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Polyploidy drives changes in tissue allocation modifying whole-plant water relations

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Abstract

Polyploid plants often display functional trait values distinct from those of diploids, influencing their stress tolerance and adaptive capacity. These differences shape how polyploids interact with their environment, a factor that is crucial to their evolutionary success. Here, we investigated the species complex *Dianthus broteri*, where ploidy level is known to correlate with water availability, as a model system to understand the possible link between ploidy and whole-plant water relations. We quantified allocation between leaves, xylem, and roots in 4 different ploidies of *D. broteri* (2x, 4x, 6x, 12x), and examined its relationship with hydraulic efficiency (K_{r-s}), water potential regulation, and stomatal conductance (g_c) in response to varying leaf-to-air vapor pressure deficits (VPD_L). A gradient in tissue allocation to roots and xylem, resulting in higher K_{r-s} and g_c and lower water potential gradients. Despite these differences, g_c responses to VPD_L were largely consistent across ploidies. In *D. broteri* 12x, the significant investment in water uptake and transport without a proportional increase in leaf area appeared suboptimal, incurring high xylem costs per unit water transport. However, this trade-off also led to increased water uptake and transport efficiency, potentially advantageous under water-limited conditions. Overall, our results indicate that multiple rounds of genome duplication cause substantial changes in whole-plant water relations, likely impacting water stress exposure in the field.

Introduction

Phenotypic change is inherent to a macromutational process such as polyploidy, defined as the state of organisms that have more than 2 full chromosome sets acquired via whole-genome duplication (WGD). Despite many studies on flowering plants, there is a lack of consensus on the direction of trait change from diploids (2x) to polyploids (>2x) that is probably due to the intricate interplay of short- and long-term phenotypic innovations mediated by WGD. The understanding of this complexity has led in recent years to a new paradigm that reconciles the diverse (and sometimes opposing) patterns of phenotypic change found in polyploid cytotypes relative to their diploid relatives. In short, polyploidy sets the foundation for change, but any adaptive potential will most likely only be fulfilled in noncompetitive environments exposed to environmental stress in which polyploidy would provide more variation for selection (Ebadi et al. 2023).

Plant polyploids consistently show increased cell size and lower cell surface area to volume ratio (Pacey et al. 2022), which slow down the cell cycle and limit growth rates (Comai 2005; Corneillie et al. 2019). In the leaf, although changes in cell size are coordinated with changes in genome size (Brodribb et al. 2013), it is known that polyploidy makes this relationship tissue-dependent (Katagiri et al. 2016). Modifications in the structure and organization of leaf cells and tissues will have massive consequences for whole-plant physiology as they directly impact rates of transpiration and photosynthesis (Simonin and Roddy 2018). One strategy for polyploids to maintain the balance between water uptake and loss would be to alter resource allocation between aboveground and belowground plant parts (Poorter et al. 2012), thereby disrupting the allometric scaling of organ size (Harashima and Schnittger 2010; Balao et al. 2011). In this context, root biomass, depth, or architecture directly influence how effectively a plant can access and transport water, collectively determining its operating water potentials (Martínez-Vilalta and Garcia-Forner 2017). However, the effect of polyploidy on root traits has received little attention (but see e.g. Allario et al. 2011; Dudits et al. 2016) and its functional significance has not yet been explored.

As allocation ratios between plant structures involved in water uptake, transport, and loss affect water-use strategies (Flo et al. 2021), polyploids could differ from their diploid counterparts in stress tolerance and potential yield—which are traded off. Polyploids generally show wider xylem vessels than diploids (Barceló-Anguiano et al. 2021; Losada et al. 2023), which theoretically increase hydraulic conductance as described in the Hagen–Poiseuille equation (Zimmermann 1983). Although polyploids displayed largely higher hydraulic conductance than diploids in *Chamerion angustifolium* (Maherali et al. 2009), the opposite trend was observed in *Atriplex canescens* (Hao et al. 2013) and atemoya (Losada et al. 2023). Similarly, greater embolism

