

1 Growth and grain yield of eight maize hybrids is aligned with water transport, stomatal conductance, and
2 photosynthesis in a semi-arid irrigated system

3
4 Sean M. Gleason^{1,2}, Lauren Nalezny¹, Cameron Hunter^{1,2}, Robert Bensen³, Satya Chintamanani⁴, Louise H.
5 Comas¹

6
7 ¹Water Management and Systems Research Unit, United States Department of Agriculture, Agricultural Research
8 Service, Fort Collins, CO 80526, USA

9 ²Department of Biology, Colorado State University, Fort Collins, CO 80523, USA

10 ³Syngenta Biotechnology Inc., Stanton, MN 55018, USA

11 ⁴Syngenta Seeds Inc., Slater, IA 50244, USA

12
13 **Abstract**

14 There is increasing interest in understanding how trait networks can be manipulated to improve the performance
15 of crop species. Working towards this goal, we have identified key traits linking the acquisition of water, the
16 transport of water to the sites of evaporation and photosynthesis, stomatal conductance, and growth across eight
17 maize hybrid lines grown under well-watered and water-limiting conditions in Northern Colorado. Under well-
18 watered conditions, well-performing hybrids exhibited high leaf-specific conductance, low operating water
19 potentials, high rates of midday stomatal conductance, high rates of net CO₂ assimilation, greater leaf osmotic
20 adjustment, and higher end-of-season growth and grain yield. This trait network was similar under water-limited
21 conditions with the notable exception that linkages between water transport, midday stomatal conductance, and
22 growth were even stronger than under fully-watered conditions. The results of this experiment suggest that
23 similar trait networks might confer improved performance under contrasting climate and soil conditions, and that
24 efforts to improve the performance of crop species could possibly benefit by considering the water transport
25 pathway within leaves, as well as within the whole-xylem, in addition to root-level and leaf-level traits.

26
27 **Abbreviations:**

28 Ψ_{MD} = leaf water potential during midday hours (1200 - 1400 hrs)

29 Ψ_{PD} = leaf water potential during predawn hours (0500 - 0630 hrs)

30 Ψ_s, Ψ_L = water potential of soil and leaf, respectively

31 π_o = leaf osmotic potential at full turgor

32 π_{tip} = leaf osmotic potential at turgor loss

33 ϵ = cell wall modulus of elasticity

34 L_A = leaf area

- 35 A_N = light-saturated, net CO₂ assimilation rate
36 D = the leaf-to-atmosphere vapor pressure deficit
37 E = transpiration
38 g_s = stomatal conductance
39 g_{s_max} = maximum achievable stomatal conductance
40 g_{s_MD} = stomatal conductance during the middle of the day (1400 hrs)
41 $g_s \sim VPD_slope$ = slope of the $g_s \sim VPD$ function when VPD is equal to 3.0 kPa
42 $g_s \sim VPD_turn$ = VPD value where the slope of the $g_s \sim VPD$ function becomes negative
43 k_{leaf_max} = maximal leaf-specific hydraulic conductance
44 K_x = xylem-specific conductivity
45 L = path-length between soil water and the sites of evaporation within the leaf
46 P_{50} = leaf water potential resulting in a 50% loss of maximal leaf conductance

47

48

49 **Keywords:** xylem; hydraulic conductance; water potential; crop traits; crop improvement; drought tolerance

50

51

52 **Introduction**

53

54 Crop performance is an outcome of the coordinated functioning of many physiological processes. The need for a
55 holistic understanding of the mechanisms underlying plant performance is becoming increasingly recognized,
56 especially for complex responses such as growth and grain production under drought (Tardieu et al. 2018).
57 Crop improvement could likely be facilitated by considering multiple physiological traits together, as well as the
58 connections between these traits and how these connections shift under different soil and climate scenarios
59 (Gleason et al. 2019). Although the idea that selection for multiple traits might result in better outcomes has
60 been suggested previously (Campos et al. 2004; Condon 2020), what is becoming more clear is the need to
61 include linkages connecting soil water, its transport to (near) the stomata, and the photochemistry that these
62 processes support (Turner et al. 2014; Brodribb et al. 2015; Gleason et al. 2017a). Several physiological traits
63 have been found to affect crop performance when studied in isolation of one another. For example, root (Comas
64 et al. 2013; White 2019), xylem (Tombesi et al. 2010; Ryu et al. 2016; Gleason et al. 2017a; Cardoso et al.
65 2018), stomatal regulation (Zaman-Allah et al. 2011; Messina et al. 2015), and photochemistry (Rocher et al.
66 1989; Galic et al. 2019), have all been shown to influence water use, water use efficiency, and growth. Given the
67 efficacy of each of these traits to affect plant functioning, as well as the clear and well-understood physiological

68 linkages among them, it is possible that crop performance could be improved by selecting for specific trait
69 combinations matched with different climate and soil scenarios.

70 Here, we focus on traits conferring improved water transport, and “drought resistance”, i.e., the ability of a
71 plant to maintain growth and reproductive fitness when the soil and xylem water potentials are low (Passioura
72 2006; Volaire 2018). Linkages among aridity (atmosphere and soil), leaf area, hydraulic conductance, and
73 photosynthesis, can be understood via the Penman-Monteith equation and Darcy’s law, as modified by
74 Whitehead and Jarvis (Whitehead et al. 1984; Whitehead 1998) (hereafter the Whitehead and Jarvis
75 proportionality):

$$76 \quad E \propto (D \cdot g_s) \propto \frac{K_x}{L_A} \cdot \frac{(\Psi_S - \Psi_L)}{L}, \quad \text{Eqn 1.}$$

77
78 where E = transpiration, D = the leaf-to-atmosphere vapor pressure deficit, g_s = stomatal conductance, K_x =
79 xylem-specific conductivity, L_A = leaf area, $(\Psi_S - \Psi_L)$ = water potential gradient between soil and leaf, and L =
80 path-length between soil water and the sites of evaporation within the leaf.

81 Eqn. 1 represents an approximation of how we might expect leaf area, xylem-specific conductance, and the
82 driving force (pressure gradient) to relate to one another (Whitehead et al. 1984). For example, if we accept that
83 CO₂ must pass through the stomata before it can be “fixed” by either pep-carboxylase (C4) or rubisco (C3, C4),
84 then we must also accept that water will pass out the stomata as a consequence (i.e., the left side of the
85 proportionality) (Whitehead 1998). This water that is spent to obtain CO₂ must be delivered to the stomata via
86 the vasculature (K_x) (Brodribb et al. 2007). If we wish to double the stomatal conductance of a given plant or
87 leaf, then we must also double K_x , or the driving force $(\Psi_S - \Psi_L)$, or decrease either L_A or L by one half (Gleason
88 et al. 2012). Each of these “choices” comes with a cost/risk, the magnitude of which depends on the climate,
89 soil, and competitive interactions with neighboring plants.

90 It is clear from Eqn. 1 that xylem-specific conductivity (K_x) is well-positioned to provide hydraulic balance
91 in the face of greater atmospheric and/or soil aridity, albeit with added investment in vasculature (Gleason et al.
92 2012, 2019). This may explain why xylem-specific conductivity (K_x) varies enormously across species and
93 habitats (nearly three orders of magnitude), far more than any other trait in the Whitehead-Jarvis proportionality
94 (He et al. 2019; Liu et al. 2019). Xylem-specific conductivity, as well as the susceptibility of the xylem to
95 failure, have recently been reported as important traits conferring drought resistance in monocotyledon crop
96 species (Guha et al. 2018; Wang et al. 2018; Gleason et al. 2019). A such, it has also been suggested that efforts
97 to improve crop performance in drought-prone environments might benefit by explicitly considering xylem traits
98 and water transport between the soil and leaf (Brodribb et al. 2015; Gleason 2015).

