Field Crops Research 234 (2019) 119–128

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Stomatal conductance, xylem water transport, and root traits underpin improved performance under drought and well-watered conditions across a diverse panel of maize inbred lines

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ARTICLE INFO

Keywords:
Xylem
Nested association mapping population
Hydraulic safety
Hydraulic efficiency
Crop performance
LASSO regression
Structural equation modeling

ABSTRACT

We evaluated sixteen traits related to water acquisition and transport, stomatal conductance, and photosynthesis within a diverse panel of maize inbred lines, founders of the U.S. maize nested association mapping (NAM) population, with the aim to determine which traits confer improved growth under water deficit and well-watered conditions. Lasso regression revealed that three key traits explained meaningful and independent proportions of variation in total end-of-season biomass under deficit irrigation (multiple $r^2 = 0.86$): 1) the maximal net CO_2 assimilation rate ($P = 0.007$), 2) the achievable stomatal conductance during the hottest part of the day ($P = 0.005$), and 3) the width-to-depth ratio of the root system at the seedling stage ($P = 0.060$), i.e., initial deep root system development facilitated growth. Under well-watered conditions, maximal stomatal conductance in the morning ($P = 0.014$) and the width-to-depth ratio of the root system ($P = 0.043$) were identified as key traits contributing to improved performance (multiple $r^2 = 0.68$). Structural equation models revealed that growth under water deficit was linked more strongly to stomatal conductance occurring during the middle of the day (std coef = 0.75; $P = 0.006$), rather than the maximal stomatal conductance (std coef = −0.25; $P = 0.368$). In turn, the maintenance of stomatal conductance through the middle of the day depended on the capacity of the xylem tissue to supply water (per unit cross-sectional area) (std coef = 0.48; $P = 0.046$). Aligned with the transport of water to the stomata and growth, root system depth ($r = 0.77$; $P = 0.003$) and width-to-depth ratio ($r = -0.55$; $P = 0.064$) at seedling stages were also correlated with the capacity of the xylem to transport water, thus suggesting close coordination between root, xylem, and stomatal traits to achieve greater growth under water deficit and well-watered conditions. We propose that maize performance under the drought conditions considered here, could likely be improved via lower stomatal sensitivity to hydraulic and atmospheric cues, greater xylem conductivity, and a deeper, but not necessarily more extensive, root system.

1. Introduction

Enhancing the performance of crop species under drought is a primary goal of agriculture and a requisite outcome to increase the productivity of marginal lands, particularly in the developing world. Projected world population growth and increasing food insecurity over the next half century will not only place a premium on plant traits conferring higher productivity in the absence of drought, but also on traits conferring higher productivity in the presence of drought. Evidence from both wild and domesticated plant species (crops) suggest that a broad range of general and specific traits may confer drought tolerance under various scenarios of management and climate variation (Chaves et al., 2003).

We consider here the traits and processes affecting a plant's access to water and its transport to the stomata, which remain important and understudied components of drought stress in crop species (Brodribb

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https://doi.org/10.1016/j.fcr.2019.02.001
Received 8 September 2018; Received in revised form 30 January 2019; Accepted 2 February 2019
0378-4290/ Published by Elsevier B.V.

et al., 2015; Gleason, 2015). Water acquisition begins at the root system, which may be modified to extract water at greater depth/extent, or water that is held at lower potential (Ali et al., 2015; Comas et al., 2013; Pace et al., 2015; Zurek et al., 2015). After water is extracted by the roots, it is then transported long distances through pipe-like conduits, which is both a risky and expensive undertaking (McCulloh and Sperry, 2006; Tyree and Zimmermann, 2002). As such, the capacity of xylem tissue to transport water (hereafter "hydraulic efficiency"), as well as its resistance to failure (hereafter "hydraulic safety"), have been closely coordinated by natural selection with the requirements for gas exchange and plant growth (Anderegg et al., 2016; Choat et al., 2012; Gleason et al., 2018a; Guha et al., 2018; Li et al., 2009). Once water finally arrives at the leaves, only then can it be exchanged for CO_2. The CO_2 exchange rate (water use efficiency; WUE) is the ratio between CO_2 assimilation and transpiration, and as such, can be improved either via increasing the rate of CO_2 assimilation (Flexas and Medrano, 2002; Gilbert et al., 2011; Salas Fernandez et al., 2015) or reducing the rate of transpiration (Medina et al., 2017; Ryan et al., 2016). It is also important to consider the daily and seasonal timing of water use. For example, the frugal use of soil water when it is in surplus, particularly under conditions of high vapor pressure deficit (VPD), may allow crops to conserve water for times when it is most needed (e.g., during reproductive development) (Berger et al., 2016; George-Jaeggli et al., 2017; Sinclair, 2018).

Plant traits affecting water extraction, transport, and use should be viewed, not as independent traits operating in isolation of one another, but rather as a connected network of traits that have been closely coordinated via natural and artificial selection. As such, "tuning" one part of the network should result in a cascade of consequences elsewhere in the network. For example, biomass produced per unit soil water (i.e., water held in the soil profile) may be enhanced by either increasing the integrated transpiration efficiency (seasonal net CO_2 assimilation per unit transpiration), or by increasing the total amount of transpiration. Although WUE can be improved without adversely impacting xylem functioning, increasing the rate of transpiration would require investment in xylem tissue capable of operating at lower water potentials, or else more extensive/deeper root systems (Brodribb et al., 2015; Comas et al., 2013; Pita et al., 2005).

Although the important role of soil water extraction in maintaining photosynthesis during moderate drought and delaying mortality under severe drought is now widely recognized (Anderegg et al., 2016; Blum, 2009; Brodribb et al., 2015; Choat et al., 2012; Sinclair, 2012; Tardieu, 2012; Turner et al., 2014), the xylem traits conferring functioning at low water potential have rarely been evaluated in crop species (Brodribb et al., 2015; Gleason, 2015; Pita et al., 2005; Sinclair et al., 2017). Water transport occurs under large tension (negative pressure) within xylem conduits. If this tension becomes too large, the adhesion and cohesion forces holding the water column together (and to the conduit wall) may break, forming a quickly expanding embolism, and thus rendering the conduit useless for water transport until it can be repaired or replaced. The capacity of xylem tissue to resist embolism formation and spread (hereafter "hydraulic safety") is therefore an important trait for all vascular species. Of equal importance is the clearly established link between the efficiency of xylem tissue to conduct water and productivity among vascular plant species (Feild et al., 2009; Gleason et al., 2018b; Hajek et al., 2014; Kotowska et al., 2015; Sterck et al., 2012). Here, we define xylem efficiency as the rate of water transport normalized by driving force, stem length, and cross-sectional area (hereafter "hydraulic efficiency").