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resistance was found in polyploids of Betula and A. canescens (Hao et al. 2013; Zhang et al. 2017), but other studies failed to find differences between ploidies (Maherali et al. 2009; De Baerdemaeker et al. 2018). With respect to gas exchange, the larger and less densely packed stomata in polyploids compared with diploids have traditionally been proposed to reduce maximum stomatal conductance, net photosynthesis (Simonin and Roddy 2018; Roddy et al. 2020) and transpiration rates (Del Pozo and Ramirez-Parra 2014; Tossi et al. 2022). Yet, no consistent findings have been reported when comparing operational stomatal conductance among ploidy levels (2x, 3x and 4x) for a range of tree species (Ræbild et al. 2024). Therefore, not only has greater stress tolerance been attributed to plant polyploids, but also higher gas exchange rates and biomass production (Greer et al. 2018, and especially in crops; see Renny-Byfield and Wendel 2014; Trojak-Goluch et al. 2021).

In this regard, a confounding factor often overlooked in most studies is that comparisons are usually restricted to those between diploids and their direct low polyploid descendants (i.e. 3x or 4x), ignoring high polyploids (i.e. >4x). These latter lineages undergo multiple rounds of genome duplication, which may increase their potential to develop complex phenotypic novelties. Additionally, post-WGD processes like exposure to selection are also crucial in defining the outcome for any functional trait (Bomblies 2020). Given this context, there is a knowledge gap as to whether trait linkages that seem to have evolved to optimize plant water relations are altered by polyploidy. For example, if reduced transpiration capacity is not linked to reduced hydraulic conductance, polyploid plants could operate at higher water potentials. This could potentially lower the risk of xylem embolism (Anderegg et al. 2017) and contribute to the association of polyploid species with stressful environments (Van De Peer et al. 2021).

The characterization of stomatal behavioral traits has been investigated in response to leaf-to-air vapor pressure deficit (VPD_I). Changes in VPD_L are known to affect carbon acquisition and water use on timescales much shorter than those of soil moisture changes (Grossiord et al. 2020), likely reflecting how plants adapt differently to environmental conditions (Brodribb and Jordan 2008; Scoffoni et al. 2016). In recent years, the simultaneous measurement of stem water potential (Ψ_{stem}) using the "dendrometric" approach and whole-plant transpiration (E_c) through gravimetric methods has permitted tracking in situ the dynamics of root-stem hydraulic conductance (K_{r-s} ; here referring to the flow path from the root surface to the stem xylem) and canopy stomatal conductance (g_c) in response to VPD_L (Bourbia et al. 2022, 2023; Bourbia and Brodribb 2024). To date, studies have focused on comparing phylogenetically distant species with contrasting morphological and anatomical identities (i.e. woody vs herbaceous, angiosperms vs gymnosperms), but whole-plant physiology under different VPD_L levels remains unexplored for diploid-polyploid plant systems.

Considering the above, Dianthus broteri (Caryophyllaceae) was selected to address our primary goal of testing the effect of ploidy on core water relations. This diploid-polyploid complex of perennial herbaceous carnations consists of 4 ploidy levels (2x, 4x, 6x, and 12x; Balao et al. 2009) with independent evolutionary trajectories (Balao et al. 2010). Interestingly, these ploidies exhibit distinct resource-use strategies along the acquisitive-conservative tradeoff (López-Jurado et al. 2022) and different responses to abiotic stressors such as extreme temperatures (López-Jurado et al. 2020, 2024). D. broteri ploidy levels also have non-overlapping geographical distributions and differ in their environmental niches. Higher polyploids (6x and 12x) are found in drier, warmer conditions typical of harsher Mediterranean climates, whereas 2x and 4x ploidy levels occupy less arid environments (López-Jurado et al. 2019). The presence of naturally occurring diploid, low polyploid, and high polyploid cytotypes in *D. broteri*, each associated with distinct habitats, makes this species complex an ideal model for examining the functional links that may connect ploidy and fitness. We hypothesized that increasing ploidy would lead to changes in tissue allometry that enhance water acquisition and transport to the canopy, possibly explaining improved performance of high ploidy varieties in areas of water shortage. The methods that we used allowed us to determine whole-plant stomatal sensitivity to VPD_L and to measure the hydraulic conductance of the root-stem portion of the plant.