99 There are, of course, other traits that confer improved performance under limited water availability. For
100 example, much research over the last two decades has focused on the improvement of transpiration efficiency,

101 either via higher photosynthesis (preferred) or reduced transpiration (less desirable) (Zhu et al. 2010; Gilbert et
102 al. 2011; Messina et al. 2015; Sinclair 2018). This can be understood in the context of Eqn. 1, as carbon income
103 per unit water that has been invested (“E”; left side of the equation) to obtain this carbon, i.e., the seasonally-
104 integrated $\text{CO}_2 \sim \text{H}_2\text{O}$ exchange rate. The efficacy of these traits to confer better performance under limited
105 water availability are supported by sound theoretical constructs, and should be most effective in environments
106 where either much of the received precipitation can be passed through crop stomata (in exchange for CO_2), or
107 that precipitation received early in the season can be “banked” in the soil and used conservatively until it is
108 needed later in the season (e.g., during anthesis). This strategy, and traits aligned with it, have been discussed at
109 length elsewhere (Turner et al. 2014; Vadez et al. 2014; Sinclair 2018) and therefore will not be discussed further
110 here, however, we note that it is important to realize that traits conferring soil water extraction and/or transport
111 may in some cases be incompatible with traits conferring higher transpiration efficiency (Blum 2009; Turner et
112 al. 2014). For example, if precipitation received early in the season is needed later in the season, conservative
113 stomatal behavior and reduced transpiration may be preferable to higher stomatal conductance and low operating
114 water potentials (Vadez et al. 2014; Sinclair et al. 2017). As such, the experiment described here should be
115 considered carefully in the context of the soil and climate characteristics of the study site, and importantly, we
116 might expect different trait combinations to confer improved performance under different soil and climate
117 conditions (Tardieu et al. 2018). Specifically, is necessary to evaluate the efficacy of different trait combinations
118 in the context of seasonal precipitation patterns, antecedent soil water, and other competing water “sinks”, e.g.,
119 evaporation from the soil surface, saturated and unsaturated movement in soil beyond the reach of the roots, and
120 soil water uptake by weeds.

121 We examined the efficacy of using conceptual and quantitative trait networks as tools to understand the
122 linkages between water, carbon income, and grain production across eight maize hybrids grown in the semi-arid
123 environment of the Colorado High Plains. We applied a quantitative framework (the Whitehead and Jarvis
124 proportionality) to help us choose which physiological traits were most likely to affect water extraction, water
125 transport, and water use efficiency under water-limited and non-limited scenarios. We addressed the following
126 questions: 1) Are biomass increment and grain yield dependent on water conductance traits (xylem, leaf,
127 stomata), 2) are traits that are necessary for improving growth via water conductance and use (leaf hydraulic
128 conductance, operating leaf water potential, maximal stomatal conductance, midday stomatal conductance, and
129 CO_2 assimilation) operating as a connected network, i.e., is there meaningful covariation among these traits and
130 is this covariation logical (i.e., strength and direction), and 3) are there specific traits and trait linkages which
131 appear to be good targets for crop improvement programs?

132
133

134 **Materials and methods**

135

136 Site and hybrid selection

137

138 This experiment was conducted at USDA's Limited Irrigation Research Farm near Greeley, Colorado, USA
139 (40.4486° latitude, -104.6368° longitude). The mean monthly minimum temperature during the growth season
140 (May – October, 2017) was 8.4 °C, mean monthly maximum temperature was 25.9 °C, and mean monthly
141 precipitation was 4.9 cm (Colorado Agricultural Meteorological Network 2020). Soils on the site range from
142 sandy loam to clay loam (Ustic Haplargids). Maize (*Zea mays* L.) hybrids were chosen to represent a wide range
143 of drought tolerance from experimental trials performed in La Salle, Colorado (Syngenta AG, Basel
144 Switzerland).

145

146

147 Experimental design

148

149 All maize hybrids were grown under fully-watered (hereafter “wet”) and water deficit (hereafter “dry”)
150 treatments. Wet and dry treatments were designed to deliver either 100% or 40% of the evapotranspiration
151 measured on a reference maize hybrid. Plots were watered once each week via drip irrigation to maintain these
152 target ET levels. Thus, all plots (hybrids) within each irrigation treatment (40% or 100% ET) received the same
153 amount of water during each irrigation, i.e. irrigation water was not adjusted to account for differences in
154 transpiration/evaporation among hybrids. Hybrids were planted (May 4) into a randomized complete block
155 design, with each hybrid by irrigation treatment being replicated four times. Plot size was 42 m by 9 m wide (12
156 rows; 0.76 m spacing). Plant spacing did not differ between hybrids and treatments and was 85,500 plants ha⁻¹.
157 Weed and fertility management followed standard practices for the region and plants appeared to be free of both
158 weeds and nutrient stress throughout the experiment. All plots received full irrigation until plants reached V7
159 (seven fully-expanded and “collared” leaves present) on June 26, after which irrigation was reduced in the dry
160 treatment. The dry treatment was lifted again as the plants approached VT (anthesis) on July 27, but was
161 implemented again once the plants achieved R3 (starch accumulating “milk” stage) on August 29. This was
162 done to avoid stress through the most sensitive reproductive stages.

163

164 Trait measurements

165

166 Biomass, grain yield, and leaf area

167

168 Shoot biomass samples were collected for all hybrids in both treatments (wet, dry) immediately after plants
169 reached physiological maturity (September 25 for dry and October 10 for wet treatments). Five representative
170 plants were harvested from each plot for biomass and grain yield, giving 20 total plants per hybrid by treatment
171 combination. Leaves, stems, ears, and grain were dried to constant mass and weighted to the nearest 0.01 g. The
172 fresh leaf area of each harvested plant was measured (prior to drying) using a leaf area meter (LI-3100C, LI-
173 COR, Lincoln, Nebraska, USA). Yield stability was calculated as the ratio of grain yield in the dry vs the wet
174 treatments.

175

176 Stomatal conductance

177

178 Stomatal conductance was measured on all hybrids in both treatments (wet, dry) using hand-held steady-state
179 porometers (Model SC-1, Meter Group Inc., Pullman, Washington, USA) between July 10 and July 14, 2017.
180 Stomatal conductance was measured by walking continuously through the field from 0900 to 1500 each day.
181 Four plants were measured within a single plot before moving on to the next adjacent plot. Each plot was
182 measured ca 5 times throughout each day, giving a total of ca 95 individual measurements for each hybrid by
183 treatment combination. Diurnal stomatal conductance trajectories for each hybrid by treatment combination
184 were fit with quadratic models using the ‘nlsLM’ function in the minpack.lm package developed for R (Elzhov et
185 al. 2016). Fitted maximum and midday (1400) values of stomatal conductance were then extracted from the
186 quadratic models.

187

188 Leaf water potential

189

190 Leaf water potential was measured on all hybrids in both treatments (wet, dry) at midday (1200-1400) and at
191 predawn (0500-0630) using a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp, Santa
192 Barbara, California, USA) between July 17 and September 1, 2017. This sampling resulted in ca 10 midday and
193 33 predawn measurements for each hybrid by treatment combination.

