JEAN PAULO VITOR DE OLIVEIRA

CHANGES IN AERENCHYMA, LEAF INTERCELLULAR SPACES, GROWTH, PHOTOSYNTHESIS, ANATOMY AND NUTRIENT UPTAKE IN MAIZE AND SORGHUM CAUSED BY DROUGHT

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Prof. Dr. Fabricio José Pereira
Orientador

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JEAN PAULO VITOR DE OLIVEIRA

ALTERAÇÕES NO AERÊNQUIMA, ESPAÇOS INTERCELULARES DAS FOLHAS, CRESCIMENTO, FOTOSSÍNTESE, ANATOMIA E ABSORÇÃO DE NUTRIENTES EM MILHO E SORGO CAUSADAS PELA SECA

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APROVADA em 25 de Abril de 2025.
Prof. Dr. Fabricio José Pereira – UNIFAL.
Prof. Dr. Vinicius Politi Duarte – UNIFAL.
Prof. Dr. Thiago Alves Magalhães – UFLA.
Prof. Dr. Felipe Fogaroli Correa – DBI/ICN.
Prof. Dr. Douglas José Marques – UFU.

Prof. Dr. Fabricio José Pereira
Orientador

LAVRAS – MG
2025

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Provérbios 13:20 diz: "Aquele que anda com os sábios será cada vez mais sábio, mas o companheiro dos tolos acabará mal"

Dedico

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“É necessário que as coisas acabem, para que coisas novas aconteçam...”.

Eckhart Tolle

“Irmãos, não penso que eu mesmo já o tenha alcançado, mas uma coisa faço: esquecendo-me das coisas que ficaram para trás e avançando para as que estão adiante”.

Filipenses 3,13-14

RESUMO

O déficit hídrico provoca alterações nos processos vitais das plantas como a transpiração e a fotossíntese. Isso está associado com mudanças climáticas intensas, o que favorece a ocorrência de seca. Além disso, os principais efeitos relacionam-se com a perda de massa seca reduzindo o crescimento e a produção das plantas. O desafio atual é explorar diferentes maneiras de abordar os mecanismos de tolerância ao déficit hídrico em plantas como o milho e o sorgo, pois essas plantas são consideradas sensível e tolerante à seca, respectivamente. Assim, o objetivo deste estudo foi avaliar o efeito da restrição hídrica na morfofisiologia e anatomia ao longo do eixo foliar e desenvolvimento radicular de plantas de milho e sorgo associado ao crescimento, espaços intercelulares e trocas gasosas. As plantas foram obtidas a partir de sementes e os experimentos foram conduzidos em casa de vegetação a 25±2 °C. As plantas de milho e sorgo foram submetidas à restrição hídrica. Foram realizadas análises de crescimento, trocas gasosas e análise quantitativa anatômica das folhas e raízes. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Scott-Knott com $P<0,05$. A falta de água foi um fator limitante para o milho, pois apresentou redução na área foliar, espessura foliar, biomassa, eficiência no uso da água, diâmetro do vaso do xilema, área da bainha do feixe e nas trocas gasosas, enquanto o sorgo permaneceu sem alteração, exceto para a eficiência do uso da água, diâmetro do xilema e a bainha do feixe que aumentou nas mesmas condições. Essas respostas foram correlacionadas com o crescimento dos espaços intercelulares nas folhas de sorgo. Além disso, a região média da folha de sorgo apresentou parâmetros mais altos em comparação com outras regiões, sendo que a base da folha de sorgo aumentou o ΦPSII, Fv/Fm e ETR. O sorgo apresentou manutenção estomática em comparação ao milho. Nas raízes, a zona pilífera apresentou maiores modificações em comparação à zona de maturação sob restrição hídrica. Dessa forma, o sorgo apresentou maior alongamento radicular, o que contribuiu para o aumento no aerênquima. Apesar disso, não houve efeitos na absorção de água e nutrientes, ao contrário das plantas de milho. Essas características contribuíram para a tolerância do sorgo à seca, enquanto o milho não conseguiu fazer os mesmos ajustes. Portanto, a seca foi prejudicial ao milho, porque reduziu a maioria dos parâmetros avaliados e isso não permitiu que o milho ajustasse os espaços intercelulares dentro da folha e nas raízes, sendo que estes parecem ser importantes para a tolerância à seca do sorgo. Ressalta-se que a maioria das características fisiológicas avaliadas possui variação ao longo do eixo da folha em ambas as espécies e a parte mediana da folha apresentou maior funcionalidade.

Palavras-chave: *Zea mays* L; *Sorghum bicolor* (L.) Moench; poaceae; metabolismo do tipo C_4; estresse hídrico.

ABSTRACT

Water loss causes changes in vital plant processes, such as transpiration and photosynthesis, and is associated with severe climate change, which increases the occurrence of drought. In addition, the main effects are related to the loss of dry mass, reducing plant growth and production. The current challenge is to explore different mechanisms of drought tolerance in plants such as maize and sorghum, which are considered drought-sensitive and drought-tolerant, respectively. This study aimed to evaluate the effects of water restriction on the morphophysiology, anatomy along the leaf axis, and root development of maize and sorghum in relation to growth and gas exchange. Plants were grown from seeds, and experiments were conducted in a greenhouse at 25 ± 2 °C. Maize and sorghum plants were subjected to water restriction, and growth, gas exchange, and quantitative anatomical analyses of leaves and roots were performed. Data were subjected to analysis of variance, and means were compared using the Scott-Knott test ($P < 0.05$). Water deficit was a limiting factor for maize, leading to reductions in leaf area, leaf thickness, biomass, water use efficiency, xylem vessel diameter, bundle sheath area, and gas exchange, whereas sorghum remained largely unaffected, except for water use efficiency, xylem diameter, and bundle sheath area, which increased under the same conditions. These responses were associated with an increase in intercellular spaces in sorghum leaves. Additionally, the middle region of the sorghum leaf exhibited higher physiological parameters compared to other regions, and the leaf base showed increased ΦPSII, Fv/Fm, and ETR. Sorghum maintained stomatal function compared to maize. In the roots, the piliferous zone exhibited more pronounced changes than the maturation zone under water restriction. Sorghum showed greater root elongation, contributing to an increase in aerenchyma formation. Despite this, there were no significant effects on water and nutrient absorption in sorghum, unlike in maize. These traits contributed to sorghum's drought tolerance, whereas maize failed to make similar adjustments. Overall, drought had a detrimental effect on maize, as it reduced most evaluated parameters and hindered the adjustment of intercellular spaces within leaves and roots, which appears to be crucial for drought tolerance in sorghum. Furthermore, most physiological traits varied along the leaf axis in both species, with the median leaf region exhibiting the highest functionality.

Keywords: *Zea mays* L; *Sorghum bico*lor (L.) Moench; poaceae; C_4-type metabolism; water stress.

INDICADORES DE IMPACTO

A escassez de água, além de ser uma preocupação global devido às mudanças climáticas, impacta a agricultura e afeta a economia ao reduzir o produto interno bruto (PIB). Esse problema também desperta grande interesse na pesquisa científica, especialmente no estudo de plantas cultivadas, como milho e sorgo, que apresentam, respectivamente, sensibilidade e tolerância à seca. Assim, os avanços nesses estudos contribuem para a compreensão da capacidade adaptativa das plantas, dos processos anatômicos e fisiológicos envolvidos e dos mecanismos de tolerância à seca. As diferenças nos padrões de resposta de milho e sorgo ao déficit hídrico evidenciam a complexidade desses mecanismos. Os resultados destas pesquisas oferecem uma compreensão mais aprofundada das respostas de milho e sorgo à seca correlacionada às trocas gasosas e os espaços intercelulares das folhas e raízes. De fato, são elementos essenciais para melhoria do desenvolvimento da agricultura do milho e do sorgo e de outras espécies vegetais podendo levar a novos modelos e teorias, bem como informações para elaboração de novas tecnologias para a gestão e mitigação dos impactos provocados pela escassez de água. Portanto, as pesquisas destacadas estão de acordo com os Objetivos de Desenvolvimento Sustentável (ODS) da Organização das Nações Unidas (ONU) na área temática de meio ambiente com os impactos sociais, econômicos e tecnológicos, uma vez que ressaltam a urgência de adotar medidas de preservação que assegurem a saúde ambiental e a conscientização sobre as mudanças climáticas. O trabalho contribui para a ação contra a mudança global do clima – Objetivo 13, na vida terrestre – Objetivo 15, na saúde e bem-estar – Objetivo 3 e na indústria, inovação e infraestrutura – Objetivo 9, presentes na ODS.

IMPACT INDICATORS

Water loss, in addition to being a global concern due to climate change, impacts agriculture and the economy by reducing gross domestic product (GDP). This problem also arouses great interest in scientific research, especially in the study of cultivated plants, such as maize and sorghum, which are, respectively, sensitive and tolerant to drought. Therefore, advances in these studies contribute to the understanding of the adaptive capacity of plants, the anatomical and physiological processes involved, and the mechanisms of drought tolerance. The differences in the response patterns of maize and sorghum to water deficit highlight the complexity of these mechanisms. The results of these studies provide a deeper understanding of the responses of maize and sorghum to drought correlated with gas exchange and the intercellular spaces of leaves and roots. In fact, they are essential elements for improving the development of maize and sorghum agriculture and other plant species, and may lead to new models and theories, as well as information for the development of new technologies for the management and mitigation of the impacts caused by water loss. Therefore, this study aligns with the United Nations (UN) Sustainable Development Goals (SDGs) in the environmental thematic area, with social, economic, and technological implications. It underscores the urgency of adopting conservation measures to ensure environmental health and climate change awareness. Specifically, this research contributes to Goal 13 (Climate Action), Goal 15 (Life on Land), Goal 3 (Good Health and Well-being), and Goal 9 (Industry, Innovation, and Infrastructure).

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

O déficit hídrico tornado o ambiente cada vez mais seco correlacionado com alteração no clima. De fato, as alterações climáticas estão reduzindo à quantidade de chuvas no ambiente tornando mais seco (BIBI; RAHMAN, 2023) afetando diretamente as plantas, o que ameaça a segurança alimentar e a produtividade das culturas agrícolas (AL ZIHAD *et al.*, 2023). A falta de água, neste cenário atual, tem causado estresse hídrico nas plantas, o que prejudica o ganho de biomassa, desenvolvimento e a qualidade (OMOTOSO *et al.*, 2023). Segundo Bistgani, Barker e Hashemi (2024) a água é um dos principais recursos para a produtividade vegetal. Além disso, a água participa das funções de transporte das células e a sua redução pode ocasionar alterações na área foliar, fotossíntese e na transpiração (QIAO *et al.*, 2024). Sendo que modificações na transpiração pode limitar o transporte de nutrientes nas plantas, o que causa alterações no metabolismo. De acordo com Abdalla *et al.* (2023) os fluxos de água do solo para as raízes e até as folhas são impulsionados por gradientes de potencial hídrico, que são induzidos pela transpiração e, inclusive, em determinados pontos do ciclo fenológico estes efeitos podem intensificar-se (SOUZA *et al.*, 2016). Portanto, para melhor compreensão dos mecanismos envolvidos das plantas à seca buscam-se espécies que apresentaram tolerância a falta de água (MULLET, 2017).

A falta de água é um fator estressante para as plantas, principalmente para o milho (YAN *et al.*, 2023). Esta espécie, *Zea mays* L., (Poaceae), é uma espécie considerada sensível à seca por necessitar de grandes volumes de água para o seu desenvolvimento (OLIVEIRA *et al.*, 2023). Dessa forma, por ser uma espécie sensível à seca o milho pode apresentar diferentes estratégias para evitar o estresse hídrico promovendo alterações na anatomia e fisiologia das folhas. Segundo Kim e Lee (2023) o milho quando submetido ao estresse hídrico pode apresentar diversos mecanismos interessantes. De fato, a alta plasticidade das plantas de milho ao estresse pode ser evidenciada pelo aumento do comprimento radicular, do tamanho do córtex e da quantidade de aerênquima (DIAZ *et al.*, 2018; KIM; LEE, 2023).

Nas espécies tolerantes à seca, como o *Sorghum bicolor* (L.) Moench., (Poaceae) (OLIVEIRA *et al.*, 2023) possuem estratégias que permitem sobreviver sob déficit hídrico. Estas espécies como o arroz (*Oryza sativa* L.) ocorreu redução na transpiração e a falta de água promoveu alocação de maior biomassa para as raízes (ILYAS *et al.*, 2021; PANDA; MISHRA; BEHERA, 2021). É importante ressaltar que o sorgo é uma espécie tipicamente mais tolerante a falta de água em comparação com outras plantas do tipo C_4 (LI *et al.*, 2010) correlacionada à alta plasticidade do sorgo como redução do número de folhas e aumento da densidade estomática (OLIVEIRA *et al.,* 2022).

As modificações anatômicas e fisiológicas das plantas de milho e de sorgo diante da falta de água estão correlacionadas com o aumento da eficiência no uso da água. De fato, o aumento da eficiência do uso da água é uma característica crucial à seca, que reflete na produtividade e na resistência das plantas e pode ser estudada em nível de folha, planta e dossel (SHARMA *et al.,* 2023). Neste contexto, os espaços intercelulares foliares podem atuar otimizando as trocas gasosas. De acordo com Oliveira *et al.* (2022, 2023) os espaços intercelulares exercem um papel fundamental na regulação das trocas gasosas, principalmente na transpiração, pois à medida que o nível da água diminuiu os espaços intercelulares reajustam-se para evitar a perda de água através dos estômatos.

Vale destacar que a área foliar também exerce um papel significativo para o controle da perda de água, pois maior ou menor expansão foliar pode modificar a densidade estomática ajustando as trocas gasosas (ANYIA; HERZOG, 2004; LIU *et al.*, 2019; OLIVEIRA *et al.,* 2025). Porém, a área foliar e outras características morfofisiológicas podem variar ao longo do eixo longitudinal da folha promovendo diferentes respostas nas porções apical, mediana e basal. Segundo Ford *et al.* (2008) e Normand *et al.* (2009) as plantas apresentam estratégias de crescimento espaciais particulares e, por causa disso, existem uma correlação entre a posição arquitetônica, morfologia dos eixos e o comportamento funcional das plantas, que geralmente são mal caracterizadas. Além disso, a raiz é o primeiro órgão em contato com os fatores abióticos (OLIVEIRA *et al.,* 2022b), sendo que a interferência desses fatores como a água podem provocar alterações significativas nos espaços intercelulares radiculares refletindo na absorção de água e nutrientes causando impactos no crescimento das plantas (SOUZA *et al.,* 2016; DIAZ *et al.,* 2018). Portanto, entender as características dos espaços intercelulares e a variação morfofisiológicas ao longo do eixo foliar e nas raízes das plantas de milho e sorgo podem explicar respostas da dinâmica na eficiência no uso da água e nas trocas gasosas e compreender estes mecanismos torna-se essencial para a sobrevivência das plantas em ambientes secos.

Dessa forma, a hipótese deste trabalho é que as características morfofisiológicas variam ao longo do eixo foliar e nas raízes das plantas de milho e sorgo correlacionada com o desenvolvimento dos espaços intercelulares submetidos ao déficit hídrico. Assim, o objetivo deste estudo foi avaliar o efeito da restrição hídrica na morfofisiologia e anatomia ao longo do eixo foliar e nas raízes de plantas de milho e sorgo associado ao crescimento, espaços intercelulares e trocas gasosas.

2 REFERENCIAL TEÓRICO

2.1 A seca em plantas e a agricultura

O volume hídrico vem alterando-se constantemente e a sustentabilidade da vida na Terra está sendo ameaçada correlacionada com as mudanças climáticas induzidas pelo homem (WANG *et al.*, 2023) e as perdas de rendimentos das culturas variam de 7% a 23% por causa das mudanças climáticas (REZAEI *et al.*, 2023). No cenário atual essas alterações vêm causando o aumento de estiagem e ondas de calor mais severas e prolongadas (KIM; LEE, 2023). O estresse hídrico tornou-se mais intenso nas últimas décadas e estima-se que a população mundial exceda 9,7 bilhões com mais de 65% das pessoas dependendo da agricultura em 2050, porém prevê que o estresse hídrico cause prejuízos em mais da metade das terras agrícolas do mundo até 2050 (CHIEB; GACHOMO, 2023; HAIDER *et al.*, 2024).

A falta de água ocasiona eventos severos de seca e a sua intensidade vem aumentando à medida que ocorre o crescimento populacional e industrial (ARIAS *et al.*, 2024). A seca ocorre quando à perda progressiva de água no solo até o nível crítico pelas plantas (HAIDER *et al.*, 2024), o que causa modificações nos vegetais devido ao estresse hídrico (CHIEB; GACHOMO, 2023). De fato, o déficit hídrico é provocado pelas mudanças climáticas afetando severamente o crescimento e o desenvolvimento das plantas (FARHAN *et al.*, 2024). Portanto, a redução do volume hídrico é uma grande ameaça para as espécies cultivadas, pois afeta sua produção e, consequentemente, a segurança alimentar de milhões de pessoas devido ao prejuízo durante a colheita das plantas.

A redução da disponibilidade de água vem afetando várias regiões e países como o Punjab, Paquistão e Ásia (FARHAN *et al.*, 2024). Dessa forma, a seca vem causando prejuízos na produção agrícola, o que afeta a vida das pessoas (QIAO *et al.*, 2024). O Brasil também sofre com déficit hídrico, principalmente nas regiões do Nordeste e do Sudeste Brasileiro (MARENGO *et al.*, 2018, 2019), sendo que no Brasil a redução no volume de água é o fator chave de perdas significativas na agricultura (WALKER *et al.*, 2024). Por essa razão, é crucial o desenvolvimento de pesquisas para obter plantas com rendimentos eficientes e reduzir os impactos da seca (YADAV *et al.*, 2021).

Portanto, tornam-se necessárias ações para minimizar os prejuízos causados pela seca no futuro. Ações como o racionamento de água, a redução do período de irrigação e a captação de água da chuva tornam-se importantes. Apesar disso, compreender as respostas de plantas a fatores abióticos, como a seca, e os mecanismos que denotam tolerância é uma das estratégias mais relevantes. Para isso, é necessário avaliar a anatomia e a fisiologia das plantas para compreender e preencher as lacunas sobre os mecanismos morfofisiológicos.

2.2 Modificações morfofisiológicas de plantas sob déficit hídrico

A água é um recurso fundamental para o desenvolvimento e crescimento das plantas e a sua disponibilidade na natureza depende das condições climáticas (REZAEI *et al.,* 2023). De fato, a perda de água na natureza é um fator para a distribuição da vegetação nos ecossistemas (QIAO *et al.,* 2024). As alterações no clima estão transformando o ambiente tornando mais quente e seco e espera-se que esses eventos intensifiquem nos próximos anos, o que afetará diretamente as plantas e a segurança alimentar global (CHIEB; GACHOMO, 2023; HAIDER *et al.,* 2024). Isso corrobora modelos climáticos que indicam redução das chuvas e aumento das secas gerando preocupação para os agricultores, pois apenas 346.895 mil hectares de área terrestre estão sob irrigação e o restante depende totalmente da precipitação natural (NYAUPANE *et al.,* 2024). Para Sheoran *et al.* (2016) cerca de 1,8 mil milhões de pessoas, ou seja, 65% da população mundial enfrentarão uma escassez absoluta de água. Neste contexto, a perda de água ocasiona perdas e limitações significativas nas plantas, pois causa modificações morfofisiológicas afetando sua produtividade (NAIKWADE, 2023).

O estresse hídrico causa uma série de modificações morfológicas e fisiológicas nas plantas, que advém da aclimatação sob condições de seca, sendo que essas modificações ocorrem nas raízes e nas folhas (MENG; ZHANG; YIN, 2023; YAO *et al.,* 2024; YAVAS *et al.,* 2024). A redução na disponibilidade hídrica pode reduzir severamente o crescimento das plantas (QIAO *et al.,* 2024). Isso tem relação com a diminuição da divisão celular e o turgor, uma vez que o estresse hídrico dificulta a divisão e a extensão celular (LONG; DENG, 2019; MENG; ZHANG; YIN, 2023). Além disso, a redução do crescimento das plantas em condições de seca pode estar relacionada com a diminuição da área foliar e conteúdo de clorofila (YAVAS *et al.*, 2024). Segundo Qiao *et al.* (2024) a seca causa modificações significativas na eficiência fotossintética nos fotossistemas II (PS II) e I (PS I), uma vez que as reações de luz são altamente dependentes da água, principalmente o PS II, resultando na redução na eficiência da conversão de energia luminosa. Qiao *et al.* (2024) destaca ainda que a seca provoca modificações no Ciclo de Calvin, pois a falta de água reduz a atividade de enzimas, fixação de carbono e dificuldades na exportação de produtos fotossintéticos. Esses fatores reduz a produtividade das plantas afetando o crescimento. Outros fatores estão associados ao estresse hídrico como redução na germinação de sementes, alteração na morfologia radicular, restrição da transpiração, fechamento estomático, redução no transporte de elétrons, folhas pequenas, sistema radicular profundo e aceleração da senescência e abscisão das folhas, redução dos espaços intercelulares e do tamanho das células do mesofilo (AMOUZOU *et al.*, 2019; MENG; ZHANG; YIN, 2023; NAIKWADE *et al.*, 2023; OLIVEIRA *et al.*, 2023; QIAO *et al.*, 2024; SHEORAN *et al.*, 2015; TORRE *et al.*, 2021; YADAV *et al.*, 2021; YAVAS *et al.*, 2024).

Outro aspecto interessante é a falta de água causa reduções no potencial hídrico do xilema, a condutância estomática e a transpiração relacionada com a difusão dos gases através dos estômatos (LI *et al.*, 2023; TORRE *et al.*, 2021; YAVAS *et al.*, 2024). Além disso, o déficit hídrico estimula várias respostas ao longo do eixo foliar nas porções apical, mediana e basal, o que pode afetar o funcionamento estomático e fotossintético. De fato, à seca pode causar distúrbios severos nas plantas, o que compromete o estado fisiológico (MUNNÉ-BOSCH; VILLADANGOS, 2023) levando a mortalidade (MCDOWELL *et al.*, 2022). Segundo Hemati *et al.* (2022) os efeitos mais comuns do estresse hídrico é reduzir a pressão de turgor nas células de crescimento afetando raízes, caule, folhas e frutos, aumento precoce da diferenciação celular, diminuição da proporção de folhas, aumento da senescência foliar, redução do conteúdo relativo de água e do espaço intercelular durante o murchamento. Nas raízes a falta de água pode aumentar a espessura da exoderme, aumento das lacunas de aerênquima, maior diâmetro e número de metaxilema e a hidropadronização, isto é, plasticidade na formação de raízes laterais em direção à água disponível (SHOAIB *et al.*, 2022) e a grande parte das mudanças anatômicas causadas pela escassez de água está relacionada ao sistema vascular devido ao transporte de água e evitar a embolia (FANG; XIONG, 2015; GUHA *et al.*, 2018; SHOAIB *et al.*, 2022).

Outra alteração importante das plantas em condições de seca é o aumento dos espaços intercelulares. O estresse hídrico pode induzir a formação de espaços intercelulares auxiliando na difusão dos gases (OLIVEIRA *et al.*, 2022). De fato, a formação dos espaços intercelulares está diretamente relacionada às condições impostas pelo ambiente (HEMATI *et al.*, 2022; OLIVEIRA *et al.*, 2023). As modificações destacadas podem estar associadas com a melhor eficiência no uso da água pelas plantas. De fato, o desenvolvimento de culturas com melhor eficiência de uso de água será crítico para adaptar estratégias agrícolas sob climas futuros previstos (PETRIK *et al.*, 2023). As estratégias na melhor eficiência do uso da água pelas plantas incluem principalmente o ajuste estomático como mudanças na densidade estomática, anatomia estomática e mecanismos de controle estomático, o que permite o controle do potencial hídrico, déficit de pressão de vapor atmosférico e concentração de CO_2, transpiração e maior espessura do mesofilo (PETRIK *et al.*, 2023). Portanto, quantificar as variáveis fisiológicas e anatômicas em estudos envolvendo as relações hídricas no sistema solo-planta-atmosfera é necessário, pois auxilia na compreensão das estratégias de aclimatação das espécies vegetais, propor um planejamento e um manejo mais adequado e uma melhor compreensão das restrições morfofisiológicas para desenvolver efetivamente espécies de culturas mais resistentes à seca.

2.3 Milho (*Zea mays* L. (Poaceae))

É conhecido que o estresse hídrico restringe o crescimento, desenvolvimento e a produção da cultura do milho. De fato, a seca prejudica várias funções morfológicas, fisiológicas e bioquímicas do milho (RASHEED *et al.*, 2023). O estresse hídrico causou cerca de 15-20% das perdas de produção de milho globalmente, pois é uma espécie que precisa de grande volume de água para o seu crescimento e os efeitos da falta de água incluem redução no potencial hídrico foliar relativo, o tamanho das folhas, a fotossíntese, a condutância estomática e a concentração subestomática de CO_2 (ADEWALE *et al.*, 2018; NGUYEN *et al.*, 2024; RASHEED *et al.*, 2023). O milho, *Zea mays* L., possui altura variando de um a quatro metros com caule do tipo colmo e o sistema radicular fasciculado, sendo uma planta sazonal e pertence à família Poaceae (gramíneas) (AHMED *et al.*, 2023). Além disso, o milho é originário do México e espalhou-se pelo mundo (ZHAO *et al.*, 2023). De acordo com Yang *et al.* (2022) estudos que visam elucidar mecanismos de tolerância à seca no milho é significativo para garantir a segurança alimentar mundial.

As plantas de milho apresentam o metabolismo do tipo C_4 (HIREL *et al.*, 2005). Segundo Nguyen *et al.* (2024) essa espécie é conhecida como uma planta isoídrica. Este comportamento proporciona regulação nos movimentos estomáticos para reduzir a perda de água pela transpiração, por meio de ajustes na condutância estomática foliar prevenindo a embolia e atingindo uma alta eficiência no uso da água (ZHOU *et al.* 2021).

O milho é a safra de grãos mais importantes do mundo vindo atrás do trigo e do arroz e é amplamente cultivado e consumido como alimento, ração e matéria-prima industrial (AHMED *et al.*, 2023; RASHEED *et al.*, 2023). Dessa forma, é uma das culturas mais amplamente cultivadas. Apesar da produção do milho ser amplamente distribuída pelo mundo a escassez hídrica associada às mudanças climáticas está influenciando negativamente esta produção nos últimos anos. De fato, até 2050 há uma previsão global de dobrar a produção agrícola para atender à crescente demanda de alimentos, porém a seca está tornando esse objetivo desafiador, sendo uma ameaça à segurança alimentar, pois está afetando florestas e pastagens (ANWAR *et al.,* 2024). Portanto, o milho possui grande importância econômica mundial e entender os mecanismos causados pelo estresse hídrico poderá auxiliar no aumento da produtividade da planta e na preservação da biodiversidade. Para Mansour *et al.* (2023) a falta de água é um desafio decisivo que afeta as funções fisiológicas e a produtividade do milho e mais pesquisas são cruciais para melhorar a tolerância do milho ao estresse da seca, pois sua produção deve atender ao aumento global de consumo devido à crescente população.

