Title

Low stomatal sensitivity to vapor pressure deficit in irrigated common, lima and tepary beans

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Title. Low stomatal sensitivity to vapor pressure deficit in irrigated common, lima and tepary beans

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ABSTRACT

A limited transpiration rate under high vapor pressure deficit (VPD) could be used to conserve soil water for later use under drought conditions. Many crops show this behavior either as limited transpiration or decreases in stomatal conductance. However, little work has been done in *Phaseolus*. Four experiments evaluated stomatal closure across a range of VPD for well-watered plants, each experiment using varying combinations of genotypes of common (15), lima (6) and tepary beans (7 genotypes). A two-year experiment found genotypic variation in average stomatal conductance, but genotypes only had 14% stomatal closure between a VPD of 1 to 4 kPa. In comparison, soybean, which is known to close stomata, had a 40% decrease for similar conditions in Davis, CA, USA. In a second field experiment and outdoor pot experiments, genotypes from the three species displayed, on average, a 34, 50 to 45% increase in stomatal conductance with increasing VPD. Six genotypes were statistically indistinguishable from a 40% decrease, but all had low probability (p<0.21) of having 40% closure, and some showed little closure in other experiments. The VPD range measured in this study was large relative to the range for hot, arid California, thus the results are generalizable: most *Phaseolus* beans are not expected to have appreciable stomatal closure under well-watered conditions. Thus, there is limited evidence that *Phaseolus* has somegenetic diversity in stomatal responses to VPD, relative to that shown in other species. However, there was constitutive genetic variation in species and genotypic stomatal conductance under low VPD conditions.

Keywords: soil water deficit avoidance, drought, *P. vulgaris, P. lunatus, P. acutifolius,* limited transpiration

Abbreviations: C, common bean; g_{H2O}, stomatal conductance to water vapor; L, lima bean; P, cowpea; T, tepary bean; T_{air}, air temperature; VPD, vapor pressure deficit.

1. Introduction

A limited transpiration rate at high vapor pressure deficit (VPD) for well-watered conditions has been used as a mechanism to breed water conservative crops (Sinclair et al., 2010; Sinclair et al., 2016). Using the technique of weighing pots under high VPD, many crops have been found to have this behavior, including legumes such as: chickpea (Zaman-Allah et al., 2011), cowpea (Belko et al., 2012), peanut (Devi et al., 2010), and soybean (Sadok and Sinclair, 2009), and other crops like corn (Yang et al., 2012; Gholipoor et al., 2013), sorghum (Gholipoor et al., 2010), tall fescue (Sermons et al., 2012), and wheat (Schoppach and Sadok, 2012). An alternative technique can be used for field evaluations, where measurements of stomatal closure under high VPD would correspond to limited transpiration, and has been applied, for example, to soybean (Gilbert et al., 2011; Medina and Gilbert, 2016) and peanut (Shekoofa et al., 2015). However, little work has determined whether this behavior is found in common bean (*Phaseolus vulgaris* L.) or other domesticated *Phaseolus* species. One greenhouse study did find that varieties of common bean had stomatal closure when exposed to the VPDs at the extreme of those experienced during growth (Comstock and Ehleringer, 1993).

Finding such stomatal closure in beans in the field would be useful, as stomatal closure early in the season would lead to a decrease in water use, and thus soil water conservation for later periods of drought, as evidenced by delayed wilting in soybean (King et al., 2009). Common bean

may benefit from conservative water use as they are often grown in drought prone environments(Singh, 2001; Polania et al., 2016). Despite a large quantity of drought related work on *P. vulgaris*, and a dry origin for many genotypes (e.g. Northwestern Mexico and Chile), limited transpiration or stomatal closure behavior remains obscure in this species.

Less drought research has been done on lima bean (*P. lunatus* L.), despite the wide adaptation range of this species (Debouck, 1999; Maquet et al., 1999; Gepts, 2001; Freytag and Debouck, 2002; Delgado Salinas and Gama López, 2015). Tepary bean (*P. acutifolius* A. Gray) is the "archetypal" drought tolerant crop – growing in the agriculture system with the least annual rainfall in the world (Freeman and Station, 1912; Nabhan, 1990; Rainey and Griffiths, 2005). However, tepary drought tolerance may derive from a fast completion of its lifecycle, thereby avoiding soil water deficit. The species may also rely on just one monsoonal rainfall season; this would not allow provision of future rainfall, which water conservative behavior would benefit from. Thus, including the broadly adapted lima bean and the extreme arid environment tepary would increase the chances of finding alternative stomatal behaviors.

The objective was to determine if genotypes and species in domesticated *Phaseolus* had variation in stomatal closure at high evaporative demand under well-watered conditions. The hypothesis was that genotypes/species from arid environments would have the greatest closure under high evaporative demand, conserving the most water. Similarly, the least commercially improved species, tepary bean, would have more drought-adapted traits, such as water conservation and lower stomatal conductance.

2. Material and methods

Four experiments were undertaken on combinations of species and genotypes (Table 1): Experiment A: a field trial on eight common bean genotypes, and some other selections in 2013 and 2014; Experiment B: an outdoor pot experiment in 2014 on the same eight genotypes as in Experiment A; Experiment C: a parallel pot experiment to B on three genotypes from each of the three bean species; Experiment D: a field trial in 2015 on four to five genotypes from each of the three bean species.

Table 1. Genotypes of beans used in the pot and field experiments

Species	Genotype	Type/origin	Growth habit[a]	Experiment			
				A	B	C	D
Vigna unguiculata	CB46	CA, UCD	II	Y2013[b]			
P. vulgaris	SEA5	M, CIAT[c]	II	Y	Y		
	UCD9634	D/J, UCD	II	Y	Y		
	Flor de Mayo Eugenia (FDM)	J, INIFAP	III	Y	Y		Y
	Matterhorn	D, MSU	II	Y	Y		
	Victor	D/J, USDA, WSU	III	Y	Y		Y
	Pinto San Rafael (PSR)	D, INIFAP	III	Y	Y		
	L88-63	M, MSU	II	Y	Y		
	SER118	M, CIAT	II	Y	Y		
	SXB405	M, CIAT	II	Y			
	Tio Canela 75 (TC75)	M, EAP	II	Y2013			
	ICA Bunsi	M, ICA	II	Y			

	BAT477	M, CIAT	III			Y
	CA Early Light Red Kidney (CELRK)	CA, UCD	I		Y	
	UCD Cran 0801	CA, UCD	I		Y	
	Jalo EEP558	A, Brazil, UCD	I		Y	
P. acutifolius	Yellow	Tucson, Arizona	III			Y
	Yoeme Brown	South Sonora Coast	III			Y
	Paiute White	Southern Utah	III			Y
	San Pablo Balleza (SP.Balleza)	Chihuahua, Mexico	III			Y
	G40010	El Salvador, CIAT	III		Y	Y
	T-241 White	USA (WA), USDA	III		Y	
	WYOMING 27905	USA (WY), USDA	III		Y	
P. lunatus	UC 92	A, UCD	bush	Y2013		
	UC Haskell	M, UCD	vine	Y2013	Y	Y
	G26451	M, UCD	vine		Y	Y
	Pima Orange	M, Gila River, AZ	vine			Y
	G27360	M, Mexico, CIAT	vine			Y
	Dompe 95	A, UCD	bush		Y	

[a] Growth Habit: I, determinate bush; II, indeterminate bush; III, indeterminate prostrate; IV, indeterminate climbing.

[b] included in experiment in 2013 but not 2014.

[c] Abbreviations: A, Andean; CA, California; CIAT, International Center for Tropical Agriculture; D, Durango; EAP, Escuela Agrícola Panamericana - Zamorano, Honduras; J, Jalisco; ICA, Instituto Colombiano de Agricultura M, mesoamerican; UCD, University of California Davis; USDA, United States Department of Agriculture.

The selection of common bean and lima bean genotypes used for these experiments included representatives of Andean and Mesoamerican centers of domestication and, for common bean, included diverse eco-graphic races from Mexico (Durango and Jalisco). Tepary bean accessions were selected from geographically distinct areas (Table 1), although less is known about tepary diversity (Schinkel and Gepts 1988, 1989; Blair et al. 2012).

All field trials were performed during the summer on the Plant Sciences Research Station of the University of California Davis (38.53N, -121.78E). This Central Valley site receives no rainfall in the summer (<0.25cm) due to a hot, arid Mediterranean climate (Csa, Köppen climate classification). The soil type for the 2013 and 2014 field experiments was a Yolo silty loam, fine silty, mixed, nonacid, thermic Mollic Xerofluvents, and in 2015 a similar adjacent Reiff very fine sandy loam.

