



**YASMINI DA CUNHA CRUZ**

**GROWTH, ANATOMY AND ECOPHYSIOLOGY OF *Typha*  
*domingensis* Pres. UNDER DROUGHT AND SEASONAL  
VARIATION OF RAINFALL**

**LAVRAS – MG  
2021**

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

Prof. Dr. Fabricio José Pereira  
Orientador

Prof. Dr. Evaristo Mauro de Castro  
Coorientador

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**2021**

*À minha mãe Patrícia, minha fortaleza.  
Às minhas irmãs, Jordane e Tamara, por tanto amor e cumplicidade.  
Dedico.*

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*“(...) A vida inventa! A gente principia as coisas, no não saber por que, e desde aí perde o poder de continuação, porque a vida é mutirão de todos, por todos remexida e temperada. O mais importante e bonito, do mundo, é isto: que as pessoas não estão sempre iguais, ainda não foram terminadas, mas que elas vão sempre mudando. Afinam ou desafinam.”*

*(João Guimarães Rosa em “Grande Sertão: Veredas”, 1956).*

## RESUMO

As plantas em seu ambiente natural experimentam frequentemente períodos de baixa precipitação, altas temperaturas e mudanças no estado hídrico do solo. O déficit hídrico é um importante fator de estresse em plantas que afeta diretamente o crescimento através de modificações anatômicas, morfofisiológicas e bioquímicas. Em resposta a esse fator as plantas podem desenvolver adaptações que denotam tolerância ou sensibilidade. As respostas adaptativas dependem da duração, frequência, intensidade e período do desenvolvimento da planta quando sujeita ao estresse hídrico. O regime de precipitação influencia o nível de água nas áreas úmidas e expondo as macrófitas a variações sazonais de disponibilidade hídrica. *Typha domingensis* têm potencial invasivo e cresce em uma ampla faixa de variação no nível de água que vai de 0 a 115 cm. Por isso, a espécie sobrevive em ambientes parcialmente secos, com uma redução de cerca de 50% do potencial hídrico do solo, e em ambientes alagados com uma inundação de até 1,5 m da folha. A alta plasticidade fenotípica de *T. domingensis* denota o desenvolvimento características adaptativas que possibilitam a colonização e sobrevivência em locais considerados adversos para muitas espécies. Além disso, espécie apresenta alta capacidade de reprodução clonal e de dispersão, colonizando grandes áreas onde podem causar danos à biodiversidade. Nesse trabalho, estudamos as características anatômicas, fisiológicas e nutricionais que permitem o crescimento de plantas de *T. domingensis* cultivadas em sob condições de seca controlada e natural pela variação sazonal de chuvas.

**Palavras-chave:** Macrófitas. Déficit Hídrico. Fotossíntese. Trocas Gasosas. Absorção de Nutrientes.



## ABSTRACT

Plants in their natural environment experience periods of low rainfall, high temperatures and variations in the water status of the soil. Water deficit is an important stress factor in plants that directly affects growth through anatomical, morphophysiological and biochemical changes. In response to this factor, plants can develop adaptations that denote tolerance or sensitivity. Adaptive responses depend on the duration, frequency, intensity and period of plant development when subjected to water stress. The precipitation regime influences the flooding level in wetlands exposes macrophytes to seasonal variations in water availability. *Typha domingensis* have invasive potential and grows in a wide range of variation from 0 to 115 cm. Therefore, the specie survives in partially dry environments, with a reduction of about 50% of the soil water potential, and in environments with a flood of 1.5 m from the leaf. The high phenotypic plasticity of *T. domingensis* denotes the development of adaptive traits for colonization and survival in environment considered adverse for many species. In addition, the specie has a high capacity for clonal reproduction and dispersion, colonizing large areas where it can cause damage to biodiversity. In this study, availed the anatomical, physiological and nutritional traits that condition the growth of *T. domingensis* plants grown under controlled and natural drought conditions, due to the seasonal variation of rainfall.

**Keywords:** Macrophytes. Water Deficit. Photosynthesis. Gas exchange. Nutrient Uptake.

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## PRIMEIRA PARTE

### 1 INTRODUÇÃO GERAL

As plantas em seu ambiente natural experimentam frequentemente períodos de baixa precipitação, altas temperaturas e mudanças no estado hídrico do solo (LIPIEC et al., 2013), essas condições caracterizam períodos secos. Variações naturais no regime de precipitação, na temperatura e, conseqüentemente, no estado hídrico do solo, produzem períodos sazonais secos e úmidos (COELHO; CARDOSO; FIRPO, 2015).

Existe um consenso de que as ações antrópicas vêm alterando a temperatura global, refletindo no regime de precipitação e modificando o comportamento sazonal dos elementos climáticos em diferentes escalas em cada região do planeta, produzindo eventos extremos do clima (MARENGO, 2008). Dessa forma, muitos locais podem apresentar estações úmidas e secas mais longas e severas, além disso, o fluxo de rios e o nível de água em áreas úmidas pode ser afetado influenciando a vegetação local (CECÍLIO et al., 2010).

Em áreas úmidas há uma flutuação sazonal no nível de água relacionada ao regime de precipitação (MELLO et al., 2008). As modificações dos eventos do clima que produzem, por exemplo, períodos secos mais frequentes e prolongados (COELHO; CARDOSO; FIRPO, 2015), pode ser crítica em áreas úmidas, reduzindo consideravelmente o nível de água e expondo as macrófitas ao déficit hídrico. As respostas adaptativas a esses eventos estão relacionadas a plasticidade de cada espécie, mas em linhas gerais, o déficit hídrico prolongado é limitante ao crescimento e desenvolvimento das plantas (FANG; XIONG, 2015, CRUZ et al., 2019).

O déficit hídrico é um importante fator de estresse em plantas que condiciona modificações anatômicas, morfofisiológicas e bioquímicas (TAIZ et al., 2017). As plantas podem ser tolerantes ou sensíveis a esse fator e as respostas adaptativas dependem da duração, frequência, intensidade e do período de desenvolvimento da planta. Espécies tolerantes ao déficit hídrico desenvolvem adaptações para manter um alto potencial de água nos tecidos, prevenindo a desidratação (VADEZ, 2014). Nesse contexto, a água é o principal limitante ao crescimento de plantas. Além disso, a redução do conteúdo hídrico do solo, diminui sua solução e a disponibilidade de nutrientes em formas absorvíveis pelas plantas, influenciando as características fisiológicas e morfológicas que estimulam a produção de mecanismos de tolerância (MALAVOLTA, 2006).

As macrófitas, incluindo *Typha domingensis*, estão expostas a variações sazonais do nível de água nas áreas úmidas. Em virtude disso, apresentam adaptações que possibilitam a

sobrevivência nessas condições, como a presença de aerênquima constitutivo foliar e radicular (VIVIAN et al., 2014). O aerênquima representa uma adaptação de sobrevivência ao alagamento, que condiciona um ambiente hipóxico (VIVIAN et al., 2014). Por outro lado, essa estrutura está relacionada à resistência radicular em plantas tolerantes a seca, reduzindo os custos metabólicos na raiz (FANG; XIONG, 2015, CRUZ et al., 2020).

Plantas de *T. domingensis* ocupam áreas com uma ampla faixa de variação no nível de água, que oscila entre 0 e 115 cm (CHEN; ZAMORANO; IVANOFF, 2013). A espécie sobrevive e mantém seu crescimento populacional em ambientes parcialmente secos, com uma redução de cerca de 50% do potencial hídrico do solo (VIVIAN et al., 2014; CRUZ et al., 2019; CRUZ et al., 2020); e em ambientes alagados com uma inundação de até 1,5 m da folha, alterando a alocação de biomassa foliar (SORRELL; HAWES, 2010; CHEN; ZAMORANO; IVANOFF, 2013; VIVIAN et al., 2014).

*Typha domingensis* exibe alta plasticidade fenotípica, colonizando e sobrevivendo em ambientes considerados adversos para muitas espécies, como locais contaminados por metais (HEGAZY; ABDEL-GHANI; EL-CHAGHABY, 2011) e enriquecidos nutricionalmente (CORRÊA et al., 2017). A espécie exibe alta capacidade de reprodução clonal e taxas de dispersão elevadas, ocupando grandes áreas onde pode se tornar invasora. O potencial invasivo de *T. domingensis* associado ao seu caráter competitivo resultam no seu crescimento descontrolado, que trazem danos a biodiversidade local e prejuízos as atividades antrópicas que envolvem o uso da água (HEGAZY; ABDEL-GHANI; EL-CHAGHABY, 2011). Por isso, compreender a biologia da espécie é essencial para a definição de estratégias de controle e manejo adequados.

Considerando que os parâmetros anatômicos e fisiológicos que condicionam tolerância ao déficit hídrico em uma espécie aquática para sobrevivência e manutenção do crescimento populacional em ambientes secos não estão claramente compreendidos e, que conhecer as relações hídricas da espécie é essencial na definição de estratégias de manejo e controle; acreditamos que apesar de se comportar com as plantas tolerantes, a seca influencia negativamente esses parâmetros e, conseqüentemente, o crescimento da taboa. Nosso objetivo foi avaliar o crescimento, as características anatômicas, ecofisiológicas e nutricionais em plantas de *T. domingensis* submetidas ao déficit hídrico controlado e à variação sazonal de chuvas.

## **2 REFERENCIAL TEÓRICO**

### **2.1 Clima e variação sazonal de umidade**

O clima pode ser definido como um conjunto de condições do tempo em determinado período e em determinada área. O clima de uma região é condicionado pela interação de elementos abióticos como radiação, temperatura, velocidade de direção do vento, umidade do ar e precipitação. Essas variáveis, por sua vez, são influenciadas pela altitude, latitude, topografia, características do solo e da vegetação (ALVARENGA, 2012).

A precipitação representa um importante elemento climático que exibe variação sazonal influenciando diretamente o ciclo hidrológico e outros elementos do clima (ALVARENGA, 2012). A precipitação é o fator do clima mais afetado pelas possíveis mudanças no clima (MARENGO et al., 2015). As mudanças climáticas globais influenciadas por ações antrópicas e suas implicações apresentam previsões com base em modelos interpretativos passivos de muitas discussões (GRANDIS; GODOI; BUCKERIDGE, 2010). Contudo, há um consenso de que essas mudanças influenciam diretamente o regime de precipitação em detrimento do aumento da temperatura global e, conseqüentemente, a duração e a intensidade das estações úmida e seca.

Em linhas gerais, o aumento da evaporação da água superficial em função da temperatura elevada denota uma maior precipitação atmosférica em diferentes intensidades e regiões do planeta (CECÍLIO et al., 2010). Por isso, muitos locais tendem a experimentar estações secas mais frequentes e severas, enquanto outros podem apresentar estações úmidas prolongadas; caracterizando os eventos extremos do clima como a estiagem no sudeste brasileiro em 2014/2015 (COELHO; CARDOSO; FIRPO, 2015) e muitos outros eventos de seca e inundações em diversas regiões do país.

Na região Sudeste do Brasil, a precipitação exibe dois períodos bem característicos: um que concentra maior volume de chuvas de dezembro a fevereiro durante o verão, e outro com menor volume de precipitação, entre junho e agosto no inverno (MARENGO et al., 2015). O estado de Minas Gerais apresenta um ciclo anual de precipitação e temperatura bem característico, com inverno frio e seco e verão quente e chuvoso (NATIVIDADE; GARCIA; TORRES, 2017).

Alterações no regime de chuvas podem modificar o fluxo de rios, níveis de lagos e áreas úmidas em geral (MELLO et al., 2008), afetando a vegetação dessas áreas. A variação sazonal de umidade impõe a maioria das plantas limitações ao seu crescimento e desenvolvimento (RAMOS et al., 2010). Em condições tropicais muitas plantas apresentam crescimento vegetativo na maior parte do seu ciclo de vida em função das temperaturas

elevadas e da baixa disponibilidade hídrica. Enquanto outras espécies em condições subtropicais apresentam crescimento intenso durante a primavera e o verão, reduzindo durante o outono e inverno. Sugerindo que o crescimento tende a acompanhar a variação sazonal do clima. Em períodos de baixa temperatura e disponibilidade hídrica o crescimento pode reduzir, e nos períodos de temperatura e disponibilidade hídrica elevados ele pode ser retomado (STENZEL et al., 2005).

## **2.2 Estresse hídrico em plantas – alagamento e déficit hídrico**

As plantas se adaptam ao estresse hídrico no ambiente por meio de uma variedade de mecanismo fisiológicos, celulares e moleculares. Esses mecanismos incluem desde respostas transitórias à baixa umidade do solo até os principais mecanismos de sobrevivência por meio do florescimento precoce na ausência de chuvas sazonais (BASU et al., 2016). A intensidade dessa resposta é modulada pela intensidade, duração e taxa de progressão da supressão hídrica (PINHEIRO; CHAVES, 2011).

Abordagens distintas são utilizadas quando se trata de estresse por disponibilidade hídrica, mas de forma geral, qualquer fator que interrompe, restringe ou acelera os processos naturais de uma planta ou em suas partes é considerado um fator de estresse, podendo a planta apresentar-se resistente ou não a esse fator (SILVA et al., 2011). Contudo, se os limites de tolerância são excedidos e a capacidade adaptativa da planta é suplantada o resultado são danos permanentes ou frequentemente a morte (SILVA et al., 2011; BASU et al., 2016).

