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Investigating the role of residual conductance and xylem vulnerability to embolism in drought resistance

Talitha Soares Pereira
Doctor Scientiae

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Thesis submitted to the Plant Physiology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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Assent:

Talitha Soares Pereira
Author

Samuel Cordeiro Vitor Martins
Adviser

To my mother (in memoriam), who awaited and cheered for this achievement more
than anyone else.

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ABSTRACT

PEREIRA, Talitha Soares, D.Sc., Universidade Federal de Viçosa, October, 2024. **Investigating the role of residual conductance and xylem vulnerability to embolism in drought resistance**. Adviser: Samuel Cordeiro Vitor Martins. Co-advisers: Fabio Murilo da Matta and Amanda Ávila Cardoso.

Drought is one of the most significant challenges facing plant production, and its impacts are expected to be intensified due to climate change. While much research has focused on woody plants, this thesis explores the mechanisms of drought tolerance and avoidance in herbaceous species, with particular emphasis on crops and wild *Solanum* species. Across several studies, it becomes clear that, unlike woody plants, stomatal safety margin (SSM) and xylem embolism resistance (P_{50}) are not reliable indicators of drought survival for herbaceous species. Instead, traits related to drought avoidance—such as early stomatal closure, reduced nighttime transpiration, high water storage, capacitance (C), and minimum conductance (g_{min})—emerge as key factors for enhancing survival under extreme drought conditions. In the case of *Solanum* species, *S. pennellii* demonstrated superior drought resistance, underscoring the importance of traits such as high C and g_{min}, which allow for better water retention and efficient use during stress. Despite varied geographical origins, a narrow range in P_{50} was observed across domesticated and wild species, suggesting that breeding for drought resistance in cultivated tomatoes should focus on traits beyond xylem vulnerability. Additionally, nighttime transpiration was identified as a significant factor in plant water use during prolonged drought. The regulation of nighttime water loss varied across plant groups, with ferns relying on passive stomatal closure, gymnosperms combining passive and ABA-mediated closure, and angiosperms using ABA-dependent mechanisms. These findings highlight the multifaceted nature of drought responses and suggest that integrating drought tolerance and avoidance strategies could significantly enhance crop resilience to water stress. This thesis provides valuable insights into the hydraulic and physiological traits that underpin drought survival in herbaceous plants, with important implications for crop improvement and resilience under future climate conditions.

Keywords: abscisic acid; capacitance; drought avoidance; herbaceous plants; hydraulic traits; minimum conductance; nighttime transpiration; plant water use; tomato; xylem embolism resistance

RESUMO

PEREIRA, Talitha Soares, D.Sc., Universidade Federal de Viçosa, outubro de 2024. **Investigando o papel da condutância residual e da vulnerabilidade do xilema à embolia na resistência à seca**. Orientador: Samuel Cordeiro Vitor Martins. Coorientadores: Fabio Murilo da Matta e Amanda Ávila Cardoso.

A seca é um dos maiores desafios enfrentados pela produção vegetal, e seus impactos devem se intensificar devido às mudanças climáticas. Embora grande parte das pesquisas tenha se concentrado em plantas lenhosas, esta tese explora os mecanismos de tolerância e evasão à seca em espécies herbáceas, com ênfase em culturas agrícolas e espécies silvestres do gênero *Solanum*. Ao longo de vários estudos, fica claro que, ao contrário das plantas lenhosas, a margem de segurança estomática (SSM) e a resistência ao embolismo do xilema (P_{50}) não são indicadores confiáveis de sobrevivência à seca em espécies herbáceas. Em vez disso, características relacionadas à evasão da seca—como fechamento estomático precoce, redução da transpiração noturna, alta capacidade de armazenamento de água, capacitância (C) e condutância mínima (g_{min})—emergem como fatores chave para aumentar a sobrevivência em condições de seca extrema. No caso das espécies de *Solanum, S. pennellii* demonstrou uma resistência superior à seca, destacando a importância de características como alta capacitância e gmin, que permitem melhor retenção de água e uso eficiente durante o estresse. Apesar das diferentes origens geográficas, observou-se uma faixa estreita de P_{50} entre as espécies, sugerindo que o melhoramento genético para resistência à seca em tomates cultivados deve se concentrar em características além da vulnerabilidade do xilema. Além disso, a transpiração noturna foi identificada como um fator significativo no uso de água pelas plantas durante longos períodos de seca. A regulação da perda de água noturna variou entre os grupos de plantas, com samambaias dependendo do fechamento estomático passivo, gimnospermas combinando o fechamento passivo e mediado por ABA, e angiospermas utilizando mecanismos dependentes de ABA. Esses resultados ressaltam a natureza multifacetada das respostas à seca e sugerem que a integração de estratégias de tolerância e evasão à seca poderia melhorar significativamente a resiliência das culturas ao estresse hídrico. Esta tese oferece *insights* valiosos sobre os traços hidráulicos e fisiológicos que fundamentam a sobrevivência à seca em plantas herbáceas, com importantes implicações para o melhoramento de culturas e a resiliência sob condições climáticas futuras.

Palavras-chave: ácido abscísico; capacitância; plantas herbáceas; condutância mínima; resistência à seca; resistência ao embolismo do xilema; transpiração noturna; uso de água pelas plantas

SUMMARY

CHAPTER 1

Linking water-use strategies with drought resistance across herbaceous crops**

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Abstract

Woody plants minimize xylem embolism formation during drought essentially by closing stomata at higher water potentials and/or by increasing the xylem resistance to embolism. Both of these mechanisms result in a higher stomatal safety margin (SSM), which is the water potential difference between stomatal closure and embolism formation. Here we investigated whether increasing SSM represents a drought resistance mechanism for herbaceous plants and how the different water-use strategies impact their survival. For that, we exposed four herbaceous crops, with contrasting drought resistance, to severe water deficit to assess drought-induced damage and mortality. Unlike woody species, SSM was not associated with plant survival for herbaceous crops. Soybean, which presented the largest SSM across the four crops (1.67 MPa), exhibited the earliest mortality of leaves and whole plants as well as the highest rate of plant mortality (100%) at the end of the drought period. Cowpea, with a SSM of 0.63 MPa, was the most drought-resistant species, with the latest leaf damage and the highest plant survival (100%). The most effective traits ensuring survival in herbaceous crops under severe drought were those related to drought avoidance mechanisms such as (i) early stomatal closure, (ii) very low residual transpiration post-stomatal closure, and (iii) high capacitance pre- and post-turgor loss.

Keywords: cowpea, drought-induced mortality, embolism resistance, leaf capacitance, soybean, stomatal closure, sunflower, tomato.

Introduction

Understanding plant mortality in response to drought has been a recurrent topic in plant science, especially considering the growing number of die-off events being reported throughout the world (Hammond *et al.*, 2022). Most studies, however, have focused on woody-plant mortality, either because of their perennial life cycle or because of the critical ecosystem services they provide to humanity (Martinez-Vilalta *et al.*, 2019; Trugman, 2022). Nevertheless, herbaceous plants are fundamental for food security. First, because the majority of crops used for food production are herbaceous, and, second, because grasses constitute an important plant group for livestock feeding (Dhankher and Foyer, 2018).

Drought-induced mortality of plants results from a series of mechanisms involving feedback between fluxes and pools of water and carbon within plants (McDowell *et al.*, 2022*a*). In the last instance, mortality of all organs and individuals involves cellular failure and the underlying factor behind this failure is severe tissue dehydration (McDowell *et al.*, 2022*a*). Dehydration results from a major impairment in the water supply as water-transporting xylem cells lose their function in response to drought, a phenomenon that has been called plant hydraulic failure. This occurs when the xylem tension surpasses a threshold water potential, leading to major events of embolism, thus resulting in water transport within plants becoming irrecoverable (Sperry and Tyree, 1988; Kaack *et al.*, 2021).

The ubiquitous consequence of hydraulic failure to plant mortality (Hammond et al., 2019) led to a growing number of studies deepening our knowledge of the components involved in the water transport system in plants, particularly, hydraulic conductance and xylem resistance to embolism. Xylem resistance to embolism is commonly represented by the water potential inducing 50% embolism (P_{50}) in the xylem or inducing 50% loss in hydraulic conductance (hydraulic P_{50}) (Choat *et al.*, 2012*a*). In turn, despite the P_{50} has been commonly associated with drought resistance, the stomatal safety margin (SSM) is considered a more robust parameter to such end, once it involves P_{50} and the water potential at stomatal closure (Ψg_{s90}) (Chen *et al.*, 2021). Nonetheless, to precisely determine the time range for a plant to reach these threshold water potentials, information on water fluxes and pools is necessary (Blackman *et al.*, 2016*a*, 2019*b*).

Plant water balance is a function of water loss and absorption. During drought, when the soil-root resistance considerably increases (Abdalla *et al.*, 2021), the plant water potential is essentially determined by the water pool inside the plant and the rate of water loss. Early during drought, water loss rate is considerably minimized due to stomatal closure (Choat *et al.*, 2018*a*; Volaire, 2018*a*; Blackman *et al.*, 2019*b*; Zia *et al.*, 2021). As drought persists, plants continue to slowly lose water from leaves, especially through the cuticle and partially open stomata. This residual transpiration of leaves after complete stomatal closure is also known as minimum leaf conductance (g_{min}) (Blackman *et al.*, 2019*b*; Duursma *et al.*, 2019*a*). There is growing recognition that g_{min} plays an important role in estimating water fluxes in plant canopies (Barnard and Bauerle, 2013) and that it can directly influence the time it takes for plants to reach lethal water potential (Ψ_w) during a severe drought. Therefore, g_{min} has been used in plant drought response models, such as the hydraulic model developed by (Blackman et al., 2016), which allows the determination of the time that trees take to desiccate from stomatal closure to lethal levels of drought stress. The g_{min} has also been appointed as a key trait to be selected and bred to increase drought resistance in crops (Duursma *et al.*, 2019*a*).

Besides g_{min}, the time for plant desiccation from stomatal closure to lethal levels is also dependent on the area for water loss (total leaf area), the amount of water in the plant (water pool), and plant capacitance (Blackman *et al.*, 2016*a*; Li *et al.*, 2022). The role of capacitance in plant hydraulics is still underexplored, although some studies have demonstrated its importance for plant hydraulics, especially in species presenting passive control of stomatal movements (Martins *et al.*, 2016*a*). Because capacitance determines the extent to which the internal water pool buffers the xylem water potential during drought, capacitance would be expected to be an influential parameter in the time to reach critical thresholds of hydraulic failure and plant deaf desiccation. Even though (Blackman et al., 2019) did not find any correlation between the time for desiccation to lethal levels and shoot capacitance in *Eucalyptus* species, additional studies including those on other species are still necessary to confirm (or not) the importance of plant capacitance for drought resistance.

According to Levitt (1980), drought resistance consists of three not mutually exclusive strategies: drought escape, avoidance, and tolerance. Briefly, the drought escape strategy is based on plants avoiding exposure to stress mainly through adjustments in phenology; on the other hand, drought avoidance and tolerance are strategies that involve plant exposure to stress, but with contrasting mechanisms regarding variation in plant water potential. Drought avoiders are plants that seek a homeostasis in water potential through minimization of water loss and/or maximization of water absorption. Drought tolerance involves withstanding decreases in water potential as plants accumulate solutes to decrease their turgor loss point and/or build xylem conduits more resistant to embolism (Delzon, 2015).

A prominent role of xylem embolism resistance for drought tolerance in woody species was demonstrated by (Blackman et al., 2019), as given by the strong correlation between the time observed to desiccation and stem P_{50}. Although the studies with woody plants have emphasized the role of P_{50} in plant mortality, the hydraulic architecture of herbaceous species may reveal that different components of water relations might be involved in drought-induced mortality in this functional group. (Lens et al., 2016) have shown a significant overlap between the range of P_{50} of woody and herbaceous plants. However, variation in other components such as g_{min} and capacitance might be as important as P_{50} in defining the mortality of herbaceous plants. These plants are known for their relatively high water content (Xiong and Nadal, 2020*a*), and given the expected correlation between water content and capacitance, we also expect these plants to present considerably higher capacitance than trees and shrubs. Nonetheless, the marked importance of P_{50} in drought resistance of woody species seems to be aligned with this group consistently adopting drought tolerance mechanisms (e.g., Lenz et al., 2006; Mitchell et al., 2008; Huang et al., 2024). Interestingly, evidence from herbaceous systems is less consistent, with studies reporting both drought tolerance and avoidance mechanisms in this group (Kramp et al., 2022).

Given the substantial lack of information regarding water-use strategies influencing hydraulic failure and plant mortality across herbaceous species during drought, the central objective of this study was to evaluate a series of hydraulic characteristics in herbaceous crops. Finding the variables that can be related to the mortality of these plants will allow more concise predictions to assist in the improvement of drought resistance in this group. Here we used four species of economic importance and with different degrees of drought resistance to explore different combinations of mechanisms for water-use under drought. These are cowpea (*Vigna unguiculata* (L.) Walp.), sunflower (*Helianthus annuus* L.), tomato (*Solanum lycopersicum* L.) and, soybean (*Glycine max* (L.) Merr.). Cowpea is a drought-resistant plant and an important source of protein for the semi-arid tropics of Asia, Africa, and Latin America (Singh et al., 2003; Agbicodo et al., 2009; Merwad et al., 2018). Sunflower is considered a moderate drought-resistant crop due to its drought escape behavior (Hussain et al., 2018) and its highly explorative root system (Moschen et al., 2017). On the other hand, soybean, and tomato, two of the most largely cultivated crops around the world, demand a high amount of water to grow and reproduce and are highly susceptible to drought (Rigano et al., 2014, Arya et al, 2021, Campobenedetto et al., 2021).

Materials and Methods

Plant material

Plants of tomato (*Solanum lycopersicum* L. ‘M82’), soybean (*Glycine max* (L.) Merr. ‘EMBRAPA 48’), cowpea (*Vigna unguiculata* (L.) Walp. ‘Pingo de Ouro1-2’), and sunflower (*Helianthus annuus* L. ‘garden sunflower – ISLA’) were cultivated from seeds in 5.5 L-plastic pots filled with clay soil, sand, and commercial substrate (2:1:1). Plants were maintained inside a greenhouse (Viçosa, 20° 45’ S, 42° 15’ W, Brazil) under natural light (maximum photosynthetic photon flux density (PPFD) of c. 900 μmol m^{-2} s^{-1}) and temperature (c. 29°C) until they were c. 40-day-old. During cultivation, plants received daily watering to full soil capacity and a weekly application of fertilizer using a commercial NPK formulation (10:10:10). A total of 10 individuals per species were cultivated and each individual was placed inside an individual pot.

Crop responses to drought

Drought was imposed in five well-watered plants by withholding water until all species started to show signs of leaf death, which happened after 15 days. During the experiment, measurements of leaf water status, gas exchange, and chlorophyll fluorescence were performed in well-watered and stressed plants. Following 15 days of drought, plants were rehydrated, and new measurements were taken for the plants that survived the drought for two additional days. Leaves were considered dead when dry and brown. Plants were considered dead when they experienced browning of stem and foliage; in addition, these plants were kept at field capacity for two additional weeks and resprouting was not observed.

Predawn and midday leaf water potential ($\Psi_{predawn}$ and Ψ_{midday}, respectively) were measured on the third, seventh, and fifteenth days of water withholding. For that, leaves were collected at 5:00 and 13:00, bagged with damp paper towels for 10 min to ensure whole leaf equilibration, and measured using a Scholander pressure chamber (Model 1000, PMS Instruments, Albany, OR, USA). Leaf gas exchange was measured on the days zero, one, three, five, seven, nine and fifteen of water withholding and after re-watering, and whole-plant transpiration were assessed daily due to the non-destructive nature of the methods. Leaf gas exchange was measured from 10:00 to 14:00 using an infrared gas analyzer (LI-6400XT, LI-COR, Lincoln, NE, USA). Conditions in the chamber were set at 400 ppm of CO_2 and PPFD of 1000 µmol m^{-2} s^{-1}, and measurements were performed under ambient conditions of temperature (c. 29°C) and VPD (c. 1.5 kPa). To simplify and facilitate the comparison of changes in photosynthesis (A) and stomatal conductance (g_s) among the four species throughout the experiment, A and g_s were normalized using the values of irrigated plants as a reference. Whole-plant transpiration was quantified gravimetrically, and, to correct the water loss by evaporation, pots filled with the same amount and type of substrate (without plants) were used as a control. Briefly, we allowed these pots to dry to find the relationship between the substrate water content vs evaporation rate, then, for a given substrate water content, we subtracted the evaporation rate (given by the pots without plants) from the evapotranspiration rate (given by the pots with plants). The maximum quantum efficiency of the photosystem II (F_v/F_m) – a common index of leaf damage and mortality (Guadagno *et al.*, 2017) – was measured in a dark-adapted leaf of 5 individuals per treatment of each specie on days 5, 11, and 15 of water withholding using an infrared gas analyzer described above. For that, leaves were illuminated with weak modulated measuring beams (0.03 µmol m^{-2} s^{-1}) and with saturating white light pulses (8000 µmol m^{-2} s^{-1}). F_v/F_m was next calculated as $F_v/F_m = [(F_m - F_0)/F_m)]$, where F_0 is initial fluorescence and F_m is maximum fluorescence emission. All physiological parameters were also assessed on the second day following rehydration when leaves/plants were alive. In addition, during the drought and recovery time, visual analyses were made to register the beginning of leaf and plant mortality (that was considered when all leaves were dead).

Leaf morphology and hydraulics

Fully expanded leaves were collected from the middle third of shoots of well-watered plants and characterized in terms of specific leaf area (SLA), g_{min}, saturated water content (SWC), turgor loss point (Ψ_{tlp}), capacitance before and after the turgor loss point (C_{ft} and C_{tlp}, respectively), and P_{12}, P_{50}, and P_{88}.

The SLA for the five individuals per species was measured from one leaf per plant ($n = 5$). Leaf area was measured using a flatbed scanner and ImageJ (National Institute of Health, USA), and leaf dry mass was assessed after leaves were oven-dried at 70°C until constant weight. Finally, SLA was calculated as projected leaf area divided by leaf dry mass.

The g_{min} was measured using the mass loss of detached leaves method (Duursma et al., 2019). Leaves taken from different individuals ($n = 5$) of each species in well-watered conditions were collected early in the evening, taken to the lab, and subsequently recut under water to allow rehydration (c. 12 h). The fully hydrated leaves had their petiole end completely sealed with parafilm and then placed on the stand to allow slow dehydration while weighted every 15 min for 4 h. For each plant, g_{min} was determined from the slope of the linear part of the relationship between leaf mass versus time after stomatal closure and normalized by leaf area, VPD (obtained from air temperature and humidity measurements during the dehydration period), and atmospheric pressure (P). The calculations were performed using the spreadsheet provided by (Sack & Scoffonni, 2011).

One mature leaf taken per individuals of tomato ($n = 8$), cowpea ($n = 8$), sunflower ($n = 6$), and soybean ($n = 6$) were utilized to construct pressure–volume curves (Tyree and Hammel, 1972). Leaves were collected early in the evening, scanned for leaf area measurements, recut under water, and allowed to rehydrate overnight until water potential was higher than 0.1 MPa. Leaf fresh weight and water potential were measured over time during slow bench dehydration until leaf water potential stopped falling due to leaf cell damage. Using leaf dry mass of oven-dried leaves (at 70°C until constant weight), the relative water content was calculated and plotted against the inverse of leaf water potential. SWC was estimated by extrapolating the regression of water mass vs water potential to estimate the water mass at 0 MPa and subsequently dividing it by the dry mass. The Ψ_{tlp} was determined by the inflection point between the pre-turgor loss and post-turgor loss portions of the curve. C_{ft} and C_{tlp} were obtained from the linear slopes of the plot (before and after the turgor loss point, respectively) and normalized by leaf area.

Leaf vulnerability curves for each species were assessed by the optical method (Brodribb *et al.*, 2016). Well-watered potted individuals of each species [tomato ($n = 5$), cowpea ($n = 6$), sunflower ($n = 5$), and soybean ($n = 6$)] were taken to the lab early in the morning and plants had the soil washed out from the roots and kept with the roots submerged in water until launching the scanner. Subsequently, a fully mature leaf from the middle third of shoots was placed under either a flatbed scanner (V800, Epson, Tokyo, Japan) to record the development of embolism in the leaves with images taken every c. 3 min. While embolism was recorded, plants were maintained under low light conditions (PPFD < 100 μmol m^{-2} s^{-1}) to allow whole plant equilibration. The leaf water potentials for tomato, soybean, and cowpea were periodically measured until complete leaf xylem embolism using a Scholander pressure chamber (Model 1000, PMS Instruments, Albany, USA). For sunflower, due to its reduced number of leaves, leaf discs were collected to quantify RWC, and Ψ_w was estimated from the linear equation between Ψ_w and RWC obtained through pressure-volume curves. Embolism events were determined when visible changes in color in the xylem were observed. A small leaf section covering major and minor veins was selected and the area of embolized xylem (%) was quantified over time by image subtraction and the data was plotted against leaf water potential. For further details on this method, see the open-source website www.opensourceov.org. The optical P_{12}, P_{50}, and P_{88} for each species were calculated as the mean value obtained from all curves. The Ψg_{s90} (i.e., water potential at 90% of stomatal closure) were assessed simultaneously to the leaf vulnerability curves by measuring the declines in g_s in neighboring leaves as plants dehydrated using an infrared gas analyzer (LI-6400XT, LI-COR, Lincoln, NE, USA) with the same chamber's conditions mentioned above. Finally, for each species, the SSM was calculated as defined by Ψ_{gs90} minus P_{50}.

Statistical analysis

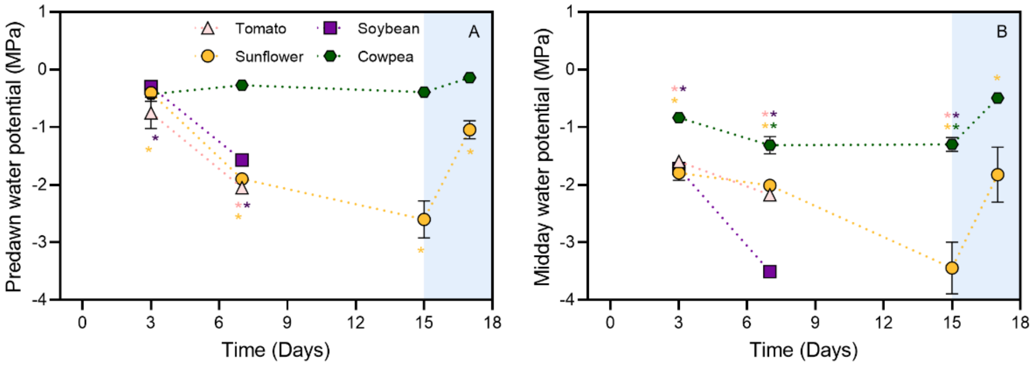
Differences between control and water deficit treatments were tested independently for each species with one-way ANOVA and post-hoc test t (<0.05). Statistical analyses for pressure-volume curve results and hydraulics features among species were performed using the SAS Learning Edition 4.1® software (SAS Institute, 2006). The data were submitted to an analysis of variance and the significant differences between the means were determined by the Fisher's least significant difference (LSD) test for multiple comparisons with a probability level of ≤0.05.

