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Diversity in stomatal and hydraulic responses to post-flowering drought in common (*Phaseolus vulgaris*) and tepary (*P. acutifolius*) beans

Thomas N. Buckley[1] | **Troy S. Magney**[1] | **Jorge C. Berny Mier y Teran**[1] | **Colleen Mills**[1] | **Antonia Palkovic**[1] | **Travis A. Parker**[1] | **Marshall A. Pierce**[2] | **Yasmin Wadhwani**[1] | **Christopher Y. S. Wong**[1,3] | **Paul Gepts**[1] | **Matthew E. Gilbert**[1]

[1]Department of Plant Sciences, University of California, Davis, California, USA

[2]Department of Agriculture, Plant Science Faculty, Ventura College, Ventura, California, USA

[3]Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick, Canada

Correspondence

Thomas N. Buckley, Department of Plant Sciences, University of California, Davis, CA 95616, USA.
Email: tnbuckley@ucdavis.edu

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Abstract

Plants differ widely in how soil drying affects stomatal conductance (g_s) and leaf water potential (ψ_{leaf}), and in the underlying physiological controls. Efforts to breed crops for drought resilience would benefit from a better understanding of these mechanisms and their diversity. We grew 12 diverse genotypes of common bean (*Phaseolus vulgaris* L.) and four of tepary bean (*P. acutifolius*; a highly drought resilient species) in the field under irrigation and post-flowering drought, and quantified responses of g_s and ψ_{leaf}, and their controls (soil water potential [ψ_{soil}], evaporative demand [Δw] and plant hydraulic conductance [K]). We hypothesised that (i) common beans would be more "isohydric" (i.e., exhibit strong stomatal closure in drought, minimising ψ_{leaf} decline) than tepary beans, and that genotypes with larger ψ_{leaf} decline (more "anisohydric") would exhibit (ii) smaller increases in Δw, due to less suppression of evaporative cooling by stomatal closure and hence less canopy warming, but (iii) larger K declines due to ψ_{leaf} decline. Contrary to our hypotheses, we found that half of the common bean genotypes were similarly anisohydric to most tepary beans; canopy temperature was cooler in isohydric genotypes leading to smaller increases in Δw in drought; and that stomatal closure and K decline were similar in isohydric and anisohydric genotypes. g_s and ψ_{leaf} were virtually insensitive to drought in one tepary genotype (G40068). Our results highlight the potential importance of non-stomatal mechanisms for leaf cooling, and the variability in drought resilience traits among closely related crop legumes.

KEYWORDS

drought stress tolerance, stomata, terminal drought, vapour pressure deficit (VPD)

1 | INTRODUCTION

A growing human population requires ever more food, yet climate change and limited arable land constrain crop production. In particular, soil water deficit suppresses yield globally (Leng & Hall, 2019). The frequency and severity of droughts have increased in recent decades in many regions (Spinoni et al., 2014), and this may continue as climate change increases atmospheric demand on plant water (Grossiord et al., 2020; Vicente-Serrano et al., 2022), effectively depleting soil moisture available for crops. Thus, there is a need to identify, understand, and harness genetic variation in drought resilience of yield in crops. Yield suppression in drought occurs through several mechanisms, including depression of photosynthesis due to stomatal closure, and depression of sink strength in harvestable components, such as seed number. Even when yield suppression is caused by reduced sink strength rather than energy supply (Hageman & Van Volkenburgh, 2021; Rodrigues et al., 2019) (but see Farooq et al., 2017), breeding to alleviate sink limitation would simply shift the limitation to energy supply. Thus, stomatal closure will always ultimately limit crop production in drought. It is therefore essential to understand how and why stomatal drought responses vary in agronomic species.

Legumes in particular have exceptional but unrealised potential for improving water-use efficiency (WUE; photosynthesis per unit transpiration, or yield per unit applied water) (Adams, Buchmann, et al., 2018; Adams, Buckley, et al., 2018). A given rate of photosynthesis can, in principle, be achieved with either a low WUE (e.g., if stomatal conductance is high but photosynthetic capacity is low) or a high WUE (if stomatal conductance is low but photosynthetic capacity is high). That is, water and nitrogen (N) are partially substitutable as resource inputs for photosynthesis (Farquhar et al., 2002; Miller et al., 2001; Taylor & Eamus, 2008). In low-nitrogen soils, legumes have an intrinsic advantage over non-legumes, due to their ability to fix atmospheric N. This ability has been shown to translate into greater N investment in photosynthetic enzymes, and hence greater photosynthetic capacity (Adams, Buckley, et al., 2018; Adams et al., 2016), giving legumes the theoretical potential for greater WUE than non-legumes. However, elite legume crops have largely been bred to maximise growth and yield under full irrigation, resulting in exceptionally high stomatal conductances that cannot be sustained under drought (Adams, Buckley, et al., 2018). Thus, there is an opportunity to improve legume WUE and drought resilience.

An ideotype for improving both WUE and yield resilience in water-limited conditions would combine water-conservation and drought-insensitivity; that is, stomatal conductance (g_s) would be lower in wet soils than in current elite varieties, but less sensitive to soil drying. Experimental evidence (Adams, Buckley, et al., 2018) suggests that, due to legumes' N advantage, g_s could be substantially reduced in elite varieties with little effect on photosynthesis, greatly enhancing WUE. Many possible traits and trait syndromes could help achieve this ideotype. To visualise these traits, consider a heuristic (Figure 1) based on two quantitative constraints linking stomatal conductance and leaf water potential (ψ_{leaf}). One constraint is the requirement for transpiration to be balanced by water flow from soil to leaves; that is, $g_s \cdot \Delta w \approx K \cdot (\psi_{\text{soil}} - \psi_{\text{leaf}})$, where Δw is the leaf-to-air water vapour mole fraction difference, ψ_{soil} is soil water potential, and K is the plant hydraulic conductance. Note that Δw is determined by both atmospheric vapour pressure and leaf temperature, and the latter is influenced by evaporative cooling and hence by g_s. The other constraint is the fact that g_s is metabolically regulated in relation to leaf water status (Buckley, 2019; McAdam & Brodribb, 2016; Sack et al., 2018); for simplicity, we represent that constraint here and in Figure 1 by a linear response of g_s to turgor: $g_s = \alpha \cdot (\psi_{\text{leaf}} + \pi_{\text{leaf}})$, where α represents the sensitivity of g_s to leaf turgor and π_{leaf} is bulk leaf osmotic pressure. The operating point—the actual values of g_s and ψ_{leaf} under given conditions—is the intersection of these two constraints (Figure 1a). Thus, net changes in g_s during soil water deficit result from four types of underlying changes: (i) direct effects of ψ_{soil} decline itself (Figure 1b), (ii) amplification of those direct effects by decline in K or increase in Δw (hydraulic or thermal amplification; Figure 1c), (iii) effects of osmotic adjustment on leaf turgor (Figure 1d), and (iv) a decline in g_s for a given leaf turgor, represented here by decline in α (Figure 1e). We describe pattern (iv) as "stomatal downregulation," by analogy to "photosynthetic downregulation" under elevated CO_2 (i.e., downwards acclimation in photosynthetic capacity).

Predominance of stomatal downregulation during drought leads to "isohydric" behaviour—strong decline in g_s and little change in ψ_{leaf}—whereas strong osmotic adjustment could lead to maximally "anisohydric" behaviour—little change in g_s and strong decline in ψ_{leaf}. Amplification of stomatal closure, either by hydraulic conductivity loss (K decline) or by temperature increase (Δw increase), would cause coordinated declines in both g_s and ψ_{leaf} (Figure 1f). Thus, in a drought-insensitive ideotype, g_s would be sustained in drought through some combination of osmotic adjustment, minimal stomatal downregulation, minimal hydraulic decline (low hydraulic vulnerability), and minimal Δw increase (little increase in leaf temperature).