194

195 Light-saturated net CO₂ assimilation and stomatal response to VPD

196

197 These traits were measured only on hybrids in the wet treatment using two portable gas-exchange systems
198 (Model LI-6400-40, LI-COR Biosciences, Lincoln, Nebraska, USA). Measurements were taken between June
199 27 and July 7, 2017. Briefly, each morning, one plant of each hybrid would be randomly selected from the field,
200 severed at its base, wrapped in white plastic, and brought back to the laboratory, thus giving at least six replicates
201 of each hybrid (~one of each hybrid per day). Plants were then re-cut under water (leaving the severed end

202 submerged) in a climate-controlled room (temp = 25 °C; relative humidity > 60%). Light-saturated net CO₂
203 assimilation rate was measured on the top-most, fully-expanded leaf under light saturated conditions (1800 μmol
204 m⁻² s⁻¹). Chamber temperature and VPD were kept below 30 °C and 1.5 kPa for at least 20 minutes prior to
205 recording maximal measurements. After maximal measurements were recorded, stomatal conductance was
206 measured under increasing VPD, from 1.5 kPa to 3.0 kPa in 0.2 kPa steps. Stomatal response to VPD was
207 mostly flat, with a slight decline between 2.5 kPa and 3.0 kPa. To quantify the change in slope between 2.5 kPa
208 and 3.0 kPa, spline models were fit to g_s~VPD data using the ‘loess’ function in R and differences in slope
209 extracted from these fitted models.

210

211 Pressure-volume curves

212

213 Pressure-volume data were measured only on hybrids in the wet treatment. The theory and assumptions of
214 pressure-volume data have been discussed at length elsewhere (Schulte and Hinckley 1985; Ding et al. 2014).
215 Here, we report how we obtained the necessary data to build pressure-volume curves and the metrics we
216 extracted from them. Pressure-volume data were measured on six days between August 8 and August 17, 2017.
217 Briefly, on each day, one leaf from each hybrid was collected randomly from the wet treatment during predawn
218 hours (0500 – 0600), immediately placed in a sealable plastic bag and brought back to the laboratory (six
219 replicates per hybrid). Leaves were repeatedly weighted to the nearest 0.0001 g and their leaf water potential
220 measured with a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp, Santa Barbara,
221 California, USA). At least nine pressure-volume points were obtained in this way for each leaf. The reciprocal
222 of pressure (1/MPa) was plotted against one minus relative water content (1 – RWC). Cell wall elasticity (ε),
223 water potential at turgor loss (π_{tip}), osmotic potential at full turgor (π_o), and leaf capacitance (C_{Leaf}) were extracted
224 from each curve. Leaf capacitance was estimated from the initial slope of the pressure volume curve prior to
225 turgor loss.

226

227 Maximal leaf hydraulic conductance and leaf hydraulic vulnerability

228

229 Maximal leaf hydraulic conductance (K_{leaf_max}) and leaf hydraulic vulnerability were measured only on hybrids in
230 the wet treatment using the Rehydration Kinetics Method (Brodribb and Holbrook 2003) between July 14 and
231 July 28, 2017. Briefly, on each day, ca four plants of each hybrid were cut at the base in the field during
232 predawn hours (0500 – 0600), immediately placed in a white plastic bag, and brought back to the laboratory,
233 where they were re-cut under water (in a large bucket), leaving their canopies still wrapped in plastic. Plants
234 were removed from the bucket and dried down to a range of water potentials between -0.3 MPa and -4.0 MPa
235 under a box fan. Once a plant had dried down to the desired water potential, two adjacent leaves near the top of

236 the canopy were cut off. The water potential of one leaf was immediately measured in a Scholander pressure
237 chamber (Model 3005, Soil Moisture Equipment Corp, Santa Barbara, California, USA). The other leaf was re-
238 cut underwater, and the cut end allowed to re-hydrate (underwater) between 5 and 20 seconds whilst illuminated
239 (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF). The water potential of the “re-hydrated” leaf was then immediately measured in the
240 pressure chamber. Leaf conductance (K_{leaf}) was calculated from its change in water potential and the increase in
241 water volume during re-hydration, after Brodribb and Holbrook (2003):

242

$$243 \quad K_{\text{leaf}} = \frac{C_{\text{leaf}} \ln \left[\frac{\Psi_0}{\Psi_f} \right]}{t}, \quad \text{Eqn 2}$$

244

245 where K_{leaf} = the leaf conductance, C_{Leaf} = leaf capacitance, i.e., the change in leaf water content per change in
246 water potential prior to turgor loss (obtained from the initial slope of pressure-volume curves), Ψ_0 and Ψ_f are the
247 leaf water potentials before re-hydration and after re-hydration, and t = the re-hydration time. K_{leaf} was measured
248 in this way for at least 28 plants of each hybrid such that the decline in K_{leaf} could be plotted against leaf water
249 potential to develop a “vulnerability curve”. $K_{\text{leaf}} \sim \Psi_{\text{leaf}}$ data were then fit with sigmoidal models after Pammenter
250 and Vander Willigen (1998) using the ‘nlsLM’ function in the minpack.lm package developed for R (Elzhov et
251 al. 2016). From this curve, we ranked the susceptibility of the hybrids to hydraulic failure according to their loss
252 of K_{leaf} per unit decline in leaf water potential. For this purpose, we use the leaf water potential at which 50% of
253 the maximal leaf conductance was lost (P_{50}).

254

255 Statistical analysis

256

257 All analyses, model fitting, and graphics were done in R 3.5.1. (R Core Team 2015). Bivariate correlation and
258 multivariate analyses were done on hybrid mean data using the ‘lm’ function in base R and the ‘principal’
259 function in the ‘psych’ package for R, respectively. Given the small sample size (eight hybrids), bootstrapping
260 was used to estimate the stability of fitted principal components and trait loadings (Babamoradi et al. 2013). If
261 the standard deviation across bootstrapped samples was greater than 0.5 (range = -1 to +1), the loading was
262 noted as “unstable”. Varimax rotation (an orthogonal method) was used to obtain more interpretable principal
263 components and the ‘gggraph’ package for R was used to plot the results. The data used in this study are available
264 in csv format (Appendix S1). All analyses and figures can be reproduced using these data. Additionally, the R
265 code written to perform all analyses and figures are available from the first author upon request.

266

267

268 **Results**

269

270 We present first the main axes of variation across the traits, i.e. trait groupings (Fig. 1). We note that because our
271 emphasis was focused on performance under irrigated conditions, more traits were measured on fully irrigated
272 plants (wet treatment) than on plants growing under water stress (dry treatment). Thus, these two data-sets (wet,
273 dry) were analyzed independently.