The founder lines of the US maize Nested Association Mapping (US-NAM) population were chosen as our subjects of study (McMullen et al., 2009; Yu et al., 2008). The maize NAM population was designed to facilitate the linking of genes to desired phenotypic traits. It consists of 25 recombinant inbred line (RIL) families, with 200 lines within each family. RILs were constructed by crossing one founder line with a fully-sequenced reference line (B73) (McMullen et al., 2009). The NAM founder lines are often used for initial screening of germplasm because they are a relatively small group of genotypes that represent much of the genetic diversity existing in maize. This facilitates phenotyping of labor-intensive measurements, e.g., xylem functioning, while still capturing much of the phenotypic variation within the species.

Here, we used 22 of the 26 inbred founder lines plus Mo17, another widely used public inbred line that complements the NAM panel (Morgante et al., 2005), to address three key objectives. First, we sought to determine which traits were the most important in conferring higher end-of-season biomass under water-deficit as well as under well-watered conditions. Secondly, we wanted to know if there was a re-ranking of trait importance under well-watered versus water-deficit scenarios. Lastly, considering just the most dominant traits that influence growth, we wanted to quantify the variation in these traits among the NAM founder lines.

2. Materials and methods

2.1. Genotype selection

Seed for the NAM founder lines was obtained through the North Central Regional Plant Introduction Station (Ames, Iowa, USA). In 2015, we grew 24 genotypes: B73‡, B97‡, CML103‡, CML247, CML277, CML322‡, CML333, CML52, CML69‡, HP301, Ki11‡, Ky21‡, M162 W, M37 W, Mo17‡, Mo18W‡, Ms71‡, NC350‡, NC358, Oh43, Oh7B‡, P39, Tx303‡, Tzi8. "‡" denote the 13 genotypes that were also grown in 2016. These genotypes represent a wide range of tropical and temperate inbred lines, including yellow dent, white dent, white flint, yellow flint, popcorn (2015 only), and sweet corn (2015 only) (Foley, 2012). Geographic regions of origin included Mexico, North America (Iowa, Indiana, Kentucky, Missouri, Michigan, North Carolina, Ohio, and Texas), Thailand, South Africa, and Nigeria (2015 only). More data on each of the maize lines included in this study can be found in previously published reports (Flint-Garcia et al., 2005; Gore et al., 2009) and on the Maize Genetics Cooperation Stock Center website (http://maizecoop.cropsci.uiuc.edu/nam-rils.php).

2.2. Site and field conditions

NAM lines were grown during the 2015 and 2016 field seasons at the Limited Irrigation Research Farm, USDA-ARS, Greeley, CO, USA (40.45 °N, 104.64 °W, 1428 m asl). Mean monthly precipitation during the growth seasons (May – October) in 2015 and 2016 was 43.4 and 27.5 mm, respectively. The mean daily maximum/minimum temperatures during the 2015 and 2016 growing seasons were 26.2/8.0 °C and 26.9/8.7 °C, respectively. Soils on the site range from sandy loam to clay loam (Ustic Haplargids) (USDA-NRCS, 2015).

In 2015, 24 maize inbred lines were grown and evaluated on the site. In 2016 the total number of lines was reduced to the 13 that exhibited the highest rates of emergence in 2015 (ca > 80%). In both years, genotypes were hand-planted the first week of May into four well-watered plots and four deficit plots. Water was applied through a surface drip irrigation system twice every week, with well-watered plots receiving 100% ET requirements of a reference maize crop grown in the same field, whereas deficit plots received only 40% ET of the same reference crop.

Each of the eight main plots (four well-watered and four deficit plots) was 44 m long and separated in the field from one another by at least 12 rows of border plants, consisting of a dominant hybrid that performs well in the area (Dekalb DK 52-04). Within *each* of these main plots, either 24 (2015) or 13 (2016) subplots were established, depending on the number of genotypes planted that year; 24 genotypes in 2015 and 13 genotypes in 2016. As such, within each main plot, one genotype was assigned one subplot and planted into this subplot. Subplots were two rows wide, with 15 cm between plants and 76 cm between rows. In 2015 the larger number of genotypes (24) required smaller subplots (2 rows x 150 cm), whereas in 2016 the fewer genotypes planted (13) allowed for larger subplots (2 rows x 300 cm). As such, each subplot in 2015 contained 20 plants, whereas in 2016 each subplot contained 40 plants. This planting design allowed for each genotype to be replicated four times (four subplots) within each irrigation treatment. Plants were harvested on September 28 (2015) and on October 13 (2016). Although the plots in this study were small, they were similar in size to many field trails and within the proposed range identified for maize (Chaves and de Miranda Filho, 1992). We also note that since the completion of this study, we have completed a much larger plot study (plot size = 9 m x 23 m) at the same location with eight maize genotypes, giving similar trait-performance results (unpublished data). We are therefore confident that plot size did not adversely affect the outcome of the study.

A late-season hail storm occurred on August 19, 2016 prior to harvest. This storm resulted in leaf shredding and ca < 10% leaf area loss, with similar effects in both fully-watered and deficit treatments. The genotype rankings did not appear strongly affected by the storm, as the across-genotype correlation in total end-of-season biomass between the two years (i.e., plotting 2015 and 2016 data against one another) exhibited strong correlation ($r = 0.74$; $p < 0.001$).

2.3. Trait measurements

Eight gas exchange, hydraulic, and root traits were included in the study. Here, we provide a brief description of each trait measurement, but direct the reader to Appendix A in the supplemental information for the full and detailed description of each method.

Diurnal trajectories of stomatal conductance (G_S) were measured on all genotypes in both years using a steady-state diffusion leaf porometer (Model SC-1, Decagon Devices, Pullman, WA, USA). Measurements were taken by randomly selecting one plant from each subplot (i.e., genotype), measuring one leaf from this selected plant, and then moving on to the next subplot/genotype. Data collection occurred continuously from ca 0800 to 1500 h on each day that G_S was measured (6 days in 2015, 9 days in 2016) between July 6 and August 3 in both years. Maximal (G_{S_max}) and midday (G_{S_MD}) stomatal conductance was extracted from these trajectories (measured between 1330 and 1430 h). Maize growth stage ranged from V10-V21, depending on the date and the genotype-specific rate of development. The total number of leaves produced by the genotype was also recorded and expressed per unit thermal time. Thermal time was calculate after Ritchie (1998). Additionally, the number of days required to reach anthesis (anth_d) were taken from a previous report (Foley, 2012).

Xylem embolism resistance of stems was measured using the standard centrifuge technique (Alder et al., 1997) between August 22 and September 23, 2016. Genotypes were sampled and measured evenly across this time period to ensure that differences among genotypes were not confounded with sampling time. Five replicate plants were measured for each genotype in the fully-watered treatment (plants grown under deficit were not measured). Stems from each genotype were trimmed appropriately to ensure nearly all vessels were “closed” prior to measurement (Fig. S1, methods SI). Loss of conductance was measured using a custom made 15-cm diameter rotor (Michigan State University Machine Shop) and a Sorvall superspeed centrifuge (RC2-B, Sorvall inc., Norwalk, USA) (see Alder et al., 1997). The percent loss of stem-specific conductivity (PLC), relative to the maximal observed value for each stem, was plotted against xylem tension and fit with exponential sigmoid models after Pammenter and Vander Willigen (1998) using the ‘nlsLM’ function in the minpack.lm package developed for R (Elzhov et al., 2016). The water potentials associated with 12, 50, and 88% loss of conductivity were solved from the fitted models for each stem, i.e., “P_{12}”, “P_{50}”, “P_{88}”. PLC values were also estimated for midday conditions (PLC_{MD}) by solving fitted models for the leaf water potentials measured during midday hours (described below).