Our objective was to quantify variation in these physiological trajectories of drought response in *Phaseolus* bean plants. Our overarching question was, how do stomatal conductance, its response to post-flowering drought, and the underlying biophysical controls vary across diverse genotypes of common bean (*Phaseolus vulgaris*) and tepary bean (*P. acutifolius*)? Common bean is widely grown in the United States, the Americas and Africa, often in drought conditions. Drought resilience varies greatly across diverse genotypes of wild and domesticated common bean (Beebe et al., 2008, 2013; Cortés & Blair, 2018; Berny Mier y Teran et al., 2019). However, the physiological basis of that variation is poorly understood, with little past focus on genetic variation in photosynthesis and water relations. Although several physiological traits have been shown to influence yield under drought in a few genotypes of common bean, we do not know how such traits vary across diverse germplasm, nor how they interact to explain observed variation in yield resilience—as is the case with many crops. Breeding for drought resilience could thus be better informed by physiological understanding. Examination of the ecophysiology of drought responses in tepary beans may also provide useful understanding, as this species is known to be highly drought resilient, exhibiting less stomatal closure than common beans (Medina et al., 2017; Mwale et al., 2020). Tepary bean traits can be introgressed into common bean using embryo rescue, congruity backcrossing, or interspecific bridging lines (Barrera et al., 2022).

We explored patterns of change in g_s and ψ_{leaf}, and the underlying physiological controls, in 12 genotypes of *P. vulgaris*, including the eight parents of a Multi-parent Advance Generation Intercross (MAGIC) population described in Berny Mier y Teran (2018), as well as four genotypes of *P. acutifolius* (Table 1), grown in the field under conditions of full irrigation (control) and drought (irrigation withheld after flowering, often called "terminal drought" in agronomic

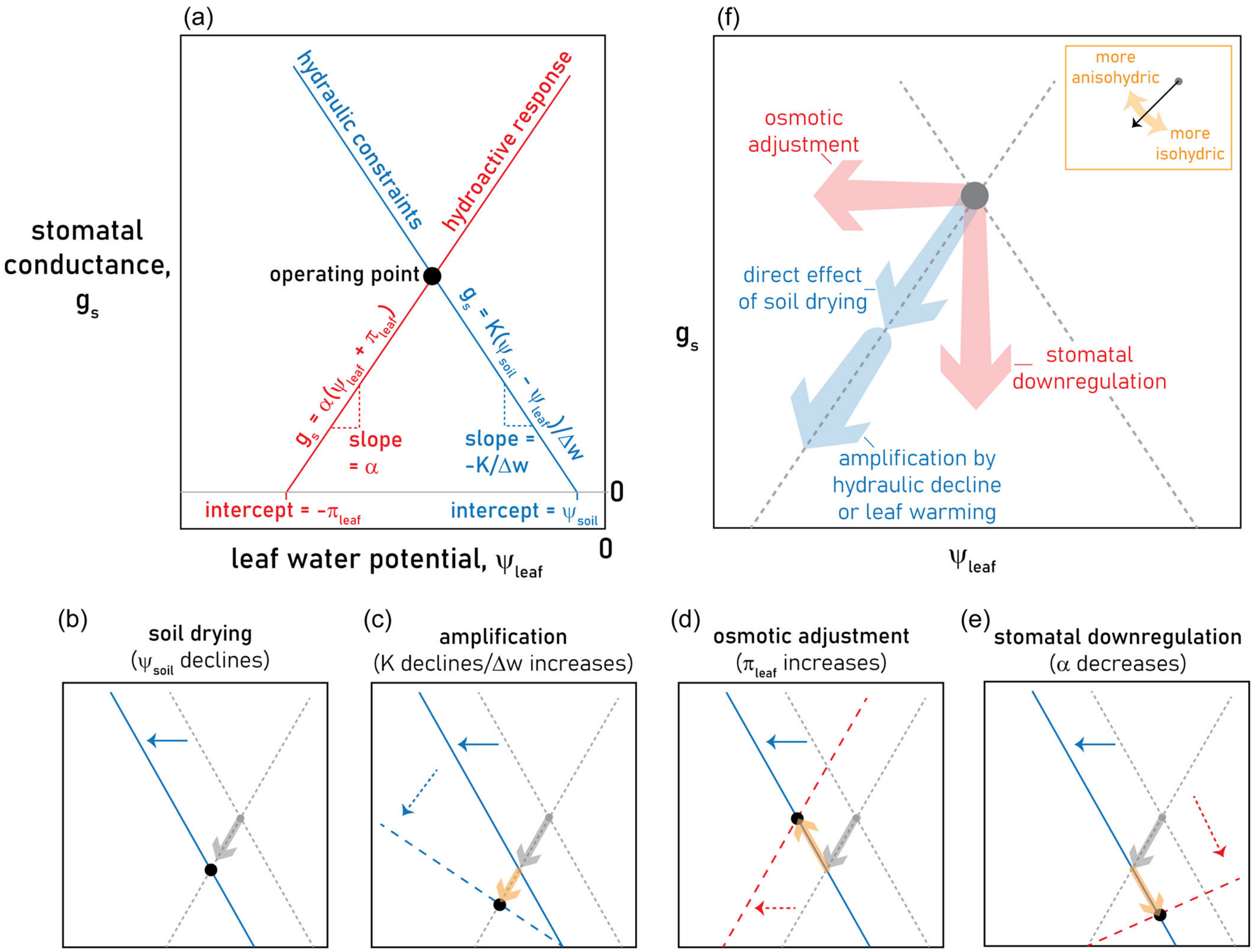

FIGURE 1 Changes in stomatal conductance (g_s) and leaf water potential (ψ_{leaf}) in response to soil drying can be understood as the net result of several distinct influences, each of which can be visualised using the heuristic shown here. The operating point, that is, the realised stomatal conductance and leaf water potential, is defined by the intersection of two relationships that constrain g_s and ψ_{leaf} with respect to one another (a). One, shown by blue lines in (a–e), is the requirement for transpiration rate ($g_s \Delta w$) to equal the rate of water flow into the leaf ($K(\psi_{soil} - \psi_{leaf})$) at steady-state ($\Delta w$ is the leaf to air water vapour mole fraction difference, ψ_{soil} is soil water potential, and K is whole plant hydraulic conductance on a leaf area basis). This leads to one equation for stomatal conductance: $g_s = K(\psi_{soil} - \psi_{leaf})/\Delta w$. The other constraint is the "hydroactive response", that is, active regulation of stomatal conductance in relation to leaf water content. The simplest way to illustrate this regulation is to assume g_s is proportional to leaf turgor ($g_s = \alpha(\psi_{leaf} + \pi_{leaf})$, where α is a proportionality coefficient representing the sensitivity of metabolic regulation of g_s in relation to leaf turgor, and π_{leaf} is leaf osmotic pressure); in reality the relationship may not be linear, and may include an offset such that stomata close before or after turgor loss, but the linear relationship suffices to illustrate the main points here. The effect of a change in any of the underlying controls (ψ_{soil}, K, Δw, π_{leaf}, or α) can be visualised graphically as shown here. For example, changes in ψ_{soil} or the ratio $K/\Delta w$ change the x-intercept (b) and slope (c), respectively, of the hydraulic constraint, and changes in π_{leaf} and α change the x-intercept (d) and slope (e), respectively, of the hydroactive constraint. Limiting cases for the patterns of change in g_s and ψ_{leaf} (f) correspond to dominance of one or more of these particular controls. The inset in (f) indicates how trajectories of g_s and ψ_{leaf} decline map onto the continuum between "isohydric" and "anisohydric" behaviour.

research). We tested three hypotheses: (1) that common beans would exhibit stronger stomatal closure in drought than tepary beans, resulting in a more nearly isohydric pattern; and that genotypes displaying greater ψ_{leaf} decline (more anisohydric genotypes) would (2) exhibit smaller increases in evaporative demand, because their tendency to keep stomata more open would sustain evaporative cooling by transpiration, but (3) would experience larger declines in hydraulic conductance due to their greater drop in water potential.

2 | METHODS

2.1 | Plant material

We examined 12 genotypes of common bean (*P. vulgaris*) including eight parents of a MAGIC population (Table 1) and four other randomly selected progeny genotypes from that population. The MAGIC population consisted of eight Mesoamerican landraces selected from

TABLE 1 List of genotypes used in this study: parent genotypes of the common bean (*Phaseolus vulgaris*) MAGIC population, tepary beans (*P. acutifolius*), and members of the MAGIC population other than the parents.