Results

Crop responses to drought

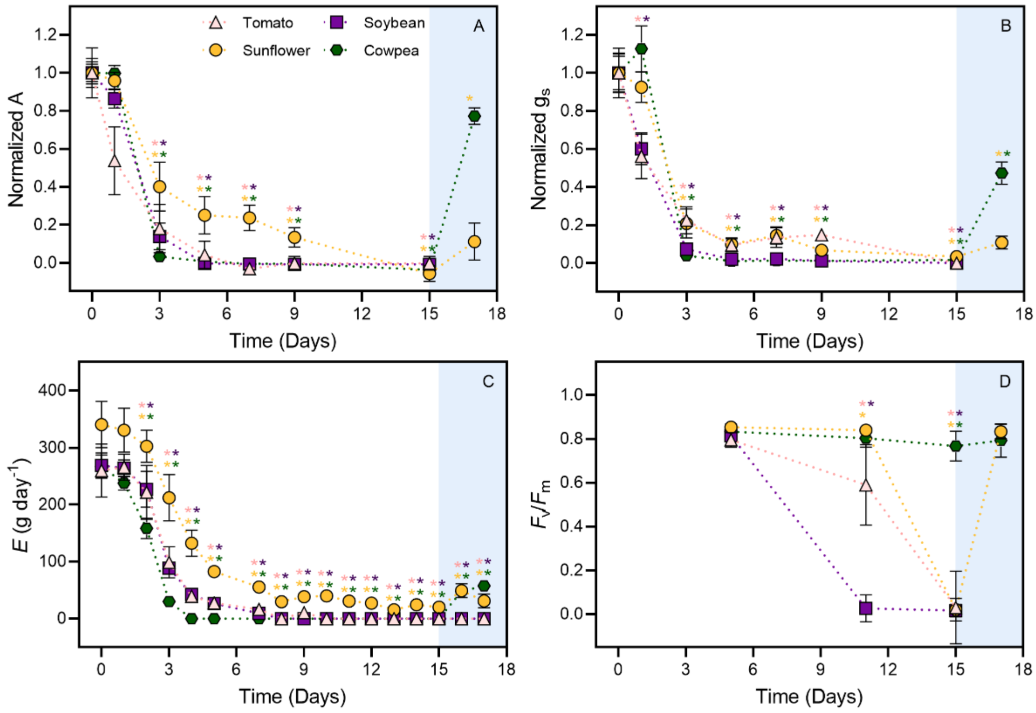
Over the 15 days of drought, cowpea plants maintained their $\Psi_{predawn}$ around -0.30 MPa (Fig 1A), while the Ψ_{midday} decreased to around -1.30 MPa after seven days of stress and was maintained at similar levels until the 15th day of sustained drought (Fig 1B); on the other hand, the $\Psi_{predawn}$ and Ψ_{midday} of the remaining crops decreased to -0.48 and -1.75 MPa, respectively, at the third day of drought. After seven days, tomato and sunflower plants achieved -2.00 MPa for both $\Psi_{predawn}$ and Ψ_{midday} whereas soybean plants achieved -1.57 MPa for $\Psi_{predawn}$ and -3.50 MPa for Ψ_{midday}. After 15 days of drought, sunflower $\Psi_{predawn}$ dropped to -2.60 MPa and Ψ_{midday} to nearly -3.50 MPa, while tomato and soybean could not be measured as plants of these crops were already dead (Fig 1A-B, Table 1). When rehydrated for two days, the Ψ_{midday} of cowpea plants returned to control levels, while for sunflowers, it remained lower (c. -2.00 MPa) than the control (c. -0.81 MPa) (Fig 1 B; Table S1).

Fig. 1. Changes in predawn (A) and midday (B) water potential (MPa) during 15 days of sustained water withholding and two days of rehydration (blue) across four herbaceous crops [soybean (purple square), tomato (pink triangle), sunflower (yellow circle), and cowpea bean (green hexagon)]. Values are mean ± SE (n = 5). Due to intense desiccation and browning of all leaves of soybean and tomato plants, the water potentials could only be assessed until day seven. Because of the mortality of sunflower plants, n on day 15 is equal to 4, and n on day two after rehydration is equal to 3. Asterisks denote statistical differences among water regimes within species according to the t-test ($p < 0.05$).



The *A* and g_s declined in response to drought in all crops (Fig 2A-B). Overall, *A* and g_s declined to their lowest levels on the third day of drought in cowpea and soybean, on the fifth day in tomato, and, on the ninth day, sunflower still sustained nearly 20% of its maximum *A*. Alike leaf gas exchange, whole-plant transpiration decreased significantly, reaching very low levels, if not becoming undetectable, as the drought progressed (Fig 2C). Cowpea, sunflower, and tomato plants transpired similar amounts of water per day during well-watered conditions, but a faster decline in transpiration was observed for cowpea over the other two crops. For instance, transpiration ceased to occur after three days of drought in cowpea plants, and only after eight days of drought in soybean and tomato. Sunflower plants exhibited the highest rate of whole-plant transpiration, which, despite reaching its lowest rate after seven days of drought, did not cease completely even after 15 days of water restriction. Two days after rehydration, cowpea and sunflower partially restored leaf gas exchange and whole-plant transpiration – cowpea at a much higher extension, though – while soybean and tomato plants could not have their leaf gas exchange measured as all individuals were dead at this point (Table 1; Fig 3).

Fig. 2. Changes in photosynthesis (A; µmol m^{-2} s^{-1}) (A), stomatal conductance (g_s; mol m^{-2} s^{-1}) (B), whole-plant transpiration (E_{plant}; g day^{-1}) (C), and maximum quantum efficiency of the photosystem II (F_v/F_m) (D) during 15 days of sustained water withholding and two days after rehydration (blue) across four herbaceous crops [soybean (purple square), tomato (pink triangle), sunflower (yellow circle), and cowpea bean (green hexagon)]. Values are mean ± SE (n = 5). Due to the mortality of all soybean and tomato plants during the drought imposition, data was not assessed following rehydration. Because of the mortality of sunflower plants, n on day 15 is equal to 4, and n on day two after rehydration is equal to 3. Asterisks denote statistical differences among water regimes within species according to the t-test ($p < 0.05$).



After five days of drought, the leaves of all crops exhibited high F_v/F_m (*c.* 0.8), which was similar to well-watered plants (Fig 2D; Table S1). The majority of cowpea plants continued to exhibit similar values of F_v/F_m even after 15 days of drought and also after two days of recovery (Fig 2D). A few leaves of cowpea, however, were damaged or dead after 15 days of drought (Table 1; Fig 3). Sunflowers maintained higher values of F_v/F_m until 11 days of drought

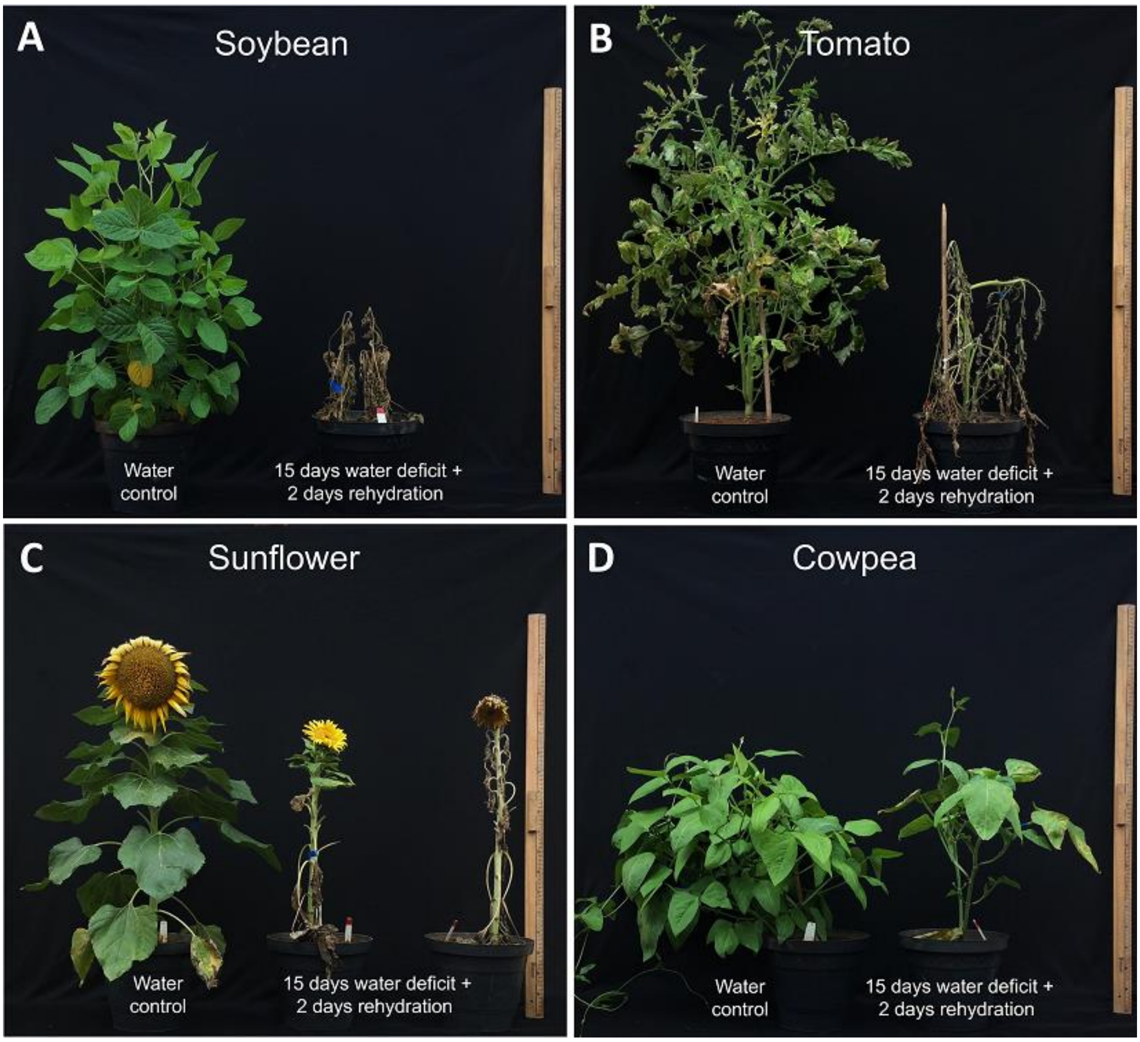
(Fig 2D). On the 14th day of drought, leaf mortality was observed on sunflower plants (Table 1) and on the 15th day, nearly all leaves were dead (confirmed by the very low values of F_v/F_m (Fig 2D; Fig 3). Plants of both cowpea and sunflower (the 60% that survived) exhibited leaves that completely restored their F_v/F_m following two days of rewatering (Fig 2D). Soybean and tomato leaves started to exhibit mortality after nine days of drought and very low values of F_v/F_m (close to zero) after 11 and 15 days of drought, respectively (Fig 2D). As stated above, all individuals of soybean and tomato plants were dead on the second day of rehydration following 15 days of sustained water withholding (Table 1, Fig 3), thus the recovery of F_v/F_m was not assessed (Fig 2D).

Table 1. Beginning of visual leaf and plant mortality and the percentage of plant mortality on the second day of rehydration following 15 days of sustained water withholding. The approximate day on which leaf and plant mortality started to be observed for each species is also presented.

Species	Leaf mortality	Plant mortality	Plant mortality (%)
Soybean	Yes (Day 9)	Yes (Day 9)	100
Tomato	Yes (Day 9)	Yes (Day 11)	100
Sunflower	Yes (Day 14)	Yes (Day 17*)	40
Cowpea	Yes (Day 15)	No	0

*Day 17 means 15 days of drought plus two days of rewatering.

Fig. 3. Well-watered plants (left) and plants on the second day of rehydration after a 15-day-period of sustained water withholding (middle and right) of four herbaceous crops [soybean (A), tomato (B), sunflower (C), and cowpea (D)].



Leaf morphology and hydraulics

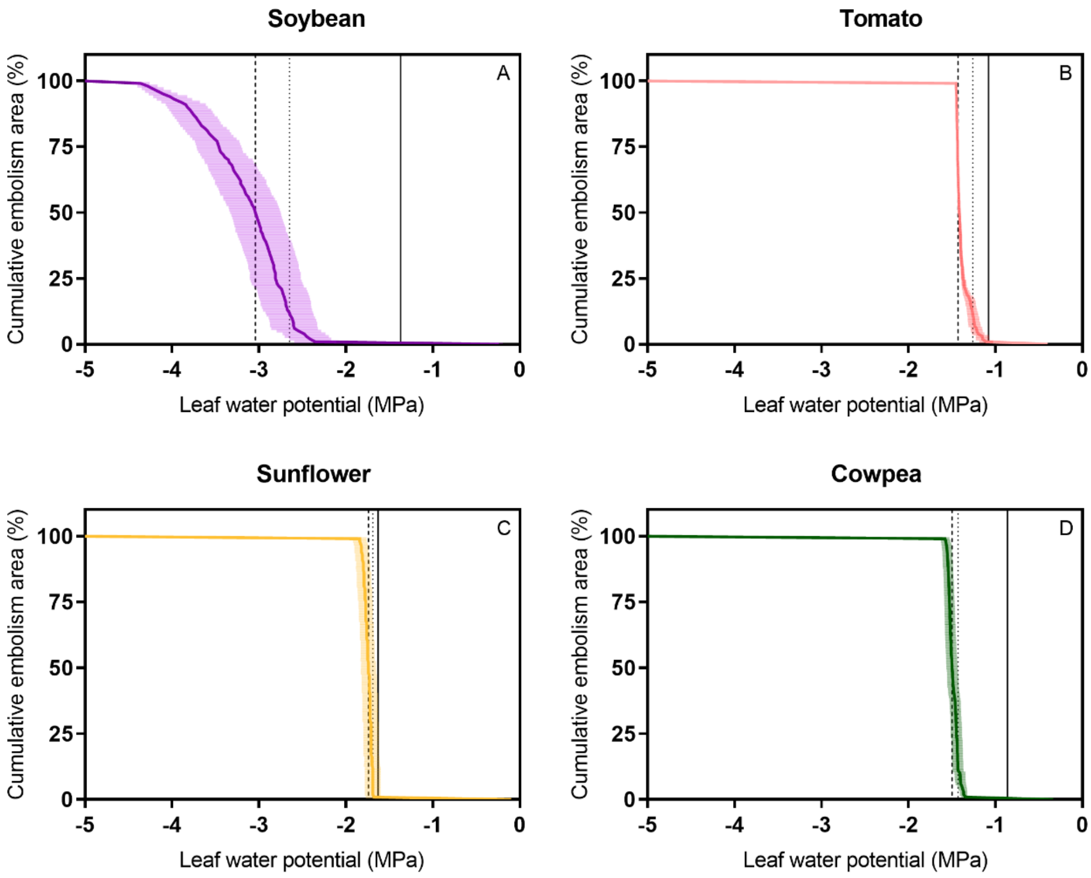
The SLA of soybean, sunflower, and tomato were significantly (varying from 200 to 212 cm^2 g^{-1}), while the SLA of cowpea was smaller than the remaining species (c. 172 cm^2 g^{-1}) (Table 2). A much larger variation across crops was found for the g_{min}, which ranged from 5.35 mmol m^{-2} s^{-1} in cowpea up to 22.45 mmol m^{-2} s^{-1} in tomato. Sunflower presented the second lowest g_{min} (c. 11.4 mmol m^{-2} s^{-1}). Regarding the SWC, cowpea and tomato presented very similar values (c. 5.51 and 5.44 g, respectively), while soybean presented the lowest (c. 2.27 g) and sunflower had the highest SWC (c. 7.59 g). Besides having the lowest SLA and g_{min}, cowpea plants also had the highest leaf capacitances (both before and after the TLP), which were respectively, two and five times higher than the values presented by soybean (the species with the lowest capacitances). Again, sunflower had the second-lowest leaf capacitances.

The Ψg_{s90} and Ψ_{tlp} were similar to each other for soybean, tomato, and cowpea, but Ψg_{s90} was considerably higher than the Ψ_{tlp} in sunflower (Table 2). Cowpea and tomato presented the two highest Ψg_{s90} and Ψ_{tlp} (varying between *c*. -0.86 to -1.08 MPa) and soybean presented Ψg_{s90} and Ψ_{tlp} of c. -1.40 MPa. Sunflower exhibited a Ψ_{tlp} of -1.03 MPa and a Ψg_{s90} of 1.63 MPa. The leaf xylem resistance to embolism, which was assessed through optical vulnerability curves (Fig 4), varied considerably across individual leaves and crops (Table 2). Tomato and cowpea had the least resistant xylem (P_{50} of c. -1.45 MPa), sunflower had slightly more resistant xylem than tomato and cowpea (P_{50} of c. -1.74 MPa), and soybean exhibited incredibly resistant xylem (P_{50} of c. -3.04 MPa). Due to its considerably low P_{50}, the SSM of soybean was the highest (1.67 MPa), followed by cowpea (0.63 MPa), tomato (0.34 MPa), and finally sunflower (0.11 MPa).

Table 2. Specific leaf area (SLA), minimum leaf conductance (g_{min}), saturated water content (SWC), capacitance at full turgor and following turgor loss point (C_{ft} and C_{tlp}, respectively), turgor loss point (Ψ_{tlp}), water potential at 50% cumulative embolism (P_{50}), and stomatal safety margin (SSM) across four herbaceous crops. Values are mean ± SE (*n* = 8 for tomato and cowpea, and *n* = 6 for soybean and sunflower). Different letters denote statistical differences among genotypes according to the LSD test ($P<0.05$).

Species	SLA (cm^2 g^{-1})	g_{min} (mmol m^{-2} s^{-1})	SWC (g/g)	C_{ft} (mol m^{-2} MPa^{-1})	C_{tlp} (mol m^{-2} MPa^{-1})	Ψ_{tlp} (MPa)	$\Psi(g_{s90})$ (MPa)	P_{50} (MPa)	HSM (MPa)
Soybean	200.0 ± 6.0a	17.21 ± 1.09b	2.27 ± 0.213c	0.8 ± 0.06b	1.3 ± 0.10c	-1.45 ± 0.08b	-1.37 ± 0.14b	-3.04 ± 0.29b	1.67
Tomato	210.5 ± 7.9a	22.45 ± 1.08a	5.51 ± 0.333b	1.0 ± 0.11b	3.5 ± 0.39b	-0.91 ± 0.01a	-1.08 ± 0.09a	-1.42 ± 0.01a	0.34
Sunflower	212.6 ± 3.8a	11.4 ± 1.10c	7.59 ± 0.176b	1.8 ± 0.13a	4.8 ± 0.58a	-1.03 ± 0.05a	-1.63 ± 0.06b	-1.74 ± 0.09a	0.11
Cowpea	171.9 ± 13.4b	5.35 ± 0.58d	5.44 ± 0.500a	1.9 ± 0.05a	6.8 ± 0.73a	-1.01 ± 0.05a	-0.86 ± 0.03a	-1.49 ± 0.06a	0.63

Fig. 4. Cumulative embolized xylem area (%) during leaf dehydration across four herbaceous crops [soybean (brown), tomato (red), sunflower (yellow), and cowpea bean (green)]. Each curve is represented by mean (solid lines) ± SE (shaded area) (*n* = 5 for tomato and sunflower, and *n* = 6 for soybean and cowpea). The vertical solid, dotted, and dashed lines indicate the mean water potential at 90% of stomatal closure (gs90), the threshold of 12, and 50% cumulative embolism, respectively.



Discussion

Here we exposed four crops to severe drought by withholding irrigation until events of leaf mortality were visibly identified in all plant species. Under such drought intensity, roots likely became disconnected from the soil (Carminati et al., 2013; Rodriguez-Dominguez & Brodribb, 2019), and, consequently, plant water-use defined their rate of survival. Contrary to some findings in woody plants (Chen et al., 2019; Oliveira et al., 2021), we found that the SSM was poorly associated with plant survival in herbaceous crops. This finding was also observed when comparing different tomato genotypes with contrasting levels of abscisic acid, in which plants with similar SSM exhibit very different times for hydraulic failure and mortality (Haverroth et al., 2023). Rather, drought avoidance mechanisms, i.e., (i) early stomatal closure (i.e. high Ψg_{s90}), (ii) very low residual transpiration post-stomatal closure (i.e. low g_{min}), and (iii) high pre- and post-turgor loss capacitance rendered the most effective strategy in ensuring plant survival under severe drought in herbaceous crops.

Contrasting crop responses to drought due to differences in water-use strategy

We assessed leaf water-use strategies through stomata (g_{min} and Ψ_{gs90}), water storage (by using capacitance), capacity for turgor maintenance (Ψ_{tlp}), and xylem resistance to embolism (P_{50}). Classically, a drought avoidance strategy (in the sense of minimizing both water loss and variation in water potential) would associate with (i) sensitive stomata to water deficit (high Ψ_{gs90}), (ii) minimal residual transpiration (low g_{min}), and (iii) strong buffering against water potential variation per unit of water released (high capacitance). On the other hand, a drought tolerance strategy would associate with (iv) the ability to sustain turgor at lower water potentials (lower Ψ_{tlp}) and (v) a xylem highly resistant to embolism (low P_{50}). Therefore, we speculated whether an ideotype of drought-resistant plant (aiming at survivability under severe drought, i.e., increasing xylem safety) would combine the mechanisms of drought avoidance and tolerance.