2.1. Experiment A (Field 2013 and 2014)

A field experiment was conducted in 2013 and 2014 on eight diverse genotypes of common bean and some other common and lima bean genotypes in some of the years (Table 1; planted: 5 Jun 2013 and 8 Jun 2014, harvested: 10 Sep 2013 and 12 Sep 2014). The experiment consisted of three blocks/replications of the genotypes planted in random order, a randomized complete block design (RCBD) in 2013, and in 2014 a staggered design was used where initial measurements were done on three well-watered blocks, subsequently the second block was subject to terminal drought starting on 14 Jul 2014. Thus, the two years did not have a consistent blocking design, and block effects were not accounted for. The genotype plots consisted of one single row bed, 6.1 m long, with 0.76 m spacing between rows. A small alley at the end of the plot separated plots (~1m),

otherwise all plots were either bordered by other genotypes, or a five row field border planting. Plants were sampled for gas exchange more than 1m into the plot. The seeds were machine planted, 10 cm apart, flood pre-irrigated, and later maintained with four flood irrigation events. Each flood irrigation brought the rooting volume to field capacity. Pest and diseases were controlled using conventional chemical controls. Gas exchange measurements on well-watered plants were made on all blocks in random order within five days of flood irrigation. Water deficit blocks (2014) were measured on the same days as well-watered plants in random order, alternating between well-watered and water deficit blocks.

2.2. Experiment B (Outdoor pots 2014)

Plants were grown in large pots (11.4L) in an open field at the UC Davis Orchard Park Greenhouse facility during the summer of 2014 in a RCBD. Four blocks were planted with random order of genotypes within the blocks, and each well-watered pot had a water deficit pot adjacent to it. A border, one pot wide, of a common bean genotype (BAT477) was planted around the entire experiment. Eight common bean genotypes from Experiment A were measured in this experiment (Table 1). Pots had a custom mix of sand, topsoil, pumice, fir bark and peat moss, 3:3:2:1:1, by volume. Pots were whitewashed and the grow area covered with 50% shade cloth to prevent pot heating. Three seeds of a genotype were planted per pot along with those of another genotype, BAT477. After emergence, seedlings were thinned so that there was only one seedling per genotype per pot. Measurement of BAT477 acted as a within pot control for variation between pots across space and time. All plants were fertigated with a modified Hoagland solution using a pressure compensating dripper. After establishment (two weeks), two stakes were placed to

provide anchorage and support for each plant per pot, and the overhead shade removed when the developing plant canopy was considered to prevent pot overheating. The experiment extended from planting (11 Aug 2014) to harvest of biomass (19 Sep 2014). Manual weeding and pesticides were applied as needed. Water was withheld from water deficit pots for seven days from 22 days after planting, leading to rapid dry-down in comparison to the field experiments.

2.3. Experiment C (Outdoor pots 2014)

Plants were grown in the same arrangement and at the same time as described in B, but consisted of a different grouping of genotypes, in this case three genotypes of common, lima and tepary beans (Table 1). Different sampling days and a separate LICOR6400 to the other experiments was used to measure these genotypes (Table 2).

Table 2.

Weather summary for the days of measurement

Experiment	Date (m/dd/yyy)	T_{air} range (^{o}C)	Max. VPD (kPa)
A (field)	7/18/2013	11.6-33.6	4.1
	8/6/2013	9.8-28.1	2.4
	8/16/2013	16.5-34.4	3.8
	7/17/2014	16.7-28.7	2.1
	7/31/2014	15.9-36.2	4.5

B (pots)	9/4/2014	11.8-34.8	5.5
	9/6/2014	11.3-32.2	4.6
C (pots)	9/7/2014	11.5-32.4	4.6
B and C (pots)	9/10/2014	17.9-36.5	6.2
	9/12/2014	12.8-37.5	6.9
	9/14/2014	15.3-35.2	5.4
	9/16/2014	12.7-32.5	4.5
D (field)	7/28/2015	16.3-38.6	5.8
	8/5/2015	10.6-32.5	3.4
	8/17/2015	14.2-39.2	5.8
	8/24/2015	13.1-33.9	4.0
	8/31/2015	12.9-33.3	3.8

153

154 *2.4. Experiment D (Field 2015)*

155 A field experiment was conducted on four genotypes of common and lima bean, and five
156 of tepary bean selected based on indeterminate growth habit to control for differences among the
157 species (Table 1; planted: 6/21/2015, harvested: 10/22/2015). The experiment consisted of five
158 blocks/replications of the 13 genotypes planted in random order, a RCBD. Genotype plots
159 consisted of two 1.50 m wide and 3.05 m long double row beds, with 0.76 m spacing between
160 rows (i.e. a plot was four rows). The seeds were hand planted, 10 cm apart, sprinkler irrigated for
161 establishment, and later maintained with 20 cm deep, subsurface drip. Each block was split by a
162 two row border and irrigation was withheld from the second plot 30 days after planting. The
163 experiment was surrounded by a two row border (east-west) or 3.05m long plot (north-south) of
164 varying genotypes. Gas exchange data was only collected from the two middle rows at least 1m

from each end of the row. Pest and diseases were controlled with using conventional chemical controls.

2.5. Climate

Daily weather was obtained from the Davis station in the California Irrigation Management Information System (CIMIS, 2015) for the period of 1983 to 2015. The weather station is within 1500 m of all the field and outdoor pot sites. The daily maximum air temperature and minimum relative humidity was used to calculate the maximum daily VPD percentiles for the months of the year. Historically, Davis CA has the majority of days in a June to September growing season with maximum daily VPD between 2.4 and 4.4 kPa (Fig. 1). At the extreme, 95% of days during the growing season have maximum daily VPD's between 1.1 and 6.1 kPa. These are extreme values, as the majority of daylight hours have lower VPD's than the daily maximum. Thus, the range of VPD's measured in these experiments (up to 5 or 6 kPa; Table 2), is representative of the extremes of the hot, arid climate of California.

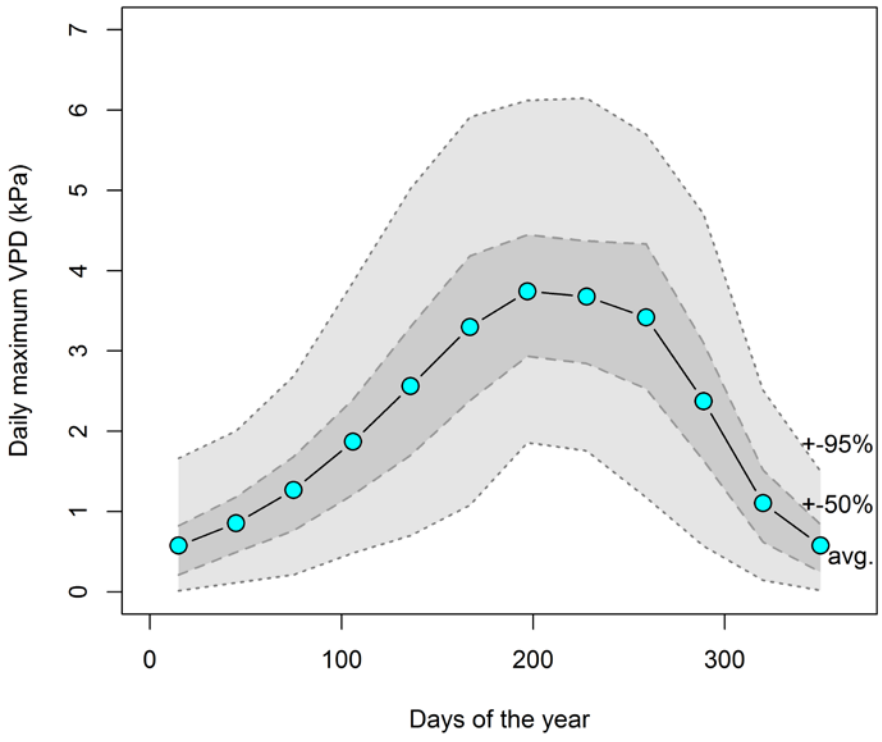

Fig. 1. Variation in the daily maximum VPD for Davis CA for the period 1983 to 2015. Values are the average daily maximum VPD for each month, or the interval containing 50 or 95% of daily maximum VPD values.

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2.6. Gas exchange measurements

A LICOR6400 with 2 cm^2 fluorometer attachment, or the standard LED-lit, 6 cm^2 chamber (LI-COR, Lincoln, NE) were used to measure stomatal conductance (g_{H2O}). Measurements were made by measuring all genotypes in a block, including both irrigation treatments, and then advancing to another block. The order of block measurement was randomized each day. Measurements were on sunny days starting between 8:30 – 11:00AM and ending between 4:10 –

6:00PM allowing a wide range of VPD to be measured in one day. In Davis, VPD typically increases due to increasing air temperature, not vapor pressure, during the day until late afternoon; thus measurements represent stomatal response to increasing VPD. Time-of-day effects could not be distinguished from VPD effects as the two co-vary strongly. A trifoliolate leaf was selected for measurement based upon being in the sun and fully expanded. Chamber conditions were: saturating PPFD, ranging between experiments from 1600 to 2000 μmol m^{-2} s^{-1}, and set at a particular level for the entirety of an experiment; flow, 250 to 400 μmol s^{-1} for the fluorometer chamber and 500 to 700 μmol s^{-1} for the large chamber and chamber CO_2, 400 μmol mol^{-1}.