Group	Name (source)	Ecogeographic race, seed type	Area of cultivation	Reason for selection	Biotic resistances	Growth habit
MAGIC parents	SEA 5 (CIAT)	Mesoamerica, Cream	Tropical lowlands	Deep rooting, low stomatal conductance	Charcoal root rot, Fusarium root rot, BCMV (I)	III
	Victor (USDA ARS-WSU)	Durango, Pink	Great Plains	High-yielding and stable in BeanCAP drought trials	BCMV, Curly top virus, Fusarium root rot	II
	L88-63 (MSU)	Mesoamerica, Small black	Tropical lowlands	High-yielding in tropics & Michigan, deep rooting	Charcoal root rot	II
	Pinto San Rafael (INIFAP)	Durango, Pinto	Dry highlands	High-yielding		III
	Flor de Mayo Eugenia (INIFAP)	Jalisco, Flor de Mayo	Humid highlands	High-yielding, high stomatal conductance	Rust, anthracnosis	III
	SER 118 (CIAT)	Mesoamerica, Small red	Tropical lowlands	Yield production efficient		II
	Matterhorn (MSU)	Durango, Great Northern	Great Plains	High-yielding and stable in BeanCAP drought trials	BCMV (I), rust (Ur-3)	II
	UCD 9634 (UC Davis)	Durango, Pink	California	High-yielding in California trials	BCMV (I)	II
Tepary beans	Tepary Big Fields[a]	White	Arizona	Heat and drought		III
	G40068	Yellow	Arizona	Heat and drought		III
	G40159	White	Sonora, Mexico	Heat and drought		III
	TEP 22[b]	White	Puerto Rico	Heat and drought	Bacterial blight, seed weevils	III
	Genotype name	**Cytoplasm donor**[c]				
MAGIC crosses	M4.66	SER118				
	M7.87	L88-63				
	M6.77	Victor				
	M3.94	Flor de Mayo Eugenia				

Note: Growth habit: II = indeterminate bush, III = indeterminate prostrate.

Abbreviations: CIAT, Centro Internacional de Agricultura Tropical; INIFAP, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias; MAGIC, Multi-parent Advance Generation Intercross; MSU, Michigan State University; UCD, UC Davis; USDA-ARS-WSU, USDA-ARS at Washington State University.

[a] Tepary Big Fields was sourced from https://www.nativeseeds.org/products/pt109.

[b] Registration of TEP 22 is given by Porch et al. (2013).

[c] The MAGIC crosses are crosses of all eight parents, differing in the cytoplasm donor.

a broad range of environmental conditions (Berny Mier y Teran, 2018). We also examined four landraces of tepary bean (*P. acutifolius*) selected based on prior evidence that seed yield in these genotypes is highly resilient to post-flowering drought, and on their origin in the desert southwest US and northwestern Mexico (Mwale et al., 2020, 2022; Nabhan & Felger, 1978; Pratt & Nabhan, 1988; Rao et al., 2013).

The 16 genotypes examined here were among 320 genotypes planted together in the field; for the sake of replicability and context, details of the broader field configuration are given in Supporting Information: Methods S1. The drought and control treatments were conducted in adjacent regions of the field, separated by a road 12 m wide. In both treatments, subsurface (50 cm depth) driplines were centred beneath each planting bed and water was supplied daily. Additionally, pairs of surface driplines (each positioned 5 cm from a planting row, closer to the centre of the plot) were used in each bed to augment irrigation during establishment (1–31 days after sowing, DAS). Drought was imposed by ceasing irrigation at 52 DAS (26 July 2021), when 36% of the plots had begun flowering. No rain occurred at the site between 25 May and 22 October 2021.

2.2 | Measurement of stomatal conductance and water potential

We conducted physiological measurements in three 5-day campaigns: once before (31–35 DAS) and twice after flowering and imposition of drought (70–74 and 84–88 DAS). In each campaign, we measured stomatal conductance using a porometer (AP4, Delta-T Instruments) on the abaxial surface of one leaflet from each of 5–8 leaves per plot for each genotype listed in Table 1. We repeated these measurements four times daily, beginning at 0800, 1000, 1200 and 1400 h (US Pacific Daylight Time). In most cases, each measurement cycle took approximately 45 min. We recalibrated the porometer before each cycle.

In each campaign, we also measured pre-dawn and mid-day leaf water potentials using a Scholander pressure chamber with a digital pressure gauge (model 1505D; PMS Instruments). We collected two leaves per plot for each genotype using sharp secateurs at approximately 0540–0600 h (pre-dawn) and 1300–1320 h (mid-day). Each leaf was immediately enclosed in a plastic bag that had been exhaled into to minimise transpiration after excision, and the bag was quickly but gently appressed to remove excess air, sealed, and placed in a cooler containing ice. After collection, we returned leaves to the laboratory (~5 min) and measured their water potentials using continuous pressurisation at a rate of approximately 0.01 MPa s^{-1}. We estimated ψ_{soil} as predawn leaf water potential. This assumes that water flow within the plant at pre-dawn was negligible, which in turn assumes transpiration rate and capacitive recharge of water stores in the plant were both negligible. Transpiration was likely negligible given the occurrence of mild dewfall at predawn during all campaigns and both treatments, and capacitive flows at dawn were likely negligible given the small stature of bean plants.

As a further check on the effect of withholding irrigation on soil moisture, we measured soil water content in both treatments at a series of depths in the soil (20, 50, 80, 100, 120 and 140 cm) using a neutron backscatter detector soil moisture probe (InstroTek Inc., CPN 503 ELITE Hydroprobe). Steel access tubes (152.4 cm long, 5.25 cm inner diameter, 5.58 cm outer diameter; part number 100400411; Home Depot) plugged at the lower end with plastic bungs were installed in vertical holes cored in the centre of each plot before planting, and covered with aluminium beer cans to prevent rain intrusion between measurements. Measurements were made in conjunction with each physiological measurement campaign (at 31, 77 and 88 DAS, between 1000 h and 1300 h). At the start of each sampling day, a standard count was measured at 1 m above the ground. Results are presented as uncalibrated count ratios (soil count/standard count), where higher ratios indicate higher soil moisture content.

2.3 | Canopy temperature and leaf-to-air vapour gradient

On the second day of each measurement campaign (32, 71 and 85 DAS), we measured thermal infra-red emission from each plot using a Zenmuse XT-radiometric thermal camera (640 × 512 px, 9 mm lens; DJI) on a DJI Matrice 100 drone. We inferred canopy temperature from the resulting images as (TIF VALUE)·0.04–273.15. Extraction of canopy data were conducted in QGIS v3.10 according to the methods of Parker et al. (2020). To calculate Δw (leaf to air water vapour mole fraction gradient), we estimated the vapour pressure inside the leaf as saturation vapour pressure (in Pa, calculated from canopy temperature T, in °C, as $611.2 \cdot \exp[17.62 \cdot T/(243.12 + T)]$), subtracted the ambient vapour pressure at the time of the drone flight (measured by a CIMIS station adjacent to the field [38.5358° N, −121.7764° E]; https://cimis.water.ca.gov/, last accessed 05 February 2024), and divided the resulting vapour pressure difference by atmospheric pressure to give a mole fraction (mol mol^{-1}). Values presented in figures were multiplied by 1000 to give units of mmol mol^{-1}.

2.4 | Plant hydraulic conductance

We calculated whole-plant hydraulic conductance on a leaf area basis (K, mmol m^{-2} s^{-1} MPa^{-1}) for each plot by multiplying mean stomatal conductance (g_s, mmol m^{-2} s^{-1}, measured for each plot by porometry as described earlier) at each of 1200 h and 1400 h by Δw (mol mol^{-1}) to give transpiration rate, and dividing that value by the difference between predawn and midday leaf water potentials (MPa): $K = g_s \cdot \Delta w/(\psi_{soil} - \psi_{leaf})$. This assumes leaf boundary layer resistance is negligible compared to stomatal resistance, soil water potential at midday is similar to leaf water potential at pre-dawn, and transpiration and water transport through the plant are in steady-state. We did not attempt to quantify adaxial stomatal conductance, so our estimates of K should only be considered proportional to the true values that would be obtained had we measured whole-leaf g_s; thus, we do not draw any conclusions from absolute differences in K itself across genotypes, but only from changes in K in drought within each genotype. As K is calculated from g_s, these two variables are statistically conflated, so we also did not draw inferences from correlations between K and g_s.