“Calculated” stem-specific conductivity (K_{S_calc}) across all 13 genotypes in 2016 was measure from stained stem cross-sections (5 replicates per genotype) taken from the fully-watered treatment. Although stem-specific conductivity was obtained empirically during the measurement of embolism resistance (described above), we decided to also estimate this trait from the measured anatomy for two reasons. Firstly, stem-specific conductivity scales strongly with vessel diameter and number (Gleason et al., 2015; Sperry et al., 2007), and as such, these xylem traits may serve as good breeding targets. Secondly, the embolism resistance measurements revealed a ˜5% decrease in conductance between the first and second centrifuge spins, but sigmoidal loss of conductance thereafter. Considering that this initial drop in conductance was likely caused by aquaporins and/or ion-mediated changes in xylem conductance, we had more confidence in stem-specific conductivity values that were calculated directly from vessel anatomy.

Leaf water potentials were measured at predawn (ca 0500 h; Ψ_{PD}) and at “solar-noon” midday (ca 1400 h; Ψ_{MD}) using a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp, Santa Barbara, CA, USA). Predawn measurements were carried out August 8–10, 2016 and midday measurements were carried out August 15–17, 2016 (G_S was measured in the preceding two weeks). All 13 genotypes in each irrigation treatment were measured. On each measurement day, three individual plants of each genotype within each of three blocks per irrigation treatment were measured, giving nine replicates per genotype within each irrigation treatment.

Maximal net CO_2 assimilation (A_{max}) and stomatal density data for the same NAM founder lines were taken from a previously published report (Foley, 2012). Briefly, A_{max} values were measured on greenhouse-grown plants (four weeks old), whereas stomatal density data were obtained from field-grown plants (at V14) at the Purdue Agronomy Center for Research and Education, West Lafayette, IN (40.47 °N, -86.99 °W, 216 m asl) in 2010 and 2011.

Root morphological traits of the NAM founder lines were taken from a previous report (Zurek et al., 2015). This included 20 different root traits that were measured on seedlings (at 3, 6, 9, and 12 d after planting) of the founder lines under non-drought conditions using a gel-based root imaging platform. Details of the site, plants, and imaging system are described in Zurek et al. (2015) and Topp et al. (2013). The root width-to-depth ratio (RWDR), specific root length (SRL), total root volume (root_vol), and root depth (root_depth) were taken from these previous analyses.

2.4. Analyses

Mean genotype trait values were used in most analyses because our primary aim was to understand *across-genotype* variation in traits conferring faster growth. However, we report all observations (including within-genotype observations) in the supplemental information (Tables S1-S5). Traits collected in 2016 (on 13 genotypes) served as our primary dataset for the analyses because a more extensive range of traits was measured in 2016. However, the end-of-season biomass used in these analyses for genotypes measured both years includes values averaged across both years. We did this because the previously described hail storm that occurred near the end of the 2016 growing season (August 19), significantly shredded the leaves and resulting in some biomass loss. As such, we feel the mean biomass data better reflect plant performance overall. However, we do mention where these analyses differed meaningfully between years.

The predictive capacity of plant traits and developmental stage on growth (end-of-season biomass) were evaluated using Pearson correlation, lasso regression (least absolute shrinkage and selection operator) (Tibshirani, 2011, 1996), and structural equation modeling. Mean genotype values were used for all these analyses. Lasso is primarily a variable selection method that minimizes the inclusion of spurious covariates (i.e., avoids inflated type 1 error), whilst also reducing bias and improving statistical power over more traditional methods (e.g., step-wise regression). Lasso does this by applying a regularization parameter (λ) that “shrinks” the model coefficients towards zero, thus eliminating some variables from the model. Lasso was used to identify traits with meaningful predictive capacity on end-of-season biomass. Initially 14 physiological, morphological, and developmental traits were included in the model, as informed by bivariate correlation, and previous studies. The regularization parameter λ was then increased (shrinking variable coefficients towards zero), resulting in the elimination of variables in sequence of their coefficient size (from smallest to largest), until the mean standard error of the model increased to within 1 standard error of the full model. As such, the selected model represented the most highly regularized model that was still within 1 standard error of the full model. The R package “glmnet” was used to perform the lasso analyses (Friedman et al., 2010).

After choosing the most parsimonious regression model for the prediction of biomass growth, we then wished to better understand how the hydraulic traits in this model, i.e., the traits associated only with the transport and use of water, were coordinated to achieve higher growth under water deficit. Firstly, we plotted individual bivariate correlations among all trait variables to understand trait-by-trait association. We then tested different structural equation models (SEM) to determine which traits contributed meaningfully to growth and under what circumstances (i.e., well-watered or deficit treatments). To do this we first developed a conceptual path model to describe the relationships among hydraulic and stomatal traits, and how these traits might relate to growth. The structure of this model was informed by the lasso regression, the bivariate correlations, as well as previous reports (Fan et al., 2012; Hoeber et al., 2014; Kondoh et al., 2006; Sterck et al., 2012). Secondly, key connections between traits were removed and the model fit again. The total explained variance by both of these models (with and without these key connections) was then compared via ANOVA. This procedure was repeated for the deficit and well-watered datasets individually, to determine if some traits were more important than others under deficit conditions vs well-watered conditions. Owing to the small number of observations for all measured traits (13 genotypes), Monte Carlo simulation was used to determine statistical power, bias, and the coverage obtained by the SEM models (Muthén and Muthén, 2002). Results from the Monte Carlo simulation resulted in the scaling back of the original SEM models to four or fewer variables. SEM models were fit with the ‘lavaan’ package for R (Rosseel, 2012). Monte Carlo simulation was performed using the ‘simsem’ package for R (Jorgensen et al., 2018). All data were scaled to zero mean and unit variance prior to fitting SEM and lasso regression models.

