Plasticity in stomatal behaviour across a gradient of water supply is consistent among field-grown maize inbred lines with varying stomatal patterning

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Abstract

Stomata regulate leaf CO\textsubscript{2} assimilation (A) and water loss. The Ball–Berry and Medlyn models predict stomatal conductance (\textit{g}\textsubscript{s}) with a slope parameter (\textit{m} or \textit{g}\textsubscript{1}) that reflects the sensitivity of \textit{g}\textsubscript{s} to A, atmospheric CO\textsubscript{2} and humidity, and is inversely related to water use efficiency (WUE). This study addressed knowledge gaps about what the values of \textit{m} and \textit{g}\textsubscript{1} are in C\textsubscript{4} crops under field conditions, as well as how they vary among genotypes and with drought stress. Four inbred maize genotypes were unexpectedly consistent in how \textit{g}\textsubscript{s}, \textit{m} and \textit{g}\textsubscript{1} decreased as water supply decreased. This was despite genotypic variation in stomatal patterning, A and \textit{g}\textsubscript{s}. \textit{m} and \textit{g}\textsubscript{1} were strongly correlated with soil water content, moderately correlated with predawn leaf water potential (\textit{Ψ}\textsubscript{pd}), but not correlated with midday leaf water potential (\textit{Ψ}\textsubscript{md}). This implied that \textit{m} and \textit{g}\textsubscript{1} respond to long-term water supply more than short-term drought stress. The conserved nature of \textit{m} and \textit{g}\textsubscript{1} across anatomically diverse genotypes and water supplies suggests there is flexibility in structure-function relationships underpinning WUE. This evidence can guide the simulation of maize \textit{g}\textsubscript{s} across a range of water supply in the primary maize growing region and inform efforts to improve WUE.

KEYWORDS

Ball–Berry and Medlyn models, drought stress, genotypes, maize, stomatal conductance

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INTRODUCTION

Stomata on leaf surfaces regulate the exchange and trade-off of carbon and water between plants and the atmosphere while responding to environmental and physiological signals (Berry et al., 2010; Hetherington & Woodward, 2003). Stomatal conductance ($g_s$) is a key determinant of leaf, plant, canopy, ecosystem, regional and global fluxes of water, carbon and energy (Bonan et al., 2014; Franks et al., 2017). Therefore, stomatal conductance is a key regulator of crop performance as well as the biogeochemistry of natural and managed ecosystems (Leakey et al., 2019). And, a mathematical representation of $g_s$ is one fundamental component of models of plant and ecosystem function (Lawrence et al., 2019; Oleson et al., 2010; Sellers et al., 1997).

Two of the most widely used models of $g_s$ are the Ball–Berry (BB) model (Ball et al., 1987) and Medlyn (MED) model (Medlyn et al., 2011). Both the BB and MED models describe $g_s$ as a function of the rate of net photosynthetic carbon dioxide assimilation ($A$) and atmospheric carbon dioxide concentration at the leaf surface ($C_a$), along with either atmospheric relative humidity ($H_r$) or vapour pressure deficit ($D_v$) at the leaf surface. Stomatal behaviour in response to these drivers is captured in terms of a slope parameter ($m$ or $g_1$) and intercept parameter ($g_0$ or $g_{0mb}$). The slope parameter $m$ reflects the sensitivity of $g_s$ to changes in $A^*/H_r/C_a$ (hereafter referred to as the BB Index) and $g_1$ to $A/(C_a\sqrt{D_v})$ (hereafter referred to as the MED Index). Biologically, $m$ and $g_1$ are an inverse of intrinsic water use efficiency (iWUE, $A/g_s$) for given set of environmental conditions, for example, fixed $H_r$ or $D_v$ and $C_a$ (Leakey et al., 2006; Wolz et al., 2017). As an extension of these basic models, there have been numerous formulations to incorporate the effects of water stress on $g_s$, all of which are based on empirical functions describing the response of $g_s$ model parameters to variation in soil or plant water status (Damour et al., 2010).

