estresse quanto para o alto rendimento (GRIFFITHS; PAUL, 2017). Dessa forma, estudar a expressão do caráter de resistência da espécie durante o crescimento vegetativo pode ser uma forma de evitar essa competição.

Muitas pesquisas sobre a adaptação das plantas sob déficit hídrico têm se concentrado somente na eficiência do uso da água, eficiência da transpiração e outras características relacionadas ao ganho de carbono assimilado na fotossíntese que por outro lado acaba perdendo água com a abertura estomática (VADEZ et al., 2014). No entanto, estudos mostram que características anatômicas radiculares como a formação de aerênquima e a redução da área de células corticais vivas podem reduzir os custos metabólicos para a exploração do solo contribuindo para a aquisição de água e gerando um impacto significativo no rendimento da cultura sob seca (LYNCH; CHIMUNGU; BROWN, 2014; CHIMUNGU et al., 2015; LYNCH, 2015). Além disso, a capacidade da planta de manter o turgor celular sob déficit hídrico pelo ajuste osmótico reflete diretamente no aumento da produtividade do genótipo (PIRES et al., 2020).

Baseado na importância das culturas de soja e milho e os impactos negativos do déficit hídrico em suas produções, tem ocorrido uma busca por genótipos tolerantes a fim de mitigar os efeitos de ambiente desfavorável para a produção e garantir a segurança alimentar no futuro. Estudar e identificar características associadas a tolerância a seca ajuda na compreensão do processo de tolerância além de subsidiar os trabalhos dos Programas de melhoramento genético responsáveis pela criação de espécies de acordo com as demandas atuais. O Objetivo do trabalho foi avaliar como as características estruturais e fisiológicas do milho e da soja estão relacionadas com a tolerância à seca. Adicionalmente, foi avaliado os mecanismos moleculares e hormonais envolvidos na formação de aerênquima em raízes de milho.

2 REFERENCIAL TEÓRICO

2.1 Déficit hídrico em plantas

De acordo com o Painel Intergovernamental sobre mudanças climáticas – IPCC, a seca agrícola refere-se aos déficits de umidade de mais ou menos um metro da superfície do solo e que impacta as safras. Muitos dos episódios de seca que ocorrem em todo o mundo são provenientes do fenômeno El Niño/Oscilação Sul, que afeta diversos países incluindo o nordeste do Brasil (TRENBERTH et al., 2014). Por causa das mudanças climáticas houve um aumento na intensidade e frequência dos veranicos, fazendo com que ocorra secas cada vez mais intensas e duradouras (MORA et al., 2017). Em 2015 a região Centro oeste do Brasil foi fortemente afetada com a falta de chuvas com uma redução de 40% na safra de soja 2014/2015 sendo uma das piores secas já registradas (RIBEIRO et al., 2018).

O déficit hídrico é considerado um dos estresses que mais afeta os processos fisiológicos das plantas e consequentemente o crescimento e a produtividade. Em relação à adaptação ao déficit hídrico, as plantas podem ser classificadas em três tipos: plantas xerófitas (que ocorre principalmente em regiões áridas), plantas mesófitas (que ocorrem principalmente em ambientes semi-áridos e sub-úmidos) e plantas hidrófitas que estão distribuídas principalmente em ambientes com umidade ou água em excesso (WARMING et al., 1909). A maioria das plantas cultivadas pertencem ao grupo das plantas mesófitas, o que faz com que a deficiência hídrica seja um problema para essas plantas.

As plantas respondem de diversas formas ao estresse hídrico como mudanças na condutância estomática, crescimento, acúmulo de osmólitos e expressão de determinados genes (FURLAN et al., 2012). No entanto toda e qualquer resposta irá depender da intensidade e duração do estresse, interação com outros estresses, estágio de desenvolvimento da espécie (MENESES et al., 2006). As respostas das plantas à seca são classificadas em quatro tipos básicos de estratégias sendo de escape, de evitação, de tolerância e às plantas que recuperam da seca (FANG; XIONG, 2015).

Os dois principais mecanismos de resistência a seca apresentados pelas plantas são as que evitam e as que toleram a seca (YUE et al., 2006). As plantas que evitam a seca são caracterizadas por manter um elevado potencial hídrico e aumentar a eficiência do uso da água (EUA) através da redução da transpiração foliar e do desenvolvimento de um sistema radicular com enraizamento mais profundo (BLUM, 2005; LUO, 2010; FANG; XIONG, 2015). Já a tolerância a seca refere-se à capacidade das plantas em sustentar suas atividades fisiológicas, mesmo que em nível baixo, sob condições de déficit hídrico (LUO, 2010; FANG; XIONG, 2015). E algumas das formas de tolerância ocorre através do aumento de moléculas

osmorreguladoras na célula para manter o turgor celular e da expressão de genes de proteínas e metabólitos de defesa para reduzir o acúmulo de substâncias danosas ao seu metabolismo (HU; XIONG, 2014; FANG; XIONG, 2015).

Apesar das diferentes formas adotadas pelas plantas para resistirem á seca, a deficiência hídrica pode atuar como um agente de mortalidade. Segundo McDowell et al. (2008) a mortalidade causada pelo déficit hídrico pode passar por alguns mecanismos de como por exemplo a falta de carbono. A hipótese da falta de carbono prevê que o fechamento estomático, diminui a absorção de carbono, como resultado da demanda metabólica contínua por carboidratos.

Características radiculares como crescimento das raízes, arquitetura, anatomia e morfologia são apontadas como promissoras para uma nova e necessária revolução verde (LYNCH, 2007; DEN HERDER et al., 2010). Para Lynch et al. (2015) mecanismos que influenciam a eficiência metabólica do crescimento radicular e da exploração do solo, devem ser componentes importantes da tolerância à seca e da eficiência nutricional pois melhoram o crescimento da planta sob estresse. Isso inclui: Uma produção de um número ótimo de raízes; maior alocação de biomassa para classes de raízes que são menos exigentes metabolicamente; e redução dos custos metabólicos do tecido radicular através da formação de aerênquima e da redução da área de células corticais vivas.

2.2 Cultura da soja sob déficit hídrico

A soja [*Glycine max* (L.) Merrill] é uma planta herbácea eudicotiledônea da família Fabaceae, é uma importante cultura agrícola, utilizada para consumo humano, animal e na produção de biocombustível. Possui um elevado teor de proteína e óleo nos seus grãos e é a segunda maior fonte para a produção de óleo vegetal (DAY, 2013). A soja é umas das quatro plantas mais cultivadas no mundo e a primeira entre as oleaginosas (VALLIYODAN et al., 2017). Além disso o “Complexo Soja” (grão, farelo e óleo), tem um papel muito importante no desenvolvimento da economia brasileira. (ABIOVE, 2021).

Os danos da seca provocados na cultura da soja irão depender da duração do estresse e o estágio de desenvolvimento em que a planta se encontra. No entanto, para a soja a fase mais sensível é a de floração e enchimento dos grãos (NAOE et al., 2015). O déficit hídrico, consequentemente, reduz o conteúdo de água nas células limitando a abertura estomática que irá causar uma queda na assimilação de CO₂, diminuindo assim o crescimento e a produção das plantas (CASTRO et al., 2019).

Alguns estudos têm mostrado que algumas características conferem maior tolerância à seca em plantas de soja. Um trabalho sobre as condições climáticas atuais e futuras no sul do Brasil mostrou que a maior produção de soja foi atingida quando características como maior profundidade do sistema radicular, transpiração limitada em relação ao déficit de pressão de vapor e menor sensibilidade durante o enchimento dos grãos, evidenciando ganhos superiores em relação ao efeito de uma única característica (BATTISTI et al., 2017).

Plantas de soja podem adaptar-se à seca por meio do desenvolvimento de raízes que podem alcançar a água e nutrientes em camadas mais profundas do solo (MANAVALAN et al., 2009) e um dos principais fatores que influenciam a profundidade de enraizamento da soja é a taxa de alongamento da raiz. Como a raiz principal é a primeira a ser formada, a identificação de genótipos com raízes principais que se alongam rapidamente em condições ótimas, pode permitir a determinação de capacidade de enraizamento mais profundo (MANAVALAN et al., 2009).

Algumas características morfofisiológicas influenciam na tolerância da soja ao déficit hídrico, sejam elas contribuindo para o aumento da absorção de água ou reduzindo a sua perda para a atmosfera (FRANCHINI et al., 2017). As variedades de soja tolerantes à seca normalmente atingem determinadas respostas em condições de escassez de água. Stolf et al. (2009), mostrou que o comprimento das plantas submetidas ao déficit hídrico foi maior em uma variedade tolerante comparada a uma outra sensível. Outra resposta também foi encontrada por Franchini et al. (2017) para o sistema radicular de possíveis variedades tolerantes à seca, ao notarem comprimento e área radicular maior do que outras cultivares sob déficit hídrico nas camadas mais superficiais do solo.

Prince et al. (2017) mostrou que a adaptação à seca de linhagens de soja de alto rendimento tiveram alteração nas características anatômicas da raiz. Eles observaram que sob condições de seca houve redução da área de seção transversal da raiz e aumento do metaxilema, o que diminui o custo metabólico para que as raízes atingissem maiores profundidades no solo a fim de otimizar o transporte de água.

A mudança dos mecanismos adaptativos da folha e da raiz durante a seca é essencial para que não haja perdas de produtividade. A seleção de características anatômicas radiculares em plantas de soja, como por exemplo, o aumento do número de vasos de metaxilema poderia facilitar a seleção de genótipos adaptados a ambientes com déficit hídrico (PRINCE et al., 2017). Apesar de haver diferenças entre os genótipos e até mesmo a existência de variedades que toleram mais a seca, ainda faltam estudos sobre os alvos fisiológicos e estruturais que irão

contribuir para o melhoramento e conseqüentemente o rendimento dessa espécie nessas condições.

2.3 Cultura do milho sob déficit hídrico

O milho (*Zea mays* L.) é uma espécie pertencente à família Poaceae. Sua domesticação ocorreu há cerca de sete a dez mil anos na América Central. É considerada uma das plantas cultivadas mais antigas e um dos vegetais mais estudados, possuindo caracterização genética mais detalhada dentre as plantas cultivadas. No Brasil, o milho tem grande importância econômica tendo em vista as diversas formas de consumo, desde a alimentação humana e animal até a indústria de alta tecnologia e utilização na produção de biocombustíveis (FORNASIERI FILHO, 2007). É cultivado praticamente em todo o Brasil e a maior parte da produção nacional concentra-se nas regiões Sul, Centro-Oeste e Sudeste, correspondendo a 90% do total produzido no país (CONAB, 2021).

Sob o estresse causado pela seca, as plantas alocam mais recursos para o crescimento das raízes em relação ao crescimento da parte aérea, o que pode aumentar a aquisição de água (SHARP; DAVIES, 1989). O sistema radicular deve lidar com a disponibilidade de nutrientes, excesso e falta de umidade, juntamente com outros fatores abióticos como a textura e a compactação do solo (HAUCKET al., 2015). Segundo Nielsen (2013), para entender melhor o crescimento e os problemas associados à restrição hídrica, é importante conhecer o desenvolvimento das raízes do milho.

Variações dos genótipos para as características anatômicas da raiz, tem sido estudado, uma vez que essas características podem melhorar a exploração dos estratos profundo do solo. De acordo com Saengwilai, Tian e Lynch (2014), os genótipos de milho com poucas raízes nodais têm maior aquisição de N em solos com baixo teor de N. Gao e Lynch (2016) demonstraram que a baixa proporção de raiz nodal no milho melhora a tolerância à seca, aumentando a profundidade do enraizamento e a aquisição de água a partir do subsolo. Segundo Zhan, Schneider e Lynch (2015) a redução da densidade de ramificação da raiz lateral do milho pode melhorar a tolerância à seca, reduzindo os custos metabólicos da exploração do solo, permitindo maior alongamento da raiz axial, maior profundidade de enraizamento e, portanto, maior aquisição de água em solo seco. As características anatômicas específicas que podem contribuir para a profundidade de enraizamento e reduzir os custos metabólicos radiculares, incluem formação de aerênquima cortical, tamanho da célula cortical (LYNCH, 2013) e componentes da área cortical viva (JARAMILLO et al., 2013).