3. Results

3.1. Traits correlated with higher end-of-season biomass

Fourteen traits were initially evaluated as predictors of growth: maximal stomatal conductance (G_{S_max}), midday stomatal conductance (G_{S_MD}), calculated stem-specific conductivity (K_{S_max}), leaf water potential at predawn (Ψ_{PD}), leaf water potential at midday (Ψ_{MD}), the xylem water potential associated with 50% loss of stem-specific conductivity (P_{50}), the percent loss of conductivity at midday (PLC_{MD}), the hydraulically-weighted vessel diameter (D_H), maximal net CO_2 assimilation rate (A_{max}), number of growth days to anthesis (anth_d), the width-to-depth ratio of the root system (RWDR), the root length-to-mass ratio (SRL; specific root length), total root volume (root_vol), and the depth of the root system (root_depth). Of these fourteen variables initially included in the lasso models, most traits had little independent predictive capacity on end-of-season biomass. The final lasso models included only maximal stomatal conductance (G_{S_max}) and the root width-to-depth ratio (RWDR) for the fully-watered treatment ($R^2 = 0.68$; $P < 0.001$) and maximal net CO_2 assimilation rate (A_{max}), stomatal conductance at midday (G_{S_MD}), RWDR, and SRL for plants grown under deficit irrigation ($R^2 = 0.88$, $P < 0.001$) (Table 1). This does not mean that other traits, which also exhibited significant correlation with growth (Fig. 1), were not important, but only that their contributions to growth were closely aligned with the selected traits. Considering the importance of gas exchange in the lasso analysis, we then narrowed our focus to determine the direct and indirect contributions of xylem and stomatal traits on growth.

Table 1
Lasso regression results for fully-watered and deficit treatments. End-of-season biomass was fit as the dependent variable in all models. Initial models started with 14 xylem, stomatal, developmental, and gas-exchange traits as the independent variables. Only variables remaining after regularization (i.e., with coefficients > 0) were included in the final model. Variable names are the same as in Fig. 1.

Lasso regression models	df	R^2	RMSE	P	AIC
Fully-watered treatment					
G_{S_max}	10	0.60	0.718	0.014	−6.33
+ RWDR	9	0.68	0.595	0.043	−9.62
Deficit treatment					
A_{max}	*10*	0.44	0.747	0.007	−5.47
+ G_{S_MD}	9	0.78	0.492	0.005	−13.82
+ RWDR	8	0.86	0.413	0.060	−16.96

3.2. Re-ranking of trait importance under well-watered versus deficit scenarios

There was a clear shift in the predictive capacity of some plant traits on end-of-season biomass from the fully-watered to the deficit treatment. The fully-watered treatment exhibited strong correlation between maximal gas-phase conductance (G_{S_max}) and biomass ($r = 0.64$, $P = 0.018$), whereas the deficit treatment did not ($r = 0.22$, $P = 0.472$). Stomatal conductance during the hottest and driest part of the day (G_{S_MD}) was positively correlated with biomass (Fig. 1a,c), but there were subtle differences between years, with stronger correlation exhibited in the fully-watered treatment in 2016 and stronger correlation in the deficit treatment in 2015 (Fig. 2). SEM revealed that the ability to maintain stomatal conductance through midday (G_{S_MD}) was a key trait linked to higher biomass production in the deficit treatment, but not the fully-watered treatment, i.e., the removal of this path in the SEM resulted in a significantly poorer fitting model in the deficit treatment ($P = 0.015$), but not the fully-watered treatment ($P = 0.961$) (Fig. 1b,c; Table 2). Furthermore, when grown under fully-watered conditions, the supply of water through the plant stem (K_{S_max}) to the stomata during morning hours (G_{S_max}) was strongly linked to higher biomass production, and the removal of this path from the SEM resulted in a poorer fit ($P = 0.083$). In contrast, water supply through the stem to the stomata during the morning hours was not an important path leading to higher growth in the deficit treatment ($P = 0.990$), whereas water supply to the stomata during the middle of the day was, i.e., the removal of this path resulted in a poorer fitting model ($P = 0.062$). This is not to say that midday stomatal conductance was not an important trait in the fully-watered treatment. In fact, bivariate correlation between biomass and midday stomatal conductance were stronger in the fully-watered treatment than in the deficit treatment in 2016 (Fig. 2a). The calculated conductive capacity of stem tissue (K_{S_calc}) was conferred mainly via larger diameter vessels ($r = 0.93$; $P < 0.001$), rather than the total number of vessels ($r = -0.30$; $P = 0.314$), and as such, both stem-specific conductivity and the hydraulic diameter were correlated with midday stomatal conductance (Fig. 3a,b).

Although we restrict SEM to traits we measured in this study, bivariate correlation suggested there may have been a shift in the importance of root traits from the fully-watered to deficit treatment. The depth of the seedling root system, and specific root length (length/mass) were significantly correlated with both maximal and midday stomatal conductance under fully-watered conditions ($P < 0.1$ in all