Recent studies have highlighted the importance of understanding how the slope parameters of $g_s$ models vary among plants and across environmental gradients (Franks et al., 2018; Lin et al., 2015; Miner & Bauerle, 2017; Wolz et al., 2017). But, despite the importance of $g_s$ and models of $g_s$, knowledge gaps remain about: (1) how the slope parameters of stomatal conductance models vary within crop species; (2) whether there are significant genotype by environment interactions; and (3) how stomatal patterning on the epidermis may influence model representations of $g_s$.

There is clear evidence for substantial variability in the parameters of stomatal conductance models within and across different plant functional types (Franks et al., 2018; Miner & Bauerle, 2017; Wolz et al., 2017). And, the limited available data suggest that intraspecific variation can be as great as interspecific variation (Miner et al., 2017). $g_s$ is determined by the stomatal dynamics (opening and closing of stomatal aperture) as well as maximum conductance via epidermal stomata patterning (stomatal density, size and distribution) (Dow et al., 2014; Lawson & Matthews, 2020; Leakey et al., 2019; Xie et al., 2021). Stomatal patterning more broadly covaries with vein density, leaf width and canopy temperature (Brodribb & Holbrook, 2003; Brodribb & McAdam, 2011; Cano et al., 2019; Prakash et al., 2021). But, complexity in the relationships between these traits means that structural-functional relationships controlling $g_s$ are still not easily predicted. For example, the relationship between $g_s$ and stomatal density can be positive (Li et al., 2017; Xu & Zhou, 2008), undetectable (Zhao et al., 2015), or negative (Bresta et al., 2018) across different species or pools of intraspecific variation. Likewise, the relationship between $g_s$ and stomatal complex area can be positive (Galvés et al., 2013; Li et al., 2017), undetectable (Xu & Zhou, 2008; Zhao et al., 2015), or dependent on the shape of the stomatal complex rather than its size (Xie et al., 2021). Therefore, it is of interest to investigate if variation in stomatal patterning contributes to intraspecific variation in the slope parameters of $g_s$ models.

When vegetation is sampled across biomes at a global scale, $m$ or $g_1$ are lower when water is less available (Lin et al., 2015). This evidence is in line with theoretical expectations (Damour et al., 2010; Lin et al., 2015; Miner & Bauerle, 2017), and the widespread observation that plants have greater iWUE when drought-stressed compared to being well-watered (Leakey et al., 2019; Miner et al., 2017). Leaf, vegetation and land surface models often feature a function that lowers $m$ or $g_1$ as a function of plant or soil water status (Anderegg et al., 2017; Klein, 2014; Wolf et al., 2016). Models have even been developed linking variation in $m$ to abscisic acid concentration in the xylem (Gutschick & Simonneau, 2002). However, there are also numerous examples where the slope parameters of $g_s$-models have been insensitive to variation in plant or soil water status in the field, unless water stress was extreme (Gimeno et al., 2016; Misson et al., 2004; Xu & Baldocchi, 2003). Miner and Bauerle (2017) reported significantly lower $m$ when maize was water-stressed in pots. But, the relationships between $m$ and plant or soil water status were weak. $m$ did not vary over time in field-grown maize, but it is not clear if plant water status changed over the experimental period or not. Structure-function relationships can underpin the physiological plasticity of plants across environmental gradients. For example, acclimation to humidity modifies the link between leaf size and the density of veins and stomata (Carins Murphy et al., 2014). The integration of the BB model of $g_s$ with a model predicting maximum $g_s$ from stomatal anatomical traits was a valuable recent advance (Dow et al., 2014). But, studies of variation in stomatal patterning among crop varieties and studies parameterizing $g_s$ models have generally occurred in isolation of one another. This study aimed to address knowledge gaps about genetic variation in $g_s$ model parameters across a gradient of water stress by investigating four inbred lines of maize (Zea mays L.) with a range of stomatal densities.