2.5 | Osmotic pressure

Samples were collected in 2021 for osmotic pressure measurement but were subsequently destroyed by a freezer failure. We replanted the eight MAGIC parent genotypes in 2022, following procedures mostly as described earlier, but in a different field several hundred metres west of the 2021 field. For each plot, we collected two leaves at pre-dawn and returned them to the lab as described earlier. We then rehydrated these leaves by recutting their petioles under water and leaving the petioles in falcon tubes filled with water for 24–36 h in a dark room. We removed approximately 4 cm^2 of tissue from the middle leaflet of each rehydrated leaf, avoiding the midrib, sealed it in a Whirl-Pak bag, dipped the bag in liquid nitrogen to eliminate turgor pressure by fracturing cell membranes, and stored the bags at −80°C. For measurement, each sample was placed in a 0.6 mL microcentrifuge tube with a 1.6 mm hole drilled into the end, which itself was placed into a 2 mL microcentrifuge tube and centrifuged at 15,000 rpm for approximately 2 min to express sap from the leaf tissue. Expressed sap was kept frozen at −20°C until measurement, at which time it was thawed and then transferred using a pipettor to a filter-paper disk in the sample well of a Wescor Vapro vapour pressure osmometer (Model 5600; ELITechGroup Inc.). The osmometer was recalibrated every 1–3 h using standards (100, 290 and 1000 mmol kg^{-1} osmolality) provided by the manufacturer.

2.6 | Statistical analysis

For most measured quantities, we tested and quantified differences between control (irrigated) and drought treatments by analysis of variance in linear mixed models fitted using *lmer*() in R (package lme4) for all data combined for both post-drought campaigns, with genotype × treatment as a fixed effect and plot nested within campaign (second or third) as a random effect. For stomatal conductance, we also included cycle (measurement cycle, i.e., time of day: 0800, 1000, 1200 or 1400 h) as a fixed effect. For Δw and K, there was no replication within plots (because Δw was estimated using plot-level average canopy temperature measured by drone, and estimation of K depended on Δw), so the only random effect was campaign. We made two estimates for K in each plot (based on g_s measured at 1200 h and 1400 h, because leaves were collected at approximately 1300 h for midday water potential), so we also included cycle (1200 h or 1400 h) as a fixed effect in the model for K. We assessed the magnitude and significance of drought vs control differences within genotypes using the function *emmeans*() (package emmeans); within-treatment values, control-drought differences, and standard errors reported for each variable are estimated marginal means and their standard errors reported by *emmeans*().

2.7 | Partitioning ψ_{leaf} decline into contributions from changes in ψ_{soil}, g_s, Δw and K

At steady state, transpiration rate ($\approx g_s\Delta w$) is equal to the rate of water flow from the soil to the leaf ($K[\psi_{\text{soil}} - \psi_{\text{leaf}}]$), giving ψ_{leaf} as

$$\psi_{\text{leaf}} = \psi_{\text{soil}} - \frac{g_s\Delta w}{K}, \tag{1}$$

so the change in ψ_{leaf} under post-flowering drought, as compared to the control, is

$$\delta\psi_{\text{leaf}} = \delta\psi_{\text{soil}} - \delta\left(\frac{g_s\Delta w}{K}\right), \tag{2}$$

where δ denotes a difference between drought and control conditions. Thus, ψ_{leaf} declines due to a combination of effects of changes in ψ_{soil}, g_s, Δw, and K. To quantify the effects of changes in each of these variables, the third term in Equation (2) can be broken into components attributable to changes in g_s, Δw and K using calculus:

$$\delta\left(\frac{g_s\Delta w}{K}\right) = \int_c^d d\left(\frac{g_s\Delta w}{K}\right) = \int_c^d \frac{\Delta w}{K} dg_s + \int_c^d \frac{g_s}{K} d\Delta w - \int_c^d \frac{g_s\Delta w}{K^2} dK, \tag{3}$$

where c and d denote control and drought conditions, respectively. Each integrand on the right-hand side of Equation (3) can be estimated by its average between treatments:

$$\delta\left(\frac{g_s\Delta w}{K}\right) \approx \left\langle\frac{\Delta w}{K}\right\rangle \int_c^d dg_s + \left\langle\frac{g_s}{K}\right\rangle \int_c^d d\Delta w - \left\langle\frac{g_s\Delta w}{K^2}\right\rangle \int_c^d dK = \left\langle\frac{\Delta w}{K}\right\rangle \delta g_s + \left\langle\frac{g_s}{K}\right\rangle \delta\Delta w - \left\langle\frac{g_s\Delta w}{K^2}\right\rangle \delta K, \tag{4}$$

where angled brackets denote averages between control and drought conditions. The contributions (C) of changes in ψ_{soil}, g_s, Δw and K to the total decline in ψ_{leaf} are then given, respectively, by

$$\begin{aligned} C(\psi_{\text{soil}}) &\equiv \delta\psi_{soil}, \quad C(g_s) \equiv -\left\langle\frac{\Delta w}{K}\right\rangle \delta g_s, \\ C(\Delta w) &\equiv -\left\langle\frac{g_s}{K}\right\rangle \delta\Delta w, \quad C(K) \equiv \left\langle\frac{g_s\Delta w}{K^2}\right\rangle \delta K. \end{aligned} \tag{5}$$

(The last three terms are reversed in sign from Equation (4) because the effect of $g_s\Delta w/K$ on ψ_{leaf} is negative; cf. Equation (2)). We applied these calculations using within-treatment marginal means for each variable, estimated as described earlier under Section 2.6.

3 | RESULTS

3.1 | Changes in water potential in response to post-flowering drought

Cessation of irrigation resulted in a significant decline in soil water content (Supporting Information: Figure S1) and soil water potential (ψ_{soil}, estimated as predawn leaf water potential) ($F(1,144) = 75.77$, $p < 0.0001$), which itself differed significantly across genotypes ($F(15,144) = 2.64$, $p = 0.0015$), ranging from insignificant change (+0.02 ± 0.05 MPa; SEA 5; $p = 0.68$) to a decrease of 0.32 ± 0.07 MPa (TEP 22; $p < 0.0001$) (Figure 2a,c). Midday leaf water potential (ψ_{leaf}) also declined significantly ($F(1,144) = 41.19$, $p < 0.0001$), and the decline differed significantly across genotypes ($F(15,144) = 2.53$, $p = 0.0023$) (Figure 2b,d), ranging from insignificant change under drought (+0.01 ± 0.10 MPa; Flor de Mayo Eugenia, $p = 0.89$) to a decrease of 0.54 ± 0.12 MPa (Tepary Big Fields, $p < 0.0001$). Notably, the decline in ψ_{leaf} was larger than that in ψ_{soil} in most cases (cf. Figure 2c,d).

3.2 | Changes in stomatal conductance under post-flowering drought

Stomatal conductance (g_s) differed significantly across genotypes ($F(14,147) = 1.88$, $p = 0.03$) and measurement cycles ($F(1,4221) = 51.94$, $p < 0.0001$), and decreased during post-flowering drought ($F(1,147) = 304.4$, $p < 0.0001$) (Figure 3a). Moreover, the degree of g_s decline in drought differed significantly across genotypes ($F(15,147) = 2.21$, $p = 0.0083$) (Figure 3b), ranging from 101 ± 63 mmol $\text{m}^{-2}\,\text{s}^{-1}$ (G40068) to 438 ± 51 mmol $\text{m}^{-2}\,\text{s}^{-1}$ (M4.66).