O aerênquima cortical radicular (ACR) é uma característica anatômica com potencial para melhorar a eficiência de aquisição de recursos e a tolerância ao estresse abiótico em culturas de campo (LYNCH, 2011). Esse tecido é comum em membros da família Poaceae e tem sido mostrado sua formação em uma variedade de classes radiculares (HE; MORGAN; DREW, 1992; FAN et al., 2003; STRIKER et al., 2007).

Burton, Lynch e Brown (2013) observaram o desenvolvimento da raiz de milho, mostrando que as raízes de terceira ordem possuíam maior proporção de aerênquima. Ainda segundo Burton, Lynch e Brown (2013) maior quantidade desse tecido foi observada na posição média do que na posição basal de todas as raízes, exceto a raiz primária, enquanto as raízes laterais e a base das raízes não formaram aerênquima. Em outro estudo realizado por Zhu, Brown e Lynch (2010), foi observado a variação de aerênquima entre sete genótipos de milho em condições de estresse hídrico. Os resultados obtidos mostram que o aerênquima aumenta a tolerância à seca reduzindo os custos metabólicos, permitindo assim maior crescimento da raiz para a aquisição de água a partir de um solo seco.

Segundo Lynch (2013), o tamanho das células corticais vivas reduziu os custos metabólicos do crescimento e manutenção das raízes tanto em termos do custo de carbono da respiração radicular como do teor de nutrientes dos tecidos vivos. Chimungu et al. (2015), estudando o tamanho das células corticais vivas na raiz do milho em estresse hídrico, demonstram que o tamanho da célula cortical viva de grande porte melhora a tolerância à seca no milho e reduz os custos metabólicos da exploração do solo, portanto, reduzindo então a respiração da raiz, obtendo raízes mais profundas para melhorar a exploração da água no solo, maior taxa fotossintética e maior biomassa e produção de grãos do que linhagens com células corticais pequenas.

A implantação de fenótipos de raiz com maior eficiência metabólica da exploração de solo representa novo e inexplorado paradigma para desenvolver culturas com maior eficiência de recursos e resiliência (LYNCH; CHIMUNGU; BROWN, 2014). O Brasil se destaca como grande produtor mundial de milho e dessa forma, o estudo com variedades de milho amplamente cultivadas no Brasil, oferece o potencial para a criação de variedades mais tolerantes às nossas condições climáticas, visto que, existe uma grande variação nas respostas adaptativas das plantas a seca.

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SEGUNDA PARTE

**ARTIGO 1: YIELD INDICATORS IN SOYBEAN GENOTYPES UNDER WATER
DEFICIT**

YIELD INDICATORS IN SOYBEAN GENOTYPES UNDER WATER DEFICIT

Bethânia Silva Morais de Freitas Pedrini, Evaristo Mauro de Castro

ABSTRACT

Water deficit limits the development and yield of soybean plants; therefore, there is a need for tolerant genotypes. The objective of this study was to determine the anatomical and physiological changes in the 7739 M and “Desafio” varieties under water deficit and whether the traits found may indicate a greater or lesser degree of tolerance that justifies the higher yield observed in the field for the 7739 M genotype compared to genotype Desafio when a drought occurs during the crop cycle. The two soybean genotypes were grown in pots in a greenhouse under two water regimes: well-watered (WW - 100% fc) and water deficit (WD - 30% fc). The Desafio genotype showed a reduction in leaf thickness under water deficit, and the 7739 M genotype showed no difference for this trait between treatments, indicating that the osmotic adjustment maintained cell turgor and tissue thickness, important adaptive responses to drought conditions. A large reduction in root, stem and leaf dry mass was observed when comparing the well-watered treatment with the water deficit treatment in the 7739 M genotype, a finding that may be related to the greater capacity of this genotype to allocate photoassimilates to the grain. The high phenotypic plasticity of the morphological traits, such as root dry mass, plant height and leaf water potential, observed in the 7739 M genotype are related to drought tolerance and increased water absorption. In conclusion, the traits observed in the 7739 M genotype indicate greater tolerance to water deficit and greater productive stability.

Keywords: Tolerance. *Glycine max* (L.) Merrill. 7739 M. Desafio. Anatomy.

1 INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is considered one of the most important crops in the world due to its oil and protein production capacity (FAO, 2020). In the 2019/2020 crop, Brazil had a record production of 135 million tons, placing the country as the world's largest soybean producer, with an area of 38.25 million hectares and an average yield of 3,528 kg/ha (CONAB, 2020). The Central-West region is the main producer in the country, accounting for approximately 45% of the planted area (CONAB, 2020). However, climatic adversities, such as short periods of water deficit can occur even during the rainy season (MARCUIZZO; CARDOSO, 2012), limiting grain development and yield (MERTZ-HENNING et al., 2018).

Water deficit affects the biochemical, physiological and morphological processes in plants, and the damage may vary based on the duration and phenological stage during which stress occurs (BUEZO et al., 2019). One of the changes observed is stomatal closure to reduce transpiration, which decreases CO₂ uptake (LI et al., 2017) and consequently affects plant growth and production. However, plants have drought tolerance mechanisms, allowing some physiological and anatomical adjustments to address water scarcity.

The tolerance mechanisms against water stress include greater water use efficiency (BUEZO et al., 2019), maintenance of turgor through cellular osmotic adjustment (BLUM, 2005) (BASU et al., 2016), and modifications in the root system, such as a reduction in lateral root formation (DEAK; MALAMY, 2005) and an increase in the main root length (JUMRANI; BHATIA, 2019). In addition, root anatomical traits, such as root cortical aerenchyma, have been shown to be excellent attributes for the uptake of soil resources through reductions in tissue metabolic costs (LYNCH, 2015).

To guarantee production in the face of rainfall instability, farmers have increasingly sought to use varieties that can tolerate drought periods and maintain good yield. However, there are great difficulties in identifying tolerant genotypes due to the strong interactions between genotype and environment and little knowledge about the function and tolerance mechanisms (NAGHAVI; ABOUGHADAREH; KHALILI, 2013). The soybean variety 7739 M has been considered by farmers in southwestern Goiás as a soybean cultivar more tolerant to short drought periods because it presents a lower yield reduction when compared to other soybean cultivars, such as Desafio, under these conditions.

Thus, this study was conducted to determine the anatomical and physiological changes in the 7739 M and Desafio varieties as a function of water deficit and whether the traits found indicate or justify the greater or lesser degree of tolerance of a given genotype to better understand this behavior in the field.

2 MATERIAL AND METHODS

2.1 Plant material, cultivation conditions and experimental design

The M7739 IPRO variety (Monsoybean Seeds, São Paulo, Brazil), referred to in this study as “7739 M”, is in maturity group 7.7 and has a semi-determinate growth habit (MONSOY, 2020), and the 8473 RSF variety (Brasmax Seeds, Cambé, Brazil), referred to as “Desafio”, is in maturity group 7.4 and has an indeterminate growth habit (BRASMAX, 2020). Both varieties are widely cultivated in the Central-West region of Brazil, with high production potential.

The experiment was conducted in the greenhouse of the Departamento de Biologia da Universidade Federal de Lavras. The seeds were sown in rhizotron pots filled with substrate composed of washed sand and commercial substrate at a 1:1 ratio. Each pot received three seeds, and after germination, the plants were thinned, leaving only one plant per pot. In the treatment under water deficit, moisture was maintained at 30% of field capacity, which corresponds to 0.13 g of watercompost⁻¹, and the treatment under normal irrigation conditions was maintained at 100% of field capacity, with 0.42 g of watercompost⁻¹. On the first day for both treatments, the pots were irrigated with 40% Hoagland and Arnon nutrient solution (1950). The plants were kept in a greenhouse with controlled temperature (25 ± 2 °C), and soil moisture was monitored using resistive moisture sensors with an LM393 comparator module and an Arduino Mega 2560 microcontroller. The experiment was conducted for 45 days.

The experimental design was completely randomized in a 2 x 2 factorial scheme, with two soybean genotypes (7739 M and Desafio) and two irrigation regimes, i.e., watered plants and plants under water deficit, totaling eight replicates, with each replicate consisting of one plant.

2.2 Anatomical analyses

At 45 days after sowing, the plants were removed from the rhizotron pots, and the roots of the lower region were collected by removing the substrate and then washed. A fully expanded third trifoliate leaflet was collected. Both were fixed in FAA [70% (v/v) formaldehyde + acetic acid + 70% ethanol (v/v), 0.5: 0.5: 9.0 (v/v/v)] for 72 h (JOHANSEN, 1940) and then stored in 70% alcohol until further analysis. The roots and leaves were embedded to perform the cross-sectioning. For this purpose, the material was dehydrated in a series of ethanol (alcohol) solutions with increasing concentrations (70, 80, 90 and 100%) at

room temperature, in accordance with Johansen (1940). Subsequently, the material was immersed for 24 h in a preinfiltration solution composed of 100% ethanol and base resin (1:1) using a HistoResin kit (Leica Microsystems, Heidelberg, Germany) following the instructions of the manufacturer. After this period, the base resin was infiltrated for another 24 h at 4 °C. For polymerization, a HistoResin kit (Leica Microsystems, Heidelberg, Germany) was used. The 8- μm -thick cross-sections were obtained in a semiautomatic rotary microtome and then stained in a 1% toluidine blue solution, pH 6.7 (FEDER; O'BRIEN, 1968).

The slides were photographed with an AxioCam Erc 5s camera (Zeiss, Oberkochen, Germany) coupled to the Axio Lab.A1 microscope (Zeiss, Oberkochen, Germany). To obtain cross-section quantitative data, the images were analyzed using ImageJ software. The parameters analyzed in the roots were cross-sectional area, root cortex thickness, vascular cylinder area and metaxylem vessel diameter. The parameters analyzed in the leaves were adaxial and abaxial epidermis thickness, palisade and spongy parenchyma thickness, total leaf thickness, midrib area, xylem area, phloem area, xylem vessel diameter and number of vascular bundles.

2.3 Analysis of plant growth

Growth evaluations were performed on the 39th day after germination. A tape measure was used to measure the height of the plant from the substrate to the highest point of the upper highest point of the stem. The stem diameter was measured using a caliper at a height of 5 cm above the substrate. The roots were washed to remove the substrate before sampling. Stems, leaves and roots were dried at 60 °C until reaching constant weight, and then, the dry mass was determined on an analytical balance (AY220, Shimadzu, São Paulo, Brazil). The root/shoot ratio (g g^{-1}) was calculated.

2.4 Physiological analyses

Gas exchange data were obtained using an infrared gas analyzer (IRGA; model LI-6400XT, Li-COR Biosciences, Lincoln, Nebraska, USA), a 6 cm^2 cuvette and a red/blue LED light source (LI6400-02B, Li-Cor, Lincoln, Nebraska, USA). The evaluations were performed on the leaflet of the third fully expanded trifoliate leaf. The measurements were made between 8:00 am and 11:00 am, and the photosynthetic photon flux density (PPFD) was standardized to 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the measuring cuvette. In addition, the mixing function used carbon dioxide capsules to provide 380 ppm CO_2 during the analyses. The net photosynthesis rate (A), stomatal conductance for water vapor (gsw), transpiration rate (E),

intercellular CO₂ carbon (C_i), intercellular and atmospheric carbon ratio (C_i/C_a) and water use efficiency (WUE) were calculated.

Leaf water potential (LWP) was obtained using a portable Scholander pressure chamber (model 1000; PSM Company Instrument, Corvallis, Oregon, USA). The pressure chamber used N₂ gas to apply the necessary pressure. The evaluations were performed on the leaflet of the third fully expanded leaf. The equilibrium pressure required to bring water to the cut made in the midrib was recorded as the LWP.

The maximum quantum yield of photosystem II was obtained using a MINI-PAM modulated fluorometer (Walz, Effeltrich, Germany). The same leaves that were used in the gas exchange analysis were preadapted to the dark with tweezers for 30 minutes before the fluorescence measurements. The leaf area under the tweezers was then subjected to a low intensity pulse of modulated red light (0.03 μmol m⁻² s⁻¹) and then to a pulse of 0.8 s of saturating actinic light (> 6000 μmol m⁻² s⁻¹). The potential quantum yield of photosystem II was calculated as follows: F_v/F_m = (F_m-F_o)/F_m, where F_o is the minimum fluorescence obtained by the application of low intensity light and F_m is the maximum fluorescence obtained by applying the saturating actinic light pulse.