Notably less data describing $g_s$ model parameters is available from C₄ crops than other functional groups (Lin et al., 2015; Miner et al., 2017). This is despite the importance of maize, sugarcane, sorghum, switchgrass and other species as sources of food, fuel, fibre and feed (Leakey, 2009). Maize is a model plant for studying the genomics, genetics and physiology of complex traits in C₄ plants (Buckler et al., 2009). The development of a machine-learning tool to automatically analyse microscopy images of the leaf epidermis facilitated detailed...
analysis of variation in stomatal patterning across genetically and anatomically diverse maize inbred lines that were consistent over two growing seasons (Xie et al., 2021). Relative to diversity in the species as a whole, B73 and MS71 were identified as inbred lines with moderate (106 mm⁻²) and low stomatal density (88 mm⁻²), respectively (Xie, 2021). When these lines were crossed and self-pollinated, the resulting recombinant inbred lines (RILs) displayed significant transgressive segregation. The RILs with extremely high stomatal density (111 mm⁻²) and extremely low stomatal density (74 mm⁻²) were selected and designated RIL2 and RIL1, respectively.

The parameters of the BB and MED models of gs were measured for the four genotypes of maize (B73, MS71, RIL1 and RIL2) under five levels of water supply. Water availability treatments were generated using an in-field rain-out shelter facility located in the Midwest United States, which is the world’s primary region of maize production (USDA, 2020). The following predictions were tested: (1) the m and g₁ parameters of gs models will be lower when water supply is restricted; and (2) the plasticity of m and g₁ in response to drought stress will vary among genotypes with distinct stomatal patterning.

2 | MATERIALS AND METHODS

2.1 | Field site and experimental treatments

The study was conducted at a field rain-out shelter facility (Supporting Information: Figure S1) on the University of Illinois at Urbana-Champaign research farm in Champaign, IL, USA (www.igb.illinois.edu/soyface/, 40°02′N, 88°14′W) in 2019. The soil type at this site is Drummer–Flanagan series (fine-silty, mixed, mesic Typic Endoa-quoll). It is an organically rich, highly productive Corn Belt soil. The field is tile-drained and has been in continuous cultivation of arable crops for decades. The rain-out shelter had an automatically retractable roof and walls (A-Frame, Cravo Equipment Ltd.) that were used to exclude precipitation from a field plot with dimensions of ~76 × 9 m. A weather station integrated with the control system (Igrow 1400; Link4 Corporation) automatically closed the roof and walls within 2 min of precipitation being detected by an optical rain sensor. The roof and walls automatically reopened after no precipitation was detected for 10 min. To prevent lateral percolation of water into the soil covered by the rain-out shelter, a plastic barrier impermeable to water was buried vertically from the soil surface to a depth of 1.2 m around the perimeter of the rain-out shelter.

Ten rows of crops were planted along the length of the facility at a spacing of 0.76 m. The outer rows of plants were treated as a border and not sampled, leaving the eight central rows for analysis of variation in stomatal patterning across genetically and anatomically diverse maize inbred lines that were consistent over two growing seasons (Xie et al., 2021). Relative to diversity in the species as a whole, B73 and MS71 were identified as inbred lines with moderate (106 mm⁻²) and low stomatal density (88 mm⁻²), respectively (Xie, 2021). When these lines were crossed and self-pollinated, the resulting recombinant inbred lines (RILs) displayed significant transgressive segregation. The RILs with extremely high stomatal density (111 mm⁻²) and extremely low stomatal density (74 mm⁻²) were selected and designated RIL2 and RIL1, respectively.

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The response of steady-state gs to variation in photosynthetic photon flux density (PPFD) was measured on the youngest fully expanded leaves near the top of the canopy from 27 August to 3 September 2019, using six portable photosynthetic gas exchange systems (Model Li-6800; Li-Cor Inc.) in the field rain-out shelter. A variation on the approach of Ball et al. (1987), Leakey et al. (2006) and Wolz et al. (2017) was used, but on attached leaves in the field. Leaves were acclimated in the chamber with a target leaf temperature of 30.0°C, relative humidity of 60%, reference cuvette CO₂ concentration of 400 μmol mol⁻¹, PPFD of 2000 μmol m⁻² s⁻¹ and flow rate of 400 μmol s⁻¹ for approximately 30 min. Once gs had attained steady-state rates, incident PPFD was decreased stepwise to 1500, 1200, 900, 700, 500, 400, 300, 200, 100 and 50 μmol m⁻² s⁻¹. At each light level, gs were allowed to reach steady-state before results were recorded (less than 30 min) and the next stepwise change was initiated (Supporting Information: Figure S3a). A full gs-response curve took a minimum of 120 min with 11 points of light levels. Measurements were started around 7 AM and continued no later than 2 PM. A total of 71 leaves were measured providing three to four samples per genotype in each of the five levels of water supply.