3.3 | Joint trajectories of stomatal conductance and water potential

Although g_s and midday ψ_{leaf} both systematically declined, on average, their joint patterns of change under post-flowering drought differed notably across genotypes and species (Figure 4). Six of the common bean genotypes (Victor, Pinto San Rafael, SER 118, Matterhorn, UCD 9634 and M6.77) exhibited archetypal "anisohydric" behaviour, in which both g_s and ψ_{leaf} decline substantially in response to drought (Figure 4a). By contrast, the other six common beans (SEA 5, L88-63, Flor de Mayo Eugenia, M4.66, M7.87 and M3.94) exhibited "isohydric" behaviour, in which ψ_{leaf} remains nearly unchanged under drought, as compared to a continuously irrigated control (Figure 4b). Among four tepary bean genotypes, three were clearly anisohydric, whereas both g_s and ψ_{leaf} were nearly insensitive to drought in the fourth genotype, G40068 (Figure 4c).

3.4 | Hydraulic and thermal amplification of ψ_{leaf} and g_s decline

All of the anisohydric common beans and two of the tepary beans showed clear amplification of declines in both ψ_{leaf} and g_s. That is, ψ_{leaf} declined substantially more than soil water potential, which means that the depression of ψ_{leaf} below soil water potential by transpiration ($\psi_{\text{soil}} - \psi_{\text{leaf}} = g_s{\cdot}K/\Delta w$) was greater under drought, even though g_s itself had declined. This in turn means that the ratio of hydraulic conductance (K) to evaporative demand (Δw) must have increased, due either to hydraulic decline in response to leaf dehydration, leaf warming, or both (Figure 5). The thin lines in Figure 4 show the actual joint trajectories of g_s and ψ_{leaf}—that is, how g_s and ψ_{leaf} actually changed—whereas the thick semi-transparent lines overlaid on the thin lines show how g_s and ψ_{leaf} *would have declined* in the absence of amplification—that is, if ψ_{leaf} had declined only, and exactly, as much as ψ_{soil}, and assuming a unique linear relationship between g_s and ψ_{leaf}. Thus, the extent to which the thin lines extend beyond the thick lines is a visual measure of the magnitude of hydraulic or thermal amplification of the declines of ψ_{leaf} and g_s caused by soil drying. Expressed as a percentage, amplification ranged from 21% (TEP 22) to 279% (SER 118) among the anisohydric genotypes.

3.5 | Contributions of changes in underlying variables to total declines in leaf water potential

The total declines in ψ_{leaf} reported earlier resulted from several distinct effects, of which three tended to reduce ψ_{leaf} (namely, decline in ψ_{soil} itself, increase in Δw, and decline in K), and one—stomatal closure—tended to increase ψ_{leaf}, that is, counteract the declines in ψ_{leaf} caused by changes in ψ_{soil}, Δw and K. The magnitude of these four effects differed between the three groups of bean genotypes described earlier (Figure 6). In all groups, the largest single effect was that of stomatal closure, and the second-largest was thermal amplification (increase in Δw). The four effects roughly cancelled each other out in the isohydric common beans, leaving ψ_{leaf} nearly unchanged under drought (hence the description of these genotypes as "isohydric"). In the anisohydric common beans, the effects of changes in ψ_{soil}, K, and Δw were larger than in the isohydric beans (by 1.4–2.7-fold), leading to a substantial overall decline in ψ_{leaf}. Notably, this occurred despite a larger positive effect of stomatal closure on ψ_{leaf} in the anisohydric beans as compared to the isohydric beans.

The pattern in tepary beans was similar to that in the anisohydric common beans, except that the direct effect of ψ_{soil} itself was greater and each of the other effects were smaller. Due to the small number of tepary bean genotypes examined, however, these differences were not statistically significant.

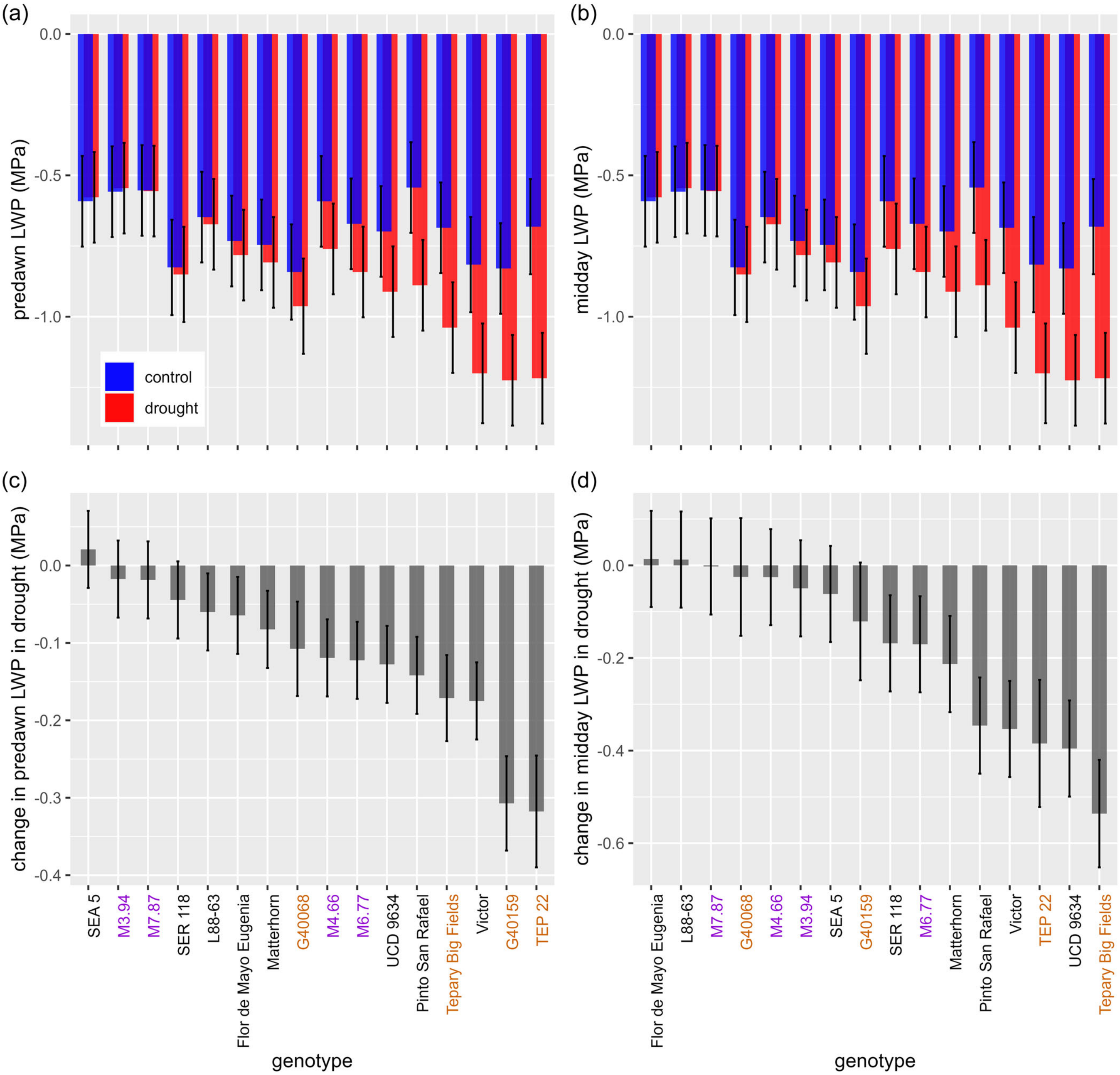

FIGURE 2 Soil water potential (a,c; estimated as predawn leaf water potential) and midday leaf water potential (b and d) declined in response to post-flowering drought (red bars) as compared to irrigated control (blue bars) in 12 genotypes of common bean and four genotypes of tepary bean. Note that the *y*-axis ranges differ for each plot. Genotypes in (a) and (b) are ordered by differences shown in (c) and (d). Genotype names are coloured by species/origin (black: common bean MAGIC parents; violet: MAGIC crosses; orange: tepary bean). Values shown are marginal means in each treatment (a, b) and their differences between treatments (c and d), and error bars are SEs of these values, estimated from linear mixed models as described in Section 2. MAGIC, Multi-parent Advance Generation Intercross. [Color figure can be viewed at wileyonlinelibrary.com]