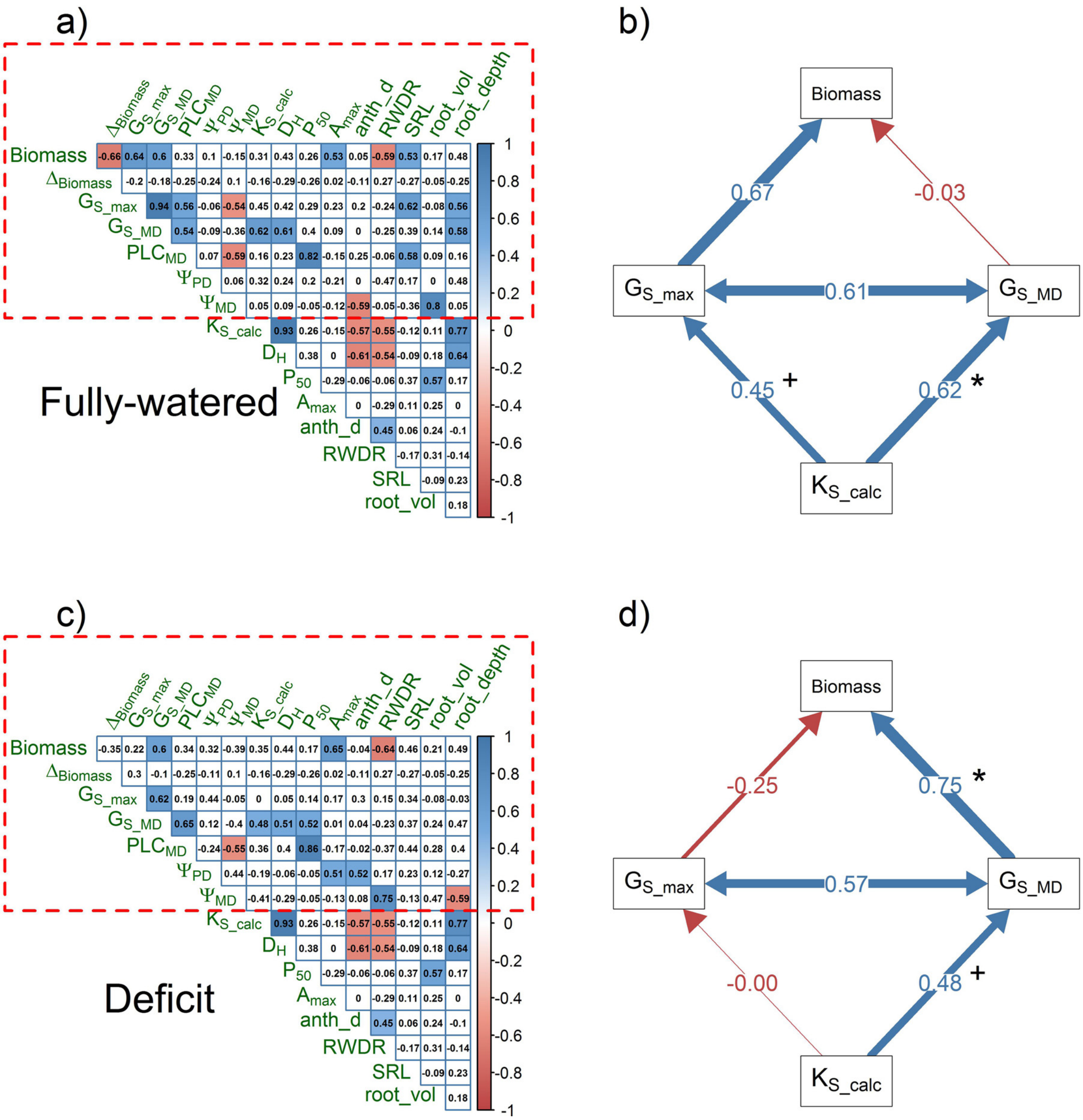

Fig. 1. Pearson's correlation matrices (a, c) and structural equation models (SEM) (b, d) for well-watered (a, b) and deficit (c, d) treatments. Significant ($\alpha = 0.10$) correlation coefficients are denoted by shading, with color denoting direction (blue = +; red = -). Functional relationships in the SEM are represented as single-headed arrows, whereas correlative relationships are represented with double-headed arrows. Standardized path coefficients are shown and also represented by arrows, with color denoting direction similar to the correlation matrix, and the thickness indicating relative coefficient size, with thicker arrows denoting larger coefficients. "*" and "+" denote statistical significance ($P < 0.05, 0.10$) of individual paths, where their removal from the SEM resulted in a significantly poorer fit. All traits were measured in 2016 except for total biomass, which represents the mean of both years. Biomass = end-of-season biomass; $\Delta_{Biomass}$ = the fractional change in biomass from the fully-watered to the deficit treatment; G_{S_max} = maximal daily stomatal conductance; G_{S_MD} = midday (1400 h) stomatal conductance; PLC_{MD} = the percent loss of stem-specific conductivity at midday (1400 h); Ψ_{PD} = leaf water potential at predawn; Ψ_{MD} = leaf water potential at midday (1400 h); K_{S_calc} = calculated stem-specific conductivity; D_H = hydraulically-weighted vessel diameter; P_{50} = xylem water potential corresponding to a 50% loss of stem-specific conductivity; A_{max} = maximal net CO_2 assimilation; anth_d = growing days required between germination and anthesis; RWDR = the root width-to-depth ratio of the root system; SRL = specific-root-length (root length / dry root mass); root_vol = convex hull volume of the root system.

cases), but not under deficit conditions. However, considering that root traits were not measured under deficit conditions (the genotype-specific response is unknown), there may have been a re-ranking of root traits scores across genotypes, and this may be responsible for the apparent lack of correlation between some root traits and performance under deficit.

Aside from the observed shift in the correlation matrix from fully-watered to deficit, as described above, there were remarkable similarities between treatments as well. High stomatal conductance during the middle of the day was a primary correlate with end-of-season biomass under both fully-watered ($r = 0.60$; $P = 0.030$) and deficit ($r = 0.60$; $P = 0.031$) conditions. Similarly, the calculated supply of water through the stem xylem (K_{S_calc}) was correlated with the maintenance of midday stomatal conductance in fully-watered ($r = 0.62$; $P = 0.024$) and deficit ($r = 0.48$; $P = 0.094$) treatments. This result, as well as the strong correlation between root depth at the seedling stage and the K_{S_calc} ($r = 0.77$; $P = 0.003$), suggests that plant performance under both fully-watered and deficit conditions depends critically not only on capacities of the roots, stems, and leaves to transport water to the stomata, but also on the close coordination of these systems.

Counterintuitively, the percent loss of stem-specific conductivity at midday was *positively* associated with G_{S_MD}, i.e., stems that had lost a

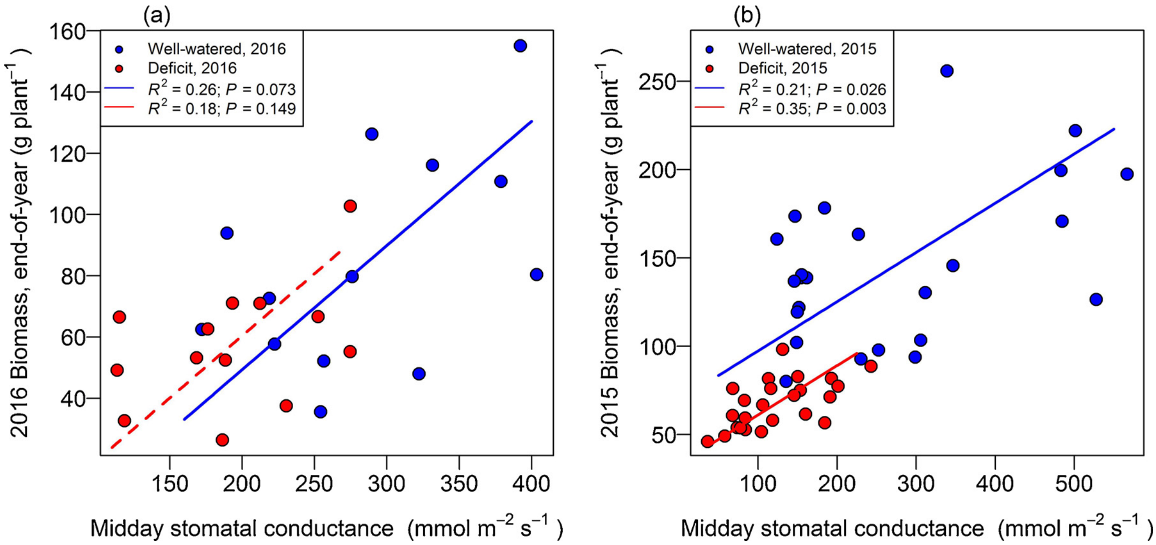

Fig. 2. Relationship between the end-of-season biomass and the achievable stomatal conductance during the middle of the day in 2016 (a) and 2015 (b). Standard major axes models were fit independently to well-watered and deficit data when the fitted coefficients differed significantly between treatments (e.g., 2015). Each symbol represents a mean genotype value in either the well-watered (blue) or deficit (red) treatments.

greater percentage of their water transporting capacity were associated with *higher* levels of stomatal conductance during the middle of the day in both fully-watered (r = 0.54; $P <$ 0.056) and deficit (r = 0.65; $P <$ 0.016) treatments (Fig. 1a,b).

3.3. Trait variation among the NAM founder lines

Owing to the large range of genotypes and traits measured in this study, we do not attempt to report all genotype comparisons nor all treatment interactions here, but rather, highlight the key differences among the genotypes. We note that all observations for each trait have been included as csv files (Tables S2-S5) as well as the genotype summary data, which were used in the bivariate regression, lasso regression and structural equation models (Table S1).