2.4 Sorgo (*Sorghum bicolor* (L.) Moench. (Poaceae))

A falta de água e a exposição prolongada à seca têm efeitos deletérios no crescimento, desenvolvimento e na produtividade das plantas cultivares (FAHAD *et al.,* 2017). No entanto, existe cultivares, como o sorgo, que são tolerantes à seca sendo uma opção de cultivo em ambientes áridos. De fato, a resiliência do sorgo ao estresse por calor e seca, o uso eficiente da água e a alta produção de biomassa o tornam adequado para ambientes áridos (TAVAZOH *et al.,* 2024).

O sorgo, *Sorghum bicolor* (L.) Moench., é uma espécie da família Poaceae e é um grão popular em todo o mundo, principalmente na África devido ao seu uso tradicional na alimentação (RASHWAN *et al.,* 2021). É importante destacar que, aproximadamente 50% a 70% das terras de cultivo de sorgo na África Subsaariana são propensas à secas recorrentes (OTIENO, 2024; PUTRI *et al.,* 2023). Dessa forma, em regiões com escassez de água extrema, o cultivo de culturas tolerantes à seca, como o sorgo, torna-se uma opção mais viável em comparação ao milho devido à sua capacidade superior de prosperar com água limitada (NDOU *et al.,* 2023; OLIVEIRA *et al.,* 2023; TAVAZOH *et al.,* 2024).

O sorgo é uma planta nativa dos países africanos (DEVNARAIN *et al.,* 2019) e possui grande importância econômica (JENKINS *et al.,* 2020) e destaca-se na alimentação básica de muitos países (IMPA *et al.,* 2019; RATHER *et al.,* 2023). Isso está relacionado com a alta produtividade do sorgo, pois esta espécie é uma cultivar produtora de grãos classificada em quinto lugar no mundo e em segundo na África, depois do milho (NDOU *et al.,* 2023) e o seu grão é usado como fonte de alimento para humanos na África e Ásia, enquanto os caules e folhas são usados para ração animal e produção de energia em outros países como Brasil e Estados Unidos da América (SATISH *et al.,* 2016).

Segundo Calzadilla *et al.* (2013) o setor agrícola, que usa 70% da água do mundo, está mais suscetível às mudanças climáticas e as plantas de sorgo demonstram-se uma ampla adaptação às mudanças climáticas devido ao cultivo global, o que proporciona ao sorgo crescer em regiões com baixa disponibilidade de água. Portanto, nota-se que o plantio desta cultura pode ser uma solução sustentável para os agricultores no combate às mudanças climáticas. De fato, a tecnologia verde é primordial para mitigar os efeitos do estresse da seca no sistema de cultivo agrícola (PUTRI *et al.*, 2023).

Neste contexto, o sorgo é a principal cultura cultivada em condições de seca (NDOU *et al.*, 2023; TAVAZOH *et al.*, 2024) e é considerado tolerante ao déficit hídrico (IMPA *et al.*, 2019). Por essa razão, o sorgo é uma promessa significativa como uma cultura forrageira sustentável, principalmente em regiões que priorizam tanto a quantidade quanto a qualidade da forragem, especialmente aquelas com recursos de irrigação limitados como a África e Ásia (BHATTARAI *et al.*, 2020; RATHER *et al.*, 2023). Apesar da tolerância à seca o sorgo pode apresentar modificações que afeta sua produtividade. De fato, o estresse hídrico influenciou negativamente as características morfológicas e relacionadas ao rendimento do sorgo (SARSHAD *et al.*, 2021). No entanto, estas modificações podem ser mecanismos para melhorar o desempenho das plantas em condições de seca. Portanto, estudar os mecanismos que regem a tolerância à seca pelas plantas de sorgo pode auxiliar em programas de melhoramento genético e no entendimento das principais estratégias destas plantas que denotam tolerância em ambientes secos.

2.5 Estratégias de tolerância das plantas à seca

No cenário de mudanças climáticas à seca afeta diretamente as plantas gerando respostas contrastantes, isto é, espécies tolerantes e sensíveis à seca, como o sorgo e o milho, respectivamente (OLIVEIRA *et al.*, 2023). É importante destacar que no Brasil o milho atingiu um rendimento de 5,7 toneladas por hectare em uma área de 21.500 mil hectares e o sorgo atingiu um rendimento de 3,1 toneladas por hectare em uma área de 1.541 mil hectares entre os anos de 2023-2024 (QIAO *et al.*, 2024). Segundo o mesmo autor, as plantas de milho e sorgo são as principais culturas em vários países, pois destacam-se pelo rendimento e a importância alimentar.

Neste contexto, a falta de água é uma preocupação mundial na agricultura, sendo que a resposta mais comum de tolerância das plantas à seca envolve a diminuição da perda de água (OLIVEIRA *et al.*, 2023; QIAO *et al.*, 2024). Dessa forma, o déficit hídrico estimula diversas respostas das plantas, como alterações morfológicas, fisiológicas, bioquímicas e moleculares, que acabam resultando em perturbações no funcionamento das plantas (KAPOOR *et al.*, 2020). Dentre as respostas destacam-se modificações na anatomia foliar que incluem maior densidade estomática e redução na abertura dos estômatos conservando a água na folha pela otimização da transpiração (FANG; XIONG, 2015; YAVAS *et al.*, 2024).

Outras alterações importantes nas plantas para tolerância em condições de seca envolvem folhas mais espessas e uma maior proporção de tecidos do mesofilo, bem como o aumento de osmólitos e atividades de enzimas antioxidantes (ZHU *et al.*, 2021). Pode ocorrer rápida senescência dos órgãos vegetativos diminuindo o custo metabólico e, portanto, possuem maior capacidade de acumulação de biomassa (CHATURVEDI *et al.*, 2021; IMPA *et al.*, 2019). A grande parte dessa biomassa é acumulada nas raízes o que aumenta a profundidade do sistema radicular elevando a probabilidade de obtenção de água (SOUZA *et al.*, 2016). De fato, a regulação da estrutura da raiz é outro mecanismo crítico de adaptação pelo qual as plantas aumentam a absorção de água sob seca, que incluem sistema radicular profundo, redução da densidade de ramificação lateral da raiz e aumento da proporção raiz-parte aérea (LUCOB-AGUSTIN *et al.*, 2021). Além disso, as plantas tolerantes apresentam mudanças nos vasos foliares, principalmente no xilema por causa da embolia, pois a falha hidráulica durante a seca pode levar a mortalidade das plantas (LENS *et al.*, 2022).

O papel de tolerância à seca nas plantas também incluem regulação do movimento estomático e da condutância estomática das folhas (ZHOU *et al.*, 2021), da regulação do sistema fotossintético e transpiratório (HEMATI *et al.*, 2022; QIAO *et al.*, 2024). Isso está relacionado com a quantidade e tamanho dos estômatos, pois são responsáveis pelas trocas gasosas (QIAO *et al.*, 2024), pelas alterações nos espaços intercelulares foliares, pois são responsáveis pelo armazenamento de gases como dióxido de carbono e a água (OLIVEIRA *et al.*, 2023) e nas reações dependentes de luz da fotossíntese, que respondem pela maior parte da eficiência fotossintética (QIAO *et al.*, 2024).

Portanto, o déficit hídrico é limitante ao crescimento, desenvolvimento e sobrevivência das plantas. Dessa forma, as plantas evoluíram mecanismos eficientes para resistir sob tais condições, como ajustes na anatomia foliar e no controle das trocas gasosas, que reflete na melhor eficiência no uso da água. De fato, as plantam empregam varias estratégias para melhorar sua eficiência no uso da água para sobreviver em condições de seca (QIAO *et al.*, 2024; ROWLAND *et al.*, 2023) e estes ajustes são limitados pelos estômatos, uma vez que a captação de CO_2 e a perda de água ocorrem por eles (OLIVEIRA *et al.*, 2022 e 2023). Neste contexto, espécies contrastantes à seca como o milho e o sorgo empregados como modelos em estudos de déficit hídrico podem oferecer respostas interessantes para entender as diferentes respostas das espécies em condições de estresse hídrico e permitir compreender a relação da seca com as trocas gasosas e com a anatomia foliar. Segundo Wang *et al*. (2023) há um consenso entre especialistas sobre estratégias de adaptação às mudanças climáticas e essas estratégias incluem, por exemplo, desenvolvimento de culturas resistentes à seca para combater a escassez de água.

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SEGUNDA PARTE

ARTIGO 1 CONTRASTING LEAF INTERCELLULAR SPACE DEVELOPMENT IN SORGHUM AND MAIZE MODULATES DIFFERENT TOLERANCE CAPACITY TO WATER LIMITATION

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Abstract

The objective of this study was to evaluate the relationship between intercellular spaces and leaf gas exchange and the effect of total intercellular space on the growth of maize and sorghum under water restriction. The experiments were conducted in a greenhouse in a 2 × 3 factorial arrangement (two plant types and three water conditions: field capacity (FC = 100%), 75%FC, and 50%FC) with 10 replicates. The lack of water was a limiting factor for maize because it showed reductions in leaf area, leaf thickness, biomass, and gas exchange parameters, while sorghum remained unchanged, maintaining its water-use efficiency. This maintenance was correlated with the growth of intercellular spaces in sorghum leaves because the increased internal volume led to better CO_2 control and prevented excessive water loss under drought stress. In addition, sorghum had more stomata than maize. These characteristics contributed to the drought tolerance of sorghum, while maize could not make the same adjustments. Therefore, changes in intercellular spaces promoted adjustments to avoid water loss and may have improved CO_2 diffusion, characteristics that are important for drought-tolerant plants.

Keywords: Cell area, CO_2 absorption, Gas diffusion, Mesophyll, Water relations, Water stress

Introduction

Drought directly affects plants (Chhaya et al. 2021) and can impair plant growth, development, and yield (Pareek et al. 2010). This leads to morphophysiological changes in the leaves, such as reduced leaf area (Talbi et al. 2020), stomatal closure (Carminati and Javaux 2020), and decreased stomatal conductance and photosynthesis and transpiration parameters (Wu et al. 2021). In response to drought, different plants, such as maize and sorghum, may exhibit different behaviours, reflecting different leaf anatomical and physiological responses. Maize (*Zea mays* L.) is a drought sensitive species (Gong et al. 2015), and sorghum (*Sorghum bicolor* (L.) Moench.) is a drought-tolerant species (Impa et al. 2019).

To withstand drought, plants must make anatomical and physiological adjustments in their leaves. Little has been reported about the role of intercellular spaces as modulators of responses to sensitivity or tolerance to drought in plants. In fact, water stress can induce the formation of intercellular spaces, which can aid in the diffusion of gases (Ni et al. 2014; Terashima et al. 2006). According to Oliveira et al. (2022) increased stomatal cavities are related to improved gas exchange and the drought-tolerance in sorghum. Therefore, common responses of plants to drought, such as reduced leaf thickness and leaf area, may result in adjustments to intercellular spaces and contribute to better control of gas exchange.

Plants exchange CO_2 and H_2O with the atmosphere through their stomata. The gases that diffuse into the mesophyll are retained in the substomatal cavity, which is the first connection of the stomatal pore, and after the gases are absorbed, they are distributed within the leaf by small intercellular spaces (Willmer and Fricker 1996). Therefore, intercellular spaces play an important role in the control of gas exchange together with stomata because the increase in their internal volume can improve CO_2 uptake and thus optimize biomass gain and plant growth under dry conditions.

Understanding new drought tolerance characteristics is essential for agricultural production and species conservation because drought-tolerant plants can maintain their morphophysiological structures during prolonged periods of stress (Avramova et al. 2016; Kreibich et al. 2019). This ability is related to leaf adjustments the plants make to decrease water loss and absorb more CO_2, and these adjustments may be correlated with the growth of intercellular spaces in the mesophyll. The tolerant species *Sorghum bicolor* was capable of changing the stomatal chamber which may have contributed to its drought tolerance (Oliveira et al. 2022). We hypothesized that larger leaf intercellular spaces promote gas exchange, growth, and drought tolerance in plants and that drought-tolerant plants have the plasticity to enlarge their leaf intercellular spaces, while drought-sensitive plants do not. The objective of this study was to evaluate the relationship of intercellular space size with leaf gas exchange and the effect of this space size on the growth of *Zea mays* and *Sorghum bicolor* under water restriction.

Materials and methods

Plant material and experimental design

The experiment was carried out in a greenhouse located at the Federal University of Lavras (Universidade Federal de Lavras), state of Minas Gerais, Brazil (21° 13′ 17″ S and 44° 57′ 47″ W). *Sorghum bicolor* and *Zea mays* plants were obtained from seeds provided by Embrapa's National Research Centre for Maize and Sorghum, located in Sete Lagoas, Minas Gerais, Brazil.

Seeds were sown in 5.0L plastic pots containing 2.0L of sand and 800 mL of nutrient solution (Hoagland and Arnon 1950) at 40% of its ionic force. Pots were then placed in a germination chamber under constant light at 25°C for approximately 7 days, after which the seedlings had three leaves and were 10 cm in height. Seedlings displaying good phytosanitary conditions and of similar size were individually transplanted into plastic pots containing 3.0 L of sand and nutrient solution (Hoagland and Arnon 1950) at 40% of its ionic force. These pots were kept in a green house at 25 ± 2 °C under 50% relative humidity and a 12h photoperiod.

The plants were then subjected to three water conditions according to the method used by Díaz et al. (2018): (1) field capacity (FC), (2) 75%FC, and (3) 50%FC. The field capacity was considered the maximum volume of water retained by 1.0 L of sand without becoming waterlogged. The volume of water applied to achieve FC was 310.0 mL water L^{-1} sand, and for 75%FC and 50%FC, the amounts of water in the substrate were 232.5 and 155.0 ml water L^{-1} sand, respectively. The water lost by evapotranspiration was monitored by the daily difference in the weight of each pot. Water was replaced daily, and the nutrient solution was administered weekly. Each plant remained in the test condition for 60 days. The experimental design was completely randomized, with three treatments and 10 replicates ($n = 30$). Each replicate comprised one plant. All the data from the analyses where multiple assessments were performed were then averaged per replicate (maintaining $n = 30$ and avoiding artificial replication).

Plant growth analysis

The plants were sampled at the end of the experiment and then separated into leaves, stems, and roots. Leaf area was measured using a CI-203 handheld area metre (CID Bio Science, Camas, WA, USA). The fresh mass of each plant part was measured on an AY220 analytical scale (Shimadzu, Kyoto, Japan). The tolerance index was calculated according to Wilkins (1978); for this, the following equation was used: $Ti = (SDM/CDM) * 100$ where Ti is the tolerance index, SDM is the dry mass of stressed plants (from 75 and 50%FC) and CDM is the dry mass from control plants (FC). The roots, leaves, and stems were oven-dried at 60 °C to constant weight, and the dry mass was assessed on an analytical scale. All water applied to the system was measured to calculate the water-use efficiency.

Gas exchange analysis

At the end of the experiment, leaf gas exchange was assessed with an infrared gas analyser (LI-6400XT, LI-COR Bio sciences, Lincoln, NE, USA) coupled to an LI-6400-02B cuvette with a red/blue LED light source (LI-COR). Measurements were taken from two fully developed leaves per plant between 08:00 and 10:00 a.m., with the photon flux density fixed at 1,000 µmol m^{-2} s^{-1} and a mean temperature of 28.1°C. CO_2 was obtained from the atmosphere, its mean concentration was 420.1 µmol mol^{-1} air, and the pump flow was kept at 500 µmol s^{-1}. The net photosynthesis (A), transpiration rate (E), stomatal conductance to water (gsw), and stomatal conductance to CO_2 (gtc) were assessed. Whole plant photosynthesis (Awp), whole-plant transpiration (Ewp), and whole-plant conductance (gswp) were estimated with the following equations: Awp = A × (leaf area); Ewp = E × (leaf area); and gswp = gs × (leaf area). The parameters obtained directly by the analysis of the infrared gas analyser were given in moles (CO_2 or water) per square metre per second. Thus, by multiplying by the measured leaf area of the plant (much lower than 1 m^2 at the sampling age), the calculated parameters are given in moles (CO_2 or water) per second.

Water-use efficiency

At the end of the experiment, the cumulative water-use efficiency (WUEc) was calculated (Kramer and Boyer 1995). WUEc was calculated as the ratio of the total plant dry mass to the total water consumed during the period.

Anatomical analysis

One fully developed leaf was removed and fixed in 70% ethanol (Johansen 1940). Cross-sections were obtained using a steel blade at the median leaf region. The sections were clarified with 50% sodium hypochlorite and washed twice in distilled water for 10 min. Then, the sections were stained with Safrablau (1% safranine and 0.1% astra blue at a ratio of 1:7) and mounted on slides with 50% glycerol (Johansen 1940). Paradermal imprints were taken from both the abaxial and adaxial leaf surfaces using a cyanoacrylate resin and then mounted on slides. The paradermal imprints were taken in the morning from 6 to 8 a.m., when the stomata were expected to be open. The slides were observed, and images were taken under a CX31 light microscope (Olympus, Tokyo, Japan). The total image area was 103,191.53 μm^2 and the area from transversal sections evaluated was average 67,748.51 μm^2. It was possible to see between 3 and 5 stomata per image. One slide was made per leaf, three sections and four fields were analysed per slide, and these data were averaged for each replicate. The images were analysed with ImageJ (Wayne Rasband National Institutes of Health, United States of America).

The following anatomical characteristics of the cross sections were observed: leaf thickness, mesophyll area, the area of each substomatal cavity, the area of each narrow intercellular spaces in the mesophyll. The sum of substomatal cavity areas and narrow intercellular as well as the total intercellular spaces area were calculated. The proportion of the stomatal cavity, narrow intercellular spaces and total intercellular spaces in the mesophyll were calculated as follows: IS% = (IS/MA) * 100 where IS% is the proportion of each intercellular space type, IS is the sum of measured areas from each intercellular space type and MA is the mesophyll area. The detailed method for the measurement of leaf intercellular spaces is shown in the supplementary material. In the paradermal sections, the following structures were analysed: section area, number of stomata and number of regular epidermal cells. The stomatal density (SD) was calculated as follows: SD = number of stomata × (106/section area). The number of stomata per plant (NSP) was calculated as follows: NSP = stomatal density × leaf area. Both were measured in square millimetres.

Statistical analysis

The data were subjected to two-way ANOVA, and the means were compared by the Scott–Knott test at $P < 0.05$ using SIS VAR 5.0 software (Ferreira 2011). Before parametric analysis, the data were tested for normality with the Shapiro–Wilk test, and all variables showed a normal distribution. Linear correlation analysis was performed and was considered significant when $R2 \geq 0.6$.

Results

Summarized data from ANOVAs are shown in Table 1. All variables showed significant interaction.

Lower water levels reduced the leaf area in maize, but they had no significant effect on this variable in sorghum (Fig. 1a). The maize plants showed higher leaf area than sorghum under the FC and 75%FC treatments, but under 50%FC, both species showed similar leaf areas (Fig. 1a). The 50%FC treatment reduced maize dry mass but promoted no significant changes in the total dry mass of sorghum (Fig. 1b). The maize plants showed higher dry mass than sorghum under all treatments (Fig. 1b). The tolerance index was similar for both species under 75%FC and higher in sorghum under 50%FC; the 50%FC treatment reduced the tolerance index in maize but had no effect in sorghum (Fig. 1c).

The net photosynthesis was higher in maize than in sorghum grown at FC and 75%FC, but this pattern was inverted at 50%FC (Fig. 2a). The 50%FC treatment increased the net photosynthesis in sorghum but had no effect on maize plants (Fig. 2a). The transpiration (Fig. 2b), stomatal conductance to water (Fig. 2c), and total conductance of CO_2 (Fig. 2d) showed similar patterns: They showed higher means in maize under FC and 75%FC, but sorghum outperformed maize under 50%FC. Lower water availability had no effect on transpiration or stomatal conductance in maize plants, but 50%FC increased these parameters in sorghum plants.

The gas exchange parameters assessed at the whole-plant level indicated significant limitations in maize plants since whole-plant photosynthesis, transpiration, and stomatal conductance were reduced by water limitation, while sorghum plants remained unaffected (Fig. 3a–c). Maize plants demonstrated higher whole-plant photosynthesis, transpiration, and stomatal conductance than sorghum plants under FC and 75%FC, but under 50%FC, sorghum plants showed similar results as maize plants (Fig. 3a, c). Transpiration remained lower in sorghum plants under all treatments (Fig. 3b). Water limitation promoted different effects on the water-use efficiency in maize and sorghum plants: Maize increased this parameter at 75%FC but reduced it at 50%FC, whereas sorghum plants showed no significant change (Fig. 3d).

Water limitation had no significant effect on the leaf thickness of sorghum but reduced this parameter in maize. The sorghum showed thicker leaves than maize under all treatments (Figs. 4a, 5). Lower water availabilities increased the substomatal cavity proportion in sorghum but caused no significant modification in maize (Figs. 4b, 6). Additionally, the sorghum plants showed substomatal cavities with larger proportion than maize plants under 50%FC (Figs. 4b, 6). The proportion of narrow intercellular spaces increased in maize under 75%FC and in sorghum in the 50%FC treatment, while 50%FC reduced this parameter in maize (Fig. 4c). Maize and sorghum showed similar means of the proportion of the narrow intercellular spaces under FC and 75%FC, but sorghum plants showed a higher mean of this variable under 50%FC (Fig. 4c). Lower water availability promoted no changes in the proportion of total intercellular spaces in maize but increased this parameter in sorghum (Fig. 4d). Maize and sorghum had similar proportions of total inter cellular spaces under FC, although under lower water avail abilities sorghum plants showed higher means than maize plants (Fig. 4d). Lower water availability increased the area of the stomatal cavity in sorghum but had no effect in maize (Figs. 4e, 6); this parameter was higher in sorghum under 50%FC compared with maize (Figs. 4e, 6).

Lower water availability had no significant effect on the stomatal density of maize (Fig. 7a, b). Sorghum showed a reduction in the 75%FC treatment on the abaxial side and in the 50%FC treatment on the adaxial side (Fig. 7a, b). Sorghum plants showed higher stomatal density than maize under all water treatments (Fig. 7a, b). Water limitation reduced the number of stomata per plant in the abaxial and adaxial leaf surfaces of maize; however, sorghum showed no significant modifications of this parameter in the abaxial surface, though 50%FC reduced it in the adaxial surface (Fig. 7c, d). Maize plants had more stomata under the FC and 75%FC treatments in the abaxial surface and under the FC treatment in the adaxial surface (Fig. 7c, d), whereas the number of stomata was similar between sorghum and maize under 50%FC on both leaf sides and 75%FC on the adaxial side (Fig. 7c, d).

Net photosynthesis showed a significant correlation with the substomatal cavity area and the total area of intercellular spaces in both maize and sorghum plants (Fig. 8a, c), whereas the area of narrow intercellular spaces showed no significant correlation with net photosynthesis (Fig. 8b). Transpiration showed a significant correlation with the stomatal cavity area of maize and sorghum but was not correlated with narrow intercellular spaces (Fig. 8d–f). The stomatal conductivity and intercellular CO_2 concentration showed similar results and were both correlated with the stomatal cavity area and the area of total intercellular spaces, although no significant correlation was found with the narrow intercellular spaces (Fig. 8g–i).

Table 1. Summarized ANOVA results for all variables analyzed, including mean square values, *F* test results, and *P* values.

Variable	*CV %*	*Mean square values*	*F test value*	*P-value*
*Total dry mass (P)	34.7	3374.7	47.2	<0.0001
*Total dry mass (W)	34.7	210.6	2.9	0.0627
^{ns}Water content in the plant (P)	8.0	345.2	10.2	0.0022
^{ns}Water content in the plant (W)	8.0	22.9	0.7	0.5108
Leaf area (P)	30.6	4329655.8	61.8	<0.0001
Leaf area (W)	30.6	992285.9	14.1	<0.0001
*Whole plant photosynthesis (P)	36.2	4.9	38.0	<0.0001
*Whole plant photosynthesis (W)	36.2	0.8	6.3	0.0034
Whole plant Transpiration (P)	33.4	0.1	77.5	<0.0001
Whole plant Transpiration (W)	33.4	0.006	6.3	0.0032
Whole plant stomatal conductance for water (P)	31.8	0.0001	59.6	<0.0001
Whole plant stomatal conductance for water (W)	31.8	0.00002	9.5	0.0003
Net photosynthesis rate (P)	20.4	13.9	2.4	0.1276
Net photosynthesis rate (W)	20.4	69.3	11.7	<0.0001
Transpiration rate (P)	20.3	0.2	3.4	0.0700
Transpiration rate (W)	20.3	0.6	12.4	<0.0001
Stomatal conductance to water vapor (P)	22.2	0.0001	0.8	0.3792
Stomatal conductance to water vapor (W)	22.2	0.001	11.1	<0.0001
Total conductance to CO_2 in leaf (P)	22.8	0.00007	1.2	0.2671
Total conductance to CO_2 in leaf (W)	22.8	0.0003	5.6	0.0048
Accumulated water use efficiency (P)	31.1	0.00007	57.0	<0.0001
Accumulated water use efficiency (W)	31.1	0.000009	6.8	0.0026
Leaf thickness (P)	5.7	4255.0	81.6	<0.0001
Leaf thickness (W)	5.7	453.5	8.7	0.0007
Substomatal cavity (P)	26.3	392063.9	4.4	0.0405
Substomatal cavity (W)	26.3	175527.5	1.9	0.1482
Narrow intercellular spaces in the mesophyll (P)	21.9	14219.4	7.9	0.0071
Narrow intercellular spaces in the mesophyll (W)	21.9	7401.1	4.1	0.0223
Total intercellular spaces in the mesophyll (P)	22.8	555607.7	6.1	0.0174
Total intercellular spaces in the mesophyll (W)	22.8	240844.2	2.6	0.0819
*Abaxial stomatal density (P)	11.9	14897.3	72.7	<0.0001
*Abaxial stomatal density (W)	11.9	275.7	1.3	0.2680
*Adaxial stomatal density (P)	24.9	12572.9	49.9	<0.0001
*Adaxial stomatal density (W)	24.9	607.7	2.4	0.0985
*Number of stomata per plant in the adaxial surface (P)	36.7	9.1	2.5	0.1211
*Number of stomata per plant in the adaxial surface (W)	36.7	4.3	11.6	0.0001
Number of stomata per plant in the abaxial surface (P)	31.4	2.2	22.5	<0.0001
Number of stomata per plant in the abaxial surface (W)	31.4	1.1	11.3	0.0001

CV% = coefficient of variation. *P-value* limit of the software is 0.0001, results lower than this limit are indicated as $p<0.0001$. (P) = Plants (sorghum and maize); (W) = Water conditions (Field capacity (FC), 75%FC and 50%FC). All variables showed significant interaction to $p<0.05$, except for those indicated by ns= not significant interaction or *= significant interaction to $p<0.1$.