O estresse hídrico por alagamento altera as propriedades químicas, físicas e biológicas do solo, modificando o crescimento, a morfologia e anatomia das plantas sob essa condição. O alagamento provoca de imediato uma redução nos níveis de O<sub>2</sub> disponível submetendo as raízes à hipóxia, diminuindo sua permeabilidade e condutividade hidráulica (DALMOLIN et al., 2012). Em ambientes constantemente alagados, como as áreas úmidas naturais, as plantas superam constantemente os efeitos do solo hipóxico desenvolvendo adaptações anatômicas como o aumento da formação de aerênquima foliar e radicular; e fisiológicas, alterando as trocas gasosas, aumentando a resistência estomática, reduzindo a fotossíntese e a transpiração (PAREEK; SOPORY; BOHNERT, 2011; SCREMIN-DIAS; LORENZ-LEMKE; OLIVEIRA, 2011; CRUZ et. al, 2019; CRUZ et al., 2020).

O déficit hídrico é um importante fator abiótico que pode regular e limitar o crescimento e desenvolvimento, afetando as plantas a nível morfológico, bioquímico e molecular (VADEZ, 2014). A sobrevivência sob essas condições está diretamente relacionada a capacidade da planta em perceber o estímulo e produzir mecanismos de tolerância que

permitam manter o potencial hídrico nos tecidos e prevenir de desidratação excessiva (FANG; XIONG, 2015).

A fotossíntese tem o déficit hídrico como seu principal redutor, uma vez que ele exerce efeito sobre componentes estomáticos e não estomáticos. Para manter os processos fisiológicos sob baixa disponibilidade hídrica, umas das primeiras estratégias para reduzir a perda de água por transpiração e manter o turgor celular é o fechamento dos estômatos. Contudo, a redução da condutância estomática reflete em limitações na assimilação de CO<sub>2</sub> e, conseqüentemente, na biomassa (ANJUM et al., 2011; LIPIEC et al., 2013). Essa é uma dualidade enfrentada por plantas sob condições de deficiência hídrica e é muito bem relatada na literatura.

As respostas fisiológicas e bioquímicas ao déficit hídrico inicialmente resultam na aclimatação, que está relacionada a plasticidade fenotípica da planta em resposta a variações ambientais (TAIZ; ZEIGER, 2009). Posteriormente, com o aumento da severidade do déficit hídrico ocorrem danos funcionais (BASU et al., 2016). As estratégias ou mecanismos de resistência ao déficit hídrico vêm sendo classificadas em três categorias: escape, evitação e tolerância (TAIZ; ZEIGER, 2009; FANG; XIONG, 2015).

No escape a planta completa seu ciclo de vida antes do início de períodos secos, modulando seu crescimento vegetativo e reprodutivo em função da disponibilidade de água por dois mecanismos diferentes: rápido desenvolvimento fenológico e plasticidade de desenvolvimento. Essa é uma estratégia observada em plantas anuais (FANG; XIONG, 2015). Na evitação, plantas sob seca moderada e leve exibem a habilidade de manter maior teor de água nos tecidos, apesar do teor reduzido de água no solo (TAIZ; ZEIGER, 2009). Isso é obtido por meio de uma variedade de características adaptativas que envolvem a minimização da perda de água e a otimização da captação de água (BASU et al., 2016); como por exemplo, o desenvolvimento de um sistema radicular mais responsivo à seca capaz de explorar recursos em camadas mais profundas do solo (JARAMILLO et al., 2013), o fechamento estomático e o desenvolvimento de uma cutícula mais espessa (LIPIEC et al., 2013). O mecanismo de tolerância em plantas sob restrição hídrica considerável no solo, envolve o desenvolvimento de características adaptativas que permitem a sobrevivência (FANG; XIONG, 2015).

### **2.3 Aspectos gerais de *Typha domingensis* Pers.**

A família Typhaceae (Poales) é representada por espécies de distribuição cosmopolita que ocupam de regiões tropicais a temperadas (CARVALHO et al., 2014). Atualmente são reconhecidos dois gêneros na família: *Typha* L. e *Sparganium* L. (APG III, 2009).

*Sparganium* apresenta cerca de 19 espécies que não ocorrem nos neotrópicos, distribuídas principalmente no hemisfério norte (KUN; SIMPSON, 2010). *Typha* é um gênero nativo da América do Sul com distribuição cosmopolita, que apresenta cerca de 15 espécies morfológicamente semelhantes (REITZ, 1984), das quais 3 ocorrem no Brasil: *Typha angustifolia*, *Typha domingensis* e *Typha latifolia* (BOVE; PAZ, 2010).

As espécies do gênero *Typha* com ocorrência no Brasil são popularmente conhecidas como taboa, mas possuem diversos sinônimos como erva-de-esteira, pau-de-lagoa, paineira-do-brejo, paina-de-flecha, entre outros. Na língua inglesa são conhecidas como cattail (rabo-de-gato) devido à inflorescência em espiga característica dessas plantas (REITZ, 1984). *Typha* sp. são monocotiledôneas helófitas, cujo sistema caulinar e radicular está em contato com a água e o solo e as folhas são aéreas. O sistema radicular apresenta raízes adventícias que se originam do caule formando o sistema radicular fasciculado. O caule é um rizoma e as folhas são verticais, paralelódromas e com mesófilo simétrico (BOEGER; DE OLIVEIRA PIL; BELÉM FILHO, 2007).

Amplamente distribuída no Brasil *Typha domingensis* Pres é uma planta perene, herbácea, aquática, que pode atingir até 3 m de altura e exibe alta propagação por sementes e vegetativa. A espécie forma agrupamentos densos nas margens de lagos, reservatórios, canais de drenagem e várzeas (CANALLI; BOVE, 2017). A alta capacidade de propagação e competição da espécie podem resultar no seu crescimento descontrolado e dominância em áreas úmidas onde o nível de água costuma flutuar. Nesses ambientes, o crescimento descontrolado da taboa pode causar danos à biodiversidade local e às atividades antrópicas que envolvem o uso da água (HEGAZY; ABDEL-GHANI; EL-CHAGHABY, 2011).

Anatomicamente as plantas de *T. domingensis* apresentam folhas anfiestomáticas e epiderme unisseriada com cutícula delgada. Abaixo da epiderme são observadas cerca de três camadas de parênquima paliçádico com células densas em função da grande quantidade de cloroplastos. As câmaras de aerênquima ocupam a maior parte do mesófilo e estão associadas ao parênquima da trabécula. Esse tecido é composto por células parenquimáticas que circundam as câmaras de aerênquima e apresentam baixo custo estrutural (BOEGER; DE OLIVEIRA PIL; BELÉM FILHO, 2007). Os feixes vasculares são colaterais fechados, com xilema e floema localizados entre o parênquima paliçádico das faces abaxial e adaxial (SANTOS et al., 2015).

O crescimento de *T. domingensis* é influenciado pela variação do nível de água em áreas úmidas que expõe as populações a períodos de alagamento (CHEN; ZAMORANO; IVANOFF, 2013; MIAO; ZOU, 2012; VIVIAN et al., 2014), e seca (CRUZ et al., 2019;



VIVIAN et al., 2014), fatores nutricionais (SANTOS et al., 2015) e o adensamento populacional (CORRÊA et al., 2015). A espécie cresce em uma ampla faixa de variação no nível de água que vai de 0 a 115 cm. Nos períodos em que a profundidade de inundação é maior o alagamento das folhas reduz a área fotossintética ativa e o crescimento (CHEN; ZAMORANO; IVANOFF, 2013). Nessas condições, há um aumento na alocação de biomassa para a parte aérea, ou seja, ocorre um investimento em área foliar a fim otimizar a captação de luz (MIAO; ZOU, 2012).

Períodos de menor disponibilidade de água nas áreas úmidas expõe as populações de taboa a profundidades de inundação reduzidas com cerca de 20 a 30 cm e em alguns casos o solo pode ficar saturado, sem alagamento das folhas (VIVIAN et al., 2014; CRUZ et al., 2019). Curiosamente, apesar de sua natureza aquática, as plantas de *T. domingensis* toleram uma redução de 50% do potencial hídrico do solo mantendo o crescimento populacional, entretanto uma redução de cerca de 25% é limitante a sobrevivência (CRUZ et al., 2019).

Bastante empregada na fitorremediação *T. domingensis* coloniza áreas contaminadas por diversos tipos de resíduos promovendo a purificação de águas residuais (HEGAZY; ABDEL-GHANI; EL-CHAGHABY, 2011). A espécie é bastante utilizada na construção de áreas úmidas artificiais para tratamento conhecidas como ‘constructed wetlands’ (GOMES et al., 2014). Estudos experimentais têm revelado que a taboa exibe alta capacidade em absorver fósforo, por isso exibe adaptações típicas para ambientes enriquecidos nutricionalmente (CORRÊA et al., 2017; HEGAZY; ABDEL-GHANI; EL-CHAGHABY, 2011; SANTOS et al., 2015). Por esse motivo, a taboa é competitivamente mais eficiente em ambientes eutrofizados.

Além das aplicações mencionadas, os rizomas da taboa apresentam potencial nutricional semelhante ao do milho e da batata, por isso ela é comumente utilizada no forrageio animal. A espécie é empregada como fonte de matéria prima para obtenção de celulose para a fabricação de papel e no artesanato, na construção de cestos e esteiras representando uma fonte de renda (JAHAN et al., 2007). Apesar dos aspectos positivos, os danos causados por espécies do gênero *Typha* não devem ser negligenciados. A planta é invasora de áreas úmidas e em condições favoráveis ao seu desenvolvimento se torna dominante, transformando essas áreas em tabuais e acelerando o processo de colmatação (HEGAZY; ABDEL-GHANI; EL-CHAGHABY, 2011). Por isso é fundamental conhecer a biologia de espécie para obtenção de dados que auxiliem no seu controle e manejo.

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**SEGUNDA PARTE**

**ARTIGO 1: SEASONAL RAINFALL VARIATION IN THE POPULATION  
GROWTH AND ECOPHYSIOLOGY OF *Typha domingensis***

**MANUSCRIPT SUBMITTED TO THE JOURNAL OF PLANT RESEARCH**

## Seasonal rainfall variation in the population growth and ecophysiology of *Typha domingensis*

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Ecology/Ecophysiology/Environmental Biology

### ABSTRACT

Precipitation is an important climatic element that defines the hydrological regime, and its seasonal variation produces annual dry and wet periods in some areas. This seasonality changes wetland environments and leverages the growth dynamics of macrophytes present, including *Typha domingensis* Pers. This study aimed to evaluate the influence of seasonal variation in precipitation on the growth, anatomy and ecophysiology of *T. domingensis* specimens from native populations. Thus, biometric, anatomical and ecophysiological traits of *T. domingensis* were evaluated over one year at four-month intervals. Reductions in photosynthesis were evidenced at the end of the wet periods and during the dry periods, and these reductions were associated with thinner palisade parenchymas. Increased stomatal indexes and densities as well as thinner epidermis observed at the beginning dry periods can be associated with higher transpiration rates during this period. The plants maintained their water contents during the dry periods, which may be related to the storage of water in leaf parenchymas. In addition, increasing proportions of aerenchymas were evidenced during the wet periods, which may be related to a compensation mechanism for soil waterlogging. Therefore, the growth, anatomy and ecophysiology of *T. domingensis* plants change throughout the year to adjust to both the dry and wet periods, providing conditions for the survival of the plants and modulating population growth.

**Keywords:** Macrophytes. Cattail. Precipitation. Photosynthesis. Anatomy.



## Introduction

The climate of a region is determined by atmospheric elements such as temperature, air humidity and precipitation over a given period of time. These variables are influenced by altitude, latitude, topography, and soil and vegetation characteristics (Alvarenga 2012). Precipitation is an important climatic element that affects the hydrological cycle and other elements of the climate, and in some regions, seasonal variations in precipitation produce dry and wet periods. The southeastern region of Brazil, where this work was carried out, has a well-defined annual cycle with two characteristic periods: a wet period, in which the highest concentration of precipitation volume occurs, lasts from December to February, and a dry period of lower precipitation occurs between June and August (Marengo et al. 2015).

Wetlands are constantly modified by hydrological regimes that alter the water level after extreme weather events such as prolonged periods of rain or drought (Vivian et al. 2014). In response to these variations, aquatic plants exhibit changes in their growth, development, distributions, abundances and compositions, as has been shown by both field or laboratory studies (Miao and Zou 2012; Chen et al. 2013; Vivian et al. 2014; Cruz et al. 2019).

Seasonal variations in rainfall impose limitations on the growth and development of most plants (Ribeiro et al. 2010). Under tropical conditions, some plants have vegetative growth during most of their life cycles due to high temperatures and low water availabilities. In contrast, some species in subtropical conditions show intense growth during spring and summer and decreased growth during autumn and winter (Stenzel et al. 2005). Growth tends to accompany the seasonal variations in the climate. In periods of low temperatures and water availabilities, growth may decrease, while in periods of high temperatures and water availabilities, growth may resume (Stenzel et al. 2005).