274 Across all hybrids within the wet treatment, water transport capacity ($K_{\text{leaf_max}}$), midday stomatal conductance
275 (g_s), and photosynthesis (A_N) appeared bundled together as a single axes of variation (principal component 1;
276 PC1) (Fig. 1 “Wet”). Beyond this, there was also strong alignment among maximal stomatal conductance
277 (g_{s_max}), growth (biomass increment), and grain yield, which manifested as a separate principal component (PC3),
278 but with linkages to photosynthesis and water transport capacity (PC1) via midday and maximal stomatal
279 conductance (Fig. 1 “Wet”). Interestingly, more negative hydraulic status, including lower osmotic adjustment
280 (π_o), leaf water potential at turgor loss (π_{tip}), and the operating water potentials during both midday (Ψ_{MD}) and
281 predawn hours (Ψ_{PD}) were associated with greater yield along the second principle component (PC2),
282 suggesting that higher yielding hybrids removed more water from the soil than poorer yielding hybrids (Fig. 1
283 “Wet”). Additionally, there was alignment between the VPD required to initiate stomatal closure
284 ($g_s \sim \text{VPD}_{\text{turn}}$), the rate of stomatal closure when VPD was equal to 3.0 kPa ($g_s \sim \text{VPD}_{\text{slope}}$), and the first axis
285 of variation (PC1; $K_{\text{leaf_max}}$, g_{s_MD} , and A_N). The direction (inverse) and alignment of this variation suggests that
286 early-closing (at low VPD) and fast-closing stomata (steeper $g_s \sim \text{VPD}$ slope) were associated with higher midday
287 stomatal conductance, higher leaf conductance, and greater CO_2 assimilation (Fig. 1 “Wet”). Also in alignment
288 with this axis (PC1) was the rate at which leaf conductance was lost (per unit Ψ_{leaf}) (P_{50}), such that hybrids with
289 higher stomatal conductance at midday also had leaves that were more susceptible to hydraulic failure at a given
290 water potential. Bootstrapping revealed that the small sample size (eight hybrids per trait) resulted in relatively
291 unstable loadings for yield stability (with PC1), $K_{\text{leaf_max}}$ and A_N (PC1), π_o and π_o (PC2) in the wet treatment, and
292 water potential (Ψ_{PD} , Ψ_{MD}) (PC2) in the dry treatment. This means that some hybrids exhibited specifically high
293 leverage on the loading factor, and when they were removed from the analysis the value of the loading decreased
294 meaningfully. As such, these linkages should be interpreted with caution.

295 An important difference between the wet and dry treatments was the much stronger alignment among
296 growth, grain yield, and midday stomatal conductance in the dry treatment (Fig. 1 “Dry”). Indeed, the bivariate
297 correlations between midday stomatal conductance and biomass ($r^2 = 0.69$; $p = 0.010$) and grain yield ($r^2 = 0.91$;
298 $p < 0.001$) were markedly high in the dry treatment (Fig. 2 a,b), suggesting very close coordination between the
299 achievable stomatal conductance in the middle of the day and growth in the dry treatment (Fig. 2B). Midday
300 stomatal conductance was also strongly correlated with light-saturated net CO_2 assimilation (symbol size) in the
301 wet treatment ($r^2 = 0.58$; $p = 0.029$) (Fig. 2B, 3A).

302 Although the bivariate models generally supported the multivariate analyses, there were a few differences
303 worth noting. Specifically, there were strong correlations among traits that in some cases loaded on different
304 principal components. For example, although the leaf's capacity for water transport ($K_{\text{leaf_max}}$) and the stomatal
305 conductance at midday ($g_{\text{s_MD}}$) loaded primarily on PC1, these traits also correlated strongly with plant growth
306 (biomass increment) and grain yield (components of PC2 and PC3) (Fig. 3 "Wet"). This suggests strong
307 coordination between the liquid water conductance, gas-phase conductance, net CO_2 assimilation, and growth,
308 but especially in the dry treatment (Fig. 3 "Dry"). The PCA and bivariate results suggest close linkage between
309 the osmotic potential (π_o) and grain yield, that is not likely manifesting through either A_N or biomass increment
310 (weak linkages), suggesting a more proximal relationship between these traits (Fig. 3 "Wet").

311 Considering both the multivariate and bivariate analyses, there appears to be strong and logical linkages that
312 exist across hybrids. Although the strength of these linkages shift somewhat between the wet and dry treatments,
313 they appear to be underpinned by the same physiological processes, that is, the acquisition of water, its transport
314 to the sites of evaporation/photosynthesis, gas-exchange, growth, and finally, grain yield.

315

316

317 **Discussion**

318

319 Traits conferring improved performance under wet and dry conditions

320

321 Taken together, the results of the multivariate and bivariate analyses suggest a logical and tightly coordinated
322 bundle of traits (processes) leading to improved performance under wet and dry treatments. Importantly, even
323 though fewer traits were measured in the dry treatment than in the wet treatment, the traits and linkages
324 conferring better performance were similar in both cases, as has been reported before (Gleason et al. 2019).
325 High positive correlation between maximal stomatal conductance and grain yield under both wet and dry
326 conditions suggests that, at least under the soil and climate of the study site and across the hybrids examined
327 here, the maintenance of stomatal conductance throughout the day appears to be required for supporting daily net
328 biomass accumulation. This result is supported by previous efforts to understand whole-plant functioning in
329 crops, as well as wild species, in that water transport ($\sim K_{\text{leaf}}$) to the stomata (g_s) drives gas-exchange ($\sim A_N$), and
330 therefore, improved growth and yield (Blum 2009; Brodrigg et al. 2015; Gleason et al. 2017a, 2019; Xiong and
331 Nadal 2020).

332 Given the apparent importance in maintaining water transport and stomatal conductance, we might also
333 expect better performing hybrids to be more resistant to embolism (lower P_{50}) than poorer performing hybrids
334 (Ryu et al. 2016), but our results do not support this. Rather, hybrids that exhibited better performance (higher
335 midday g_s , K_{leaf} , growth, yield) under both wet and dry conditions also exhibited larger reductions in hydraulic

336 conductance at low water potential (i.e., they exhibited higher P_{50} values). This result is nearly identical to a
337 previous experiment using the parents of the Nested Association Mapped population (Gleason et al. 2019), and
338 suggests that the better performing (growth and yield) hybrids/inbreds in these studies achieved greater stomatal
339 conductance and gas exchange, not by having vasculature and/or stomata that are less sensitive to low water
340 potential, but rather, by having higher hydraulic and stomatal conductance in the first place. For example, even
341 though the hybrids/inbreds that “win the race” had more sensitive stomata and more vulnerable hydraulic
342 pathways, they were still able to achieve greater liquid and gas-phase conductances through the middle of the
343 day.

344 There are two trait combinations that, in theory, will lead to a higher sustained K_{leaf} during late morning and
345 midday hours. Firstly, xylem that is more embolism resistant (lower P_{50}) will exhibit smaller reductions in K_{leaf}
346 as water potential declines through the day. Secondly, for a given embolism resistance, plants can start out in the
347 morning with higher K_{leaf} , such that their K_{leaf} remains sufficiently high during the day. As such, these two traits
348 (higher K_{leaf} vs lower P_{50}) represent functionally equivalent strategies. It is interesting to note then that either
349 natural selection, artificial selection, or both have appeared to favor hybrids (this study) and inbreds (Gleason et
350 al. 2019) with higher K_{leaf} , rather than lower P_{50} . Given that this result has now been reported in two independent
351 maize experiments using different populations, it may be worth considering the relative costs and risks of these
352 two alternative strategies. It is also noteworthy that high K_{leaf} would likely only be beneficial if embolism in
353 maize is reversible at night via root pressure, which has been reported previously in this species (Steudle et al.
354 1987; Gleason et al. 2017b).

355 Osmotic adjustment in the leaves of the hybrids examined here was closely correlated with end-of-season
356 grain production, pre-dawn water potential, and marginally with water transport and photosynthesis, again,
357 suggesting a logical network of traits leading to improved performance (Figs. 1 & 3, “wet”). Although osmotic
358 adjustment is a known beneficial drought response in vascular species, including maize (Ashwini et al. 2019;
359 Beseli et al. 2019), our results here suggest that it might serve as a more effective breeding target if other closely
360 aligned traits, namely K_{leaf} and A_N can also be targeted.