Evaporative conditions inside a gas exchange chamber are not possible to match with the ambient environment, and no effort was made to do so here. Specifically, in order to measure stomatal conductance the chamber removes the boundary layer from the measured leaf (McDermitt, 1990). This means that even if air humidity, air and leaf temperature were equalized before and after putting the leaf in the chamber, then the evaporative conditions would still be different. Instead, the chamber was allowed to equilibrate for between 120 and 180 seconds, and stomatal conductance measured, avoiding time for the stomata to respond to the new environment. Thus, the measurements were intended to represent the stomatal conductance to the ambient conditions, not to those inside the chamber. An exception was that when leaf temperatures in the chamber exceeded ~38^{o}C, rapid stomatal closure was observed during the equilibration period. To avoid such hydropassive or damage responses, chamber wall (block) temperature was generally set at 35^{o}C when needed to avoid extreme leaf temperatures, but was set at 39^{o}C when needed in the 2014 field experiment. In effect, the chamber air temperature varied with ambient conditions until it reached a threshold after which it was controlled by the cap on block temperature.

Simultaneous measurements of air temperature and relative humidity were measured either using a HTM2500LF sensor (Measurement Specialties Inc., Toulouse, France) attached to the exterior of the LICOR6400 and covered by a unaspirated radiation shield (2013 and 2014 field experiments), or a Campbell Scientific (Logan, Utah) weather station with a unaspirated radiation-shielded HMP60 sensor for the other experiments. The weather stations were situated within the experimental fields (less than 50m from all measurements), and the air temperature sensor positioned at 2m height above ground.

2.7. Statistical analysis

The relationship between stomatal conductance (g_{H2O}) and VPD was analyzed by linear model (multiple regression) for each experiment separately. The basic model used was g_{H2O} as response variable, VPD as covariate and genotype as factor (Fig. 2). The interaction between VPD and genotype was tested to determine if there were genotype differences in slope of the g_{H2O} to VPD relationship, as is standard for testing the assumptions of ANCOVA (step A and B, Fig. 2). Thus, if the interaction term was not significant (step C, Fig. 2), then it was removed and slopes considered equal for all genotypes (Engqvist, 2005). The effect of the Day of measurement and Block effects were included in the linear model as additional factors.

Differences in slopes between/within experiments would lead to difficulty in interpreting extrapolated Y-intercepts, thus a standard value, the fitted g_{H2O} value at low VPD (i.e. VPD = 2 kPa) was used when comparing species between experiments. Due to a large variation in scale, the natural logarithmic transformation was used to compare g_{H2O} values between experiments.

Given the variability in the data, an important question is whether linear regression can detect stomatal closure. Specifically, what is the probability of avoiding a type II error, i.e., finding a regression slope of zero when a slope really exists? Firstly, a standard value of closure was used based upon the observed closure for soybean measured in Davis by the same authors (Medina and Gilbert, 2016), i.e., closure was considerable if there was a 40% decrease between 1 and 4kPa VPD. Type II errors were assessed by resampling (with replacement) the observed pairs of g_{H2O} and VPD data within a genotype within an experiment (R Core Team, 2016). Fitting lines to each of these bootstraps generated a confidence interval of the g_{H2O} to VPD relationship for each genotype, and a probability that the data could represent a standard 40% stomatal closure could be approximated. Bootstrapping was chosen to avoid undue bias of the results by outliers and single points.

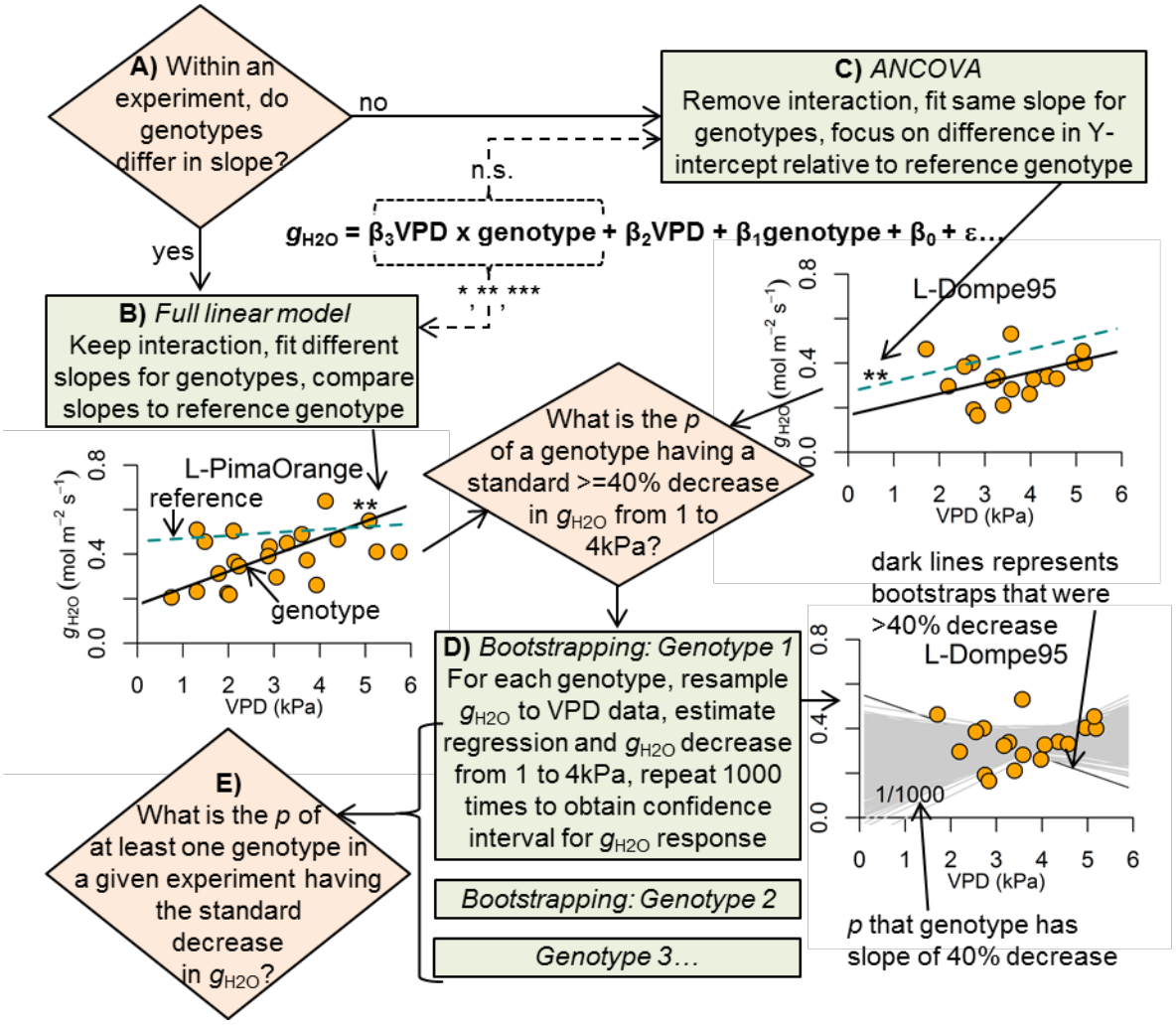

Fig. 2. Statistical procedure used to evaluate whether the relationship of g_{H2O} to VPD of genotypes varied within an experiment (step A, B or C), evaluation of the probability that a genotype had at least a 40% decrease in g_{H2O} over the VPD range 1 to 4kPa (step D), similar to that observed in Medina and Gilbert (2016), and the evaluation of experiment-wise probability of a standard decrease in at least one genotype (step E).