*Typha domingensis* Pers. grows over a wide range of flood depths, from 0 to 115 cm (Chen et al. 2013). In periods of high water availability, the leaves of *T. domingensis* become flooded, reducing its growth parameters (Miao and Zou 2012; Chen et al. 2013; Vivian et al. 2014) and photosynthesis (Chen et al. 2013) and changing its biomass allocation and nutrient uptake (Miao and Zou 2012; Chen et al. 2013). To survive such excessive flooding periods, the leaves of *T. domingensis* develop internal aeration tissue known as aerenchyma to increase the oxygen supply (Corrêa et al. 2017). However, periods with low water availability expose *T. domingensis* to shallow flooding (20-30 cm tall) or even drought situations (Vivian et al. 2014; Cruz et al. 2019). *T. domingensis* can survive under up to a 50% reduction in water potential in the substrate; however, restrictions to growth and photosynthesis are found under these conditions (Cruz et al. 2019).

The excessive growth of aquatic macrophytes, including *T. domingensis*, may be related to seasonal variations in precipitation and to the water level in wetlands (Pompêo 2017). Most climate change models predict reductions in the volume and frequency of precipitation events that may cause reductions in water levels in natural wetlands (Barros and Albernaz 2014). The invasive potential of *T. domingensis* can be related to prolonged periods of low water levels (Lishawa et al. 2010). Periods of higher macrophyte growth coincide with the management of this species when low water levels are found in wetlands (Pompêo, 2017). Considering that wetlands are strongly changed by the water regime and that the growth of aquatic macrophytes is regulated by seasonal variations, it is essential to define control and management strategies for uncontrolled growth based on the seasonal growth of these plants. The hypothesis of this work is that seasonal rainfall variation influences the growth of *T. domingensis*. Thus, the objective of this study was to evaluate the growth dynamics, leaf anatomy and ecophysiology of *T. domingensis* plants over one year.

## **Materials and methods**

### **Study area, plant material and experimental design**

The plants used in this study were collected in the city of Alfenas (MG) in a native population of *Typha domingensis* Pres. (Fig. 1). The sampled population is located in an isolated wetland with no direct connection to a drainage network or a river basin. The municipality of Alfenas (21° 25' 46" S, 45° 56' 50" W) is located in the southern region of the state of Minas Gerais, Brazil, at a mean altitude of 888 m (Fig. 1). The predominant climate in the study area is classified as Cwb (Mendes Júnior et al. 2018) a warm and rainy temperate climate type, with dry winters and warm summers according to the Köppen-Geigen (1928) and Alvares et al. (2013) classifications more information is given in Fig. 2A and 2B.

The collection of plant material was carried out over a period of one year at four-month intervals in April 2018, August 2018, December 2018 and April 2019. We defined four rainfall conditions based in the accumulated precipitation during the four months previous to sampling dates. Based on the climatic information of precipitation (Fig. 2) obtained by SISMET (2020), we considered that plants sampled in April 2018 were collected under highest precipitation (HP), as the accumulated precipitation was 738,1 mm. In August 2018 the accumulated precipitation was 83 mm and this was the lowest precipitation (LP) in the sampling period. Precipitation increased from August to December 2018 as the accumulated precipitation was 564 mm and we considered this period as increased precipitation (IP). In

April 2019 the accumulated precipitation was 654,6 mm and we considered this as a decreased precipitation (DP) as compared with the same period in 2018.

The water potential of the soil was measured with a tensiometer, and in all samples, the water potential was recorded as 1 MPa. However, the soil was saturated during the dry periods and waterlogged during the wet periods with a layer of water above the soil surface (a few centimeters tall, but the water depth varied greatly in the area). In each sampling, 25 plants were collected with fully expanded leaves in the vegetative stage, and each plant was at least 1.5 m in height. The experimental design was completely randomized with four treatments and 25 replicates of one plant.

### **Biometric analysis**

The height and number of leaves of each plant were evaluated at the sampling site. The sampled individuals were taken to the Federal University of Lavras, where the fresh leaves were scanned to obtain images that were used to measure the leaf area with ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). Subsequently, the leaves were weighed on an analytical scale (Shimadzu, Japan) to obtain the fresh mass and then oven-dried at 60°C for 72 hours. After this period, the plants were weighed on an analytical balance to obtain the dry mass. The water content (WC) of the plants was obtained by the following equation:  $WC = [(FM - DM)/FM] * 100$ , and the results are expressed as percentages, where FM is fresh mass and DM is dry mass.

### **Gas exchange analysis**

Gas exchange analysis was performed using an infrared gas analyzer model LI-6400XT (LI-COR Biosciences, Lincoln, Nebraska, USA). The analyses were carried out at the middle section of the third mature leaf. The evaluations were carried out between 8 am and 10 am, and the density of photosynthetic photon flow (PPFD) fixed in the device chamber was 1000  $\mu\text{mol m}^2 \text{s}^{-1}$ . This PPFD value was defined based on previous tests of light saturation in *Typha domingensis* plants to avoid photoinhibition or lack of radiation (Santos et al. 2015). The water use efficiency (WUE) of each plant was obtained by the photosynthetic rate/transpiration ratio (Pn/E). Whole plant photosynthesis (WPP) and whole plant transpiration (WPT) were obtained for each plant using the product photosynthetic rate and leaf area (Pn \* leaf area) and the transpiration rate and leaf area (E \* leaf area), respectively, according to the method outlined in Cruz et al. (2019). The total stomatal conductance (Tgs) of each leaf was calculated by the product between the punctual stomatal conductance (gs)

and leaf area, according to the equation:  $Tgs = (gs * \text{leaf area})$ . Chlorophyll content analysis was performed using SPAD-502 (Konica Minolta Sensing, USA). The readings were taken at two points in the terminal region of the third leaf from the center on each plant by repetition.

### **Anatomical analysis**

Fragments (10 cm in length) were taken from the middle section of the third mature leaf of each plant, fixed in a solution of formaldehyde, acetic acid and 70% ethanol (F.A.A. 70%), and further preserved in 70% ethanol (Kraus and Arduin 1997). Paradermal leaf sections were obtained from the abaxial and adaxial surfaces of each leaf using steel blades. Then, the sections were clarified with 50% sodium hypochlorite and washed with distilled water three times for 10 minutes. The sections were then stained with safranin solution and mounted on slides with 50% glycerol (Johansen 1940). The slides were photographed with a Lumenera Infinity 1-1M camera attached to a Nikon eclipse E100 light microscope (Nikon, Tokyo, Japan), and the photomicrographs obtained were used to evaluate the anatomical leaf characteristics using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). For each replicate, five sections and five fields were measured, and data were averaged for each replicate. The following anatomical traits were evaluated: the number of stomata (SN), the number of epidermal cells (EC), the length (EL) and width (EW) of stomata and the EL/EW ratio. The stomatal index (SI) was calculated as follows:  $SI = [SN / (SN + EC)] * 100$ . The stomatal density (SD) and epidermal cell density (ECD) were expressed as the number of each structure per square millimeter.

For the transverse sections, the samples were dehydrated in a crescent ethanol series of 70, 80, 90 and 100% ethanol (Johansen 1940). After dehydration, the material was immersed in a preinfiltration solution composed of 100% ethanol and base resin in a 1:1 ratio for 24 hours according to the manufacturer's instructions (Leica Microsystems, Wetzlar, Germany). Then, the base resin was infiltrated for 24 hours at 40°C. After polymerization, the samples were sectioned on a semiautomatic rotary microtome model YD-335 (Jinhua Yidi Medical Appliance CO, Zhejiang, China). The sections obtained were approximately 10  $\mu\text{m}$  thick, and the sections were stained in a 1%  $\text{m v}^{-1}$  toluidine blue solution (Feder and O'brien 1968) and mounted on slides that were then photographed under a light microscope (Nikon eclipse E100, Nikon, Tokyo, Japan) coupled to a camera (Lumenera Infinity 1-1 M). Micromorphometry analysis was performed using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). One slide was made for each replicate and five fields, and five sections were placed on each slide. The following anatomical characteristics were

evaluated: the thicknesses of the epidermis and palisade parenchyma, the diameter of the trabecular parenchyma, the area of the leaf section (LSA) and the sum of the area of each aerenchymal chamber (ACA). The proportion of aerenchyma (AE%) was calculated as follows:  $AE\% = (ACA/LSA) * 100$ , and the results are expressed as percentages.

### **Statistical analysis**

The experimental design was completely randomized with four treatments and 25 replicates of one plant. All obtained data were averaged for each replicate. The data were submitted to analysis of variance (ANOVA), and the means were compared using Scott-Knott's test to  $p < 0.05$  using Sisvar statistical software (Ferreira 2011). The data showed a normal distribution according to the Shapiro-Wilk test.

### **Results**

The number of leaves was higher at LP than at other periods; no significant differences were found among the other periods (Fig. 3A), and the plant height (Fig. 3B) was higher at HP than at other periods. The fresh (Fig. 3C) and dry (Fig. 3D) masses were higher at IP, and the fresh mass had lower means in the DP and HP samples. The leaf area showed the highest mean at HP and the smallest at IP (Fig. 3E). The water content was higher in the dry periods than in the wet periods (Fig. 3F).

The photosynthetic rate (Pn) and whole plant photosynthesis (WPP) were at their highest values at IP, were decreased at DP and HP but increased again at DP (Fig. 4A and 4B). The transpiration rate (E) and whole-plant transpiration (WPT) showed lower means during LP and higher means at HP (also at IP for E), with intermediate values during the wet periods (Fig. 4C and 4D). Stomatal conductance (gs) and total stomatal conductance (Tgs) showed higher means in the IP sample period (also at IP for gs) (Fig. 4E and 4F). The water use efficiency (WUE) was highest at LP and gradually decreased at IP, HP and LP (Fig. 4G). The chlorophyll content did not show significant differences throughout the year (Fig. 4H).

The stomatal index on the abaxial surfaces of the leaf samples was reduced throughout the whole dry periods, and it was also lower in IP and higher in DP and HP (Fig. 5A and 5B). The stomatal and epidermal cell densities showed similar responses on both the adaxial and abaxial surfaces of leaves, and these values were lower at IP and LP and increased at DP and HP (Fig. 5C-F and Fig. 6). The stomatal length from the adaxial surface showed little variation throughout the year, and the means were slightly lower at IP (Fig. 5G); however, on the abaxial surface, the stomatal length was more responsive to seasonal variations, as the

highest means were found at HP, the values decreased at LP and increased again during the wet periods (Fig. 5H). The stomatal widths on both sides of each leaf were large at HP and decreasing at LP and during the wet periods (Fig. 5I and 5J). Regarding the stomatal length/width ratio on the adaxial and abaxial sides (Fig. 5K and 5L), the ratio exhibited the lowest mean at HP and increased at LP and during the wet periods on the abaxial surface (Fig. 5L), and no significant variation of the ratio on the adaxial surface was found among LP, IP and DP (Fig. 5K).

The epidermal thicknesses of the adaxial and abaxial surfaces were larger at LP and IP and decreased at DP and HP (Fig. 7A and 7B). The diameter of the trabecular parenchyma was larger at LP, decreased during the wet periods and then increased again at HP (Fig. 7C and 7D). In addition, the cell morphology of the trabecular parenchyma was flattened in the wet periods and rounded in the dry periods, which suggests differences in cell turgor (Fig. 8). The adaxial palisade parenchyma was thicker at IP, and no significant differences were found among the other periods (Fig. 7E and Fig. 8). However, the abaxial palisade parenchyma showed higher means at LP, and IP and greatly decreased values at DP followed by an increase at HP (Fig. 7F and Fig. 8). The proportion of aerenchyma showed the highest mean at DP and decreased in the dry periods until IP (Fig. 7G).

## Discussion

Results showed that higher precipitation permits a recovery period for *T. domingensis* plants that uses the favorable conditions provided by this periods to avoid stress situations during the dry periods. The higher biomass production observed in the wet periods showed that growth investment starts when precipitation increases (IP) and is noticeable when plants are under the highest precipitation (HP). This effect may be related to the higher investment in photosynthetic area that is provided by the higher number of leaves and larger leaf areas measured for plants under increasing precipitation. It is important to note that the leaf area showed a gradual increase during the wet periods (HP, DP and LP) which accumulated 1475,1 mm corresponding to 72% of annual precipitation, with a peak at the beginning of the dry periods (corresponding to IP sampling), which is a transition period. Furthermore, at the lowest precipitation sampling (LP), the leaf area of *T. domingensis* decreased again to reduce excessive transpiration; however, reduced photosynthesis rates were also observed. Most plants submitted to some level of water limitation reduce growth parameters such as plant height and leaf area (Clauw et al. 2015; Olson et al. 2018). In addition, reductions in leaf traits limit photosynthetic efficiency but reduce the area available for water loss by transpiration,

favoring survival (Chen et al. 2013; Clauw et al. 2015; Cruz et al. 2019). In fact, the transpiration (E) and whole plant transpiration (WHT) of *T. domingensis* were higher in higher precipitation samples (IP, DP and HP) and were reduced significantly at the LP sample. Thus, *T. domingensis* responds to lower rainfall by reducing its leaf area, permitting its survival at the cost of growth capacity.