361

362 Water transport vs transpiration efficiency

363

364 It is clear from our current understanding of plant physiology and drought that the improvement of individual
365 plant traits in isolation of one another will not result in the best crop performance outcomes. Although the
366 results here suggest that higher water transport efficiency might be a good strategy for water limited
367 environments, there is evidence that traits conferring higher transpiration efficiency might also confer enhanced
368 performance under similar conditions (Zaman-Allah et al. 2011; Vadez et al. 2014; Messina et al. 2015; Sinclair
369 et al. 2017). It is possibly that different strategies may produce advantages depending on the availability of soil

370 water across time and space, as well as the balance between atmospheric and soil aridity (Eqn. 1). Although this
371 idea has yet to be tested rigorously, it should be understood that any attempt to increase either instantaneous or
372 seasonally-integrated transpiration efficiency by reducing transpiration (even in the middle of the day) would
373 have likely reduced the performance of the hybrids we evaluated here, as well as elsewhere (Jordan et al. 1983;
374 Gowda et al. 2011; Gleason et al. 2019; Palta and Turner 2019). In our view, the efficacy of both of these broad
375 crop strategies (water extraction and transport, transpiration efficiency) to confer improved performance under
376 drought are supported by sound theoretical principles, and as such, this important research question remains low-
377 hanging fruit for both experimentalists and modelers.

378

379 Conclusions

380

381 Given that photosynthesis relies directly on water transport and stomatal conductance, we suggest that these
382 traits could, in theory, be manipulated to improve crop species. However, this remains a difficult task for several
383 reasons. Firstly, xylem traits are time-consuming, expensive, and require specific expertise to measure, and are
384 therefore currently not well suited for high through-put methods. Additionally, the field of hydraulic physiology
385 is relatively new, and recent advances in this field have not yet been transferred to other disciplines. However,
386 even given these difficulties and the narrow scope of the present study (eight hybrids grown at a single site), the
387 results we report here suggest that xylem is a common failure point in the water transport pathway (soil water -->
388 xylem --> gas-exchange), and its performance correlates strongly with both gas exchange and growth (Brodrigg
389 2009; Gleason et al. 2017a; Martin-StPaul et al. 2017; Xiong and Nadal 2020). As such, we suggest that xylem
390 functioning, as well as the regulation and loss of conductance both within and outside the xylem (Scoffoni et al.
391 2017; Xiong and Nadal 2020), might be good candidates for breeding programs if these traits can be measured
392 quickly and at the appropriate scale. Furthermore, considering traits as connected networks that manifest as
393 effective crop strategies, as done here across a small group of hybrids, can help to identify novel avenues for
394 crop improvement.

395

396

397 Author Contributions

398

399 SMG and LHC developed the original idea and design for the experiment. SMG did the analyses and wrote the
400 first manuscript draft. SMG, LHC, LN, and CH performed the trait measurements. RB and SC contributed
401 germplasm and assisted with experimental design. All authors contributed equally to manuscript revisions.

402

403 Acknowledgments

404

405 We warmly thank the scientists, technicians, collaborators and students that assisted with this study. We would
406 like to especially thank Nora Flynn, Ryan Barton, Kelly Nelson, Jerry Buchleiter, Ross Stewart, and Garrett
407 Banks for their kind assistance and enthusiastic support in the field.

408

409

410 **References**

411

412

Ashwini S, Chandrakala N, Ravikumar RL (2019) Genetic variability for osmotic adjustment in pollen grains and its association with field tolerance to moisture stress in maize inbred lines. *Curr Sci* 116:279–285. <https://doi.org/10.18520/cs/v116/i2/279-285>

Babamoradi H, Van Den Berg F, Rinnan Å (2013) Bootstrap based confidence limits in principal component analysis - a case study. *Chemom Intell Lab Syst* 120:97–105. <https://doi.org/10.1016/j.chemolab.2012.10.007>

Beseli A, Hall AJ, Manandhar A, Sinclair TR (2019) Root osmotic potential and length for two maize lines differing in leaf osmotic potential. *J Crop Improv* 33:429–444. <https://doi.org/10.1080/15427528.2019.1607642>

Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *F Crop Res* 112:119–123

Brodribb TJ (2009) Xylem hydraulic physiology: the functional backbone of terrestrial plant productivity. *Plant Sci* 177:245–251

Brodribb TJ, Feild TS, Jordan GJ (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol* 144:1890–1898

Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol* 132:2166–2173

Brodribb TJ, Holloway-Phillips M, Bramley H (2015) Improving water transport for carbon gain in crops. In: Sadras VO, Calderini DF (eds) *Crop physiology applications for genetic improvement and agronomy*. Elsevier, London, pp 251–281

Campos H, Cooper M, Habben JE, et al (2004) Improving drought tolerance in maize: a view from industry. In: *Field Crops Research*. pp 19–34

Cardoso AA, Brodribb TJ, Lucani CJ, et al (2018) Coordinated plasticity maintains hydraulic safety in sunflower leaves. *Plant Cell Environ*. <https://doi.org/10.1111/pce.13335>

Colorado Agricultural Meteorological Network (2020) CoAgMet Station Summary Access. <https://coagmet.colostate.edu/>

- Comas L, Becker S, Cruz VM, et al (2013) Root traits contributing to plant productivity under drought. *Front Plant Sci* 4:. <https://doi.org/10.3389/fpls.2013.00442>
- Condon AG (2020) Drying times: plant traits to improve crop water use efficiency and yield. *J Exp Bot* DOI: 10.10: <https://doi.org/10.1093/jxb/eraa002>
- Ding Y, Zhang Y, Zheng QS, Tyree MT (2014) Pressure-volume curves: revisiting the impact of negative turgor during cell collapse by literature review and simulations of cell micromechanics. *New Phytol* 203:378–387. <https://doi.org/10.1111/nph.12829>
- Elzhov T V, Mullen KM, Spiess A-N, Bolker B (2016) minpack.lm: R interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, plus support for Bounds. R package version 1.2-1.
- Galic V, Franic M, Jambrovic A, et al (2019) Genetic correlations between photosynthetic and yield performance in maize are different under two heat scenarios during flowering. *Front Plant Sci* 10:566. <https://doi.org/10.3389/fpls.2019.00566>
- Gilbert ME, Zwieniecki MA, Holbrook NM (2011) Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *J Exp Bot* 62:2875–2887
- Gleason SM (2015) Evolutionary outcomes should inform strategies to increase drought tolerance. *Nat Plants* 1:15114. <https://doi.org/10.1038/nplants.2015.114>
- Gleason SM, Butler DW, Ziemińska K, et al (2012) Stem xylem conductivity is key to plant water balance across Australian angiosperm species. *Funct Ecol* 26:343–352
- Gleason SM, Cooper M, Wiggans DR, et al (2019) 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. *F Crop Res* 234:119–128. <https://doi.org/10.1016/j.fcr.2019.02.001>
- Gleason SM, Wiggans DR, Bliss CA, et al (2017a) Coordinated decline in photosynthesis and hydraulic conductance during drought stress in *Zea mays*. *Flora Morphol Distrib Funct Ecol Plants* 227:1–9. <https://doi.org/10.1016/j.flora.2016.11.017>
- Gleason SM, Wiggans DR, Bliss CA, et al (2017b) Embolized stems recover overnight in *Zea mays*: the role of soil water, root pressure, and nighttime transpiration. *Front Plant Sci* 8:662. <https://doi.org/10.3389/fpls.2017.00662>
- Gowda VRP, Henry A, Yamauchi A, et al (2011) Root biology and genetic improvement for drought avoidance in rice. *F. Crop. Res.*
- Guha A, Chhajed SS, Choudhary S, et al (2018) Hydraulic anatomy affects genotypic variation in plant water use and shows differential organ specific plasticity to drought in *Sorghum bicolor*. *Environ Exp Bot* 156:25–37
- He P, Gleason SM, Wright IJ, et al (2019) Growing-season temperature and precipitation are independent drivers of global variation in xylem hydraulic conductivity. *Glob Chang Biol*. <https://doi.org/10.1111/gcb.14929>
- Jordan WR, Dugas WA, Shouse PJ (1983) Strategies for crop improvement for drought-prone regions. *Agric Water Manag* 7:281–299. [https://doi.org/10.1016/0378-3774\(83\)90090-2](https://doi.org/10.1016/0378-3774(83)90090-2)