The lasso regression and structural equation results emphasize the importance of water transport, water use, and photosynthetic capacity on maize biomass accumulation. Examining each NAM genotype on a case-by-case basis revealed clear differences in these traits. Total biomass in 2015 was correlated with total biomass in 2016 across both treatments (r = 0.74; $P <$ 0.001). The strength of this correlation improved in the well-watered treatment (r = 0.77; P = 0.002) and weakened in the deficit treatment (r = 0.48; $P <$ 0.099). Nevertheless, the leading genotypes achieving maximal biomass in the deficit treatment were similar in both years, with CML103, B97, and Ms71 being among the top five genotypes in both years (Fig. S2, Table S2). Similarly, NC350 was the poorest yielding genotype in 2016 ($\bar{x}$ = 26.4 g plant^{-1}; SD = 8.76) and the second poorest yielding genotype in 2015 ($\bar{x}$ = 46.0 g plant^{-1}; SD = 12.3) (Table S2).

Results for midday stomatal conductance were similar, with significant correlation between years when pooling treatments (r = 0.57; P = 0.002), but with weaker correlation in the well-watered treatment (r = 0.38; P = 0.200) and deficit treatment (r = 0.44; P = 0.14), individually. CML322 exhibited the highest midday stomatal conductance under deficit irrigation in 2015 and the second highest in 2016, whereas Mo17 exhibited the lowest midday stomatal conductance in 2015 and the second lowest in 2016 (Fig. S3), which is consistent with a previous report (Foley, 2012). The complete raw data, i.e., all stomatal conductance measurements on all genotypes and all years, are reported in Table S3.

Calculated stem-specific conductivity differed 7-fold across genotypes (Table S1, S4). The highest calculated stem-specific conductivities were exhibited by B97 ($\bar{x}$ = 2.08 kg m^{-1} s^{-1} MPa^{-1}; SD = 0.62), Ms71 ($\bar{x}$ = 1.37 kg m^{-1} s^{-1} MPa^{-1}; SD = 0.58), and CML322 ($\bar{x}$ = 1.07 kg m^{-1} s^{-1} MPa^{-1}; SD = 0.27), whereas the lowest were exhibited by Mo18W ($\bar{x}$ = 0.30 kg m^{-1} s^{-1} MPa^{-1}; SD = 0.08), Ki11 ($\bar{x}$ = 0.54 kg m^{-1} s^{-1} MPa^{-1}; SD = 0.30), and CML69 ($\bar{x}$ = 0.56 kg m^{-1} s^{-1} MPa^{-1}; SD = 0.21) (Table S4).

Hydraulic safety ranked similarly across genotypes at all three K-loss thresholds (12%, 50%, 88%) (Fig. S4). Mo18W, Mo17, Ky21, and B97 exhibited the lowest P_{12}, P_{50}, and P_{88} values (i.e., safest from

Table 2
Fit statistics for all structural equation models (SEM). SEM were fit to well-watered and deficit datasets. For each full model, key paths were removed (e.g., "w/o Gs_{MD} ~ Ks_{max}") and the model fit again. Important paths, i.e., those resulting in a significantly poorer global fit after removal, are denoted with bold text *chi-sqr* values and associated test result ("+" and" *" denoting α values of 0.1 & 0.05, respectively). Model fit improves with increasing P, CFI (comparative fit index), and with decreasing chi-sqr, RMSEa and AIC values. The predictive capacity on growth (Biomass R^2) is also reported. Trait abbreviations are the same as given in Fig. 1.

Model	chi-sqr	P	CFI	RMSEa	RMSEa -CL	RMSEa + CL	AIC	Biomass R^2
Well-watered treatment, full model	0.04	0.844	1.000	0.000	0.000	0.421	79	0.412
w/o Biomass ~ Gs_{MD}	0.04	0.980	1.000	0.000	0.000	0.000	77	0.412
w/o Biomass ~ Gs_{max}	1.16	0.561	1.000	0.000	0.000	0.468	78	0.359
w/o Gs_{MD} ~ Ks_{calc}	**6.35***	0.042	0.887	0.409	0.068	0.786	121	0.474
w/o Gs_{max} ~ Ks_{calc}	**3.04**+	0.218	0.973	0.200	0.000	0.622	118	0.414
Deficit treatment, full model	0.01	0.937	1.000	0.000	0.000	0.192	104	0.397
w/o Biomass ~ Gs_{MD}	**5.94***	0.051	0.700	0.389	0.000	0.769	108	0.048
w/o Biomass ~ Gs_{max}	0.79	0.673	1.000	0.000	0.000	0.418	103	0.359
w/o Gs_{MD} ~ Ks_{calc}	**3.48**+	0.176	0.888	0.239	0.000	0.648	144	0.364
w/o Gs_{max} ~ Ks_{calc}	0.01	0.997	1.000	0.000	0.000	0.000	140	0.397

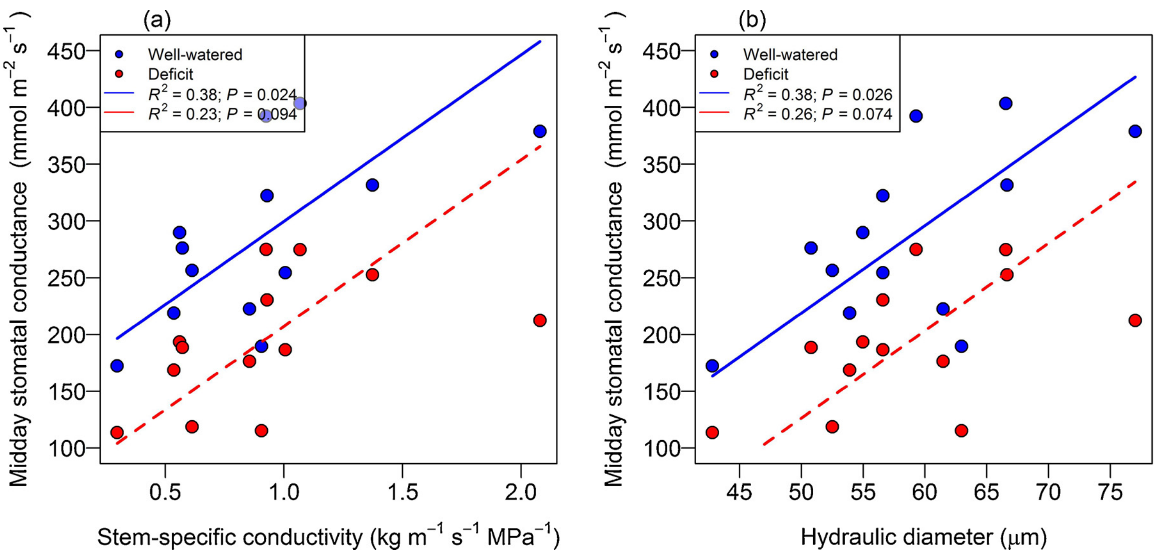

Fig. 3. Relationship between the achievable stomatal conductance during the middle of the day in 2016 and calculated stem-specific conductivity (a), and hydraulically-weighted vessel diameter (b). Standard major axes models were fit independently to well-watered and deficit data when the fitted coefficients differed significantly between treatments. Each symbol represents a mean genotype value in either the well-watered (blue) or deficit (red) treatments.