2.2 | gs-response curves

After in situ gas exchange measurements were completed each day, the same leaves were collected to measure midday leaf water
potential ($\Psi_{m0}$) using a pressure chamber (1505D; PMS Instrument Company). Then, four-leaf discs (approximately 7.1 cm² per plant) were removed and dried in an oven at 70°C to constant weight and weighed for calculation of SWC. At predawn the next day, a neighbouring leaf from the same plants was collected to measure predawn leaf water potential ($\Psi_{m0}$).

Access tubes were installed within crop rows using a tractor-mounted, customized hydraulic soil corer (Rajurkar et al., 2022) at four locations in each subplot to allow measurement of SWC twice per week at four depths from 5 to 83 cm, with 5–24, 25–43, 44–63 and 64–83 cm, respectively, using the TRIME-PICO TDR system (IMKO GmbH).

2.4 Parameterization of stomatal conductance models

Data gathered from the $g_s$-response curves were used to estimate parameters of the BB ($m$) and MED models ($g_1$) for each leaf by the least-squares and nonlinear regressions of the following functions (Supporting Information: Figure S3b):

$$g_s = m \frac{H_s}{C_s} + g_0.$$ (1)

$$g_s = 1.6 \left(1 + \frac{g_1}{\sqrt{D_1}} \right) \frac{A}{C_s} + g_{om}.$$ (2)

$g_1$ is defined as:

$$g_1 = \sqrt{\frac{3\Gamma^* \lambda}{1.6}},$$ (3)

where $\Gamma^*$ is the CO₂ compensation point for photosynthesis without dark respiration (µmol mol⁻¹) and $\lambda$ the marginal water cost of carbon gain (µmol water µmol⁻¹ CO₂). For similar conditions of temperature and over a moderate range of relative humidity (~40%–80%), $m$ and $g_1$ are approximately related by the following forms:

$$m = \frac{1.6}{H_s} \left(1 + \frac{g_1}{\sqrt{D_1}} \right).$$ (4)

$H_s$ and $C_s$ were calculated as:

$$H_s = 1 - \frac{T_r P}{e_{sat} g_b},$$ (5)

where $T_r$ is transpiration rate (µmol m⁻² s⁻¹), $P$ is air pressure (Pa) and $e_{sat}$ is the saturated vapour pressure at the substomatal cavity related to leaf temperature (Pa).

$$C_s = C_a - A g_b^{-1.37},$$ (6)

where $C_a$ is the CO₂ concentration in the sample chamber (µmol mol⁻¹), $g_b$ is the leaf boundary conductance (µmol m⁻² s⁻¹) and 1.37 is the ratio of the molecular diffusivities for H₂O to CO₂ at the leaf surface.

The slope parameters $m$ and $g_1$ were obtained by linear and nonlinear fitting to leaf gas exchange data for Equations (1) and (2), respectively. The intercepts, $g_0$ and $g_{om}$, are often thought to represent the cuticular $g_0$ or the conductance with closed stomata. Similar to previous studies (Franks et al., 2018; Lin et al., 2015; Wolz et al., 2017; Wu et al., 2020), we did not fit $g_0$ and $g_{om}$ and took them as zero. Quality assurance was performed by evaluating the goodness-of-fit between the BB model and measured data, with data from all leaves passing the criteria of an $R^2 \geq 0.9$ (Supporting Information: Table S1 and Figure S3c).