- Liu H, Gleason SM, Hao G, et al (2019) Hydraulic traits are coordinated with maximum plant height at the global scale. *Sci Adv*. <https://doi.org/10.1126/sciadv.aav1332>
- Martin-StPaul N, Delzon S, Cochard H (2017) Plant resistance to drought depends on timely stomatal closure. *Ecol. Lett.* 20:1437–1447
- Messina CD, Sinclair TR, Hammer GL, et al (2015) Limited-transpiration trait may increase maize drought tolerance in the US corn belt. *Agron J* 107:1978–1986
- Palta JA, Turner NC (2019) Crop root system traits cannot be seen as a silver bullet delivering drought resistance. *Plant Soil* 439:31–43. <https://doi.org/10.1007/s11104-018-3864-6>
- Pammenter NW, Vander Willigen C (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiol* 18:589–593
- Passioura J (2006) Increasing crop productivity when water is scarce - From breeding to field management. In: *Agricultural Water Management*. pp 176–196
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Rocher JP, Prioul JL, Lecharny A, et al (1989) Genetic Variability in Carbon Fixation, Sucrose-P-Synthase and ADP Glucose Pyrophosphorylase in Maize Plants of Differing Growth Rate. *Plant Physiol* 89:416–420. <https://doi.org/10.1104/pp.89.2.416>
- Ryu J, Hwang BG, Kim YX, Lee SJ (2016) Direct observation of local xylem embolisms induced by soil drying in intact *Zea mays* leaves. *J Exp Bot* 67:2617–2626. <https://doi.org/10.1093/jxb/erw087>
- Schulte PJ, Hinckley TM (1985) A comparison of pressure-volume curve data analysis techniques. *J Exp Bot* 36:1590–1602. <https://doi.org/10.1093/jxb/36.10.1590>
- Scoffoni C, Albuquerque C, Brodersen CR, et al (2017) Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. *Plant Physiol* 173:1197–1210. <https://doi.org/10.1104/pp.16.01643>
- Sinclair TR (2018) Effective Water Use Required for Improving Crop Growth Rather Than Transpiration Efficiency. *Front Plant Sci* 9:1442. <https://doi.org/10.3389/fpls.2018.01442>
- Sinclair TR, Devi J, Shekoofa A, et al (2017) Limited-transpiration response to high vapor pressure deficit in crop species. *Plant Sci*. <https://doi.org/https://doi.org/10.1016/j.plantsci.2017.04.007>
- Stedle E, Oren R, Schulze E-D (1987) Water Transport in Maize Roots. *Plant Physiol* 84:1220–1232. <https://doi.org/10.1104/pp.84.4.1220>
- Tardieu F, Simonneau T, Muller B (2018) The physiological basis of drought tolerance in crop plants: a scenario-dependent probabilistic approach. *Annu Rev Plant Biol* 69:733–759. <https://doi.org/10.1146/annurev-arplant-042817-040218>
- Tombesi S, Johnson RS, Day KR, Dejong TM (2010) Relationships between xylem vessel characteristics, calculated axial hydraulic conductance and size-controlling capacity of peach rootstocks. *Ann Bot* 105:327–331. <https://doi.org/10.1093/aob/mcp281>

- Turner NC, Blum A, Cakir M, et al (2014) Strategies to increase the yield and yield stability of crops under drought – are we making progress? *Funct Plant Biol* 41:1199–1206. <https://doi.org/10.1071/FP14057>
- Vadez V, Kholova J, Medina S, et al (2014) Transpiration efficiency: new insights into an old story. *J Exp Bot* 65:6141–6153. <https://doi.org/10.1093/jxb/eru040>
- Volaire F (2018) A unified framework of plant adaptive strategies to drought: Crossing scales and disciplines. *Glob Chang Biol* 24:2929–2938. <https://doi.org/10.1111/gcb.14062>
- Wang X, Du T, Huang J, et al (2018) Leaf hydraulic vulnerability triggers the decline in stomatal and mesophyll conductance during drought in rice. *J Exp Bot* doi:10.109:
- White PJ (2019) Root traits benefitting crop production in environments with limited water and nutrient availability. *Ann. Bot.* 124:883–890
- Whitehead D (1998) Regulation of stomatal conductance and transpiration in forest canopies. *Tree Physiol* 18:633–644
- Whitehead D, Edwards WRN, Jarvis PG (1984) Conducting sapwood area, foliage area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. *Can J For Res* 14:940–947
- Xiong D, Nadal M (2020) Linking water relations and hydraulics with photosynthesis. *Plant J.* 101:800–815
- Zaman-Allah M, Jenkinson DM, Vadez V (2011) A conservative pattern of water use, rather than deep or profuse rooting, is critical for the terminal drought tolerance of chickpea. *J Exp Bot* 62:4239–4252. <https://doi.org/10.1093/jxb/err139>
- Zhu X-G, Long SP, Ort DR (2010) Improving photosynthetic efficiency for greater yield. *Annu Rev Plant Biol* 61:235–261

413

414

415 **Supporting information**

416 **S1** Mean values and standard deviations for all hybrid and treatment combinations (gleason_et_al.csv)

417

418

419 **Data availability statement**

420 The data that supports the findings of this study are available in the supplementary material of this article

421

422

423 **Figure Legends**

424

425 **Figure 1.** Orthogonality rotated principal components (colored circles) and trait groupings (colored ellipses).

426 Positive loadings and negative loadings are denoted with green and red connections, with wider connections

427 indicating larger standardized coefficients (0.5 to 1.0). Coefficients with standard deviations greater than 0.5
428 (range = -1 to +1) after bootstrapping are denoted as uncertain (UC) and should be interpreted with caution. Wet
429 (fully-watered) and dry treatments (40% of reference crop ET) are shown in the left and right panels,
430 respectively. g_{S_max} and g_{S_MD} = maximal and midday stomatal conductance (gas-phase water conductance),
431 respectively. K_{leaf_max} = the maximal rate of leaf hydraulic conductance (liquid-phase water conductance). A_N =
432 the light-saturated rate of net CO₂ assimilation. P_{50} = the leaf water potential at which 50% of K_{leaf_max} was lost.
433 $g_{S\sim VPD_slope}$ = the rate of stomatal conductance decline (mmol kPa⁻¹) when the VPD was equal to 3.0 kPa, i.e.
434 the first derivative of the $g_{S\sim VPD}$ function where VPD = 3. $g_{S\sim VPD_turn}$ = the VPD initiating stomatal closure.
435 Yield = end-of-season grain yield. Ψ_{MD} and Ψ_{PD} = the leaf water potential during the middle of the day (1200-
436 1400) and during predawn hours (0500-1630), respectively. π_{tlp} and π_o = the osmotic potential at turgor loss and
437 at full turgor, respectively. Yield_stability is the ratio of grain yield produced in the dry treatment relative to that
438 produced in the wet treatment. Biomass = the end-of-season biomass of all above-ground plant components
439 (stems, leaves, reproductive structures).