Interestingly, and contrary to our expectations, the plants that performed better in terms of survival were those presenting more drought avoidance traits (traits i, ii and iii): cowpea (100% survival, traits i, ii, iii), sunflower (60% survival, traits ii and iii), and tomato (0% survival, trait i only). In cowpea, this strategy was incredibly efficient in maintaining both plant hydration and survival over a prolonged drought period (Fig 1 and 3; Table 1). Conversely, soybean plants, which presented traits associated with drought tolerance (traits iv and v), experienced an abrupt decline to exceedingly low water potentials which culminated in the mortality of all their individuals (Fig 1 and 3; Table 1). The apparent better performance of drought avoidance mechanisms under severe drought has also been reported in wheat (Li et al., 2021), maize (Liu et al., 2023), and grasses and forbes (wild species from a temperate grassland, see Kramp et al., 2022). Noteworthy, we have to highlight that our study only analyzed one facet of drought avoidance, the water loss minimization. The other facet, the increase in water uptake (which is usually associated with deep rooting) is acknowledged as a source of drought-resistance in several crops and of particular importance for sunflower when cultivated under semi-arid conditions (Hussain et al., 2018).

Regarding drought tolerance traits, under our drought conditions (water withholding in potted plants), they seemed to be less advantageous. In any case, crops often exhibit a combination of drought resistance mechanisms due to artificial selection (Huang et al., 2024). Soybean genotypes show a marked genotypic variation for water use efficiency, capacity for water extraction, and g_{min} (Hufstetler et al., 2007) and it is possible to find fast-wilting genotypes (the cultivar here used) or slow-wilting genotypes (considered drought resistant) (Kunert and Vorster, 2020). Thus, as long as there is genetic variation available, genotypes with different water-use strategies can be developed. Modern varieties of sorghum, for example, were developed with the purpose of drought escape whereas landraces are more drought tolerant (Cavatassi et al., 2010). Cultivated tomato is considered highly demanding on water and drought-sensitive (Zsogon et al., 2017); on the other hand, long-storage tomato, cultivated under no water supply in semi-arid regions of Italy, is considered drought resistant (Patane et al., 2016). Interestingly, Patane et al. (2016), when studying 10 accessions of long-storage tomato, found a positive correlation between drought-resistance and capacity for osmotic adjustment; however, drought resistance and yield were negatively correlated. Thus, it is no surprise that breeders have been looking for other tomato species, such as *Solanum pennellii and Solanum chilense,* as sources of genetic variation for drought resistance (Zsogon et al., 2017).

Stomata sensitivity to water deficit and minimum conductance

Given that the vast majority of water loss occurs through the stomata, an early and efficient stomatal closure represents one of the most efficient mechanisms plants exhibit to delay dehydration and prevent leaf damage (Delzon, 2015; Cardoso *et al.*, 2018; Dayer *et al.*, 2020*a*; Brodribb *et al.*, 2021*a*). Therefore, partial or complete stomatal closure before the Ψ_{tlp} is considered a key drought resistance mechanism. Noteworthy, cowpea closed 90% of its stomatal aperture before its Ψ_{tlp} (Table 2), and the maximum stomatal closure was observed only three days after the drought imposition (Figs 1 and 2). All other crops closed 90% of their stomata around or after turgor loss point (Table 2). Amongst the remaining three species, tomato plants exhibited the second earliest stomatal closure, at a relatively low water potential (i.e., -1.08 MPa), but this mechanism alone was not sufficient to slow down decreases in water potential in this crop (Fig 1). Sunflower, on the contrary, presented the latest stomatal closure during drought (-1.63 MPa) and yet maintained a similar decline in water potential to tomato (Fig 1), likely to the presence of other conservative mechanisms such as the second-lowest g_{min} and second-highest leaf capacitance (Table 2).

Another intrinsic leaf trait contributing to drought resistance is the g_{min} (Duursma *et al.*, 2019*a*; Machado *et al.*, 2020). Cowpea exhibited the lowest g_{min} of all four herbaceous crops utilized in this study. Additionally, the g_{min} of cowpea (5.35 mmol m^{-2} s^{-1}) resembles the lowest values found for crops in a meta-analysis (Duursma *et al.*, 2019*a*). Associated with the early and efficient stomatal closure, the very low g_{min} likely played a crucial role in allowing cowpea plants to have an efficient decline in the whole-plant transpiration to undetectable levels within the first days of drought (Fig 2C).

At the opposite extreme, tomato plants exhibited the highest g_{min} (22.45 mmol m^{-2} s^{-1}), which is also higher than other economically important herbaceous crops such as sorghum, maize, wheat, and rice (Duursma *et al.*, 2019*a*). Such high g_{min} might be a consequence of incomplete stomatal closure, which was observed during the drought experiment (see residual stomatal conductance and residual whole-plant transpiration until nine days after drought in Figs 2 B and C). Interestingly, despite having the second-lowest g_{min}, sunflower plants exhibited the highest residual transpiration for entire plants (Fig 2C), which might indicate water loss through other parts of the plant, especially flowers (Zhang and Brodribb, 2017; Bourbia *et al.*, 2020) and stems. Given that most studies only assess stem transpiration in woody plants (Wolfe, 2020; Lintunen *et al.*, 2021), further studies on stem transpiration of herbaceous plants might bring us new insights into the water conservation mechanisms of crops.

Role of leaf capacitance for drought resistance

In addition to an early and efficient stomatal closure and a low g_{min}, a high leaf capacitance is a key drought resistance trait avoiding abrupt declines in leaf water potentials for a certain amount of water lost. Before turgor loss, a higher leaf capacitance buffers against changes in Ψ_w during periods of high transpiration or high vapor pressure deficit. After turgor loss, increased capacitance may lengthen organ and plant survival by minimizing transient fluctuations in water potentials and preventing embolism after stomatal closure (Sack *et al.*, 2003*a*; Blackman and Brodribb, 2011; Bryant *et al.*, 2021). Thus, it is likely that the high leaf capacitance of cowpea played an important role in maintaining high leaf water potentials during drought. On the other hand, in sunflower, which had the second-highest capacitance, this trait buffered the decrease in water potential, but xylem tension still reached critical threshold in leaves and some plants died. Interestingly, as observed in other studies, we found a weak correlation between the saturated water content (SWC) and capacitance (Hao et al, 2008; Blackmand &Brodribb, 2011; Nadal et al., 2018). This can be seen when comparing tomato and cowpea which have nearly the same SWC but contrasting capacitances. Considering the capacitance at full turgor (C_{ft}), differences are expected as this capacitance is directly impacted by the cell wall elasticity. Conversely, the capacitance after turgor loss (C_{tlp}) depends on the amount of water released and the osmotic potential. In this study, we demonstrate the independence of these two capacitances when comparing cowpea and sunflower plants (or even tomato and soybean) which had the same C_{ft} but different C_{tlp}. Different types of water-storage cells and their localization impact leaf capacitance and, given the importance of this trait for plant survival, understanding what makes some leaves better capacitors than others might be an important avenue for improving drought resistance (Luo et al., 2021).

Xylem resistance to embolism in leaves

As drought progresses, the water potential within xylem conduits can reach a threshold at which embolism occurs and water transport is compromised. Cowpea plants were so efficient in minimizing water loss that, even after 15 days of water withholding, the lowest mean midday water potential (-1.29 MPa) did not surpass the P_{50} of this species (-1.49 MPa). Given the direct impact of xylem embolism on leaf physiology and survival (Cardoso *et al.*, 2020; Brodribb *et al.*, 2021*a*), the maintenance of leaf water potential above the P_{50} might explain why very few leaves of cowpea were damaged during drought (Table 1; Fig 3). Additionally, because of its efficiency in maintaining high water potentials during drought, cowpea was the most efficient crop to recover its function from drought (Figs 1, 2, and S1).

Tomato and soybean, on the contrary, reached P_{50} after three and seven days of drought, respectively (Fig 1), likely explaining the abrupt declines in F_v/F_m as well as the extensive mortality of leaves and whole plants of these two crops during this experiment (Table 1; Fig 3). Interestingly, soybean had the most resistant xylem to embolism and the largest SSM (Table 2), however, its poor water conservation strategy (i.e. late stomatal closure, high g_{min}, and low leaf capacitance) resulted in a fast decline in water potential below the xylem embolism threshold (Fig 1). Finally, sunflower plants also reached very low water potentials during drought (Fig 1), which were much lower than the P_{50} of this species, and because of that, we also observed a major decline in F_v/F_m and considerable mortality of leaves across the sunflower crown 14 to 15 days after the drought imposition (Table 1; Fig 3). Additionally, 40% of the sunflower plants died, likely because, in addition to extensive embolism in the xylem of leaves, stems also experienced high amounts of embolism, ultimately resulting in whole plant death (Brodribb & Cochard, 2009). In this sense, regarding the plants that survived, they could have achieved this through two ways, by (i) presenting higher stem resistance to embolism and/or by (ii) minimizing xylem tension. Our data suggest that option (ii) seems more likely as we have seen a higher variation in leaf water potential (see Fig. 1) suggesting that some plants passed the point of no return in relation to stem embolism.

At last, we asked how the information gathered in this study could be useful in the context of crop management. First and most important, for irrigation purposes, special attention should be paid to crops with leaves presenting a low g_{s90}, high g_{min}, low capacitance, and low SWC. Such crops might display fast decreases in water potential and, as a result, irrigation frequency should be higher. For breeding purposes from a water relations standpoint and focusing on ensuring survival under drought, firstly, breeders should aim at decreasing the water loss (selecting for high g_{s90} and low g_{min}) and, secondly, at slowing the water potential decline (selecting for high capacitance). This strategy is based on cowpea plants showing that avoiding the water potential decline seems to be more effective than increasing xylem resistance to embolism (see the soybean example). In addition, drought strategies minimizing water loss are of particular importance especially when considering the ongoing climate changes since the expected increases in air vapor pressure deficit will maximize water loss (McDowell et al., 2022). Obviously, for advanced steps in a breeding program, once g_{s90}, g_{min}, and capacitance were optimized, the next step would be to decrease Ψ_{tlp} and P_{50}. Decreasing Ψ_{tlp} and increasing root depth (if water is available in deep soil profiles) have been associated with enhanced water extraction (Varshney et al., 2021) which, in turn, correlates with the other facet of drought avoidance, maximization of water absorption.

Conclusion

The SSM was poorly associated with the survival rate and demonstrated that the ability to resist drought in herbaceous species goes beyond investing in xylem resistance to embolism, rather, drought avoidance traits involved in minimizing water loss were the most efficient mechanisms in ensuring survival in herbaceous crops.

Author contributions

TSP, AAC, and SCVM conceived the study; TSP, LAO, EJH, and MTA carried out experiments; TSP, AAC, and SCVM analyzed the data and wrote the manuscript; All authors read and approved the final manuscript.

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References

Abdalla, M., Carminati, A., Cai, G., Javaux, M., & Ahmed, M. A. (2021). Stomatal closure of tomato under drought is driven by an increase in soil–root hydraulic resistance. Plant Cell and Environment, 44(2), 425–431. https://doi.org/10.1111/pce.13939

Agbicodo, E. M., Fatokun, C. A., Muranaka, S., Visser, R. G. F., & Linden Van Der, C. G. (2009). Breeding drought tolerant cowpea: Constraints, accomplishments, and future prospects. Euphytica, 167(3), 353–370. https://doi.org/10.1007/s10681-009-9893-8

Arya, H., Singh, M. B., & Bhalla, P. L. (2021). Towards Developing Drought-smart Soybeans. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.750664

Barnard, D. M., & Bauerle, W. L. (2013). The implications of minimum stomatal conductance on modeling water flux in forest canopies. Journal of Geophysical Research: Biogeosciences, 118(3), 1322–1333. https://doi.org/10.1002/jgrg.20112

Blackman, C. J., & Brodribb, T. J. (2011). Two measures of leaf capacitance: Insights into the water transport pathway and hydraulic conductance in leaves. Functional Plant Biology, 38(2), 118–126. https://doi.org/10.1071/FP10183

Blackman, C. J., Pfautsch, S., Choat, B., Delzon, S., Gleason, S. M., & Duursma, R. A. (2016). Toward an index of desiccation time to tree mortality under drought. Plant Cell and Environment, 39(10), 2342–2345. https://doi.org/10.1111/pce.12758

Bourbia, I., Carins-Murphy, M. R., Gracie, A., & Brodribb, T. J. (2020). Xylem cavitation isolates leaky flowers during water stress in pyrethrum. New Phytologist, 227(1), 146–155. https://doi.org/10.1111/nph.16516

Brodribb, T. J., & Cochard, H. (2009). Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiology, 149(1), 575–584. https://doi.org/10.1104/pp.108.129783

Brodribb, T. J., Bienaimé, D., & Marmottant, P. (2016). Revealing catastrophic failure of leaf networks under stress. Proceedings of the National Academy of Sciences of the United States of America, 113(17), 4865–4869. https://doi.org/10.1073/pnas.1522569113

Brodribb, T., Brodersen, C. R., Carriqui, M., Tonet, V., Rodriguez Dominguez, C., & McAdam, S. (2021). Linking xylem network failure with leaf tissue death. New Phytologist, 232(1), 68–79. https://doi.org/10.1111/nph.17577

Bryant, C., Fuenzalida, T. I., Brothers, N., Mencuccini, M., Sack, L., Binks, O., & Ball, M. C. (2021). Shifting access to pools of shoot water sustains gas exchange and increases stem hydraulic safety during seasonal atmospheric drought. Plant Cell and Environment, 44(9), 2898–2911. https://doi.org/10.1111/pce.14080

Campobenedetto, C., Agliassa, C., Mannino, G., Vigliante, I., Contartese, V., Secchi, F., & Bertea, C. M. (2021). A biostimulant based on seaweed (*Ascophyllum nodosum* and *Laminaria digitata*) and yeast extracts mitigates water stress effects on tomato (*Solanum lycopersicum* L.). Agriculture (Switzerland), 11(6), 1–16. https://doi.org/10.3390/agriculture11060557

Cardoso, A. A., Batz, T. A., & McAdam, S. A. M. (2020). Xylem embolism resistance determines leaf mortality during drought in persea americana. Plant Physiology, 182(1), 547–554. https://doi.org/10.1104/pp.19.00585

Cardoso, A. A., Brodribb, T. J., Lucani, C. J., DaMatta, F. M., & McAdam, S. A. M. (2018). Coordinated plasticity maintains hydraulic safety in sunflower leaves. Plant Cell and Environment, 41(11), 2567–2576. https://doi.org/10.1111/pce.13335

Carminati, A., Vetterlein, D., Koebernick, N., Blaser, S., Weller, U., & Vogel, H. J. (2013). Do roots mind the gap? Plant and Soil, 367(1–2), 651–661. https://doi.org/10.1007/s11104-012-1496-9

Cavatassi, R., Lipper, L., & Narloch, U. (2011). Modern variety adoption and risk management in drought prone areas: Insights from the sorghum farmers of eastern Ethiopia. Agricultural Economics, 42(3), 279–292. https://doi.org/10.1111/j.1574-0862.2010.00514.x

Chen, Z., Li, S., Luan, J., Zhang, Y., Zhu, S., Wan, X., & Liu, S. (2019). Prediction of temperate broadleaf tree species mortality in arid limestone habitats with stomatal safety margins. Tree Physiology, 39(8), 1428-1437. https://doi:10.1093/treephys/tpz045

Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. Nature, 558(7711), 531–539. https://doi.org/10.1038/s41586-018-0240-x

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S. J., Feild, T. S., Gleason, S. M., Hacke, U. G., Jacobsen, A. L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P. J., Nardini, A., Pittermann, J., … Zanne, A. E. (2012). Global convergence in the vulnerability of forests to drought. Nature, 491(7426), 752–755. https://doi.org/10.1038/nature11688

Dayer, S., Herrera, J. C., Dai, Z., Burlett, R., Lamarque, L. J., Delzon, S., Bortolami, G., Cochard, H., & Gambetta, G. A. (2020). The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. Journal of Experimental Botany, 71(14), 4333–4344. https://doi.org/10.1093/jxb/eraa186

Delzon, S. (2015). New insight into leaf drought tolerance. Source: Functional Ecology, 29(10), 1247–1249. https://doi.org/10.2307/48576703

Dhankher, O. P., & Foyer, C. H. (2018). Climate resilient crops for improving global food security and safety. Plant Cell and Environment, 41(5), 877–884. https://doi.org/10.1111/pce.13207

Duursma, R. A., Blackman, C. J., Lopéz, R., Martin-StPaul, N. K., Cochard, H., & Medlyn, B. E. (2019). On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. New Phytologist, 221(2), 693–705. https://doi.org/10.1111/nph.15395

Guadagno, C. R., Ewers, B. E., Speckman, H. N., Aston, T. L., Huhn, B. J., Devore, S. B., Ladwig, J. T., Strawn, R. N., & Weinig, C. (2017). Dead or alive? using membrane failure and chlorophyll a fluorescence to predict plant mortality from drought. Plant Physiology, 175(1), 223–234. https://doi.org/10.1104/pp.16.00581

Hammond, W. M., Williams, A. P., Abatzoglou, J. T., Adams, H. D., Klein, T., López, R., Sáenz-Romero, C., Hartmann, H., Breshears, D. D., & Allen, C. D. (2022). Global field observations of tree die-off reveal hotter-drought fingerprint for Earth’s forests. Nature Communications, 13(1). https://doi.org/10.1038/s41467-022-29289-2

Hammond, W. M., Yu, K., Wilson, L. A., Will, R. E., Anderegg, W. R. L., & Adams, H. D. (2019). Dead or dying? Quantifying the point of no return from hydraulic failure in drought-induced tree mortality. New Phytologist, 223(4), 1834–1843. https://doi.org/10.1111/nph.15922

Hao, G. Y., Hoffmann, W. A., Scholz, F. G., Bucci, S. J., Meinzer, F. C., Franco, A. C., Cao, K. F., & Goldstein, G. (2008). Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. Oecologia, 155(3), 405–415. https://doi.org/10.1007/s00442-007-0918-5

Haverroth, E. J., Oliveira, L. A., Andrade, M. T., Taggart, M., McAdam, S. A. M., Zsögön, A., Thompson, A. J., Martins, S. C. V., Cardoso, A. A. (2023). Abscisic acid acts essentially on stomata, not on xylem, to improve drought resistance in tomato. Plant, Cell & Environment, In press. https://doi.org/10.1111/pce.14676

Huang, R., Di, N., Xi, B., Yang, J., Duan, J., Li, X., Feng, J., Choat, B., & Tissue, D. (2024). Herb hydraulics: Variation and correlation for traits governing drought tolerance and efficiency of water transport. Science of the Total Environment, 907. https://doi.org/10.1016/j.scitotenv.2023.168095

Hufstetler, E. V., Boerma, H. R., Carter, T. E., & Earl, H. J. (2007). Genotypic variation for three physiological traits affecting drought tolerance in soybean. Crop Science, 47(1), 25–35. https://doi.org/10.2135/cropsci2006.04.0243

Hussain, M., Farooq, S., Hasan, W., Ul-Allah, S., Tanveer, M., Farooq, M., & Nawaz, A. (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. Agricultural Water Management, 201, 152–166. https://doi.org/10.1016/j.agwat.2018.01.028

Kaack, L., Weber, M., Isasa, E., Karimi, Z., Li, S., Pereira, L., Trabi, C. L., Zhang, Y., Schenk, H. J., Schuldt, B., Schmidt, V., & Jansen, S. (2021). Pore constrictions in intervessel pit membranes provide a mechanistic explanation for xylem embolism resistance in angiosperms. New Phytologist, 230(5), 1829–1843. https://doi.org/10.1111/nph.17282

Kramp, R. E., Liancourt, P., Herberich, M. M., Saul, L., Weides, S., Tielbörger, K., & Májeková, M. (2022). Functional traits and their plasticity shift from tolerant to avoidant under extreme drought. Ecology, 103(12). https://doi.org/10.1002/ecy.3826

Kunert, K., & Vorster, B. J. (2020). In search for drought-tolerant soybean: Is the slow-wilting phenotype more than just a curiosity? Journal of Experimental Botany, 71(2), 457–460. https://doi.org/10.1093/jxb/erz235

Lens, F., Picon-Cochard, C., Delmas, C. E. L., Signarbieux, C., Buttler, A., Cochard, H., Jansen, S., Chauvin, T., Doria, L. C., del Arco, M., & Delzon, S. (2016). Herbaceous angiosperms are not more vulnerable to drought-induced embolism than angiosperm trees. Plant Physiology, 172(2), 661–667. https://doi.org/10.1104/pp.16.00829

Lenz, T. I., Wright, I. J., & Westoby, M. (2006). Interrelations among pressure-volume curve traits across species and water availability gradients. Physiologia Plantarum, 127(3), 423–433. https://doi.org/10.1111/j.1399-3054.2006.00680.x

Levitt, J. (1980). Responses of plants to environmental stresses. Volume II. Water, radiation, salt, and other stresses. Academic Press.