Results

Allometry

Both the leaf-to-root (L/R) ratio and the Huber value (H_v), which depict the allocation of tissue in plants, varied significantly with ploidy level in *D. broteri*. The L/R ratio decreased systematically with ploidy, the 2× having the highest values and the 12× the lowest (GLM: ploidy, P<0.05; Fig. 1A), while the opposite pattern of gradual increase with ploidy was found for the H_v (GLM: ploidy, P < 0.05; Fig. 1B).

Gas exchange and VPD_L responses

Leaf width monitored with automated optical dendrometers and Ψ_{stem} measured with a pressure chamber were consistently highly correlated across individual *D. broteri* plants, always in a linear fashion ($\mathbb{R}^2 > 0.97$, P < 0.001; Supplementary Fig. S1). This allowed Ψ_{stem} to be predicted continuously (at a 5-min interval) and in situ from optical dendrometry data. Thus, Ψ_{stem} was observed to vary proportionally with E_c dynamics under VPD_L changes in each ploidy level and both stabilized after approximately 1.5 h of constant conditions in the chamber (Fig. 2, Supplementary Fig. S2). Rapid increases in E_c occurred concurrently with rapid decreases in Ψ_{stem} after each step increase in VPD_L, starting upon illumination in the morning and imposing the low VPD_L conditions (c. 1 kPa). This pattern was maintained in subsequent medium and high VPD_L (Fig. 2, Supplementary Fig. S2), meaning that E_c continued to increase despite stomatal response to VPD_L in *D. broteri* ploidies.

Notwithstanding shared patterns in the dynamics of E_c and Ψ_{stem} , their relationship differed among ploidies. The highest-order *D. broteri* cytotype (12×) exhibited the highest steady-state E_c and least negative Ψ_{stem} across VPD_L levels (Fig. 2H, Supplementary Fig. S3), which translated into a higher $K_{\text{r-s}}$ (GLM: ploidy, P < 0.05; Fig. 3, A and B, Supplementary Fig. S4) and g_c (GLM: ploidy, P < 0.05; Fig. 3C, Supplementary Fig. S5). Regardless of ploidy, increasing VPD_L did not affect in situ $K_{\text{r-s}}$ (GLM: VPD_L, P > 0.05; Fig. 3B) despite causing a significant decline in g_c (GLM: VPD_L, P < 0.05; Fig. 3C). While $K_{\text{r-s}}$ remained largely unchanged, g_c dropped on average by $26.5 \pm 3.7\%$ during transitions from low to high VPD_L (Fig. 2C). At a reference VPD_L (c. 1 kPa), g_c was highly correlated with average $K_{\text{r-s}}$ across ploidy levels ($R^2 = 0.95$, P < 0.05; Fig. 3D).

As expected, a higher VPD_L also resulted in a lower Ψ_{stern} , then increasing the water potential gradient ($\Delta \Psi$) between the wellhydrated soil and the plant (GLM: VPD_L, P < 0.05; Fig. 4A, Supplementary Fig. S6). Nonetheless, D. broteri ploidies differed in $\Delta \Psi$, being significantly higher in 2× and 4× than in 6× and 12× (GLM: ploidy, P < 0.05; Fig. 4A). Interestingly, water potential regulation was clearly tighter for D. broteri 12× throughout VPD_L treatments (0.1 MPa average difference between low and high VPD_L)



Figure 1. Tissue allocation traits measured after the different leaf-to-air vapor pressure deficit (VPD_L) treatments in the 4 ploidy levels (2x, 4x, 6x, and 12x) of *D. broteri*. **A**) Leaf-to-root (L/R) ratio on a dry weight basis. **B**) Huber value (H_{vi} , the ratio of sapwood xylem area to subtending canopy leaf area). In the box plots, horizontal lines depict the median, and boxes and whiskers show the interquartile range and the nonoutlier ranges, respectively. Raw data points correspond to individual plants used in the experiment (n = 4). Different letters indicate means that are significantly different from each other (GLM: P < 0.05, Tukey's HSD test: $\alpha = 0.05$).

compared with the 3 other ploidies (0.3 MPa average difference between low and high VPD_L). The pattern of differentiation among ploidy levels described for $\Delta\Psi$ aligned with Ψ_{osm} . On average, the highest values of Ψ_{osm} were registered in 12× individuals and gradually were more negative with increasing ploidy, but results were not significant (GLM: ploidy, P>0.05; Fig. 4B).