440

441 **Figure 2.** Bivariate plots of the linkages between grain yield and midday stomatal conductance (g_{S_MD}) (a), and
442 between biomass and midday stomatal conductance (b). Each symbol represents a single hybrid mean value
443 (biomass and grain yield; n=4). Symbol size has been scaled to the light-saturated rate of net CO₂ assimilation.

444

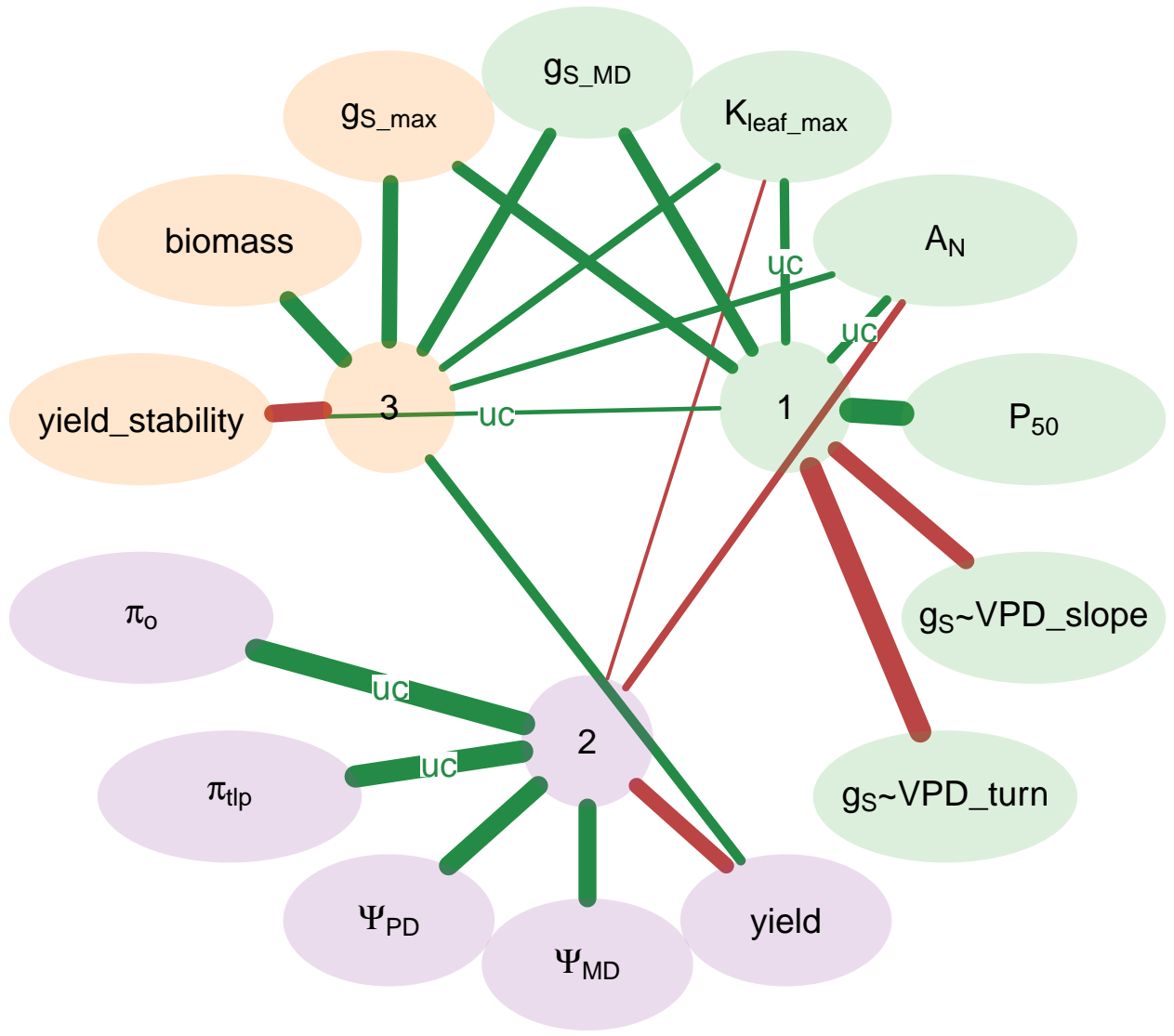
445 **Figure 3.** Correlation matrices for both wet (left panel) and dry (right panel) treatments. Correlation
446 coefficients are denoted by text and by color, with increasing color intensity indicating increasing correlation
447 strength. Variable descriptions are the same as given in Figure 1.