*Typha domingensis* under controlled drought conditions also showed reduced photosynthesis and leaf area, which limited its growth (Cruz et al. 2019). Reductions in leaf area limit the transpiration surface and, consequently, the area available for light interception, decreasing the area over which photosynthesis can occur (Weraduwege et al. 2015). The photosynthetic rate and whole plant photosynthesis of *T. domingensis* did not appear to be affected by the precipitation in its chlorophyll content, as shown in Fig. 3H, which suggests that photosynthetic and growth limitations promoted by precipitation variations may be related to structural traits. Thus, the higher photosynthesis observed at the increasing precipitation (IP) periods may be related to thinner palisade parenchyma and reduced leaf areas. The net photosynthesis can be directly related to the palisade parenchyma in *T. domingensis* (Santos et al. 2015), and a reduction in photosynthesis may be related to thinner leaves, which may in turn be related to thinner palisade parenchyma (Shipley et al. 2005). Likewise, the increases observed in photosynthetic parameters under increasing precipitation periods may be related to increased palisade parenchyma thicknesses in *T. domingensis* leaves.

Another plant response that serves to decrease water loss by transpiration consists of reducing stomatal conductance. However, closing stomata reduces the CO<sub>2</sub> supply in leaves, limiting photosynthesis and biomass production (Silva et al. 2013). This is a duality that plants may face to survive low water availabilities, and it is very well reported. Plants growing in environments with low water availabilities tend to increase their stomatal densities and indexes to improve their capacities to control transpiration (Melo et al. 2007; Moroke et al. 2011). The interpretation of this behavior is related to a quick stomatal opening sufficient to capture CO<sub>2</sub> but reducing water loss (Melo et al. 2007; Fang and Xiong 2015). Species that are not tolerant to drought may decrease their stomatal densities to avoid excessive transpiration and water loss (Anupama et al. 2019; Cruz et al. 2019); these plants may survive but at a cost to their biomass production and growth. Under severe drought conditions and despite its survival, *T. domingensis* does not show efficient stomatal control of water loss when compared with drought-tolerant plants, and this was associated with its typical behavior as an aquatic macrophyte (Cruz et al. 2019). The increases in the stomatal indexes and densities observed in the highest precipitation samples may be related to the higher transpiration

rates found during this period. This situation is out of the control of the plants, and *T. domingensis* plants may die if this condition lasts for too long. Then, new leaves are formed with improved anatomies, and at the lowest precipitation (LP) periods, transpiration is strongly reduced. These anatomical adjustments were observed in different tissues and structures. The first modifications to be highlighted are the observed reductions in both the stomatal densities and indexes, which served to reduce stomatal conductance and transpiration. These reductions increased the water use efficiency of *T. domingensis* plants in natural wetlands, permitting their survival but with lower growth capacities under limited precipitation.

The increased WUE observed at the lowest precipitation period (LP) is an important feature because it shows that *T. domingensis* plants can effectively reduce water loss but still show photosynthetic activity. At LP periods, stomatal conductance and plant transpiration were reduced, and photosynthesis started to increase, which caused an increase in water use efficiency. The control of transpiration is not exclusively related to stomatal density, as stomatal opening is also an important feature in that regard (Fang and Xiong 2015). In addition, other stomatal traits may have contributed to the reduced transpiration measured in *T. domingensis* leaves at the LP periods. For instance, at the highest precipitation (HP) periods, *T. domingensis* plants showed the highest transpiration values as well as larger stomatal sizes, as shown by the stomatal length (L), width (W) and L/W ratio values. Larger stomata at higher densities increase the stomatal conductance and transpiration; thus, at the LP periods, the observed reduction in stomatal size also promoted a certain degree of control of water loss by transpiration. This result may be related to the higher water availability in the previous period (HP). However, the increased transpiration observed at the HP periods may not be solely related to stomatal control in *T. domingensis*, as the thinner leaf epidermises measured in this period favor water loss by nonstomatic transpiration. Most plants grown under lower water availabilities, high temperatures or radiation intensities increase their cuticle production to reduce water loss (Clauw et al. 2015; Fang and Xiong 2015). However, as *T. domingensis* is an aquatic plant, its cuticle is very thin and is not usually evaluated in leaf anatomical investigations of the species (Boeger et al. 2007; Santos et al. 2015; Corrêa et al. 2017; Cruz et al. 2019). Furthermore, we did not observe detectable changes in the cuticle thickness of *T. domingensis* due to precipitation variations, which presents a problem for water use efficiency associated with reducing nonstomatic transpiration. Thus, the increased transpiration observed at the HP periods may also be related to nonstomatic water loss; to avoid dehydration, plants may have reduced their stomatal sizes and numbers. This is an



important adaptation mechanism, but this mechanism had consequences associated with reduced biomass production due to lowered photosynthesis rates. A reduction in plant biomass is a common response in most plants under water stress (Achten et al. 2010; Miao and Zou 2012; Chen et al. 2013; Silva et al. 2013; Cruz et al. 2019). Drought periods represent a reduction in the water level in wetlands, which reduces leaf flooding for emergent macrophytes (Miao and Zou 2012), reducing leaf biomass production in these plants (Miao and Zou 2012; Chen et al. 2013). It is important to note that detailed anatomical and gas exchange evaluations are rare in studies of water stress in aquatic macrophytes. Thus, as similar growth responses are usually reported for most macrophyte species under water-limited conditions, the leaf adaptations found in *T. domingensis* may also be present in other emergent aquatic plants, including reduced stomatal traits that limit transpiration but increase CO<sub>2</sub> uptake and biomass production under lower precipitations.

Despite their growth limitations, *Typha domingensis* plants showed the capacity to preserve their leaf water contents, which was important to maintain some photosynthesis and growth rates. This was an interesting and unexpected result and may be related to the trabecular parenchymas acting as seasonal aquiferous parenchymas. This work is the first to propose such a function of trabecular parenchymas because detailed anatomical functional analyses are quite rare for these plants. Aquiferous parenchymas are related to water storage and are mainly observed in plants that evolved in dry environmental conditions and have xeric traits, such as those found in Bromeliaceae species (Santos-Silva et al. 2013). Aquiferous parenchymas are permanent tissues in some xeric plants but have not been reported for aquatic plants. In fact, *T. domingensis* does not have a true aquiferous parenchyma, but the trabecular parenchyma may act in a similar way, similar to a convergent response to reduced water levels. The main feature of the aquiferous parenchyma is its capacity to store water in xerophytes. *Typha domingensis* plants not only preserved their leaf water contents during the dry periods, but these water contents were also higher in the dry periods than those during the wet periods (Fig. 3F). This water is found inside cells because intercellular spaces cannot retain this liquid inside plant organs. Additionally, most leaf tissues became thinner during the dry periods, but the trabecular parenchyma increased its cell diameter, becoming visibly rounded and suggesting higher turgidity, while in the wet periods, these cells were clearly flattened (Fig. 8). This change in the morphology associated with a higher water content during the lowest precipitation period suggests that the trabecular parenchyma has the capacity to store some water and contribute to the growth and survival of *T. domingensis* during the dry periods. The trabecular parenchyma of *T. domingensis* extends throughout the

leaf, has a structural role in the aerenchyma and shows a low metabolic cost (Boeger et al. 2007). The structural features of the trabecular parenchyma are well understood for aquatic macrophytes, but as its cells remain alive throughout the year, they may also assume secondary functions such as the water storage reported in this work for *T. domingensis* plants during low precipitation periods.

The increased proportion of leaf aerenchyma in the wet periods (HP, IP and DP) represents an important response taken to avoid hypoxia stress in *Typha domingensis* shoots. The leaves of *T. domingensis* show constitutive aerenchymas, and the development of these tissues is essential for aquatic macrophytes because flooding reduces oxygen availability (Corrêa et al. 2017; Chen et al. 2013). It is important to note that the shoots of *T. domingensis* mainly comprise leaves that show constant growth due to the presence of an intercalary meristem at their base (Corrêa et al. 2017). Oxygen limitation to the intercalary meristem of the leaf may damage its activity; thus, higher aerenchyma proportions in leaves may provide oxygen to these tissues and to other leaf tissues. At the LP periods when there is no water layer above the soils, the aerenchyma proportions in leaves decreases, as a higher investment in trabecular parenchyma is more functional under these conditions.

During the dry periods, the water content of the soil was reduced because of lower rainfall, but no noticeable impact on the population structure of *T. domingensis* was evident. The results showed that plants evaluated at the beginning of a given periods may display the accumulated effects of the previous periods. Thus, seasonal effects on *Typha domingensis* are indeed detectable with ecophysiological and anatomical methods, but the dates of sampling must consider a longer period under a given condition for these effects to become evident.

## **Conclusions**

Precipitation variations promote modifications in the photosynthesis rate, transpiration rate, growth rate and anatomy of *Typha domingensis*. The wet periods promotes higher photosynthesis rates and growth capacities of *T. domingensis*; however, the dry periods also permits a certain degree of photosynthetic rate and biomass production. *Typha domingensis* leaves are responsive to seasonal water availability, and high transpiration rates may trigger the development of smaller stomata at lower densities to reduce water loss. The limitation of *T. domingensis* photosynthesis under low precipitation periods is related to its lower stomatal capacity to uptake CO<sub>2</sub>. Some leaf tissues, particularly the trabecular parenchyma, may serve as water storage tissues during the dry periods.

### Acknowledgments

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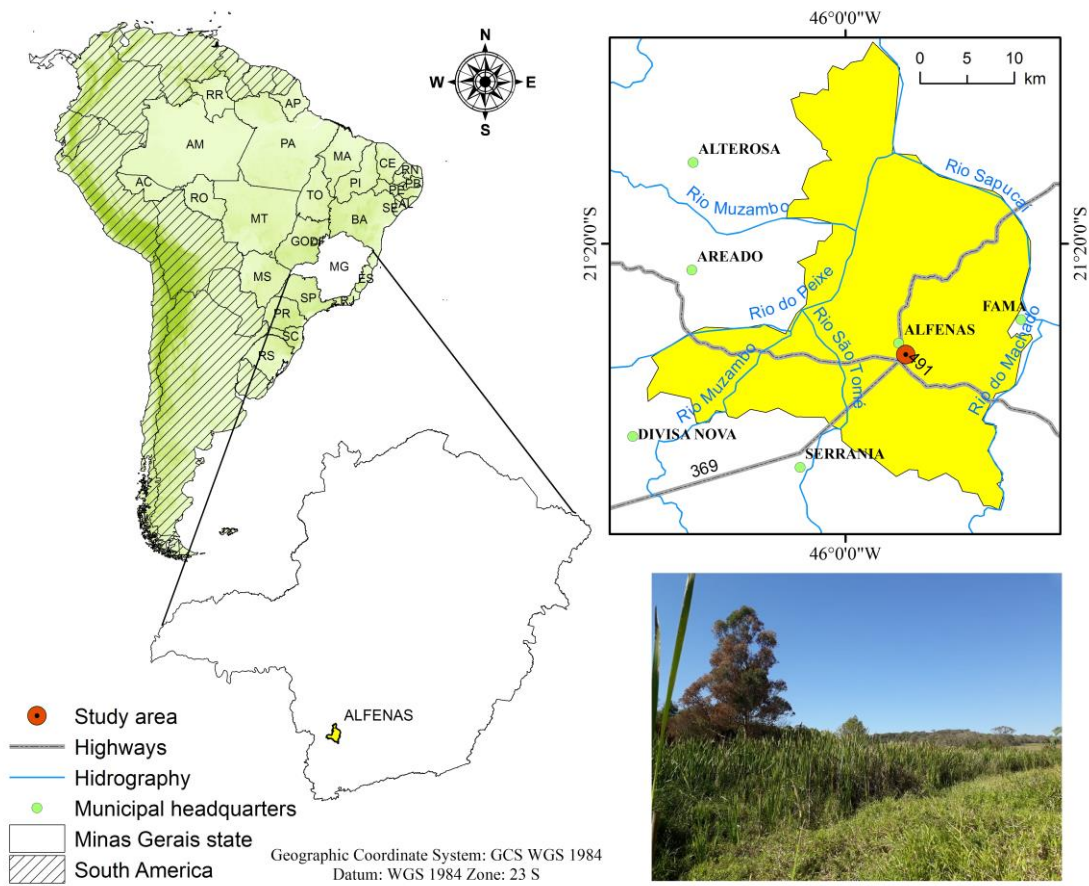


Fig. 1 Location and characterization map of municipality of Alfenas (study area).