2.5 Relative distance plasticity index

The relative distance plasticity index (RDPI) was calculated as described by (VALLADARES; SANCHEZ-GOMEZ; ZAVALA, 2006). We tested eight individuals of each soybean genotype (j = 1, 2, 3, 4, 5, 6, 7 and 8) in each water regime (i = 1, 2). The phenotypic plasticity for a given variable x may be related to the difference in x between two individuals (j and j') of the same genotype cultivated under different water regimes (i and i') (MARCHIORI et al., 2017). The RDPI was calculated as $\sum (d_{ij} \rightarrow i'j' / (xi'j' + x_{ij})) / n$, where n is the number of distances and was previously described by (VALLADARES; SANCHEZ-GOMEZ; ZAVALA, 2006). The RDPI was calculated with the data for a given variable x obtained at the end of the experimental period (i.e., 45 days, from sowing to plant harvest).

2.6 Data analysis

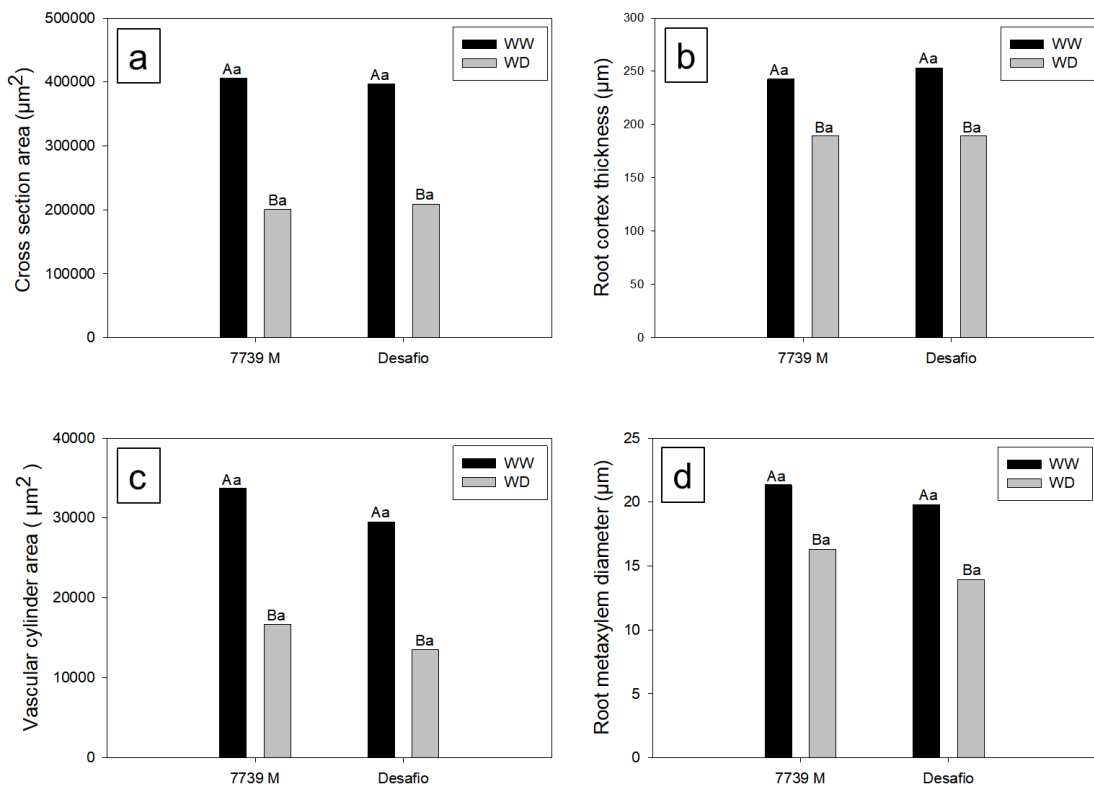
Statistical analyses were performed using SISVAR 5.0 (FERREIRA, 2011). The data were subjected to the Shapiro-Wilk normality test. Next, analysis of variance (ANOVA) was performed, and the means were compared by the Scott-Knott test at an error probability of 5%.

3 RESULTS

3.1 Root anatomical traits

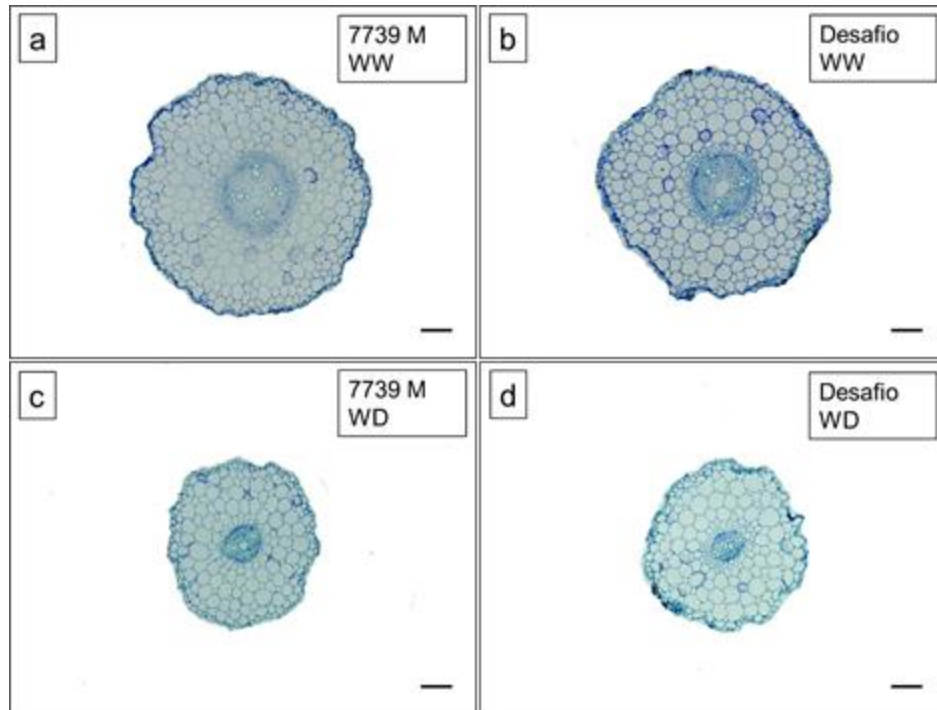
The results were similar for all anatomical variables analyzed in the roots of the two soybean genotypes, where the cross-sectional area (Fig. 1a and Fig. 2), the cortex thickness (Fig. 1b and Fig. 2), the vascular cylinder area (Fig. 1c and Fig. 2) and the metaxylem vessel diameter (Fig. 1d) were higher in the well-watered condition than in the water stress condition, with no differences between the genotypes.

Figure 1 – Root anatomical traits of two soybean genotypes (7739 M and Desafio) under well-watered (WW) and water deficit (WD) regimes. Means followed by the same letters, uppercase for the irrigation condition and lowercase for the genotype, do not differ by the Scott-Knott test at 5% probability.



Source: Author (2021).

Figure 2 – Root cross-section of soybean genotypes 7739 M and Desafio under well-watered and water deficit regimes. Bar, 100 μ m.



Source: Author (2021).

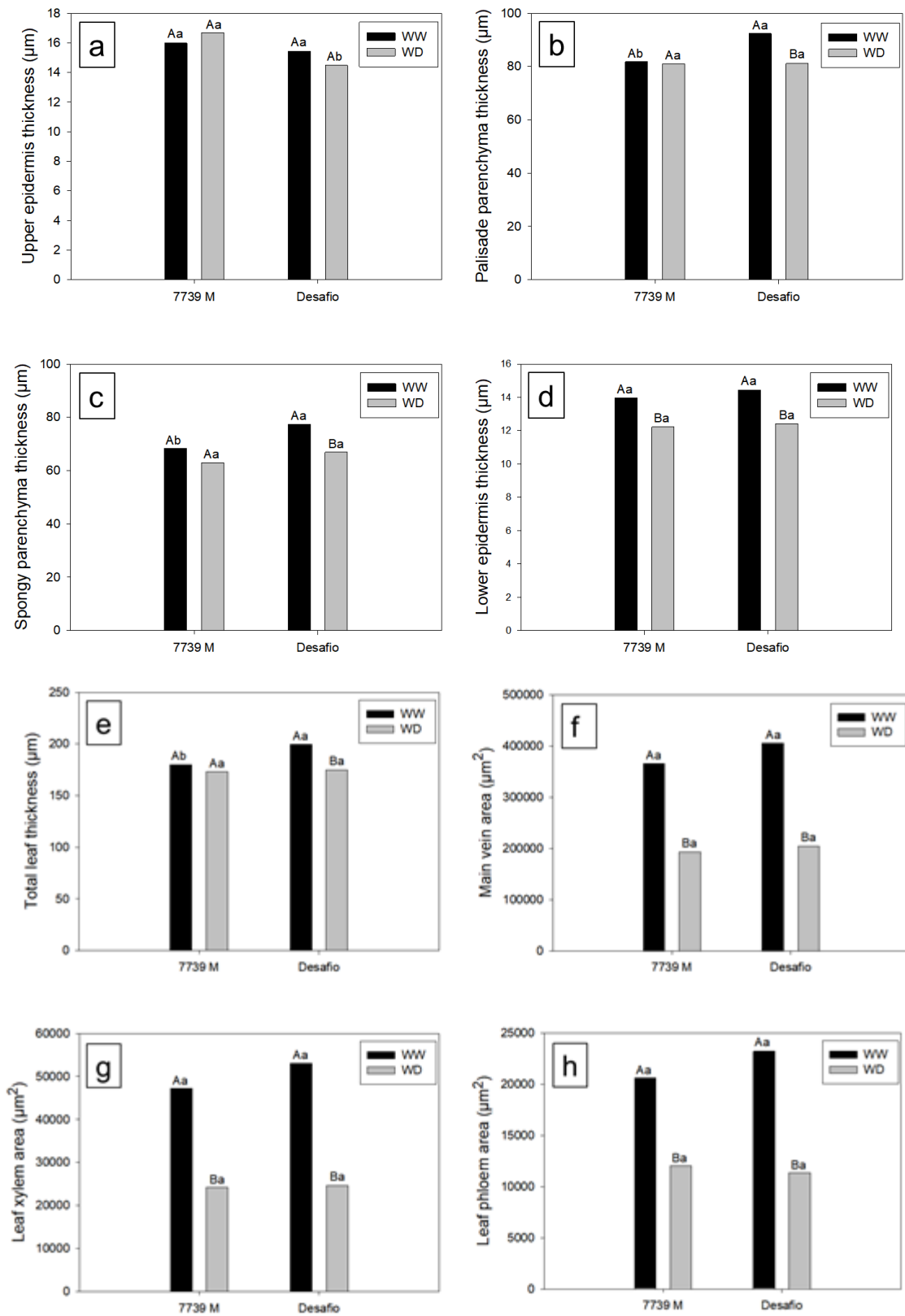
3.2 Leaf anatomical traits

Regarding leaf thickness, some differences were observed. The upper epidermis thickness under water deficit was higher in 7739 M, with no differences between treatments for the two genotypes (Fig. 3a). The palisade and spongy parenchyma thickness were higher in Desafio under the well-watered regime, whereas under water deficit, there was a reduction in this trait, which, unlike 7739 M, showed similar means for the two treatments (Fig. 3b and c).

The abaxial epidermis thickness was lower for the two genotypes under water deficit (Fig. 3d). In the well-watered regime, the total leaf thickness was higher in Desafio (Fig. 3e and Fig. 5b), and under water deficit, there was no difference between the genotypes (Fig. 3e and Fig. 5c, d). Unlike Desafio, which had a reduction in this trait under water deficit (Fig. 5d), 7739 M showed no difference between treatments (Fig. 3e and Fig. 5a, c).