448

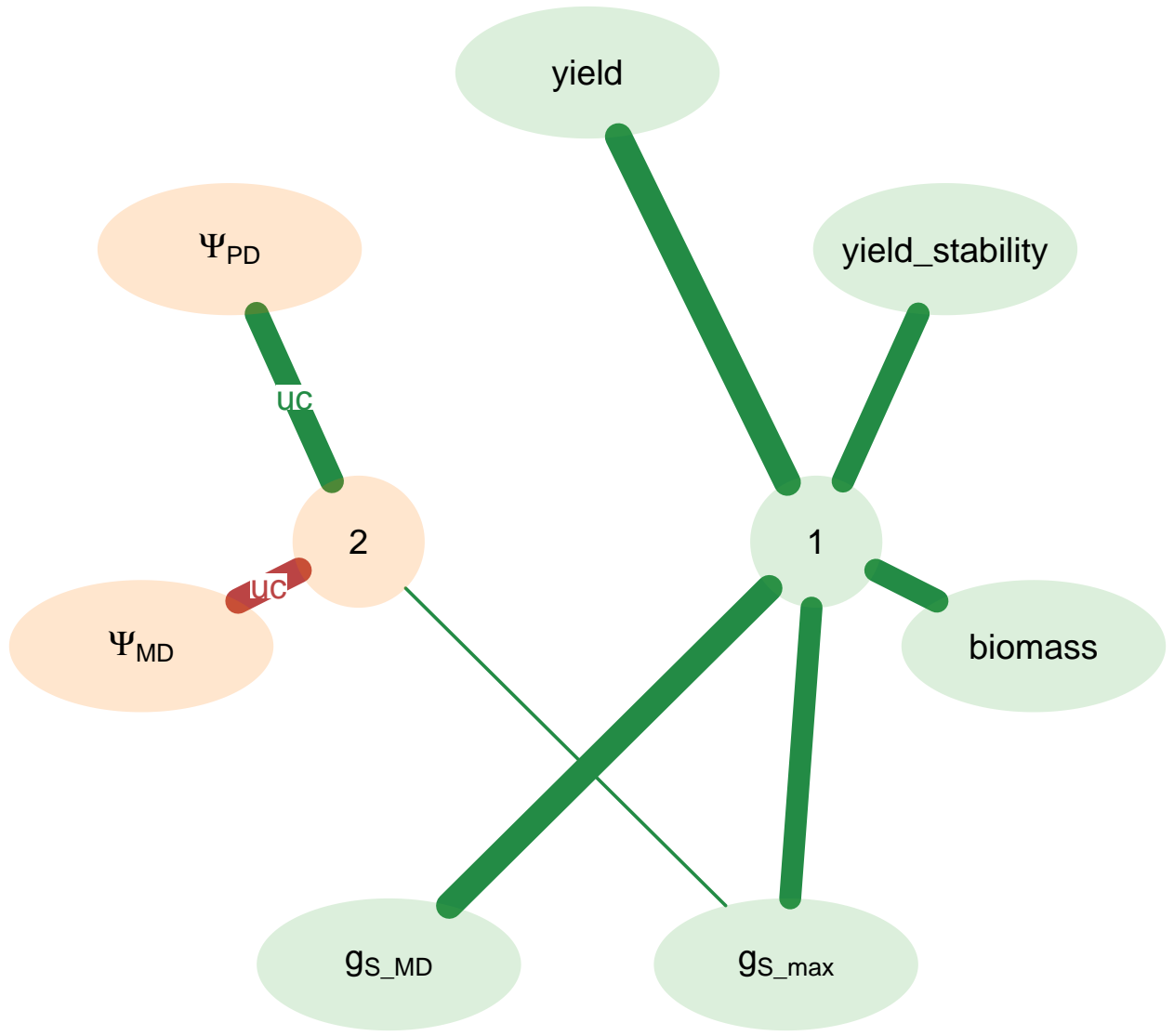
449

450

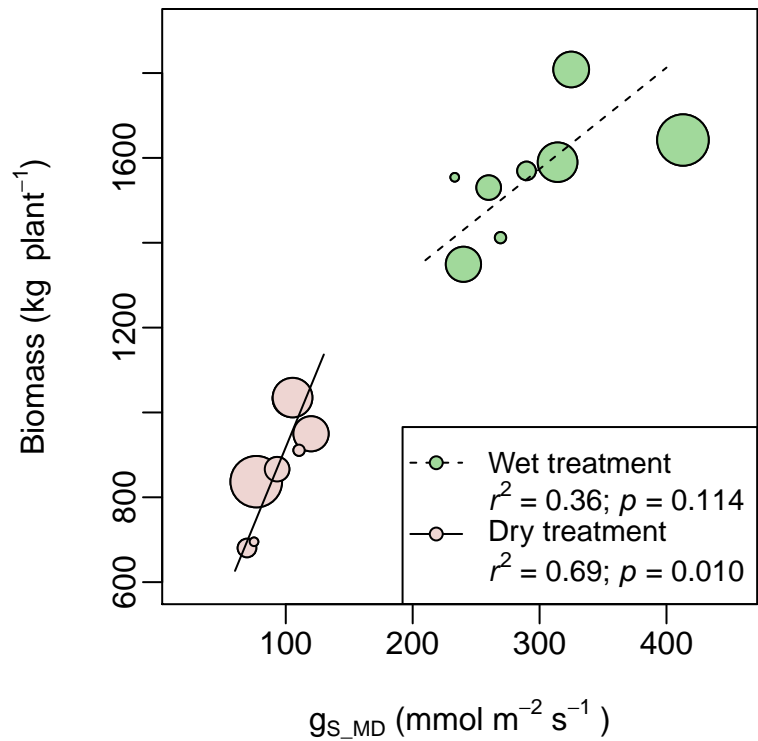
Wet



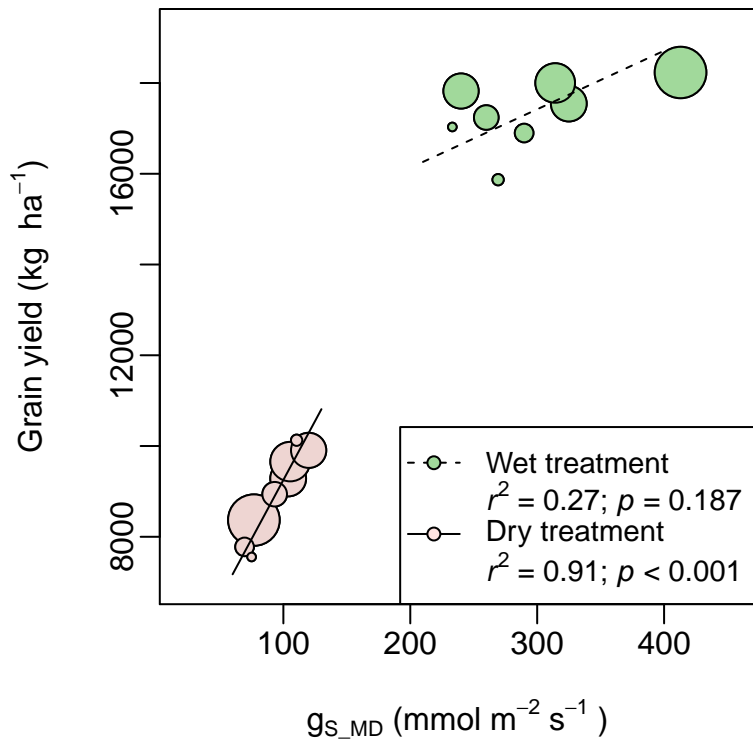
Dry



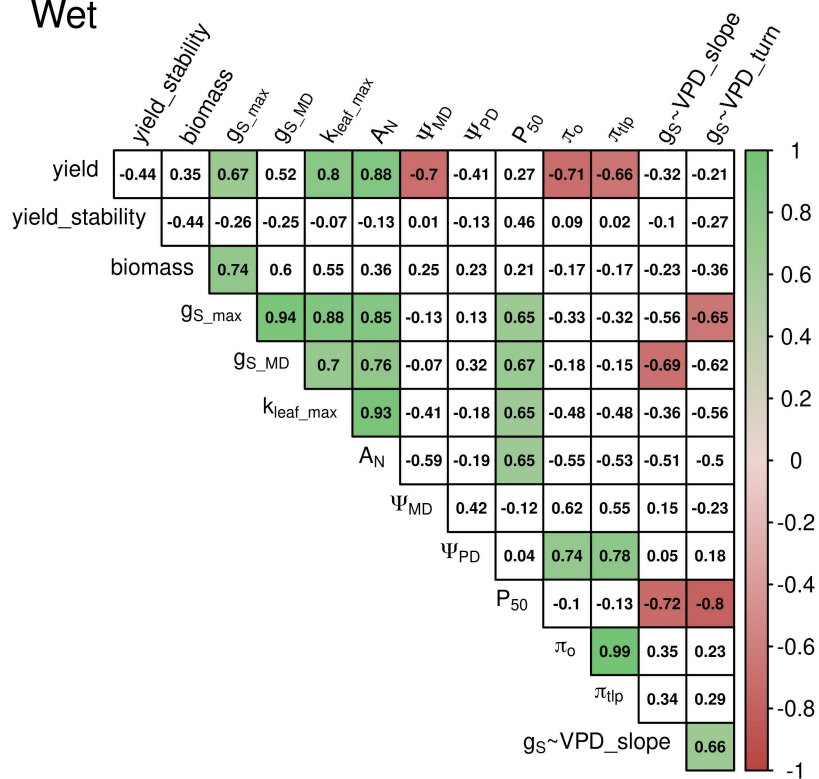
a)



b)



Wet



Dry