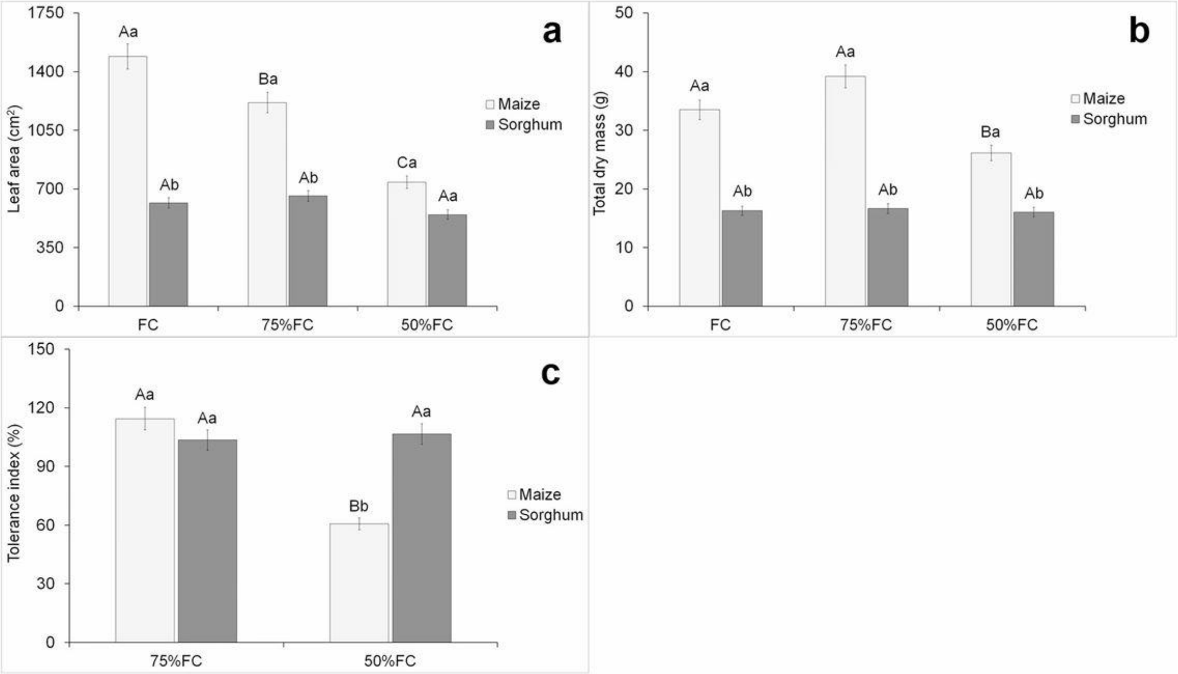

Fig. 1 Growth parameters and tolerance index of *Sorghum bicolor* and *Zea mays* under different water conditions. The lowercase letters compare plants, and uppercase letters compare water conditions. Means followed by the same letter are not significantly different according to the Scott–Knott test at $p < 0.05$. Bars = standard errors

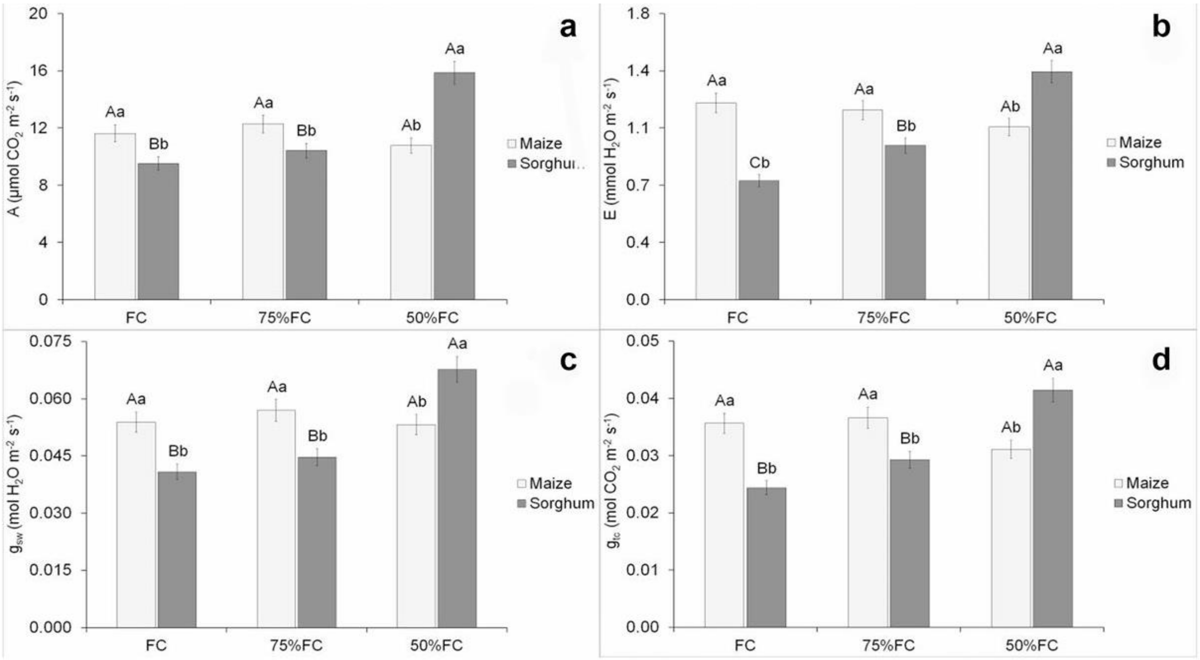

Fig. 2 Leaf gas exchange parameters of *Sorghum bicolor* and *Zea mays* under different water conditions. A net photosynthesis, E transpiration rate, gsw stomatal conductance of water vapour, gtc total conductance of CO_2. The lowercase letters compare plants, and upper case letters compare water conditions. Means followed by the same letter are not significantly different according to the Scott–Knott test at $p < 0.05$. Bars = standard errors

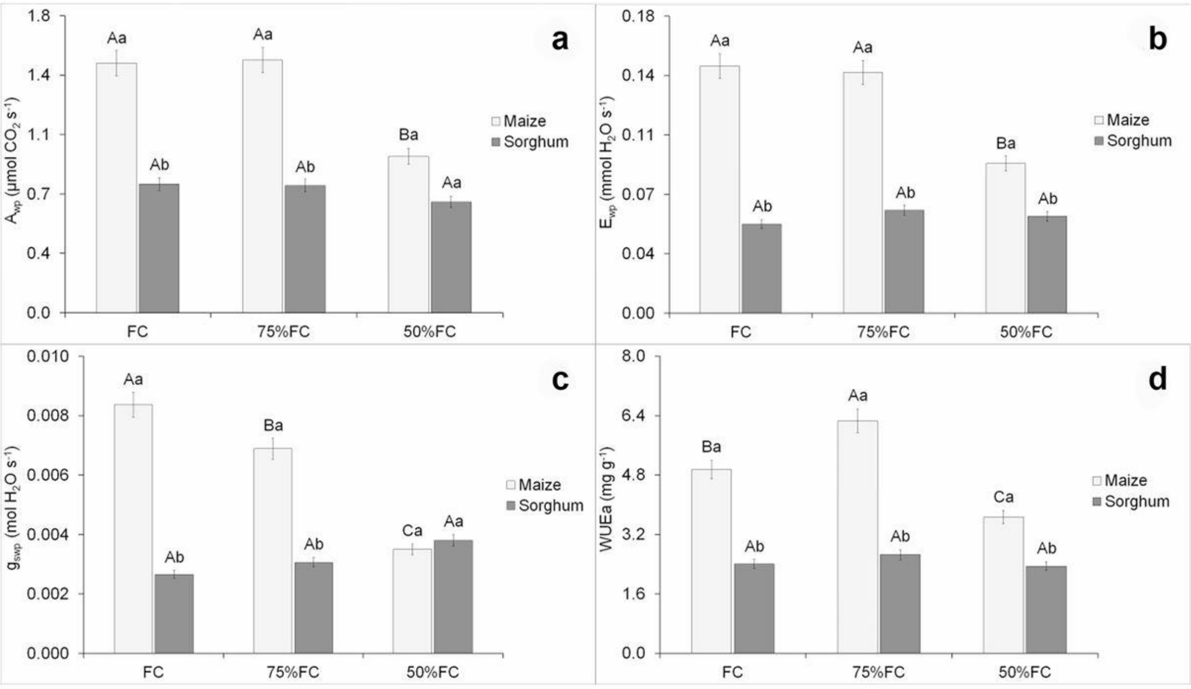

Fig. 3 Water-use efficiency and whole-plant gas exchange parameters of *Sorghum bicolor* and *Zea mays* under different water conditions. Awp whole-plant photosynthesis, Ewp whole-plant transpiration, gswp whole-plant stomatal conductance of water, WUEc cumulative water- use efficiency. The lowercase letters compare plants, and uppercase letters compare water conditions. Means followed by the same letter are not significantly different according to the Scott–Knott test at $p < 0.05$. Bars = standard errors

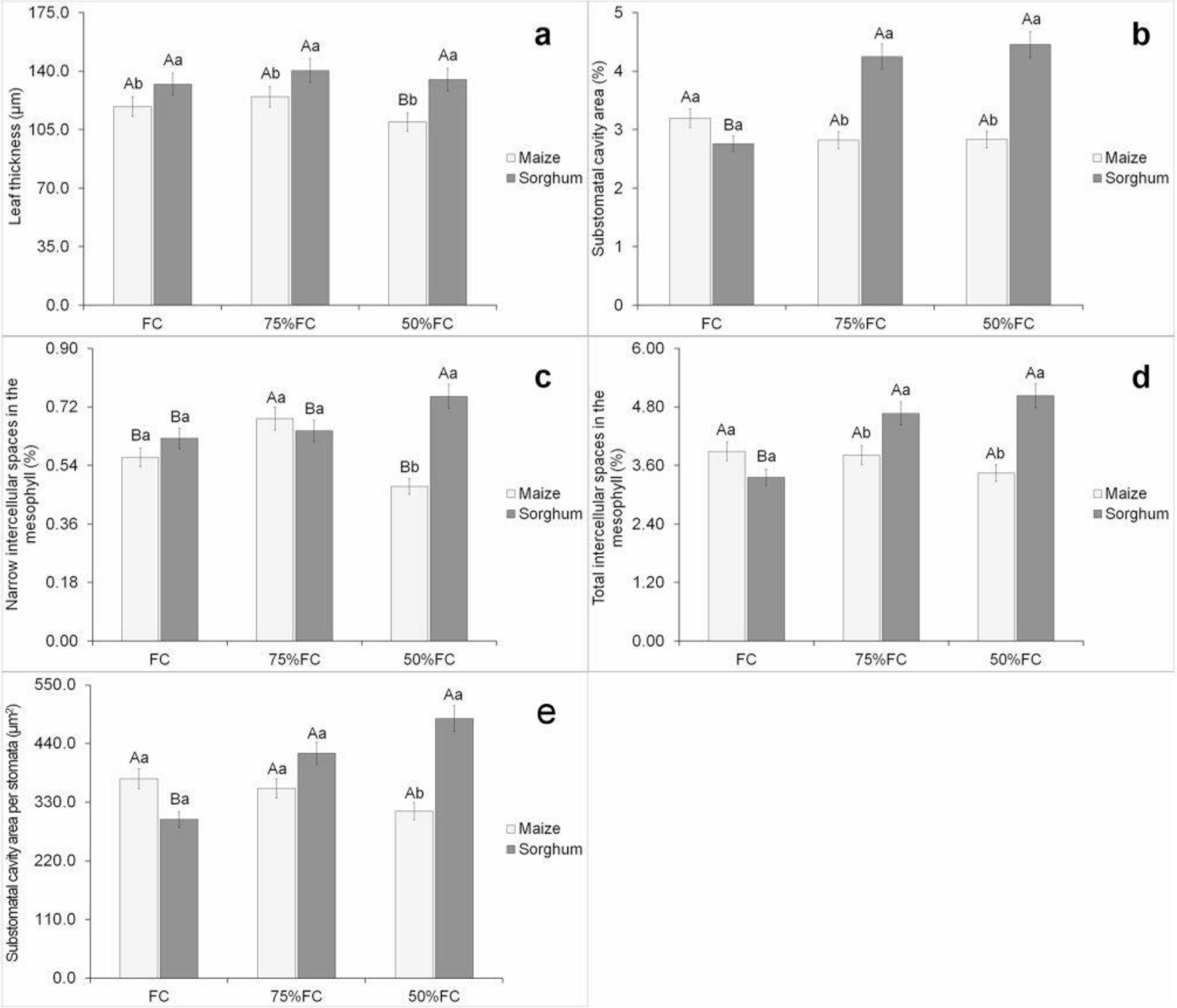

Fig. 4 Leaf anatomical characteristics of *Sorghum bicolor* and *Zea mays* under different water conditions. The lowercase letters compare plants, and uppercase letters compare water conditions. Means followed by the same letter are not significantly different according to the Scott–Knott test at $p < 0.05$. Bars = standard errors

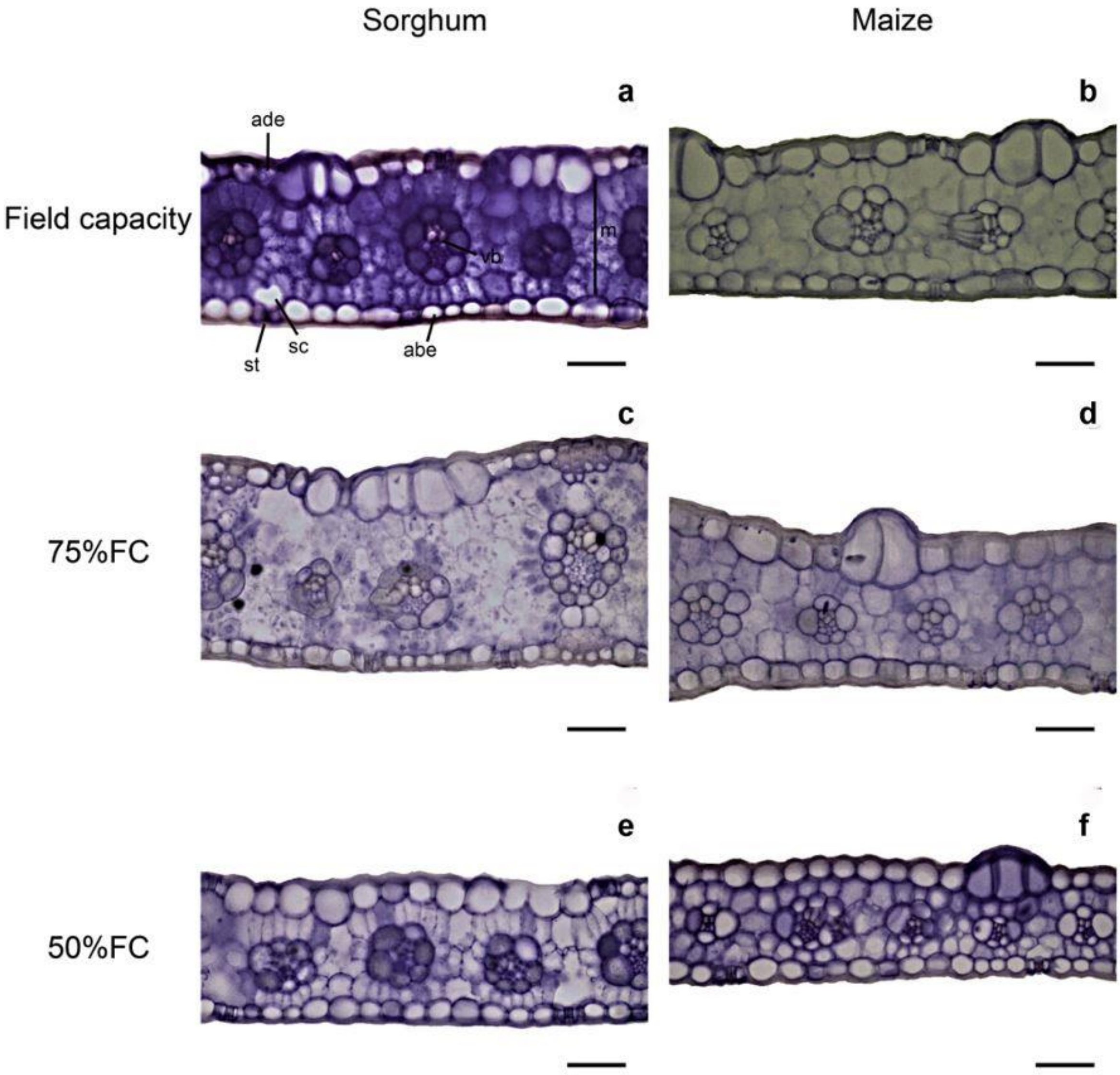

Fig. 5 Cross-sections of *Sorghum bicolor* and *Zea mays* leaves under different water conditions. ade adaxial epidermis, abe abaxial epidermis, m mesophyll, vb vascular bundle, st stomata, sc substomatal cavity. Bars = 50 µm

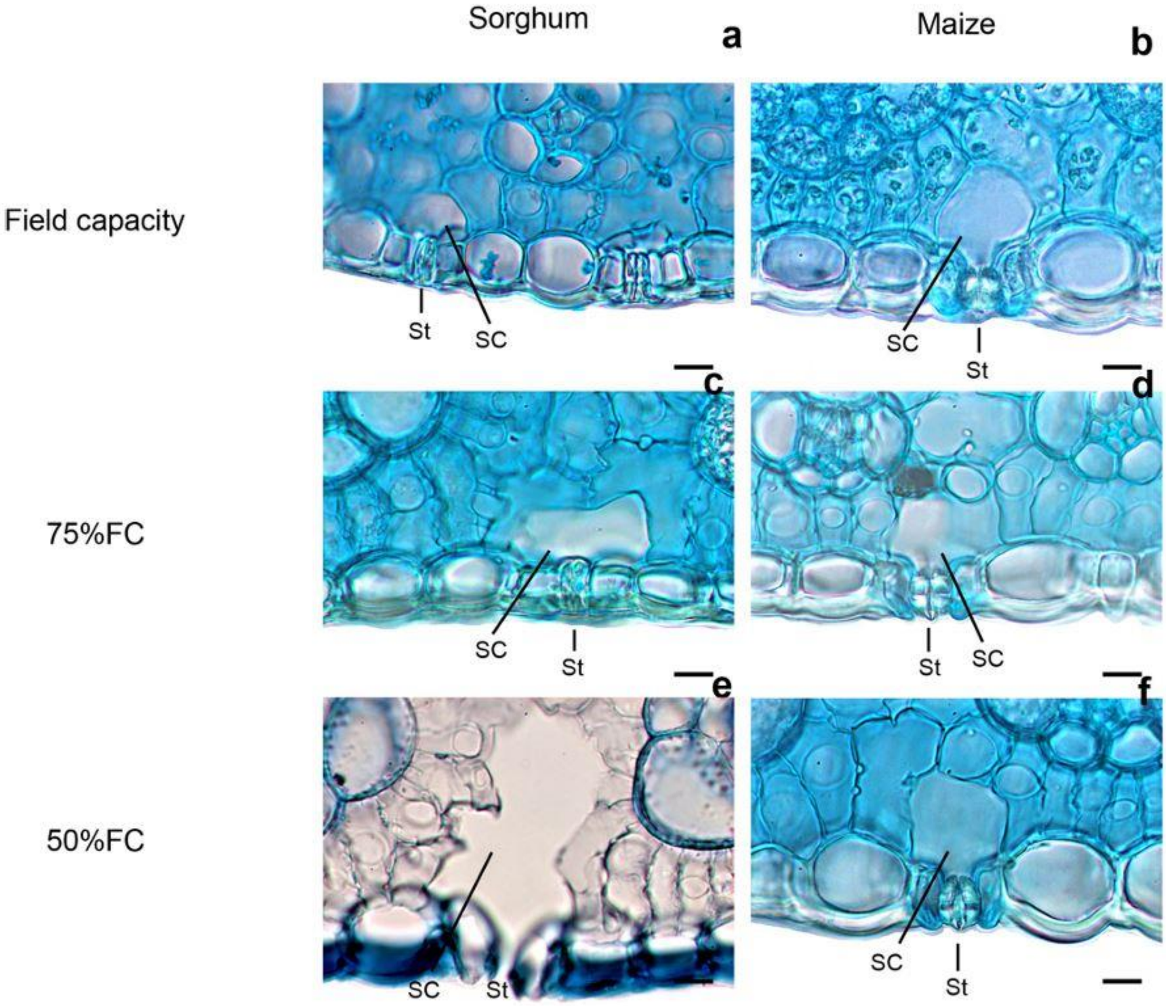

Fig. 6 Substomatal cavities of *Sorghum bicolor* and *Zea mays* leaves under different water conditions. St stomata, SC substomatal cavity. Bars = 10 µm

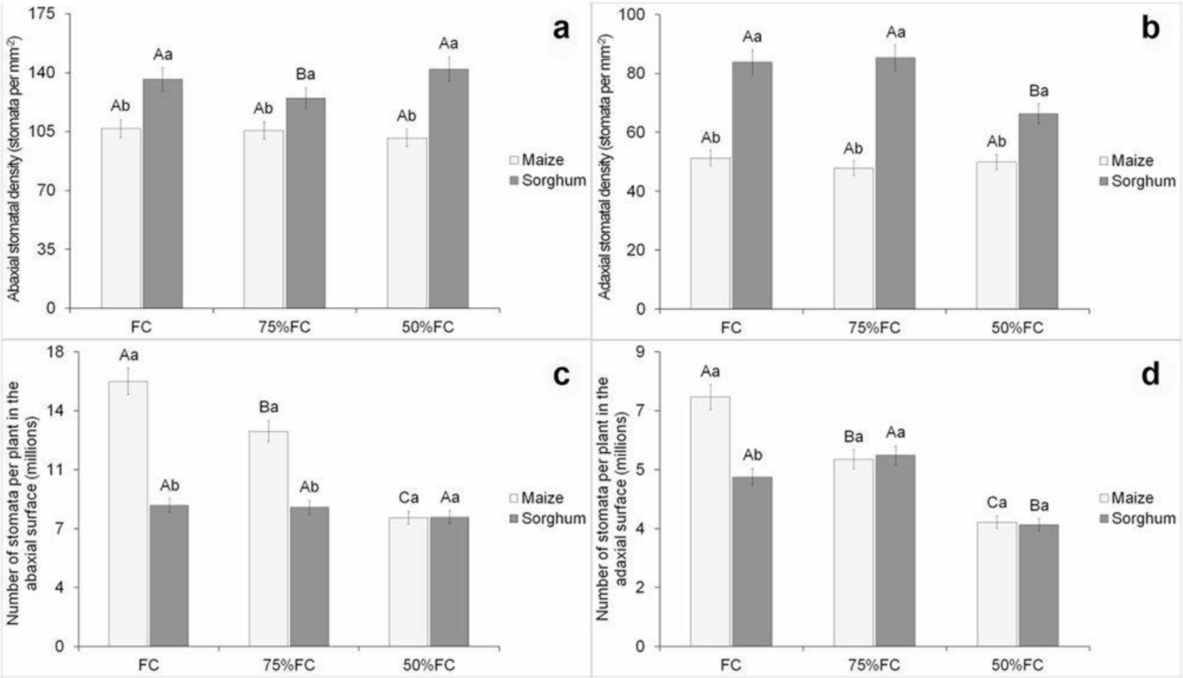

Fig. 7 Stomatal characteristics of *Sorghum bicolor* and *Zea mays* under different water conditions. The lowercase letters compare plants, and uppercase letters compare water conditions. Means followed by the same letter are not significantly different according to the Scott–Knott test at $p < 0.05$. Bars = standard errors

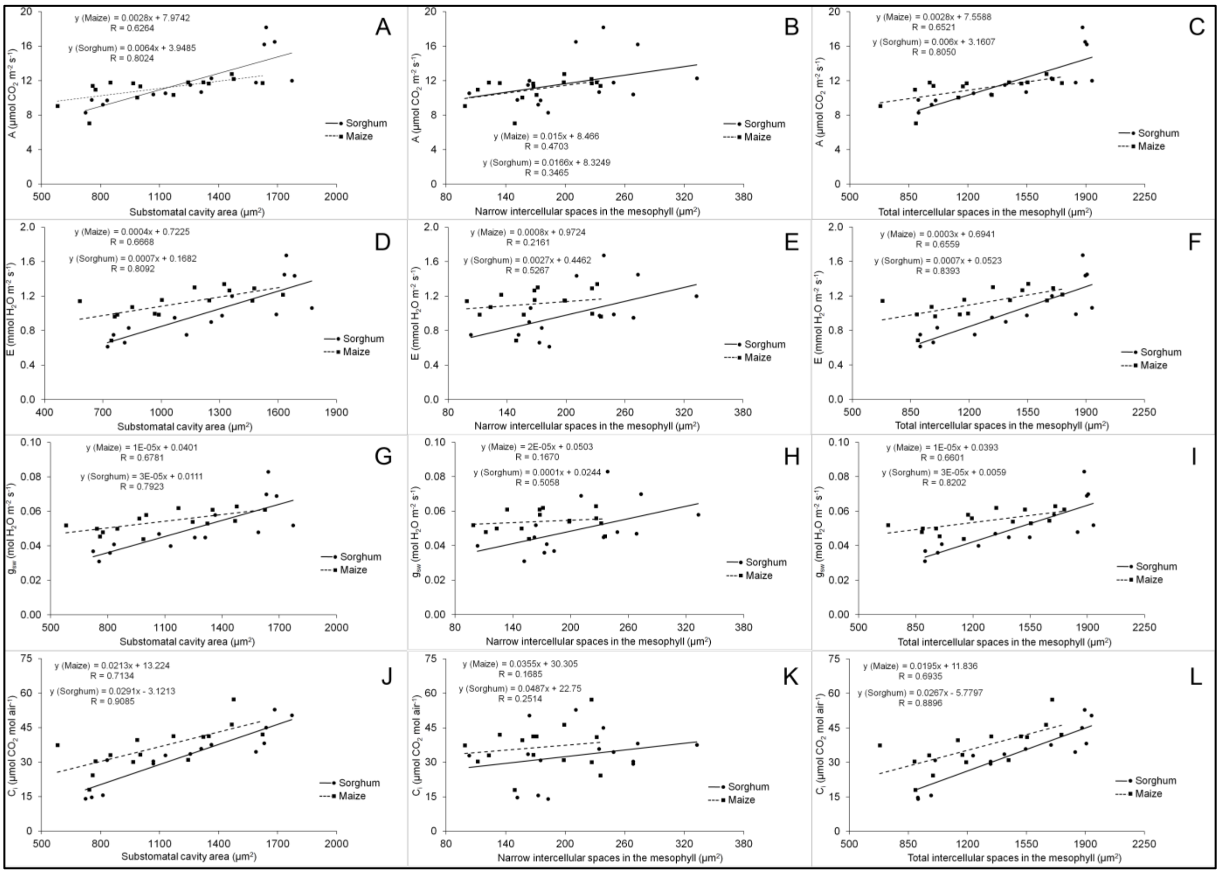

Fig. 8 Linear correlation analyses between gas exchange and leaf intercellular space parameters of *Sorghum bicolor* and *Zea mays*. *A* net photosynthesis, *E* transpiration rate, *gsw* stomatal conductance of water vapour.