Li, P., Ma, B., Palta, J. A., Ding, T., Cheng, Z., Lv, G., & Xiong, Y. (2021). Wheat breeding highlights drought tolerance while ignores the advantages of drought avoidance: A meta-analysis. European Journal of Agronomy, 122. https://doi.org/10.1016/j.eja.2020.126196

Li, X., Xi, B., Wu, X., Choat, B., Feng, J., Jiang, M., & Tissue, D. (2022). Unlocking Drought-Induced Tree Mortality: Physiological Mechanisms to Modeling. Frontiers in Plant Science, 13, 1–15. https://doi.org/10.3389/fpls.2022.835921

Lintunen, A., Preisler, Y., Oz, I., Yakir, D., Vesala, T., & Hölttä, T. (2021). Bark transpiration rates can reach needle transpiration rates under dry conditions in a semi-arid forest. Frontiers in Plant Science, 12, 1–16. https://doi.org/10.3389/fpls.2021.790684

Liu, Y., Hou, P., Zhang, W., Xing, J., Lv, T., Zhang, C., Wang, R., & Zhao, J. (2023). Drought resistance of nine maize cultivars released from the 1970s through the 2010s in China. Field Crops Research, 302. https://doi.org/10.1016/j.fcr.2023.109065

Luo, Y., Ho, C. L., Helliker, B. R., & Katifori, E. (2021). Leaf Water Storage and Robustness to Intermittent Drought: A Spatially Explicit Capacitive Model for Leaf Hydraulics. Frontiers in Plant Science, 12, 1–13. https://doi.org/10.3389/fpls.2021.725995

Machado, R., Loram-Lourenço, L., Farnese, F. S., Alves, R. D. F. B., de Sousa, L. F., Silva, F. G., Filho, S. C. V., Torres-Ruiz, J. M., Cochard, H., & Menezes-Silva, P. E. (2021). Where do leaf water leaks come from? Trade-offs underlying the variability in minimum conductance across tropical savanna species with contrasting growth strategies. New Phytologist, 229(3), 1415–1430. https://doi.org/10.1111/nph.16941

Mantova, M., Cochard, H., Burlett, R., Delzon, S., King, A., Rodriguez-Dominguez, C. M., Ahmed, M. A., Trueba, S., & Torres-Ruiz, J. M. (2023). On the path from xylem hydraulic failure to downstream cell death. New Phytologist, 237(3), 793–806. https://doi.org/10.1111/nph.18578

Martinez-Vilalta, J., Anderegg, W. R. L., Sapes, G., & Sala, A. (2019). Greater focus on water pools may improve our ability to understand and anticipate drought-induced mortality in plants. New Phytologist, 223(1), 22–32. https://doi.org/10.1111/nph.15644

Martins, S. C. V., Mcadam, S. A. M., Deans, R. M., Damatta, F. M., & Brodribb, T. J. (2016). Stomatal dynamics are limited by leaf hydraulics in ferns and conifers: Results from simultaneous measurements of liquid and vapour fluxes in leaves. Plant Cell and Environment, 39(3), 694–705. https://doi.org/10.1111/pce.12668

McDowell, N. G., Sapes, G., Pivovaroff, A., Adams, H. D., Allen, C. D., Anderegg, W. R. L., Arend, M., Breshears, D. D., Brodribb, T., Choat, B., Cochard, H., de Cáceres, M., de Kauwe, M. G., Grossiord, C., Hammond, W. M., Hartmann, H., Hoch, G., Kahmen, A., Klein, T., … Xu, C. (2022). Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. Nature Reviews Earth and Environment, 3(5), 294–308. https://doi.org/10.1038/s43017-022-00272-1

Merwad, A. R. M. A., Desoky, E. S. M., & Rady, M. M. (2018). Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application. Scientia Horticulturae, 228, 132–144. https://doi.org/10.1016/j.scienta.2017.10.008

Mitchell, P. J., Veneklaas, E. J., Lambers, H., & Burgess, S. S. O. (2008). Leaf water relations during summer water deficit: Differential responses in turgor maintenance and variation in leaf structure among different plant communities in south-western Australia. Plant, Cell and Environment, 31(12), 1791–1802. https://doi.org/10.1111/j.1365-3040.2008.01882.x

Moschen, S., Di Rienzo, J. A., Higgins, J., Tohge, T., Watanabe, M., González, S., Rivarola, M., García-García, F., Dopazo, J., Hopp, H. E., Hoefgen, R., Fernie, A. R., Paniego, N., Fernández, P., & Heinz, R. A. (2017). Integration of transcriptomic and metabolic data reveals hub transcription factors involved in drought stress response in sunflower (*Helianthus annuus* L.). Plant Molecular Biology, 94(4–5), 549–564. https://doi.org/10.1007/s11103-017-0625-5

Nadal, M., Flexas, J., & Gulías, J. (2018). Possible link between photosynthesis and leaf modulus of elasticity among vascular plants: a new player in leaf traits relationships? Ecology Letters, 21(9), 1372–1379. https://doi.org/10.1111/ele.13103

Oliveira, R. S., Eller, C. B., Barros, F. de V., Hirota, M., Brum, M., & Bittencourt, P. (2021). Linking plant hydraulics and the fast–slow continuum to understand resilience to drought in tropical ecosystems. New Phytologist, 230(3), 904–923. https://doi.org/10.1111/nph.17266

Patanè, C., Scordia, D., Testa, G., & Cosentino, S. L. (2016). Physiological screening for drought tolerance in Mediterranean long-storage tomato. Plant Science, 249, 25–34. https://doi.org/10.1016/j.plantsci.2016.05.006

Rigano, M. M., Arena, C., Di Matteo, A., Sellitto, S., Frusciante, L., & Barone, A. (2016). Eco-physiological response to water stress of drought-tolerant and drought-sensitive tomato genotypes. Plant Biosystems, 150(4), 682–691. https://doi.org/10.1080/11263504.2014.989286

Rodriguez-Dominguez, C. M., & Brodribb, T. J. (2020). Declining root water transport drives stomatal closure in olive under moderate water stress. New Phytologist, 225(1), 126–134. https://doi.org/10.1111/nph.16177

Sack L. & Scoffoni C. (2011). Minimum epidermal conductance (gmin, a.k.a. cuticular conductance). PrometheusWiki. https://prometheusprotocols.net/function/gas-exchange-and-chlorophyll-fluorescence/stomatal-and-non-stomatal-conductance-and-transpiration/minimum-epidermal-conductance-gmin-a-k-a-cuticular-conductance/

Sack, L., Cowan, P. D., Jaikumar, N., & Holbrook, N. M. (2003). The “hydrology” of leaves: co-ordination of structure and function in temperate woody species. Plant, Cell and Environment, 26, 1343–1356. https://doi.org/10.1046/j.0016-8025.2003.01058.x

Singh, B. B., Ajeigbe, H. A., Tarawali, S. A., Fernandez-Rivera, S., & Abubakar, M. (2003). Improving the production and utilization of cowpea as food and fodder. Field Crops Research, 84(1–2), 169–177. https://doi.org/10.1016/S0378-4290(03)00148-5

Sperry, J. S., & Tyree, M. T. (1988). Mechanism of Water Stress-Induced Xylem Embolism. Plant Physiology, 88(3), 581–587. https://doi.org/10.1104/pp.88.3.581

Thonglim, A., Bortolami, G., Delzon, S., Larter, M., Offringa, R., Keurentjes, J. J. B., Smets, E., Balazadeh, S., & Lens, F. (2022). Drought response in Arabidopsis displays synergistic coordination between stems and leaves. Journal of Experimental Botany, 74(3), 1004–1021. https://doi.org/10.1093/jxb/erac446

Trugman, A. T. (2022). Integrating plant physiology and community ecology across scales through trait-based models to predict drought mortality. New Phytologist, 234(1), 21–27. https://doi.org/10.1111/nph.17821

Tyree, M. T., & Hammel, H. T. (1972). The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. Journal of Experimental Botany, 23(1), 267–282. https://doi.org/10.1093/jxb/23.1.267

Urli, M., Porté, A. J., Cochard, H., Guengant, Y., Burlett, R., & Delzon, S. (2013). Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiology, 33(7), 672–683. https://doi.org/10.1093/treephys/tpt030

Varshney, R. K., Barmukh, R., Roorkiwal, M., Qi, Y., Kholova, J., Tuberosa, R., Reynolds, M. P., Tardieu, F., & Siddique, K. H. M. (2021). Breeding custom-designed crops for improved drought adaptation. Advanced Genetics, 2(3). https://doi.org/10.1002/ggn2.202100017

Volaire, F. (2018). A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. Global Change Biology, 24(7), 2929–2938. https://doi.org/10.1111/gcb.14062

Wolfe, B. T. (2020). Bark water vapour conductance is associated with drought performance in tropical trees. Biology Letters, 16(8), 1–5. https://doi.org/10.1098/rsbl.2020.0263

Xiong, D., & Nadal, M. (2020). Linking water relations and hydraulics with photosynthesis. The Plant Journal, 101(4), 800–815. https://doi.org/10.1111/tpj.14595

Zhang, F. P., & Brodribb, T. J. (2017). Are flowers vulnerable to xylem cavitation during drought? Proceedings of the Royal Society B: Biological Sciences, 284(1854), 1–8. https://doi.org/10.1098/rspb.2016.2642

Zia, R., Nawaz, M. S., Siddique, M. J., Hakim, S., & Imran, A. (2021). Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. Microbiological Research, 242, 1–16. https://doi.org/10.1016/j.micres.2020.126626

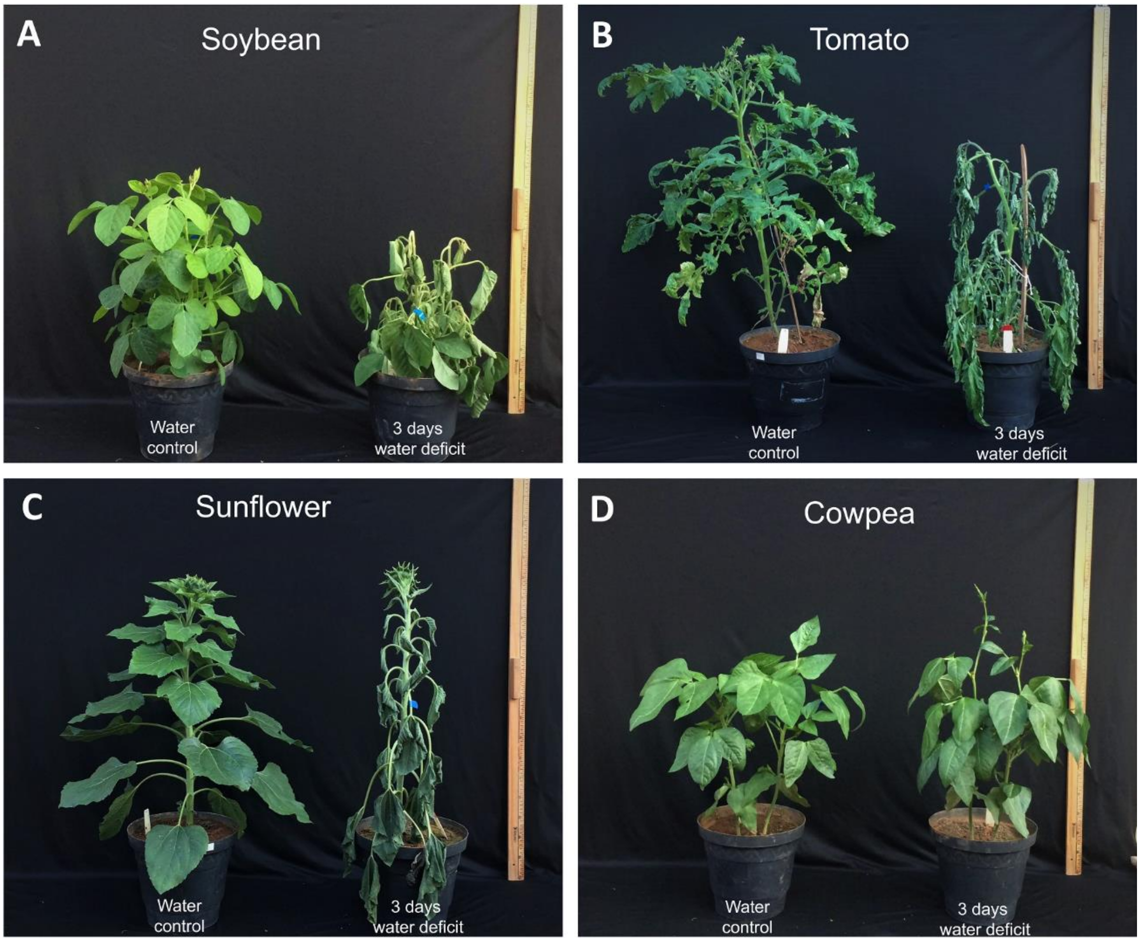
Zsogon, A., Vicente, M. H., Reartes, D. S., & Peres, L. E. P. (2017). Understanding and improving water-use efficiency and drought resistance in tomato. In Achieving sustaimble cultivation of tomatoes (pp. 117–142). Burleigh Dodds Science Publishing Limited. https://doi.org/10.19103/AS.2016.0007.01

Supporting information

Table S1. Midday water potential (Ψ_{midday}), the maximum quantum efficiency of the photosystem II (F_v/F_m), net photosynthetic rate (A), stomatal conductance (g_s), and whole-plant transpiration) across four herbaceous crop species cultivated under well-watered conditions. Values are mean ± SE ($n = 5$).

Species	$\Psi_{predawn}$ (MPa)	Ψ_{midday} (MPa)	F_v/F_m	A (µmol m^{-2} s^{-1})	gs(mol m^{-2} s^{-1})	E_{plant} (g $plant^{-1}$ day^{-1})
Soybean	-0.12 ± 0.02 a	-1.00 ± 0.11 b	0.83 ± 0.003 b	30.6 ± 0.81 a	0.64 ± 0.04 a	465.1 ± 22.80 a
Tomato	-0.12 ± 0.01 a	-0.87 ± 0.09 ab	0.81 ± 0.007 d	12.2 ± 0.74 c	0.14 ± 0.01 c	404.9 ± 18.09 b
Sunflower	-0.18 ± 0.02 b	-0.81 ± 0.07 ab	0.85 ± 0.002 a	33.2 ± 1.46 a	0.65 ± 0.04 a	524.3 ± 24.78 a
Cowpea	-0.19 ± 0.01 b	-0.73 ± 0.07 a	0.82 ± 0.004 c	23.7 ± 1.18 b	0.33 ± 0.03 b	394.2 ± 20.09 b

Fig. S1. Images of four herbaceous crop species [soybean (A), tomato (B), sunflower (C), and cowpea bean (D); control (left) water stress(right)] three days after water restriction.



CHAPTER 2

Assessing drought tolerance and avoidance in domesticated and wild tomatoes

Abstract

Drought stress poses a significant challenge to plant production and is projected to be intensified due to climate change. Plants have developed a range of drought tolerance and avoidance mechanisms to survive in water-limited environments. While much research has focused on woody plants, the mechanisms enabling herbaceous species to survive extreme drought are less explored. This study investigates the hydraulic traits of six *Solanum* species with varying abiotic stress tolerances (*S. pennellii, S. habrochaites, S. neorickii, S. galapagense, S. pimpinellifolium, and S. lycopersicum*) to evaluate drought resistance strategies within the *Lycopersicum* section. Despite the varied geographical origins of these species, our results reveal a narrow range in the water potential at which 50% of xylem hydraulic conductivity is lost (P_{50}) across genotypes, except for *S. habrochaites*, which exhibited a significantly lower P_{50}. Notably, *S. pennellii* demonstrated a prolonged time to hydraulic failure, highlighting the importance of drought avoidance traits such as high capacitance (C) and minimum conductance (g_{min}). These findings suggest that genetic breeding for drought resistance in cultivated tomatoes should prioritize traits like g_{min} and C over P_{50}. This study advocates for a more integrative approach to understanding the drought resistance of herbaceous plants and improving crop resilience to water stress studies.

Keywords: dehydration avoidance, leaf capacitance, hydraulic traits, *Solanum*, minimum leaf conductance, xylem embolism.

Introduction

Drought, one of the most limiting factors for plant production, is expected to increase in severity and frequency (Trenberth et al., 2014; IPCC. 2021). Plants have evolved a range of morphological, anatomical, and physiological mechanisms to withstand drought stress. These mechanisms can be broadly classified into two categories: (i) drought tolerance mechanisms, which allow plants to maintain physiological function despite low water potentials, and (ii) drought avoidance mechanisms, which enable plants to sustain higher water potentials during drought by maximizing water uptake and storage while minimizing water loss (e.g., stomatal control, minimum leaf conductance, leaf area, water storage, capacitance, and root depth) (Volaire, 2018*b*; Sun *et al.*, 2020; Guillemot *et al.*, 2022; Liang and Ye, 2024).

The question of what mechanisms contribute most to species-specific drought resistance has been extensively studied, particularly in tree species (McDowell *et al.*, 2008; Skelton *et al.*, 2015, 2021). There is a current consensus that plant hydraulic traits play a crucial role in determining drought tolerance, with hydraulic failure recognized as the primary cause of drought-induced mortality (McDowell *et al.*, 2008, 2022*b*). Indeed, the water potential at which 50% of a plant's hydraulic conductivity is lost (P_{50}) is commonly used as a proxy for drought tolerance (Choat *et al.*, 2012*b*, 2018*b*; Blackman *et al.*, 2016*b*). While considerable research has focused on identifying drought survival strategies in woody plants, the mechanisms enabling herbaceous species to survive and recover from extreme drought conditions remain underexplored (Choat et al., 2012; Dória et al., 2019; Lens et al., 2016; Thonglim et al., 2022; Volaire et al., 2018). Studies are also lacking in crops, despite their importance for food security.

Recently, Pereira et al. (2024) found that P_{50} was not a reliable predictor of prolonged survival in herbaceous crops under extreme drought lending support to the hypothesis that drought avoidance traits would be more important for plant survival under drought in this functional group. Furthermore, P_{50} varies considerably between tree species and habitats, with plants from xeric areas generally exhibiting greater resistance to xylem embolism compared to species from more humid environments (Choat *et al.*, 2012*b*; Lens *et al.*, 2016*b*; Skelton *et al.*, 2021). While P_{50} values across plant species range widely, from –0.5 to –19 MPa (Larter *et al.*, 2015), the variation within a single species tends to be much narrower. For example, in *Solanum lycopersicum*, leaf P_{50} values have been reported to range from –1.14 to –1.51 MPa (Haverroth *et al.*, 2023, 2024; Andrade *et al.*, 2024; Pereira *et al.*, 2024).

Adaptation to the local environment has long been considered a major factor driving morphological evolution and speciation (Nakazato *et al.*, 2010) and the tomatoes group is ideally suited to further expand this kind of integrated research. The genus *Solanum* contains the largest number of species within the *Solanaceae* family, with approximately 1,500 species, and both cultivated and wild tomatoes are classified under the section *Lycopersicon*, which consists of 13 species (Rezk *et al.*, 2021). Similar to other *Solanaceae* crops, the domestication of tomatoes involved selecting for obvious morphological traits, such as fruit size, shape, color, and productivity (Bai and Lindhout, 2007). Wild tomatoes thrive in their center of origin, the western coast of South America, where they grow in environments ranging from near sea level to altitudes of up to 3,300 meters, and from arid to rainy climates. These species often inhabit narrow, isolated valleys, adapting to specific microclimates and diverse soil types (Bauchet & Causse, 2012; Grandillo et al., 1999, 2011).

S. pennellii is a drought-adapted species and serves as an ideal experimental model for advancing the understanding of mechanisms related to drought adaptation and resistance in tomato plants (Egea *et al.*, 2018). *S. habrochaites* is resistant to low temperatures and its stomatal closure during root chilling is most likely an adaptive response to its cold habitat and might be important in the adaptation of other wild tomatoes to cold soils and other types of water stress (Easlon *et al.,* 2013; Zhang *et al.*, 2023). Chilling damage also derives in part from water stress in cold soils because chilling impedes root water absorption, which can result in shoot wilting if stomata do not close rapidly to limit water loss (Easlon *et al.,* 2013). *S. neorickii*, a species from humid regions, thriving in well-drained rocky slopes, is concentrated in the inter-Andean valleys of Peru and Ecuador, and no populations of either species are known from the west slopes of the Andes or east of the main cordilleras (Grandillo *et al.*, 2011; Ramírez-Ojeda *et al.*, 2021). Additionally, *S. galapagense* is naturally adapted to highly saline soils and may exhibit a high capacity to accumulate Na^+ and Cl^- in its leaves, enhanced hormonal signaling response, and osmotic adjustment (Bonarota et al., 2022), making these species potential candidates for evaluating hydraulic traits related to drought tolerance and mortality in herbaceous plants. *S. pennellii*, *S. galapagense*, and *S. pimpinellifolium* also exhibit higher tolerance to salt stress (Bonarota et al., 2022) and these species could maintain the root hydraulic conductance under salt stress conditions, whereas the cultivated tomato M82 could not, suggesting that the wild tomatoes could maintain better root water uptake under this stress (Han *et al.*, 2021) which is also an interesting feature for drought resistance. Finally, *S. lycopersicum*, the highly inbred tomato cultivar M82, which has traditionally been used as a check variety in breeding programs and as a reference cultivar for scientific research as a drought-sensitive cultivar, in contrast with the drought-tolerant *S. pennellii* (Egea *et al.*, 2018; Galdon-Armero *et al.*, 2018).

Given the above, the present study aims to evaluate six *Solanum* species (*S. pennellii, S. habrochaites, S. neorickii, S. galapagense, S. pimpinellifolium,* and *S. lycopersicum*) with different abiotic stress tolerances and from different habits to evaluate the extent that drought tolerance and avoidance traits vary in the *Lycopersicum* section and to determine whether the origin environment and domestication influenced the drought resistance strategies of tomato plants.

Materials and Methods

Plant material

Individuals of tomato of the *Solanum* genus *S. pennellii* (LA716), *S. habrochaites* (LA1777)*, S. neorickii* (LA2133), *S. galapagense* (LA1401)*, S. pimpinellifolium* (LA1589)*,* and *S. lycopersicum* (cv. M82) were cultivated from seeds in 5.5 L-plastic pots filled with clay soil, sand, and commercial substrate (2:1:1). One plant per pot was maintained inside a greenhouse (Viçosa, 20° 45' S, 42° 15' W, Brazil) under natural light (maximum photosynthetic photon flux density (PPFD) of c. 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and temperature (c. 30°C) until they were c. 45-day-old. During cultivation, plants received daily watering to full soil capacity and a weekly application of fertilizer using a commercial NPK formulation (10:10:10).

Leaf minimum conductance and water relation

Fully expanded leaves were collected from the middle third of shoots of well-watered plants and characterized in terms of, g_{min}, saturated water content (SWC), relative water content (RWC, modulus of elasticity (ε) turgor loss point (Ψ_{tlp}), capacitance before and after the turgor loss point (C_{ft} and C_{tlp}, respectively), and specific leaf area (SLA).

The g_{min} was measured using the mass loss of detached leaves method (Duursma et al., 2019). Leaves taken from different individuals ($n = 6$) of each species in well-watered condition were collected early in the evening, taken to the lab, had their petiole end completely sealed with parafilm, and then placed on the stand to allow slow dehydration while weighted every 15 min for 4 h. For each plant, g_{min} was determined from the slope of the linear part of the relationship between leaf mass versus time after stomatal closure and normalized by leaf area, VPD (obtained from air temperature and humidity measurements during the dehydration period), and atmospheric pressure (P).

Pressure–volume curves (Tyree and Hammel, 1972) were constructed using mature leaves taken from individuals of each species (n = 8). Leaves were collected early in the evening, scanned for leaf area measurements, recut under water, and allowed to rehydrate overnight until water potential higher than 0.1 Mpa. Leaf fresh weight and water potential were measured over time during slow bench dehydration until leaf water potential stopped falling due to leaf cell damage. Using leaf dry mass of oven-dried leaves (at 70°C until constant weight), the relative water content was calculated and plotted against the inverse of leaf water potential. Saturated water content (SWC) was estimated by extrapolating the regression of water mass vs water potential to estimate the water mass at 0 MPa and subsequently dividing by the dry mass. The Ψ_{tlp} was determined by the inflection point between the pre-turgor loss and post-turgor loss portions of the curve. *RWC* at turgor loss point was visually estimated from $\Psi_{tlp.}$ The modulus of elasticity at full turgor was calculated as change in turgor potential over the change in total RWC (ε). C_{ft} and C_{tlp} were obtained from the linear slopes of the plot (before and after the turgor loss point, respectively) and normalized by leaf area. The SLA was calculated as projected leaf area divided by leaf dry mass. For leaf minimum conductance, water relations, and drought response, we do not have data for *S. neorickii* plants, as this species did not germinate.