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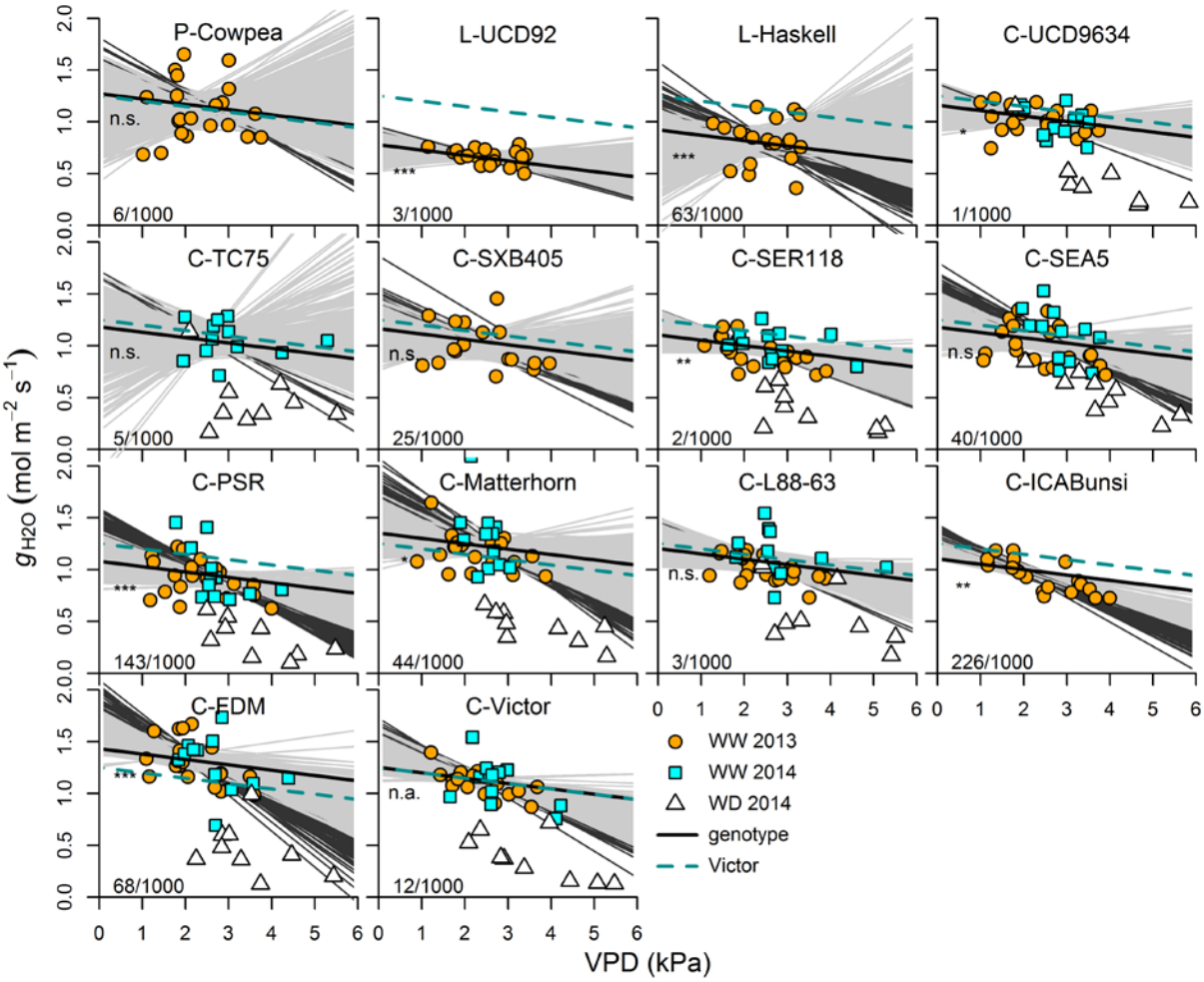
A second analysis directly tested the power of detecting stomatal closure. Three datasets were available that contained a large number of observations of the g_{H2O} versus VPD relationship: the original dataset of Medina and Gilbert (2016) for two very similar genotypes of soybean showing a 40% decrease in g_{H2O}; and in experiment B and C, described above, measurements of genotypes were paired with measurements of a common bean (BAT477) grown as a control in the same pot. A random resampling (with replacement) of pairs of g_{H2O} and VPD was performed 10000 times for each dataset using *PopTools* (Hood, 2010). The number of samples resampled from each dataset was varied from ten samples to the total number in the dataset. For each sample, linear regression was used to estimate the percentage change in g_{H2O} with a shift from 1 to 4kPa VPD. Then for each sample size, for each dataset, the 95% confidence interval was found of the percentage change. From this the sample size was found that had sufficient power to reliably distinguish the standard closure from those of BAT477 in Experiment B or C. A parametric equivalent to this analysis was also performed using the *pwr* package of R (Champely et al., 2016), asking: What is the probability (power) of avoiding a type II error? Thus, for the correlation coefficient of the Medina and Gilbert (2016) dataset and an alpha of 0.05, the power could be found for varying sample sizes.

3. Results

3.1. Experiment A (Field 2013 and 2014)

The responses of g_{H2O} to VPD were comparable between the years of measurements measured in the field on diverse common beans (Fig. 3), with the two years having the same slope

277 (VPD x Year: $F_{1,234} = 0.513$, $p = 0.474$; with analysis limited to genotypes common to both years),
278 but was 0.130 mol m^{-2} s^{-1} higher in Y-intercept in 2014 (Year: $F_{1,234} = 18.1$, $p < 0.001$). All
279 genotypes had the same statistical slope of g_{H2O} response to VPD (slope = -0.052 mol m^{-2} s^{-1} kPa^{-1};
280 Genotype x VPD: $F_{13,334} = 1.05$, $p = 0.404$; VPD: $F_{1,347} = 51.9$, $p < 0.001$). These data represent
281 a 14% decrease in g_{H2O} between VPD's of 1 and 4 kPa for the average genotype. Year and Day of
282 measurement had large effects (Year: $F_{1,347} = 15.3$, $p < 0.001$; Day: $F_{3,347} = 8.27$, $p < 0.001$). Y-
283 intercept differed between genotypes (range 0.654 mol m^{-2} s^{-1}; Genotype: $F_{13,347} = 18.9$, $p < 0.001$).
284 Relative to Victor (C-Victor), the common bean used as a reference, the two lima beans in 2013
285 had a lower Y-intercept as did a number of common beans (Pinto San Rafael, SER118, and ICA
286 Bunsi; *p*-values given in figures) while two common beans had higher g_{H2O} (Matterhorn and Flor
287 de Mayo Eugenia). The terminal drought led to considerable stomatal closure in the 2014
288 experiment, indicating that had there been considerable closure at high VPD in the well-watered
289 treatment, it would have been observed.



290

291 **Fig. 3.** Stomatal conductance (g_{H2O}) to vapor pressure deficit (VPD) responses for the 2013 and
292 2014 field trial (Experiment A). The dashed line represents the reference genotype Victor, and
293 solid line the linear model fit for the genotype of interest. Significance values (*** etc) represent
294 the difference between the Y-intercept of the genotype of interest and the reference genotype. Light
295 and dark lines represent 1000 bootstrap fits to a genotype, with dark lines and values (e.g. 6/1000)
296 representing the fits that had closure of at least 40% between 1 and 4kPa, similar to closure
297 observed previously in soybean (Medina and Gilbert, 2016). C, common bean; L, lima bean; P,
298 cowpea.

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3.2. Experiment B (Outdoor pots 2014)

The same core selection of eight common beans as in Experiment A were measured in outdoor pots (Fig. 4). There were no significant differences in the slope of the g_{H2O} to VPD relationship (Genotype x VPD: $F_{7,196} = 0.512$, $p = 0.825$) and the general slope was positive (slope = 0.095 mol m^{-2} s^{-1} kPa^{-1}; VPD: $F_{1,203} = 11.0$, $p = 0.001$). Day of measurement had large effect (Day: $F_{6,203} = 10.5$, $p < 0.001$) while Block had little effect (Block: $F_{3,203} = 1.68$, $p = 0.173$). Flor de Mayo Eugenia (C-FDM) had a higher g_{H2O}, consistent with Experiment A (Genotype: $F_{7,203} = 5.97$, $p < 0.001$). Extreme stomatal closure was present on the two days of greatest water deficit for the water deficit (WD) pot measured immediately after the well-watered pot (WW).