Discussion

The treatments applied were sufficient to stimulate drought responses in maize and sorghum plants but did not cause mortality, as all plants survived. This is important because we aimed to analyse the relationship of intercellular spaces with leaf gas exchange under water deficit, and severe stress could cause physiological disorders that could affect proper stomatal functioning or the development of plant tissues and organs. In fact, drought-sensitive plants may exhibit severe physiological disturbances to the point of causing high mortality (Cruz et al. 2019). Preserving adequate leaf development, stomatal functioning, and gas exchange was an essential issue because serious problems could distort the interpretation of the results. The results obtained show that the applied 50%FC treatment was sufficient to promote stress in maize, while sorghum showed great leaf plasticity that allowed tolerance (Fig. 1). Leaf area limitation was one of the main factors restricting water deficit in maize, and intercellular spaces (Fig. 1a), our results also indicate that substomatal cavities may be important to drought tolerance in sorghum (Figs. 4, 5, 6).

As the amount of water was reduced, the leaf area decreased in maize (Fig. 1a), so the photosynthetic area and growth were reduced. Sorghum maintained its leaf area (Fig. 1a), which allowed it to grow normally under drought. The reduction in leaf area may be related to changes in cell production or cell expansion, which are limited under drought. In fact, water deficit causes a reduction in leaf area, a limitation that has been seen in different plant species (Cruz et al. 2019; Talbi et al. 2020) and may be correlated with disturbances in cell division or loss of turgor (Talbi et al. 2020). Drought-sensitive plants respond by reducing the leaf area because this reduces the total number of stomata in the plant, allowing better control of water loss and, due to the smaller number of cells, a decrease in water consumption, which improves water-use efficiency. This explanation is corroborated by Mantoan et al. (2020) and Scoffoni et al. (2017). However, a smaller leaf area can lower photosynthesis, affecting plant biomass and development (Li et al. 2020; Oliveira et al. 2018). Therefore, the maintenance of the leaf area maintained the photosynthetic area and growth capacity in sorghum as a result of the leaf morphophysiological adjustments that maize was unable to perform under 50%FC.

Maize had a higher leaf area than sorghum under well irrigated conditions, so the maize plants had greater bio mass gain and growth thanks to the higher photosynthetic rate under these conditions. In fact, a larger leaf area allows increased photosynthesis, which is reflected in the biomass (Cruz et al. 2019). This increased growth capacity of maize was limited by its smaller leaf area, hindering its photosynthesis and growth and keeping these parameters close to those shown by sorghum, which maintained its leaf area. The reduction in leaf area is a response of drought-sensitive plants (Leuschner 2020; Talbi et al. 2020). Because maize reduced its leaf area and sorghum did not, both species had a similar leaf area under 50%FC (Fig. 1a). The ability of sorghum to maintain its leaf area under 50%FC allowed these plants to maintain their photosynthetic rate and even have a higher photosynthetic rate than maize under 50%FC. We must emphasize that the maintenance of the leaf area was only possible due to other morphophysiological changes, which allowed adequate photosynthetic rates and were related to the leaf intercellular spaces.

Regarding gas exchange, sorghum increased its photosynthetic rate under 50%FC, while maize did not adjust its rate (Fig. 2). The increase in photosynthesis in sorghum may be related to the increased entry of CO_2 by the leaves, which in turn is related to the increase in intercellular spaces under 50%FC, particularly by the increase in the substomatal cavity, which favoured higher uptake of CO_2 in the mesophyll. According to Terashima et al. (2006), CO_2 diffuses from the atmosphere to the leaves through the stomata, and CO_2 diffuses in the mesophyll through the intercellular spaces. This is important because the intercellular spaces in the sorghum leaves grew in total size and in proportion (Fig. 4), while in maize the same adjustment was not observed. The larger proportion of the intercellular spaces may have favoured the increase in the intercellular carbon concentration in the sorghum leaves. In addition, the increase in stomatal conductance of sorghum under drought may have also favoured the increase in intercellular carbon, which may also have been favoured by the larger intercellular spaces, corroborating the positive correlation between the intercellular spaces in the mesophyll and intercellular carbon and photosynthesis (Fig. 8).

Maize had a higher photosynthetic rate than sorghum under well-irrigated conditions (Figs. 2, 3), but this situation was reversed under 50%FC, demonstrating that the specific changes in the sorghum leaves allowed this species to resist drought stress. Maize decreased photosynthesis as a function of the reduction in stomatal conductance under drought. Stomatal conductance is influenced by water deficit (Wu et al. 2021), and drought-tolerant species generally have higher stomatal conductance (Orek et al. 2020). The specific modifications in sorghum were related to its expansion of intercellular spaces, as the same did not occur in maize. This indicates that larger intercellular spaces in sorghum allowed it to absorb more CO_2 destined for the mesophyll. In addition, sorghum undergoes C_4 metabolism and reaches the CO_2 saturation point rapidly (Lawson and Vialet-Chabrand 2019; Monson et al. 1999). This characteristic combined with the growth of intercellular spaces may have allowed sorghum leaves to absorb more CO_2 in a shorter time and may have also favoured better control of water loss during transpiration. Therefore, the increase in total intercellular space is an important mechanism of drought tolerance.

One result that supports the role of intercellular spaces in drought tolerance is the ability of sorghum to increase transpiration and stomatal conductance at 50%FC, as the literature shows that drought reduces transpiration and stomatal conductance (Qayyum et al. 2021; Talbi et al. 2020). Sorghum showed higher stomatal conductance, allowing greater uptake of CO_2, which favoured photosynthesis and better control of water loss. These results corroborate the results of Oliveira et al. (2022) that showed higher gas exchange parameters in sorghum under lower water availabilities. The reduction in water loss and improved CO_2 uptake can be considered responses to drought tolerance (Talbi et al. 2020). These responses in sorghum were only possible due to the increase in the intercellular spaces at 50%FC, which was attributed to a greater leaf volume, allowing an increase in CO_2 absorption, unlike maize, which did not show the same adjustment in the mesophyll.

Regarding the gas exchange adjustments at the whole plant level (Fig. 3), all of these parameters—photosynthesis, transpiration, and stomatal conductance to water vapour—decreased in maize under drought, whereas sorghum maintained their values. Reduced photosynthesis, transpiration, and stomatal conductance are responses of drought-sensitive plants (Ren et al. 2020). Sorghum maintained its photosynthetic rate in all treatments, but it was lower than maize's under well-irrigated conditions, and under 50%FC both species showed similar photosynthetic rates. This result is related to the larger leaf area in maize under well-irrigated conditions compared to sorghum, and this larger leaf area is due to genetic characteristics. Under 50%FC, there was a reduction in leaf area in maize and maintenance of leaf area in sorghum, which led both species to have the same photosynthetic rate. This reasoning can also be applied to stomatal conductance and transpiration. Therefore, sorghum showed greater control of water loss and better CO_2 absorption, which are related to the increase in intercellular spaces in the mesophyll.

The results described above show that sorghum increased the internal volume of the mesophyll, facilitating the diffusion of gases, which maintained its water-use efficiency under drought. The adjustments in the intercellular spaces conferred sorghum better control of gas exchange because drought can lead to alterations in gas exchange, modifying water-use efficiency (Zelitch and Waggoner 1962; Zhou et al. 2021). In this context, maize in the well-irrigated treatments showed better water-use efficiency, while this pattern was inverted under

50%FC, which can be attributed to the lack of adjustment of the intercellular spaces in maize. According to Huang et al. (2020), plants under drought should be able to make morphophysiological adjustments to allow the maintenance of water-use efficiency to maximize CO_2 assimilation and, at the same time, decrease water loss. Therefore, it is evident that the increase in intercellular spaces can be considered a key response by sorghum to maintain its water use efficiency.

Maize in all treatments showed higher water-use efficiency than sorghum (Fig. 3). This was because maize reduced stomatal conductance, whereas sorghum maintained it (Figs. 2, 3). The reduction in stomatal conductance pro tects plants against water loss and improves their water-use efficiency (Correia et al. 2018; Elferjani and Soolanaya kanahally 2018). However, reducing stomatal conductance also slows photosynthesis, which hindered the growth of maize, unlike sorghum, which kept up its photosynthetic rate. The decrease in photosynthesis is caused by the reduction in stomatal conductance (Talbi et al. 2020). In fact, the higher water-use efficiency in maize seemed to be related to the greater sensitivity of these plants to drought and not to tolerance, since sorghum plants maintained all their variables, while the maize plants reduced most of the parameters evaluated in this study.

According to Talbi et al. (2020), leaf changes such as reductions in leaf area and stomatal conductance are strategies to prevent plants from drying out. In this context, leaf plasticity is an important response to drought, as clearly demonstrated by sorghum and its regulation of intercellular spaces, particularly by increasing the size of the substomatal cavity. The water diffusion rate is higher than the CO_2 diffusion rate (Jarman 1974). Thus, the direct contact of the stomata with the substomatal cavity allowed sorghum to regulate the relationship between the water efflux and CO_2 influx by the stomata and allowed a greater amount of gases, such as CO_2, to be absorbed, which is an essential characteristic of drought tolerance. This capacity of sorghum was showed and discussed in Oliveira et al. (2022), based on Fick's law, the diffusion of CO2 occurs from the atmosphere to the substomatal cavity, and upon entering the substomatal cavity, CO2 follows the flow to intercellular spaces. In this study we showed that an increase in the volume of the sub stomatal cavity may result in greater CO_2 uptake, favouring movement to the mesophyll and increasing the intercellular carbon concentration, which are all correlated (Fig. 8).

Drought can cause the chlorophyll parenchyma and leaf thickness to shrink, affecting the diffusion of gases in the intercellular spaces (Evans et al. 2009; Lobato et al. 2020). In this study, leaf thickness decreased in maize (Figs. 4, 5), which limited its adjustment of intercellular spaces. Sorghum maintained its leaf thickness at 50%FC, and sorghum leaves were thicker than maize leaves. Therefore, sorghum increased its internal leaf volume, contributing to the maintenance of leaf thickness and better control of gas diffusion, which reinforced its drought tolerance. This study shows that drought stimulates the formation of intercellular spaces in sorghum.

Another important aspect observed were the changes in stomatal characteristics. Maize maintained its stomatal density, while sorghum decreased it on the adaxial side at 50%FC (Fig. 7). Stomata respond to environmental changes, especially under water restriction (Buckley 2019). Even so, sorghum showed a higher stomatal density than maize in all treatments. A higher number of stomata is a response of drought-tolerant plants (Impa et al. 2019). This is important because a higher number of stomata allows sorghum to reduce their opening time, while the gases that diffuse through the stomata can fill the largest internal leaf volume, increasing the concentration of intercellular carbon. According to Franks and Farquhar (2007), an increase in stomatal density increases stomatal conductance, allowing greater entry of gases. For maize to fill the same amount of gas volume as sorghum, it has to leave the stomata open for a longer time, but this increases transpiration, so the increase in intercellular spaces together with the higher number of stomata in sorghum is an essential characteristic that allowed greater absorption of gases in less time, reducing water loss.

The calculation of the total number of stomata is important in the interpretation of stomatal adjustments under drought because this calculation involves the leaf area of the plant; therefore, the reduction in leaf area can influence this parameter. In this study, the total number of stomata decreased on both sides, abaxial and adaxial, in maize, while sorghum showed small changes on the adaxial side under 50%FC. Nevertheless, this modification is compensated for in sorghum leaves because, as a C_4 species, sorghum is adapted to dry climates and already has many stomata and an adequate stomatal distribution (Moroke et al. 2011). In addition, when expanding the calculation to the whole plant, maize had more total stomata than sorghum under the well-irrigated treatments because it has a larger leaf area. However, drought reduces the leaf area of maize, decreasing the total number of stomata and slowing photosynthesis, so both maize and sorghum ended up with similar numbers of stomata.

Therefore, intercellular spaces act directly on drought tolerance in sorghum plants due to the greater gas diffusion capacity, which can increase the CO_2 concentration in the mesophyll, resulting in growth. In fact, the cavity and total spaces were significant in both plants. The small intercellular spaces showed no significant correlation with other parameters, which indicates that the substomatal cavities are most relevant in the total intercellular space in these plants. In addition, intercellular carbon has a high correlation with the substomatal cavity, which corroborates the data presented in this study and indicates that the intercellular car bon concentration is directly proportional to the size of the substomatal cavity (Fig. 8). This mechanism demonstrates that drought-tolerant plants necessarily modulate the substomatal cavity first rather than the small intercellular spaces in the mesophyll.

Therefore, drought was harmful to maize because it reduced most of the evaluated parameters, and this did not allow maize to adjust its intercellular spaces within the leaf, while sorghum adjusted its leaf dynamics, as observed by the lack of change in the output variables. Therefore, this study shows that sorghum, a drought-tolerant species, expands the intercellular spaces of its mesophyll to allow greater CO_2 diffusion, which together with its larger number of stomata plays an important role in regulating water loss by transpiration and maintaining growth.

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ARTIGO 2 ANATOMICAL VARIATION ALONG THE LEAF AXIS MODULATES PHOTOSYNTHETIC RESPONSES OF SORGHUM AND MAIZE UNDER DIFFERENT WATER AVAILABILITIES

Artigo Submetido no periódico Plant Biology

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Abstract

- Water limitation causes several alterations in plants which may develop tolerance responses. Maize and sorghum are both C4 crops which may contrast in drought tolerance, although several aspects of leaf anatomy and physiology remain unclear. This work aimed to investigate the effect of drought on anatomical and photosynthetic traits along the leaf axis in maize and sorghum under drought.
- Experiments were conducted in the greenhouse where maize and sorghum were exposed to three irrigation conditions (field capacity (FC), 75%FC, and 50%FC and three leaf regions were accessed for photosynthetic and anatomical parameters (leaf base, middle, and tip parts).
- Water limitation promoted reductions in maize plants in terms of water use efficiency, leaf thickness, xylem vessel diameter, and the area of the bundle sheath; however, sorghum increased these variables under the same conditions. The middle region of the leaf showed higher parameters in comparison to other leaf parts for most parameters, although sorghum plants showed increased ΦPSII, Fv/Fm, and ETR at the leaf base. Photochemical parameters increased in both species under water limitation. Maize showed increased stomatal density along the leaf axis compared to sorghum which caused higher transpiration rates.
- Thus, anatomical and photosynthetic traits vary along the leaf axis and are more reduced in maize than sorghum under drought; the middle region of the leaf is more responsive to these changes in both species.

Keywords: Mesophyll; Water relations; Leaf parts; Chlorophyll fluorescence; Gas exchange

Introduction

Climate changes have promoted more frequent water limitation events, reducing plant productivity (McDowell et al. 2022). Water limitation has severe impacts on crop production, with agricultural losses that can reach US$30 billion (Gupta et al. 2020). Cop production during drought events has become a global concern (Kim et al. 2019; Zafar et al. 2023).

Drought promotes water stress to plants which causes several modifications in anatomical and physiological traits in leaves. According to Munné-Bosch and Villadangos (2023), water limitation reduces vegetative growth, chlorophyll fluorescence parameters, content of photosynthetic parameters, and gas exchange due to stomatal limitation. Thus, water limitation is a severe menace to crop production and food safety (Rehman et al. 2023) leading to the need to find tolerant crops and understand tolerance mechanisms in plants.

Zea mays L. is a high water-demanding crop being considered a drought-sensitive species (de Oliveira et al. 2023; Shahzad et al. 2023); nonetheless, *Sorghum bicolor* (L.) Moench. is regarded as a drought-tolerant crop and more efficient among C4 plants (Impa et al. 2019; de Oliveira et al. 2023). Sorghum is often used as a model for the study of plants under water limitation (Yang et al. 2020). According to de Oliveira et al. 2023, water limitation reduces the leaf area, leaf thickness, dry mass, and gas exchange in both maize and sorghum, but these changes were more intense in maize and sorghum seems to show a higher proportion of stomatal cavity which helps in the control of water loss.

Even under water limitation, maize plants show higher leaf area compared with sorghum and this is one of the main parameters limited under such conditions (Oliveira et al. 2023). Plants show growth spatial variation that is related to drought tolerance because of a correlation between the axis morphology and its functional traits (Ford et al. 2008; Normand et al. 2009). Maize and sorghum are both C4 species with long and lanceolate leaves and some degree of variation may be expected in anatomical and physiological traits; nonetheless, these parameters often are overlooked in drought studies with crops.

Maize and sorghum share several traits like the leaf morphology and Kranz anatomy (de Oliveira et al. 2023), and C4 metabolism (Weber and von Caemmerer 2010) but have differences in size and drought tolerance (de Oliveira et al. 2023). Both species also originated from dry and hot environments, with high radiation intensity (Sage 2004). It is important to note that C4 species with Kranz anatomy show a spatial separation in the photosynthetic tissues with the phosphoenolpyruvate carboxylase (PEPCase) in mesophyll cells (chlorophyll parenchyma) that produces a C4 acid which is then transported to bundle sheath cells which contain the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and where the Calvin-Benson cycle is found (Sage 2004; Weber and von Caemmerer 2010). Water stress promotes biochemical and anatomical limitations reducing photosynthesis (de Oliveira et al. 2023; Zahra et al. 2023). The anatomical variation along the leaf axis of maize and sorghum is little understood and may influence photosynthesis.

The middle part of the leaf is often used in most anatomical and physiological studies for being considered as more functional (Taratima et al. 2020; Della Torre et al. 2021); however, it is difficult to find works that evaluated anatomy, gas exchange, or chlorophyll fluorescence at different leaf parts in a given experiment. It is known that some leaf anatomy parameters change along the leaf axis, such as the shape and amount of vascular tissues (Gavilanes et al. 2016). In addition, anatomical traits are variable in leaves like stomatal density (Hsie et al. 2015; Mansoor et al. 2019), stomatal index (Silva et al. 2009; Della Torre et al. 2021), and mesophyll thickness (Wyka et al. 2019; Della Torre et al. 2021; de Oliveira et al. 2022). Thus, it is expected that these and other anatomical and physiological traits vary along the leaf axis, despite not being evaluated in most works with maize and sorghum under drought. Because the photosynthesis is spatially separated between the mesophyll and bundle sheath cells in C4 plants (Weber and von Caemmerer 2010), the anatomical evaluation of photosynthetic tissues must include the bundle sheath in some competence; nonetheless, these cells are mostly neglected in leaf anatomical analysis of maize and sorghum under drought.

The hypotheses of this work are: 1) there are anatomical variations in the photosynthetic tissues and in stomatal traits along the leaf axis of maize and sorghum which may be related to gas exchange and photochemical responses. 2) Water limitation promotes different modifications at specific leaf parts which may help to improve drought tolerance in maize and sorghum. Thus, this work aimed to evaluate the drought effects in the anatomy, gas exchange, and photochemical responses at the base, apex, and middle regions of maize and sorghum.

Material and methods

Plant material and experimental design

The experiment was carried out in a greenhouse located at the Universidade Federal de Lavras (UFLA), state of Minas Gerais, Brazil (21º13'17"S and 44º57'47" W). *Sorghum bicolor* (L.) Moench and *Zea mays* L. plants were obtained from seeds provided by the Embrapa’s National Research Center for maize and sorghum, state of Minas Gerais, Sete Lagoas, Brazil.

Seeds were sown in 5.0 L plastic pots containing 2.0 L of sand and 800 ml of nutrient solution (Hoagland and Arnon 1950) at 40% of its ionic force. Pots were then placed in a germination chamber under constant light at 25ºC for seven days, after which the seedlings had three leaves and were 10 cm tall. Further, seedlings displaying good phytosanitary condition and of similar size were individually transplanted into 5.0 L plastic pots containing 3.0 L of sand. These pots were kept in a greenhouse at 25±2°C, under 50% relative humidity, and under a 12 h photoperiod with an average of 730 µmol m^{-2} s^{-1}. Experiments using maize or sorghum were conducted side by side in the greenhouse, exposing the plants to the same conditions.

The plants were then subjected to three water conditions according to the method used by Díaz et al. (2018). The water conditions used in the experiments were (1) field capacity (FC), (2) 75% field capacity (75% FC), and (3) 50% field capacity (50% FC). Field capacity was considered as the maximum volume of water retained by 1.0 L of sand without becoming waterlogged. The volume of water applied to achieve FC was 310.0 ml water L^{-1} sand, and for 75% FC and 50% FC, the amounts of water in the substrate were maintained at 232.5 and 155.0 ml water L^{-1} sand respectively. The first irrigation was performed with nutrient solution (Hoagland and Arnon 1950) at 40% of its ionic force and then, the water lost by evapotranspiration was monitored by the daily difference in the mass of each pot. Water was replaced daily and the nutrient solution was weekly. The plants remained in these conditions for 60 days. The experimental design was completely randomized, with three treatments and ten replicates (*n*= 30) each replicate comprises one plant per pot. All the data obtained from analyses where multiple assessments were performed (for instance, two leaves per plant in gas exchange analyses), was then averaged for each replicate (maintaining *n* = 30 and avoiding artificial replication).

Gas exchange and chlorophyll content analyses

The gas exchange was assessed at three leaf regions (apex, middle, and base) with an infrared gas analyzer model LI-6400XT (LI-COR Biosciences, Lincoln, USA) coupled to an LI-6400-02B cuvette with red/blue LED light source (LI-COR, Lincoln, USA).

Measurements were taken from two fully developed leaves per plant/replicate between 08:00 and 10:00 a.m., with the photon flux density fixed at 1000 µmol m^{-2} s^{-1}, and a mean temperature of 28.1ºC; CO_2 was obtained from the atmosphere and, its mean concentration was 420.1 µmol mol^{-1} air, and the pump flow kept at 500 µmol s^{-1}. The net photosynthesis (A) and transpiration rate (E) were assessed. The chlorophyll content was assessed using a chlorophyll meter SPAD-502 (Konica-Minolta, Japan) in the leaf base, median and apex regions. At the end of the experiment, the instantaneous water-use efficiency (WUE) was calculated as the ratio of the net photosynthesis (A) to the transpiration rate (E) (A/E).

Chlorophyll fluorescence analysis

The chlorophyll fluorescence parameters were assessed with the portable modulated fluorometer MINI-PAM (Heinz Walz GmbH, Effeltrich, Germany). The maximum photochemical yield of the PSII (Fv/Fm), the electron transport rate (ETR), and the effective photochemical yield of the PSII (ΦPSII) were assessed. Dark measurements were performed using aluminum clips (DLC-8) coupled to the optic fiber cable of the MINI-PAM at three leaf regions (apex, middle, and base) of two fully expanded leaves per plant/replicate. Dark evaluations are taken after at least 30 min of acclimation. Calculations for ΦPSII, Fv/Fm, and ETR were performed according to the Mini-Pam II user's manual (Heinz Walz GmbH, 2002). Fv/Fm was calculated as follows: Fv/Fm = [(Fm)max – (F_0)max]/(Fm)max where (Fm)max is the maximum possible Fm value and Fm is the maximum fluorescence level elicited by a pulse of saturating light (Saturation Pulse) which closes all photosystem II reaction centers, (F_0)max is the maximum F_0 which is the minimum fluorescence level excited by very low intensity of measuring light to keep photosystem II reaction centers open. ΦPSII was calculated as follows: ΦPSII = (Fm' – F)/Fm' where Fm' is the maximum fluorescence of an illuminated sample, and F is the momentary fluorescence level of an illuminated sample shortly before application of a saturation pulse. ETR is calculated as follows: ETR(II) = PAR x ETR-Factor x (PPS2/PPS1+2) x ΦPSII, where PAR is the quantum flux density of photosynthetically active radiation (PAR) impinging on the sample, ETR-Factor is the sample absorptance (= 1 – transmittance), and PPS2/PPS1+2 is the relative distribution of absorbed PAR to photo-system II.

Anatomical analysis

Two fully developed leaves per plant/replicate were removed and fixed in 70% ethanol (Johansen 1940). Sampled leaves were the same ones where we evaluated gas exchange, photochemical, and chlorophyll parameters. Transversal sections were performed using steel blades at the apex, middle, and base leaf regions. The sections were clarified with 50% sodium hypochlorite and washed twice in distilled water for 10 min. Further, the sections were stained with a safrablau solution (1% safranine and 0.1% astra blue at a proportion of 1:7) and then mounted on slides with 50% glycerol (Johansen 1940). Paradermal imprints (Segatto et al. 2004) were taken from both abaxial and adaxial leaf sides at the apex, middle, and leaf base. These imprints were obtained by covering the surface of the leaf with a thin layer of a cyanoacrylate resin which was removed after polymerization and then mounted on slides with coverslips. The paradermal imprints were taken in the morning from 6-8 a.m. when the stomata were expected to be open. The slides were observed and images were taken under a CX31 light microscope (Olympus, Tokyo, Japan). One slide per leaf was made and, three sections and four fields were analyzed for each slide, and the data were averaged for each replicate. The images were analyzed with ImageJ software version 1.45s (Wayne Rasband National Institutes of Health, USA).

The following anatomical characteristics of the transversal sections were: leaf thickness, proportion of mesophyll cells, bundle sheath, proportion of the vascular tissues, and xylem vessel diameter. For the paradermal sections, the following structures were analyzed: the adaxial and abaxial stomatal index and the abaxial and adaxial stomatal density (SD). The SD was calculated as follows: SD = number of stomata * (10^6 / section area).

Statistical analysis

The data were subjected to two-way ANOVA, and the means were compared by the Scott-Knott test as $p<0.05$ using SISVAR 5.0 software (Ferreira 2011). Before parametric analysis, the data were tested for normality using the Shapiro-Wilk test, and all the variables showed a normal distribution.