Crop responses to drought

Drought was imposed in five well-watered plants by withholding water in all species. Transpiration (TR) values (g H_2O plant^{-1}) were normalized (NTR) by calculating the ratio between water loss of the drying pot and the average of the water loss from the few days the pot was under well-watered conditions. A second correction was made to account for differences in transpiration that occurred during the days. Thus, the daily NTR of each plant was divided by the mean NTR during the period when the soil was still well-watered. Water was withheld from plants until they reached NTR below 0.10. Daily FTSW was calculated as the indicator of intensity of soil water deficit.

$$\mathrm{FTSW}=\frac{\text{daily pot weight}-\text{final pot weight}}{\text{initial pot weight}-\text{final pot weight}} \qquad (1)$$

Leaf hydraulics vulnerability

Leaf vulnerability curves for each species were assessed by the optical method (Brodribb *et al.*, 2016). Well-watered potted individuals of each species [*S. pennellii* (*n*=7), *S. habrochaites* (*n*=4)*, S. neorickii* (*n*=6), *S. galapagense* (*n*=4)*, S. pimpinellifolium* (*n*=6)*,* and *S. lycopersicum* (*n*=4)] were taken to the lab early in the morning and plants had the soil washed out from the roots to enhance the rate of soil drying. Subsequently, a fully mature leaf from middle third of shootswas placed under either a flatbed scanner (V800, Epson, Tokyo, Japan) or a custom built OpenSourceOV clamp (OSOV) to record the development of embolism in the leaves with images taken every c. 3 min. Due to their prolonged time of desiccation, *S. pennellii* plants were set up with 10 minutes between images. While embolism was recorded, plants were maintained under low light condition (PPFD < 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to allow whole plant equilibration. The leaf water potentials were periodically measured using a Scholander pressure chamber (Model 1000, PMS Instruments, Albany, USA). Embolism events were determined when visible changes in color in the xylem were observed. A small leaf section covering major and minor veins was selected and the area of embolized xylem (%) was quantified over time by image subtraction and the data was plotted against leaf water potential. For further details on this method, see the open-source website http://www.opensourceov.org. The optical P_{50} and P_{88} for each species was calculated as the mean value obtained from all curves. For each species, the SSM was calculated as defined by Ψ_{tlp} minus P_{50}. Time to achieve P_{50} and P_{88} (TP_{50}, TP_{88}) was measured in hours from the beginning of the leaf vulnerability curve.

Statistical analysis

Statistical analyses for PV and curves result and hydraulics features among species were performed using the R v.4.2.1 (R Core Team, 2022). The data were submitted to an analysis of variance and the significant differences between the means were determined by the Tukey`s test for multiple comparisons with probability level of ≤0.05, and Kruskal-Wallis test was used to non-normal data.

Results

The leaf xylem resistance to embolism, which was assessed through optical vulnerability curves (Figure 1), varied across species with the P_{50} ranging from -1.14 (*S.*

lycopersicum) to -1.54 MPa (*S. habrochaites*). The P_{88} ranged from -1.17 (*S. galapagense*) to -1.54 MPa (*S. habrochaites*) (Table 1), which did not variate significantly across the six species. However, the time to achieve those parameters was higher for *S. pennellii* (107.3 ± 0.010 h), which is in line with its presumed drought resistance. In addition, the species *S. galapagense, S. pimpinellifolium, and S. lycopersicum,* in which leaf embolism formation precedes leaf turgor loss, also presented smaller SSM, -0.02, 0.02, and 0.15 MPa, respectively.

Figure 1. Cumulative embolized xylem area (%) during leaf dehydration across across six *Solanum* species. Each curve is represented by mean (solid lines) ± SE (shaded area) [*S. pennellii* (*n*=7), *S. habrochaites* (*n*=5), *S. neorickii* (*n*=6), *S. galapagense* (*n*=4), *S. pimpinellifolium* (*n*=6), and *S. lycopersicum* (*n*=6)]. The vertical solid and dashed lines indicate the mean of the threshold of 50% cumulative embolism and water potential at turgor lost point (Ψ_{TLP}).

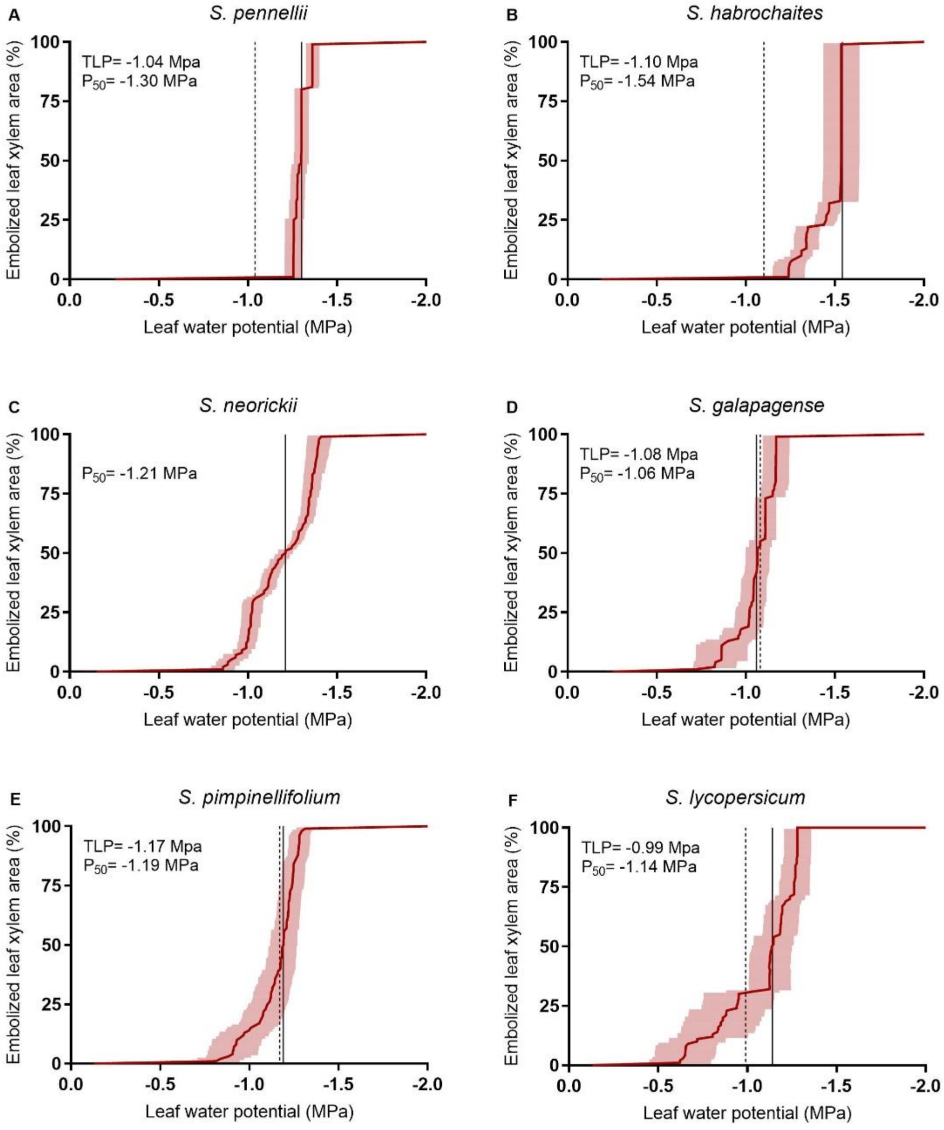


Table 1. Water potential at 50% and 88% cumulative embolism ($P_{50,} P_{88}$), time to achieve P_{50} and P_{88} ($TP_{50,} TP_{88}$) and stomatal safety margin (SSM) across six *Solanum* species. Values are mean ± SE) [*S. pennellii* (*n*=7), *S. habrochaites* (*n*=4), *S. neorickii* (*n*=6), *S. galapagense* (*n*=4), *S. pimpinellifolium* (*n*=6), and *S. lycopersicum* (*n*=4)]. Letters denote statistical Tukey`s test for multiple comparisons with probability level of ≤0.05, and Kruskal-Wallis test was used to non-normal data among genotypes.

	S. pennellii	***S. habrochaites***	***S. neorickii***	***S. galapagense***	***S. pimpinellifolium***	***S. lycopersicum***
P_{50} (MPa)	-1.30 ± 0.042 a	-1.54 ± 0.101 b	-1.21 ± 0.042 a	-1.06 ± 0.068 a	-1.19 ± 0.076 a	-1.14 ± 0.179 a
P_{88} (MPa)	-1.36 ± 0.038 a	-1.54 ± 0.101 a	-1.38 ± 0.061 a	-1.17 ± 0.074 a	-1.27 ± 0.060 a	-1.28 ± 0.181 a
TP_{50} (h)	55.2 ± 12.15 a	43.75 ± 10.93 a	21.8 ± 3.95 a	24.7 ± 6.89 a	22.17 ± 1.92 a	29.2 ± 6.73 a
TP_{88} (h)	107.3 ± 0.010 a	66.75 ± 18.17 b	41.8 ± 5.79 b	37. 0 ± 6.529 b	30.7 ± 2.95 b	51.2 ± 9.25 b
Ψ_{TLP} (MPa)	-1.04 ± 0.033 b	-1.10 ± 0.016 b	N.A.	-1.08 ± 0.023 b	-1.17 ± 0.020 a	-0.99 ± 0.026 b
SSM (MPa)	0.26	0.46	N.A.	-0.02	0.02	0.15

Among pressure-volume curve parameters, *S. pennellii* exhibited the highest SWC at 10.1 ± 0.19 significantly higher than the other species (Table 2). The Ψ_{TLP} showed minimal variation across genotypes, ranging from -0.99 (*S. lycopersicum*) to -1.17 MPa in (*S. pimpinellifolium)*. *S. pennellii* and *S. habrochaites* had the lowest relative water content at turgor loss point (RWC_{TLP}), averaging 81%, while *S. galapagense* and *S. lycopersicum* presented the highest values at 89%. The modulus of elasticity (ε) values also varied significantly among the species: *S. pimpinellifolium* and *S. galapagense* displayed the highest values (9.9 ± 0.70 and 9.0 ± 0.65 MPa, respectively); in contrast, *S. pennellii* had the lowest ε value (5.6 ± 0.76 MPa). C_{FT} was highest in *S. pennellii* (2.19 mol m^{-2} MPa^{-1}), whereas *S. galapagense* had the lowest value (0.89 mol m^{-2} MPa). Similarly, *S. pennellii* had the highest CT_{TLP}, at 6.66 mol m^{-2} MPa^{-1}, twice that of *S. habrochaites*, which had the lowest CT_{TLP} value (3.33 mol m^{-2} MPa^{-1}). In terms of minimum conductance (g_{min}), *S. lycopersicum* showed the highest value (6.09 ± 0.972 mmol m^{-2} s^{-1}), which was 2.4 times greater than the values for the other species, whose average g_{min} was 2.52 mmol m^{-2} s^{-1}. Additionally, *S. lycopersicum* had the smallest specific leaf area (SLA; 339 ± 9.2 cm^2 g^{-1}) indicating thicker leaves compared to the other species, which ranged from 390 ± 24.7 cm^2 g^{-1} in *S. galapagense* to 414 ± 18.9 cm^2 g^{-1} in *S. habrochaites* (Table 2).

For most genotypes, the NTR vs. FTSW plots were well represented by a two-segment linear model. The initial phase of soil drying was depicted by a plateau, followed by a linear decrease once FTSW dropped below a certain threshold. A higher threshold indicated greater sensitivity to soil drying. The genotype *S. galapagense* showed the highest sensibility represented by the breakpoint at FTSW equal to 0.65 (Figure 2 C), while all other species ranged from 0.38 to 0.46 (Figure 2 A, B, D, and E). These results were further supported by the visual wilting observed in *S. galapagense* after just three days of stress (Figure 3).

Table 2. Saturated water content (SWC), turgor loss point (Ψ_{tlp}), modulus of elasticity (ε), capacitance at full turgor and following turgor loss point (C_{ft} and C_{tlp}, respectively), specific leaf area *(*SLA*)*, minimum leaf conductance (g_{min}), across five *Solanum* species. Values are mean ± SE (*n* = 8 for pressure–volume curves features, and *n* = 6 for g_{min}). Letters denote statistical tests ($p \leq 0.05$) conducted among genotypes. Letters denote statistical Tukey`s test for multiple comparisons with probability level of ≤0.05, and Kruskal-Wallis test was used to non-normal data among genotypes.

	S. pennellii	***S. habrochaites***	***S. galapagense***	***S. pimpinellifolium***	***S. lycopersicum***
SWC (g g^{-1})	10.07 ± 0.18 a	6.29 ± 0.101 c	5.75 ± 0.204 c	8.41 ± 0.199 b	8.17 ± 0.190 b
Ψ_{TLP} (MPa)	-1.04 ± 0.033 b	-1.10 ± 0.016 b	-1.08 ± 0.023 b	-1.17 ± 0.020 a	-0.99 ± 0.026 b
RWC_{TLP} (%)	80.1 ± 1.65 b	81.9 ± 1.64 b	88.8 ± 0.58 a	86.6 ± 1.07 ab	89.0 ± 0.57 a
ε (MPa)	5.64 ± 0.761c	5.91 ± 0.725 b	8.98 ± 0.655 a	9.86 ± 0.705 a	9.37 ± 0.510 a
C_{FT} (mol m^{-2} MPa^{-1})	2.19 ± 0.236 a	1.35 ± 0.168 b	0.89 ± 0.051 c	1.08 ± 0.138 b	1.25 ± 0.066 b
C_{TLP} (mol m^{-2} MPa^{-1})	6.66 ± 0.445 a	3.33 ± 0.165 b	3.55 ± 0.400 b	3.46 ± 0.439 b	5.09 ± 0.482 b
SLA (cm^2 g^{-1})	398.4 ± 9.84 a	414.3 ± 18.94 a	390.2 ± 24.66 a	411.5 ± 20.41 a	338.9 ± 9.22 a
g_{min} (mmol m^{-2} s^{-1})	2.52 ± 0.382 b	2.40 ± 0.123 b	2.27 ± 0.196 b	2.91 ± 0.329 b	6.09 ± 0.972 a

Figure 2. Normalized transpiration response during drought. (A) *S. pennellii,* (B) *S. habrochaites,* (C) *S. galapagense,* (D) *S. pimpinellifolium,* and (E), *S. lycopersicum.* The two-segmented regression was used to fit these data and the threshold for the decline is noted in the figures.

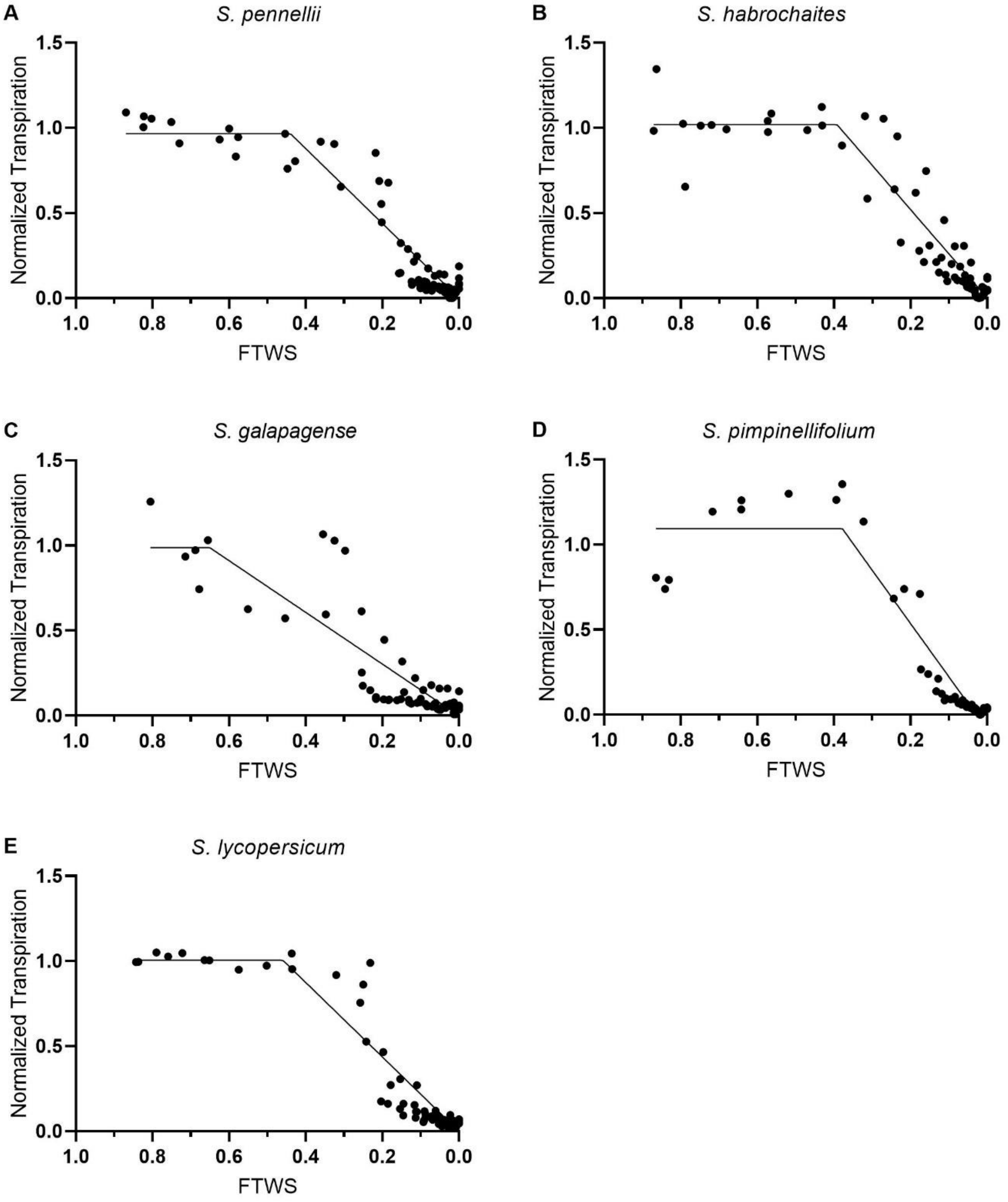
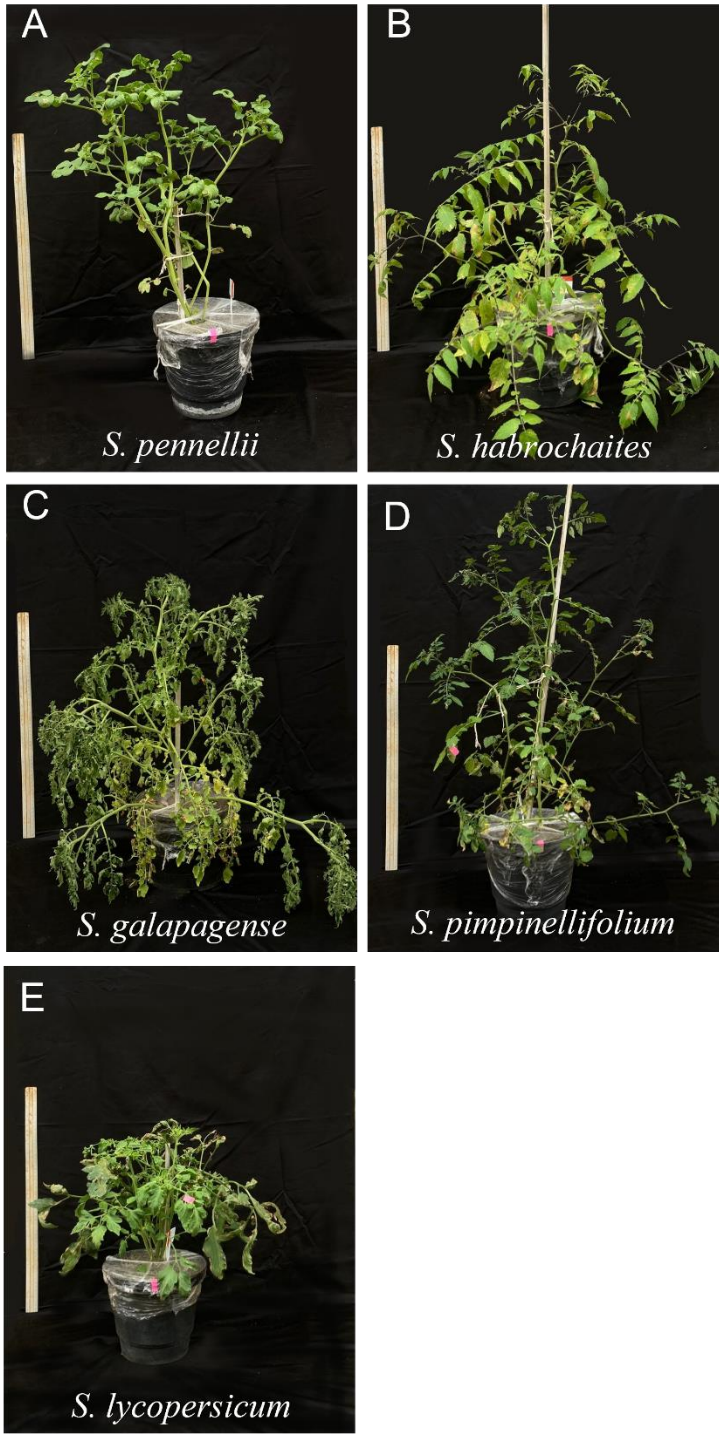


Figure 3. Plants on the third day of sustained water withholding of five tomatoes species (A) *S. pennellii,* (B) *S. habrochaites,* (C) *S. galapagense,* (D) *S. pimpinellifolium,* and (E)*, S. lycopersicum.*



Discussion

In this study, we characterized six *Solanum* species in terms of water relations and leaf xylem resistance to embolism. Our findings revealed conserved Ψ_{TLP}, leaf P_{50}, and P_{88} values across the genotypes, except for *S. habrochaites* which presented a significantly lower P_{50}. Interestingly, the time to achieve P_{88} was longer in *S. pennellii,* highlighting the importance of traits commonly associated with greater drought avoidance (higher SWC, C_{FT}, C_{TLP}, and lower ε and g_{min}) exhibited by this species.