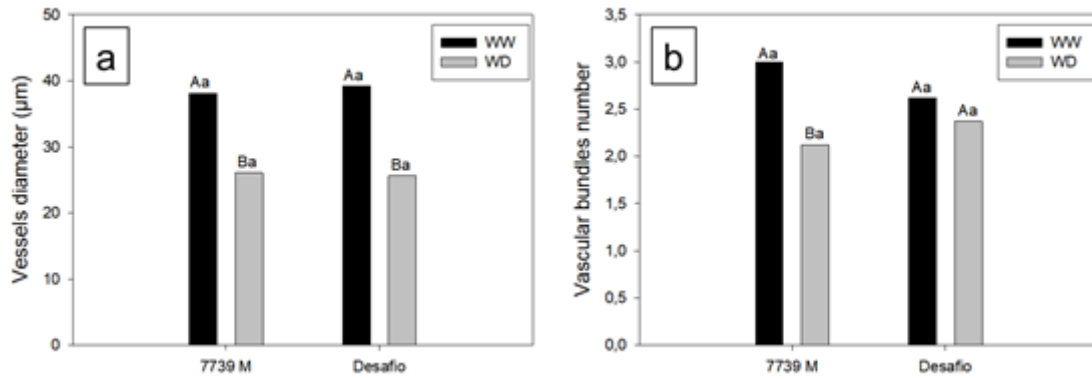
The other vascular anatomical traits of the leaf, such as midrib area (Fig. 3f), xylem area (Fig. 3 g), phloem area (Fig. 3 h) and vessel diameter (Fig. 4a), showed differences only between the treatments with higher means in the well-watered treatment (Fig. 6a, b). Regarding the number of vascular bundles, there was no difference between the genotypes; however, 7739 M had a lower mean under water deficit than under the well-watered treatment (Fig. 4b).

Figure 3 – Leaf anatomical traits of two soybean genotypes (7739 M and Desafio) under well-watered (WW) and water deficit (WD) regimes. Means followed by the same letters, uppercase for the irrigation condition and lowercase for the genotype, do not differ by the Scott-Knott test at 5% probability.



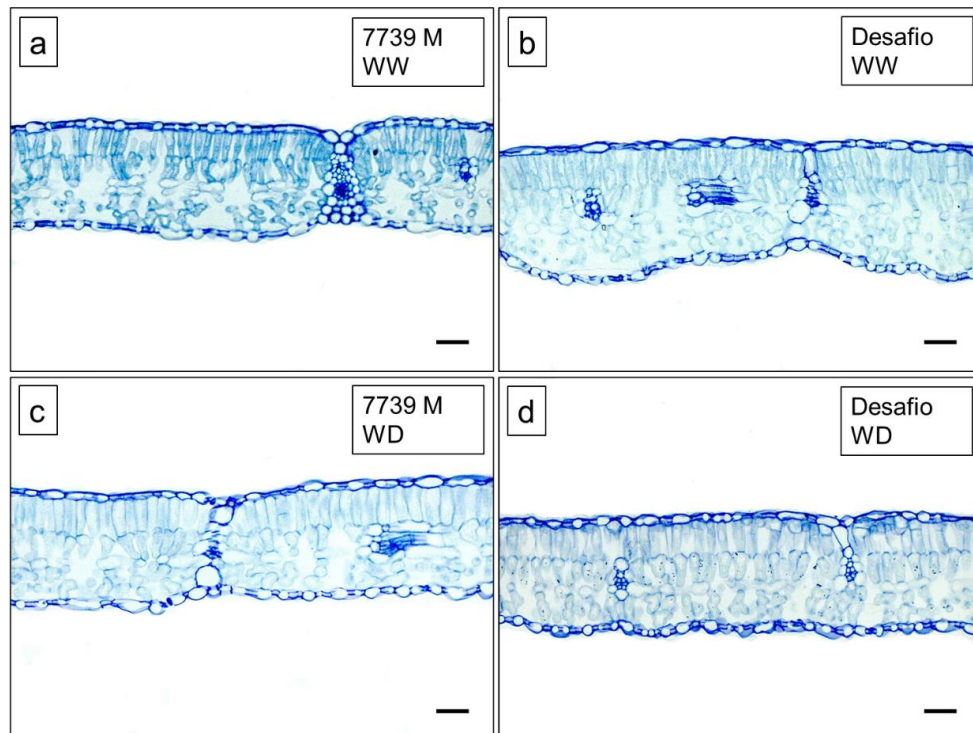
Source: Author (2021).

Figure 4 – Leaf anatomical traits of two soybean genotypes (7739 M and Desafio) under well-watered (WW) and water deficit (WD) regimes. Means followed by the same letters, uppercase for the irrigation condition and lowercase for the genotype, do not differ by the Scott-Knott test at 5% probability.



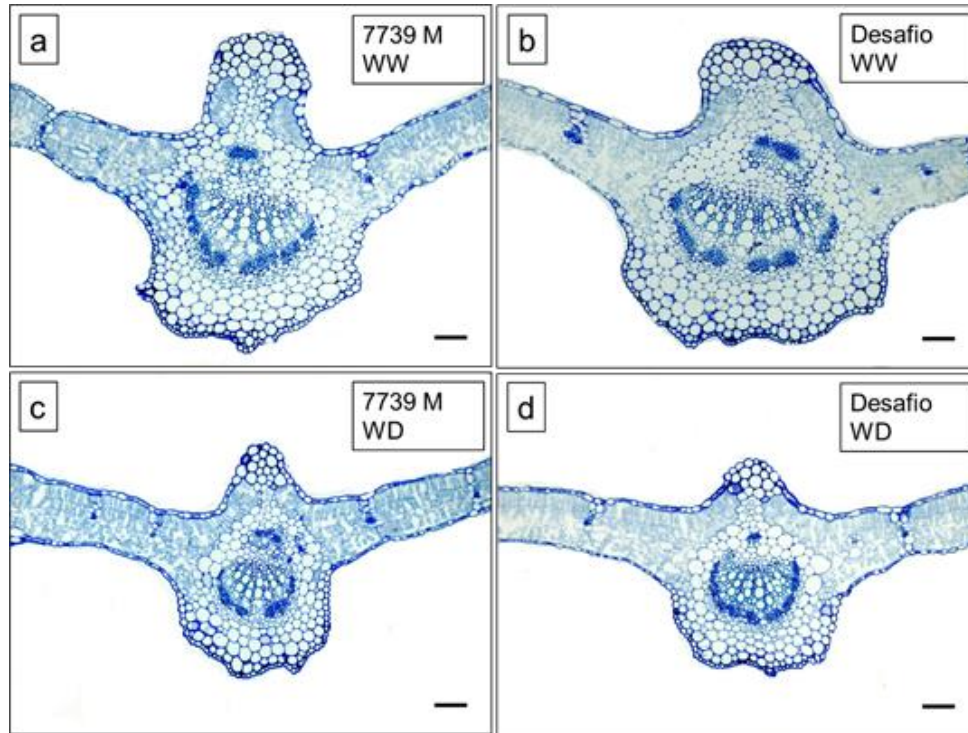
Source: Author (2021).

Figure 5 – Leaf cross-section of soybean genotypes 7739 M and Desafio under well-watered and water deficit regimes. Bar, 50 µm.



Source: Author (2021).

Figure 6 – Cross-section of the midrib of the soybean genotypes 7739 M and Desafio under well-watered and water deficit regimes. Bar, 100 μ m.



Source: Author (2021).

3.3 Plant growth

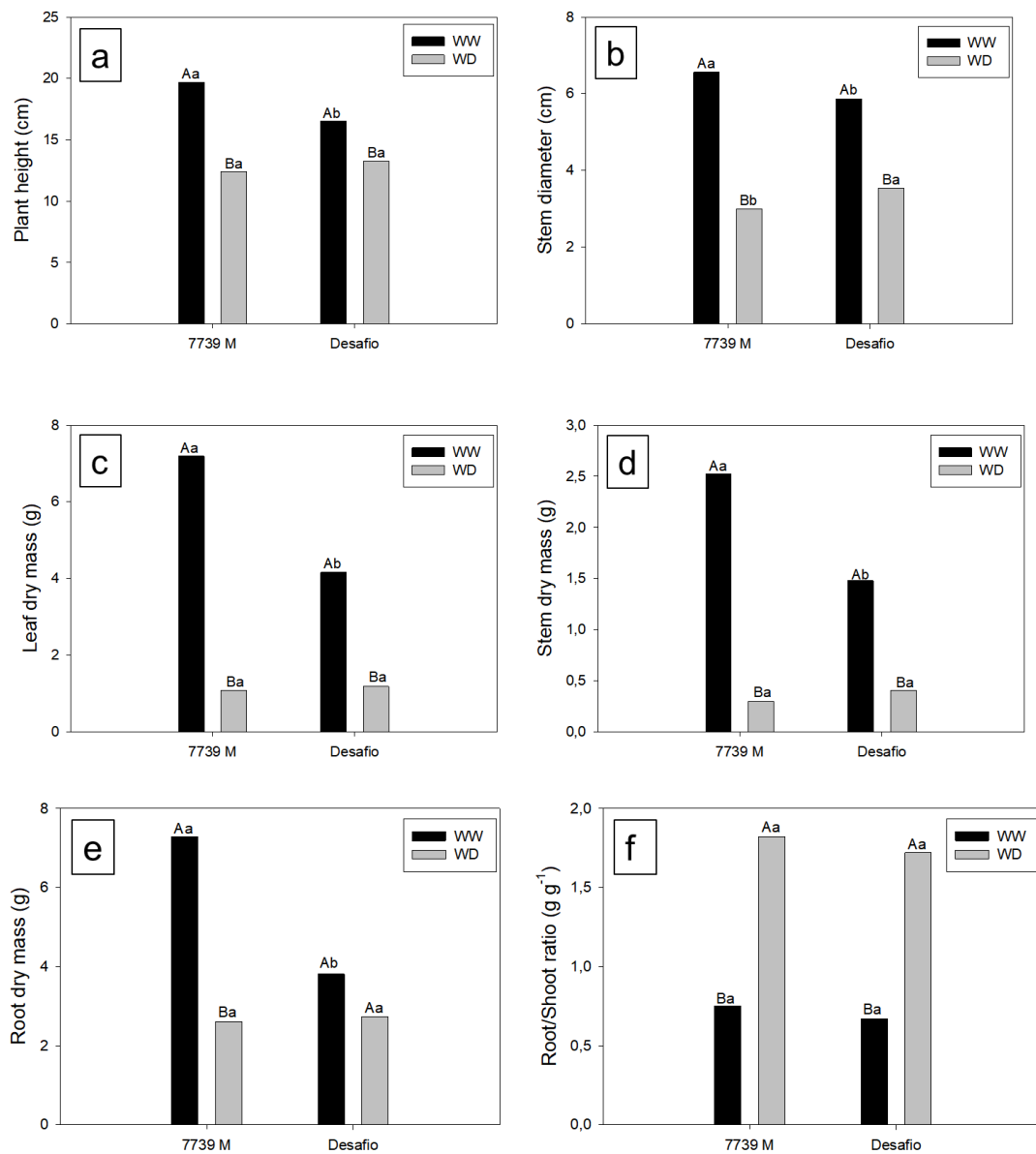
At 36 days after sowing, Desafio plants under water deficit began flowering, called reproductive stage R1, and two days later, flowering started for 7739 M plants. At the end of the experiment, i.e., 45 days after sowing, some Desafio plants in this same water condition were in reproductive stage R3, when pod formation began.

Regarding the plant growth data, most plants presented a reduction in the analyzed traits under the water deficit regime compared with the well-watered regime. Compared with Desafio plants, 7739 M plants had a taller height under the well-watered treatment, and under water deficit, there was no difference between the genotypes (Fig. 7a). In turn, the stem diameter was larger in 7739 M plants under the well-watered regime and lower under the water deficit regime (Fig. 7b).

Similar results were observed for the dry mass of the leaves, stems and roots, and under the well-watered regime, 7739 M plants had higher means than did Desafio plants, without a significant difference under water deficit between the genotypes (Fig. 7c, d, e). However, there was a large reduction in the dry mass under the well-watered regime compared water deficit regime for 7739 M plants, with an 85% reduction in leaf dry mass, 88% reduction in stem dry mass and 64% reduction in root dry mass; for Desafio plants, the

reductions were 71%, 72% and 28%, respectively. For root dry mass, Desafio plants did not show a reduction in this trait under water deficit, with equal means between treatments (Fig. 7e). The root-to-shoot ratio was not different between the genotypes for any of the treatments, with the highest means observed under water deficit (Fig. 7f).