Discussion

Polyploidy impacts tissue allocation, water transport capacity, and gas exchange

In this study, polyploidy was found to have a significant influence on the allocation ratios between key functional plant tissues. As depicted by L/R and H_v, D. broteri ploidies were ordered in a continuum from higher resource investment in leaves (water use) in the 2x cytotype to higher resource investment in roots (water uptake) and xylem (water transport) in the 12x cytotype. First, this trend indicates that cell proliferation promoted by polyploidy occurs in a tissue-specific manner, which is in accordance with previous results in the Arabidopsis ploidy series (Del Pozo and Ramirez-Parra 2014; Robinson et al. 2018). Second, the differential development of those vegetative structures seems to be nonrandom in D. broteri, suggesting post-WGD adaptive changes (Bomblies 2020). Higher ploidy levels, especially 12x, inhabit xeric environments characterized by high atmospheric demand and low soil moisture (López-Jurado et al. 2019). Under such conditions, a lower L/R ratio would be beneficial by enabling the exploration of larger soil volumes (Poorter et al. 2012) while reducing evaporative surface area and water potential gradients for water extraction (DeLucia et al. 2000; Martínez-Vilalta and Garcia-Forner 2017). A high H_v (i.e. enhanced production of sapwood xylem per unit leaf area) would similarly reduce the water potential load on the vascular system under elevated VPD_L. As ploidy increases, the greater investment in roots and xylem contributes to the high K_{r-s} values observed. Consistent with findings from polyploid tree crops (Barceló-Anguiano et al. 2021; Losada et al. 2023), larger vessels in higher D. broteri ploidies (López-Jurado et al., unpublished data) may further enhance K_{r-s}. This suggests that water can be transported effectively at low xylem tension, an

adaptive trait for plants vulnerable to embolism (Flo et al. 2021), although this has yet to be specifically investigated in D. broteri. Furthermore, higher K_{r-s} supported higher g_c , in line with several studies that have reported strong coordination between canopy conductance and hydraulic efficiency across species (Brodribb and Feild 2000; Xiong and Nadal 2020). While higher-ploidy plants typically have larger and less dense stomata-traits that might reduce responsiveness to environmental fluctuations and slow gas exchange adjustments (Drake et al. 2013; Roddy et al. 2020)we show here that the coordination between water supply and transpiration demand is preserved across ploidy levels within the same species. This coordination may arise through compensation effects, where larger stomata enable sufficient q_c to match increased K_{r-s} (Lunn et al. 2024), and is further supported by the "passive dilution' mechanism (Carins Murphy et al. 2016; Brodribb et al. 2017), in which differential leaf cell expansion results in proportional co-variation of vein and stomatal density.

Stomatal regulation to changing VPD_L was also found to be largely conserved among ploidies of the species complex studied here. Stomata closed progressively (gc decrease) with higher evaporative demand, dampening the decline in Ψ_{leaf} and the risk of xylem embolism during daytime transpiration (Buckley 2005; Buckley 2019; Bourbia et al. 2023). Importantly, significant stomatal closure occurred in all ploidies at high VPD_L without any decline in K_{r-s}. This indicates that stomatal responses are triggered by changes in plant water potential and leaf turgor and not by declining hydraulic conductance (Bourbia et al. 2022; Bourbia and Brodribb 2024). The similar stomatal VPD_L response seen across all cytotypes was surprising considering the large differences in operational $\Delta \Psi$ between them. This could be explained by the higher Ψ_{osm} observed in higher ploidy levels, which would have the effect of increasing the stomatal sensitivity to water potential due to earlier triggering of mesophyll turgor loss and abscisic acid production (McAdam and Brodribb 2018).