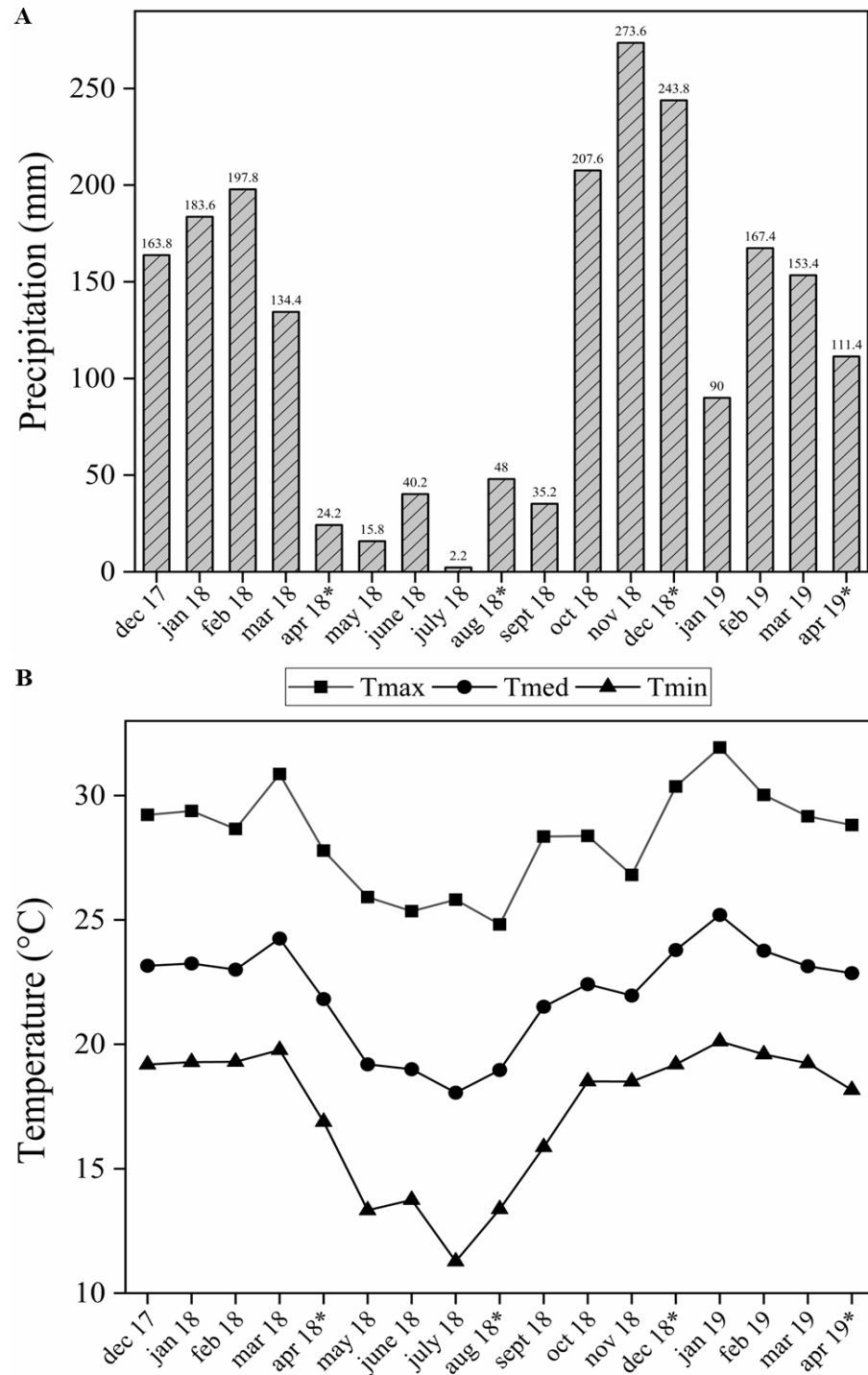


Fig. 2 Climatogram of the study area in the sampling period. A: Average values of precipitation throughout the sampling period; B: Average maximum, mean and minimum temperatures throughout the sampling period. Months followed by \* represent plants sampling periods.

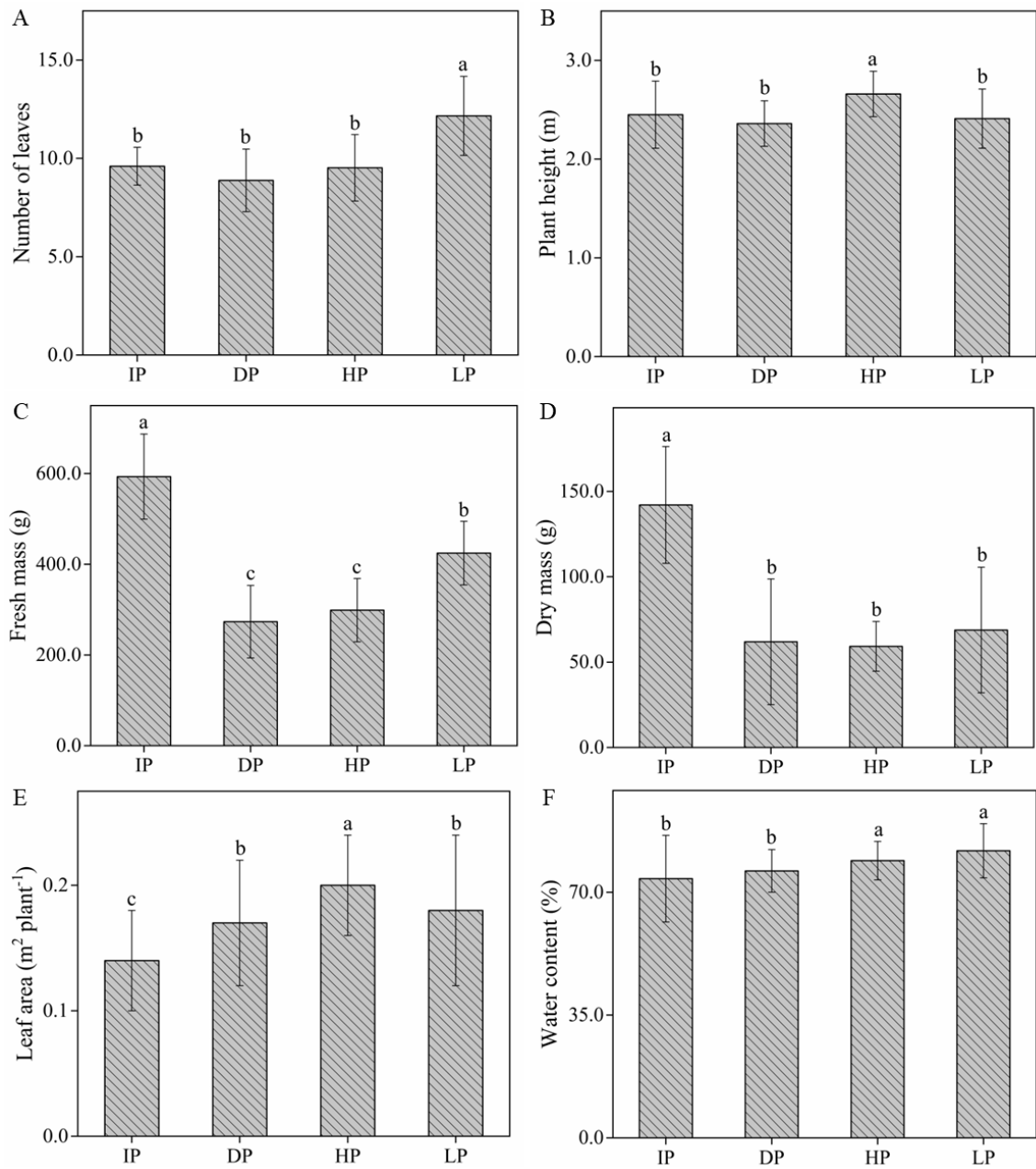


Fig. 3 Growth parameters of *Typha domingensis* under seasonal rainfall variation. IP: increased precipitation; DP: decreased precipitation; HP: highest precipitation; LP: lowest precipitation. The bars correspond to the standard errors. Means followed by the same letter do not differ according to Scott–Knott's test ( $p \leq 0.05$ ).

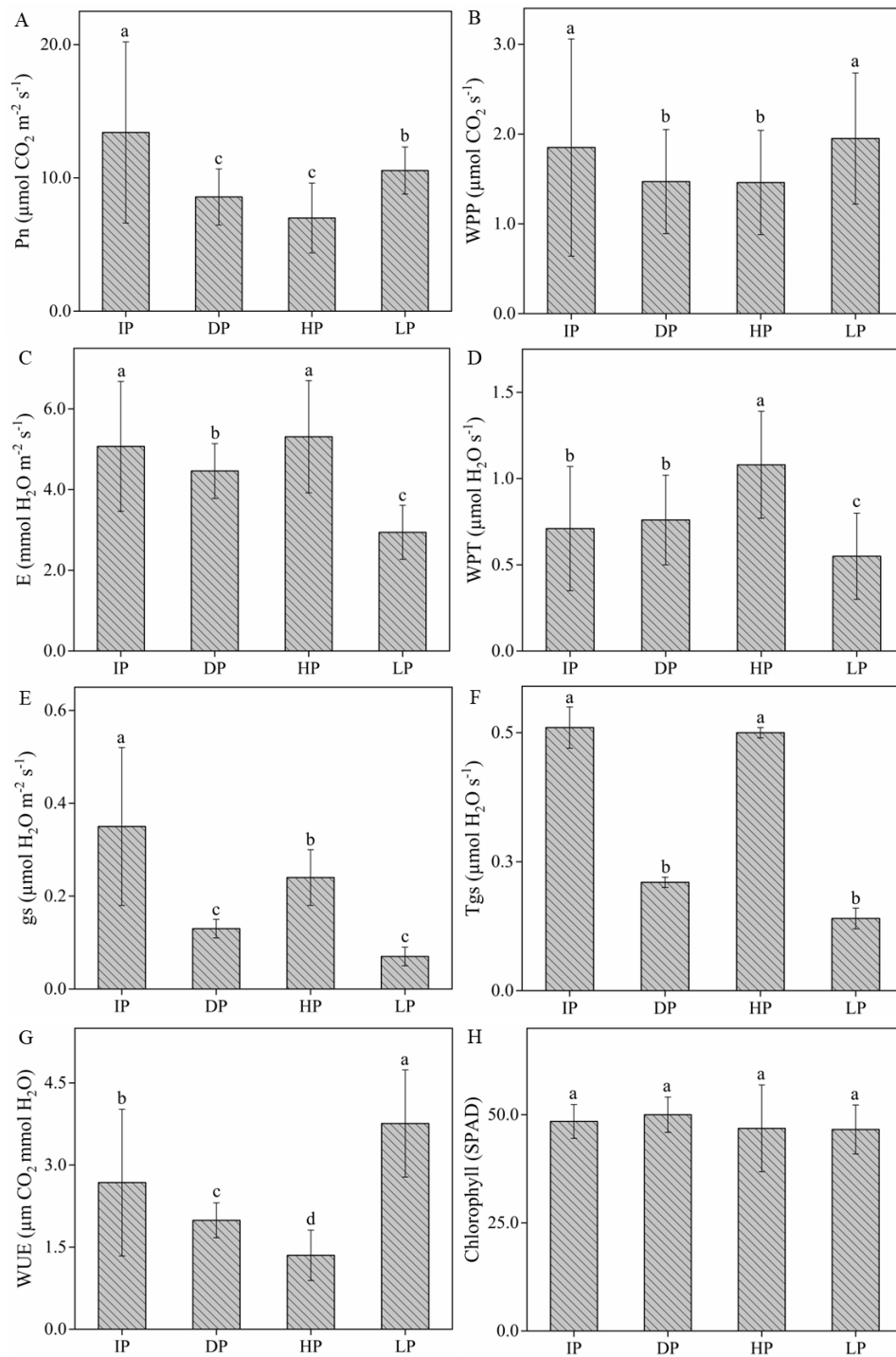


Fig. 4 Gas exchange parameters and chlorophyll contents in *T. domingensis* plants under seasonal rainfall variation. A:  $P_n$  (photosynthetic rate); B: WPP (whole plant photosynthesis); C: E (transpiration rate); D: WPT (whole plant transpiration); E: gs (stomatal conductance); F: Tgs (total stomatal conductance); G: WUE (water use efficiency). IP: increased precipitation; DP: decreased precipitation; HP: highest precipitation; LP: lowest precipitation. The bars correspond to the standard errors. Means followed by the same letter do not differ according to Scott–Knott’s test ( $p \leq 0.05$ ).