Figure 7 – Plant growth traits of two soybean genotypes (7739 M and Desafio) under well-watered (WW) and water deficit (WD) regimes. Means followed by the same letters, uppercase for the irrigation condition and lowercase for the genotype, do not differ by the Scott-Knott test at 5% probability.



Source: Author (2021).

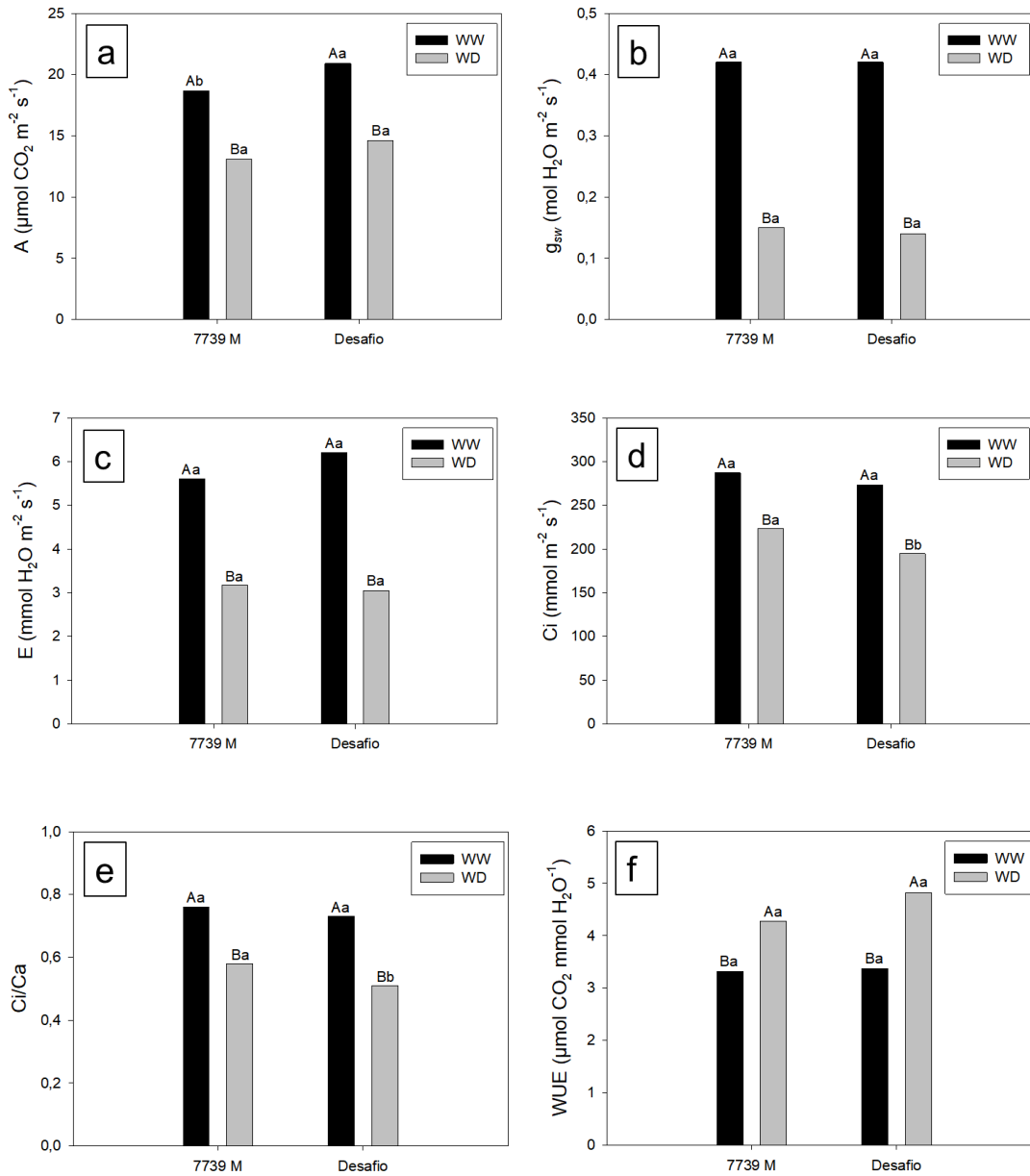
3.4 Physiological traits

Leaf gas exchange was influenced by water deficit in both genotypes, with no significant difference between them, with a lower net assimilation rate (A), stomatal conductance (g_{sw}) and transpiration (E) compared with those under the well-watered treatment (Fig. 8a, b and c). However, compared with 7739 M plants, Desafio plants showed a higher net assimilation rate (A) under the well-watered regime (Fig. 8a).

The intercellular CO_2 concentration (C_i) and the intercellular CO_2 concentration/atmospheric CO_2 concentration (C_i/C_a) were lower under water deficit than under the well-watered treatment for both genotypes. However, under water deficit, 7739 M plants had a higher mean than did Desafio plants (Fig. 8d and e). In turn, the water use efficiency (WUE) was higher for both genotypes under the water drought regime compared with the well-watered regime, with no differences between them (Fig. 8f).

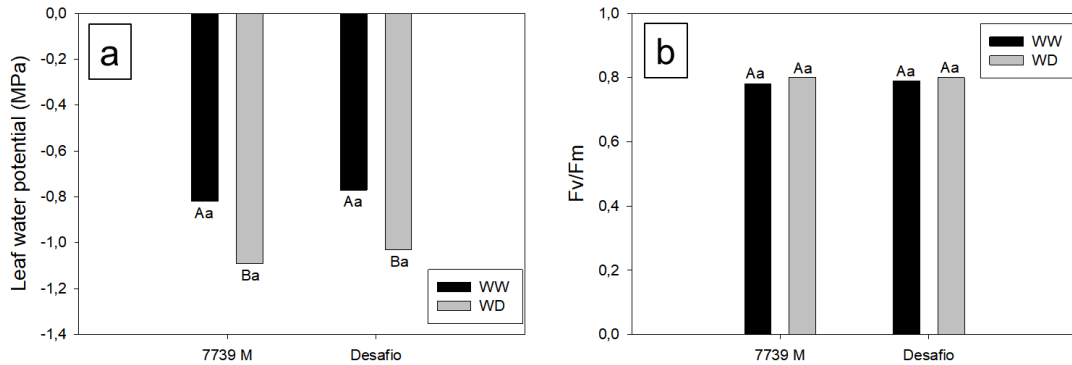
The leaf water potential (LWP) did not differ between the genotypes under any of the conditions analyzed; however, there was a decrease in this trait under water deficit (Fig. 9a). There was no variation in the maximum quantum yield between the genotypes, which maintained values considered optimal in both water conditions (9b).

Figure 8 – Physiological traits of two soybean genotypes (7739 M and Desafio) under well-watered (WW) and water deficit (WD) regimes. Means followed by the same letters, uppercase for the irrigation condition and lowercase for the genotype, do not differ by the Scott-Knott test at 5% probability.



Source: Author (2021).

Figure 9 – Leaf water potential (a) and maximum quantum yield of photosystem II (b) in two soybean genotypes (7739 M and Desafio) under well-watered (WW) and water deficit (WD) regimes. Means followed by the same letters, uppercase for the irrigation condition and lowercase for the genotype, do not differ by the Scott-Knott test at 5% probability.

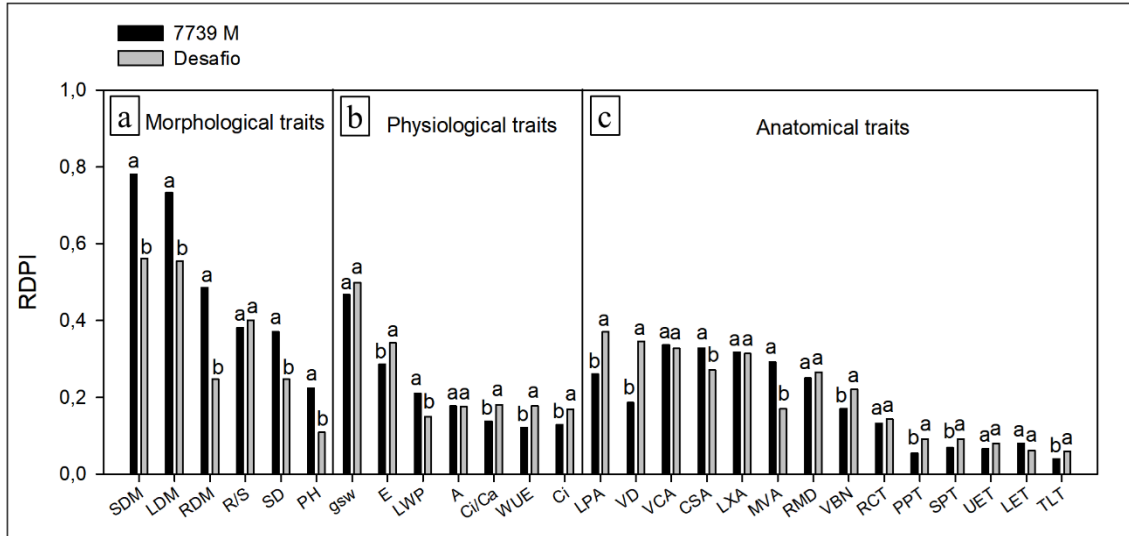


Source: Author (2021).

3.5 Phenotypic plasticity

Desafio plants had a higher RDPI for most of the variables analyzed, a finding that was observed for the physiological and anatomical traits (Fig. 10b and c). For the morphological traits, the 7739 M genotype showed a higher RDPI for 90% of the variables analyzed, with the highest RDPI values found for plant dry mass (Fig. 10a). Some traits, such as the root/shoot ratio, photosynthesis, vascular cylinder area, leaf xylem area, root cortex thickness and upper and lower epidermis thickness, did not differ between genotypes.

Figure 10 – Relative distance plasticity index in two soybean genotypes (7739 M and Desafio). (a) Morphological traits, (b) physiological traits, (c) anatomical traits. Means with the same letters between the genotypes are not significantly different from each other by the Scott-Knott test at 5% probability. SDM: stem dry mass, LDM: leaf dry mass, RDM: root dry mass, R/S: root-to-shoot ratio, SD: stem diameter, PH: plant height, g_{sw} : stomatal conductance, E: transpiration, LWP: leaf water potential, A: net assimilation rate, Ci/Ca: internal carbon/external carbon ratio, WUE: water use efficiency, Ci: internal carbon, LPA: leaf phloem area, VD: vessel diameter, VCA: vascular cylinder area, CSA: cross-sectional area, LXA: leaf xylem area, MVA: main vein area, RMD: root metaxylem diameter, VBN: number of vascular bundles, RCT: root cortex thickness, PPT: palisade parenchyma thickness, SPT: spongy parenchyma thickness, UET: upper epidermis thickness, LET: lower epidermis thickness, TLT: total leaf thickness.



Source: Author (2021).

4 DISCUSSION

The reduction in root anatomical traits under water deficit, observed here for two genotypes, is consistent with the strategy used by some crops to reduce metabolic costs and improve water transport under drought conditions (LYNCH, 2015). In the sections analyzed at eight centimeters above the root apex, no changes were found that indicate more tolerance or sensitivity; some studies have shown that traits such as a higher proportion of root cortical aerenchyma (CHIMUNGU et al., 2015; ZHU; BROWN; LYNCH, 2010) and smaller xylem vessel diameter (HENRY et al., 2012; SOUZA et al., 2013) contribute to water deficit tolerance. However, we cannot state that such root changes do not occur for soybean because in corn for example, it has been shown that aerenchyma formation occurs more than 10 cm from the root apex (BURTON; LYNCH; BROWN, 2013).

Although the 7739 M genotype had greater adaxial epidermis thickness under water deficit, the gas exchange data showed that transpiration and WUE were the same for both genotypes under this condition, thus indicating that although there is a greater barrier to prevent water loss, this condition did not influence it.

There was a reduction in the palisade and spongy parenchyma thickness and, consequently, in mesophyll thickness in Desafio plants under water deficit. Gonçalves et al. (2017) observed a lower thickness of these tissues under water deficit in transgenic and nontransgenic soybean plants, and Polizel et al. (2011) also observed this reduction in a drought-sensitive soybean genotype.