Coordinated functional changes with ploidy lead to different whole-plant water relations

2x and 4x cytotypes of *D*. *broteri*, which are distributed in relatively humid areas (López-Jurado et al. 2019), showed similar water-use



Figure 2. Diurnal changes in stem water potential (Ψ_{stem}) and whole-plant transpiration (E_c) measured in one representative plant from each ploidy level of D. broteri (2x, 4x, 6x, and 12x) subjected to 3 leaf-to-air vapor pressure deficits (VPD_L). **A**, **B**, **E**, **F**) Variation in VPD_L conditions (black line). **C**, **D**, **G**, **H**) Variation in Ψ_{stem} (black circle) inferred from foliar width variation and E_c (orange circle) measured gravimetrically, both at 5-min interval. The gray background indicates nighttime with PPFD = 0 μ mol m⁻² s⁻¹, while the white background indicates daytime with PPFD = 500 μ mol m⁻² s⁻¹. PPFD, photosynthetic photon flux density.

strategies as they did not significantly differ in their tissue allocation ratios. A downregulation of stomatal function was observed in these ploidies, with both g_c and E_c being lower compared with

the 12x. However, the reduced K_{r-s} in 2x and 4x D. broteri required the plants to operate at greater xylem tensions than their higher ploidy counterparts. 2x and 4x cytotypes would then rely on a



Figure 3. Stomatal and hydraulic responses in the 4 ploidy levels (2x, 4x, 6x, and 12x) of *D. broter* it o different leaf-to-air vapor pressure deficits (VPD_L). **A)** Steady-state root-to-stem hydraulic conductance (K_{r-s}) data pooled across VPD_L treatments. In the boxplots, horizontal lines depict the median, and boxes and whiskers show the interquartile range and the non-outlier ranges, respectively. **B)** Steady-state K_{r-s} at each VPD_L level. **C)** Steady-state whole-plant stomatal conductance (g_c) at each VPD_L level. **D)** Relationship between K_{r-s} and g_c at medium VPD_L (approx. 1.5 kPa). Colors correspond to the 4 different *D. broteri* ploidy levels. Results are presented as mean values \pm SE (n=4). Significant effects of ploidy, VPD_L, or both are indicated by asterisks (GLM: P < 0.05). Lowercase and uppercase letters denote significant differences among ploidy levels and VPD_L levels, respectively (Tukey's HSD: $\alpha = 0.05$).

higher $\Delta \Psi$ that compensates for lower $K_{r,s}$ to meet the transpirational demands of their highly developed canopy (Sack and Holbrook 2006).

Contrastingly, $6 \times D$. broteri displayed a safe whole-plant wateruse strategy under increasing evaporative demands. It was able to maintain lower $\Delta \Psi$ than 2× and 4×, avoiding xylem tensions that could damage its hydraulic system under the elevated VPD_L characteristic of its arid climate of origin (López-Jurado et al. 2019). Such an advantageous feature was not accompanied by either a higher investment in resource-costly roots and xylem or an increased E_c compared with lower ploidies. This particularly efficient behavior highlights the prominent role of polyploidy in enhancing the adaptive capacity of plants to environmental stress (Van De Peer et al. 2021; Ebadi et al. 2023). The slightly higher K_{r-s} recorded in the 6× cytotype would allow for a lower $\Delta \Psi$ by buffering the water status against changes in VPD_L, keeping an adequate hydraulic supply to the leaf epidermis (Brodribb and Jordan 2008; Zhang et al. 2013).

Such a trend toward safer water-use traits with increasing ploidy, also matching a gradient of increasing aridity, was further pronounced in the 12x cytotype. Yet, the mentioned benefits of

this strategy incurred significant costs in this ploidy. First, progressively higher atmospheric evaporative demands could cause substantial stomatal closure and hence limit carbon assimilation, as previously found in 12× D. broteri under soil drought (López-Jurado et al. 2016). More importantly, adopting an increasingly "conservative" behavior in water potential must be inefficient in terms of xylem costs per unit water transport. This cytotype had the highest E_c, meaning more water loss per unit leaf area because of its high g_c regardless of VPD_L. To offset this serious disadvantage in a xeric environment, water conservation at the whole-plant level was prioritized and probably driven by strong selective pressures post-WGD (Zhang et al. 2019). The 12× cytotype thereby decreased total canopy leaf area which, together with its maximized water uptake, indicates a dehydration avoidance strategy to cope with high atmospheric evaporative demand (Volaire 2018). Nonetheless, this lower allocation to leaf tissue was not accompanied by a decrease in xylem or root investment, leading to a mismatch between modest water demand and excessive water supply. In fact, a water-spender strategy has been previously discussed for this ploidy in López-Jurado et al. (2022) and attributed to species with large genomes such as high polyploids (Veselý et al. 2012). Although