2.5 Statistical analyses

We used a principal component analysis (PCA) to identify the major axes and explore the relationships among different traits. PCA was carried out using the OriginPro 2021 (OriginLab Corporation). Because the traits had different units, they were scaled to unit variance and zero mean using a correlation matrix before the analysis. The first three principal components (PCs) were retained (Supporting Information: Table S2).

To evaluate differences in the slopes and intercepts of the linear regressions among four genotypes of maize, we compared two models using "Compare Linear Fit Parameters and Datasets" in the OriginPro 2021 (OriginLab Corporation). For the null model, the regression parameter values are assumed to be the same across genotypes. For the model testing the hypothetical interaction effect of water availability x genotype, the regression parameter values could vary among genotypes. An $F$-statistic was constructed as described by Sokal and Rohlf (1995):

$$F = \frac{(RSS_1 - RSS_2)/(d_1 - d_2)}{RSS_2/d_2},$$ (7)

where $RSS_1$, $RSS_2$, $d_1$ and $d_2$ are the sum of the residual sum of squares (RSS) and the sum of degrees of freedom (df) of the null and test model, respectively. After the $F$ value was computed, the associated $p$ value was used to determine statistical significance. Independent regression lines were fit for each genotype when either the slopes or intercepts of the relationship between two traits were significantly different among genotypes. Regressions lines were fit across all genotypes when both the slopes and intercepts of the relationship between two traits were not significantly different among genotypes. The regression equations for all relationships are reported in Supporting Information: Table S3. All raw data is provided in Supporting Information: Table S4.

We report the $p$ value resulting from each statistical test, with $p$ values of less than 0.05 being considered significant.

3 RESULTS

3.1 PCA

The first two PCs had eigenvalues greater than 1.0 and together explained 77.5% of the overall variation in the data (Figure 1,
Supporting Information: Figure S4). The first PC appeared to correspond with the gradient of water availability, with strong loadings for SWC, water potential and leaf gas exchange traits (Figure 1, Supporting Information: Table S2). The second PC appeared to describe genotypic variation, with a strong loading for SLA. Notably, the vectors for $A_{sat}$ and $g_{sat}$ loaded roughly equally onto PC1 and PC2, while variation in $m$, $g_1$ and measures of water potential were more closely associated with PC1 and less associated with PC2. iWUE clearly varied in a manner opposite to SWC, water potential, $m$ and $g_1$. Pairwise analyses were, therefore, performed to characterize the interactive effects of water availability and genotype in more detail.

3.2 | Responses of $g_s$-model parameters to varying water availability and plant water status

The slope parameters ($m$ or $g_1$) of $g_s$-models were lower in value when the average SWC at soil depth profile of 5–83 cm was lower (Figure 2a,b; $p \leq 0.002$; Supporting Information: Table S1). But, there were no significant differences among genotypes in the relationship between SWC and $m$ or $g_1$ (Figure 2a,b; $p \geq 0.662$). This consistency in $m$ and $g_1$ among genotypes as SWC varied with irrigation rate was observed regardless of the soil depth at which SWC was measured (Figure 2c–j; $p \geq 0.557$). However, the proportion of variance in $m$ or $g_1$ explained by SWC varied from being strongest for intermediate soil depths (44–63 cm; $R^2 = 0.50$–0.52; Figure 2g,h) followed by deeper soil layers (64–83 cm; $R^2 = 0.43$–0.44; Figure 2i,j) and shallower soil layers (5–24 and 25–43 cm; $R^2 = 0.36$–0.37; Figure 2c–f). And, this corresponded with $m$ or $g_1$ being more sensitive to a given change in SWC at intermediate and deeper soil depths than the equivalent changes in SWC in shallower soil layers (Figure 2).

Plant water status across the range of SWC was characterized in terms of leaf water potential both predawn ($\Psi_{pd}$) and during the midday period ($\Psi_{md}$). $m$ and $g_1$ both were lower when $\Psi_{pd}$ was more negative (Figure 3a,b; $p \leq 0.014$). But, the relationships of $m$ or $g_1$ with $\Psi_{md}$ were not significant (Figure 3c,d; $p = 0.094$–0.112) and $\Psi_{pd}$ did not explain as much variation in $g_s$-model slope parameters as SWC. There was also no variation among genotypes in these relationships between $m$ or $g_1$ and $\Psi_{md}$ (Figure 3; $p \geq 0.555$).