embolism) (Fig. S5). Mo18W had particularly low P_{50} (-2.37 MPa; *SD* = 0.20) and P_{88} (-3.50 MPa; *SD* = 0.25) values (Fig. S5). These values were significantly lower than that exhibited by any other genotype in the study, except for Ky21 ($P < 0.05$). In comparison, the mean P_{88} value among the other 12 genotypes measured (omitting Mo18W) was -2.37 MPa (*SD* = 0.33), revealing that the P_{88} value for Mo18W was more than a whole MPa unit lower than the mean value among the other genotypes. The genotypes exhibiting the highest P_{12} values (least safe from embolism) were CML103, Ms71, and B73, whereas the genotypes exhibiting the highest P_{50} and P_{88} were NC350, CML103, and B73 (Fig. S5). K-loss models and fitted parameters are reported in Table S5.

Predawn water potentials were similar across all genotypes in both the well-watered and deficit treatments ($P > 0.288$), whereas midday water potentials differed significantly among some genotypes in both the well irrigated and deficit treatments (Table S1). Most notably, B97 ($\bar{x}$ = -1.90 MPa; *SD* = 0.139), B73 ($\bar{x}$ = -1.84 MPa; *SD* = 0.202), and Mo18W ($\bar{x}$ = -1.82 MPa; *SD* = 0.223) had the lowest midday leaf water potentials under deficit irrigation, whereas Mo17 ($\bar{x}$ = -1.42 MPa; *SD* = 0.180), Ki11 ($\bar{x}$ = -1.48 MPa; *SD* = 0.200), and Oh7B ($\bar{x}$ = -1.50 MPa; *SD* = 0.213) had the highest leaf water potentials under deficit. The rankings among genotypes changed somewhat between the deficit and well-watered treatments during midday, although Mo18W was among the lowest three genotypes and Mo17 exhibited the highest leaf water potential in both treatments. B97 not only exhibited the lowest midday water potentials under deficit, but also exhibited the highest stem-specific conductivity and a high degree of hydraulic safety from embolism. In contrast to this, Mo18W exhibited low midday leaf water potentials under drought, as well as the highest embolism resistance, but had the lowest stem-specific conductivity.

For those interested in gas exchange and root trait differences among the genotypes we report mean values in the genotype-specific trait data (Table S1), but direct the reader to Foley (2012) and Zurek et al. (2015) for the original data.

4. Discussion

4.1. *Water transport, stomatal conductance, and root architectural traits as a functional assemblage*

We report here for the first time, empirical evidence linking the performance of maize, under both well-watered and deficit conditions, to root and xylem traits conferring greater stomatal conductance. These results are well aligned with our present understanding of how physiological systems are connected and organized, not just in maize, but in vascular species more generally. For example, the rate of CO_2 assimilation increases monotonically with stomatal conductance, and water expenditure at the stomata must be continuously suppled via the vascular and root systems. Therefore, the structure and functioning of these systems must be coordinated via natural and artificial selection, and therefore, efforts to improve crop species would likely benefit from considering the functioning of these traits as a connected assemblage.

Lasso regression identified three significant predictors of biomass production under deficit irrigation conditions: 1) the maximal net CO_2 assimilation rate, 2) the maximum achievable stomatal conductance during the hottest part of the day, and 3) the width-to-depth ratio of the seedling root system, i.e., deeper, rather than wider root system development facilitating growth. Taken together, gas exchange (i.e., exchange of water for CO_2), the delivery of the water to the stomata, and the access to water via the root network appear to be the core processes sustaining growth under the drought conditions provided in this experiment.

Three important results emerged from the SEM analyses. Firstly, the maintenance of high stomatal conductance during the hottest part of the day was associated with high stem-specific conductivity, even though there was no direct bivariate correlation between stem-specific conductivity and growth (fully watered and deficit conditions). This suggests that when evaluating growth˜trait coordination, it is important to work from a plausible causal model, and evaluate both direct and indirect paths. The second key insight from these analyses is that, to a large extent, the traits conferring faster growth under deficit were the same traits conferring faster growth under well-watered conditions, as reported by others (Lopes et al., 2011). The important caveat to this is that the effect of greater calculated stem-specific conductivity and traits aligned with it (e.g., root depth, and root width-to-depth ratio) influenced biomass accumulation more strongly via *maximal* stomatal conductance when plants were grown under fully-watered conditions, but via *midday* stomatal conductance when grown under water deficit.

The linkage we report here between water transport, stomatal conductance and growth is supported by empirical results in cotton, maize, wheat, cacaco, poplar, and eucalyptus, (Dabbert and Gore, 2014; Fischer et al., 1998; Gleason et al., 2017a; Hajek et al., 2014; Kotowska et al., 2015; Pita et al., 2005; Schreiber et al., 2011). Functional linkage between water transport capacity and growth appears to have arisen not only within species, but also across species, for which the evidence is now quite clear (Brodribb et al., 2007; Fan et al., 2012; Gleason et al., 2018b; Hoeber et al., 2014). Taken together, we suggest that it is logical to expect the growth rates of vascular species to align with root and xylem traits that improve access to water and its delivery to the stomata. We should probably expect this, except in cases where water conservation is both possible (i.e., using soil as a storage reservoir) and necessary (Messina et al., 2015; Reyes et al., 2015; Sinclair et al., 2005; Zaman-Allah et al., 2011a).

4.2. Traits conferring better performance in contrasting drought scenarios

We should not expect the same plant traits to confer improved performance under drought in all cases. For example, limited transpiration during high-VPD hours would likely lead to improved performance under some conditions, whereas greater soil water extraction would likely lead to improved performance under other conditions (Chenu et al., 2013; Tardieu, 2012; Turner et al., 2014). Although we do not attempt to review the broad literature on this topic here, we feel it is necessary to address the theoretical and empirical support for both of these crop strategies (water conservation vs water extraction), and to what extent they are compatible with one another.

Limited transpiration crops are desirable in some situations, not because they use less water per se, but rather, by using less water (particularly at high VPD) the efficiency and timing of their water use can be improved, either seasonally integrated or instantaneous (Sinclair et al., 2005; Sinclair and Muchow, 2001). For example, in many parts of the world planting coincides with adequate precipitation and soil moisture, but hot dry summers result in water-limited growth later in the season, often when reproductive structures are developing. In this scenario, if plants can be designed/bred to use water more frugally (via limited transpiration) early in the season, then water may be banked in the soil and used later in the season when it is needed. This idea is supported strongly by theory (Berger et al., 2016; Sinclair, 2018; Sinclair et al., 2017), simulation (Messina et al., 2015; Sinclair et al., 2010, 2005), as well as direct and indirect empirical results (Khan et al., 2007; Reyes et al., 2015; Schoppach et al., 2017; Zaman-Allah et al., 2011b, 2011a). However, the assumption soil water not used by crops early in the season will remain in the soil for several months (at accessible water potential), and not be lost to evaporation and capillary movement appears questionable, especially in situations where unsaturated flow can be considerable (Green and Anapalli, 2018).