Figure 4. Water and osmotic potential responses in the 4 ploidy levels (2x, 4x, 6x, and 12x) of *D. broteri* to different leaf-to-air vapor pressure deficits (VPD_L). **A)** Steady-state water potential gradient in stem xylem, $\Delta \Psi = \Psi_{soil} - \Psi_{stem}$. Results are presented as mean values \pm SE (*n*=4). **B)** Leaf osmotic potential at full turgor (Ψ_{osm}) measured after the VPD_L treatments. In the boxplots, horizontal lines depict the median, and boxes and whiskers show the interquartile range and the nonoutlier ranges, respectively. Colors correspond to the 4 different *D. broteri* ploidy levels. Significant effects of ploidy, VPD_L, or both are indicated by asterisks (GLM: P < 0.05). Lowercase and uppercase letters denote significant differences among ploidy levels and VPD_L levels, respectively (Tukey's HSD: $\alpha = 0.05$).

adaptive adjustments in plant tissue allocation are known to be driven by environmental changes (e.g. Prescott et al. 2020), this can be challenged in polyploids due to their increased genetic diversity. Polyploids might be excessively responsive to selective pressures, especially during establishment, leading to maladaptive resource allocation ratios (Poorter et al. 2012).

By combining tissue allocation and in situ physiological traits measured in a complex ploidy series including diploid, low polyploid, and high polyploid cytotypes, our results support a large effect of polyploidy on multiple aspects of water relations that are closely coordinated with plant morphology and anatomy. Ploidy increase in D. broteri systematically altered the linkage between leaf, xylem, and root investment, resulting in contrasting wateruse strategies. Our work adds a new mechanistic dimension to previous studies on the impact of ploidy increase on climate adaptation and ecological tolerance (i.e. Napier et al. 2022). While WGD from 2x to 6x can confer physiological plasticity and fitness advantages (Guo et al. 2023), our results suggest that additional rounds of WGD in high polyploids might cause suboptimal allocation of tissue and lead to inefficient plant-environment interactions. Ultimately, this could alter competition effects between diploids and polyploids and decide the fate of newly evolved polyploid lineages in a changing climate.

Materials and methods Plant material and growth conditions

Plants of D. broteri (including the 4 ploidy levels) were grown from seeds in glasshouse facilities at the University of Tasmania. All individuals were grown in 2 L pots containing potting mix (medium 7: 4 mix of composted fine pine bark and coarse-washed river sand) and watered daily. Conditions in the controlled glasshouse cell were: unfiltered natural light (from 0 μ mol photons m⁻² s⁻¹ during the night to a peak of 1000 μ mol photons m⁻² s⁻¹ during the daytime), 25/15 °C day/night temperature, and 40/70% day/night relative humidity (RH). By the onset of the experiment,

D. broteri plants were 1 year old and total leaf area ranged from 40 to 205 cm^2 .

Temperature and humidity conditions defining VPD_L treatments

On the evening preceding measurements, pots of 4 plants per D. broteri ploidy level were watered to field capacity and allowed to drain excess water. The surface of each pot was then covered with aluminum foil to prevent direct soil evaporation. Subsequently, plants were transferred to a well-ventilated controlled-environment chamber where they were exposed to different steady-state VPD_L levels. VPD_L was calculated with the Arden Buck equation (Buck 1981) using leaf temperature and air RH data. Air temperature and RH were monitored at 1-min intervals with a temperature/humidity sensor data logger (HOBO model MX2302A; Onset Computer Corporation, Bourne, MA, USA) placed close to the canopy of the measured plants. Leaf temperature was measured during stable conditions of air temperature using a fine-wire thermocouple placed on the underside of different leaves of each individual and was logged on a datalogger (CR1000; Campbell Scientific, Logan, UT, USA).