We observed that the values and variation in P_{50} between wild tomatoes and certain genotypes of *S. lycopersicum* were consistent with previous studies, ranging from -1.1 to -1.5 MPa (Haverroth *et al.*, 2023, 2024; Andrade *et al.*, 2024; Pereira *et al.*, 2024). This was somewhat unexpected considering the different geographical origins of the species used here (https://tgrc.ucdavis.edu/) and current literature showing that species native to arid environments tend to exhibit more negative P_{50} values, reflecting greater embolism resistance, compared to those from humid regions (Choat *et al.*, 2012*b*; Larter *et al.*, 2015; Lens *et al.*, 2016*b*; Dória *et al.*, 2019; Skelton *et al.*, 2021; Huang *et al.*, 2024). For example, Volaire et al. (2018) observed that, in 12 populations of the grass *Dactylis glomerata*, stem P_{50} ranged from –3.1 to –6.4 MPa, and P_{88} from –5.1 to –11.6 MPa. However, when analyzing the functional range of Ψ_w in wild and domesticated tomatoes under severe drought (Dariva *et al.*, 2020; Alves *et al.*, 2021; Andrade *et al.*, 2024; Pereira *et al.*, 2024), we noticed that the lowest Ψ_w values are c. -2.0 MPa; thus, it seems reasonable to expect that P_{50} values should be higher than this value. If -2.0 and -1.1 MPa (variation in Ψ_{TLP}) are good candidates for the lower and higher thresholds for P_{50}, respectively, such conserved values naturally constrain the variation in xylem resistance in tomatoes.

At first glance, such a narrow range for P_{50} in tomatoes seems to undermine the importance of the parameter, however, Andrade et al. (2024) showed that a 0.25 MPa difference in P_{50} when comparing *S. lycopersicum* and the insensitive auxin mutant *diageotropica* (*dgt*) was sufficient to change their response to drought recovery. In any case, the conserved and limited window of variation for drought tolerance traits in tomatoes highlights the importance of drought avoidance traits in prolonging the time to hydraulic failure (THF) and, consequently, drought survival. Ziegler et al. (2024) evaluated drought survival across saplings of 12 tropical rainforest tree species with contrasting hydraulic strategies and observed that variations in g_{min} were translated into variations in THF, indicating that a water-saving, drought-avoidance strategy is the most effective way to delay hydraulic failure. Additionally, no correlation between traits related to xylem vulnerability to embolism and THF was observed (Martin-StPaul *et al.*, 2017; Blackman *et al.*, 2019*a*; Duursma *et al.*, 2019*b*; Machado *et al.*, 2021; Ziegler *et al.*, 2024). The previous results were similar to the findings of Pereira et al. (2024) when studying herbaceous crop plants with different degrees of drought resistance (tomato, sunflower, cowpea, and soybean). The most important take-home message from these studies is that the independence of drought tolerance and avoidance traits implies that genetic breeding can look for variation in one of them without necessarily compromising the other.

An important strategy to increase THF is to increase capacitance (*C*), which is the case for *S. pennellii*. Plants with higher *C* can store water in tissues to prevent steep decreases in xylem water potential during dry periods when compared with plants with lower *C* (Sack *et al.*, 2003*b*; Xiong and Nadal, 2020*b*). Luo et al. (2021), using a grass leaf model, showed that a large *C* could help delaying the decrease in water potential by increasing the time scale, and, as a result, stabilizing the plant hydraulics and reducing the variation of water content. These observations indicate that an effective strategy for a plant to be more resilient under water stress would be to increase its water-storage capacitance rather than to increase the resistance of pathways connecting xylem and capacitors (Luo *et al.*, 2021). *S. pennellii* has been used as a source of alleles for conferring drought resistance in *S. lycopersicum*; indeed, several introgression lines (ILs) have been generated (Eshed and Zamir, 1995) and Coneva et al. (2017) generated a double IL with higher leaf thickness which was shown to present increased saturated water content and drought resistance (Rosa, 2022).

In our study, the narrow range for P_{50} and conserved values for Ψ_{TLP} showed that these parameters could not explain the differential drought resistance across the domesticated and wild species. Therefore, it seems that traits related to avoiding damage at the cellular level and maintaining, as long as possible, metabolic activity could be an important facet of drough resistance in tomato plants (Abate *et al.*, 2021). Indeed, Abdellatif et al. (2023) conclude that tomato mutants exhibited drought resistance by inhibiting oxidative damage, preventing membrane damage, and conserving the plant water status. A similar response was found by Spormann et al. (2024) by comparing the responses of wild tomato species under exposure to conditions of soil salinity and low irrigation. Overall, the main findings of the previous study suggested osmoregulatory and antioxidant mechanisms in *S. galapagense* and *S. habrochaites* might be the ones related to the enhanced crop resilience of these wild species under environmental stress.

Wild tomatoes occur in habitats from extremely dry to moist areas, which may have resulted in the adaptation of populations or species to differences in soil moisture availability.

For instance, it is possible to find populations of *S. pimpinellifolium* in a wide range of water availability, from a mean annual precipitation of 49 to 2800 mm (Martínez-Cuenca *et al.*, 2020), indicating a high plasticity within the same species, once it can survive in wet and dry areas. This plasticity could explain why tomatoes presented a similar hydraulic response under drought when grown in a common garden in this study. The literature also has shown no difference in leaf physiological responses to soil drought within or between tomato species grown in a common garden, even with accessions originated from locations with vastly different climatic conditions, which would suggest strong potential for differential adaptation to drought (Easlon and Richards, 2009).

Conclusion

The response to water restriction is highly multifaceted, influenced by a complex interplay of plant attributes such as stomatal behavior, hydraulic architecture, morpho-anatomical traits, leaf properties, root traits, and hormonal signaling. This study highlights the need to go beyond drought tolerance traits and emphasizes the need for an integrated approach to understanding drought resistance. Notably, g_{min} and C have been frequently cited as critical mechanisms for plant survival during severe drought and were related to the high THF in *S. penellii*. Despite varying geographical origins, P_{50} values and sensibility to soil drying were consistent across species, when growing in common garden. More studies to explore the influence of plant plasticity on hydraulic response are needed, and efforts to improve *S. lycopersicum* drought resistance should focus on both drought avoidance and tolerance traits.

References

Abate E, Nardini A, Petruzzellis F, Trifilò P. 2021. Too dry to survive: Leaf hydraulic failure in two *Salvia* species can be predicted on the basis of water content. Plant Physiology and Biochemistry **166**, 215–224.

Abdellatif IMY, Yuan S, Yoshihara S, Suzaki T, Ezura H, Miura K. 2023. Stimulation of tomato drought tolerance by PHYTOCHROME A and B1B2 mutations. International Journal of Molecular Sciences **24**.

Alves FM, Joshi M, Djidonou D, Joshi V, Gomes CN, Leskovar DI. 2021. Physiological and biochemical responses of tomato plants grafted onto *Solanum pennellii and Solanum peruvianum* under water-deficit conditions. Plants **10**.

Andrade MT, Cardoso AA, Oliveira LA, Pereira TS, Haverroth EJ, Souza GA, DaMatta FM, Zsögön A, Martins SCV. 2024. Enhanced drought resistance in tomato via reduced auxin sensitivity: delayed dehydration and improved leaf resistance to embolism. Physiologia Plantarum **176**.

Bai Y, Lindhout P. 2007. Domestication and breeding of tomatoes: What have we gained and what can we gain in the future? Annals of Botany **100**, 1085–1094.

Bauchet G, Causse M. 2012. Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. Genetic Diversity in Plants. 133–162.

Blackman CJ, Creek D, Maier C, *et al.* 2019. Drought response strategies and hydraulic traits contribute to mechanistic understanding of plant dry-down to hydraulic failure. Tree Physiology **39**, 910–924.

Blackman CJ, Pfautsch S, Choat B, Delzon S, Gleason SM, Duursma RA. 2016. Toward an index of desiccation time to tree mortality under drought. Plant Cell and Environment **39**, 2342–2345.

Bonarota MS, Kosma DK, Barrios-Masias FH. 2022. Salt tolerance mechanisms in the *Lycopersicon* clade and their trade-offs. Oxford University Press.

Brodribb TJ, Skelton RP, McAdam SAM, Bienaimé D, Lucani CJ, Marmottant P. 2016. Visual quantification of embolism reveals leaf vulnerability to hydraulic failure. New Phytologist **209**, 1403–1409.

Choat B, Brodribb TJ, Brodersen CR, Duursma RA, López R, Medlyn BE. 2018. Triggers of tree mortality under drought. Nature **558**, 531–539.

Choat B, Jansen S, Brodribb TJ, *et al.* 2012. Global convergence in the vulnerability of forests to drought. Nature **491**, 752–755.

Coneva V, Frank MH, de Luis Balaguer MA, Li M, Sozzani R, Chitwood DH. 2017. Genetic architecture and molecular networks underlying leaf thickness in desert-adapted tomato *Solanum pennellii*. Plant Physiology **175**, 376–391.

Dariva FD, Copati MGF, Pessoa HP, Alves FM, Dias F de O, Picoli EA de T, da Cunha FF, Nick C. 2020. Evaluation of anatomical and physiological traits of *Solanum pennellii* Cor.

associated with plant yield in tomato plants under water-limited conditions. Scientific Reports **10**.

Dória LC, Meijs C, Podadera DS, Del Arco M, Smets E, Delzon S, Lens F. 2019. Embolism resistance in stems of herbaceous Brassicaceae and Asteraceae is linked to differences in woodiness and precipitation. Annals of Botany **124**, 1–14.

Duursma RA, Blackman CJ, Lopéz R, Martin-StPaul NK, Cochard H, Medlyn BE. 2019. On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. New Phytologist **221**, 693–705.

Easlon HM, Asensio JSR, St.Clair DA, Bloom AJ. 2013. Chilling-induced water stress: Variation in shoot turgor maintenance among wild tomato species from diverse habitats. American Journal of Botany **100**, 1991–1999.

Easlon HM, Richards JH. 2009. Drought response in self-compatible species of tomato (Solanaceae). American Journal of Botany **96**, 605–611.

Egea I, Albaladejo I, Meco V, Morales B, Sevilla A, Bolarin MC, Flores FB. 2018. The drought-tolerant *Solanum pennellii* regulates leaf water loss and induces genes involved in amino acid and ethylene/jasmonate metabolism under dehydration. Scientific Reports **8**.

Eshed Y, Zamir D. 1995. An Introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics **141**, 1147–1162.

Flores-Saavedra M, Plazas M, Vilanova S, Prohens J, Gramazio P. 2023. Induction of water stress in major *Solanum* crops: A review on methodologies and their application for identifying drought tolerant materials. Elsevier B.V.

Galdon-Armero J, Fullana-Pericas M, Mulet PA, Conesa MA, Martin C, Galmes J. 2018. The ratio of trichomes to stomata is associated with water use efficiency in *Solanum lycopersicum* (tomato). Plant Journal **96**, 607–619.

Grandillo S, Chetelat R, Knapp S, *et al.* 2011. *Solanum* sect. *Lycopersicon*. Wild Crop Relatives: Genomic and Breeding Resources. Springer Berlin Heidelberg, 129–215.

Grandillo S, Zamir D, Tanksley SD. 1999. Genetic improvement of processing tomatoes: A 20 years perspective. Euphytica **110**, 85- 97,

Guillemot J, Martin-StPaul NK, Bulascoschi L, *et al.* 2022. Small and slow is safe: On the drought tolerance of tropical tree species. Global Change Biology **28**, 2622–2638.

Han W, Jia J, Hu Y, Liu J, Guo J, Shi Y, Huo H, Gong H. 2021. Maintenance of root water uptake contributes to salt-tolerance of a wild tomato species under salt stress. Archives of Agronomy and Soil Science **67**, 205–217.

Haverroth EJ, Oliveira LA, Andrade MT, Taggart M, McAdam SAM, Zsögön A, Thompson AJ, Martins SCV, Cardoso AA. 2023. Abscisic acid acts essentially on stomata, not on the xylem, to improve drought resistance in tomato. Plant Cell and Environment **46**, 3229–3241.

Haverroth EJ, Rimer IM, Oliveira LA, de Lima LGA, Cesarino I, Martins SCV, McAdam SAM, Cardoso AA. 2024. Gradients in embolism resistance within stems driven by secondary growth in herbs. Plant Cell and Environment **47**, 2986–2998.

Huang R, Di N, Xi B, Yang J, Duan J, Li X, Feng J, Choat B, Tissue D. 2024. Herb hydraulics: Variation and correlation for traits governing drought tolerance and efficiency of water transport. Science of the Total Environment **907**.

IPCC. 2021**.** Climate Change 2021. In The physical science basis. Contribution of Working Group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge University Press.

Larter M, Brodribb TJ, Pfautsch S, Burlett R, Cochard H, Delzon S. 2015. Extreme aridity pushes trees to their physical limits. Plant Physiology **168**, 804-807,

Lens F, Picon-Cochard C, Delmas CEL, ***et al.*** 2016. Herbaceous angiosperms are not more vulnerable to drought-induced embolism than angiosperm trees. Plant Physiology **172**, 661–667.

Liang X, Ye Q. 2024. Integrating dehydration tolerance and avoidance in drought adaptation. Journal of Plant Ecology **17**.

Luo Y, Ho CL, Helliker BR, Katifori E. 2021. Leaf water storage and robustness to intermittent drought: A spatially explicit capacitive model for leaf hydraulics. Frontiers in Plant Science **12**, 1–13.

Machado R, Loram-Lourenço L, Farnese FS, Alves RDFB, de Sousa LF, Silva FG, Filho SCV, Torres-Ruiz JM, Cochard H, Menezes-Silva PE. 2021. Where do leaf water leaks come from? Trade-offs underlying the variability in minimum conductance across tropical savanna species with contrasting growth strategies. New Phytologist **229**, 1415–1430.

Martínez-Cuenca MR, Pereira-Dias L, Soler S, López-Serrano L, Alonso D, Calatayud Á, Díez MJ. 2020. Adaptation to water and salt stresses of *Solanum pimpinellifolium* and *Solanum lycopersicum* var. cerasiforme. Agronomy **10**.

Martin-StPaul N, Delzon S, Cochard H. 2017. Plant resistance to drought depends on timely stomatal closure. Ecology Letters **20**, 1437–1447.

McDowell N, Pockman WT, Allen CD, ***et al.*** 2008. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytologist **178**, 719–739.

McDowell N, Sapes G, Pivovaroff A, ***et al.*** 2022. Mechanisms of woody-plant mortality under rising drought, CO_2 and vapour pressure deficit. Nature Reviews Earth and Environment **3**, 294–308.

Nakazato T, Warren DL, Moyle LC. 2010. Ecological and geographic modes of species divergence in wild tomatoes. American Journal of Botany **97**, 680–693.

Pereira TS, Oliveira LA, Andrade MT, Haverroth EJ, Cardoso AA, Martins SCV. 2024. Linking water-use strategies with drought resistance across herbaceous crops. Physiologia Plantarum **176**.

Ramírez-Ojeda G, Peralta IE, Rodríguez-Guzmán E, Chávez-Servia JL, Sahagún-Castellanos J, Rodríguez-Pérez JE. 2021. Climatic diversity and ecological descriptors of wild tomato species (*Solanum* sect. *lycopersicon*) and close related species (*Solanum* sect. *juglandifolia* y sect. *lycopersicoides*) in Latin America. Plants **10**.

Rezk A, Abhary M, Akhkha A. 2021. Tomato (*Solanum lycopersicum* L.) breeding strategies for biotic and abiotic stresses. Advances in Plant Breeding Strategies: Vegetable Crops. Springer International Publishing, 363–405.

Rosa BL. 2022. The genetic basis of drought resistance in tomato. Universidade Federal de Viçosa.

Sack L, Cowan PD, Jaikumar N, Holbrook NM. 2003. The 'hydrology' of leaves: co-ordination of structure and function in temperate woody species. Plant, Cell and Environment **26**, 1343–1356.

Sack L, Tyree MT. 2005. Leaf hydraulics and its implications in plant structure and function hydraulic conductance in the whole-plant system. In: Holbrook M, Zwieniecki M, eds. Physiological Ecology: Vascular Transport in Plants. 93–114.

Skelton RP, Anderegg LDL, Diaz J, Kling MM, Papper P, Lamarque LJ, Delzon S, Dawson TE, Ackerly DD, Performed PP. 2021. Evolutionary relationships between drought-related traits and climate shape large hydraulic safety margins in western North American oaks. Proceedings of the National Academy of Sciences **118**.

Skelton RP, West AG, Dawson TE. 2015. Predicting plant vulnerability to drought in biodiverse regions using functional traits. Proceedings of the National Academy of Sciences of the United States of America **112**, 5744–5749.

Spormann S, Soares C, Azenha M, Martins V, Fidalgo F. 2024. A look into osmotic, ionic, and redox adjustments in wild tomato species under combined salt and water stress. Plant Stress **13**.

Sun S, Jung EY, Gaviria J, Engelbrecht BMJ. 2020. Drought survival is positively associated with high turgor loss points in temperate perennial grassland species. Functional Ecology **34**, 788–798.

Thonglim A, Bortolami G, Delzon S, Larter M, Offringa R, Keurentjes JJB, Smets E, Balazadeh S, Lens F. 2022. Drought response in Arabidopsis displays synergistic coordination between stems and leaves. Journal of Experimental Botany **74**, 1004–1021.

Trenberth KE, Dai A, Van Der Schrier G, Jones PD, Barichivich J, Briffa KR, Sheffield J. 2014. Global warming and changes in drought. Nature Climate Change **4**, 17–22.

Tyree MT, Hammel HT. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. Journal of Experimental Botany **23**, 267–282.

Volaire F. 2018. A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. Global Change Biology **24**, 2929–2938.

Volaire F, Lens F, Cochard H, Xu H, Chacon-Doria L, Bristiel P, Balachowski J, Rowe N, Violle C, Picon-Cochard C. 2018. Embolism and mechanical resistances play a key role in dehydration tolerance of a perennial grass *Dactylis glomerata* L. Annals of Botany **122**, 325–336.

Xiong D, Nadal M. 2020. Linking water relations and hydraulics with photosynthesis. The Plant Journal **101**, 800–815.

Zhang KP, Zhao XY, Bi MX, Zhang HD, He Y, Cui YQ, Qi MF. 2023. Membrane lipid metabolism and scavenging of reactive oxygen species are essential for cold resistance of *Solanum habrochaites* (LA1777): Lipidomics and transcriptomes analysis. Environmental and Experimental Botany **209**.

Ziegler C, Cochard H, Stahl C, *et al.* 2024. Residual water losses mediate the trade-off between growth and drought survival across saplings of 12 tropical rainforest tree species with contrasting hydraulic strategies. Journal of Experimental Botany **75**, 4128–4147.

CHAPTER 3

Reduced nighttime transpiration during drought is driven by divergent pathways across vascular plant lineages

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Abstract

Stomatal closure in response to water deficit is crucial for maintaining plant water balance. While the mechanisms driving daytime stomatal closure under drought are well studied, the mechanism driving progressive declines in nighttime transpiration (E_{night}) during drought remains less understood. To investigate whether either abscisic acid (ABA) or declining leaf water status drives progressive declines in nighttime transpiration during drought in vascular plants, we conducted experiments in a representative fern, gymnosperm, and woody and herbaceous angiosperm species, *including a severe* ABA-deficient mutant. These species spanned a spectrum of stomatal control by ABA ranging from ABA insensitive (fern) to ABA reliant (herbaceous angiosperm) for stomatal closure. We found that reductions in E_{night} during drought are driven by passive stomatal closure in ferns and gymnosperms, transitioning to ABA regulation in gymnosperms under severe stress, and in herbaceous angiosperms being driven entirely by ABA. In all species, the proportion of night transpiration relative to whole day transpiration increased as stomata closed during the drought. The reduction of nighttime transpiration during drought appears to be a convergent stomatal response across vascular land plants, but is driven by diverse regulatory mechanisms linked to evolutionary history and ecological strategy.

Introduction

Water use by plants is directly linked to primary productivity because land plants exchange water for carbon dioxide uptake from the atmosphere through stomata (Zeppel et al., 2014; Buckley, 2019). Stomatal closure is critical for reducing water loss when conditions are not optimal for photosynthesis or when plant survival is threatened by limited water availability, with a near universal closure of stomata during night and during drought in vascular plants (Costa et al., 2015; Tombesi et al., 2015; Martin-StPaul et al., 2017; Yang et al., 2021). The timing and extent of stomatal closure is critical for understanding limitations to agricultural production, as well as predicting the timing of plant mortality (Yang et al., 2021), and to correctly model vegetation-atmosphere fluxes (Wankmüller and Carminati, 2022).

While stomata generally close at night to prevent water loss, nighttime transpiration (E_{night}) can still account for 5 to 40% of total daily transpiration (E_{daily}) (Caird et al., 2007). This nocturnal water loss, which varies widely across plant types, biomes, and climates (Dawson et al., 2007; Resco de Dios et al., 2019; Yu et al., 2019), can impact water use efficiency, particularly under water-limited conditions. Adaptive explanations for a partial nocturnal stomatal opening have been proposed, suggesting potential functional benefits to incomplete closure (Caird et al., 2007; Zeppel et al., 2014; Meng et al., 2024). These include to enhance evaporative cooling, the maintenance of a transpiration stream for nutrient uptake, oxygen delivery, CO_2 degassing, capacitance refilling, or a preemptive circadian rhythm for early morning stomatal opening (Wang et al., 2021). Despite its relevance, nighttime transpiration is commonly underestimated or ignored in models of plant gas exchange during drought, as it is assumed that stomata close completely at night in non-crassulacean acid metabolism plants (Zeppel et al., 2014; Resco de Dios et al., 2015; Hoshika et al., 2018). Models based on optimization predict that when there is no carbon fixation during nighttime that stomata will be closed (Wang and Dickinson, 2012; Wang et al., 2021).