Results

The ANOVA summarized data for maize and sorghum are shown in Tables 1 and 2 respectively. Both maize and sorghum leaves increased their length during the entire experimental period (Fig. 1). Sorghum leaves showed similar elongation under all water conditions, but 75%FC surpassed other treatments at the end of the experiment (Fig. 1A). Nonetheless, water limitation under 50%FC significantly reduced the leaf length of maize plants after 40 days of the experiment (Fig. 1B).

There was a positive interaction for all the gas exchange and chlorophyll content parameters (Tables 1 and 2). Water limitation caused no modifications in the photosynthesis of leaf apex and middle regions of sorghum leaves but 75%FC reduced this parameter at the leaf base (Fig. 2A). The photosynthesis of sorghum is similar at different leaf parts under FC and 50%FC but is lower at the leaf base under 75%FC (Fig. 2A). Photosynthesis in maize was similar under FC and 50%FC; however, it was reduced under 75%FC at the leaf apex (Fig. 2B). Leaf middle and base parts show similar photosynthesis under FC and 50%FC, but it is lower at the leaf apex of maize plants under 75%FC (Fig. 2B).

Water limitation increased transpiration at the apex of sorghum leaves but promoted no effects at the middle part and 75%FC reduced this parameter at the base (Fig. 2C). Transpiration of sorghum leaves was lower at the leaf apex under FC, but was lower at the leaf base of 75%FC and showed similar means at all leaf parts under 50%FC (Fig. 2C). Drought reduced transpiration in maize leaves at the apex only at 75%FC; however, it was increased by 50%FC at the middle region and by 50 and 75%FC at the leaf base (Fig. 2D).

Drought reduced the water use efficiency at the apex of sorghum leaves but remained unaffected at the middle and base parts (Fig. 2E). The apex of sorghum leaves shows higher water use efficiency under FC, but no significant differences were found in water limitation (Fig. 2E). Drought reduced the water use efficiency of maize leaves at the apex under 75%FC only and base under 50%FC; however, 75%FC increased this parameter at the middle part (Fig. 2F). Under FC conditions the water use efficiency of maize leaves was higher at the base but 75%FC increased this parameter in both leaf base and middle parts and there were no significant differences for this parameter under 50%FC (Fig. 2F).

The chlorophyll content showed no interaction (Tables 1 and 2) and was not affected by the water limitation or leaf parts in both maize and sorghum plants (Fig. 2G and 2H). Photochemical parameters did not show a significant interaction between leaf parts and water availability (Tables 1 and 2). The water limitation increased the effective photochemical yield of the PSII (ΦPSII) in sorghum leaves but this parameter was lower at the apex (Fig. 3A). Drought promoted no significant effects in the ΦPSII of maize but this parameter was lower at the leaf apex (Fig. 3B). Water limitation increased the maximum photochemical yield of the PSII (Fv/Fm) of sorghum leaves but there is no significant variation between leaf parts (Fig. 3C). Drought did not promote significant modifications in Fv/Fm in maize leaves but the apex showed lower means compared with other parts (Fig. 3D). Drought increased the electron transport rate (ETR) in sorghum leaves but this parameter was lower at the leaf apex (Fig. 3E). The 50%FC increased the ETR in maize leaves and it was lower at the leaf apex (Fig. 3F).

For the anatomical traits from transversal sections, only the area of the bundle sheath cells and the xylem vessel diameter showed significant interaction (Tables 2 and 3). The 75%FC increased the leaf thickness of sorghum but it was thinner at the apex compared with other parts (Fig. 4A and Fig. 6). The 50%FC reduced the leaf thickness in maize and the middle part of the leaf is the thicker part of the leaf and the apex the thinner (Fig. 4B and Fig. 7). Water limitation promoted no significant changes in the proportion of mesophyll cells in sorghum leaves but its base shows lower proportion of the tissue compared with other regions (Fig. 4C and Fig. 6). The proportion of mesophyll cells was not significantly modified by water limitation or leaf parts in maize (Fig. 4D and Fig. 7).

The 75%FC increased the area of bundle sheath cells at the middle region of the leaf but had no effect in other parts (Fig. 4E and Fig. 6). The middle region of the sorghum leaf shows larger bundle sheath cells under all water treatments compared with other leaf parts (Fig. 4E and Fig. 6). Water limitation at the 50%FC reduced the size of the bundle sheath cells in maize leaves (Fig. 4F and Fig. 7). Maize leaves show larger bundle sheath cells at its middle region while the apex shows the smaller cells (Fig. 4F and Fig. 7). The proportion of vascular tissues in sorghum leaves was not significantly modified by the water availability or the leaf regions (Fig. 4G and Fig. 6). The water limitation promoted no significant effect on the proportion of vascular tissues in maize leaves but these tissues showed bigger proportion at leaf base (Fig. 4H and Fig. 7).

Drought increased the xylem vessel diameter at the leaf apex (50%FC) and middle regions (75%FC) but did not affect the leaf base in sorghum (Fig. 4I and Fig. 6). The middle region of sorghum leaves shows larger xylem vessels at FC and 75%FC treatments but under 50%FC the leaf apex showed larger vessels (Fig. 4I and Fig. 6). The 75%FC reduced the xylem vessel diameter in the middle region of maize leaves but had no effect in other leaf parts (Fig. 4J and Fig. 7). The vessel diameter was smaller at the apex of maize leaves but under FC conditions base region show greater vessels (Fig. 4J and Fig.7).

Data for stomatal traits showed interaction only for the adaxial and abaxial stomatal index from sorghum leaves and the abaxial stomatal index and density for maize (Tables 1 and 2). The water limitation reduced the adaxial stomatal index in sorghum leaves at all regions (Fig. 5A). The middle part of sorghum leaves shows a higher stomatal index except for plants under 50%FC which show lower means at the apex (Fig. 5A). The adaxial stomatal index showed no significant modification promoted by water treatments or leaf regions in maize (Fig. 5B). Water limitation reduced the abaxial stomatal index only at the middle region of sorghum leaves (Fig. 5C). The leaf parts show little variation for the abaxial stomatal index of sorghum but this parameter was smaller at the leaf base under the FC treatment (Fig. 5C). The 50%FC increased the abaxial stomatal index at all parts of maize leaves (Fig. 5D) and the leaf apex shows the highest means under all irrigation conditions (Fig. 5D).

Water limitation reduced the adaxial stomatal density of sorghum leaves and the leaf apex shows the lowest means for this parameter (Fig. 5E). The adaxial stomatal density was not affected by water limitation in maize leaves and the leaf base shows higher means for this parameter (Fig. 5F). Water limitation promoted no significant modifications in the abaxial stomatal density of sorghum leaves but variation of this parameter is found at different leaf parts with higher means at the base and the lowest at the apex (Fig. 5G). The 50%FC reduced the abaxial stomatal density in maize leaves at the apex and middle regions but increased this parameter at the leaf base (Fig. 5H). The leaf apex shows higher stomatal density in maize leaves under all water conditions, except for the leaf base under 75%FC which showed similar (Fig. 5H).

Table 1 – Summarized ANOVA results for all variables analyzed, including mean square values, *F* test results, and *P* values for the *Sorghum bicolor* experiment. CV% = coefficient of variation.

Variable	***CV %***	***Mean square values***	***F test value***	***P-value***
*Net photosynthesis (L)	18.7	31.5	8.4	0.0015
*Net photosynthesis (W)	18.7	16.5	4.4	0.0220
*Transpiration rate (L)	14.6	0.1	2.8	0.0764
*Transpiration rate (W)	14.6	0.4	8.1	0.0018
*Instantaneous water-use efficiency (L)	21.6	46.8	9.9	0.0006
*Instantaneous water-use efficiency (W)	21.6	2.1	0.4	0.6403
Chlorophyll content (L)	18.2	49.2	1.1	0.3496
Chlorophyll content (W)	18.2	5.8	0.1	0.8820
Actual photochemical efficiency of PSII (L)	30.2	0.02	3.6	0.0317
Actual photochemical efficiency of PSII (W)	30.2	0.04	7.1	0.0017
The maximum PSII quantum yield (L)	3.3	0.002	1.9	0.1538
The maximum PSII quantum yield (W)	3.3	0.02	12.4	<0.0001
Electron transport rate (L)	30.0	1031.5	3.0	0.0547
Electron transport rate (W)	30.0	2110.2	6.2	0.0034
Leaf thickness (L)	11.9	19985.9	38.9	<0.0001
Leaf thickness (W)	11.9	1471.0	2.8	0.0621
Proportion of mesophyll cells (L)	2.2	36.4	4.0	0.0216
Proportion of mesophyll cells (W)	2.2	27.9	3.0	0.0511
*Bundle sheath area (L)	24.6	25784100.5	46.3	<0.0001
*Bundle sheath area (W)	24.6	3499626.5	6.2	0.0022
Proportion of the vascular tissues (L)	33.4	2.2	0.2	0.7491
Proportion of the vascular tissues (W)	33.4	12.2	1.5	0.2104
*Xylem vessel diameter (L)	23.1	89.2	16.1	<0.0001
*Xylem vessel diameter (W)	23.1	14.4	2.6	0.0756
*Adaxial stomatal index (L)	11.9	156.1	21.7	<0.0001
*Adaxial stomatal index (W)	11.9	132.9	18.5	<0.0001
*Abaxial stomatal index (L)	9.3	52.8	5.8	0.0043
*Abaxial stomatal index (W)	9.3	22.5	2.4	0.0893
Adaxial stomatal density (L)	13.8	33754.7	111.5	<0.0001
Adaxial stomatal density (W)	13.8	2868.9	9.4	0.0002
Abaxial stomatal density (L)	13.0	8812.6	11.3	<0.0001
Abaxial stomatal density (W)	13.0	721.9	0.9	0.3979

P-value limit of the software is 0.0001, results lower than this limit are indicated as $p<0.0001$. (L) = Leaf regions (Leaf apex, leaf medium, and leaf base); (W) = Water conditions (Field capacity (FC), 75%FC and 50%FC); * = significant interaction at $p<0.05$.

Table 2 – Summarized ANOVA results for all variables analyzed, including mean square values, *F* test results, and *P* values for the *Zea mays* experiment. CV% = coefficient of variation.

Variable	***CV %***	***Mean square values***	***F test value***	***P-value***
*Net photosynthesis (L)	23.7	32.4	10.6	0.0004
*Net photosynthesis (W)	23.7	2.9	0.9	0.3934
*Transpiration rate (L)	13.5	0.1	0.9	0.3993
*Transpiration rate (W)	13.5	0.2	14.2	<0.0001
*Instantaneous water-use efficiency (L)	24.2	115.9	12.5	<0.0001
*Instantaneous water-use efficiency (W)	24.2	10.6	1.1	0.3348
Chlorophyll content (L)	19.7	35.2	1.6	0.2075
Chlorophyll content (W)	19.7	54.3	2.4	0.0906
Actual photochemical efficiency of PSII (L)	31.3	0.05	9.8	0.0002
Actual photochemical efficiency of PSII (W)	31.3	0.01	2.4	0.0951
The maximum PSII quantum yield (L)	2.7	0.004	5.4	0.0067
The maximum PSII quantum yield (W)	2.7	0.00001	0.01	0.9882
Electron transport rate (L)	30.1	2628.2	9.2	0.0003
Electron transport rate (W)	30.1	1171.3	4.1	0.0208
Leaf thickness (L)	12.4	12647.4	29.7	<0.0001
Leaf thickness (W)	12.4	2416.1	5.6	0.0048
Proportion of mesophyll cells (L)	2.2	4.2	0.4	0.6359
Proportion of mesophyll cells (W)	2.2	0.7	0.07	0.9270
Bundle sheath area (L)	35.7	19552146.2	27.3	<0.0001
Bundle sheath area (W)	35.7	2347019.2	3.2	0.0389
Proportion of the vascular tissues (L)	34.0	24.6	3.8	0.0255
Proportion of the vascular tissues (W)	34.0	7.9	1.2	0.2971
*Xylem vessel diameter (L)	22.2	244.4	38.6	<0.0001
*Xylem vessel diameter (W)	22.2	33.8	5.3	0.0052
Adaxial stomatal index (L)	11.6	6.2	2.1	0.1252
Adaxial stomatal index (W)	11.6	5.5	1.8	0.1578
*Abaxial stomatal index (L)	12.3	377.3	45.7	<0.0001
*Abaxial stomatal index (W)	12.3	221.2	26.8	<0.0001
Adaxial stomatal density (L)	12.2	556.6	4.9	0.0092
Adaxial stomatal density (W)	12.2	263.2	2.3	0.1019
*Abaxial stomatal density (L)	14.2	18804.2	37.3	<0.0001
*Abaxial stomatal density (W)	14.2	4191.7	8.3	0.0005

P-value limit of the software is 0.0001, results lower than this limit are indicated as $p<0.0001$. (L) = Leaf regions (Leaf apex, leaf medium, and leaf base); (W) = Water conditions (Field capacity (FC), 75%FC and 50%FC). * = significant interaction at $p<0.05$.

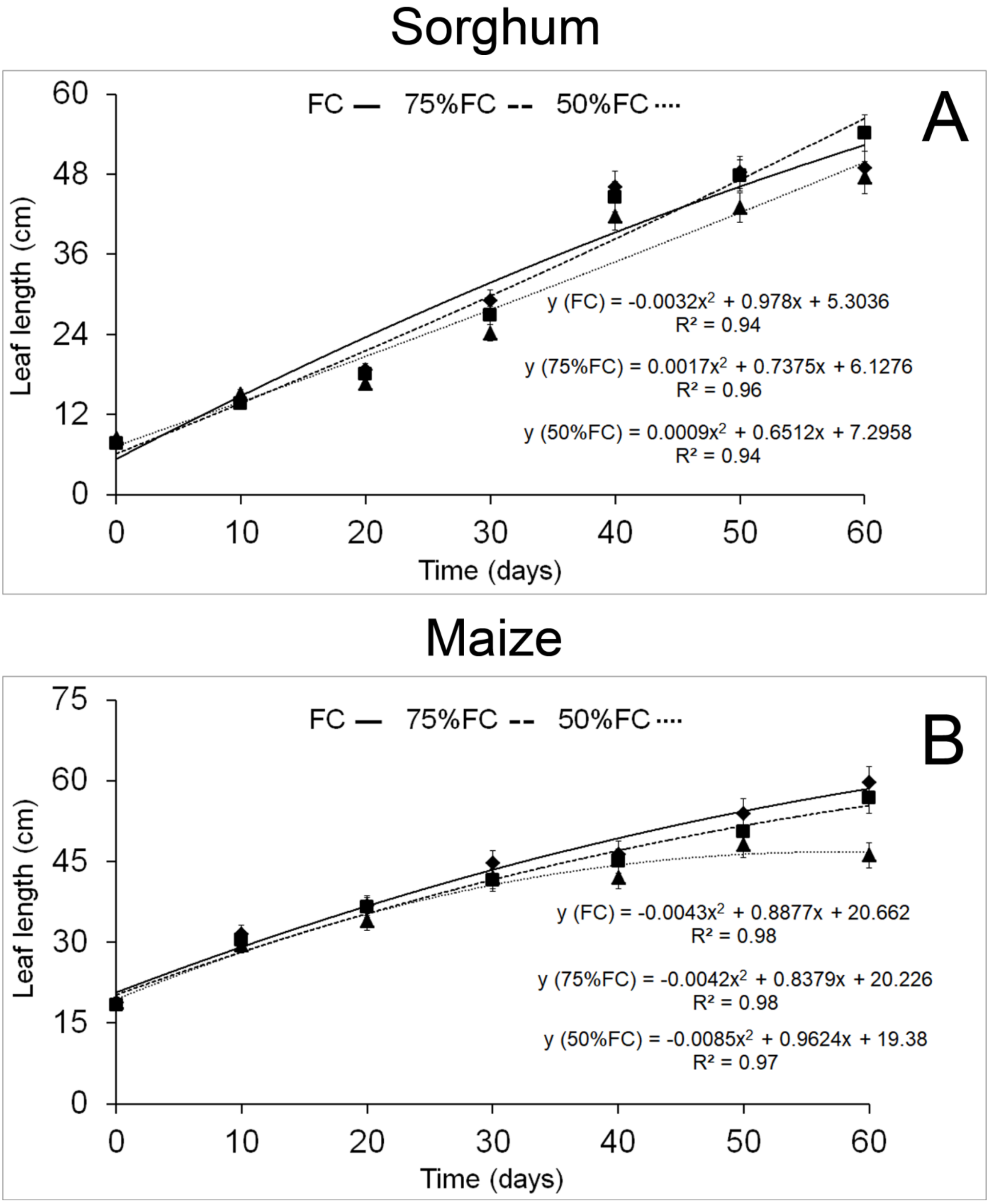

Figure 1 – Leaf length from *Zea mays* and *Sorghum bicolor* under field capacity (FC), 75% FC, and 50% FC. Bars = standard error (*n* = 30).

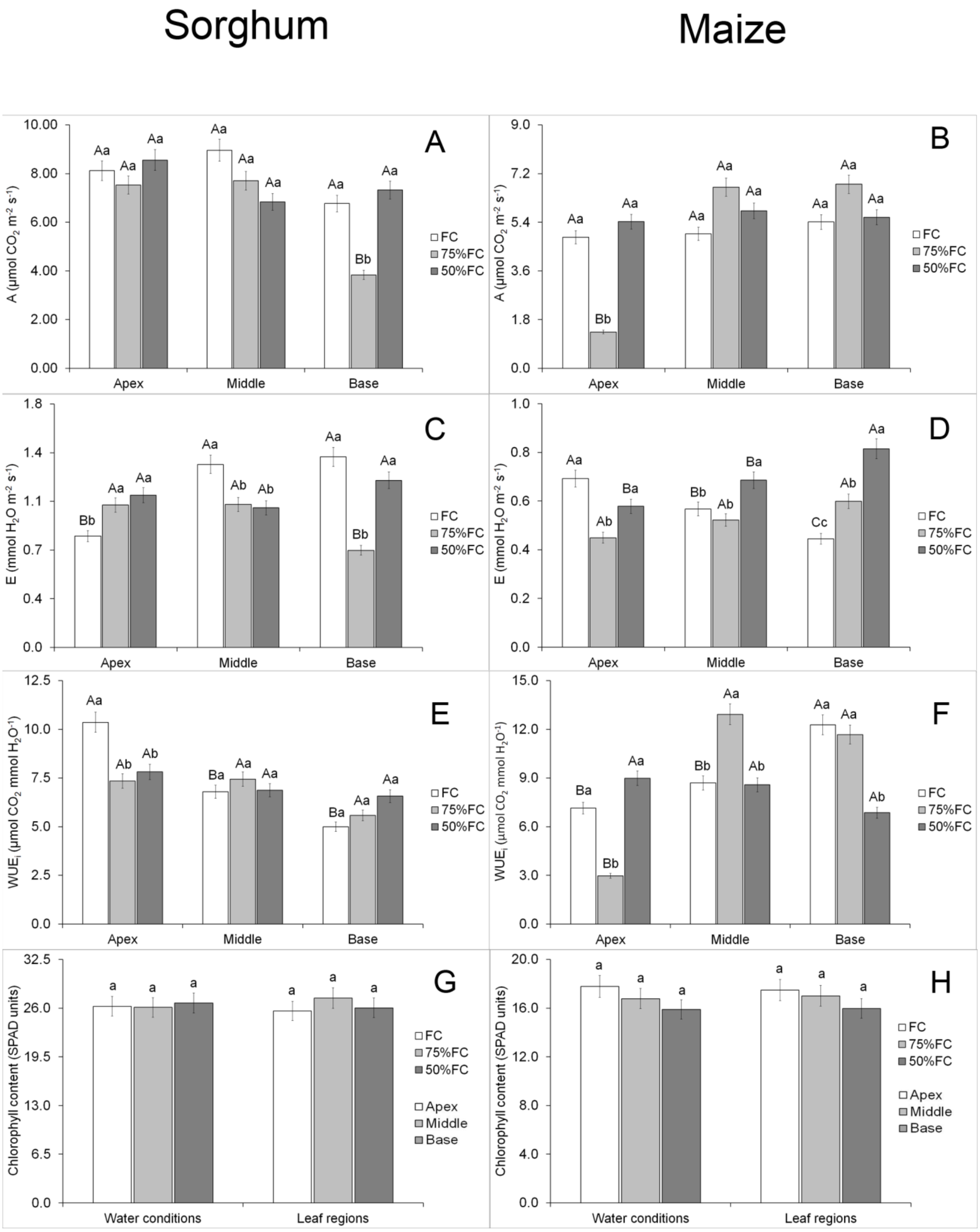

Figure 2 - Leaf gas exchange parameters and chlorophyll content of *Sorghum bicolor* and *Zea mays* under different water conditions. A = Net photosynthesis; E = Transpiration rate; WUEi = instantaneous water-use efficiency. The lowercase letters compare water conditions and uppercase ones compare leaf regions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors (n = 30).

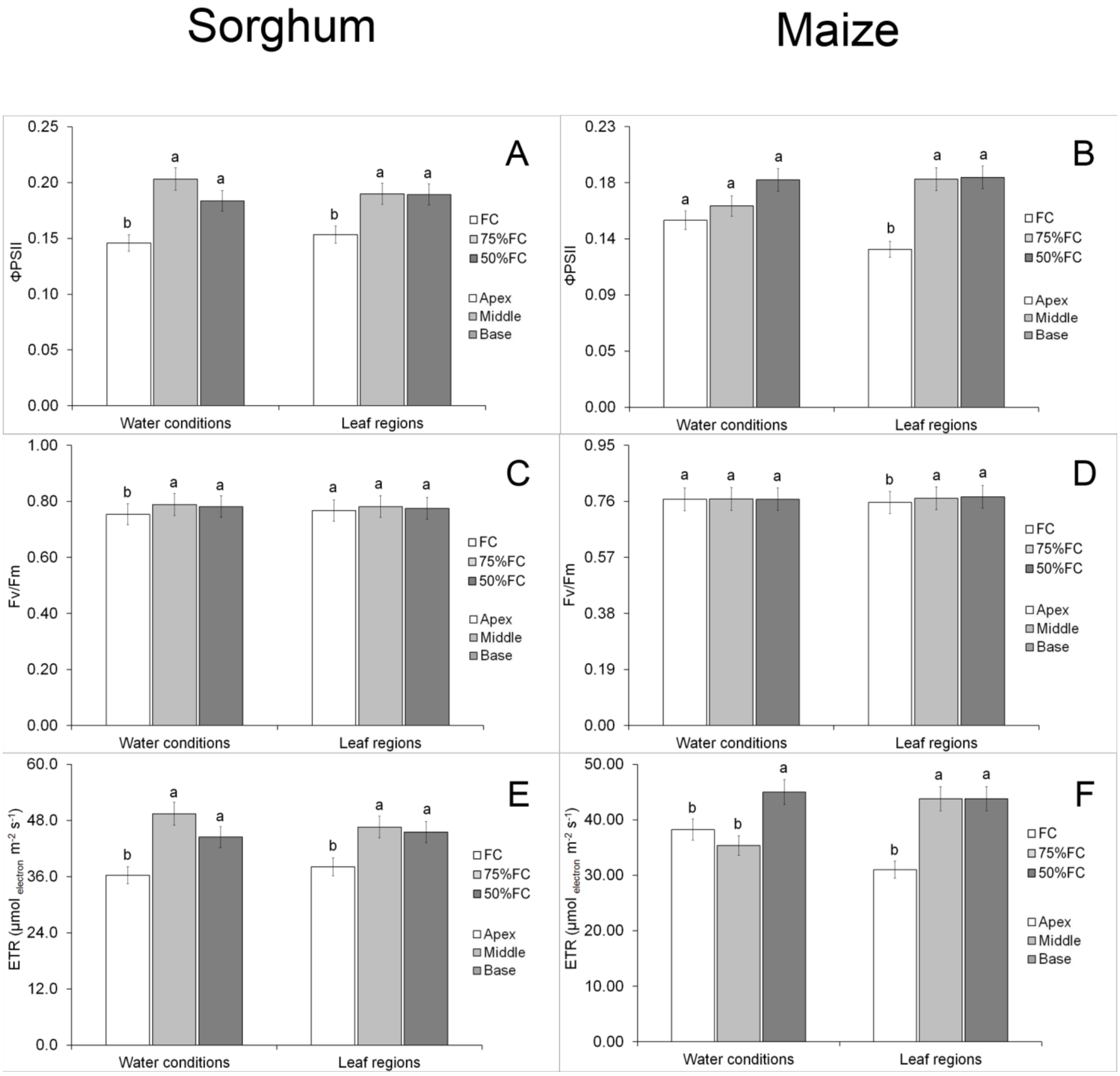

Figure 3 – Chlorophyll fluorescence parameters of *Sorghum bicolor* and *Zea mays* under different water conditions. ΦPSII = actual photochemical efficiency of PSII; Fv/Fm = the maximum PSII quantum yield; ETR = Electron transport rate. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors (n = 30).

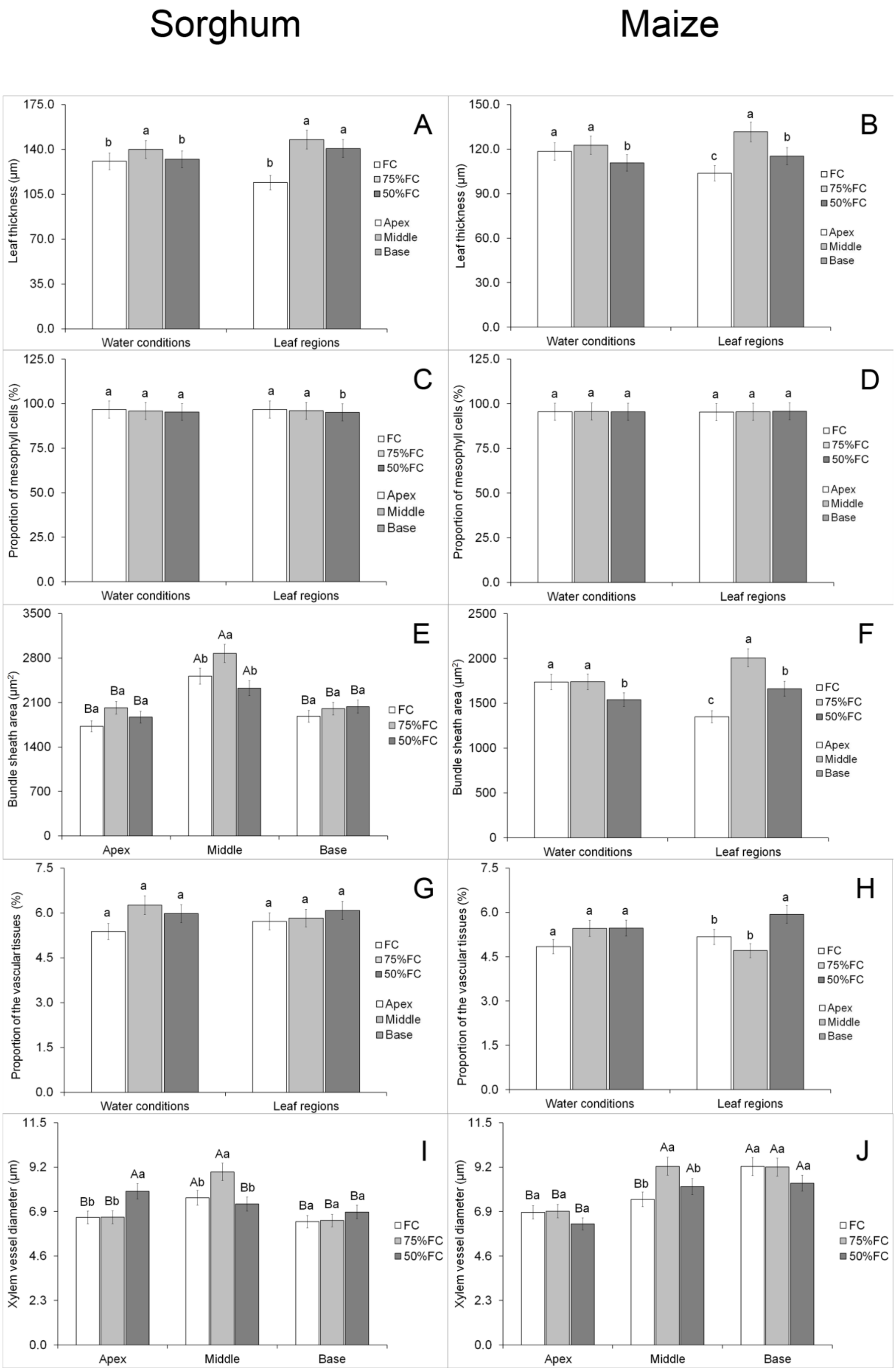

Figure 4 - Leaf anatomical characteristics of *Sorghum bicolor* and *Zea mays* under different water conditions. The lowercase letters compare water conditions and uppercase ones compare leaf regions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors (n = 30).