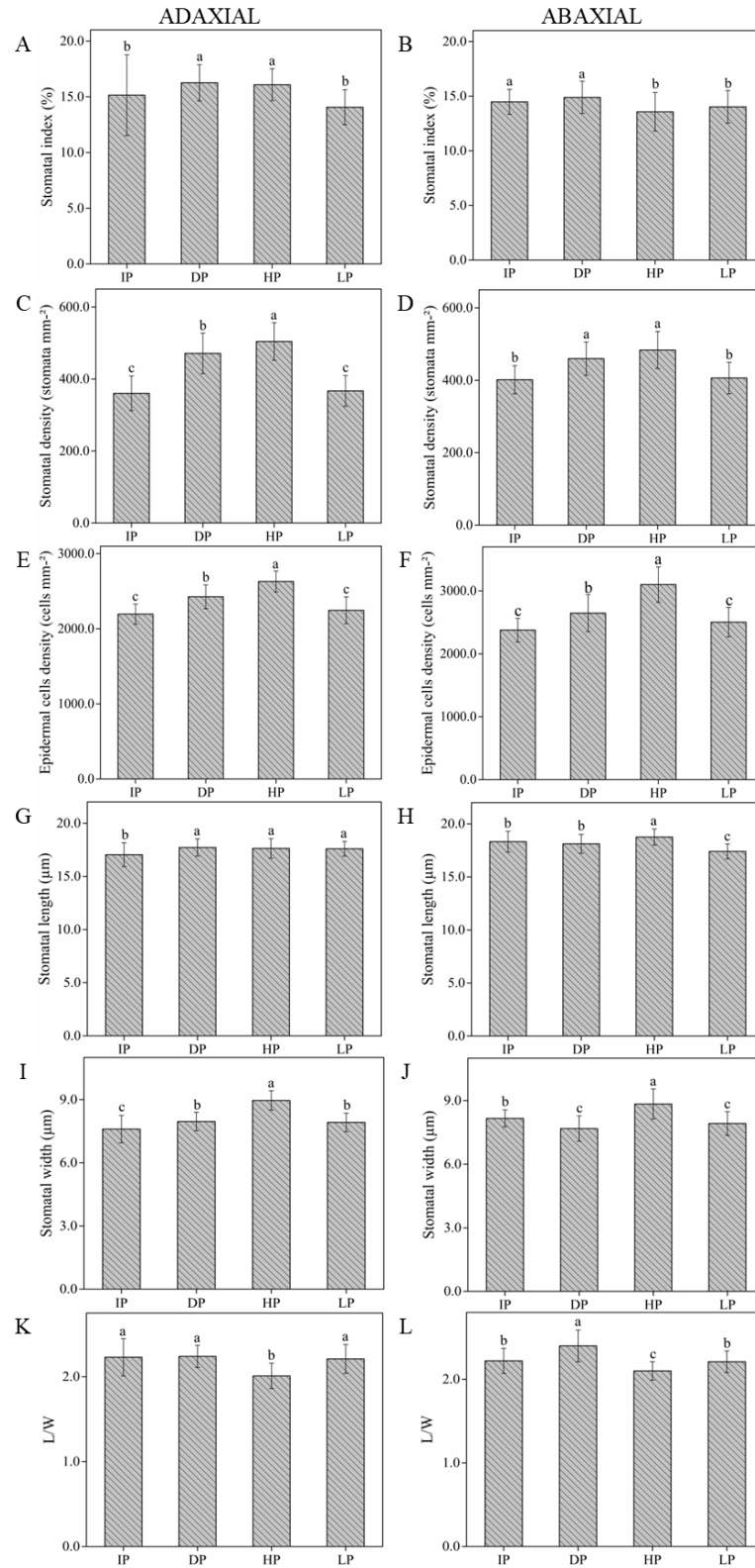


Fig. 5 Epidermal traits of *Typha domingensis* plants under seasonal rainfall variation. L: L/W (ratio between stomatal length and width). IP: increased precipitation; DP: decreased precipitation; HP: highest precipitation; LP: lowest precipitation. The bars correspond to the standard errors. Means followed by the same letter do not differ according to Scott–Knott's test ( $p \leq 0.05$ ).

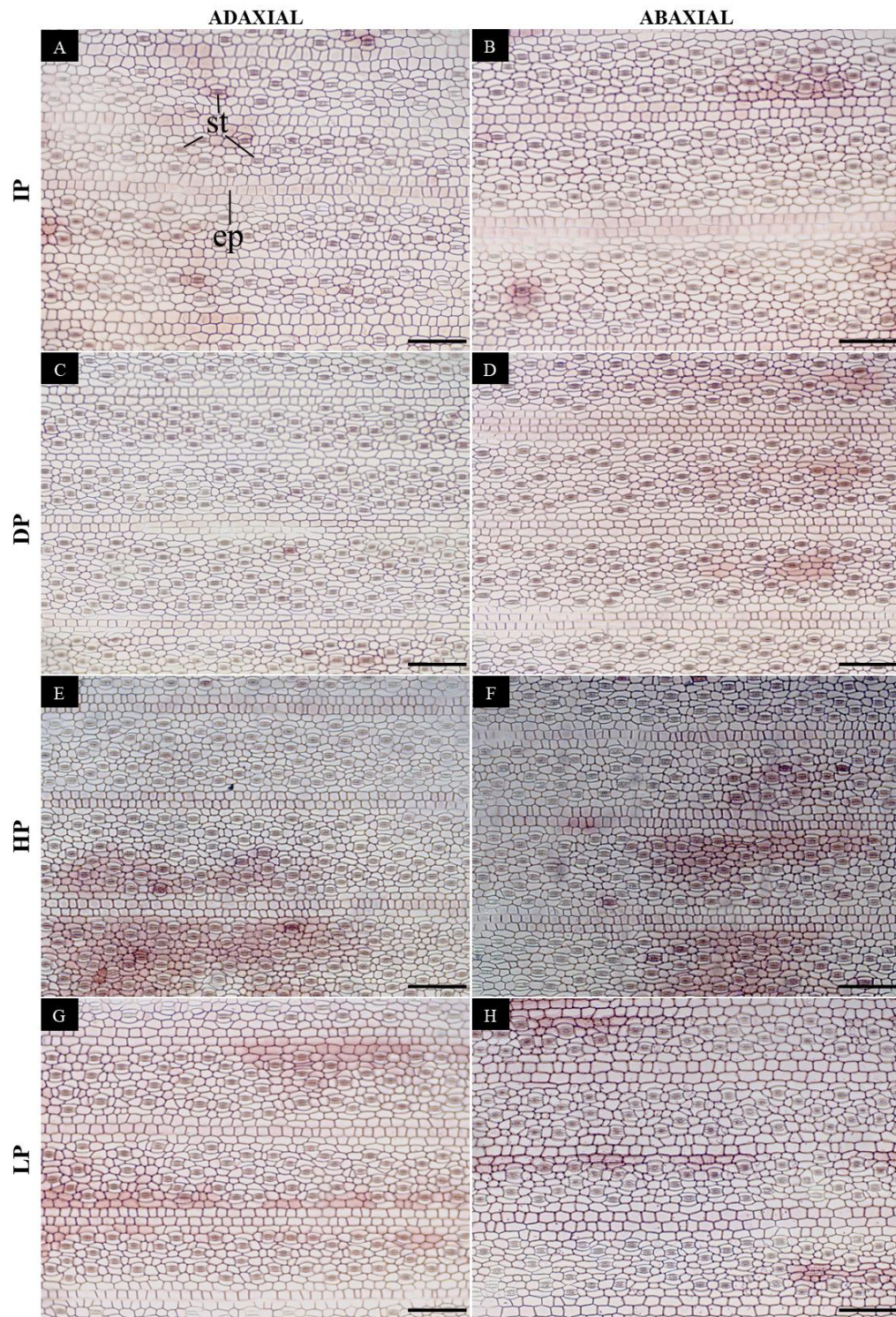


Fig. 6 Paradermal sections of the adaxial and abaxial surfaces of *Typha domingensis* leaves under seasonal rainfall variation. A and B: increased precipitation (IP); C and D: decreased precipitation (DP); E and F: highest precipitation (HP); G and H: lowest precipitation (LP). ep: epidermal cells, st: stomata. Bars = 100 µm.

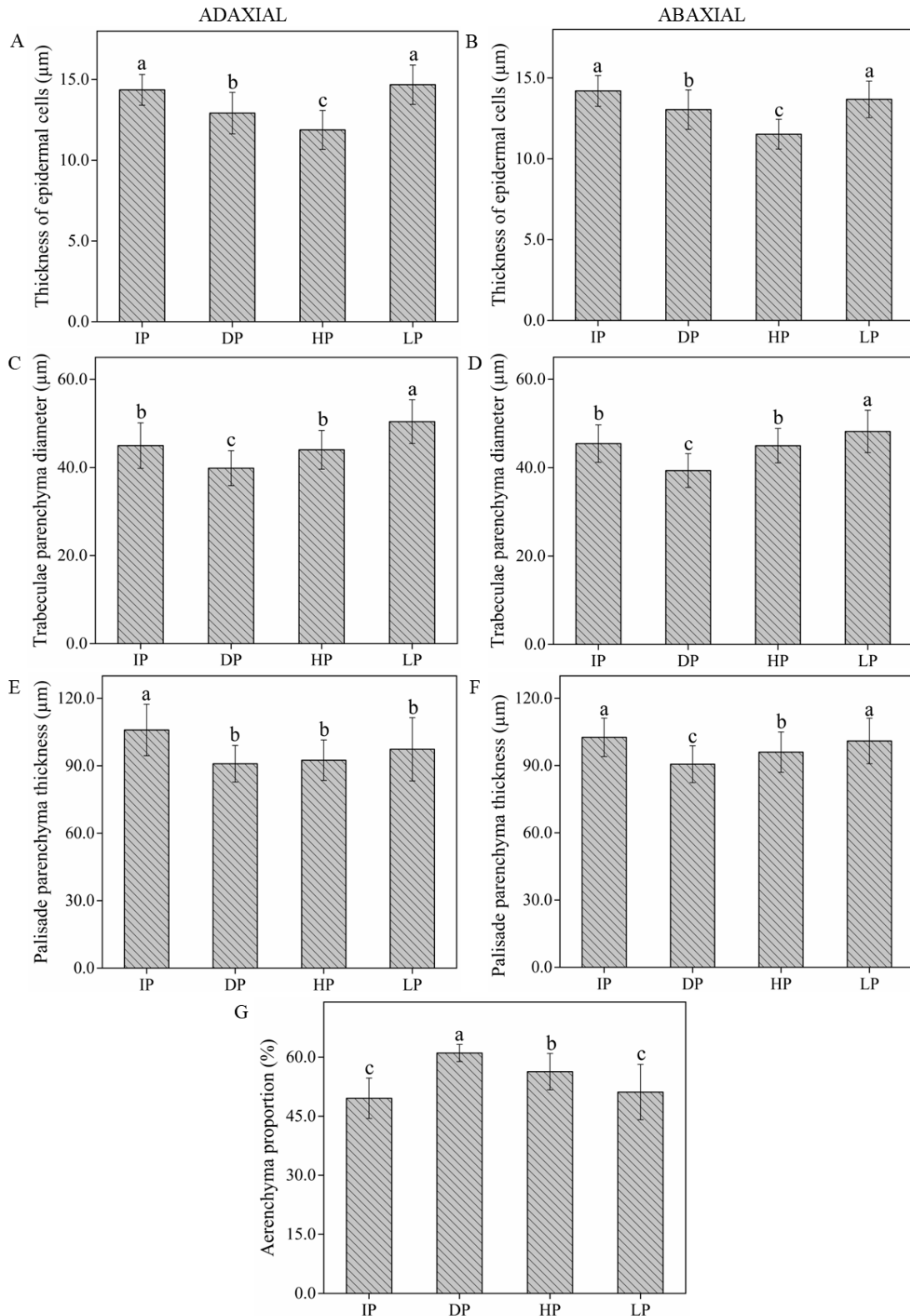


Fig. 7 Anatomical traits of *Typha domingensis* leaves under seasonal rainfall variation. IP: increased precipitation; DP: decreased precipitation; HP: highest precipitation; LP: lowest precipitation. The bars correspond to the standard errors. Means followed by the same letter do not differ according to Scott–Knott's test ( $p \leq 0.05$ ).