In vascular plants, stomatal closure in response to declining leaf water potential (Ψ_w) during drought is regulated by both hydropassive and hydro-active mechanisms (Buckley, 2019). In lycophytes and ferns, stomatal closure in response to declining leaf water status requires no metabolic input or complex signaling intermediates (Martins et al., 2016; Brodribb and McAdam, 2017). In species from these lineages, guard cell turgor is linked to leaf turgor such that as Ψ_w declines, the pore closes passively reducing leaf transpiration. In contrast, as leaf turgor declines during drought, seed plants synthesize the phytohormone abscisic acid (ABA) to trigger early stomatal closure, ensuring minimal stomatal conductance by actively reducing guard cell turgor (Mittelheuser and Van Steveninck, 1969; Leckie et al., 1998; Brodribb and McAdam, 2013). Stomatal closure in the dark occurs by a different mechanism: in contrast to light-induced opening, at night, reduced plasma membrane H^+-ATPase activity leads to membrane depolarization, promoting the extrusion of K^+ and Cl^- from guard cells into the cytoplasm and then across the plasma membrane to the extracellular space (Pemadasa, 1981; Ward and Schroeder, 1994). This ion transport reduces guard cell turgor, resulting in a reduction in stomatal aperture (Pemadasa, 1981; Ward and Schroeder, 1994; Roelfsema et al., 2001; Roelfsema and Hedrich, 2005).

Given the potential importance of nocturnal transpiration for modelling plant water use, particularly in response to drought (Cavender-Bares et al., 2007), it is essential to understand the effect of drought on stomatal closure in the dark. We hypothesized that E_{night} will decline with drought and given the differences in the mechanism driving daytime stomatal closure during drought across species from the major lineages of vascular land plants, similar differences will exist in the mechanism driving progressive reductions in E_{night} during drought. In order to test this hypothesis, we aimed to identify the potential mechanism driving progressive reductions in E_{night} during drought utilizing a diversity of vascular land plants.

To investigate whether progressive decline in E_{night} during drought is regulated by the same pathway(s) that drive stomatal closure in the day under drought, we included species and mutants that encompass a diversity of stomatal physiologies, including the fern *Cyrtomium falcatum* (L.f.) C.Presl (*Dryopteridaceae*), from a group of land plants in which stomata close passively by declining leaf water status under drought, and in which endogenous ABA, synthesized during drought, does not close stomata (McAdam and Brodribb, 2012; Cardoso et al., 2019). The gymnosperm, *Pinus taeda* L. (*Pinaceae*), representing a lineage of land plants in which stomata are regulated by ABA during drought, and from a genus that has been characterized as synthesizing high levels of ABA during a drought, in order to minimize transpiration (Brodribb and McAdam, 2013). The embolism-resistant, woody, angiosperm species *Umbellularia californica* (Hook. & Am.) Nuttall (*Lauraceae*) was included as it utilizes high levels of ABA to close stomata at the onset of drought, then as Ψ_w declines as drought progresses, ABA levels decline and stomata transition to being closed passively by low leaf water status (McAdam et al., 2024; Mercado-Reyes et al., 2024). Finally, we also included the herbaceous angiosperm *Solanum lycopersicum* cv. Rhinelands Rhum L. (*Solanaceae*) which relies on ABA to close stomata during drought (Christmann et al., 2005; Hasan et al., 2021), and a double mutant on the same background (*sitiens flacca*), which is unable to synthesize ABA and close stomata in response to dehydration (Brodribb et al., 2021).

Materials and Methods

Plant material

The *S. lycopersicum* WT and mutant plants were cultivated from seed in pots filled with mixed soil (1:1:1 sand : topsoil : course pine bark). Experiments were conducted when sufficient leaf area was present with plants that were 55 days old (WT) and 7 months old, (mutant plants). *P. taeda*, *C. falcatum* and *U. californica* were 5-year-old plants grown in the glasshouses of Purdue University, West Lafayette (Indiana, USA) (40° 25′ N, 86° 54′ W, elevation: 187 m). All plants were grown under 22/19 ºC day/night temperature under natural light. Before experiments, plants were watered daily and fertilized weekly with liquid nutrients (Miracle-Gro Water-soluble All Purpose Plant Food, Scotts Company LLC, OH, USA).

Canopy transpiration and leaf water potential

To measure whole plant transpiration, we used plants with bagged pots to eliminate soil evaporation and placed on an individual scale weighing lysimeter connected to a CR850 data logger that recorded average weight every 5 minutes. With this data, we measured daily, day, and night canopy transpiration [E_{24} (24 hours), E_{day} (dawn to dusk), and E_{night} (dusk to dawn); g H_2O plant $^{-1}$).

Transpiration values were used to calculate transpiration ratios (TR) - the ratio between water loss of the drying pot and the average of the water loss from well-watered plants on the same day. Due to plant-to-plant variation, normalize transpiration ratio (NTR) was calculated as the daily TR divided by the mean TR of the same individual during the period when the soil was still well-watered. Water was withheld from plants until they reached NTR near-equal 0.15. Night/Day ratio was calculated dividing night NTR by day NTR, each normalized by the respective night and day lengths.

Daily fraction of transpirable soil water (FTSW) was calculated as the indicator of intensity of soil water deficit (Sinclair and Ludlow, 1986).

$$\text{FTSW} = \frac{\text{daily pot weight} - \text{final pot weight}}{\text{initial pot weight} - \text{final pot weight}} \qquad (1)$$

Temperature and relative humidity measurements were recorded every minute using a HOBO MX2301A Data Logger (Onset Computer Corporation), suspended at plant height in the glasshouse. The gravimetric determination of whole plant water loss was then used to calculate average canopy stomatal conductance (g_c) during day and night. Mean vapor pressure deficit (VPD) of the atmosphere for the time during which transpiration was measured (Sadler and Evans, 1989) and g_c was then calculated using the equation 2 bellow:

$$g_c = \frac{\text{moles of water lost/(area*s)}}{\text{VPD} * \text{Patm}} \qquad (2)$$

Dawn and midday water potential (Ψ_w), and abscisic acid levels were measured through experiment. For that, healthy and mature leaves were collected, bagged with damp paper towels for 10 min to ensure whole leaf equilibration, and Ψ_w were measured using a Scholander pressure chamber (Model 1505D; PMS Instruments). After measuring Ψ_w, a subsample of tissue was then taken for hormone analysis (see below). Daily Ψ_w was estimated by plotting Ψ_w measured during experiment against FTSW.

Stomatal response to rehydration in C. falcatum

In one drought stressed *C. falcatum* plant (NTR near-equal 0.15), the stipe of the leaf was excised under water, and g_s was continuously recorded under dark conditions until it stabilized. Gas exchange was measured using an infrared gas analyzer (LI-6800). Environmental conditions in the leaf cuvette were controlled: light was turned off, temperature was set to 23°C, and CO_2 concentration was maintained at 400 μmol mol^{-1}. Samples for Ψ_w and foliar ABA quantification were collected before rehydration and at the end of the analyses.

ABA quantification

The ABA was quantified from the same leaf used for Ψ_w. Samples were weighted, covered in 80% methanol in water (v v^{-1}) with added butylated hydroxytoluene (250 mg l^{-1}), chopped in smaller pieces, and stored at -20ºC. To prepare samples for ABA quantification, the tissue was homogenized, and 15μl of ABA standard (1ng $μl^{-1}$) was added to each sample, which was then stored overnight at 4°C to allow plant tissue to settle. Subsequently, an aliquot of the supernatant was dehydrated at 40ºC. The ABA resuspended in 200 μl of 2% acetic acid in water (v v^{-1}) and ABA levels quantified using liquid chromatography tandem mass spectrometry (Agilent 6460, QQQ LCMS). Foliar ABA levels were expressed in terms of dry weight (DW), which was quantified after ABA determination by weighing the dry mass of the sample harvested and extracted for analysis. Normalizing the ABA levels to tissue DW avoids passive increases in ABA levels as cells dehydrate (McAdam, 2015).

Results

During drought daytime transpiration (E_{day}) and E_{night} rates remained constant until a certain threshold soil moisture level (fraction of transpirable soil water, FTSW), with the exception of the severe ABA biosynthetic mutant of *S. lycopersicum* (Figure 1). After this threshold FTSW was crossed, E_{day} and E_{night} progressively decreased in a linear fashion in response to declining FTSW in all species (Figure 1). In the fern *C. falcatum* and conifer *P. taeda* we observed a significant difference between the E_{day} and E_{night} FTSW breakpoints at which transpiration began to decline, with E_{night} in both species starting to decline linearly at a higher FTSW compared to when E_{day} began declining (Figure 1A and B). For both angiosperms species, *U. californica* (Figure 1C) and the wild-type plants of *S. lycopersicum* (Figure 1D), the decline in transpiration rate was coordinated for day and night at a similar FTSW.

The fern *C. falcatum,* conifer *P. taeda,* and angiosperm *U. californica* had an unchanged E_{night}/E_{day} ratio as FTSW declined, remaining constant (between 0 and 0.3) until FTSW had declined to approximately 0.2, at which point it increased (Figure 2A, B, and C). In contrast, in the wild-type plants of *S. lycopersicum,* the E_{night}/E_{day} ratio was high at around 0.55 and then decreased as FTSW declined until 0.2; after 0.2, this ratio increased to 0.9, surpassing the initial value observed when the soil was well-watered (Figure 2 D). In the severely ABA-deficient mutant plants of *S. lycopersicum sitiens flacca*, the E_{night}/E_{day} ratio remained constant throughout the entire drought (Figure 2 E). Except for the mutant, the point of increase in the E_{night}/E_{day} ratio during drought occurred, in all species, when plants started to lose a consistent and minimum amount of water in a 24 h period, indicating that daytime stomatal conductance had reached a minimum.

E_{night} in *C. falcatum* and *S. lycopersicum* accounted 21 and 24%, respectively, of 24 h canopy transpiration (E_{24}) during well-watered conditions and 31 and 33% when FTSW was below 0.1. E_{night} of both *P. taeda* and *U. californica* was 6% of E_{24} while water was not limiting, whereas it reached up to 19% of E_{24} at low FTSW (Table 1). The higher proportion of E_{night} to E_{24} under drought reflects a greater relative decline in stomatal conductance rate during the day than during the night, particularly in the woody species (Table 2).

In the fern *C. falcatum*, E_{night} progressively declined as Ψ_{w} declined during drought, but the onset of this decline was not associated with an increase foliar ABA level (Figure 3 A). In the conifer *P. taeda*, the progressive reduction in E_{night}, as Ψ_{w} declined during drought, corresponded to a substantial increase in foliar ABA levels from 0.3 to 2.52 μg g^{-1} DW (Figure 3 B). In the woody angiosperm *U. californica*, a classical peaking-type drought response in foliar ABA levels during drought was observed, with the initiation of the progressive decrease in E_{night} coinciding with the initial increase in ABA levels during drought peaking at 18.5 µg g^{-1} DW (Figure 3 C). In the herbaceous angiosperm, *S. lycopersicum*, E_{night} abruptly decreased as Ψ_w declined and coincided with an increase in foliar ABA level that reached 40.6 µg g^{-1} DW at the end of the drought (Figure 3 D). Although in *C. falcatum* endogenous ABA levels during the drought increased above levels measured under well-watered conditions, they were never higher than 0.5 µg g^{-1} DW. To test whether this increase in endogenous ABA synthesized during drought or low Ψ_w drove reductions in E_{night} in *C. falcatum,* we excised a stipe underwater while measuring stomatal conductance in the dark. Stomatal conductance in the dark increased rapidly when leaves were rehydrated despite leaves having high levels of endogenous ABA (Figure 4).

Discussion

In this study, we exposed four species from different vascular plant lineages and an ABA deficient herbaceous angiosperm mutant to drought to investigate the mechanism driving progressive declines in E_{night} under soil water deficit. The results show that water loss at the canopy level during drought can be substantial throughout the night, especially in the herbaceous angiosperm *S. lycopersicum* and fern *C. falcatum.* We also found a progressive decrease in E_{night} during drought and an increase in the ratio of total transpiration occurring at night during drought in all species, supporting the conclusion that E_{night} should not be considered a single constant when photosynthesis is absent (Duursma et al., 2019). Our findings are similar to what has been reported in *Quercus* (Cavender-Bares et al., 2007), *Helianthus* (Howard and Donovan, 2007), *Eucalyptus* (Wang et al., 2024) and *Vitis* (Coupel-Ledru et al., 2016), which also show reductions in E_{night} during drought. While we observed this phenomenon across our diverse sample of vascular plants, the mechanism driving this stomatal response is highly divergent across species, linked to broad phylogenetic differences in stomatal control during drought, from passive regulation in ferns to complete active control by ABA in angiosperm herbs.

While still debated, in vascular land plants, the progressive decline in E_{day} in response to drought appears to have evolved from a passive process directly mediated by leaf water status to an active process controlled by the extrusion of ions from guard cells driven by endogenous ABA (Brodribb and McAdam, 2011). In ferns and lycophytes, stomata predictably respond to plant water deficit as passive hydraulic valves, closing rapidly during dehydration and opening upon rehydration (McAdam and Brodribb, 2014). In contrast, in seed plants, stomatal closure in response to water deficit is mediated by increased levels of endogenous ABA (Brodribb and McAdam, 2011; McAdam and Brodribb, 2014). Here we show that in the fern *C. falcatum,* reductions in E_{night} during drought appear to be driven by passive declines in leaf water status (Brodribb and McAdam 2011; McAdam and Brodribb 2013). Two lines of evidence support this conclusion, the first is the variable but relatively low levels of endogenous ABA (lower than 0.5 µg g^{-1} DW) in the leaf during drought in this species, which did not correspond to the decline in E_{night}. The second, and more compelling, is that when a leaf in a state of water deficit and reduced E_{night} is rehydrated instantaneously through the petiole in the dark, the stomata rapidly reopened to a conductance recorded in the dark under well-watered conditions (Figure 4). The rapid stomatal opening in the dark following rehydration of a dehydrated leaf is similar to what has been reported across a diversity of lycophytes and ferns in the light during a drought (McAdam and Brodribb, 2012; Cardoso et al., 2019; Cardoso and McAdam, 2019). These results suggest that endogenous ABA synthesized during drought does not reduce E_{night} in *C. falcatum*.

In the seed plants, there is considerable evidence to suggest that increased ABA levels during drought, particularly early in a drought, drive declines in E_{day} (Manandhar et al., 2024a; Manandhar et al., 2024b). In seed plants, daytime stomatal closure in response to water deficit is usually induced by augmented levels of ABA, which leads to a depolarization of guard cell membranes triggering osmotic ion efflux and a loss of guard cell turgor (Assmann and Shimazaki, 1999; Roelfsema and Hedrich, 2005; Geiger et al., 2011; McAdam and Brodribb, 2014; Hasan et al., 2021). In the conifer *P. taeda,* we observed an increase in foliar ABA levels at a similar Ψ_{w} to when considerable declines in E_{night} occurred. In addition, when both E_{night} and E_{day} were plotted against FTSW, E_{night} started to decrease at a higher FTSW than decreases in E_{day}. Interestingly, this difference also occurred in the fern *C. falcatum,* suggesting that there might be an increased sensitivity of stomata to a passive decline in water status when guard cell turgor pressure is reduced by darkness in the conifer. This response of *P. taeda* matches a previous report in conifers in which stomatal closure in response to changes in leaf water status transitions from passive regulation under mild changes in leaf water status to regulation by ABA under prolonged water deficit (McAdam and Brodribb, 2014). McAdam and Brodribb (2014) found that only after prolonged exposure to more extreme water stress did active ABA-mediated stomatal closure become important in *Metasequoia glyptostroboides*, a gymnosperm species.

In the two angiosperm species, *U. californica* and *S. lycopersicum,* there was a clear regulation of progressive declines in E_{night} by ABA levels. *U. californica* plants exhibit a peaking-type dynamic of ABA levels with decreasing Ψ_{w}, with ABA levels rising early in drought but declining later in drought (Mercado-Reyes et al., 2024), whereby stomatal closure transitions from ABA-regulated (early in drought when Ψ_{w} is not very low) to passively regulated (late in drought when Ψ_{w} is much lower) (McAdam et al., 2024). We observed a continual decline in E_{night} in *U. californica* after the initial peak in ABA levels during drought, suggesting that in this species, the progressive reduction in E_{night} during drought is mediated by both active and passive mechanisms. *U. californica* exhibited greater reductions in E_{night} in a higher FTSW compared to *S. lycopersicum*, but in both species this reduction corresponded to both an increase in ABA levels and the onset of a reduction in E_{day}.

In *S. lycopersicum,* ABA levels increased at a threshold FTSW and Ψ_{w} at which E_{night} and E_{day} began to decline during drought. In this species, ABA levels remained high for the duration of the drought and were likely critical for driving progressive reductions in E_{night}. Compelling evidence for this is that no declines in either E_{night} or E_{day} occurred as FTSW declined during drought in the *sitiens flacca* ABA biosynthetic double mutant plants of this species were observed. These mutants are unable to synthesize ABA as Ψ_{w} declines and thus do not close stomata in response to dehydration leading to embolism formation and leaf death (Brodribb et al., 2021). There are reports in some herbaceous angiosperms that leaf water status modulates the sensitivity of stomata to ABA, with increasingly negative Ψ_{w} enhancing the closing response of stomata to increased ABA levels around the guard cells and in the xylem (Tardieu and Davies, 1992). Indeed, our data suggest that low leaf water status and high levels of ABA can enhance the degree of stomatal closure in the dark.

It is notable that stomata do not fully close at night in well-watered plants. While the functional significance of E_{night} remains equivocal, the mechanisms underlying partial stomatal opening at night are not yet fully understood. Nighttime stomatal closure is typically attributed to ion extrusion from guard cells, but the persistence of partial opening suggests that this process may be incomplete in well-watered conditions. As drought progresses, reductions in E_{night} indicate that stomatal apertures can close further compared to well-watered conditions. This response likely reflects decreased guard cell turgor in ferns, and during early drought in gymnosperms, whereas in angiosperms, it involves enhanced ion extrusion mediated by ABA.

Our data suggests that E_{night} can represent a substantial proportion of E_{24}. If water loss through partially open stomata during a non-photosynthetic period is large, especially when water resources are scarce, these conditions would lead to loss of a limited resource without any carbon gain benefit, thus reducing overall plant water use efficiency (Caird et al., 2007a; Caird et al., 2007b). The substantial water cost of E_{night} could represent a major problem for agronomic development, especially in areas where water is particularly scarce (Resco de Dios et al., 2015) and the fact that nighttime temperatures are predicted to increase at a higher rate than daytime temperatures in response to climate change (Cox et al., 2020).

Conclusion

Here we show that progressive decreases in E_{night} during soil drought occurs in plants across the euphyllophyte clade but that the mechanisms that drive this response are divergent. We observed passive stomatal closure driving progressive reductions in E_{night} in the representative fern and perhaps in the representative conifer early in a drought. On the other hand, increased ABA levels triggered progressive reductions in E_{night} in the seed plant species. In all species we observed an increase in the contribution of E_{night} to E_{24}, but only when E_{day} approached a minimum. Consequently, during well-watered conditions and short drought events, E_{night} does not appear to play a large contribution to E_{24}, however, during severe and/or long dry seasons, E_{night} and minimum conductance become significantly more important contributor to plant water economy (Dayer et al., 2020; Pereira et al., 2024).

Author contributions

TSP, AM, and SAMM conceived the study; TSP and AM carried out experiments; TSP, AM, and SAMM analyzed the data and wrote the manuscript; All authors read and approved the final manuscript.

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References

Assmann SM, Shimazaki K-I (1999) Update on signal transduction the multisensory guard cell. Stomatal responses to blue light and abscisic acid 1. Plant Physiol **115**: 809–815

Brodribb T, Brodersen CR, Carriqui M, Tonet V, Rodriguez Dominguez C, McAdam S (2021) Linking xylem network failure with leaf tissue death. New Phytologist **232**: 68–79

Brodribb TJ, McAdam SAM (2011) Passive Origins of Stomatal Control in Vascular Plants. Science (1979) **331**: 582–585

Brodribb TJ, McAdam SAM (2017) Evolution of the stomatal regulation of plant water content. Plant Physiol **174**: 639–649

Brodribb TJ, McAdam SAM (2013) Abscisic acid mediates a divergence in the drought response of two conifers. Plant Physiol **162**: 1370–1377

Buckley TN (2019) How do stomata respond to water status? New Phytologist **224**: 21–36

Caird MA, Richards JH, Donovan LA (2007a) Nighttime stomatal conductance and transpiration in C3 and C4 plants. Plant Physiol **143**: 4–10

Caird MA, Richards JH, Hsiao TC (2007b) Significant transpirational water loss occurs throughout the night in field-grown tomato. Functional Plant Biology **34**: 172–177

Cardoso AA, McAdam SAM (2019) Misleading conclusions from exogenous ABA application: A cautionary tale about the evolution of stomatal responses to changes in leaf water status. Plant Signal Behav. doi: 10.1080/15592324.2019.1610307

Cardoso AA, Randall JM, McAdam SAM (2019) Hydraulics regulate stomatal responses to changes in leaf water status in the fern *Athyrium filix-femina* $^{1[OPEN]}$. Plant Physiol **179**: 533–543

Cavender-Bares J, Sack L, Savage J (2007) Atmospheric and soil drought reduce nocturnal conductance in live oaks. Tree Physiol **27**: 611–620

Christmann A, Hoffmann T, Teplova I, Grill E, Müller A (2005) Generation of active pools of abscisic acid revealed by in vivo imaging of water-stressed Arabidopsis. Plant Physiol **137**: 209–219

Costa JM, Monnet F, Jannaud D, Leonhardt N, Ksas B, Reiter IM, Pantin F, Genty B (2015) Open all night long: The dark side of stomatal control. Plant Physiol **167**: 289–294

Coupel-Ledru A, Lebon E, Christophe A, Gallo A, Gago P, Pantin F, Doligez A, Simonneau T (2016) Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. Proc Natl Acad Sci U S A **113**: 8963–8968

Cox DTC, Maclean IMD, Gardner AS, Gaston KJ (2020) Global variation in diurnal asymmetry in temperature, cloud cover, specific humidity and precipitation and its association with leaf area index. Glob Chang Biol **26**: 7099–7111

Dawson TE, Burgess SSO, Tu KP, Oliveira RS, Santiago LS, Fisher JB, Simonin KA, Ambrose AR (2007) Nighttime transpiration in woody plants from contrasting ecosystems. Tree Physiol **27**: 561–575

Dayer S, Herrera JC, Dai Z, Burlett R, Lamarque LJ, Delzon S, Bortolami G, Cochard H, Gambetta GA (2020) The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. J Exp Bot **71**: 4333–4344

Duursma RA, Blackman CJ, Lopéz R, Martin-StPaul NK, Cochard H, Medlyn BE (2019) On the minimum leaf conductance: Its role in models of plant water use, and ecological and environmental controls. New Phytologist **221**: 693–705