3.3 | $A_{sat}$, $g_{sat}$ and SLA as drivers of variation in $g_s$-model parameters under varying SWC

Drought-induced variation in $m$ was significantly associated with variation in both $g_{sat}$ and $A_{sat}$ in a genotype-specific fashion.
The sensitivity of $m$ or $g_1$ to $g_{sat}$ or $A_{sat}$ were consistent across all genotypes (i.e., regression slopes did not significantly differ, $p \geq 0.445$), but the value of $m$ or $g_1$ for a given $g_{sat}$ or $A_{sat}$ differed between genotypes (i.e., regression intercepts significantly varied, $p \leq 0.049$). The proportion of variation in $g_{sat}$-model parameters explained by $g_{sat}$ and $A_{sat}$ was very similar, as described by goodness-of-fit, that is, $R^2$ (Figure 4). These relationships stem from lower SWC driving progressively lower $g_{sat}$ and $A_{sat}$ in a genotype-specific manner ($p \leq 0.013$, Figure 5a,b). And, the observed intercept changes in $g_{sat}$ or $A_{sat}$ were driven by the genotype-specific responses to SWC. Drought-induced variation in $g_{sat}$ and $A_{sat}$ was significantly associated with $\Psi_{md}$ ($R^2 > 0.67, p < 0.001$; Figure 5c,d).
that is, \( g_{sat} \) and \( A_{sat} \) covared with leaf water status assessed immediately after gas exchange measurements were completed. And, this response was consistent across all four genotypes \((p \geq 0.201)\). The correlations between \( g_s \) and \( \Psi_{pd} \) as well as \( A \) and \( \Psi_{pd} \) were species-specific \((p \leq 0.051)\), with genotypes having different \( g_s \) or \( A \) during the day even when the water status of the plants had been equivalent predawn (Figure 5e,f). Neither \( m \) nor \( g_1 \) was significantly correlated with SLA (Figure 6a,b, \( p \geq 0.604 \)). But, the anticipated negative relationships between the slope parameters of the \( g_s \)-models and intrinsic WUE were observed (Figure 6c,d, \( p \leq 0.048 \)).

**4 | DISCUSSION**

This study successfully addressed its aims to investigate how genotypic variation in \( g_s \)-model parameters among four anatomically distinct maize inbred lines was impacted by a gradient in water availability at a mesic site in the Midwest United States. As predicted, \( m \) and \( g_1 \) were progressively lower when plants were more drought-stressed due to withholding of water supply (Figures 1 and 2). Variation in \( m \) and \( g_1 \) showed the strongest relationships with water availability in deeper soil layers, and moderate dependency on \( \Psi_{pd} \), but no significant association with \( \Psi_{md} \) (Figures 2 and 3). Contrary to expectations, inbred genotypes of maize that significantly vary in stomatal patterning, \( g_s \) and \( A \) (Figures 4 and 5) were very consistent with respect to \( g_s \)-model parameters and their plasticity in response to drought stress (Figure 2). These findings provide new evidence to guide how models of maize should simulate \( g_s \) and its influence on plant function across a range of water status that is relevant to field conditions in the primary growing region of this major crop.