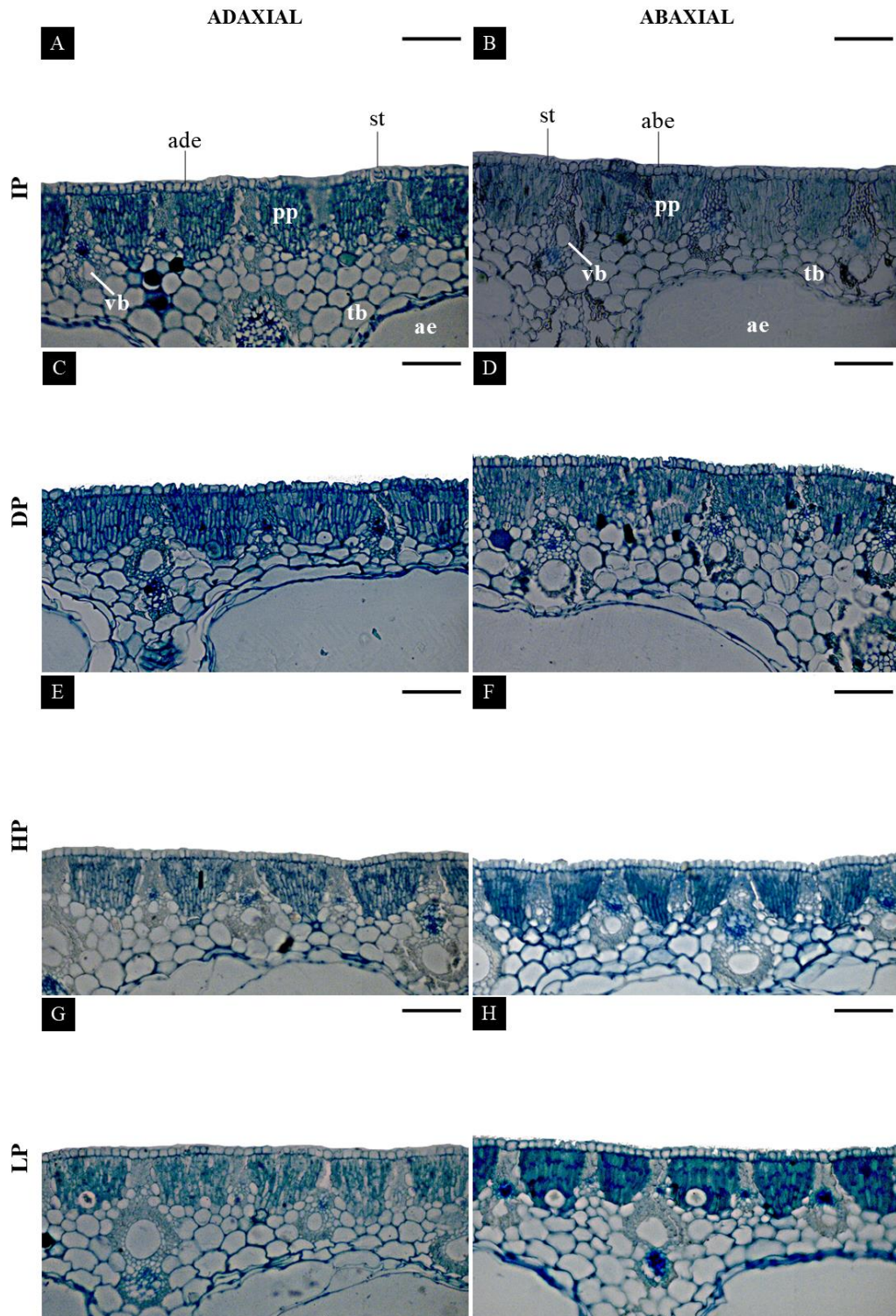


Fig. 8 Transversal sections of *Typha domingensis* leaves under seasonal rainfall variation. A and B: increased precipitation (IP); C and D: decreased precipitation (DP); E and F: highest precipitation (HP); G and H: lowest precipitation (LP). ade: adaxial epidermis, abe: abaxial epidermis, st: stomata, pp: palisade parenchyma, vb: vascular bundle, ae: aerenchyma, tb: trabeculae parenchyma. Bars = 100  $\mu\text{m}$ .

**ARTIGO 2: ROOT ANATOMY AND NUTRIENT UPTAKE OF *Typha domingensis*  
Pers. (TYPHACEAE) GROWN UNDER DROUGHT CONDITION**

**MANUSCRIPT PUBLISHED AS A SHORT COMMUNICATION IN THE JOURNAL  
RHIZOSPHERE**



## **Root anatomy and nutrient uptake of *Typha domingensis* Pers. (Typhaceae) grown under drought condition**

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### **ABSTRACT**

*Typha domingensis* is an invasive macrophyte which can be found even under low water levels. The objective of this study was to evaluate the root growth and anatomy as related to nutrient uptake and distribution of Cattail under different water availabilities. *Typha domingensis* individuals were subjected to five water conditions: waterlogged, field capacity (FC), 75%FC and 50%FC. Nutrient contents of shoots and roots as well as the root growth and anatomy were evaluated. Lower water levels reduced root length (30%) and dry mass (90%) of Cattail. Moderate drought increased aerenchyma development in root cortex but reduced the number and the diameter of xylem vessels in the vascular cylinder. Drought reduced the uptake of most nutrients; however, Cattail promoted a higher translocation of macronutrients to shoot. *Typha domingensis* showed drought tolerance with modifications in root anatomy and higher nutrient translocation to shoots allowing its survival under mild water limitation.

**Keywords:** Macrophytes. Cattail. Nutrient uptake. Nutrient Translocation. Aerenchyma.

Periods of low rainfall, high temperatures or anthropic effects may create drought events (Lipiec et al., 2013). Drought affects plants at the morphological, biochemical and molecular levels and drought tolerant species develop adaptations to maintain its water potential and prevent dehydration (Vadez, 2014). Drought stimulates root elongation as it grows deeper in the soil to improve water and nutrient uptake (Morison et al., 2008). Another important root response to drought is the aerenchyma development which reduces its metabolic cost (Jaramillo et al., 2013).

The aquatic macrophyte *Typha domingensis* Pers. (cattail) can be found under low water levels and showed tolerance to drought conditions; however, its photosynthesis and growth were reduced (Cruz et al., 2019). The root system of *Typha latifolia* was also limited under drought (Asamoah and Bork, 2010). However, drought-tolerant species show higher biomass investment in the root system. Moreover, water and nutrient uptake as well as plant hydraulic conductance depend on root anatomy (Vadez, 2014) and drought damages nutrient uptake, promoting nutritional stress (Dimkpa et al., 2019) apart from water limitation. Eutrophic conditions are often reported to promote excessive growth on cattail (Escutia et al., 2010). As drought may cause nutrient limitation and this may cause growth limitation to aquatic plants under this condition. Thus, the objective of this work was to evaluate the root growth, anatomy and nutrient uptake in Cattail grown under different water conditions.

Cattail individuals were collected in wetlands located in the Universidade Federal de Lavras, washed with tap water and then cultivated in a greenhouse using nutrient solution (Hoagland and Arnon, 1950) at 40% of its ionic strength. Plants were maintained under these conditions for 60 days, to obtain acclimated clones.

The clones were transferred to 4.0 L plastic pots containing 2.4 L of vermiculite. The field capacity (FC) of the vermiculite was determined according to Díaz et al. (2018). Vermiculite was selected against soil to permit the control of nutrient availability in a chemically inert substrate.

Plants were submitted to five water conditions as follows: Waterlogged (producing a 5 cm water layer over the surface), field capacity (FC= 100%), 75%FC, 50%FC and 25%FC. The water lost by evapotranspiration was replenished daily by the difference of masses and the nutrient solution was replaced weekly. Plants were maintained under these conditions for 60 days. The experimental design was completely randomized with five treatments and seven replicates of one plant.

Roots were oven-dried at 60°C until constant mass and weighed to obtain the dry mass. The length and diameter of the longest root were measured with a caliper. The content of

macronutrients (N, P, K, Ca, Mg and S) and micronutrients (B, Cu, Zn. and Fe) in roots and shoots were measured. For this, 500 mg of dry mass were ground using a Willey-type mill and then incinerated at 550 °C (for B determination) or acid digestion was performed (Sarruge and Haag, 1974). For acid digestion, 10 mL of HNO<sub>3</sub> were added to samples and left resting for 12 h. Digestion was performed at 150°C for 30 minutes until the volume of the nitric acid was halved. Thereafter, 1.0 mL of HClO<sub>4</sub> was added and the temperature was increased to 210°C until solution was cleared. The products of the digestion were transferred to 25 ml flasks which had its volume filled with distilled water and then taken to read at an atomic absorption spectrometer (Perkin Elmer, Waltham, USA).

To determine the total contents of macronutrients in shoots and roots, the following equation was used:  $MAC = (NL/100) * DM$ . In what: MAC= macronutrient content; NL= nutrient level in percentage and DM= organ dry mass; results were expressed in grams. The total micronutrient content was determined as:  $MIC = (NL * DM) / 1000$  were: MIC = micronutrient content, NL= nutrient level in ppm and DM= organ dry mass; results were expressed in milligrams. For nutrient translocation calculation the following calculation was used:  $NT = (NC / TNP) * 100$ , where: NT= nutrient translocation, NC= nutrient mass (MIC or MAC) and TNP= total nutrient in the plant.

Roots were collected and fixed in FAA<sub>70</sub> solution (formaldehyde, acetic acid and 70% ethanol) for 72 hours and further stored in 70% ethanol. This material was submitted to an ethanolic series for dehydration (Johansen, 1940). Subsequently, the material was immersed in a pre-infiltration solution composed of 100% ethanol and base resin (1:1) for 24 hours according to the instructions of the manufacturer (Leica, Wetzlar, Germany). Then, base resin infiltration was performed for 24 hours at 40°C and polymerization was completed after five days. Transversal sections at the pilifer zone (approximately 2.5 cm from the root apex) were performed in a rotating microtome (Jinhua Yidi, Zhejiang, China) and stained with 1% toluidine blue (Feder and O'brien, 1968).

Slides were photographed with a Zeiss Axio Lab.A1 microscope (Zeiss, Heidelberg, Germany) and the micromorphometric analyzes performed with UTHSCSA-ImageTool software. One slide, five sections and five microscopical fields were analyzed per replicate.

The following root anatomical characteristics were evaluated: thicknesses of epidermis, endodermis and cortex, number and diameter of metaxylem vessels, proportions of aerenchyma, vascular cylinder and xylem as well as the gray level of the endodermis. The proportion of aerenchyma was calculated by the ratio between the sum of the areas of all intercellular spaces and the area of the cortex multiplied by 100. The proportion of the

vascular cylinder was calculated by the ratio between the area of vascular cylinder and the total area of the root section multiplied by 100. In addition, the proportion of xylem was calculated by the ratio between the xylem area by the vascular cylinder area multiplied by 100.

Data was submitted to one-way ANOVA, and means were compared by the Scott-Knott test at 5% significance using Sisvar software.

Water deficit at 25%FC killed all plants but all other treatments permit plant survival whereas water availabilities below waterlogged significantly reduced root length, diameter and dry mass (Fig. 1A, B and C).

Drought reduced the epidermis thickness (Fig. 1D and Fig. 2) but promoted no effect to endodermis (mean= 9.6  $\mu\text{m}$ ) and cortex (mean= 534.32  $\mu\text{m}$ ) thicknesses. Furthermore, drought reduced the number (Fig. 1E and Fig. 2) and diameter (Fig. 1F and Fig. 2) of metaxylem vessels. Waterlogging and drought significantly decreased the aerenchyma proportion (Fig. 1G and Fig. 2). Drought reduced the gray level of the endodermis (Fig. 1H), but had no effect on the xylem (mean= 2.97%) and vascular cylinder (mean= 27.81%) proportions.

Drought increased shoot contents of N, Ca, P and Mg (Fig. 3A) but had no effect on K and S (Fig. 3A). Root N, Ca and P contents increased under drought conditions (Fig. 3C), however, this treatment reduced K, Mg and S levels (Fig. 3C). Furthermore, drought reduced total N, P, K, Ca and Mg contents (Fig. 3E). Translocations of K, Mg and S were increased by drought, but this condition had no effect in N and Ca translocations (Fig. 3G). Drought increased B, Cu and Fe contents in shoots but had no effect in Zn levels (Fig. 3B). Likewise, drought had no effect for Cu, Zn and Fe contents in roots whereas B level was increased (Fig. 3D). Moreover, drought reduced the total micronutrient content (Fig. 3F) and decreased B translocation, increased Cu translocation but had no effect on Zn and Fe translocations (Fig. 3H).

Root growth inhibition is a common response in plants under water deficiency. In this study, drought reduced the root system of *Typha domingensis* which can cause growth limitation. Drought-tolerant species usually increase root growth to uptake water deeper in the substrate (Anjum et al., 2011; Lipiec et al., 2013). The reduction of the root growth in *Cattail* was compensated by a decrease of its diameter, forming thinner roots and providing conditions for survival under drought. It is well known that thinner roots are more efficient for water and nutrient uptake (Postma et al., 2014), thus, thinner roots may improve water and nutrient uptakes of *Cattail* compensating the lower development of the root system.

Results of this work support the drought-tolerance of *Cattail* plants to mild drought conditions as reported by Cruz et al. (2019). According to Asamoah and Bork (2010) *Typha latifolia* shows root mortality and lower biomass under drought conditions. Drought limits photosynthesis and carbon assimilation, lowering biomass production (Anjum et al., 2011). This lower photosynthesis was found in *Cattail* (Cruz et al., 2019) and may have limited its root growth.

Anatomical data revealed no damage to root structure of *Cattail* under drought. Moreover, modifications on root anatomical parameters may have favored cattail survival. Plants submitted to 50%FC showed thinner epidermis and increased density of endodermis cell walls. Thinner epidermis favors water uptake and the thickening of the endodermis increases its function as an apoplastic barrier which prevents radial water loss (Aroca and Ruiz, 2012). *Cattail* maintained xylem characteristics under drought and this may have contributed for nutrient transport to shoots. The development of smaller xylem vessels is a common tolerance response under drought because it prevents vessel cavitation which lowers xylem conductance (Li et al., 2009). Moreover, both the size and number of xylem vessels are related to root hydraulic conductance and drought tolerance (Vadez, 2014). Higher nutrient translocation to shoots is an important compensation mechanism developed by *Cattail* since reduced root growth may have limited nutrient uptake.

Increased proportion of aerenchyma in *Cattail* under moderate drought may be related to drought tolerance. The proportion of aerenchyma is often related to drought tolerance (Jaramillo et al., 2013; Vadez, 2014; Díaz, et al., 2018). Aerenchyma development reduces the number of cortical cells and the metabolic costs for root growth (Díaz et al., 2018). Thus, higher aerenchyma proportion in *Cattail* reduces the metabolic cost for root maintenance favoring its survival under drought.

Drought reduces the nutrient availability in the soil and, consequently, the plant metabolism (Silva et al., 2011; Lipiec et al., 2013). Likewise, water is essential nutrient translocation and distribution throughout the plant (Silva et al., 2011). *Cattail* is able to uptake nutrients under drought conditions but favored its translocation to shoots which is an important drought-tolerance mechanism because of the lower nutrient acquisition.