Geiger D, Maierhofer T, Al-Rasheid KAS, Scherzer S, Mumm P, Liese A, Ache P, Wellmann C, Marten I, Grill E, et al (2011) Stomatal closure by fast abscisic acid signaling is mediated by the guard cell anion channel SLAH3 and the receptor RCAR1. Plant Biol. doi: 10.1126/scisignal.2001346

Hasan MM, Gong L, Nie ZF, Li FP, Ahammed GJ, Fang XW (2021) ABA-induced stomatal movements in vascular plants during dehydration and rehydration. Environ Exp Bot. doi: 10.1016/j.envexpbot.2021.104436

Hoshika Y, Osada Y, de Marco A, Peñuelas J, Paoletti E (2018) Global diurnal and nocturnal parameters of stomatal conductance in woody plants and major crops. Global Ecology and Biogeography **27**: 257–275

Howard AR, Donovan LA (2007) Helianthus nighttime conductance and transpiration respond to soil water but not nutrient availability. Plant Physiol **143**: 145–155

Leckie CP, Mcainsh MR, Allen GJ, Sanders D, Hetherington AM (1998) Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. Plant Biol **95**: 15837–15842

Manandhar A, Pichaco J, McAdam SAM (2024) Abscisic acid increase correlates with the soil water threshold of transpiration decline during drought. *Plant, Cell & Environment* **47**: 5067–5075

Manandhar A, Rimer IM, Soares Pereira T, Pichaco J, Rockwell FE, McAdam SAM (2024) Dynamic soil hydraulic resistance regulates stomata. New Phytol **244**: 147-158

Martins SCV, McAdam SAM, Deans RM, Damatta FM, Brodribb TJ (2016) Stomatal dynamics are limited by leaf hydraulics in ferns and conifers: Results from simultaneous measurements of liquid and vapour fluxes in leaves. Plant Cell Environ **39**: 694–705

Martin-StPaul N, Delzon S, Cochard H (2017) Plant resistance to drought depends on timely stomatal closure. Ecol Lett **20**: 1437–1447

McAdam S (2015) Physicochemical quantification of abscisic acid levels in plant tissues with an added internal standard by ultra-performance liquid chromatography. Bio Protoc. doi: 10.21769/bioprotoc.1599

McAdam SAM, Brodribb TJ (2012) Fern and lycophyte guard cells do not respond to endogenous abscisic acid. Plant Cell **24**: 1510–1521

McAdam SAM, Brodribb TJ (2014) Separating active and passive influences on stomatal control of transpiration. Plant Physiol **164**: 1578–1586

McAdam SAM, Manandhar A, Kane CN, Mercado-Reyes JA (2024) Passive stomatal closure under extreme drought in an angiosperm species. J Exp Bot **75**: 6850–6855

Meng D, Ma J, Min X, Zang Y, Sun W (2024) Nocturnal stomatal behaviour and its impact on water use strategies of desert herbs in the Gurbantunggut Desert, Northwest China. Science of the Total Environment. doi: 10.1016/j.scitotenv.2024.172749

Mercado-Reyes JA, Pereira TS, Manandhar A, Rimer IM, McAdam SAM (2024) Extreme drought can deactivate ABA biosynthesis in embolism-resistant species. Plant Cell Environ **47**: 497–510

Mittelheuser C, Van Steveninck R (1969) Stomatal Closure and Inhibition of Transpiration induced by (RS)-Abscisic Acid. Nature **221**: 281–282

Pemadasa MA (1981) Photocontrol of stomatal movements. Biological Reviews **56**: 551–588

Pereira TS, Oliveira LA, Andrade MT, Haverroth EJ, Cardoso AA, Martins SCV (2024) Linking water-use strategies with drought resistance across herbaceous crops. Physiol Plant. doi: 10.1111/ppl.14114

Resco de Dios V, Chowdhury FI, Granda E, Yao Y, Tissue DT (2019) Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. New Phytologist **223**: 1696–1706

Resco de Dios V, Roy J, Ferrio JP, Alday JG, Landais D, Milcu A, Gessler A (2015) Processes driving nocturnal transpiration and implications for estimating land evapotranspiration. Sci Rep. doi: 10.1038/srep10975

Roelfsema MRG, Hedrich R (2005) In the light of stomatal opening: new insights into “the Watergate.” New Phytologist **167**: 665–691

Roelfsema MRG, Steinmeyer R, Staal M, Hedrich R (2001) Single guard cell recordings in intact plants: Light-induced hyperpolarization of the plasma membrane. Plant Journal **26**: 1–13

Sadler EJ, Evans DE (1989) Vapor pressure deficit calculations and their effect on the combination equation. Agric For Meteorol **49**: 55–80

Tardieu F, Davies WJ (1992) Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol **98**: 540–545

Tombesi S, Nardini A, Frioni T, Soccolini M, Zadra C, Farinelli D, Poni S, Palliotti A (2015) Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Sci Rep. doi: 10.1038/srep12449

Wang K, Dickinson RE (2012) A review of global terrestrial evapotranspiration: Observation, modeling, climatology, and climatic variability. Reviews of Geophysics. doi: 10.1029/2011RG000373

Wang Y, Anderegg WRL, Venturas MD, Trugman AT, Yu K, Frankenberg C (2021) Optimization theory explains nighttime stomatal responses. New Phytologist **230**: 1550–1561

Wang Z, Du A, Liu S, Xu Y, Zhu W, Xiang W (2024) Quantification of nocturnal water use and its composition in a *Eucalyptus urophylla* × *E. grandis* plantation on the Leizhou Peninsula, South China. J Geophys Res Biogeosci. doi: 10.1029/2023JG007779

Wankmüller FJP, Carminati A (2022) Stomatal regulation prevents plants from critical water potentials during drought: Result of a model linking soil–plant hydraulics to abscisic acid dynamics. Ecohydrology. doi: 10.1002/eco.2386

Ward JM, Schroeder J (1994) Calcium-activated K^+ channels and calcium-induced calcium release by slow vacuolar ion channels in guard cell vacuoles implicated in the control of stomatal closure. Plant Cell **6**: 669–683

Yang YJ, Bi MH, Nie ZF, Jiang H, Liu XD, Fang XW, Brodribb TJ (2021) Evolution of stomatal closure to optimize water-use efficiency in response to dehydration in ferns and seed plants. New Phytologist **230**: 2001–2010

Yu K, Goldsmith GR, Wang Y, Anderegg WRL (2019) Phylogenetic and biogeographic controls of plant nighttime stomatal conductance. New Phytologist **222**: 1778–1788

Zeppel MJB, Lewis JD, Phillips NG, Tissue DT (2014) Consequences of nocturnal water loss: A synthesis of regulating factors and implications for capacitance, embolism and use in models. Tree Physiol **34**: 1047–1055

Figures

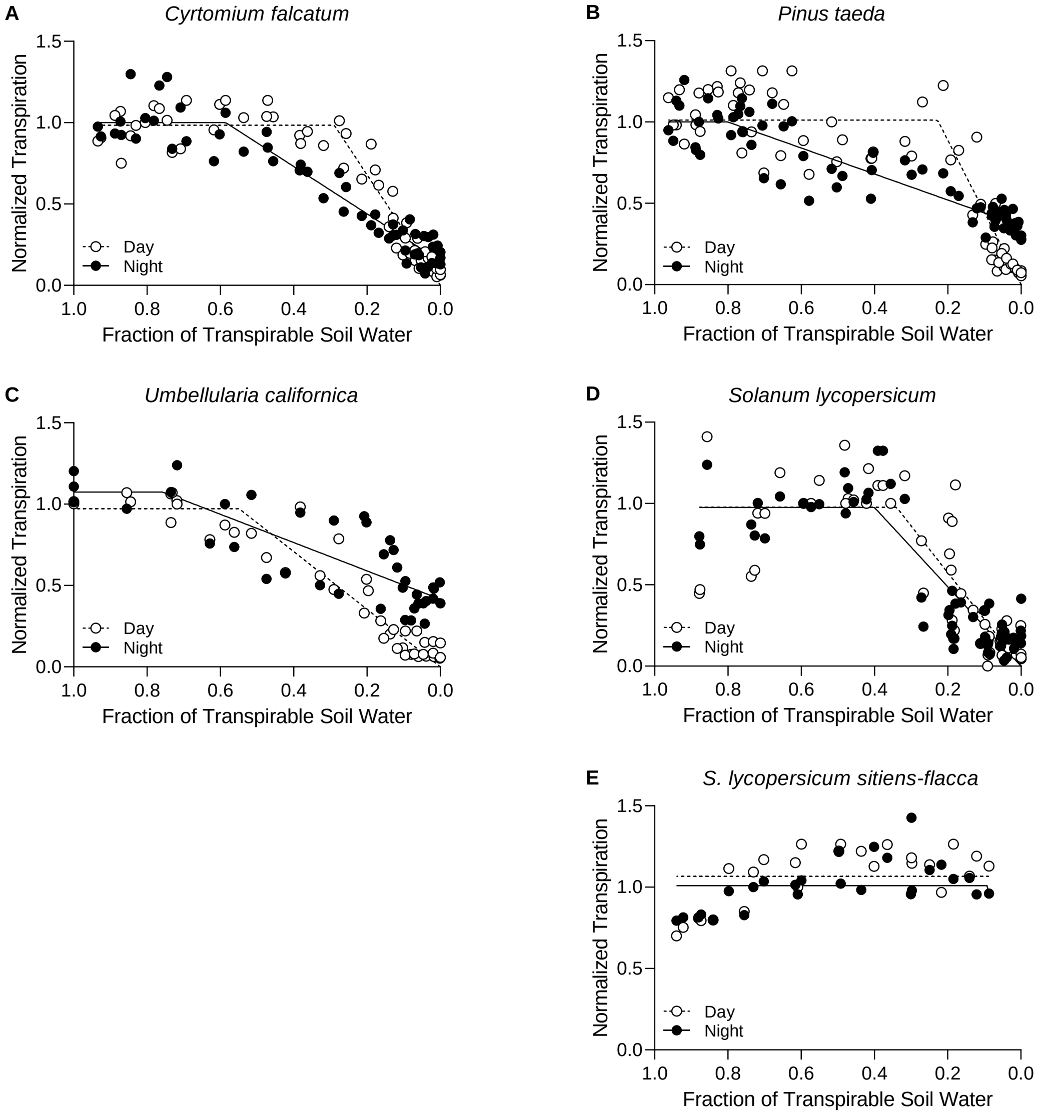

Figure 1. The day (white) and nighttime (black) normalized transpiration response to soil drought, expressed as fraction of total transpirable soil water in the fern *Cyrtomium falcatum* (A)*;* conifer *Pinus taeda* (B)*;* woody angiosperm *Umbellularia californica* (C)*;* herbaceous wild-type angiosperm *Solanum lycopersicum* (D)*;* and corresponding ABA biosynthetic mutant *sitiens flacca* (E). A two-segmented linear regression was fitted to determine the breakpoint of reduced transpiration at night and day.

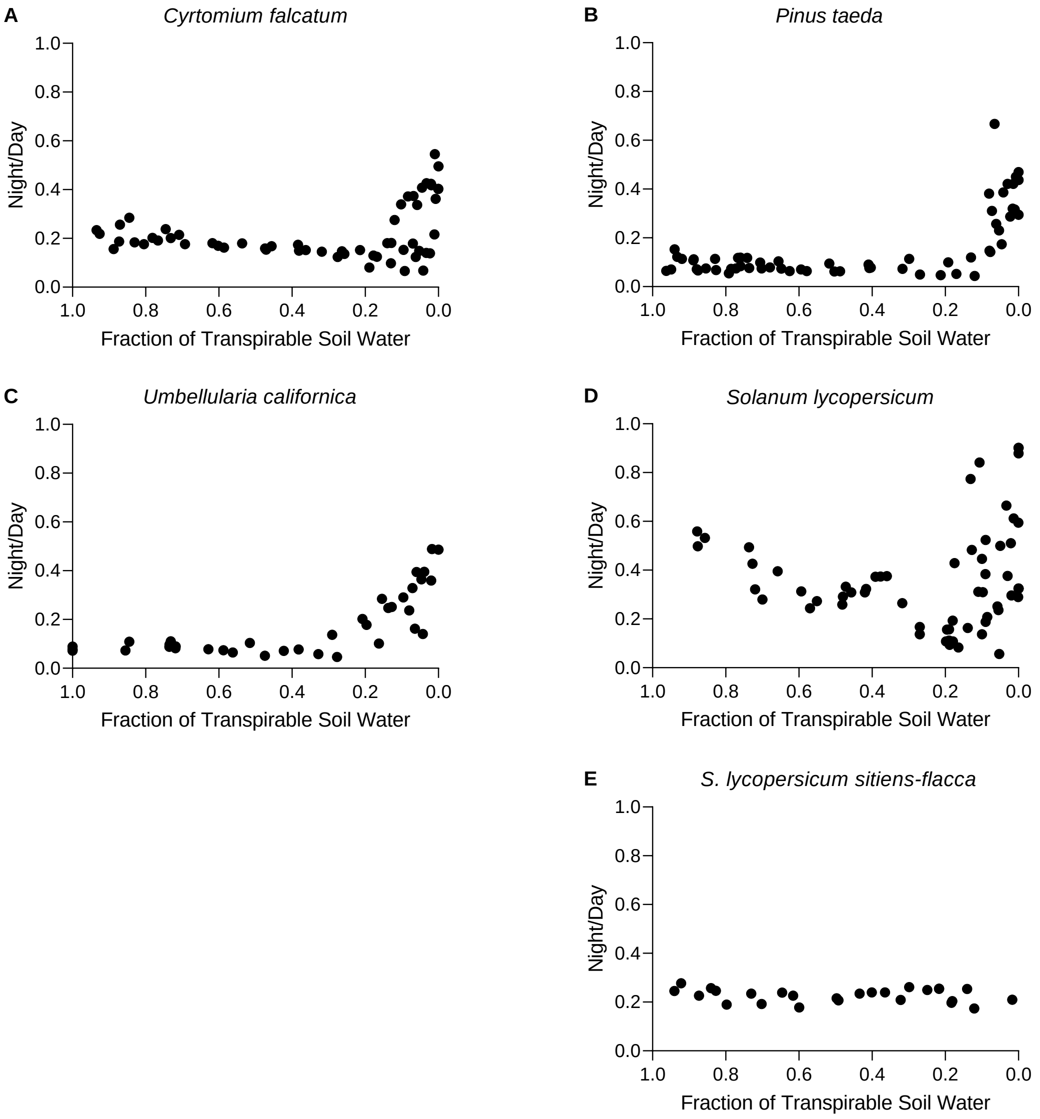

Figure 2. The ratio of nighttime to daytime transpiration as the fraction of transpirable soil water declined in the fern *Cyrtomium falcatum* (A)*;* conifer *Pinus taeda* (B)*;* woody angiosperm *Umbellularia californica* (C)*;* herbaceous wild-type angiosperm *Solanum lycopersicum* (D)*;* and corresponding ABA biosynthetic mutant *sitiens flacca* (E).

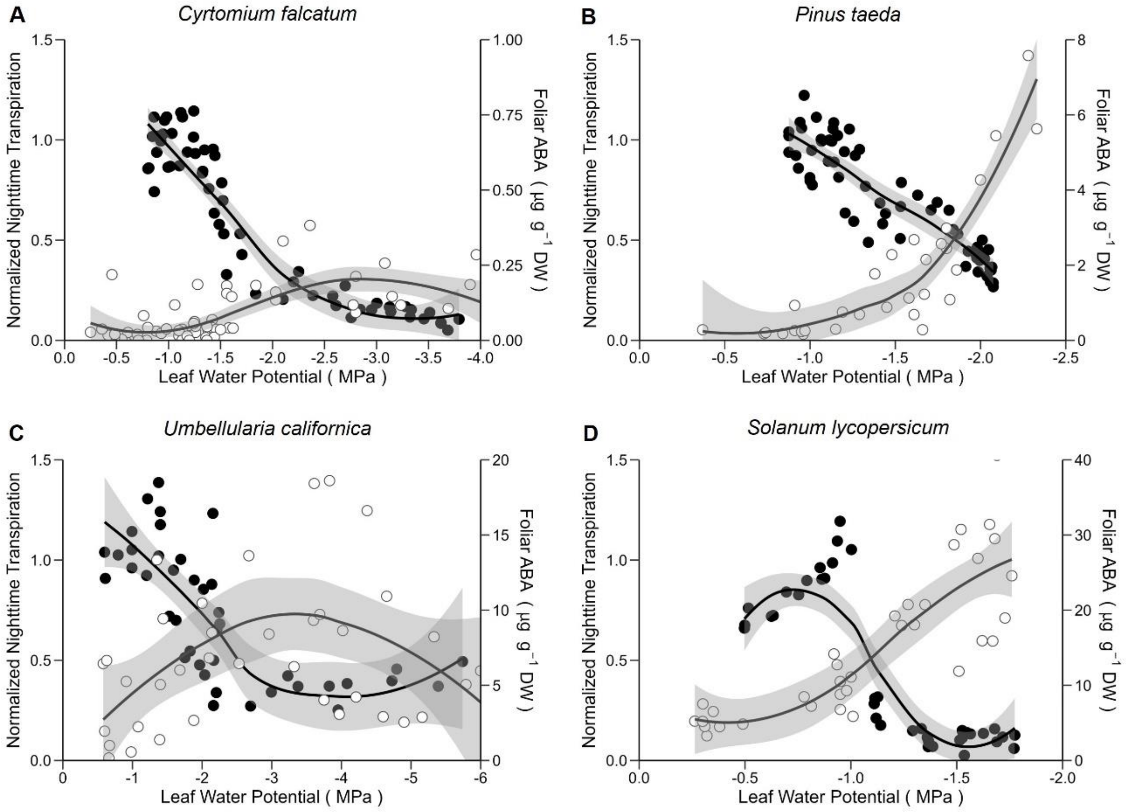

Figure 3. The relationship between normalized nighttime transpiration (filled black circles) and foliar ABA level (filled white circles) and leaf water potential (Ψ) in the fern *Cyrtomium falcatum* (A)*;* conifer *Pinus taeda* (B)*;* woody angiosperm *Umbellularia californica* (C)*;* herbaceous wild-type angiosperm *Solanum lycopersicum* (D)*;* and corresponding ABA biosynthetic mutant *sitiens flacca* (E). A loess (locally linear smoothing) regression with shaded area depicting the standard error is shown for each relationship.

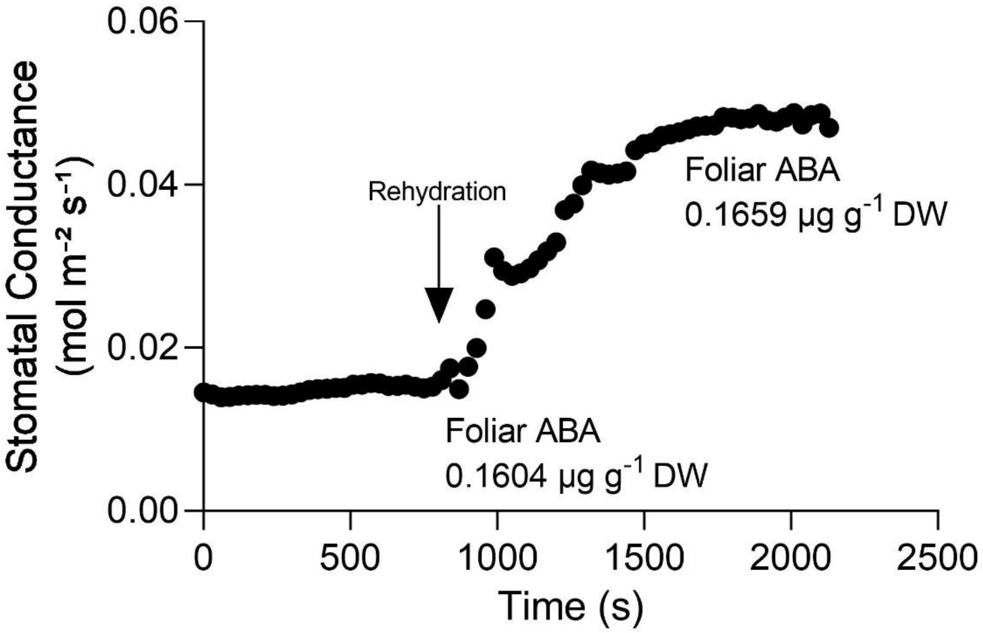

Figure 4. Stomata conductance measured in the dark on an intact leaf of the fern *Cyrtomium falcatum* under severe drought (-3.5 MPa). The leaf was excised under water, time denoted by an arrow and instantaneously rehydrated. Foliar abscisic acid (ABA) levels were measured in a neighboring pinna at the point of rehydration and in the pinna in which gas exchange was measured at the end of the experiment. Foliar ABA levels at both time points are indicated on the chart.

Tables

Table 1. Mean transpiration rate (±SE) during the day and night (*E;* g H_2O plant^{-1}) under well-watered conditions and at the lowest measured FTSW in the fern *Cyrtomium falcatum;* conifer *Pinus taeda;* woody angiosperm *Umbellularia californica* and herbaceous wild-type angiosperm *Solanum lycopersicum.*

Species	Control		Water Deficit	
	E_{day}	E_{night}	E_{day}	E_{night}
C. falcatum	78.4 ± 7.59	19.9 ± 0.85	6.6 ± 0.53	2.9 ± 0.02
P. taeda	469.0 ± 7.48	24.2 ± 3.05	27.7 ± 2.87	6.5 ± 0.82
U. californica	390.7 ± 45.04	26.2 ± 2.52	34.3 ± 3.55	7.7 ± 0.15
S. lycopersicum	169.8 ± 3.92	54.6 ± 1.80	11.9 ± 0.12	5.9 ± 0.97

Table 2. Mean canopy conductance (±SE) during the day and night (g_c*;* mmol m^{-2} s^{-1}) under well-watered conditions and at the lowest measured FTSW in the fern *Cyrtomium falcatum;* conifer *Pinus taeda;* woody angiosperm *Umbellularia californica* and herbaceous wild-type angiosperm *Solanum lycopersicum.*

Species	Control		Water Deficit	
	Day g_c	Night g_c	Day g_c	Night g_c
C. falcatum	226.4 ± 8.64	116.9 ± 10.75	6.7 ± 0.67	5.8 ± 1.28
P. taeda	58.8 ± 4.27	6.0 ± 0.58	4.9 ± 0.58	2.5 ± 0.20
U. californica	101.3 ± 7.10	21.6 ± 2.79	7.2 ± 1.65	4.6 ± 0.95
S. lycopersicum	234.0 ± 11.65	61.8 ± 1.29	3.6 ± 0.58	5.2 ± 0.69