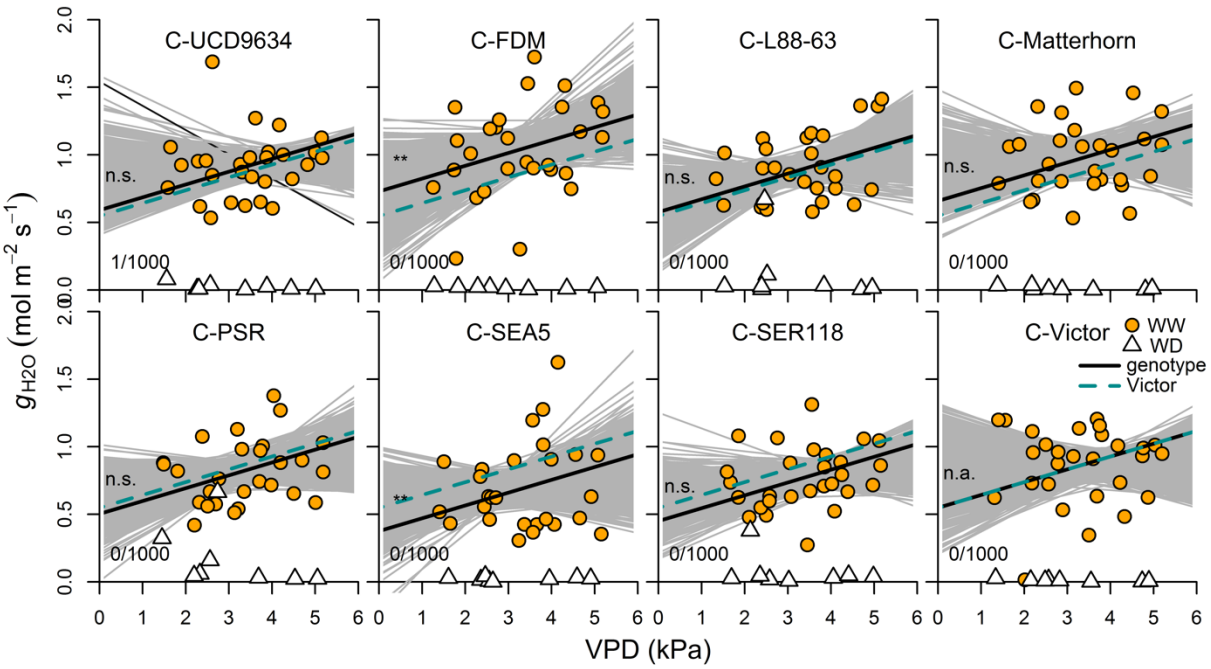

Fig. 4. Stomatal conductance (g_{H2O}) to vapor pressure deficit (VPD) responses for the 2014 outdoor pot experiment (Experiment B) with similar common bean genotypes to Experiment A. The dashed line represents the reference genotype Victor, and solid line the linear model fit for the genotype of interest. Significance values represent the difference between the Y-intercept of the genotype of interest and the reference genotype. Light and dark lines represent 1000 bootstrap fits to a genotype, with dark lines and values (e.g., 1/1000) representing the fits that had closure of at least 40% between 1 and 4kPa, similar to closure observed previously in soybean (Medina and Gilbert, 2016). C, common bean.

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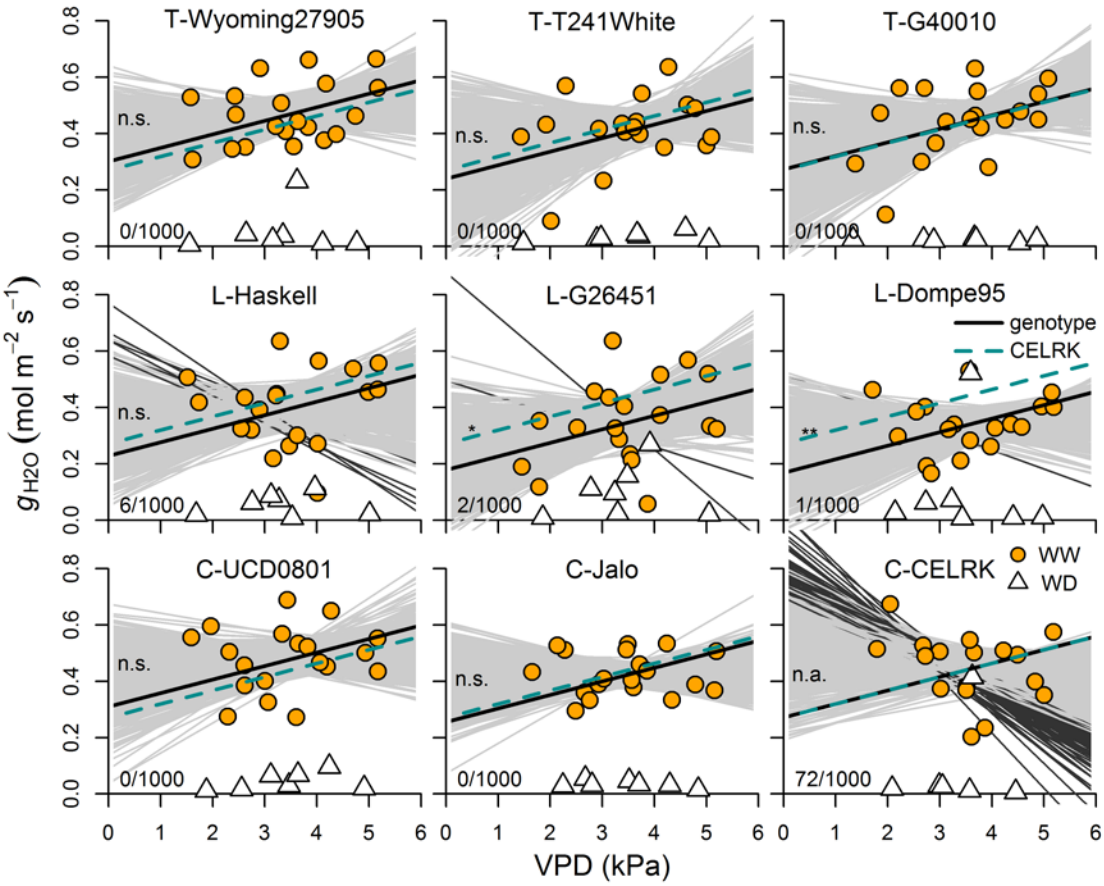
3.3. Experiment C (Outdoor pots 2014)

The absolute values of g_{H2O} measured in the Experiment C and subsequent field experiment (D) were about half of the values in experiment A and B (Fig. 3 and 4 compared to 5 and 6). It is unclear what the reason for this difference was, as Experiments A, B and D were measured with the same LICOR6400, while Experiment C used another. The first LICOR6400 was calibrated prior to experiment A, and after use in D was checked and found to be accurate, thus calibration does not explain the differences between Experiment A, B and the lower values in D. Measurements of BAT477 in Experiment B and C demonstrated that the two LICOR6400's appeared to be calibrated differently (for water or temperature, but not CO_2). Regardless, all data within an experiment were measured using the same gas exchange system, and were internally comparable. The water deficit measurements demonstrated that the systems were capable of measuring closure.

There were no significant differences in the slope of the g_{H2O} to VPD relationship in Experiment C (Fig. 5; Genotype x VPD: $F_{8,143} = 0.948$, $p = 0.479$) and the general slope was small and positive (slope = 0.078 mol m^{-2} s^{-1} kPa^{-1}; VPD: $F_{1,151} = 6.76$, $p = 0.0102$). Day of measurement had large effect (Day: $F_{4,151} = 6.19$, $p < 0.001$) while Block had a smaller effect (Block: $F_{3,151} = 3.76$, $p = 0.012$). Stomatal conductance varied between genotypes, with two lima bean genotypes, Dompe 95 and G26451 lower than the common bean California Early Light Red Kidney used as a reference (Genotype: $F_{8,158} = 3.40$, $p = 0.0012$). Extreme stomatal closure was present in the WD treatment, similar to Experiment B.

The pot experiment data for B and C were noisy, and it could be argued that visually the points appear random, despite strong statistical support. However, for every measurement of a genotype in a pot, a matching measurement was made on BAT477, a common bean, planted in all pots as a within-pot control. There were no significant effects of companion genotype on BAT477's g_{H2O} to VPD relationship (data not shown; Experiment B - Companion genotype x VPD: $F_{7,196} = 1.01$, $p = 0.425$; VPD: $F_{2,196} = 0.794$, $p = 0.374$; Companion genotype: $F_{7,196} = 1.18$, $p = 0.314$; Day: $F_{4,196} = 10.5$, $p < 0.001$; Block: $F_{3,196} = 1.68$, $p = 0.173$; Experiment C - Companion genotype x VPD: $F_{8,142} = 0.559$, $p = 0.81$; VPD: $F_{1,142} = 0.176$, $p = 0.68$; Companion genotype: $F_{8,142} = 1.90$, $p = 0.065$; Day: $F_{4,142} = 7.15$, $p < 0.001$; Block: $F_{3,142} = 2.89$, $p = 0.037$). These are a strong indication that the results of Experiments B and C were statistically robust. Outlying values of g_{H2O} for a genotype (e.g., lower than 0.2 mol m^{-2} s^{-1} at low VPD), in general, had matching low values for BAT477, indicating a potspecific reason for the outliers.



353

354 **Fig. 5.** Stomatal conductance (g_{H2O}) to vapor pressure deficit (VPD) responses for the 2014
355 outdoor pot experiment comparing species (Experiment C). The dashed line represents the
356 reference genotype California Early Light Red Kidney bean, and solid line the linear model fit for
357 the genotype of interest. Significance values represent the difference between the Y-intercept of
358 the genotype of interest and the reference genotype. Light and dark lines represent 1000 bootstrap
359 fits to a genotype, with dark lines and values (e.g. 6/1000) representing the fits that had closure of
360 at least 40% between 1 and 4kPa, similar to closure observed previously in soybean (Medina and
361 Gilbert, 2016). C, common bean; L, lima bean; T, tepary.