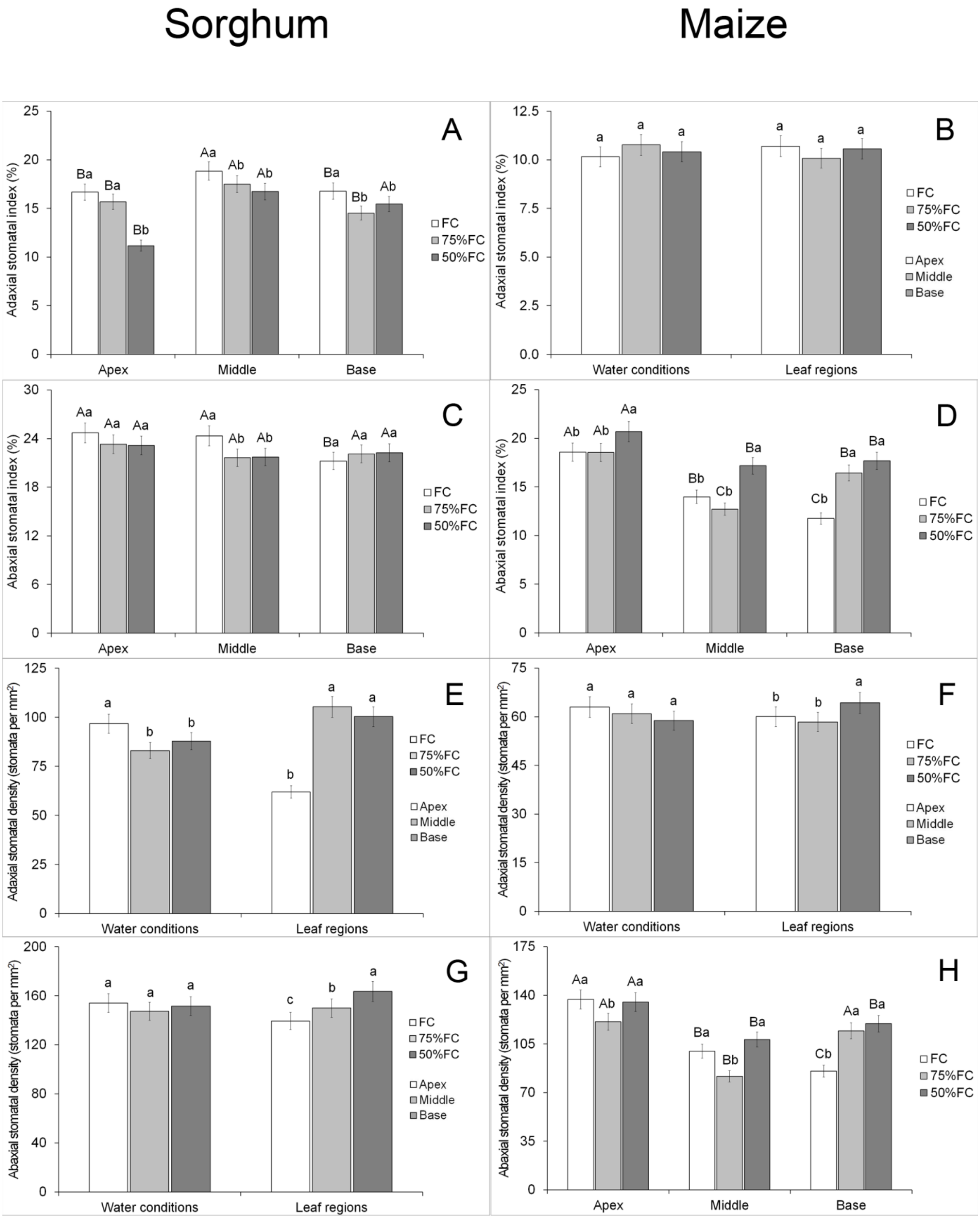

Figure 5 - Stomatal characteristics of *Sorghum bicolor* and *Zea mays* under different water conditions. The lowercase letters compare water conditions and uppercase ones compare leaf regions. The same letter shows no significant differences according to the Scott-Knott test at *$p<0.05$*. Bars= standard errors (*n* = 30).

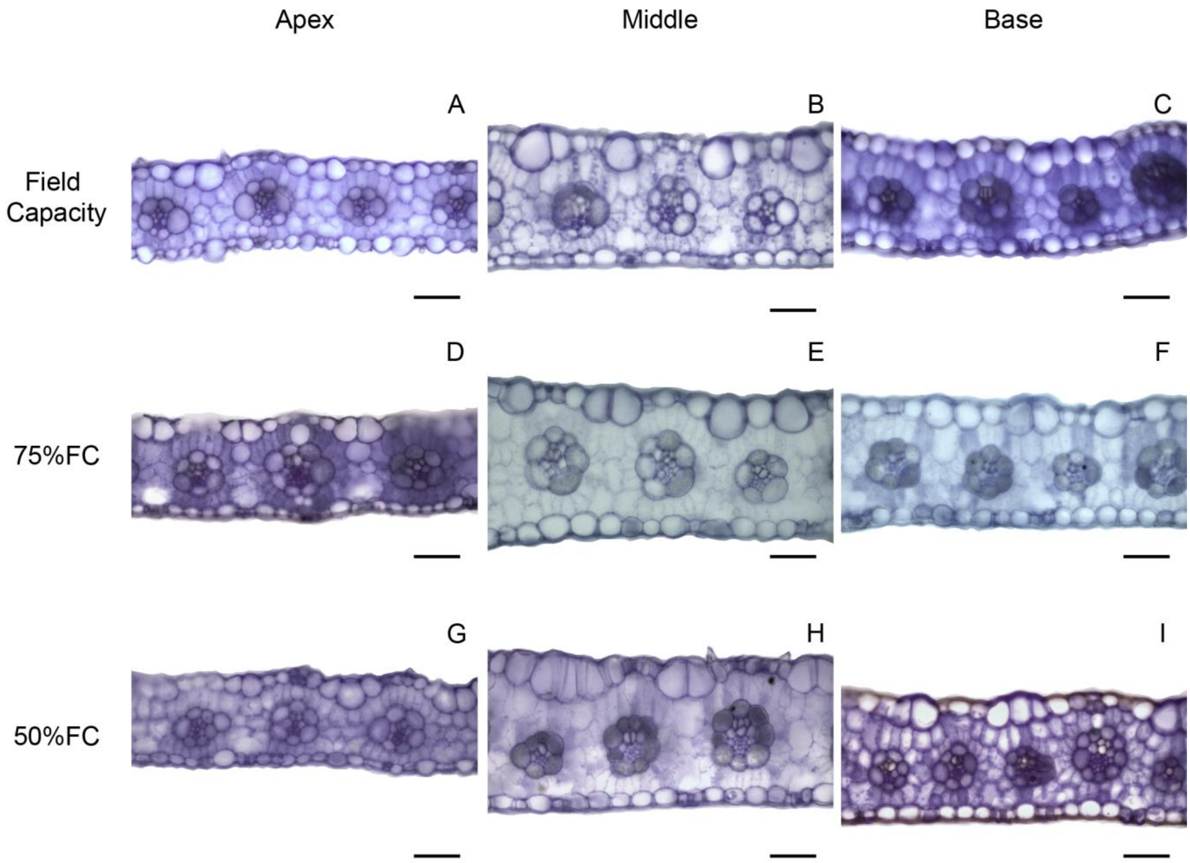

Figure 6. Transversal sections from *Sorghum bicolor* leaves at different water conditions. ade = adaxial epidermis, abe = abaxial epidermis, cp = chlorophyll parenchyma (mesophyll cells), st = stomata, phl = phloem, xl = xylem, bs = bundle sheath. Leaf parts: Apex = A, D, G, middle = B, E, H, Base = C, F, 1. Water treatments: Field capacity: A,B,C, 75%FC= D,E,F, 50%FC = G,H,I. Bars = 50 µm.

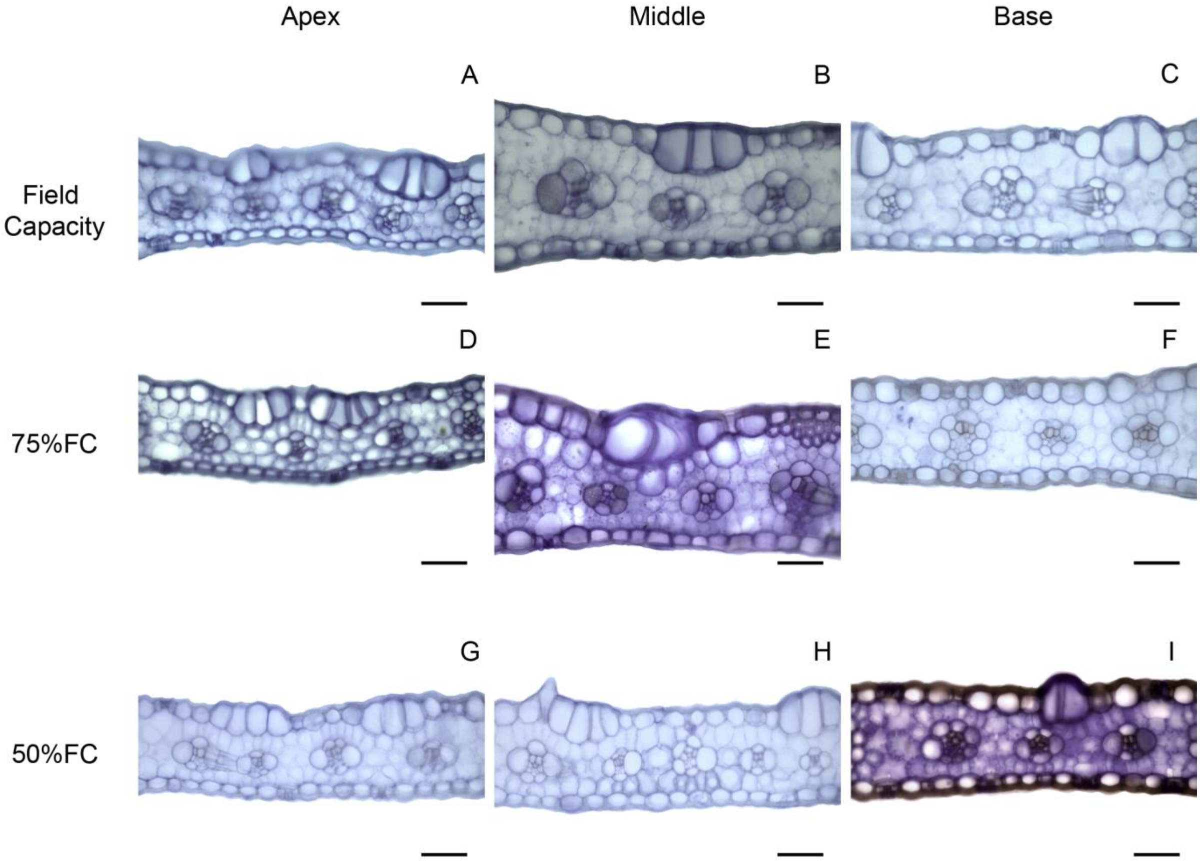

Figure 7. Transversal sections from *Zea mays* (Poaceae) leaves under different water conditions. ade = adaxial epidermis, abe = abaxial epidermis, cp = chlorophyll parenchyma (mesophyll cells), st = stomata, phl = phloem, xl = xylem, bs = bundle sheath. Leaf parts: Apex = A, D, G, middle = B, E, H, Base = C, F, 1. Water treatments: Field capacity: A,B,C, 75%FC= D,E,F, 50%FC = G,H,I. Bars = 50 µm.

Discussion

Water limitation promoted several anatomical and physiological responses in maize and sorghum leaves with some similar trends and some contrasting aspects. In this work, we used water limitation sufficient to promote physiological responses without being lethal to maize and sorghum (de Oliveira et al. 2023). This was important to keep plants alive and to register modifications found along the leaf axis within physiological conditions. Severe drought may cause serious issues in the physiology (Munné-Bosch and Villadangos 2023) causing plant mortality (McDowell et al. 2022).

Most of the variables suggest that the middle region of the leaf is the most responsive for both maize and sorghum. This part seems more functional as compared with the apex and base parts and shows higher mean values for some important tolerance traits under water limitation. The variation along the leaf axis is a key feature of abiotic responses in plants (Li et al. 2020). It is important to note that both maize and sorghum have lanceolate (long) leaves and this morphology creates the possibility of variation since the leaf apex is distant from its base. It is known that long maize leaves bend causing changes in the intercepted photosynthetic radiation (Ford et al. 2008). Since drought reduces the cell's turgor pressure in maize (Talbi et al. 2020) it is reasonable to state that these leaves suffer some degree of bending under water limitation affecting light interception photosynthesis and other anatomical and physiological parameters. According to Ford et al. (2008), the maize leaf can bend at its apex exposing the middle part to higher radiation intensities as compared with this part. The middle region of the leaf is the most used in leaf anatomical and physiological studies with maize and sorghum (Cai et al. 2020; Chen et al. 2022; de Oliveira et al. 2022; de Oliveira et al. 2023). Thus, anatomical and physiological variation along the longitudinal axis of long leaves may be caused partially by microclimates intercepting radiation differently among other factors.

Under 75%FC the photosynthesis was reduced at the base of sorghum leaves whereas maize leaves showed a reduction in this parameter at their apex. This result may be related in part to the lower amount of photosynthetic tissues found in these regions. This is supported by the thinner apex with a lower proportion of mesophyll cells and smaller bundle sheath cells in sorghum; however, in maize, there is a smaller proportion of mesophyll and bundle sheath cells (Fig. 4). The middle region is the most functional in both maize and sorghum leaves. This may be due to a natural development of these long leaves that can bend at the apex (Ford et al. 2008) which may limit their exposition to light and limit photosynthetic activity. Monocotyledon leaves can often develop short apical regions and auriculate sheaths (Ford et al. 2008; Jennifer C. Paltiyan-Bugtong et al. 2023). Water stress can promote acclimation responses in leaves of tolerant species (López-Hidalgo et al. 2023); results suggest that the apex and base of maize and sorghum leaves develop reduced tissues which may acclimate under water stress, generating more functional regions at the middle part of the leaf but keeping photosynthetic activity along the leaf axis even under stress.

Transpiration reduced with drought at the leaf apex of sorghum plants but reduced at the middle part under water limitation; however, in maize, this parameter reduced at the apex and increased at the middle and base leaf parts (Fig. 2). In both species these changes suggest compensation mechanisms along the leaf, increasing transpiration in some parts while it is reduced in other. This seems to keep the overall transpiration rate of the leaf and causes no severe damage to the leaf physiology of these species. Water limitation can cause compensation mechanisms in the stomatal responses (Zafar et al. 2023). These changes also influenced the water use efficiency in these parts, reducing it in leaf parts where transpiration increased. The water use efficiency is related to stomatal responses in maize and sorghum under drought (de Oliveira et al. 2023). Drought can reduce the cell turgor in leaves (Ghaffar et al. 2023) and cause changes in water use efficiency (Te et al. 2023). Transpiration changes in maize leaves caused a reduction of the water use efficiency in the middle and base regions of the leaf. In maize, the middle part of the leaf intercepts higher radiation compared with other leaf parts (Ford et al. 2008) and this may reduce the overall photosynthetic capacity of the leaf because the middle is the most functional leaf part; this does not affect sorghum plants though, suggesting that this species showed more stable middle parts.

Water limitation increased photochemical traits in sorghum plants which were higher at the middle and base of the leaves. The absence of modifications in the chlorophyll content suggests tolerance to the water limitation at levels tested. The stable chlorophyll content is often considered a drought-tolerance trait in many plants such as wheat (Ghaffar et al. 2023). The absence of negative effects from water limitation in chlorophyll content in both maize and sorghum plants is important to, in part, explain the increased parameters obtained from chlorophyll fluorescence parameters. Water limitation increased photochemical traits in sorghum (Fig. 3) and promoted no significant limitation to maize. In both maize and sorghum plants, the middle and base regions showed higher photochemical parameters. This may be related to its elongated morphology (lanceolate) that promotes higher light uptake at the middle part(Ford et al. 2008). Many works use the middle region of maize and sorghum plants for anatomical and physiological analyses (Romanowska-Duda et al. 2019; Cai et al. 2020; de Oliveira et al. 2022, 2023). Our results suggest the middle and base of long leaves like those of maize and sorghum may show more functional traits and corroborate its use as the sampled region in previous works.

Photochemical results suggest tolerance mechanisms in sorghum under water limitation which permit the increase of these parameters under drought; nonetheless, this response is not found in maize. This may be related to several mechanisms that protect the PSII under drought conditions in sorghum. Protected PSII responses were also reported by Lin et al. (2023) in *Erodium oxyrhinchum* M. Bieb. under drought conditions. In addition, the middle part of the leaves from both species showed increased photochemical traits. Photosynthesis shows variation along the leaf surface (Moustakas et al. 2022). Drought-tolerant species usually regulate water transport to leaves and favor the Fv/Fm parameter (Lin et al. 2023). Sorghum plants may increase water transport to leaves under drought and this may favor the synthesis of electron acceptors from PSII. Another possibility is the capacity of sorghum to protect the thylakoid membranes where PSII is found. Tylacoid membrane stability is mandatory for the efficiency of PSII operation (Romanowska-Duda et al. 2019; Stefanov et al. 2021). The protection of the PSII may be related to increased photochemical responses in sorghum under drought and it is an important tolerance trait.

In this work, we evaluated an uncommon parameter that may also help explain photosynthetic responses in C_4 species, which is the size (area) of bundle sheath cells. In C_4 species the rubisco activity is exclusive to bundle sheath cells (Weber and von Caemmerer 2010; Wang et al. 2012), but it is often overlooked in anatomical analyses. The rubisco activity is important to photochemical responses because the Calvin-Benson cycle consumes ATP and NADPH which are the products of this photosynthesis stage, and higher consumption of such metabolites may stimulate their production.

Both maize and sorghum showed adequate leaf development, without severe deformations as shown in Figs. 6 and 7 that evidence similar leaf structure under all water treatments and this may permit some degree of photosynthetic activity and growth. However, maize showed a reduction of the leaf thickness and in the bundle sheath under 50%FC while sorghum plants showed no significant limitation to these tissues being an important tolerance trait. Water limitation can reduce the leaf thickness and the chlorophyll parenchyma (Wyka et al. 2019; Della Torre et al. 2021). According to Khan et al. (2023), leaf thickness is related to biomass production and plant productivity because it is associated with photosynthesis. The capacity of sorghum plants to keep the leaf thickness and vascular bundle size is important to drought tolerance as compared to maize.

Leaf anatomy from both maize and sorghum showed significant anatomical variation along its longitudinal axis and these suggest that its middle part is more functional. Maize and sorghum both show long leaves (de Oliveira et al. 2023) and this morphology can promote the bending of the leaf apex region which reduces light uptake in this part (Ford et al. 2008). It is well documented that the leaf morphology influences its radiation uptake (Carvalho et al. 2006; Dzvene et al. 2023). The leaf apex is thinner in both maize and sorghum leaves and there are reductions in the vascular bundle and xylem cells. Smaller vascular tissue development, mainly the xylem may be caused by several environmental factors such as drought (Guha et al. 2018; Bijanzadeh et al. 2023) which may reduce vessel cavitation and favor water transport (Choat et al. 2016). The reduction of the mesophyll and bundle sheath cells may reduce photosynthetic activity because these cells are both important for C4 photosynthesis (Wang et al. 2012). These results support the previous works selecting the middle part of the leaf to sample anatomical and physiological traits.

Stomatal traits in sorghum show responses related to drought tolerance while maize stomata showed little variation to drought. Sorghum's stomatal index and density were reduced under water limitation which may limit water loss by transpiration whereas these traits from maize remain unchanged or increased. Stomata are related to the control of gas exchange in leaves and are important in regulating water loss and photosynthesis under drought (Cai et al. 2020; Zia et al. 2021). The reduction of the number of stomata is often related to lower transpiration and water loss (Gerardin et al. 2018; Niu et al. 2023). It is also important to note that most positive stomatal changes in sorghum were found in the middle and base parts of the leaves, suggesting that this leaf part is more efficient in gas exchange and the control of water loss compared to the leaf apex.

Finding new tools to access drought effects and tolerance markers is essential for crop production and screening for drought-tolerant breeds. According to Munné-Bosch and Villadangos (2023), there is an increasing necessity to find tools to monitor drought stress in crops as a worldwide demand. In addition, Kreibich et al. (2019) suggest that the lack of data limits effective actions for crop production under water limitation. In this work, results suggest that there is a relevant modification in photosynthetic capacity and anatomical traits along the leaf longitudinal axis and the middle part of the leaf of both maize and sorghum shows improved traits compared to the leaf axis and base. Long leaves like those from maize may cause bending of its apex (Ford et al. 2008) and this may reduce light interception and develop less functional physiological and anatomical features. Thus, the middle part of the leaf from both maize and sorghum is more relevant when accessing functional characteristics in studies for breeding and adaptation to water limitation conditions. It is important to note that although the leaf middle part is more functional in both maize and sorghum, there is significant variation along the leaf axis for anatomical and physiological characteristics that must be considered depending on the objective of the study, for instance in studies with the plant shoot architecture, self-shading and spacing which may change the way radiation is intercepted by these leaves. In addition, our study used one genotype for each species and further studies must investigate using other maize and sorghum materials to address this limitation. Nonetheless, the majority of works about physiological and anatomical traits in maize and sorghum use one or a few genotypes (Guha et al. 2018; Impa et al. 2019; Romanowska-Duda et al. 2019; Cai et al. 2020; Chen et al. 2022; de Oliveira et al. 2022; de Oliveira et al. 2023; Dzvene et al. 2023). The use of one genotype for native species studies is also the most common approach using non-crop species (Hsie et al. 2015; Gavilanes et al. 2016; Mansoor et al. 2019; Della Torre et al. 2021; Lin et al. 2023). Therefore, results found here can be extended for other maize and sorghum genotypes but its application can be further expanded using genotypes from different regions.

Conclusion

Sorghum higher showed drought-tolerance traits compared to maize like stable photosynthesis, transpiration, chlorophyll content, and photochemical and anatomical characteristics. Most physiological traits vary along the leaf axis in both species and the middle part of the leaf is more functional with reduced features in the apex and base. Variation in anatomical and physiological traits in maize and sorghum leaves seems to be related to the elongated morphology of these leaves.

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ARTIGO 3 ROOT CORTICAL AERENCHYMA IN SORGHUM INCREASES ITS ROOT ELONGATION AND VOLUME, FAVORING NUTRIENT UPTAKE AND GAS EXCHANGE COMPARED TO MAIZE UNDER WATER LIMITATION

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Abstract

This work investigated root anatomical traits related to its growth and nutrient acquisition capacity in maize and sorghum plants under water limitation. Experiments were conducted in a greenhouse in a factorial 2x2 scheme (two plant species maize and sorghum) and two water availabilities (well-irrigated and limited irrigation) with six replicates. Leaf gas exchange, growth, aerenchyma formation, root tissues in both maturation and piliferous regions, and macro and micronutrient uptake were analyzed. Water limitation reduced maize growth with no significant changes in sorghum. Sorghum showed higher photosynthesis and transpiration compared to maize which exhibited higher water use efficiency; nonetheless, water limitation increased the leaf water content in sorghum but not in maize. Water limitation increased the root length, volume, and aerenchyma development in sorghum plants only. Increased root elongation and volume favored macro (N, Mg, and Ca) and micronutrient (Zn, Cu, Mo, and Fe) acquisition in sorghum compared to maize. Water limitation stimulated the acquisition of B, Mn, Zn, Cu, Mg, and N in both species; but Fe and Mo contents under water limitation increased only in sorghum. Sorghum's root aerenchyma development and volume under water limitation seem related to a higher elongation capacity favoring nutrient acquisition in this species compared with maize under water limitation.

Keywords: Root water relation. Macro and micronutrients. Root volume. Water stress. *Zea mays* L. *Sorghum bicolor* (L.) Moench.

1 Introduction

In recent years crop production regions have been facing more frequent climate variations affecting the water availability and the occurrence of drought events (Haider et al. 2024). Crop yield can be severely damaged by water limitation causing significant economic losses (Rezaei et al. 2023). In sensitive plants, water limitation limits root development harming water and nutrient acquisition with negative consequences to plant growth (Díaz et al. 2018; Hemati et al. 2022). For both maize and sorghum plants limited root growth under water limitation impairs their productivity and resilience (Pereira et al. 2008; Díaz et al. 2018; Oliveira et al. 2023).

It is important to note that sorghum shows higher tolerance to water limitation, contrasting with maize (Oliveira et al. 2023), and this difference in their responses depends on physiological and anatomical modifications to overcome water stress. For instance, the development of larger leaf intercellular spaces, particularly, a larger stomatal cavity improves leaf gas exchange in sorghum compared with maize under drought (Oliveira et al. 2022, 2023). Favorable anatomical and physiological to improve drought tolerance are organ-dependent and root modifications to improve water and nutrient uptake are key features for the tolerance to water limitation in plants (Ilyas et al. 2021; Kim and Lee 2023).