3.6 | Osmotic adjustment in common bean MAGIC parents

Osmotic pressure at full turgor (π_{ft}), measured in the eight common bean MAGIC population parents in a separate field experiment in 2022, differed among genotypes, but with marginal significance (*F* (7,48.3) = 2.21, *p* = 0.05) (Supporting Information: Figure S2a). π_{ft} increased in response to post-flowering drought (Supporting Information: Figure S2b), but also with marginal significance (*F* (1,48.3) = 4.65, *p* = 0.036), and post hoc analysis found the change was not statistically significant within any given genotype (*p* = 0.10 to 0.85). Repeating the analysis with genotype as a random effect gave a

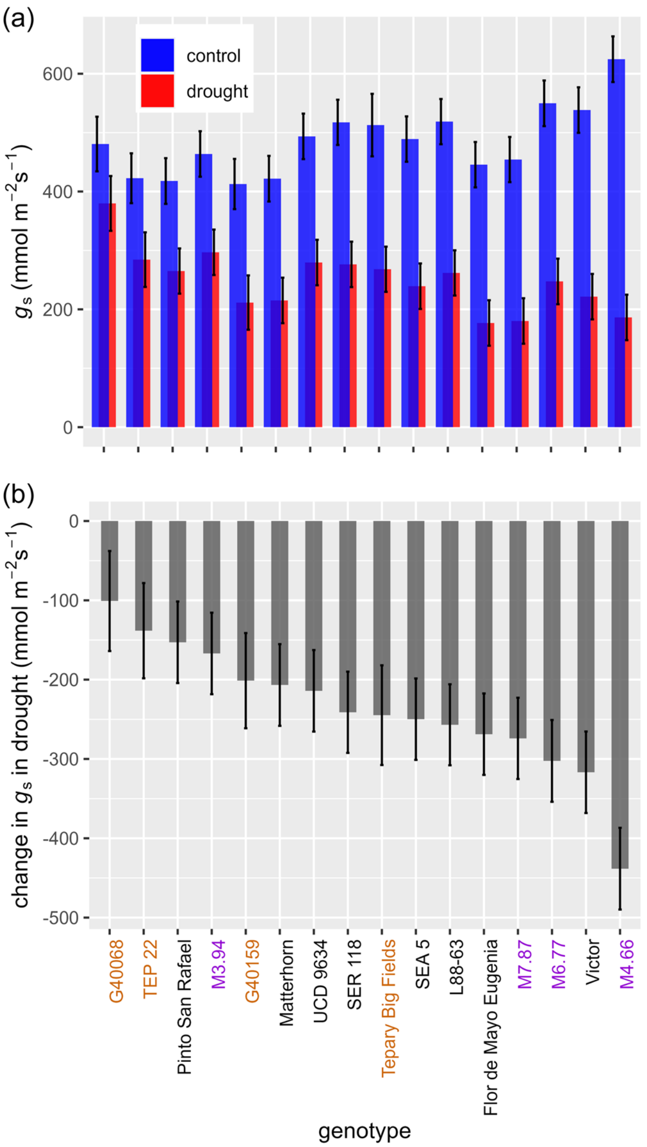

FIGURE 3 Stomatal conductance (g_s) declined in response to post-flowering drought (a), and the magnitude of decline differed nearly five-fold across genotypes (b). Genotypes in (a) are ordered by differences shown in (b). Genotype names are coloured by species/origin (black: common bean MAGIC parents; violet: MAGIC crosses; orange: tepary bean). Values shown are marginal means in each treatment (a) and their differences between treatments (b), and error bars are SEs of these values, estimated from linear mixed models as described in Section 2. MAGIC, Multi-parent Advance Generation Intercross. [Color figure can be viewed at wileyonlinelibrary.com]

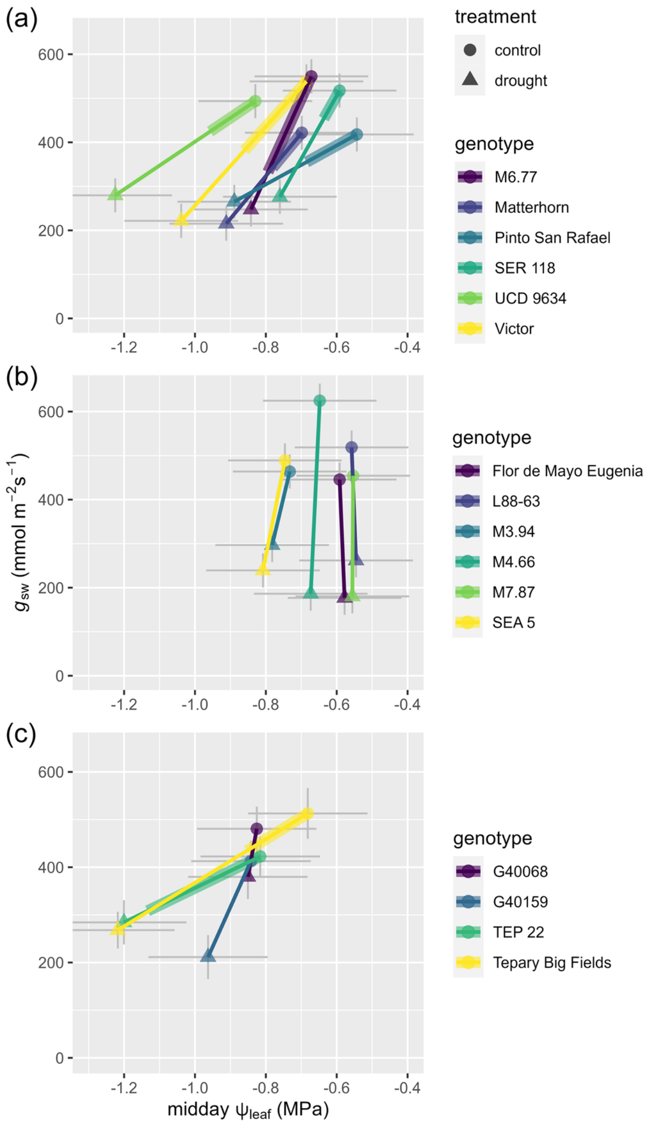

FIGURE 4 Genotypes and species differed in the pattern of concurrent changes in stomatal conductance and midday leaf water potential. Six of the common bean genotypes exhibited archetypal "anisohydric" behaviour (panel a), in which both g_s and ψ_{leaf} declined substantially in response to post-flowering drought, whereas the other six common beans (panel b) exhibited archetypal "isohydric" behaviour, in which g_s declines greatly and ψ_{leaf} remains nearly constant in drought. Among four tepary bean genotypes (panel c), three were clearly anisohydric and the fourth exhibited very small changes in both g_s and ψ_{leaf}. Moreover, all of the anisohydric common beans and two of the tepary beans showed clear amplification of ψ_{leaf} and g_s declines; that is, ψ_{leaf} declined more than soil water potential declined, due to some combination of decreased hydraulic conductance (i.e., hydraulic amplification) and increased leaf temperature (hence greater evaporative demand; i.e., thermal amplification). The thick semi-transparent lines show how g_s and ψ_{leaf} would have declined if ψ_{leaf} had declined only as much as soil water potential; thus, the extent to which the thin lines extend beyond the thick lines is a visual measure of the degree of hydraulic and/or thermal amplification of g_s and ψ_{leaf} decline. [Color figure can be viewed at wileyonlinelibrary.com]

similar result, indicating osmotic adjustment that was statistically significant ($F(1,55.2) = 4.87$, $p = 0.032$) but marginal (an increase of 0.07 ± 0.03 MPa, from 1.12 ± 0.03 MPa in the irrigated control). This degree of osmotic adjustment was similar in magnitude to the decline in ψ_{soil} in the same eight genotypes in the 2021 experiment (0.08 ± 0.02 MPa).