However, the 7739 M genotype did not show such a reduction, as the parenchyma and mesophyll thickness was equal under both the well-watered and water deficit regimes. This

behavior, i.e., the same leaf thickness maintained under well-watered and water deficit conditions, was observed in a drought-tolerant corn genotype (PIRES et al., 2020). This is due to the osmotic adjustment of cells responsible for maintaining cell turgor, which is an important adaptive response of plants to drought because the cell expansion process contributes to the growth and yield of plants during water stress (BHASKARA et al., 2017; BLUM, 2005; FARQUHARSON, 2017).

For osmotic adjustment to occur, plants develop mechanisms to maintain cell turgor through the synthesis of compatible osmoprotectants, osmolytes or solutes (PINTÓ - MARIJUAN; MUNNÉ -BOSCH, 2013). Thus, tolerant plants tend to accumulate these osmotically active compounds. Castro et al. (2019) observed a reduction in total soluble sugars, nonreducing sugars and proline in the Desafio genotype under water deficit. This would explain the lower yield in the field during the drought period compared with that for the 7739 M genotype, indicating high efficiency in osmotic adjustment.

Under the water deficit regime, physiological traits such as stomatal conductance, transpiration and WUE were the same for both genotypes, suggesting that they have similar physiological mechanisms to deal with water scarcity. The 7739 M genotype showed an increase in the internal carbon concentration (C_i), which, under conditions of water deficit, suggests damage to the photosynthetic apparatus (ZHANG et al., 2016). However, there were no changes in potential photochemical efficiency (F_0/F_m), thus indicating that there was no photoinhibitory damage under water deficit in any of the genotypes. Furthermore, the fact that there was a lower C_i/C_a ratio in Desafio plants may suggest that this genotype fixes more CO_2 molecules, contributing to the increase in photosynthesis, as found for some soybean varieties under water stress (BUEZO et al., 2019; MESQUITA et al., 2020) However, in this study, this trait did not seem to be affected in either genotype under drought, as the means for the net photosynthesis rate were similar. Castro et al., 2019 also did not observe differences in the photosynthetic rate for these same genotypes under drought, and the C_i/C_a ratio was higher in the 7739 M genotype under stress conditions.

The 7739 M genotype had higher means in the well-watered condition for all growth traits, and it was thus a more robust variety than Desafio under ideal cultivation conditions. In turn, under water deficit, there was no difference between the genotypes except for stem diameter, which was larger in Desafio plants but had greater phenotypic plasticity in 7739 M plants.

The large reduction in leaf, stem and root dry masses in the 7739 M genotype under water deficit also explains the higher yield of this genotype in the field during the drought

period, as it would be allocating photoassimilates to the grain. In addition, the Desafio genotype entered the reproductive phase earlier, and the lower production in the field under drought conditions may be due to a lower assimilate partitioning capacity. Fenta et al. (2014) found a similar result for another soybean variety with lower yield and a shorter vegetative growth period under water deficit.

The phenotypic plasticity results indicate that the different genotypes had greater plasticity in certain classes of traits, as was the case for the Desafio genotype, which despite presenting more traits with higher RDPI values, the higher values were concentrated only in physiological and anatomical traits. In turn, the 7739 M genotype, in addition to having the highest RDPI values for morphological traits, showed a higher value for LWP, which according to Pires et al. (2020) includes phenotypic traits related to drought tolerance and increased water absorption.

The high degree of plasticity of quantitative physiological traits such as photosynthesis and stomatal conductance seems to be ideal for survival under abiotic stress and for yield (DALAL; ATTIA; MOSHELION, 2017). However, because the 7739 M genotype is reported to have a higher yield potential under water deficit, the data here also suggest that high phenotypic plasticity only indicates a higher yield when phenotypic traits such as root dry mass, plant height and LWP are related to drought tolerance and increased water absorption (PIRES et al., 2020). Furthermore, a higher RDPI for anatomical traits, such as the cross-sectional area of the root and the main vein area, could also contribute to drought tolerance because they are traits related to water transport in plants.

In conclusion, the analysis of these two genotypes revealed some traits, such as the ability to maintain leaf tissue thickness through osmotic adjustment, that seem to be responsible for the higher yield of the 7739 M genotype under water deficit, representing an adaptive advantage over the Desafio genotype. In addition, the large reduction in dry mass in the 7739 M genotype indicates that this genotype is able to make better use of assimilates, explaining the greater yield in the field during the drought period. Greater phenotypic plasticity in morphological traits and in LWP also contribute to high yield in the field under drought conditions because these traits are related to water absorption and drought tolerance.

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**ARTIGO 2: ETHYLENE AND HYDROGEN PEROXIDE IN AERENCHYMA
FORMATION IN MAIZE ROOTS UNDER WATER DEFICIT**

**MANUSCRIPT FORMATED ACCORDING TO THE AUTHOR'S GUIDELINES
FROM PLANT, CELL AND ENVIRONMENT**

Ethylene and hydrogen peroxide in aerenchyma formation in maize roots under water deficit

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Abstract

Root cortical aerenchyma (RCA) is important tissue involved in the tolerance of plants under drought conditions. The aim of this study was to evaluate root anatomy and the relationship between the production of ethylene and hydrogen peroxide (H₂O₂) during aerenchyma formation under water deficit. A drought-tolerant genotype (DKB 390) and a drought-sensitive genotype (BRS 1010), grown in irrigated pots with 30% water holding capacity, were used. In the region near the root apex, the RCA showed no difference between the genotypes and between the water treatments, and a higher proportion of RCA was found in the region 12-13 cm above the root apex in plants exposed to water deficit. Ethylene production was considerably higher in plants exposed to water deficit, and higher H₂O₂ production was also found in the same treatment for genotype DKB 390. The greater development of aerenchyma in the most proximal region of the root is related to cell death regulated by ethylene and H₂O₂ and to the presence of more differentiated cells.

Keywords: Root anatomy. Drought resistance. *Zea mays* L. Genotype DKB 390. Genotype BRS 1010.

1 INTRODUCTION

Maize is the most cultivated and commercialized crop in the world. The top maize-producing countries foresee large reductions in production due to the increase in temperature with global warming (Tigchelaar, Battisti, Naylor & Ray 2018) because heat and drought are limiting abiotic stresses for production. Therefore, in recent years, one of the strategies to ensure national food security and economic equilibrium has been the development of drought-tolerant hybrids (Lunduka, Mateva, Magorokosho & Manjeru 2019).

The root system plays an important role in water uptake under water deficit conditions. The reduction in metabolic costs of roots for soil exploration is an important strategy for drought tolerance, given that more carbon resources can be allocated to root growth due to less respiration of root tissues, ensuring greater water absorption (Zhu, Brown & Lynch 2010).

One of these strategies is the formation of root cortical aerenchyma (RCA), tissue containing gas spaces formed by cell death, so-called lysigenous aerenchyma (Drew, He & Morgan 2000; Evans 2003). Some studies report that maize hybrids with more RCA show higher biomass and higher yield than do hybrids with lower RCA, showing that this characteristic improves the growth of plants under drought conditions (Zhu *et al.* 2010; Chimungu *et al.* 2015).

Ethylene is a hormone produced in response to some type of stress (Morgan & Drew 1997). Drew *et al.* (2000) and Evans (2003) have shown that ethylene is involved in aerenchyma formation under oxygen deficiency conditions. Reactive oxygen species (ROS) are also produced in response to abiotic stresses (Jaspers & Kangasjärvi 2010) and can act in the signaling of many biological processes in plants, for example, in programmed cell death that will lead to the formation of aerenchyma (Xu *et al.* 2013). In addition, cell death can be induced by ethylene via hydrogen peroxide (H₂O₂), showing that they can also act together (Steffens & Sauter 2009; Steffens, Geske & Sauter 2011). However, all these processes are described under hypoxic conditions, and the process of aerenchyma formation under drought conditions is not well understood.

Analyzing the structural characteristics in the vegetative phase correlated with tolerance to water stress can contribute to the development of stress-tolerant hybrids in breeding programs. Thus, to better understand the location and process of aerenchyma formation under drought conditions and how different genotypes respond to and tolerate drought at the root structural level, the objective of this study was to analyze root anatomy

and relate ethylene and H₂O₂ production to aerenchyma formation under water deficit in two maize genotypes that differ in terms of drought tolerance.

2 MATERIALS AND METHODS

2.1 Plant material and cultivation conditions

This experiment was conducted in a greenhouse of the Departamento de Biologia da Universidade Federal de Lavras. The maize genotypes used were DKB 390, considered drought tolerant (de Souza *et al.* 2013), and BRS 1010, considered drought sensitive (Magalhães *et al.* 2015). The plants were obtained from seeds germinated on filter paper soaked in distilled water. Next, the seeds exhibiting radicle protrusion were transferred to rhizotron pots filled with compost consisting of filtered sand and commercial substrate at a ratio of 1:1. Each pot received a seedling.

For water deficit treatment, moisture was maintained at 30% of the water holding capacity, corresponding to 0.13 g of watercompost⁻¹, and for normal irrigation treatment, moisture was maintained at 100% water holding capacity, corresponding to 0.42 g of watercompost⁻¹. On the first day, for the two treatments, the pots were irrigated with Hoagland and Arnon (1950) nutrient solution at 40% ionic strength. The plants were kept in a greenhouse with controlled temperature (25±2 °C), and soil moisture was monitored using resistive moisture sensors with an LM393 voltage comparator module and an Arduino Mega 2560 microcontroller. The experiment was conducted for 30 days.

2.2 Experimental design

The experimental design was completely randomized in a 2×2 factorial arrangement, with two maize genotypes (DKB 390 and BRS1010) and two irrigation systems: irrigated plants and plants under water deficit, comprising eight replicates, with each replicate consisting of one plant.

2.3 Anatomical analyses

The pots were emptied; the nodal roots were collected, and the substrate was removed. The roots were then washed and fixed in FAA [70% (v/v) formaldehyde + concentrated acetic acid + 70% (v/v) ethanol, 0.5:0.5:9.0 (v/v/v)] for 72 h (Johansen 1940) and then stored in 70% ethanol until further analysis. The cross sections of the roots were obtained in two different regions: 2 cm and 12 cm above the root apex, with the apex being considered the region 1 cm from the tip. The sections were cut by hand using steel blades and then cleared with 50%

(w/v) sodium hypochlorite, rinsed in distilled water, stained with safrablau solution [1% (w/v) safranin and 0.1% (w/v) Astra blue at a ratio of 7:3] and mounted on slides with 50% (v/v) glycerol.

The slides were photographed with an AxioCam Erc 5s camera (Zeiss, Oberkochen, Germany) coupled to an Axio Lab A1 microscope (Zeiss, Oberkochen, Germany). To obtain the quantitative data of the cross sections, the images were analyzed using RootScan software, which was developed to analyze root anatomy (Burton, Lynch & Brown 2013). The analyzed parameters were as follows: average size of the cortical cells, root cross-sectional area, vascular cylinder area, percentage of RCA ([aerenchyma area/total cortical area] * 100), area occupied by cortical cells (cortical area - total aerenchyma area - vascular cylinder area), number of metaxylem vessels, and metaxylem vessel area.

2.4 Production of ethylene

The ethylene produced by the roots was measured by sealing potted plants with PVC film and incubating for 1 h. Using a syringe, a sample of the internal atmosphere was collected through a rubber septum installed in the middle region of each pot, and then transferred to 9-mL vacuum blood tubes (GD090CA Biocon). The concentration of ethylene produced by the roots was determined in the Departamento de Química Analítica da Universidade Federal de Lavras. Next, 2.5 mmol of gas was removed from the tube with a Gastight syringe (Hamilton Storage Technologies, Franklin, MA, USA) and transferred to a Shimadzu GC-17^a gas chromatograph (Shimadzu do Brasil, São Paulo, SP) equipped with flame ionization detector (GC-FID). The peak areas were transformed into $\mu\text{L L}^{-1}$ using a correction factor obtained by injecting standard gas with a known ethylene concentration. The ethylene production by the roots was calculated considering the fresh root mass and the ethylene accumulation time in the pot, and the results were expressed in $\mu\text{L C}_2\text{H}_4 \text{ h}^{-1} \text{ kg}^{-1}$ fresh root mass.