Root modifications that improve drought tolerance comprehend complex changes which include distinctive traits from increased growth to cortical aerenchyma development (Díaz et al. 2018; Ilyas et al. 2021; Yao et al. 2024). Early studies associate root aerenchyma development with waterlogging due to its capacity to provide oxygen by air internal diffusion to flooded roots (Gao et al. 2025); however, the tissue was further recognized as an important drought-tolerance trait (Lenochová et al. 2009; Souza et al. 2016). Root cortical aerenchyma development under water limitation reduces the metabolic cost of roots by lowering the number of cells; this favors root growth and improves water and nutrient uptake (Lynch 2015; Schneider and Lynch 2018). Sorghum is recognized as more drought-tolerant compared to maize and understanding its capacity to develop root cortical aerenchyma and how it correlates with water and nutrient uptake is important to crop production and breeding programs.

According to Fan et al. (2007), the development of root cortical aerenchyma in roots can reduce its hydraulic conductivity. Nonetheless, aerenchyma formation in roots can increase at the maturation region, where water and nutrient uptake are already reduced being the development of this tissue limited at the piliferous region (Díaz et al. 2018). In maize plants grown under water limitation, aerenchyma formation increased in the maturation region but showed no significant changes in the piliferous region, and this improved root growth, water and phosphorus uptake in drought-tolerant maize genotypes (Díaz et al. 2018). Although sorghum is a species known for its drought tolerance, how aerenchyma develops in different root regions and how this can correlate with nutrient uptake is unclear.

Understanding how aerenchyma formation differs among different root regions in maize and sorghum grown under drought and its effects on nutrient uptake in these species can elucidate important traits for crop improvement and drought tolerance. Thus, this work hypothesizes that sorghum shows a higher capacity to improve root traits under drought compared with maize particularly the aerenchyma formation that improves root growth favoring water and nutrient uptake. This work aimed to investigate the aerenchyma development in different regions of maize and sorghum roots and its relation to growth, photosynthesis, and nutrient uptake of these species under water limitation.

2 Material and methods

2.1 Plant material and experimental design

The experiment was carried out in a greenhouse located at the Universidade Federal de Lavras, state of Minas Gerais, Brazil. *Sorghum bicolor* (L.) Moench and *Zea mays* (L.) plants were grown from seeds provided by Embrapa's National Research Centre for Maize and Sorghum, located in Sete Lagoas, Minas Gerais, Brazil.

Seeds were sown in 5.0 L plastic pots containing 2.0 L of sand and 800 mL of nutrient solution (Hoagland and Arnon 1950) at 40% of its ionic strength and the seedlings were obtained according to Oliveira et al. (2023).

The plants were then subjected to two water conditions: (1) Well Irrigated (WI) and (2) Limited irrigation (LI). The well-irrigated was considered the maximum volume of water retained by 1.0 L of sand without becoming waterlogged. The volume of water applied to achieve WI was 310.0 mL water L^{-1} sand and for LI was 155.0 ml water L^{-1} sand. The water lost by evapotranspiration was monitored by the daily difference in the weight of each pot. Water was replaced daily, and the nutrient solution was weekly. The experiment was conducted in a 2 × 2 factorial scheme with two cereal species (sorghum and maize) and two irrigation conditions (well-irrigated and limited irrigation), with four treatments and six replicates. Each replicate comprised one plant. All the data from the analyses where multiple assessments were performed were then averaged per replicate (maintaining n = 24 and avoiding artificial replication).

2.2 Analysis of plant growth

At the end of the experiment, root length was measured using a metric ruler. In addition, water content (WC) in the plant and its organs was calculated using the formula: $WC = [(FM - DM) / FM] \times 100$, where FM represents the fresh mass and DM is the dry mass of the plant/organ. After these measurements, the plants were dried at 60 °C until a constant weight was achieved, and the total dry mass was determined using an analytical balance (AY220, Shimadzu, São Paulo, Brazil). The plants were then separated into roots and aerial parts, and the ratio of root dry mass to total dry mass was calculated.

2.3 Leaf relative water content

Leaf relative water content (RWC) was measured following the methodology described by Weatherley (1950). Six leaf disk samples, approximately 0.5 cm in diameter, were taken from the median portion of the leaves. The disks were initially weighed to determine fresh mass (FM) and then placed in Petri dishes immersed in deionized water for 24 h. After this period, they were reweighed to obtain turgid mass (TM) and subsequently dried in paper bags in a forced-air oven at 60 °C until a constant weight was achieved, determining the dry mass (DM). RWC was calculated using the formula: $RWC = [(FM - DM) / (TM - DM)] \times 100$.

2.4 Gas exchange analysis

Gas exchange analysis was conducted at the end of the experiment, in the morning between 8:00 and 11:00 a.m., on the first fully expanded leaf. Measurements were carried out using an infrared gas analyzer (IRGA) LI-6400XT (Li-COR Biosciences, Lincoln, NE, USA), with one leaf per plant sampled. In the measurement chamber, photosynthetically active radiation was maintained at 1000 $\mu mol\ m^{-2}\ s^{-1}$ using a 6400-02B LED light source, with a reference CO_2 concentration of 392.3 $\mu mol\ L^{-1}$, a flow rate of 499.48 mol s^{-1}, a leaf temperature of 20.32 °C, and a vapor pressure deficit (VPD) of 0.91 kPa.

2.5 Water-use efficiency (WUE)

At the end of the experiment, instantaneous water-use efficiency (WUEi) was calculated following Kramer and Boyer (1995). WUE (µmol CO_2 $mmol^{-1}$ H_2O) was determined as the ratio of photosynthesis (A) to transpiration (E) values obtained from the IRGA analyses.

2.6 Plant Nutrient Analysis

For the evaluation of macro- and micronutrient concentrations, whole plants were separated, washed with running water, and dried in a forced-air oven at 60 °C for 72 hours. The dried material was then ground using a Willey-type knife mill, and 500 mg of dry mass was weighed on an analytical balance for nitroperchloric digestion, following the method proposed by Sarruge and Haag (1974). In this process, 10 mL of concentrated HNO_3 was added to the sample, which was left to rest for 12 hours. Digestion was then carried out at 150 °C for 30 minutes or until the nitric acid volume was reduced by half. After this step, 1.0 mL of $HClO_4$ was added, and the digestion block temperature was increased to 210 °C for 20 minutes or until the solution was clarified. The digestion product was transferred to a 25 mL volumetric flask, to which 10 mL of distilled water was added, and readings were performed using a flame atomic absorption spectrophotometer.

The following macronutrients were analyzed: phosphorus (P), nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg), and sulfur (S). The micronutrients analyzed included zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), molybdenum (Mo), and sodium (Na).

2.7 Anatomical Analysis

At the end of the experiment, root samples were collected and fixed in 70% FAA (formaldehyde, glacial acetic acid, and 70% ethanol in a 0.5:0.5:9 ratio) for 72 h (Johansen 1940) and then stored in 70% ethanol until analysis. Hand-sectioned transverse root samples were obtained using steel blades and stained with safranin solution (0.1% safranin and 1% Astra blue in a 7:3 ratio). Semi-permanent slides were prepared following the method described by Kraus and Arduin (1997), observed under a CX31 trinocular light microscope (Olympus, Tokyo, Japan) equipped with an image capture system, and digitized for further analysis using the ImageJ software. Sections were obtained from the maturation and root hair zones of maize and sorghum roots.

The following anatomical parameters were evaluated: total root area, cortical region area, cortical aerenchyma area, vascular cylinder area, epidermis thickness, exodermis thickness, endodermis thickness, cortex thickness, and xylem vessel diameter. Additionally, root volume was calculated using the formula: $RV= [(HA+MA)/2]*RL$, where, VR = root volume; HA = total area of the root hair zone; MA = total area of the maturation zone; RL = root length. All measurements were converted to square millimeters.

2.8 Statistical Analysis

The data were checked for normality using the Shapiro–Wilk test and subjected to analysis of variance (two-way ANOVA), and the means were compared by the Scott–Knott test for $p < 0.05$ using the SISVAR statistical software (Ferreira 2011).

3 Results

There was significant interaction between plant species and irrigation for the root length and plant dry mass, but no significant interaction was found for the root dry mass, root:shoot ratio, and root and leaf water contents (Fig. 1). Water limitation increased the root length from sorghum only (Fig. 1). Maize showed longer roots at well irrigated condition but under limited irrigation both maize and sorghum showed similar root length (Fig.1A). Water limitation promoted no significant changes in the root dry mass and this parameter was higher in maize at both irrigation treatments (Fig. 1B). Water limitation reduced the total dry mass only in maize that showed higher means at well irrigated treatment but under limited irrigation showed similar values to sorghum (Fig. 1C). Maize showed higher shoot:root ratio compared with sorghum and water limitation promoted no significant changes in this variable (Fig. 1D). Sorghum showed higher root water content compared to maize but water limitation promoted no significant changes in this parameter (Fig. 1E). Maize showed higher leaf water content compared to sorghum but the limited irrigation treatment promoted no significant changes in this variable (Fig. 1F).

No significant interaction between plant species and irrigation was found for photosynthesis, transpiration, and water use efficiency but a significant effect occurred for leaf relative water content (Fig. 2). Sorghum showed higher photosynthesis than maize and water limitation promoted no significant effect in this parameter (Fig. 2A). Transpiration was higher in sorghum compared to maize but irrigation promoted no significant effect in this parameter (Fig. 2B). The water use efficiency was higher in maize compared to sorghum but water irrigation promoted no significant effect in this parameter (Fig. 2C). Limited irrigation increased the leaf relative water content in sorghum only (Fig. 2D). Maize showed higher leaf relative water content than sorghum under well-irrigated conditions but under limited irrigation, both species showed similar means (Fig. 2D).

No significant interaction between plant species and irrigation was observed for the macronutrient content (Fig. 3). The P content was similar in both irrigation treatments and also between maize and sorghum (Fig. 3A). The limited irrigation treatment increased the N content and sorghum showed higher means compared with maize (Fig. 3B). Sorghum showed higher Ca content compared with maize but irrigation treatments did not modify this parameter (Fig. 3C). The K content was similar between maize and sorghum and was not modified by irrigation treatment (Fig. 3D). Limited irrigation increased the Mg content and this parameter was greater in sorghum compared to maize (Fig. 3E). The S content was not modified neither by irrigation treatments nor plant species (Fig. 3F).

Regarding the micronutrients, there was significant interaction for irrigation treatments and plant species only for Fe and Mo contents (Fig. 4). Limited irrigation increased the Zn content and this variable was higher in sorghum compared to maize (Fig. 4A). The Cu content showed similar results, increasing under limited irrigation and sorghum showing higher levels than maize (Fig. 4B). Limited irrigation increased the Mn content nut no significant differences were found between sorghum and maize (Fig. 4C). Limited irrigation increased the Fe content in sorghum but decreased this parameter in maize (Fig. 4D). Under well-irrigated conditions maize and sorghum showed similar Fe content; however, under limited irrigation sorghum Fe content surpassed maize (Fig. 4D). Limited irrigation increased the B content but no significant differences were found between sorghum and maize (Fig. 4E). Limited irrigation increased the Mo content in sorghum but reduced this parameter in maize (Fig. 4F). Sorghum showed higher Mo content than maize in both irrigation treatments (Fig. 4F). The Na content was not significantly modified neither by irrigation treatments or plant species (Fig. 4G).

There was a significant interaction between irrigation and plant species for aerenchyma area and root volume (Table 1). Limited irrigation inhibited aerenchyma formation in maize at both piliferous and maturation regions but promoted no significant effect in sorghum (Table 1, Fig. 5, and Fig. 6). Under well-irrigated conditions maize and sorghum showed similar aerenchyma areas at both maturation and piliferous regions but under limited irrigation sorghum showed higher aerenchyma area than maize (Table 1, Fig. 5, and Fig. 6). Limited irrigation decreased the average root volume from maize but increased this parameter in sorghum (Table 1, Fig. 5, and Fig. 6). Under well-irrigated conditions maize showed greater average root volume but under limited-irrigation this was inverted and sorghum showed larger root volume than maize (Table 1, Fig. 5, and Fig. 6).

There was significant interaction for irrigation and plant species for most anatomical parameters at the root piliferous region, except for epidermis and endodermis thicknesses (Fig. 7). Limited irrigation increased the total root area from sorghum but reduced this parameter in maize (Fig. 7A and Fig. 5). Under well-irrigated conditions maize showed larger total root area compared to maize, but under limited irrigation this inverted and sorghum showed higher means (Fig. 7A and Fig. 5). The area of the vascular cylinder showed similar results, being increased under limited irrigation in sorghum and reduced in maize (Fig. 7B and Fig. 5). Limited irrigation reduced the cortex thickness from maize but caused no significant effect in sorghum (Fig. 7C and Fig. 5). Under well-irrigated conditions maize and sorghum showed similar cortex thickness but under limited irrigation maize showed thinner cortex (Fig. 7C and Fig. 5). Limited irrigation increased the xylem vessel diameter from sorghum but reduced this parameter in maize (Fig. 7D and Fig. 5). Under well-irrigated conditions, maize and sorghum showed similar xylem vessel diameter but under limited irrigation sorghum showed larger xylem vessels (Fig. 7D and Fig. 5).

Still, at the piliferous root region, the epidermis thickness was not significantly modified by irrigation or plant species (Fig. 7E and Fig. 5). Limited irrigation increased the exodermis thickness from sorghum but reduced this parameter in maize (Fig. 7F and Fig. 5). Under well-irrigated conditions maize showed thicker exodermis compared to sorghum, but at limited irrigation both species showed similar means (Fig. 7F and Fig. 5). The endodermis thickness was not significantly modified neither by irrigation or plant species (Fig. 7G and Fig. 5). Limited irrigation increased the root cortex area from sorghum but reduced this parameter in maize (Fig. 7H and Fig. 5). Under well-irrigated conditions maize showed larger root cortex area but under limited irrigation this inverted and sorghum showed larger cortex area (Fig. 7H and Fig. 5).

At the root maturation region there was not a significant interaction between irrigation and plant species for most parameters, except for the cortex thickness and xylem vessel diameter (Fig. 8). There was no significant effect from irrigation or plant species for the total root area (Fig. 8A and Fig. 6) and vascular cylinder area (Fig. 8B and Fig. 6). Limited irrigation reduced the cortex thickness only from maize (Fig. 8C and Fig. 6). Under well-irrigated conditions maize and sorghum showed similar means for the root cortex thickness; nonetheless, under limited irrigation maize showed thinner cortex (Fig. 8C and Fig. 6). Limited irrigation increased the xylem vessel diameter in sorghum but had no significant effect in maize (Fig. 8D and Fig. 6). Under well-irrigated conditions maize and sorghum showed similar xylem vessel diameter but under limited irrigation sorghum showed larger xylem vessels (Fig. 8D and Fig. 6). Limited irrigation promoted no significant modification in the epidermis thickness but maize showed thicker epidermis compared sorghum (Fig. 8E and Fig. 6). At the root maturation region neither irrigation nor plant species showed significant modifications in the exodermis thickness (Fig. 8F and Fig. 6), endodermis thickness (Fig. 8G and Fig. 6), and root cortex area (Fig. 8F and Fig. 6).

Table 1 Cortical root aerenchyma area and average root volume of *Sorghum bicolor* and *Zea mays* under different water conditions. Data is shown as mean ± standard deviation.

Aerenchyma area (μm^2) from piliferous region		
	Maize	Sorghum
Well-irrigated	78796.30±61842.38Aa	108473.76±79910.89Aa
Limited irrigation	0.00±0.00Bb	115239.35±88659.44Aa
Aerenchyma area (μm^2) **from the maturation region**		
	Maize	Sorghum
Well-irrigated	111111.97±47802.69Aa	130793.22±108837.51Aa
Limited irrigation	0.00±0.00Bb	151844.57±32246.60Aa
Average root volume (mm^3)		
	Maize	Sorghum
Well-irrigated	366.49±96.64Aa	179.81±129.56Bb
Limited irrigation	196.33±57.90Bb	366.46±84.78Aa

The lowercase letters compare plants and uppercase ones compare water conditions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$.

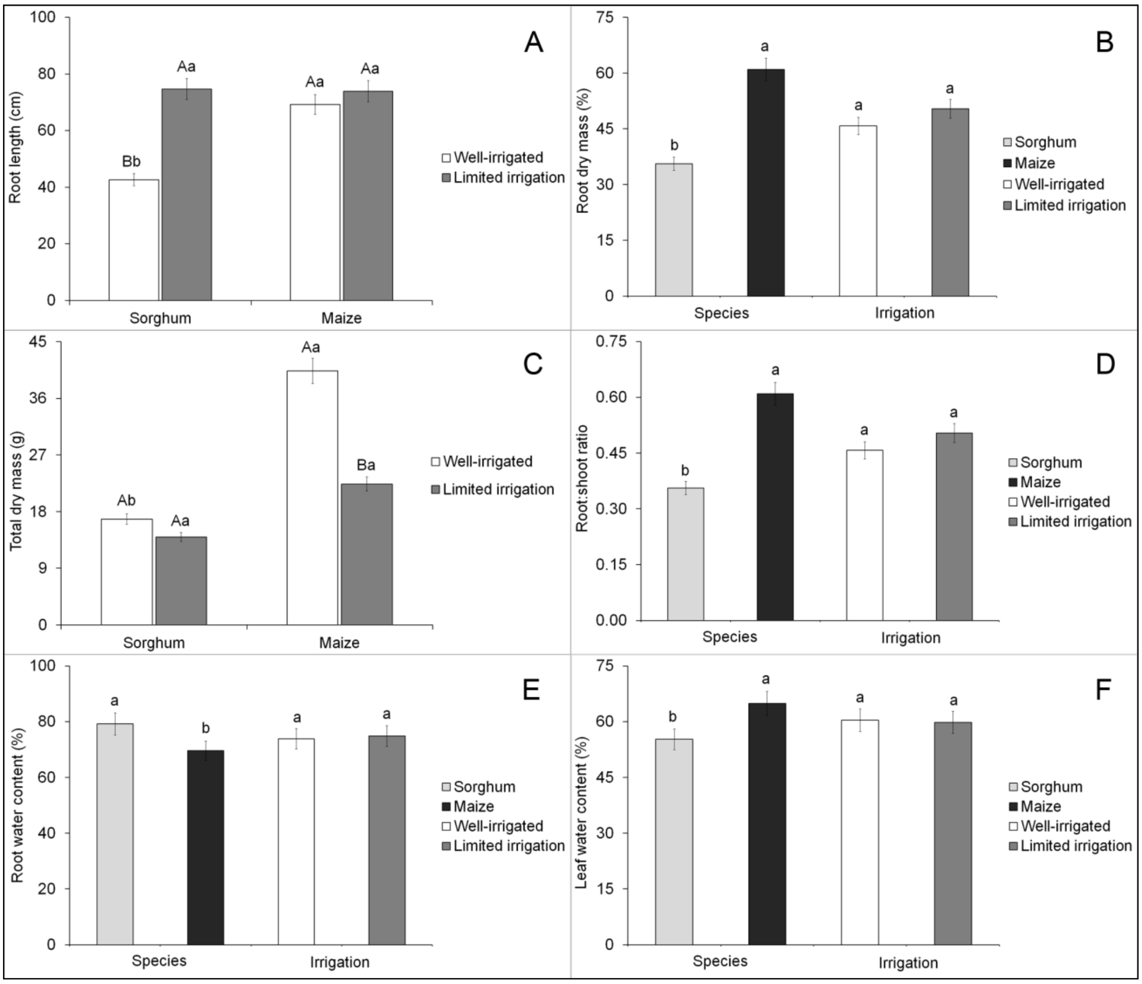

Fig. 1 Growth parameters of *Sorghum bicolor* and *Zea mays* under different irrigation conditions. The lowercase letters compare to plants and uppercase ones compare water conditions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors.

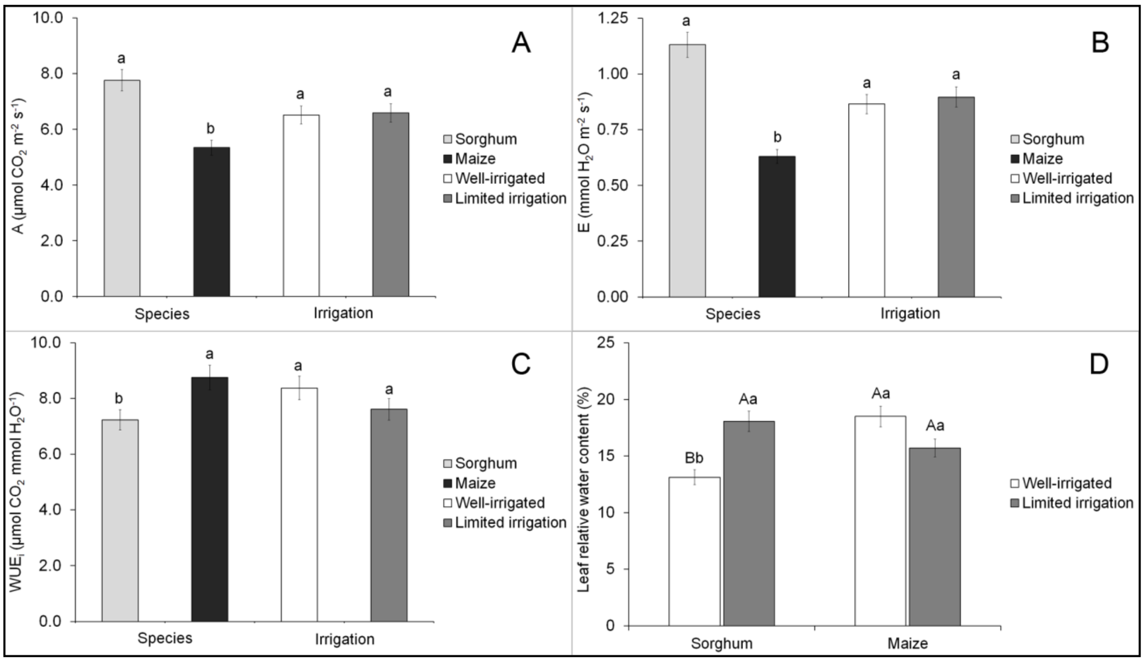

Fig. 2 Leaf gas exchange and leaf relative water content parameters of *Sorghum bicolor* and *Zea mays* under different irrigation conditions. A = Net photosynthesis; E = Transpiration rate; WUEi = water-use efficiency. The lowercase letters compare plants and uppercase ones compare water conditions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors.

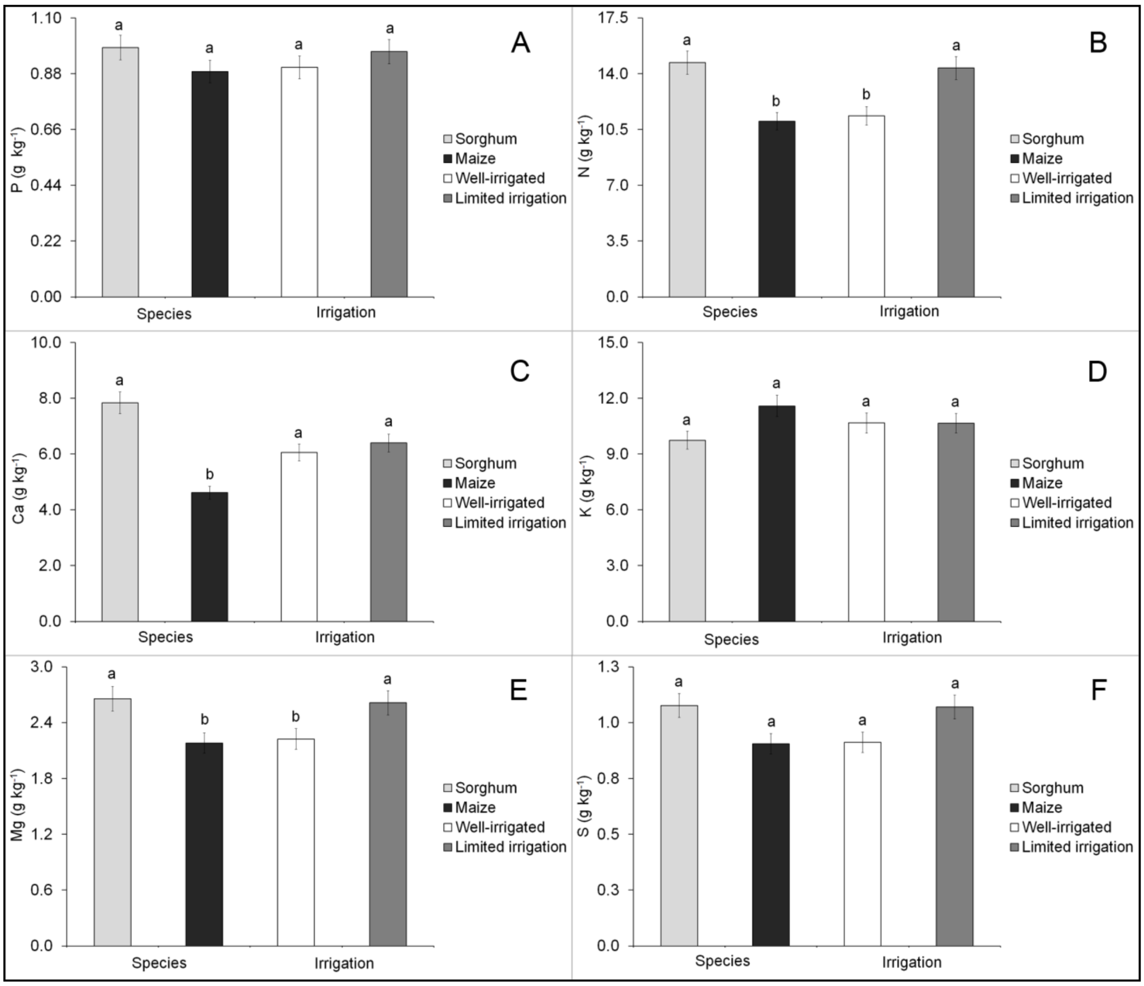

Fig. 3 Macronutrient content of *Sorghum bicolor* and *Zea mays* under different irrigation conditions. The lowercase letters compare plants and uppercase ones compare water conditions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors.