The results reported here should also be evaluated in view of the small plot size, i.e., two planted rows with borders on either side, and that only one drought scenario was evaluated. Under these conditions, traits leading to greater water acquisition (i.e., accessing water deeper in the profile or held at lower water potential) would more likely lead to greater yield potential (Blum, 2009; Brodribb et al., 2015) than would increasing transpiration efficiency (Sinclair et al., 2017). This is because the opportunity cost of reducing transpiration during high-VPD hours is that non-transpired water then becomes available to neighboring plants of a different genotype, i.e., water stored in the soil cannot be used later in the season (Rebetzke et al., 2014).

Considering the large variation in climate and soil across the world, it has been suggested that every individual situation may require a specifically designed genotype. The extent to which this is true, or that we might expect some traits to confer improved performance in most cases (Blum, 2017), remains largely untested. It is however important to recognize that limiting transpiration via stomata (Medina et al., 2017; Ryan et al., 2016) or structural characteristics (Borrell et al., 2014; George-Jaeggli et al., 2017; Sutka et al., 2016) are largely at crossed-purposes to traits conferring greater water acquisition, transport, and use (Blum, 2009). It therefore seems of critical importance to evaluate these two broad crop strategies under different drought/management scenarios in the field. Although efforts have addressed this question (Gaffney et al., 2015; Reyes et al., 2015; Tolk et al., 2016), we feel that including genotypes with well-understood and contrasting physiological and structural characteristics would assist this effort.

4.3. The relative importance of xylem efficiency and embolism resistance under well-watered and deficit conditions

The similarity between traits conferring greater growth under deficit and well-watered conditions should not be surprising considering that water transport during midday, even under well-watered conditions, is a demanding process. For example, maize commonly loses greater than 50% of its conductive capacity during the day, even when soil water potential is high, but then regains this conductance at night (Gleason et al., 2017a; McCully, 1999). As such, higher yield must be coordinated with hydraulic and stomatal functioning in response to both atmospheric and soil aridity. Most importantly, the maize genotypes that were able to maintain higher midday stomatal conductance did so largely by starting out with a higher stem-specific conductivity in the morning, rather than exhibiting a shallower trajectory of conductivity loss, i.e., greater hydraulic safety.

The rather counterintuitive correlation (i.e., positive slope coefficient) between embolism vulnerability and midday stomatal conductance in this study is difficult to interpret, and it is therefore unclear if improvement of hydraulic safety would also lead to better performance of maize under drought. On one hand, the observed positive correlation between these traits may reflect an intrinsic tradeoff between growth and survival, as has been suggested to exist in woody perennial species (Meinzer et al., 2010; Sperry et al., 2008). For example, it has long been assumed that the xylem traits that confer hydraulic safety are the exact same traits that reduce maximal hydraulic efficiency, and therefore growth (Carlquist, 1975). However, in the case of maize, which exhibits significant capacity to refill embolized vessels overnight (Gleason et al., 2017b), natural and artificial selection may have placed a premium on the ability to repair embolized stems, rather than the ability to avoid embolism in the first place. If the improvement of embolism safety and embolism repair represent alternative strategies, then we might expect inverse correlation between these two traits, as well as traits closely aligned with this axis of variation. In particular, isohydric vs anisohydric stomatal behavior may underpin the divergence in strategies aligned with either embolism repair or embolism avoidance (Attia et al., 2015; Meinzer et al., 2016). However, stem hydraulic safety (e.g., P_{50}) and hydraulic efficiency (K_{S_calc}) were not inversely correlated in this study, nor is it clear that there exists a constraint (e.g., a structural, biochemical, or developmental conflict) that would prevent the improvement of both safety from embolism and stem-specific conductivity in crop plants (Gleason et al., 2016, 2015).

4.4. Where do we go from here?

Considering the significant advancements in plant breeding and gene editing methods that have been developed over the last decade, our understanding of the physiological determinants of drought tolerance has made little progress by comparison. Central to this dilemma are three important obstacles. Firstly, it is uncertain what plant traits actually confer improved performance under drought. Secondly, it is not well understood if plant traits represent mutually exclusive alternatives to one another (i.e., tradeoffs), or if they represent orthogonal processes. Thirdly, it is not well understood if the efficacy of traits should be ranked similarly under all drought scenarios, or if different rankings should be considered under different circumstances. In this study we addressed the first of these questions as it applies to deficit irrigation in small plots that are similar in size to those used in plant breeding trials. We also addressed the second question, to the extent that it can be evaluated from correlative analyses. Our results provide evidence that stem-specific conductivity and stomatal conductance during the middle of the day are important contributing traits to the higher growth of maize under drought.

Despite the progress that has been made linking hydraulic phenotypes to performance, the genetic underpinnings of hydraulic traits remain poorly understood. We suggest that the NAM panel is uniquely designed to provide this understanding. As such, a logical next step would be to identify one or two key hydraulic traits (e.g., hydraulically-weighted vessel diameter, xylem hydraulic efficiency) and measure these traits across the 25 NAM families (i.e., all 5000 recombinant inbred lines). This would allow for a whole-genome association analysis (GWAS; genome-wide association study) and represents our best chance to elucidate the genetic architecture underpinning these hydraulic traits in maize. Towards the development of a breeding population, we suggest beneficial alleles could be identified and accessed via broad survey across extant maize collections (e.g., the USDA GRIN collection).

The inverse correlation between stomatal conductance and hydraulic safety suggests that hydraulic safety may not be necessary to achieve higher growth under moderate drought, but this has not been tested under contrasting drought scenarios, e.g., recovery from short but severe drought or more severe deficit irrigation during vegetative growth. We suggest that this largely untested question deserves more attention. Specifically, whether or not the relative importance of stem-specific conductivity and hydraulic safety differ under severe vs moderate drought requires additional study.

Acknowledgements

We warmly thank the technicians and scientists at the Water Management and Systems Research Unit, USDA-ARS, Fort Collins, CO, for their supportive contributions to this study. Specifically, we would like to thank Gerald Buchleiter, Garrett Banks, Liam Cummins, Joe Miller, Veronica Travers, Ming Han, Katie Willi, Kevin Yemoto, Ross Steward, KaMele Sanchez, Bailey Kraich, Cory Arnold, and Ben Choat for their herculean efforts maintaining the irrigation facility in 2015 and 2016. We would also like to thank the USDA National Germplasm Resources Laboratory for providing the seed for this study and Timothy Green for advice on water movement in soils.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fcr.2019.02.001.

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