The desired VPD_L levels were reached following a regular sequence from low VPD_L in the early morning to successively higher values during the day. VPD_L levels <2 kPa were achieved by varying RH only while keeping temperature relatively constant at 23 °C, but VPD_L levels >2 kPa needed a joint decrease in RH and increase in air temperature to 27 to 28 °C. Prior to VPD_L manipulation, plants were maintained at 20 to 21 °C in the dark and high RH (c.80% to 90%) using a commercial humidifier. During the next morning, plants were illuminated at a photosynthetic photon flux density of 500 μ mol m⁻² s⁻¹ at the canopy level and RH was decreased to c. 65% (VPD_L c. 1 kPa). A second VPD_L level was reached by turning off the humidifier, allowing RH to remain constant at c. 50% (VPD_L c. 1.5 kPa). Finally, RH was further reduced to c. 30% (VPD_L c. 2.5 kPa) and maintained at this level using a

Continuous measurements of E_c and Ψ_{stem}

 $E_{\rm c}$ and $\Psi_{\rm stem}$ were monitored simultaneously and continuously in each plant inside the growth chamber during step changes in VPD_L. $E_{\rm c}$ was monitored gravimetrically by placing each *D. broteri* individual on a computer-interfaced balance and weighing it every 5 min to an accuracy of ±0.01 g (VIBRA model ALE6202R; Shinko Denshi Co., Japan). $E_{\rm c}$ was finally normalized by the total leaf area of each plant, estimated using ImageJ (Schneider et al. 2012) after harvesting and scanning all the leaves at the end of the experiment.

In parallel, a high-resolution automated optical dendrometer (Cavicam Co., Hobart, Tas., Australia; for details see http://www. cavicam.co and Bourbia et al. 2021) was attached to 1 mature (nongrowing) leaf blade in each individual plant. This device was used to monitor changes in foliar width at 5 min intervals, from which the temporal dynamics of Ψ_{stem} could be inferred. Foliar width of the leaf enclosed (non-transpiring) in the dendrometer was calibrated against Ψ_{stem} measured with a pressure chamber (PMS Instruments, Albany, OR, USA) on neighboring non-transpiring leaves (following the recommendations of Rodriguez-Dominguez et al. 2022). Each Ψ_{stem} measurement was made after leaf width had reached a steady state and remained constant for at least 1 h. Calibration was then built using 4 to 5 Ψ_{stem} values for each individual plant obtained during the different transpirational steady states, encompassing the maximum range of Ψ_{stem} (1 measurement was done before switching on the lights, and the rest at the other 3 stable VPD_L treatments).

Response of $\Delta \psi$ to VPD_L

To characterize the interaction between stomatal regulation and transpiration rate as mediated by stem water potential, the water potential gradient between the soil and the stem ($\Delta\Psi$) was calculated at each corresponding steady-state VPD_L level in each plant. $\Delta\Psi$ was calculated as $\Psi_{\text{soil}} - \Psi_{\text{stem}}$. Ψ_{soil} was assumed to be always 0 MPa because plants were well watered right before measurements and pots were covered, preventing significant water loss.

Measurement of K_{r-s}

 K_{r-s} was determined in all plants at each of the 3 steady-state E_c and Ψ_{stem} (or $\Delta \Psi$) during low, medium and high VPD_L. K_{r-s} was calculated based on the normal application of Darcy's law:

$$K_{\rm r-s} = \frac{E_{\rm c}}{\Delta \Psi} \tag{1}$$

Leaf temperature was found to vary approximately 5 °C throughout measurements, from 23 °C at high RH to 28 °C at low RH. As a result, K_{r-s} in all ploidies of *D. broteri* was normalized to the viscosity of water at 20 °C according to soil temperature.