The line of best fit describing how \( m \) varies with SWC across the whole rooting zone (Figure 2a) was very similar to that for pot-grown maize (Miner & Bauerle, 2017). But, this probably is somewhat coincidental because the physical characteristics of the soils in the two experiments are very different, so the moisture release curves and effects on plant water status of the two gradients in water supply were expected to differ. This interpretation is consistent with the well-watered greenhouse-grown plants having \( \Psi_{md} \) equivalent to \( \Psi_{pd} \) in the field, but much less negative than \( \Psi_{md} \) in the field (Figure 3; Miner & Bauerle, 2017). Nonetheless, the consistency in the direction of response in the two studies, and the consistency in response among anatomically diverse inbred lines in the present study, suggests that the results do provide a reasonable first approximation of how maize stomata operate in a production setting and how that should be parameterized in models. The decrease in the value of the slope parameters of the \( g_s \)-models in response to the relatively mild drought stress imposed is equivalent to a ~15% increase in leaf-level intrinsic WUE. In absolute terms, this is a significant increase given the already high iWUE of maize. And, this is almost as large as the response of
iWUE to elevated CO₂ concentrations in maize or soybean at the same site, which had significant consequences for agronomic performance and canopy carbon and water fluxes (Bernacchi et al., 2007; Gray et al., 2016; Hussain et al., 2013; Jin et al., 2018; Markelz et al., 2011). But, this experiment did resolve relatively subtle treatment effects when contrasted against the stronger variation in the slope parameters of gs models under drought stress that are possible in more xeric locations (e.g., Héroult et al., 2013).

The data presented here are valuable because there are far fewer estimates of slope parameters for gs models (i.e., m and g₁) for C₄ species than C₃ species in general, and especially under field conditions (Lin et al., 2015; Miner et al., 2017). On average, m was 3.87 and g₁ was 0.87 kPa⁻⁰.⁵ across the four genotypes of maize under well-watered conditions (Figure 2, Supporting Information: Table S1). This sits between parameter estimates previously published for maize grown in controlled environment conditions (m = 3.06, Ball 1988; m = 3.23, Collatz et al., 1992; m = 4.53, Miner & Bauerle, 2017; g₁ = 1.281; Yun et al., 2020) and very close to a parameter estimate for maize in the field in Colorado (m = 3.72, Miner & Bauerle, 2017). The results are also comparable to measurements of Panicum virgatum (m = 3.9), Miscanthus x giganteus (m = 3.3) and Sorghum bicolor (m = 4.32) grown at nearby field sites (LeBauer et al., 2013; Li et al., 2021) as well as C₄ grasses in general (m = 4.1, Miner et al., 2017; m = 4.0, Franks et al., 2017). But, relatively subtle variation in gₛ-model parameters can significantly impact predictions of leaf, canopy, ecosystem and global water fluxes (Franks et al., 2017; Wolz et al., 2017), so additional data collection is still needed. Investigation of hybrid maize as well as maize lines that capture additional genetic and physiological diversity would be particularly valuable to aid in simulations of carbon and water fluxes for this key crop and the US Corn Belt region as a whole. And, future experiments should explore if the responses to mild drought stress reported here continue in a linear fashion as stress becomes more severe.

There is significant uncertainty surrounding the physiological mechanisms that underpin variation in m or g₁ across different growing conditions in either time or space (Damour et al., 2010; Héroult et al., 2013; Miner et al., 2017; Xu & Baldocchi, 2003). Some studies demonstrated that m and g₁ are relatively stable under drought conditions (Gimeno et al., 2016) or the inclusion of leaf water potential did not improve model performance (Wu et al., 2020). In a natural oak-grass savanna, m for blue oak remained constant through a severe summer drought (Xu & Baldocchi, 2003). But, others have found a response in gₛ-model parameters to water deficit (Anderegg et al., 2017; Damour et al., 2010; Sellers et al., 1996; Venturas et al., 2018). In a common garden experiment, m decreased under drought in two Eucalyptus species from humid regions but not in two other eucalypts from drier regions (Héroult et al., 2013). This mechanistic uncertainty is reflected in a subset of models variously using SWC, soil Ψ, plant water status, or even hormone concentrations to