362

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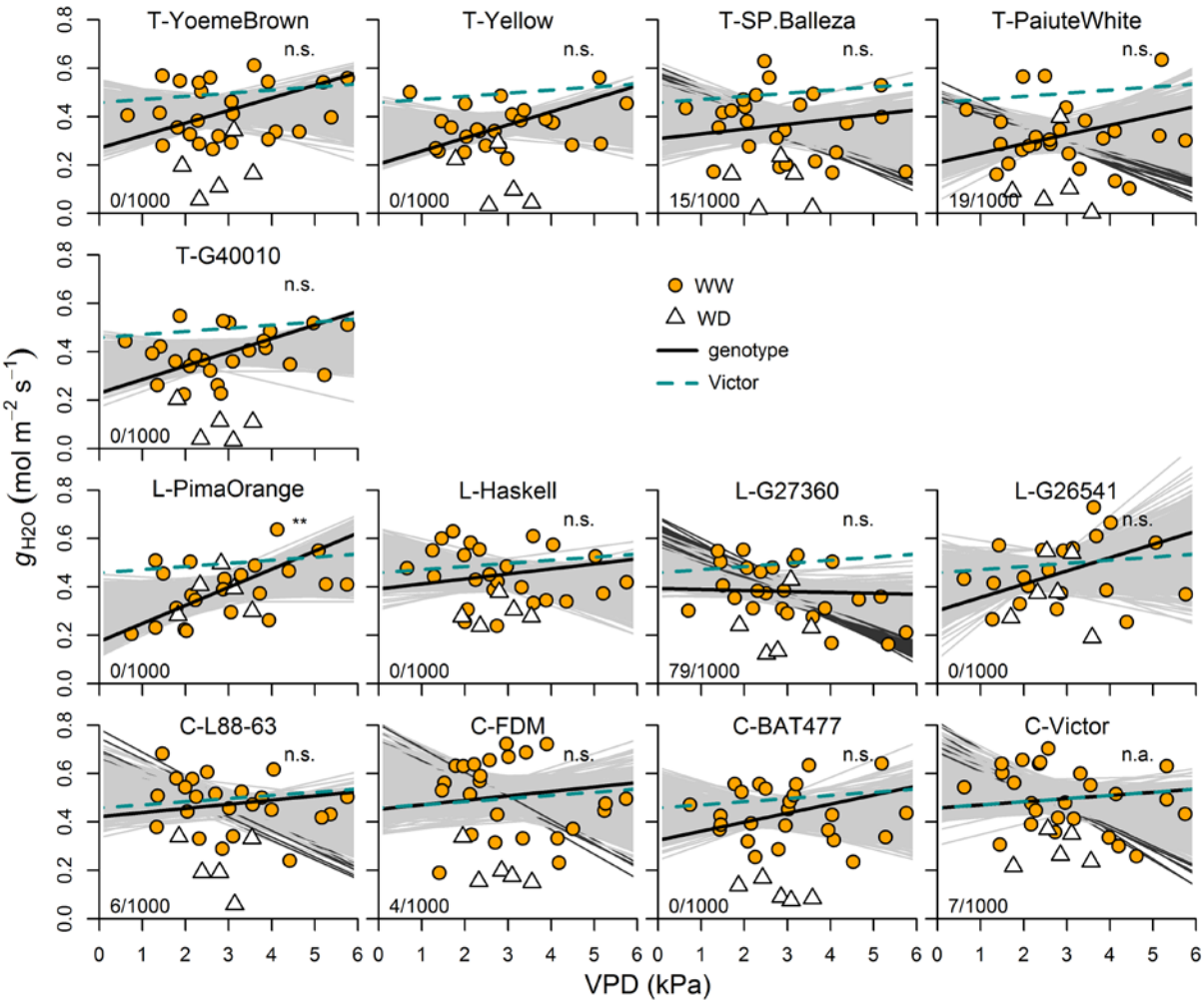
3.4. Experiment D (Field 2015)

There were differences in slope between genotypes for the g_{H2O} to VPD relationship in experiment D (Fig. 6; Genotype x VPD: $F_{12,280} = 1.93$, $p = 0.031$), and no significant main slope effect (VPD: $F_{1,280} = 0.402$, $p = 0.527$). Genotypes had variation in g_{H2O} for a given VPD (Genotype: $F_{12,280} = 7.21$, $p < 0.001$). The slope of Pima Orange was highest, and different from Victor, the reference. Although slopes varied, all five teparies and two lima beans had lower g_{H2O} for most of the VPD range relative to common bean, Victor. In all field trials, Victor had one of the highest g_{H2O}'s similar to Flor de Mayo Eugenia, which was consistently the highest. Terminal drought led to considerable stomatal closure in tepary and common bean genotypes, but not lima beans, which did not show other symptoms of stress either (stem water potentials were similar between treatments for lima bean accession's).



376

377 **Fig. 6.** Stomatal conductance (g_{H2O}) to vapor pressure deficit (VPD) responses for the 2015 field
378 experiment comparing species (Experiment D). The dashed line represents the reference genotype
379 Victor, and solid line the linear model fit for the genotype of interest. Significance values represent
380 the difference between the slope of the genotype of interest and the reference genotype. Light and
381 dark lines represent 1000 bootstrap fits to a genotype, with dark lines and values (e.g. 6/1000)
382 representing the fits that had closure of at least 40% between 1 and 4kPa, similar to closure
383 observed previously in soybean (Medina and Gilbert, 2016). C, common bean; L, lima bean; T,
384 tepary.

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386 *3.5. Evaluation of statistical power*

387 Four lines of evidence indicate that there was limited stomatal closure present in the many
388 genotypes sampled. Firstly, soil water deficit treatments led to large decreases in stomatal
389 conductance, indicating that if closure had occurred then the equipment and experimental design
390 were capable of measuring the closure. Secondly, if ~25 random pairs of g_{H2O} and VPD were
391 drawn from the large dataset of Medina and Gilbert (2016), representing the standard case of 40%
392 closure in soybean, then this sample size would be sufficient to detect a statistical difference to a
393 slope of zero. The control genotype, BAT477, in Experiment B or C showed a significant
394 difference to the standard soybean data with a sample size of 25, confirming this analysis (Fig.
395 7A). Thirdly, a similar parametric analysis also indicated that a sample size of ~25 is needed to
396 have a power of 0.95, or 95% probability of avoiding type II errors (Fig. 7B). Sample sizes, per
397 genotype, were: Experiment A between 12 and 33 (average 26, majority > 30); B between 27 and
398 28; C between 18 and 20; D between 20 and 25 (average 24, majority 25). Thus, most analyses
399 had approximately sufficient samples to result in a power to avoid type II errors of 0.9 or higher.

400 Finally, bootstraps of the slope of the relationship of g_{H2O} to VPD for each genotype
401 indicated that Experiment A had $p = 0.496$ that at least one genotype had a slope of greater than
402 40% closure, with four of fourteen genotypes being not significantly different to a slope of 40%,
403 but in all cases the average slope was shallower than 40%. Experiment B was not consistent with
404 closure ($p = 0.001$, that at least one genotype had 40% closure). Experiment C had $p = 0.081$
405 probability that at least one genotype had closure, with only one genotype (California Early Light
406 Red Kidney bean) showing any probability of closure ($p = 0.072$). Experiment D had a $p = 0.129$

that at least one genotype had closure, with only one genotype with a non-significant slope to a 40% closure ($p = 0.068$).

A broader question is why there is high variability in stomatal conductance for a given VPD and within a genotype? Day of measurement effects were considerable and further experimentation is necessary to evaluate the reasons for this. A partial explanation for variability in g_{H2O} is that at high values the equation used has a small denominator, leading to noise in primary measurements propagating large noise in g_{H2O}. That is, the primary measurement, total conductance has a denominator of leaf internal water vapor mole fraction minus ambient water vapor mole fraction (Anonymous, 2011). This difference is minimized as stomatal conductance increases, and as internal water vapor is calculated from leaf temperature, small noise in leaf temperature leads to increasing variability in conductance.

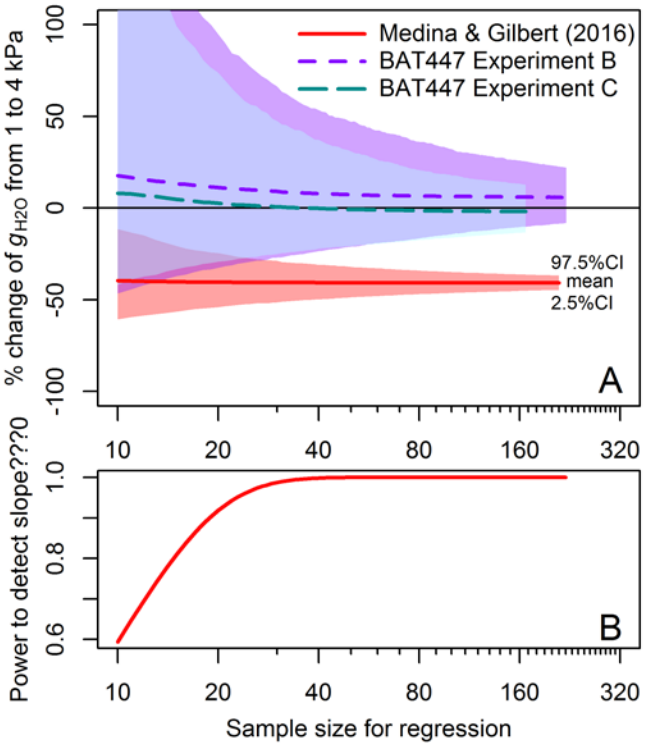

Fig. 7. Confidence intervals (95%) of the percentage change in stomatal conductance between 1 and 4kPa VPD, as estimated by linear fits to resamples of large datasets varying the size of the resample (panel A) and power to detect the standard 40% stomatal closure with varying sample sizes for the data of Medina and Gilbert (2016) (panel B). Three available large datasets representing specific genotypes were compared, each with large sample sizes and each simulated for sample sizes varying from ten to the total dataset size. See Materials and methods for details of analysis.