2.5 Production of hydrogen peroxide

Hydrogen peroxide (H_2O_2) content was determined according to Velikova, Yordanov & Edreva (2000) at the Laboratorio de Fisiologia Molecular e Bioquímica de Plantas da Universidade Federal de Lavras. For the extraction, 200 mg of fresh root mass (whole roots) was macerated in liquid nitrogen, homogenized in 1.5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 g for 15 min at 4°C. Aliquots of the supernatant were added to the reaction medium (10 mmol L^{-1} potassium phosphate buffer (pH 7) and 1.0 mol L^{-1} potassium

iodide). H_2O_2 content was determined by reading the absorbance at 390 nm and constructing a standard curve for H_2O_2 .

2.6 Statistical analysis

Statistical analyses were performed using the software SISVAR 5.0 (Ferreira 2011). The data were subjected to the Shapiro-Wilk normality test. Next, analysis of variance (ANOVA) was performed, and the means were compared by the Scott-Knott test at a 5% probability of error.

3 RESULTS

The cross-sectional area of the roots 2 cm above the apex showed no significant difference between the genotypes in the same irrigation regime. However, this variable under water deficit conditions was reduced in genotype DKB 390 and genotype BRS 1010 compared to that under irrigated conditions (Fig. 1a). A similar result was observed for the vascular cylinder area, with no significant difference between genotypes and a reduction in this variable under water deficit conditions (Fig. 1b). For root cortical aerenchyma (RCA), there was no difference between the genotypes and between the irrigation conditions (Fig. 1c). Regarding the median size of the cortical cells, for sections 2 cm above the apex, there was also no difference between the genotypes or between the irrigation conditions (Fig. 1d).

The root region 12cm above the root apex had a larger cross-sectional area in the DKB 390 genotype than in the BRS 1010 genotype under both water deficit and irrigation conditions (Fig. 2a, Fig. 3a and 3c). The plants irrigated had a larger cross-sectional root area (Fig. 2a). The vascular cylinder area decreased substantially under water deficit conditions, where a reduction was observed in genotypes DKB 390 and BRS 1010 compared to that under normal irrigation conditions (Fig. 2b, Fig. 3c ed). The vascular cylinder area for the DKB 390 genotype was 183% larger than that for genotype BRS 1010 under water deficit conditions (Fig. 2b).

Under water deficit conditions, there was an increase in the RCA 12-13 cm above the apex for both genotypes studied; however, there was no significant difference between genotypes in this irrigation regime (Fig. 2c, Fig. 3c ed). For plants irrigated, the percentage of RCA was higher for genotype BRS 1010 than for genotype DKB 390 (Fig. 2c and Fig. 3b). In the same region, the median size of the cortical cells under water deficit conditions was higher for DKB 390, which exhibited no differences from that under normal irrigation conditions

(Fig. 2d). BRS 1010 had the largest median cortical cell size under irrigation conditions compared to that under water deficit conditions (Fig. 2d).

The cortical cell area and metaxylem vessel area were reduced in genotype DKB 390 and genotype BRS 1010 under water deficit conditions relative to irrigated conditions (Fig. 4a and b). However, for these two parameters, DKB 390 had a higher mean value than did BRS 1010 (Fig. 4a and b) for both treatments, irrigated and water deficit. The number of metaxylem vessels (Fig. 4c) was higher under normal irrigation conditions for both evaluated genotypes, with no difference between them for this irrigation regime. Under water deficit conditions, there was also no difference between genotypes regarding the number of metaxylem vessels.

Ethylene production by maize roots was substantially higher under water deficit conditions than under normal irrigation conditions, with no difference between genotypes under the same irrigation regime (Fig. 5a). For H₂O₂ content (Fig. 5b), there was a significant interaction between irrigation regime and genotype, where it was observed that in the DKB 390 genotype, water deficit caused an increase in the H₂O₂ concentration relative to that under normal irrigation conditions. For the BRS 1010 genotype, the irrigation regime did not influence the production of H₂O₂ by root cells.

4 DISCUSSION

The cross-sectional area and the vascular cylinder area sectioned 1-2 cm above the root apex showed that this region, close to the meristem, does not have a well-differentiated structure because there was no difference between the genotypes, contrary to what was observed in roots sectioned at 12-13 cm above the root apex, where the DKB 390 genotype had higher mean values for these parameters than did the BRS 1010 genotype under both irrigation regimes. Therefore, the variation that may occur in the root anatomy as a function of the environment and the genotype depends on the region being analyzed.

The proportion of aerenchyma in the sections analyzed 1-2 cm above the apex was not statistically significant. These data corroborate Burton *et al.* (2013), who reported that no aerenchyma formation occurs in regions between 0-10 cm from the root apex or aerenchyma formation is not well developed. Takahashi, Yamauchi, Rajhi, Nishizawa & Nakazono (2015) also reported the formation of aerenchyma regulated by ethylene in the basal region (1-4 cm from the root-shoot junction) of primary roots of maize seedlings and did not observe the formation of this tissue near the apical region (2.5 and 3 cm from the root tip) under normal conditions.

Although all root regions are equally exposed to ethylene, aerenchyma formation is not stimulated in the region near the apex, which is well developed in the region 12-13 cm above the root apex in plants exposed to water deficit. Thus, what causes the proximal root to be more responsive to ethylene in aerenchyma formation? Takahashi *et al.* (2015) proposed that although ethylene is perceptible in all root cells, only cortical cells express genes related to ethylene-induced programmed cell death and that this process is regulated, in part, by ROS. Thus, we suggest that these genes are expressed only above the root elongation zone because as the distance from the meristematic region increases, the more differentiated the cells are and thus the more likely they will be sensitive to ethylene, i.e., express receptors that interact with signaling molecules generated by this gas. Based on this, the anatomical characteristics that will be taken into consideration for the analysis in this study and correlated with the others will be those from the most proximal region (12-13 cm).

Under water deficit conditions, the proportions of aerenchyma in the two genotypes were higher than those under normal irrigation conditions, a fact that has already been reported by several authors (Zhu *et al.* 2010; de Souza *et al.* 2013; de Souza, Magalhães, de Castro, Duarte & Lavinsky 2016; Chimungu *et al.* 2015). Greater aerenchyma formation under water deficit conditions could be related only to the high production of ethylene in this condition because this hormone mediates the formation of lysigenous aerenchyma by the same cell death mechanism previously reported by Drew *et al.* (2000) and Evans (2003) under hypoxic conditions. However, as previously reported here, it is believed that the process of aerenchyma formation does not depend only on the amount of ethylene produced but on the sensitivity of the cortical cells to ethylene. This is the first study to analyze this relationship of aerenchyma formation in response to ethylene under severe drought conditions in maize roots.

In addition to the role of ethylene in aerenchyma formation, another determining factor is H₂O₂ production. Stress tolerance is not only characterized by the development of an antioxidant system, as the production of these molecules also acts in the transduction of signals, regulating hormonal signaling pathways, such as for ethylene (Mittler *et al.* 2011; Czarnocka & Karpiński 2018). Steffens & Sauter (2009); Steffens *et al.* (2011) and Yamauchi *et al.* (2014) showed a direct relationship of H₂O₂ and ethylene in programmed cell death. This observation explains the greater aerenchyma formation in the DKB 390 genotype under water deficit conditions, exhibiting higher concentrations of H₂O₂ and ethylene, relative to aerenchyma formation in this genotype under normal irrigation conditions, where the lower concentration of H₂O₂ and ethylene may not have been sufficient to trigger a response. Thus,

the higher concentration of H₂O₂ present for aerenchyma formation may be another strategy of the DKB 390 hybrid that confers drought tolerance.

We consider the average cortical area as another important characteristic that confers drought tolerance to DKB 390 because in the region 12-13 cm above the apex, this genotype had a larger cortical cell size than did BRS 1010, and this size did not differ from that of the cells under irrigated conditions, indicating that cell turgor was maintained even under stress caused by water deficit. Pires *et al.* (2020) found a similar result for the leaf of the DKB 390 genotype, where the thickness of the mesophyll did not change with the decrease of the water regime, indicating efficiency in the osmotic adjustment, which is an important adaptive response of the plant to drought. In addition, according to (Chimungu, Brown & Lynch 2014), greater cortical cell size increases drought tolerance and reduces metabolic costs, providing greater root growth and water uptake; this hypothesis is supported by the fact that the increase in the size of vacuoles reduces respiration when compared to respiration in the cytoplasm, which requires higher metabolic activity.

A reduction in cortical cell area is considered a good indicator of drought tolerance, i.e., reduced metabolism to keep cortical cells alive (Jaramillo *et al.* 2013). The data in this study that support this process involve the correlation of the cross-sectional area of the root with the proportion of cortical aerenchyma, given that the smaller the cross-sectional area of the root and the greater the proportion of aerenchyma, the smaller is the cortical cell area. Therefore, the maize plants under water deficit conditions showed a reduction in cortical cell area due to this process. Thus, contrary to what was proposed by Jaramillo *et al.* (2013) and in agreement to Zhu *et al.* (2010), we consider RCA to be the main structural feature of the root responsible for reducing metabolic costs and increasing water deficit tolerance, in addition to the median size of cortical cells.

The area of the metaxylem vessels seems to be proportional to the area of the vascular cylinder, which is greater for the DKB 390 genotype in the two water conditions. The reduction in the number and area of metaxylem vessels under water deficit for both genotypes may also be due to the reduction of the root vascular cylinder in this condition. It is known that narrower metaxylem vessels are related to increased drought tolerance because they are less susceptible to cavitation (Hacke & Sperry 2001; Comas *et al.* 2013).

5 CONCLUSIONS

All evidence shows that aerenchyma formation under drought conditions is due to ethylene signaled by H₂O₂ and that the more proximal root region responds better to this formation because it has more differentiated and sensitive cells. In addition, genotype DKB 390 exhibits anatomical and physiological characteristics that support the tolerance of this genotype to water deficit conditions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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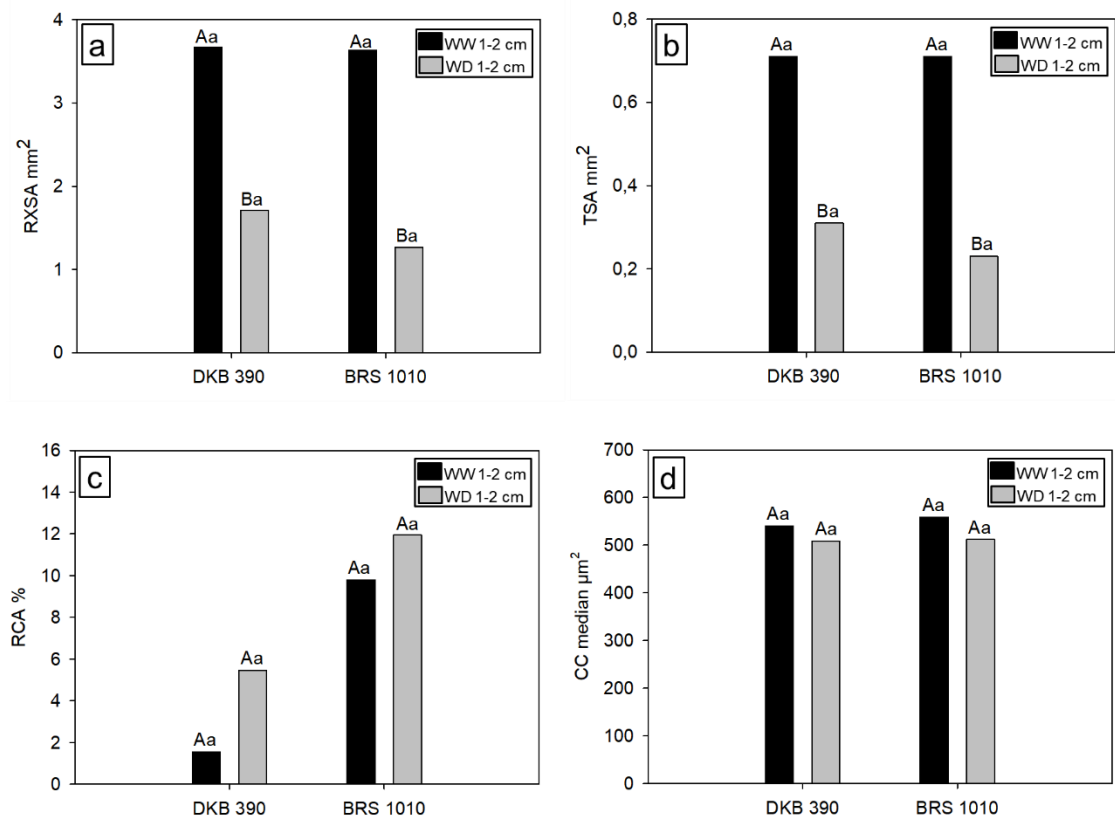


Figure 1. Root anatomical characteristics assessed 1-2 cm above the apex of two maize genotypes (drought-tolerant DKB 390 and drought-sensitive BRS 1010) under two irrigation regimes (irrigated and water deficit). RXSA: cross-sectional area, TSA: vascular cylinder area, RCA: proportion of aerenchyma, and CC median: average length of the cortical cells. Means followed by similar letters, uppercase for irrigation and lowercase for genotype, do not differ by the Scott-Knott test at 5% probability.