Response of g_c to VPD_L

 $g_{\rm c}$ was calculated in every individual plant at each steady-state $E_{\rm c}$ and concurrent steady-state VPD_L using Fick's law:

$$g_{\rm c} = E_{\rm c} \times \frac{P_{\rm atm}}{\rm VPD_{\rm L}} \tag{2}$$

where P_{atm} is atmospheric pressure (101.3 kPa). The chamber

conditions were stirred by the air conditioning fan; therefore the boundary layer conductance should have been very high and consistent between measurements.

Osmotic potential, Huber value, and tissue allocation

Leaf osmotic potential at full turgor (Ψ_{osm}) was quantified in 1 leaf of each plant used in the experiment. Well-watered plants were bagged in the evening to keep a saturated atmosphere and dark conditions overnight, therefore ensuring full turgor during sampling the following morning. Discs of c. 5 mm diameter were collected from healthy, fully expanded leaves, then wrapped in aluminum foil, and immediately frozen in liquid nitrogen to fracture cell walls and eliminate turgor pressure. Leaf discs were subsequently sealed in a stem psychrometer (PSY1, ICT International, Armidale, Australia) and leaf water potential (Ψ_{leaf}) was logged every 10 min until stable (c. 1 h). Based on the same principle applied by Bartlett et al. (2012) to vapor-pressure osmometry, Ψ_{leaf} was considered to be Ψ_{osm} here due to the absence of turgor pressure or apoplastic potential.

The Huber value (H_v ; the ratio of sapwood xylem area to subtending canopy leaf area) was also determined in every individual plant at the end of the experiment. Xylem cross-sectional area was measured at the base of the stem using ImageJ (Schneider et al. 2012) on pictures taken under the microscope. H_v was then calculated by dividing that basal xylem area by the total leaf area.

Biomass fractions of leaves and roots were determined in each individual after oven drying at 60 °C to constant weight. Biomass allocation for these herbaceous plants was then expressed as the leaf-to-root (L/R) ratio on a dry weight basis.

Statistical analysis

We used linear regressions to determine the correlation between leaf width and Ψ_{stem} in each *D. broteri* plant. Generalized linear models (GLMs) were used to analyze the effects of both ploidy and the VPD_L level on $K_{\text{r-s}}$, g_c and $\Delta \Psi$. For the variables that were not continuously monitored (Ψ_{osm} , H_v , and L/R ratio), ploidy level was the only categorical factor included in the GLMs. Effects were considered to be significant when P < 0.05. The Shapiro–Wilk test was used to check the normality of model residuals for each variable. Tukey's post-hoc HSD tests ($\alpha = 0.05$) were used to detect pairwise differences between ploidies and/or VPD_L levels. In case weight data were noisy over the 5 min interval, a linear filtering was applied to smooth E_c signal and define its steady states more clearly. R v.4.3.3 (R Core Team 2024) was used to perform all analyses and create all figures.

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Author contributions

J.L.J. and T.J.B. designed the research. J.L.J. performed the research. J.L.J. analyzed the data and wrote the paper with revisions from I.B. and T.J.B.

Supplementary data

The following materials are available in the online version of this article.

Supplementary Figure S1. Relationship between foliar width and leaf water potential (Ψ_{leaf}) in *D. broteri* ploidies.

Supplementary Figure S2. Diurnal changes in stem water potential (Ψ_{stem}) and whole-plant transpiration (E_c) under different leaf-to-air vapor pressure deficits (VPD_L) for all remaining plants from Fig. 2.

Supplementary Figure S3. Steady-state whole-plant transpiration (E_c) in *D. broteri* ploidies under different leaf-to-air vapor pressure deficits (VPD_L).

Supplementary Figure S4. Steady-state root-to-stem hydraulic conductance (K_{r-s}) in *D. broteri* ploidies under different leaf-to-air vapor pressure deficits (VPD₁).

Supplementary Figure S5. Steady-state whole-plant stomatal conductance (g_c) in *D. broteri* ploidies under different leaf-to-air vapor pressure deficits (VPD_L).

Supplementary Figure S6. Steady-state water potential gradient in stem xylem ($\Delta \Psi$) in *D. broteri* ploidies under different leaf-to-air vapor pressure deficits (VPD_L).

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Conflict of interest statement. None declared.

Data availability

The data that support the findings of this study will be available from the corresponding author upon reasonable request.

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