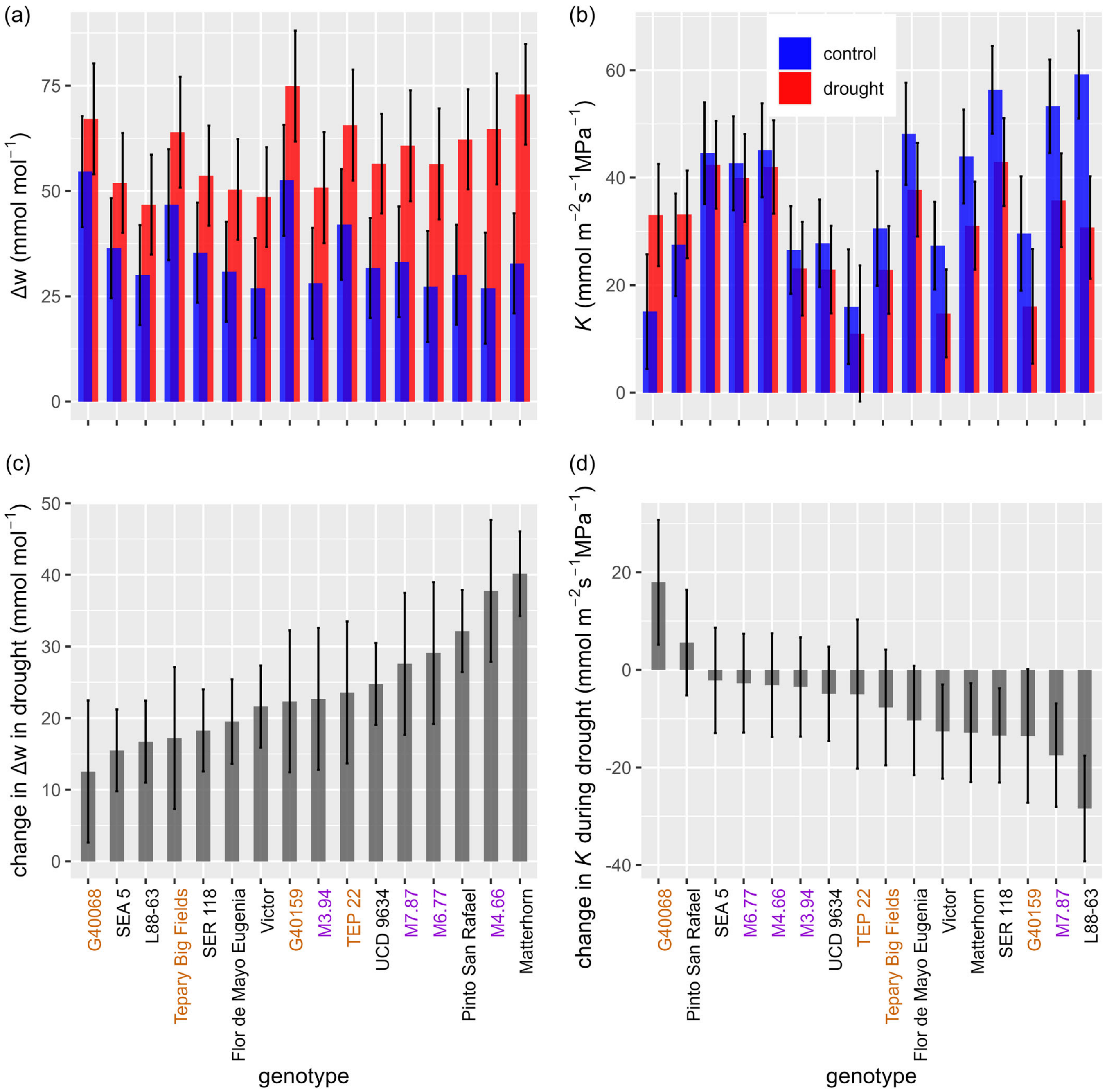

FIGURE 5 Genotype and species variation in (a) evaporative demand (Δ*w*, the leaf-to-air water vapour gradient expressed as a mole fraction) and (b) plant hydraulic conductance (*K*) in control and drought treatment, and (c) the increase in Δ*w*, or (d) the decrease in *K*, under post-flowering drought as compared to irrigated control. Genotypes in (a) and (b) are ordered by differences shown in (c) and (d). Genotype names are coloured by species/origin (black: common bean MAGIC parents; violet: MAGIC crosses; orange: tepary bean). Values shown are marginal means in each treatment (a and b) and their differences between treatments (c and d), and error bars are SEs of these values, estimated from linear mixed models as described in Section 2. MAGIC, Multi-parent Advance Generation Intercross. [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Our measurements of stomatal responses to post-flowering drought in common and tepary beans, and of the underlying biophysical controls, revealed divergent patterns among groups of genotypes, in relation to the archetypal patterns laid out in our drought-response heuristic (Figure 1). Six common bean genotypes closely matched the archetypal "isohydric" pattern, with minimal decline of ψ_{leaf} but substantial decline in g_s, while the other six genotypes exhibited anisohydric behaviour, with significant decline of ψ_{leaf}. We also found a range of patterns among four tepary bean genotypes. However, these patterns and their relation to one another did not conform to

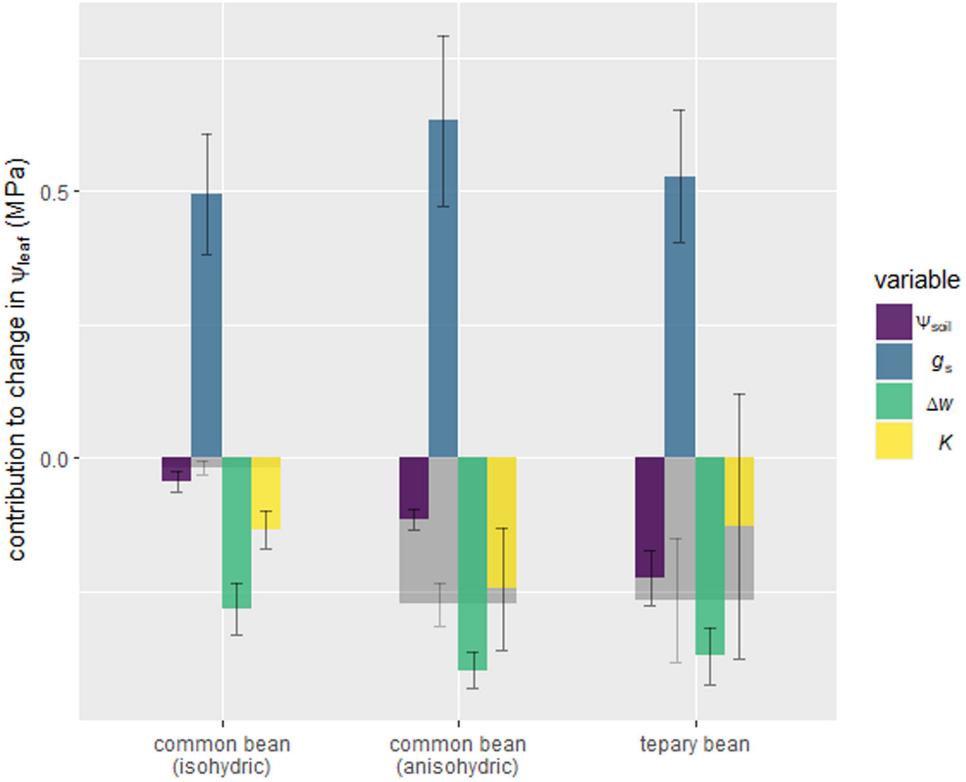

FIGURE 6 The decline in leaf water potential (ψ_{leaf}) under post-flowering drought was the net effect of four influences, namely changes in soil water potential (ψ_{soil}), stomatal conductance (g_s), evaporative demand (Δw) and hydraulic conductance (K). The semitransparent grey bars in the background indicate the total decline in ψ_{leaf}, for reference. For example, the increase in Δw in isohydric common beans would, by itself, have reduced ψ_{leaf} by over 0.25 MPa, but this effect was offset by partial stomatal closure, which reduced transpiration rate and would by itself have *increased* ψ_{leaf} by about 0.5 MPa. "*Isohydric*" common beans refers to genotypes SEA 5, L88-63, Flor de Mayo Eugenia, M4.66, M7.87 and M3.94; "*anisohydric*" refers to genotypes Victor, Pinto San Rafael, SER 118, Matterhorn, UCD 9634 and M6.77. Means and SEs shown are within each genotype group. [Color figure can be viewed at wileyonlinelibrary.com]