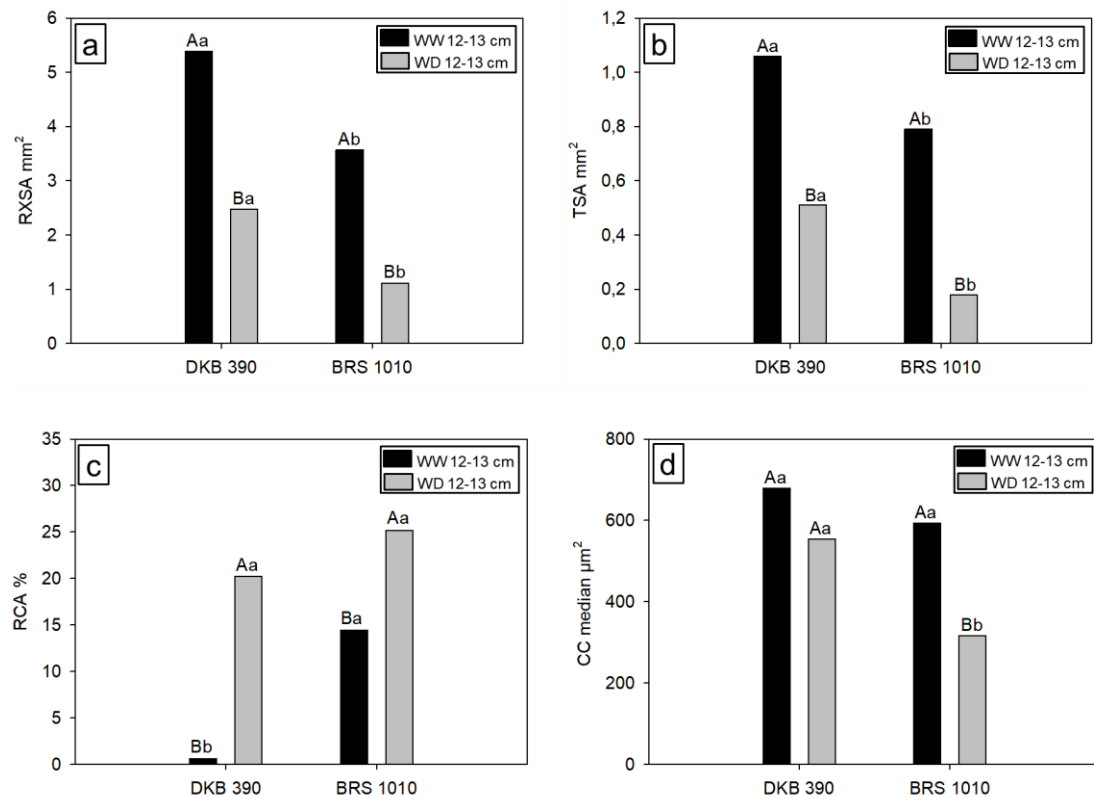


Figure 2. Root anatomical characteristics evaluated 12-13 cm above the root apex of two maize genotypes (drought-tolerant DKB 390 and drought-sensitive BRS 1010) under two irrigation regimes (irrigated and water deficit). RXSA: cross-sectional area, TSA: vascular cylinder area, RCA: proportion of aerenchyma, and CC median: median length of the cortical cells. Means followed by similar letters, uppercase for irrigation and lowercase for genotype, do not differ by the Scott-Knott test at 5% probability.

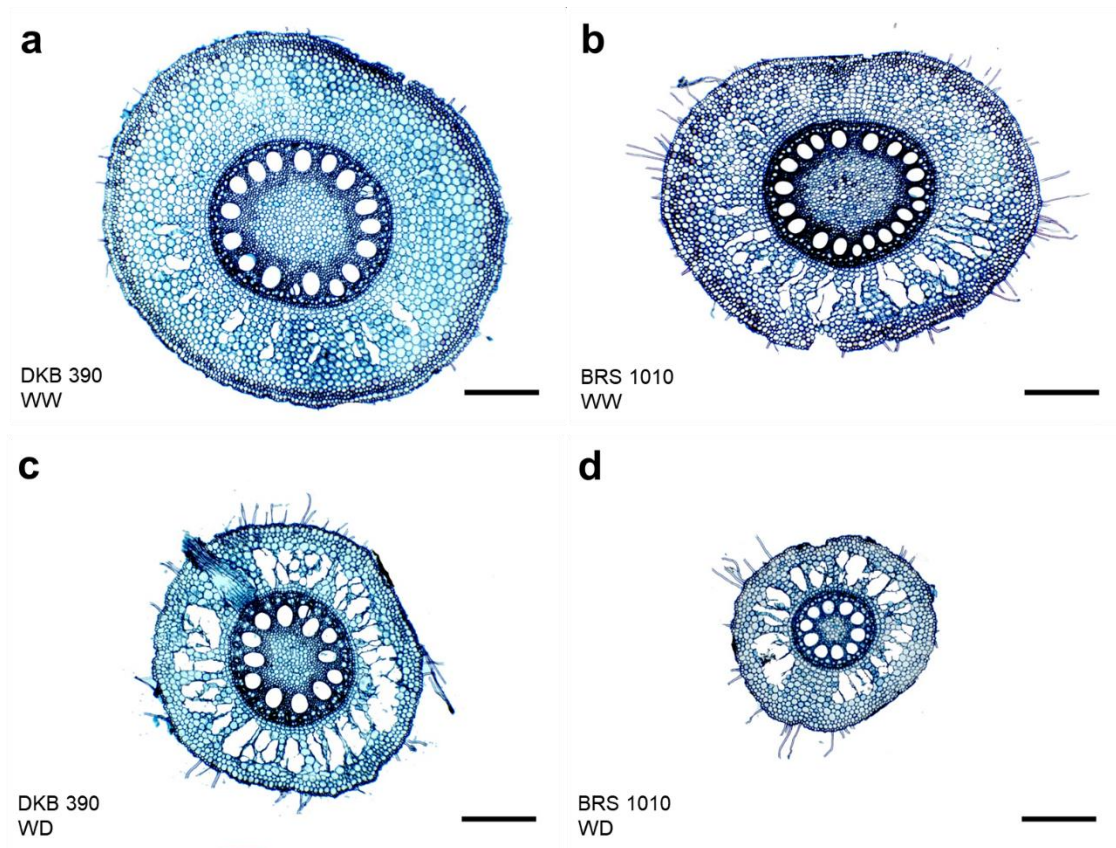


Figure 3. Proportion of aerenchyma in cross sections evaluated 12-13 cm above the apex of nodal roots of two maize genotypes (drought-tolerant DKB 390 and drought-sensitive BRS 1010) under two irrigation regimes (irrigated and water deficit). a) DKB 390 irrigated; b) BRS 1010 irrigated; c) DKB 390 under water deficit; d) BRS 1010 under water deficit. Bar: 50 µm.

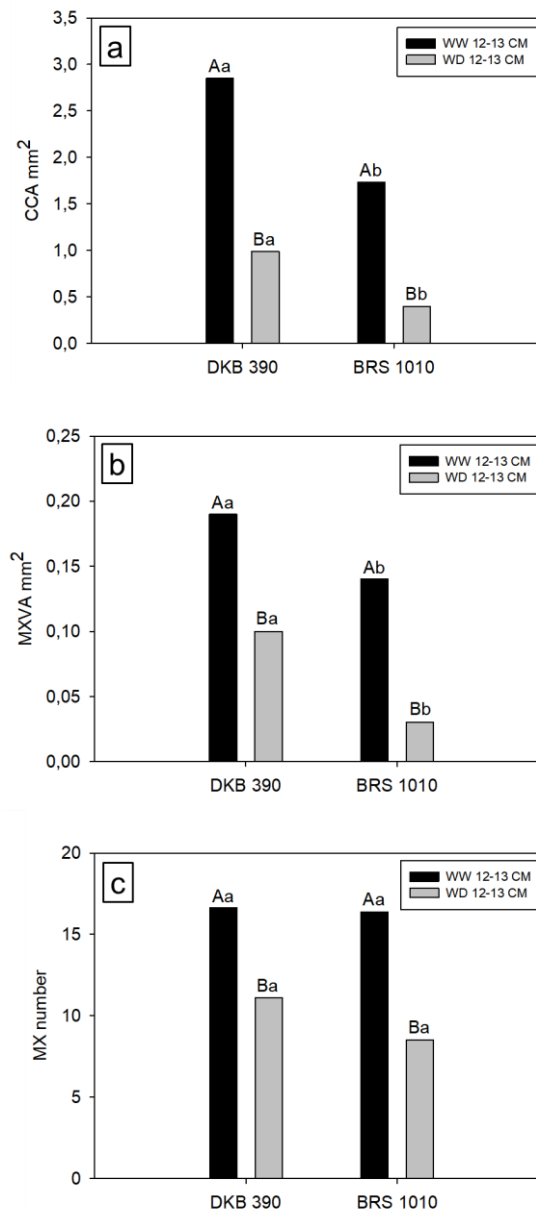


Figure 4. Root anatomical characteristics evaluated 12-13 cm above the root apex of two maize genotypes (drought-tolerant DKB 390 and drought-sensitive BRS 1010) under two irrigation regimes (irrigated and water deficit). CCA: cortical cell area, MXVA: metaxylem vessel area, and MX number: number of metaxylem vessels. Means followed by similar letters, uppercase for irrigation and lowercase for genotype, do not differ by the Scott-Knott test at 5% probability.

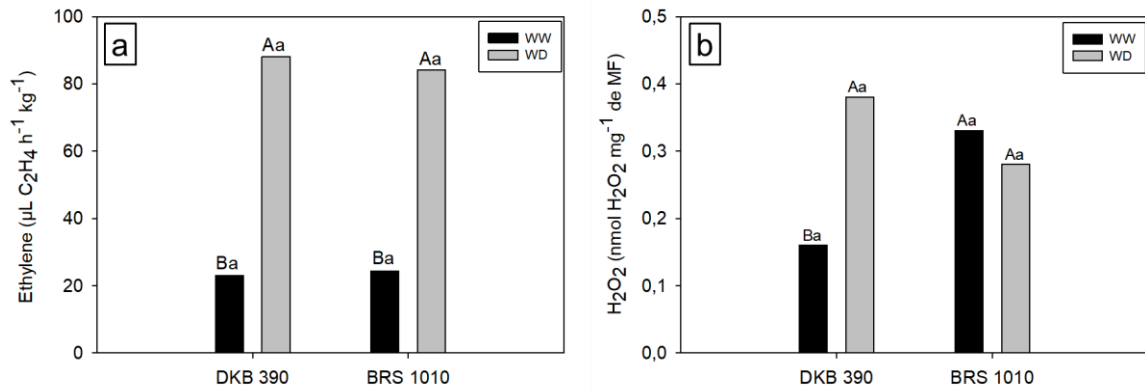


Figure 5. Production of ethylene (a) and H_2O_2 content (b) in roots of two maize genotypes that differ in terms of drought tolerance (drought-tolerant DKB 390 and drought-sensitive BRS 1010) under two irrigation regimes (irrigated and water deficit). Means followed by similar letters, uppercase for irrigation and lowercase for genotype, do not differ by the Scott-Knott test at 5% probability.