**FIGURE 4**  The relationships between the BB slope (m) and MED slope (g₁) with light-saturated photosynthesis rate (Aₛₐ₅) and stomatal conductance (gₛₐ₅) for four genotypes of maize (B73, MS71, RIL1 and RIL2) under five levels of water supply. The results of statistical tests are provided as described in Figure 2. Plotted points are genotype means at each level of SWC ± SD. BB, Ball–Berry; MED, Medlyn; RIL, resulting recombinant inbred line; SWC, soil water content.
modulate simulations of stomatal behaviour in response to drought stress (Anderegg et al., 2017; Damour et al., 2010; Oleson et al., 2010; Sellers et al., 1996; Sperry et al., 2017; Venturas et al., 2018). Variations in \( m \) or \( g_1 \) for field-grown maize most closely correlated with SWC in intermediate to deep layers of the rooting profile, were moderately correlated with \( \Psi_{pd} \), but were not correlated with \( \Psi_{md} \) (Figures 2,3). \( \Psi_{pd} \) is commonly considered to be in equilibrium with soil \( \Psi \) and the observed data indicate that the water supply treatments here caused long-term variation in soil water status that was beyond the capacity of the system to recover overnight. Nevertheless, the differences between \( \Psi_{pd} \) and \( \Psi_{md} \) indicate that significant additional short-term water stress did develop during the day as the evaporative demand of the crop was met to differing degrees at the different levels of water supply. This strong role of water stress that temporarily develops during the day is evident from the relationships of \( g_s \) and \( A \) with \( \Psi_{md} \) rather than \( \Psi_{pd} \) (Figure 5). But, crucially, the lack of relationship of \( m \) or \( g_1 \) with \( \Psi_{md} \) implies that the plasticity in parameters of \( g_s \) models is driven by long-term signals and responses rather than the short-term responses to drought within a single day. This may include changes in photosynthetic capacity, which can influence the sensitivity of \( g_s \) to atmospheric conditions (Franks et al., 2017), but further work will be needed to resolve the mechanistic details. Notably, no relationship was found between SLA and \( m \) or \( g_1 \) among the four maize inbred lines (Figures 1 and 6). This contrasts with the results of a study of tropical rainforest trees, but may reflect the consequence of studying intraspecific rather than interspecific variation in traits (Wu et al., 2020).

**FIGURE 5** The relationships between light-saturated photosynthesis rate (\( A_{sat} \)) and stomatal conductance (\( g_{sat} \)) with average SWC at a soil depth of 5–83 cm (a,b), and predawn and midday leaf water potential (\( \Psi_{pd} \) and \( \Psi_{md} \)) for four genotypes of maize (B73, MS71, RIL1 and RIL2) under five levels of water supply. The results of statistical tests are provided as described in Figure 2. Plotted points are genotype means at each level of SWC ± SD. RIL, resulting recombinant inbred line; SWC, soil water content.
The four maize inbred lines studied display significant variation in stomatal density, other aspects of stomatal patterning and anatomy (Xie et al., 2021), and $A$ and $g_s$ (Figure 5). Nevertheless, they had very similar $m$ and $g_1$ (Figures 2 and 3). And, genotype-specific plasticity in $m$ or $g_1$ in response to a gradient of water supply could not be detected. This convergence in $g_s$-model parameter values across genotypes is consistent with the trade-off between carbon gain and water use (i.e., WUE) being the most fundamental trade-off for terrestrial plant life (Boyer, 1982; Briggs & Shantz, 1917; Hetherington & Woodward, 2003). And, it indicates that there must be significant flexibility in structure-function relationships between stomatal patterning and other aspects of leaf gas exchange. Or in other words, the same WUE can be achieved with different configurations of stomata, leaf hydraulics and photosynthesis. This complements emerging frameworks for understanding the tight coordination in the photosynthetic, gas exchange and water supply capacities of leaves across the diversity of land plants (Deans et al., 2020). It is also important to recognize that flexibility in structure-function relationships of the type observed here will set constraints and maybe create opportunities for efforts to engineer or select for improved crop WUE (Leakey et al., 2019). New high-throughput phenotyping and analytical techniques are providing unprecedented detail and depth of information about the suite of traits that underpin variation in WUE within C₄ species (Ferguson et al., 2021; Pignon et al., 2021a, 2021b; Xie et al., 2021). This should then in turn allow additional studies of the type presented here to quantify $g_s$-model parameters in other genotypes and provide the parameterization data needed to inform crop improvement effort with in silico analyses (Marshall-Colon et al., 2017).

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DATA AVAILABILITY STATEMENT

All data associated with this study are available in the supplementary materials.

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