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3.6. Species differences

A consistent pattern across Experiments A, C and D was the lower g_{H2O} of lima bean relative to common bean (Fig. 8; Species: $F_{1,21} = 222.7$, $p < 0.001$; genotype g_{H2O} predicted for 2 kPa was used as replicates, and tepary beans were removed as teparies were not represented in the first experiment). The g_{H2O} of tepary bean genotypes was lower than lima bean in the field, but higher in pots (Species x Experiment: $F_{2,16} = 7.43$, $p = 0.005$; comparing just the experiments with teparies: pot experiment C and field experiment D).

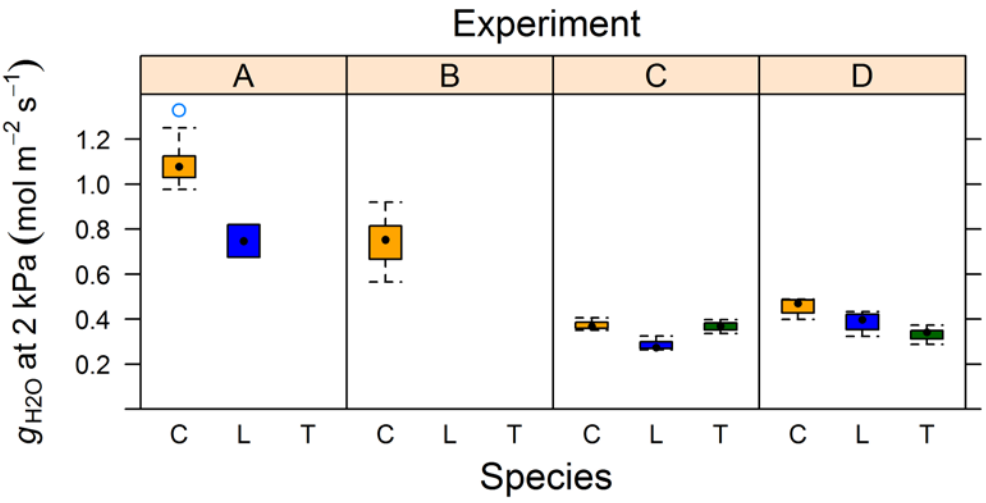

Fig. 8. Species differences in stomatal conductance (g_{H2O} predicted for 2 kPa) across the four experiments (A to D). For each box shown, three or more genotypes were sampled for each species in all experiments except lima bean in A, in which n=2 genotypes). C, common bean; L, lima bean; T, tepary. Box and whisker plots represent the median, 25 and 75 percentiles and range of the data.

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445 **4. Discussion**

446

447 *4.1. Limited VPD responses*

448 An evaluation of the genetic variation in stomatal conductance (g_{H2O}) response to vapor
449 pressure deficit (VPD) was performed for three species of *Phaseolus* including 28 genotypes of
450 diverse origins. However, relative to past literature for other crops, limited variation was found in
451 stomatal closure under high VPD.

452 Across four experiments there was genotypic variation in the slope of the g_{H2O} to VPD
453 response in one experiment (Fig. 6). Treating each genotype individually, there was some limited
454 support for the possibility of a 40% change in g_{H2O} from 1 to 4kPa VPD, with one genotype having
455 p = 0.226. Thus, within an experiment, genotypes largely shared a similar sensitivity to VPD,
456 varying between a 14% decrease to 50% increase between low and high VPD (1 to 4kPa). In
457 comparison, soybean genotypes that have a limited transpiration response to VPD display a 40%
458 decrease in stomatal conductance across the same range of VPDs for Davis, CA (Medina and
459 Gilbert, 2016). In North Carolina, the same soybean genotypes showed a ~50 to 90% decrease for
460 a less extreme range of VPD's (Gilbert et al., 2011). Past work on common bean is difficult to
461 compare to these data. Common bean, possibly including the genotype Victor, demonstrated
462 stomatal closure at VPD's of 20 to 50 mbar/bar (~2 to 5 kPa) (Comstock and Ehleringer, 1993).
463 However, the experiments were conducted in greenhouses with limited exposure to high VPD
464 during growth. To be clear, those authors did excellent hydraulic work, but it is difficult to
465 extrapolate from those data whether similar stomatal closure would occur in the field. In the current

466 experiment, the leaves were measured without time for stomata to equilibrate to the gas exchange
467 chamber and thus more closely represent the field responses to VPD.

468 The unexpected result reported here warrants the question of whether there was a
469 "machinery problem" preventing measurement of low g_{H2O} – but in all four experiments parallel
470 measurements on drought exposed plants showed moderate to severe stomatal closure, strongly
471 demonstrating that if stomatal closure at high VPD had occurred, then it would have been
472 measured.

473 One explanation for the relative lack of closure, is the recent evidence that a variety of
474 species lose the limited transpiration behavior when exposed to high temperatures (Sermons et al.,
475 2012; Yang et al., 2012; Seversike et al., 2013; Riar et al., 2015; Shekoofa et al., 2015). In those
476 studies a threshold of about 30^{o}C resulted in less limitation on transpiration possibly due to an
477 inducible mechanism. If such a mechanism was present in common bean, then the hot conditions
478 of the current experiments may result in loss of the limited-transpiration trait. Possibly *Phaseolus*
479 beans in lower temperature environments may then display stomatal closure at moderate VPD's.
480 An alternative explanation is that the lack of stomata sensitivity of cultivated *Phaseolus* species
481 represents one end of a continuum of response types (Mencuccini and Comstock, 1999), similar
482 to the extreme position of cotton (Lu and Zeiger, 1994). Unlike many natural plants (Sperry et al.,
483 2002), *Phaseolus* must have considerable investment in hydraulic structure to allow them to avoid
484 critical transpiration rates whilst maintaining high stomatal conductance at high VPD.

485 The VPD range measured here was large in comparison to other experiments but applicable
486 to the California Central Valley environment generally. Most agricultural environments are likely
487 to have extreme VPD's less than 5 kPa (e.g., for an air temperature of 40^{o}C, relative humidity must

be below 33% to result in a more severe VPD, or at 45ºC, RH must be < 50%; or at 35ºC, RH must be <12%). Thus, these three species of *Phaseolus* showed an excellent ability to maintain stomatal apertures under a very broad range of evaporative demands, and in general, are not expected to have stomatal closure under hot, well-watered field conditions.

4.2. Species differences

Tepary bean agriculture is considered as extremely drought tolerant (Nabhan, 1990). However, the drought tolerance may apply to the type of agriculture (floodplain, short season), rather than to plant hydraulic responses. Tepary did not appear to conserve water at high VPD through stomatal closure in any of the experiments here, and the maximal stomatal conductance appeared high and comparable to the other species. Thus, tepary's drought tolerance may be more closely related to a fast growth habit, early maturity and deep rooting than water conservation. These characteristics allow better water status at maturation and enhanced photosynthate partitioning to seeds (Rao et al., 2013). Diverse common beans showed considerable variation in maximum stomatal conductance and thus it seems that there is genetic variation that can be used for breeding. In particular, Flor de Mayo Eugenia consistently had the highest g_{H2O}'s in all experiments where it was included. Maximum stomatal conductance is likely related to stomatal density, patterning and stomata size (Franks and Beerling, 2009), particularly in species such as these that show little hydraulic limitation to transpiration.

Lima beans consistently had lower stomatal conductance than common beans in all three experiments where lima's were included. Such a constitutive low conductance may lead to water conservation relative to common bean under all conditions. However, the absolute values of g_{H2O}

for lima are still high relative to many crops or natural plants (Wright et al., 2004). Thus, it is unclear how large a change in canopy transpiration would occur as a result of lower g_{H2O} in lima bean relative to common bean.