Macronutrients are important for the regulation of stomatal opening and transpiration (K), cell turgor (Ca), and photosynthesis (Mg, S). Drought causes nutritional stress due to poor nutrient acquisition (Dimkpa et al., 2019). Thus, a higher nutrient translocation to shoots may be an important drought-tolerance mechanism. For instance, the reduction of P significantly limits growth of cattail because of dysfunctional photosynthetic and anatomical traits (Santos et al.,

2015). In addition, cattail growth is strongly regulated by higher N levels (Escutia et al., 2010) and Ca deficiency of *Cattail* limits Cd tolerance in *T. latifolia* (Rodriguez et al., 2015). Furthermore, the higher nutrient translocation to shoots can alleviate the effects of drought to cattail. Thus, *Typha domingensis* is tolerant to mild drought conditions and develops thinner roots with a higher nutrient translocation to shoots.

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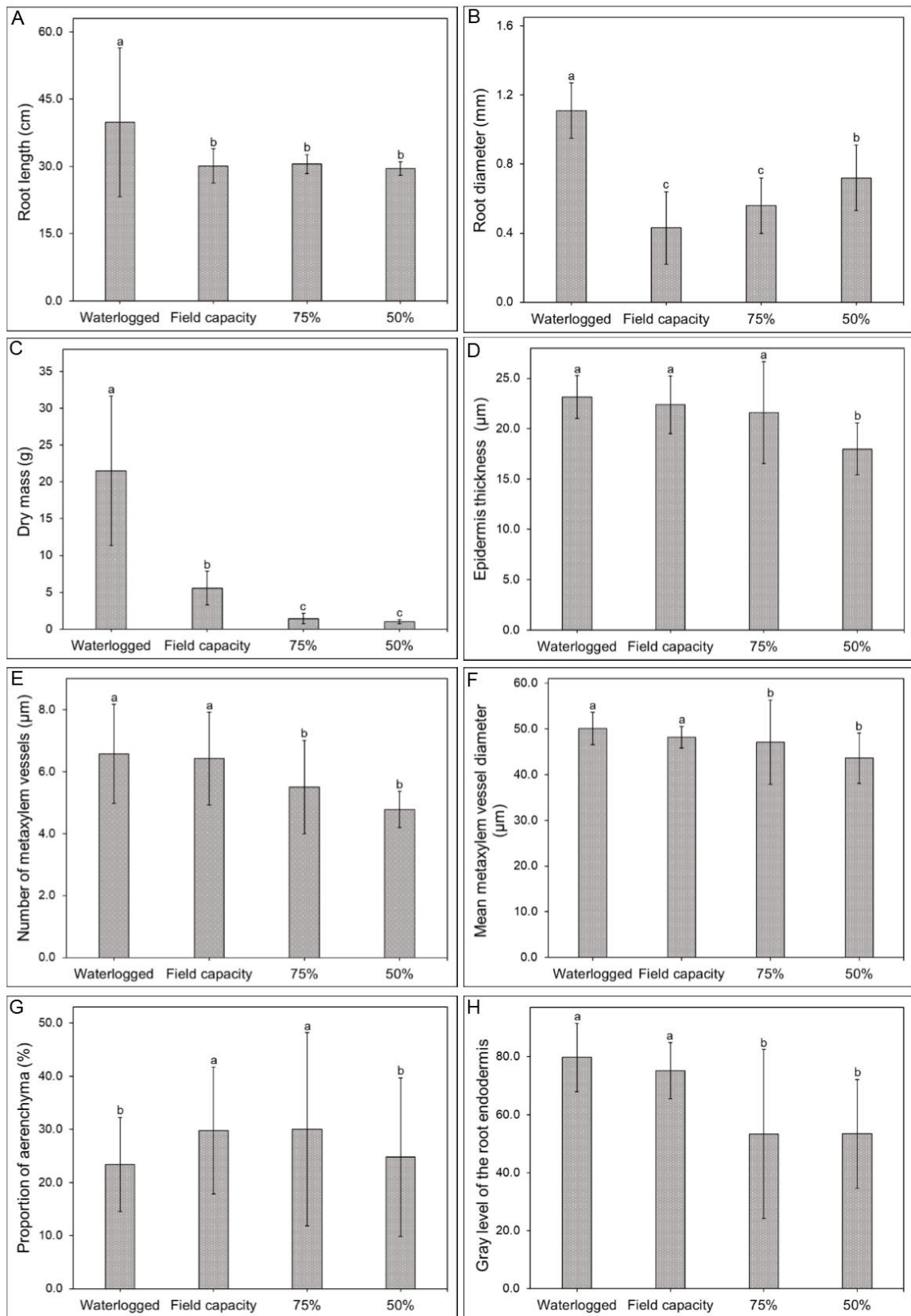
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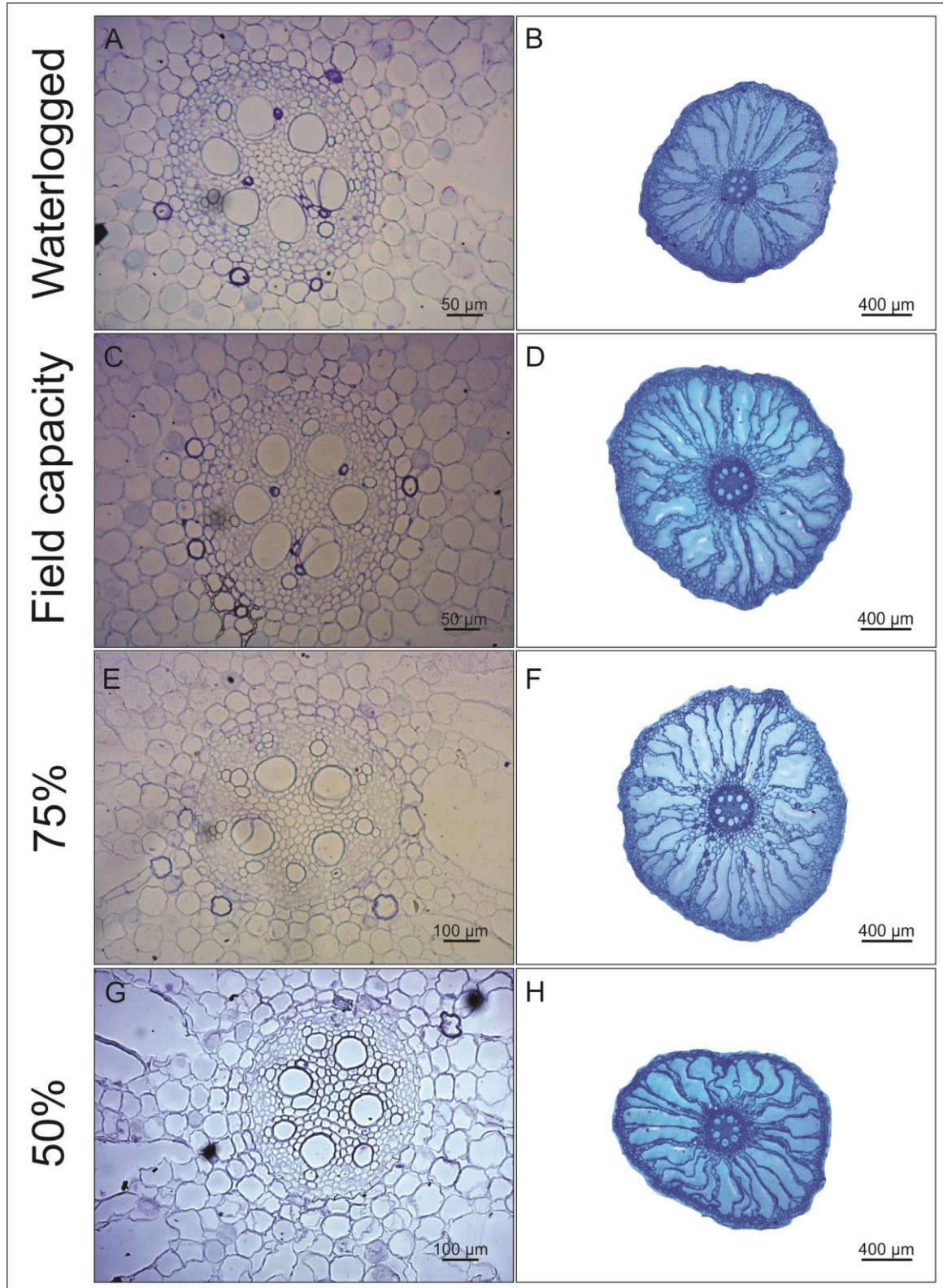
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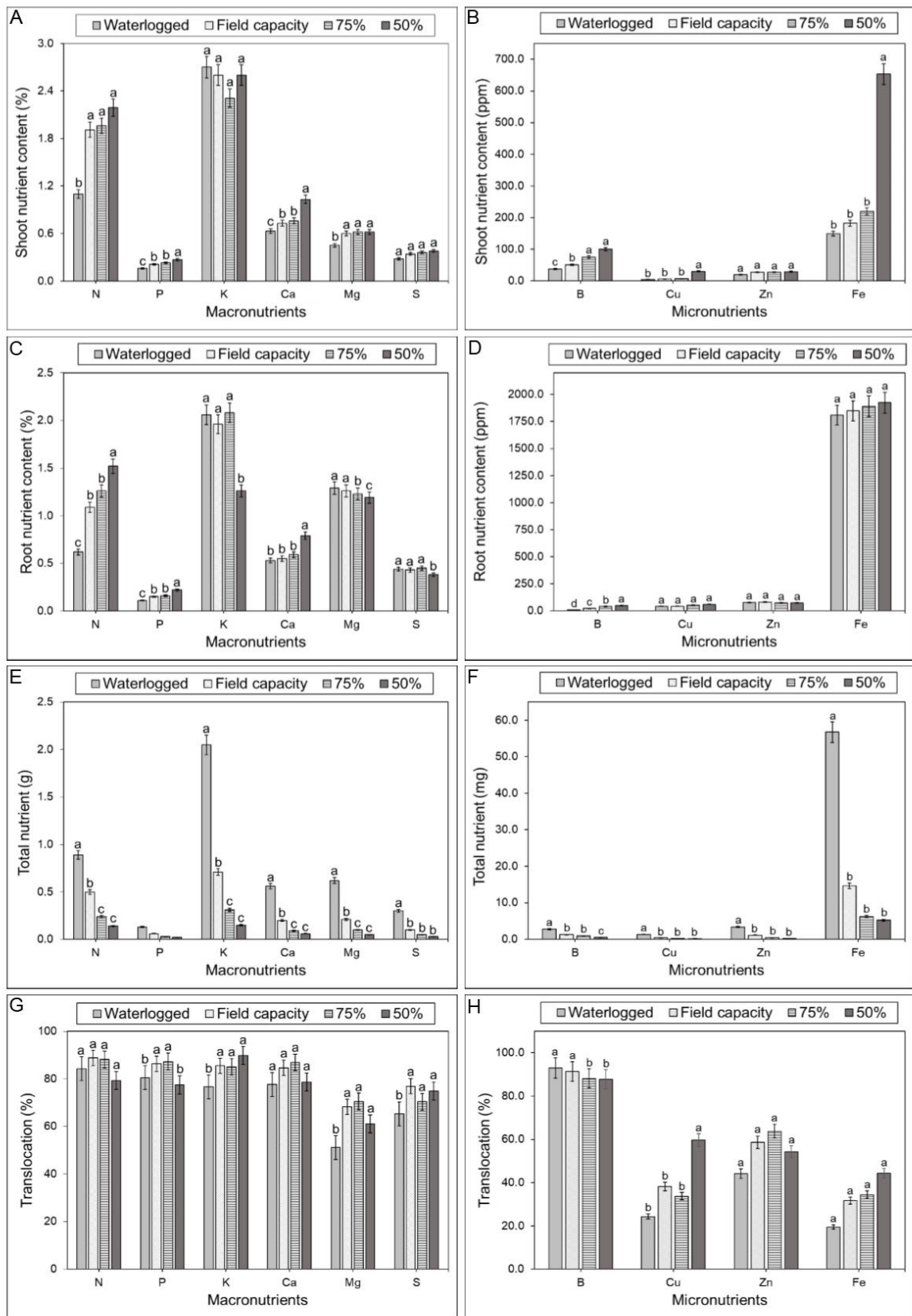




**Figure 1.** Root growth and anatomical parameters of *Typha domingensis* (cattail) plants under different water availabilities. Bars = standard error. Means followed by the same letters do not differ according to the Scott-Knott test at  $P < 0.05$  ( $n = 35$ ).



**Figure 2.** Transversal sections in the vascular cylinder (A, C, E, G) and pilifer zone (B, D, F, H) of *Typha domingensis* (cattail) roots grown under different water availabilities. A and B: waterlogged; C and D: field capacity (FC); E and F: 75%FC; G and H: 50%FC.



**Figure 3.** Macro and micronutrient contents of *Typha domingensis* (cattail) grown under different water availabilities. Bars = standard error. Means followed by the same letters do not differ according to the Scott-Knott test at  $P < 0.05$  ( $n = 35$ ).