our hypotheses. Common beans were more isohydric than tepary beans on average (Hypothesis #1), but this description masked a bimodal distribution of isohydricity among the common beans, in that half of the genotypes were strongly anisohydric. Similarly, Hypothesis #2, that stomatal closure would be greater in isohydric than anisohydric genotypes, held up well when comparing isohydric common beans to tepary beans (in which g_s declined by 55 ± 5 and 38 ± 7%, respectively), but poorly when comparing isohydric to anisohydric common beans (g_s declined by 48 ± 3% in the latter); that is, in common beans, the anisohydric pattern did not arise chiefly from lesser stomatal closure. Our third hypothesis also received mixed support: although we did observe substantial decline in hydraulic conductance under drought in most genotypes, and the degree of decline was greater in anisohydric than isohydric common beans, it was similar between isohydric common beans and (anisohydric) tepary beans. Our results thus paint a more subtle and nuanced picture about the ecophysiology of leaf response to post-flowering drought in beans than our hypotheses had predicted, while supporting the contention that iso- and anisohydry can arise from a range of diverse and interacting mechanisms (Martínez-Vilalta & Garcia-Forner, 2017).

We found several results particularly surprising. For one, although stomatal closure was similar between anisohydric and isohydric common beans and was lesser in tepary beans than isohydric common beans, our causal analysis revealed that stomatal closure was equally or more hydraulically consequential, in terms of countering the decline in ψ_{leaf} during drought, in the anisohydric and tepary beans than in the isohydric genotypes. For example, if stomatal closure had occurred in isolation, it would have increased ψ_{leaf} by a similar amount (about 0.5 MPa) in isohydric common beans and tepary beans, despite a much larger drop in g_s in the isohydric beans. This contradicts the idea that isohydric plants preserve leaf water status through strong stomatal closure, while anisohydric plants preserve stomatal opening by allowing ψ_{leaf} to decline. The contradiction arises because a third quantity—the ratio of evaporative demand to hydraulic conductance ($\Delta w/K$)—controls the sensitivity of ψ_{leaf} to g_s, and this quantity was larger in the tepary beans, due both to larger Δw and smaller K. We had expected to find precisely the converse—that is, tepary beans would be 'hydraulically overbuilt,' thus buffering them from effects of soil drying, while their sustained stomatal opening in drought would improve evaporative cooling, allowing them to experience lower Δw.

Another surprising, but illuminating, result was that the direct or passive effect of soil drying on leaf water potential was more strongly amplified by leaf warming (leading to increased Δ*w*) than by hydraulic decline (decreased *K*). The literature in water relations ecophysiology in recent decades has focused heavily on hydraulic amplification of ψ_{leaf} decline and stomatal closure (Albuquerque et al., 2020; Anderegg et al., 2018; Bartlett et al., 2016; Brodribb & Holbrook, 2006; Rodriguez-Dominguez et al., 2016; Saliendra et al., 1995; Scoffoni & Sack, 2017; Scoffoni et al., 2017, 2018; Wolf et al., 2016), and less so on thermal amplification, that is, the negative feedback of stomatal closure on itself, and on ψ_{leaf} decline, caused by increased Δ*w*. Our results suggest not only that reduced evaporative cooling due to stomatal closure can strongly amplify ψ_{leaf} decline by warming leaves, but also that this effect can be more important than hydraulic decline, and moreover it may vary significantly among genotypes of domesticated bean species. The increase in Δ*w* under drought varied widely across the 16 genotypes examined here, from 12 to 40 mmol mol^{-1} (or from < 20% to over 100%, expressed relative to the value under irrigation). Our measurements do not allow us to attribute this variation to any particular trait, but anecdotal observations suggest two traits could be particularly important: dynamic leaf orientation (we noticed, but did not formally measure, that some genotypes were much more strongly diaheliotropic than others) and trichome properties (which could reduce leaf temperature by increasing albedo, and/or by increasing boundary layer resistance, which would increase the cooling effect of transpiration by decoupling leaf temperature from air temperature; Ehleringer & Mooney, 1978). Future work should aim to quantify variation in these and other aspects of leaf structure and function that influence temperature, and thus could be harnessed as traits to reduce thermal amplification of ψ_{leaf} decline and stomatal closure.

The six isohydric bean genotypes exhibited, by definition, little decline in leaf water potential during drought. It is helpful to examine how they achieved this outcome physiologically. The decline in stomatal conductance during drought is caused by the effect on stomatal guard cells of abscisic acid (ABA), which is synthesised in drying leaves and/or roots (McAdam & Brodribb, 2016; McAdam et al., 2016). Yet, in some of the isohydric common beans, neither soil nor midday leaf water potential declined measurably during drought. That outcome could, in theory, arise if these genotypes had very deep roots that could continue accessing abundant water despite the lack of irrigation; however, that would preserve not only ψ_{leaf} but also g_s itself, whereas g_s declined sharply in these genotypes. Thus, the isohydric beans must have an extremely sensitive signalling response that engages under even mild declines in water status, causing sharp downregulation of stomatal conductance. Whatever the mechanism, it is incompatible with a drought-resilient ideotype, suggesting additional research contrasting ABA signalling and metabolism between these genotypes and their anisohydric relatives is needed.

None of the common beans completely matched the drought-resilient ideotype, in which g_s declines negligibly in drought while ψ_{leaf} declines strongly, but the anisohydric lines did differ in their proximity to the ideotype. One line in particular (Pinto San Rafael) had lower g_s under irrigation than any of the other common beans, and also had among the smallest declines in g_s under drought (Figure 3). Pinto San Rafael is a member of ecogeographic race Durango of common bean (Acosta Gallegos et al., 2016). These results are consistent with adaptation of this race to its original range in the semi-arid highlands of northern Mexico and the southwestern United States (Acosta-Díaz et al., 2009; Singh et al., 1991). The most extreme opposite of that pattern was in M4.66 (an eight-way cross of the MAGIC parents, with SER 118 being the cytoplasm donor), which had by far the highest g_s under irrigation (over 600 mmol m^{-2} s^{-1}) and the largest % decline in g_s (over 70%). One tepary bean genotype (G40068) stood out as being exceptionally resilient, with g_s dropping by just 20% under drought, consistent with the previous finding by Rao et al. (2013) that this genotype was outstanding for drought stress. G40068 is a landrace from Pima County Arizona (specifically 32.27N, −112.73W), which experiences just 229 mm of annual precipitation and average high temperatures of 39.8°C in the hottest month (Fick & Hijmans, 2017), corresponding approximately with the time of germination. The drought resilience of this line is therefore likely the result of adaptation to this extreme environment.

5 | CONCLUSION

We uncovered highly divergent patterns of stomatal and hydraulic responses to post-flowering drought across 12 genotypes of common bean and four of tepary bean, including both archetypally "anisohydric" and "isohydric" behaviour. Our hypotheses were largely refuted: common beans were not universally more isohydric than tepary beans (in fact, half were strongly anisohydric), more-anisohydric genotypes did not generally experience smaller increases in evaporative demand and larger declines in hydraulic conductance, and isohydric genotypes did not generally exhibit a greater degree of stomatal closure in drought. One tepary bean (G40068) exhibited almost no physiological response to drought. These surprising patterns suggest that non-stomatal mechanisms for leaf cooling (such as leaf movement and trichomes) and variation in hydraulic vulnerability to low water potential may be important drivers of genotypic differences in physiological resilience to drought among closely related crop legumes.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in bean_gs_ms at https://github.com/TomBuckleyLab/bean_gs_ms.

ORCID

Thomas N. Buckley http://orcid.org/0000-0001-7610-7136
Troy S. Magney http://orcid.org/0000-0002-9033-0024
Colleen Mills http://orcid.org/0009-0006-6698-397X
Matthew E. Gilbert http://orcid.org/0000-0002-6761-7975

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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