5. Conclusion

Despite sampling of diverse species and genotype origins, there was limited evidence of *Phaseolus* species having constraints on transpiration under high evaporative demands, under well-watered conditions. No genotype showed large decreases (50-90%) in stomatal conductance, despite considerable evaporative demand. A few genotypes were not distinguishable from a 40% decrease, but generally showed less sensitive responses. Thus, future searches for sensitive stomatal responses in *Phaseolus* beans will have to include wild relatives. Alternatively, the stomatal sensitivity to VPD behavior may be under inducible genetic control, and may not be expressed under in hot environments such as California's. If so, then future work may need to either genetically alter the temperature threshold for the inducible behavior, or find dry, mild temperature environments where the stomatal closure behavior is expressed and would have a water conservation advantage.

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References

Anonymous, 2011. Using the LI-6400/LI-6400XT Portable Photosynthesis System V6. LI-COR Biosciences, Lincoln, NE.

Belko, N., Zaman-Allah, M., Diop, N., Cisse, N., Zombre, G., Ehlers, J., Vadez, V., 2012. Restriction of transpiration rate under high vapour pressure deficit and non-limiting water conditions is important for terminal drought tolerance in cowpea. Plant Biology 15, 304-316.

Champely, S., Ekstrom, C., Dalgaard, P., Gill, J., Weibelzahl, S., De Rosario, H., 2016. The 'pwr' package: basic functions for power analysis. R Foundation for Statistical Computing, Vienna, Austria.

CIMIS, 2015. California Irrigation Management Information System. California Department of Water Resources, Sacramento, CA.

Comstock, J., Ehleringer, J., 1993. Stomatal response to humidity in common bean (Phaseolus vulgaris): implications for maximum transpiration rate, water-use efficiency and productivity. Functional Plant Biology 20, 669-691.

Debouck, D.G., 1999. Diversity in *Phaseolus* species in relation to the common bean. Common bean improvement in the twenty-first century. Springer, pp. 25-52.

Delgado Salinas, A., Gama López, S., 2015. Diversidad y distribución de los frijoles silvestres en México. . Universidad Nacional Autónoma de México Revista Digital Universitaria 16.

Devi, M.J., Sinclair, T.R., Vadez, V., 2010. Genotypic variation in peanut for transpiration response to vapor pressure deficit. Crop Science 50, 191-196.

560 Engqvist, L., 2005. The mistreatment of covariate interaction terms in linear model analyses of
561 behavioural and evolutionary ecology studies. Animal Behaviour 70, 967-971.

562 Franks, P.J., Beerling, D.J., 2009. Maximum leaf conductance driven by CO2 effects on stomatal
563 size and density over geologic time. Proceedings of the National Academy of Sciences 106, 10343-
564 10347.

565 Freeman, G.F., Station, U.o.A.A.E., 1912. Southwestern Beans and Teparies. University of
566 Arizona.

567 Freytag, G., Debouck, D.G., 2002. Taxonomy, distribution, and ecology of the genus *Phaseolus*
568 (Leguminosae-Papilionodeae) in North America, Mexico and Central America. . SIDA, Botanical
569 Miscellany.

570 Gepts, P., 2001. *Phaseolus vulgaris* (Beans). In Encyclopedia of Genetics.Academic Press, 1444–
571 1445.

572 Gholipoor, M., Choudhary, S., Sinclair, T.R., Messina, C.D., Cooper, M., 2013. Transpiration
573 Response of Maize Hybrids to Atmospheric Vapour Pressure Deficit. J. Agron. Crop Sci. 199,
574 155-160.

575 Gholipoor, M., Prasad, P.V.V., Mutava, R.N., Sinclair, T.R., 2010. Genetic variability of
576 transpiration response to vapor pressure deficit among sorghum genotypes. Field Crops Res. 119,
577 85-90.

578 Gilbert, M., Holbrook, N.M., Zwieniecki, M., Sadok, W., Sinclair, T.R., 2011. Field confirmation
579 of genetic variation in soybean transpiration response to vapor pressure deficit and photosynthetic
580 compensation for this effect. Field Crop. Res. 124, 85-92.

581 Hood, G., 2010. PopTools version 3.2.5.

582 King, C.A., Purcell, L.C., Brye, K.R., 2009. Differential wilting among soybean genotypes in
583 response to water deficit. Crop Sci. 49, 290-298.

584 Lu, Z., Zeiger, E., 1994. Selection for higher yields and heat resistance in Pima cotton has caused
585 genetically determined changes in stomatal conductances. Physiologia plantarum 92, 273-278.

586 Maquet, A., Vekemans, X., Baudoin, J.P., 1999. Phylogenetic study on wild allies of Lima
587 bean,*Phaseolus lunatus* (Fabaceae), and implications on its origin. Plant Systematics and
588 Evolution 218, 43-54.

589 McDermitt, D., 1990. Sources of error in the estimation of stomatal conductance and transpiration
590 from porometer data. Hortsci. 25, 1538-1548.

591 Medina, V., Gilbert, M.E., 2016. Physiological trade-offs of stomatal closure under high
592 evaporative gradients in field grown soybean. Functional Plant Biology, -.

593 Mencuccini, M., Comstock, J., 1999. Variability in hydraulic architecture and gas exchange of
594 common bean (Phaseolus vulgaris) cultivars under well-watered conditions: interactions with leaf
595 size. Functional Plant Biology 26, 115-124.

596 Nabhan, G., 1990. Gathering the Desert. University of Arizona Press, Tucson.

597 Polania, J.A., Poschenrieder, C., Beebe, S., Rao, I.M., 2016. Effective Use of Water and Increased
598 Dry Matter Partitioned to Grain Contribute to Yield of Common Bean Improved for Drought
599 Resistance. Frontiers in plant science 7.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rainey, K.M., Griffiths, P.D., 2005. Evaluation of *Phaseolus acutifolius* A. Gray plant introductions under high temperatures in a controlled environment. Genet Resour Crop Evol 52, 117-120.

Rao, I., Beebe, S., Polania, J., Ricaurte, J., Cajiao, C., Garcia, R., Rivera, M., 2013. Can tepary bean be a model for improvement of drought resistance in common bean? African Crop Science Journal 21.

Riar, M.K., Sinclair, T.R., Prasad, P.V., 2015. Persistence of limited-transpiration-rate trait in sorghum at high temperature. Environmental and Experimental Botany 115, 58-62.

Sadok, W., Sinclair, T.R., 2009. Genetic variability of transpiration response to vapor pressure deficit among soybean cultivars. Crop Science 49, 955-960.

Schoppach, R., Sadok, W., 2012. Differential sensitivities of transpiration to evaporative demand and soil water deficit among wheat elite cultivars indicate different strategies for drought tolerance. Envir. Exp. Bot. 84, 1-10.

Sermons, S.M., Seversike, T.M., Sinclair, T.R., Fiscus, E.L., Rufty, T.W., 2012. Temperature influences the ability of tall fescue to control transpiration in response to atmospheric vapour pressure deficit. Functional Plant Biology 39, 979-986.

Seversike, T.M., Sermons, S.M., Sinclair, T.R., Carter, T.E., Rufty, T.W., 2013. Temperature interactions with transpiration response to vapor pressure deficit among cultivated and wild soybean genotypes. Physiologia plantarum 148, 62-73.

621 Shekoofa, A., Rosas-Anderson, P., Sinclair, T.R., Balota, M., Isleib, T.G., 2015. Measurement of
622 Limited-Transpiration Trait under High Vapor Pressure Deficit for Peanut in Chambers and in
623 Field. Agronomy Journal 107, 1019-1024.

624 Sinclair, T., Devi, J., Carter, T., Jr., 2016. Limited-Transpiration Trait for Increased Yield for
625 Water-Limited Soybean: From Model to Phenotype to Genotype to Cultivars. In: Yin, X., Struik,
626 P.C. (Eds.), Crop Systems Biology. Springer International Publishing, pp. 129-146.

627 Sinclair, T.R., Messina, C.D., Beatty, A., Samples, M., 2010. Assessment across the United States
628 of the benefits of altered soybean drought traits. Agron. J. 102, 475-482.

629 Singh, S.P., 2001. Broadening the genetic base of common bean cultivars. Crop Science 41, 1659-
630 1675.

631 Sperry, J.S., Hacke, U., Oren, R., Comstock, J., 2002. Water deficits and hydraulic limits to leaf
632 water supply. Plant, Cell & Environment 25, 251-263.

633 Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares,
634 J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J.,
635 Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets,
636 U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas,
637 S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum.
638 Nature 428, 821-827.

639 Yang, Z., Sinclair, T.R., Zhu, M., Messina, C.D., Cooper, M., Hammer, G.L., 2012. Temperature
640 effect on transpiration response of maize plants to vapour pressure deficit. Envir. Exp. Bot. 78,
641 157-162.

642 Zaman-Allah, M., Jenkinson, D.M., Vadez, V., 2011. Chickpea genotypes contrasting for seed
643 yield under terminal drought stress in the field differ for traits related to the control of water use.
644 Functional Plant Biology 38, 270-281.

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646