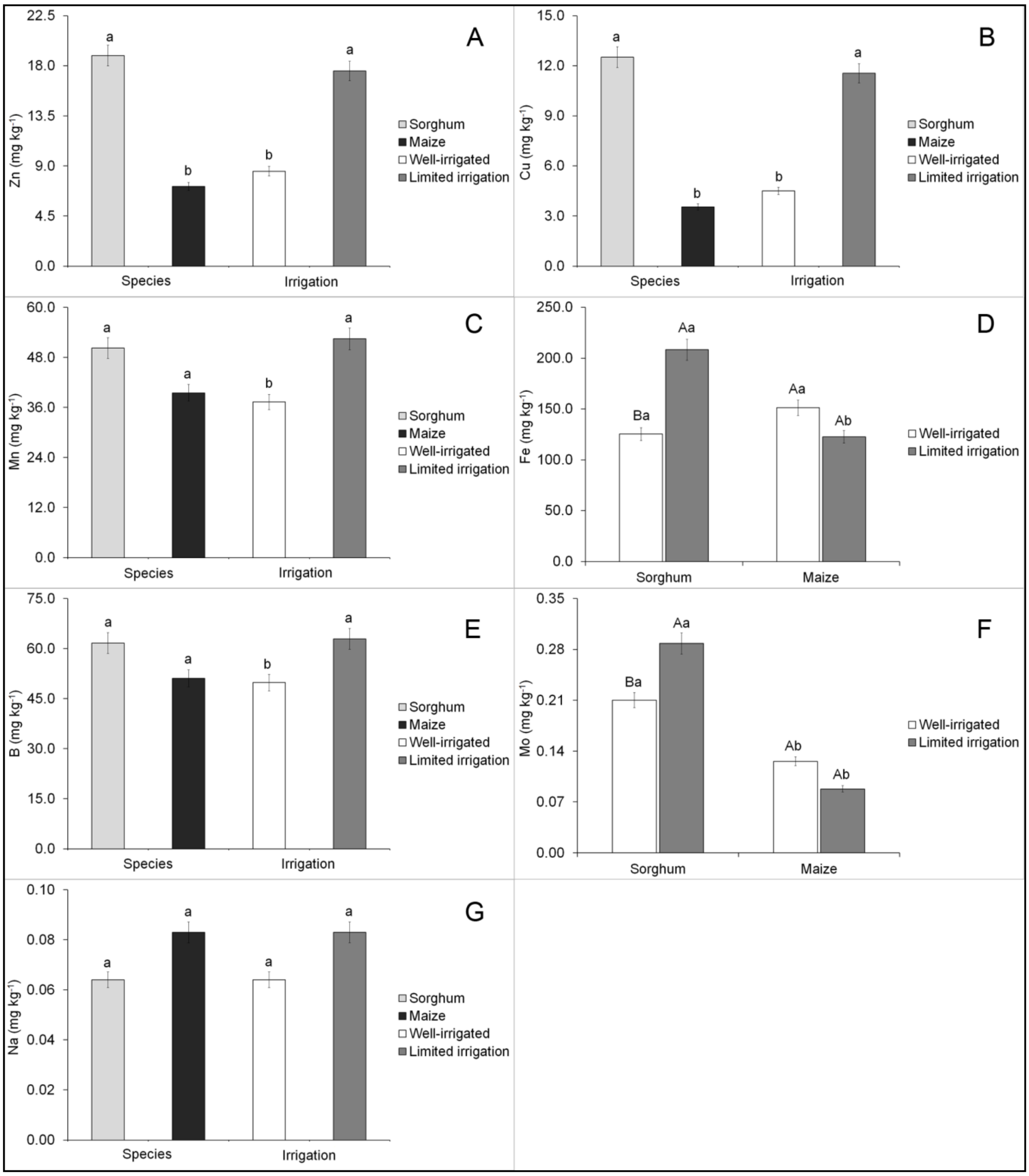

Fig. 4 The micronutrient content of *Sorghum bicolor* and *Zea mays* under different irrigation conditions. The lowercase letters compare plants and uppercase ones compare water conditions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors.

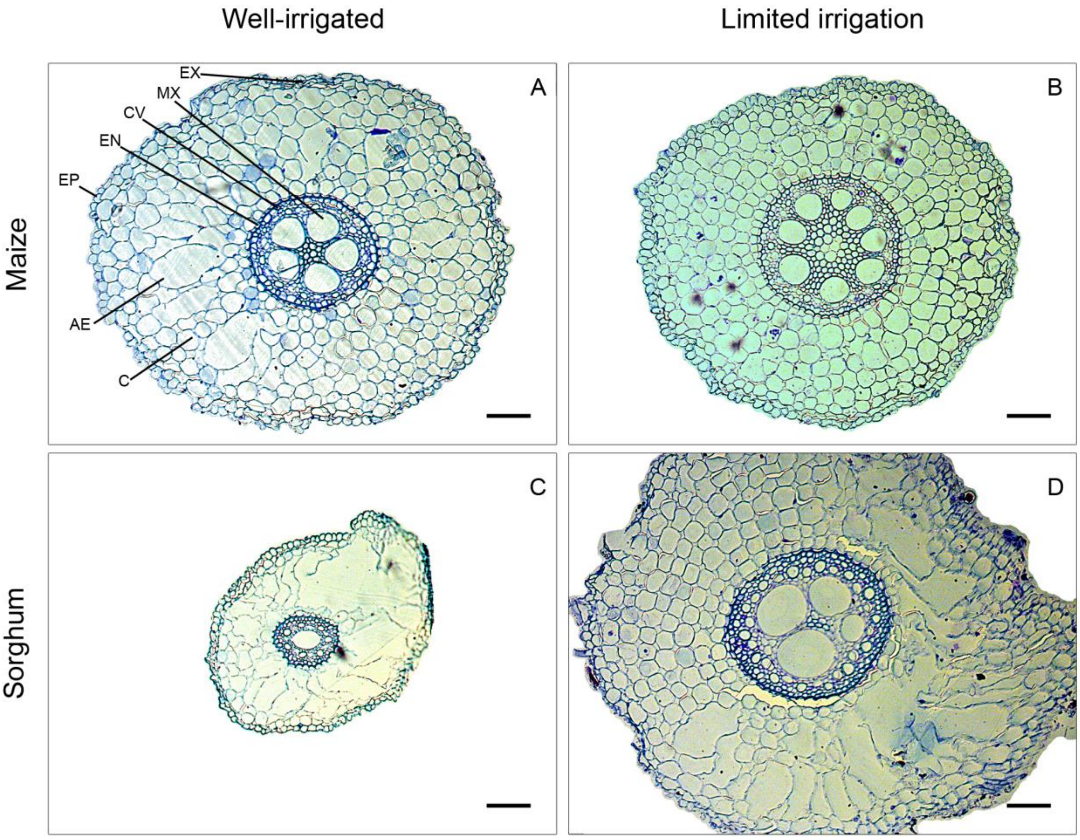

Fig. 5 Transversal sections of *Sorghum bicolor* and *Zea mays* root piliferous under different water conditions. EP = Epidermis cells; EX = Exodermis cells; AE = Cortical aerenchyma; EN = Endodermis cells; CV = Vascular cylinder; C = Córtex; MX = Metaxylem vessel; Bars = 100 µm.

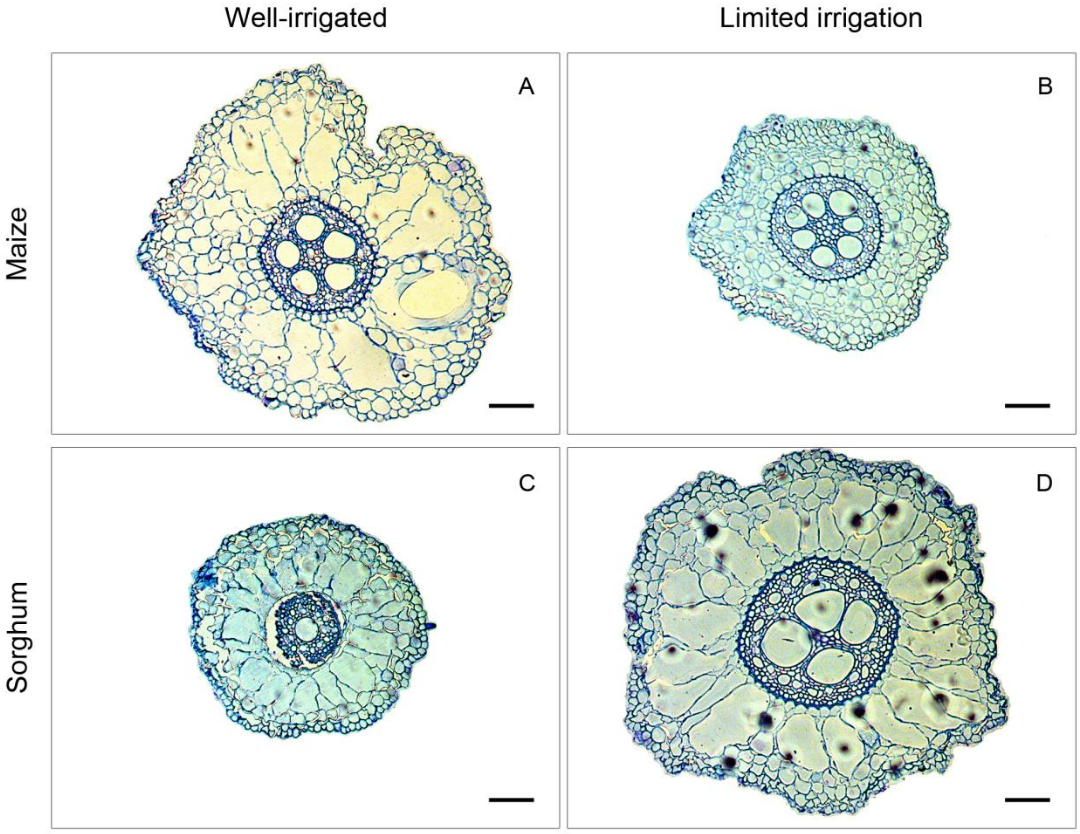

Fig. 6 Transversal sections of *Sorghum bicolor* and *Zea mays* root maturation region under different water conditions. Bars = 100 µm.

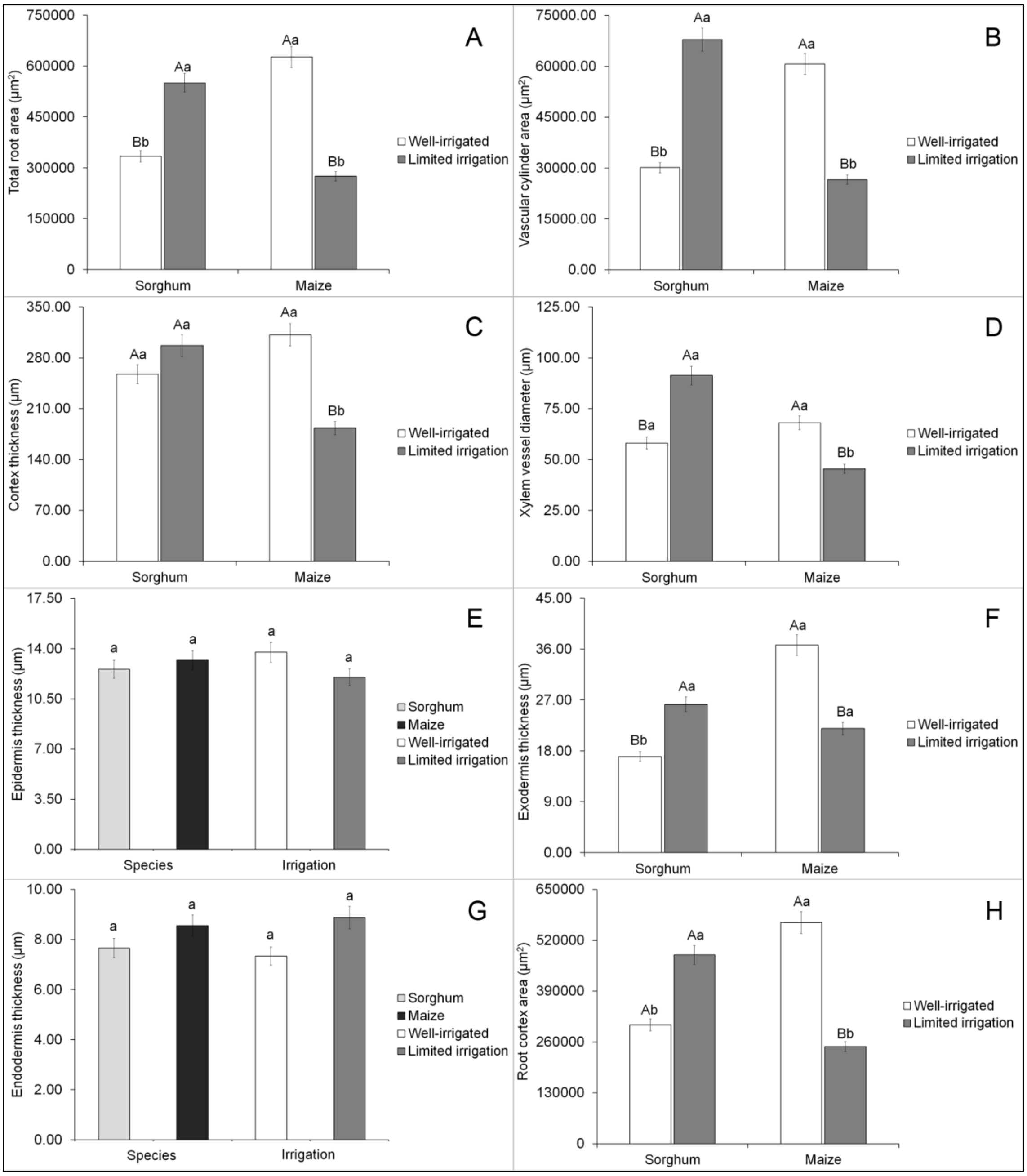

Fig. 7 Root anatomical parameters from the piliferous region of *Sorghum bicolor* and *Zea mays* under different irrigation conditions. The lowercase letters compare plants and uppercase ones compare water conditions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors.

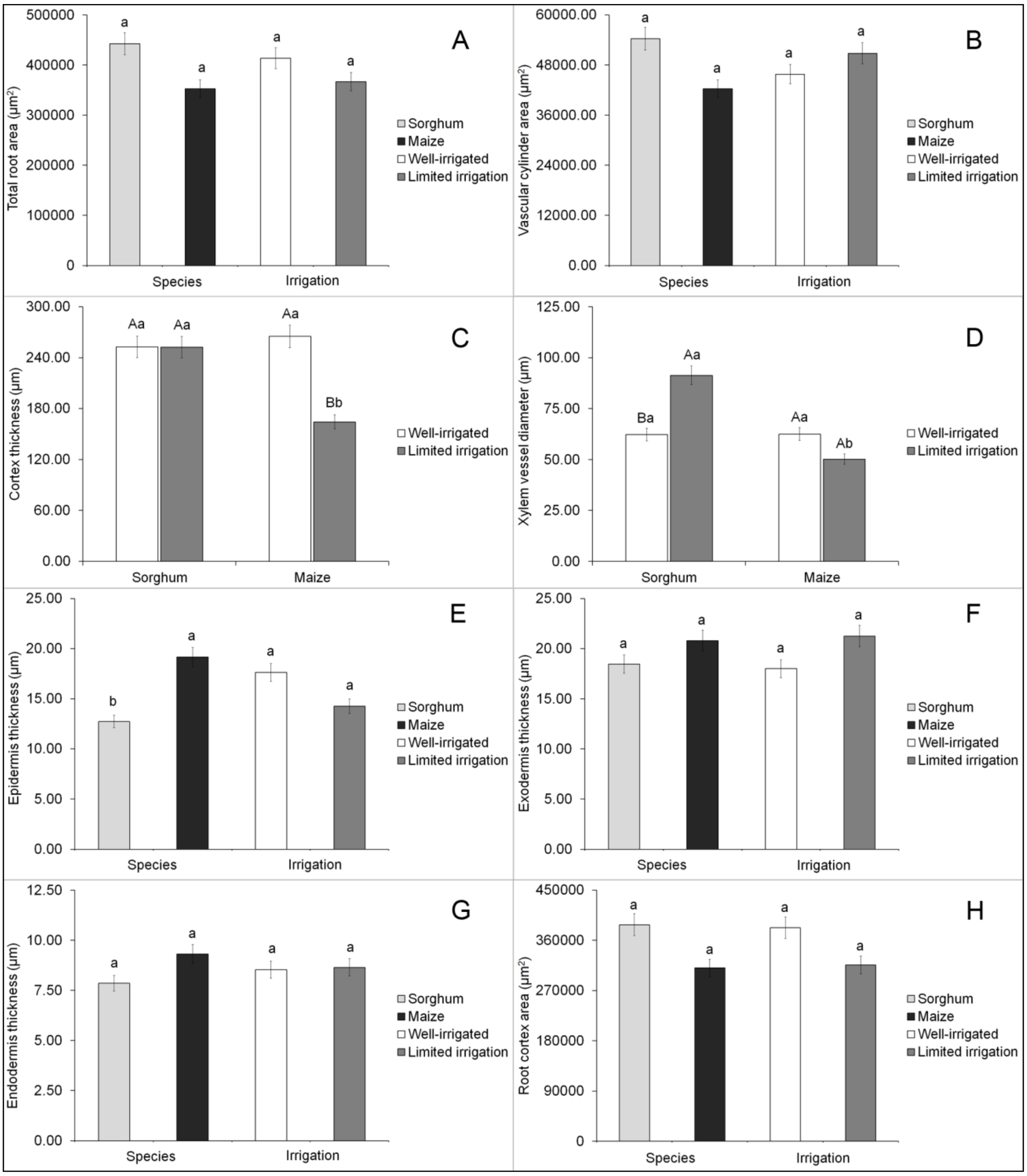

Fig. 8 Root anatomical traits from the maturation region of *Sorghum bicolor* and *Zea mays* under different irrigation conditions. The lowercase letters compare plants and uppercase ones compare water conditions. The same letter shows no significant differences according to the Scott-Knott test at $p<0.05$. Bars= standard errors.

4 Discussion

Maize and sorghum belong to the same family (Poaceae) among monocotyledon and contrast to drought tolerance (Oliveira et al. 2023). Maize plants are bigger than sorghum (Oliveira et al. 2023) and under well-irrigated conditions, these plants showed larger biomass because of these natural size differences as shown by variables related to plant dry mass (Fig. 1). Nonetheless, maize reduced its total dry mass under limited irrigation while sorghum kept its growth, this is a typical tolerance response from sorghum that is often classified as drought-tolerant species (Gong et al. 2015) and the lower growth of maize corroborate its response as a drought-sensitive crop (Impa et al. 2019). In addition to stable biomass production under drought only sorghum plants increased their root length, improving its capacity to acquire water and nutrients compared to maize.

One important variable that contributes to facilitating root growth is the aerenchyma formation, which was inhibited in maize under limited irrigation but sorghum maintained its development. Root cortical aerenchyma formation can be stimulated by flooding (Gao et al. 2025) and drought (Souza et al. 2016), being also constitutive, and always present, in aquatic plants like *Typha domingensis* Pers. (Corrêa et al. 2015; Duarte et al. 2021). Aerenchyma formation reduces the number of cells in the root cortex, reducing its metabolic cost and favoring root growth (uptake (Lynch 2015; Schneider and Lynch 2018; Kalra et al. 2024). Thus, the capacity of sorghum plants to maintain the root cortical aerenchyma development under limited irrigation favored its capacity to obtain water and nutrients compared with maize.

Aerenchyma formation despite its benefits for root growth can be a factor that reduces the root hydraulic conductance but sorghum roots did not show such limitation they improved water and nutrient acquisition under limited irrigation and sorghum showed improved nutrient uptake compared to maize. According to Fan et al. (2007) increased root cortical aerenchyma influences nutrient uptake. In addition, Díaz et al. (2018) showed that maize roots develop higher aerenchyma percentage at the maturation region compared to the piliferous region benefiting from reduced metabolic cost and avoiding a limitation to water and nutrient uptake. The three main water and nutrient uptake pathways present in roots are apoplastic, symplastic, and transcellular (Wang et al. 2023). All these pathways depend on cells for water movement, being through their cell walls (apoplastic) or cytoplasm (symplastic and transcellular). Thus, reduced cell number is expected to reduce water and nutrient uptake, but this did not occur in sorghum, suggesting that roots from this species show some compensation mechanism to avoid such limitation.

Sorghum roots were longer and showed larger area compared to maize and these parameters increased the total root volume, which may be related to a larger external area for trichome formation and water and nutrient uptake compared to maize under limited irrigation. Sorghum showed higher root water content, transpiration, and leaf relative water content compared to maize (Fig. 2) and showed higher N, Mg, Ca, Zn, Cu, Mo, and Fe compared to maize. In addition, under limited irrigation, only sorghum increased the Mo and Fe acquisition. These results indicate that sorghum roots showed no limitation for water and nutrient uptake although high aerenchyma development its nutrition and water content seem higher than these parameters in maize. Higher hydration and nutrient acquisition favored the photosynthesis in sorghum, maintaining its dry mass production, even under drought, unlike maize. Thus, results suggest that higher root volume is a relevant tolerance mechanism in sorghum for limited water availabilities and since maize showed smaller roots it limited its water and nutrient uptake.

The improved nutrient content in sorghum and better Mo and Fe acquisition under limited irrigation are key parameters for its improved photosynthesis and growth-limited irrigation. Sorghum showed higher N, Mg, Ca, Zn, Cu, Mo, and Fe compared to maize. It is important to note that N and Mg are structural elements from chlorophyll molecules being key nutrients for photosynthesis. In addition, N increases the capacity of plants to uptake water in roots and the water conservation in leaves under arid environments (Kumari et al. 2022; Kalra et al. 2024), drought tolerance is also often associated with Zn (Yavas; Unay, 2016; Kalra et al. 2024). In addition, N is part of several proteins related to the photosynthesis complex (Nelson and Ben-Shem 2004). The particular increase in the Fe and Mo under limited irrigation in sorghum deserves attention. Iron is a key micronutrient for photosynthesis, present in the iron-sulfur proteins among the electron transport chain in the Z scheme (Nelson and Ben-Shem 2004). The improved Fe uptake in sorghum under drought may have favored its photosynthesis, maintaining the dry mass production. Mo is also an essential element for photosynthesis and growth because this element is a co-factor to key enzymes in the N metabolism, such as the nitrate reductase (Kaiser et al. 2005). In addition, Mo also catalyzes the sulfite oxidase enzyme, which converts sulfite to sulfate, used in the synthesis of amino acids containing sulfur (Kaiser et al. 2005), present in the iron-sulfur proteins from photosynthesis (Nelson and Ben-Shem 2004). Thus, the improved nutrition of sorghum compared to maize and also the higher Mo and Fe acquisition under limited irrigation suggest an important response in sorghum to maintain photosynthesis under limited irrigation.

Important anatomical traits in sorghum roots related to improved water and nutrient uptake and transport to leaves were found compared to maize. It is important to note that the vascular system and particularly the xylem vessels are key parameters to transport water and nutrients from roots to leaves. The vascular system is modified under drought conditions and it is one of the main traits of drought tolerance (Kalra et al. 2024). The xylem vessel diameter is important for nutrient acquisition and can be reduced under drought in plants sensitive to water stress, limiting nutrient uptake (Cunha Cruz et al. 2020). Limited nutrient availability can increase the xylem vessel diameter (Scarpa et al. 2022), favoring its transport to shoots. Sorghum showed capacity to increase the vascular cylinder size and also the xylem vessel diameter under limited irrigation, in contrast to maize which reduced these parameters in both root regions. Thus, results indicate that the capacity to improve the vascular system under limited irrigation is a key feature of sorghum tolerance to lower water availabilities.

One final adjustment that may have improved root anatomy in sorghum under limited irrigation is its capacity to increase the root diameter maintaining the cortex thickness under such conditions in both maturation and piliferous regions. Larger roots increase its external surface, favoring water and nutrient uptake, but it is also expected to increase the cortex thickness. Sorghum roots increase their diameter and volume under limited irrigation; however, the cortex thickness remains unaltered. This is only possible because the cortical area geometry is trapezoid (Pereira et al. 2008). According to Pereira et al. (2008), the cortical area from maize increased in flooding-tolerant maize genotypes while its thickness reduced and claimed that this was possible because the cortex area can be interpreted as this equation: $CA = CT(CIP+CEP)/2$, where CA is the cortex area, CT is the cortex thickness, CIP is the cortex internal perimeter and CEP is the cortex external perimeter. This is similar to the trapezoid area in geometry it is possible to increase the area when the sum of the cortex perimeters increases, even if its thickness is reduced (Pereira et al. 2008). Results from sorghum under limited irrigation corroborate this model, increasing the total area of the root and maintaining the cortex thickness. This is important to keep the hydraulic conductivity of the root because increased cortex thickness may reduce water and nutrient uptake. This is why many species reduce their cortex thickness under drought, a response found in maize (Fig. 7 and Fig. 8). It is also important to note that the cortex is important for aerenchyma formation (Pereira et al. 2008) and by reducing its thickness aerenchyma development can be compromised, results also found for maize (Table 1). Drought-tolerant species like sorghum maintain their root circumference under drought (Yang et al. 2024). Thus, sorghum roots seem capable of increasing their size while keeping their conductivity by not modifying the cortex thickness.

5 Conclusion

Sorghum shows higher photosynthesis, growth, and water and nutrient acquisition compared to maize under limited water availability. The higher photosynthetic and growth capacity of sorghum compared to maize under limited irrigation can be related to its higher capacity to develop aerenchyma, which improved root growth favoring water and nutrient acquisition. Sorghum shows higher N, Mg, Ca, Zn, Cu, Mo, and Fe contents compared to maize and increases Fe and Mo acquisition under limited irrigation. Sorghum shows higher vascular cylinder area and xylem vessel diameter than maize under limited irrigation, favoring water and nutrient transport to shoots.

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3 CONSIDERAÇÕES FINAIS

As mudanças climáticas vêm causando redução no volume hídrico, o que provoca alterações nos ecossistemas globais. As plantas sofrem os impactos negativos da falta de água promovendo mudanças estruturais na anatomia e fisiologia e, consequentemente, no desenvolvimento e no crescimento vegetal.

Apesar dos longos períodos de seca, existem espécies de plantas que possuem tolerância ao déficit hídrico, que podem ser uma alternativa de adaptação nos ambientais propícios a tais condições climáticas. Dessa forma, pretende-se buscar com o estudo compreender as características morfofisiológicas que regem a tolerância à seca e agregar a literatura acadêmica, principalmente para as espécies cultivadas, pois estas plantas são a base da segurança alimentar de milhões de pessoas no mundo como o milho e o sorgo.

Portanto, os resultados obtidos com este trabalho auxiliarão os programas de melhoramento genético, na produção agrícola e na preservação da biodiversidade no cenário de mudanças climáticas, bem como ajudarão a compreender como as plantas tolerantes ajustam a sua eficiência no uso da água em condições de seca.