THESIS

MINIMUM STOMATAL CONDUCTANCE: IMPLICATIONS FOR DESCRIBING THE GENETIC CONTROL OF TRANSPIRATION

Submitted by

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ABSTRACT

MINIMUM STOMATAL CONDUCTANCE: IMPLICATIONS FOR DESCRIBING THE GENETIC CONTROL OF TRANSPIRATION

Minimum stomatal conductance (g_0) makes a significant contribution to the rate of water loss in plants. The influence of g_0 on water use efficiency (WUE) has implications for plant drought tolerance and adaptation, thus we propose that g_0 can be used as a trait to describe the genetic control of water use in leaf transpiration models. In the model species, *Arabidopsis thaliana*, g_0 exhibits both environmental and genetic variation. We explored one g_0 quantitative trait locus (QTL) by measuring and simulating transpiration for two *A. thaliana* accessions Kas-1 and Tsu-1, as well as recombinant inbred lines (RILs) from a reciprocal cross of the two parental lines. Using a three-dimensional spatially explicit plant process model, MAESTRA, we aimed to: (1) test the accuracy of transpiration prediction for Kas-1 and Tsu-1 using measured g_0 values, (2) parameterize MAESTRA with Tsu-1, Kas-1, and RIL g_0 values to predict transpiration of RILs containing either Tsu-1 and Kas-1 alleles at the g_0 QTL, and (3) determine if a relationship exists between g_0 values under well-watered and drought conditions in *A. thaliana*. MAESTRA accurately predicted *A. thaliana* transpiration for Kas-1 and Tsu-1 accessions when parameterized with measured g_0 values. There was no significant difference between measured and simulated transpiration estimates for both accessions, with Tsu-1 simulated transpiration 5.2% lower than the mean measured, and Kas-1 simulated transpiration 1.4% higher than measured. On average, Kas-1 transpired 73% as much water as Tsu-1. Due to the lack of specific knowledge of RIL physiology aside from g_0, simulating RIL transpiration with varying g_0 values yielded non-significant results. However, based on the simulated means

for RIL transpiration using RIL, Kas-1, and Tsu-1 g_0 values, we show that g_0 parameterization predicts daily transpiration when all other parameters are held constant at Tsu-1 or Kas-1 measured and presumed physiology. This further points to the importance of g_0 for transpiration predictions. Data on additional g_0 QTL could aid in predicting transpiration from novel genotypes such as RILs containing multiple combinations of alleles from parental genotypes. We found that accessions with relatively high well-watered g_0 values showed sharper declines in g_0 during drought compared to accessions with lower g_0 values under well-watered conditions ($p < 0.0001$). The use of plant physiological models for predicting transpiration of novel genetic lines will benefit from the further knowledge of the genetic control of g_0.

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INTRODUCTION

The distribution, survival, and fecundity of plant species depends on the timing and availability of rhizospheric water (Lu et al., 1998; Heschel et al., 2002; Donovan et al., 2007). The frequency and severity of drought stress and extreme weather patterns, however, are predicted to increase in many locations worldwide (Sangakkara et al., 2001; Dai et al., 2004). Consequently, rhizospheric water deficits will intensify, potentially reducing crop yields and plant fitness (Araus et al., 2002; Kumar et al., 2008; Chenu et al., 2009). Concurrently, added demands will be placed on irrigation water as food crop production increases in an attempt to match world population growth (Howell, 2001). These factors combine to make enhancing the drought tolerance of crops a vital component of contending with future limited water resources (Araus et al., 2002; Tardieu, 2003; Campos et al., 2004).

As an adaptive response to soil water deficits, plants alter their development, morphology, molecular composition, or physiological traits over time (Passioura, 1997; McKay et al., 2003, 2008). One such physiological trait that varies in response to vapor pressure deficit (VPD) and rhizospheric moisture is minimum stomatal conductance (also commonly referred to as nighttime stomatal conductance and g_{night}, but here we refer to it as g_0) (Caird et al., 2007; Christman et al., 2008). Generally, plants in more drought-prone environments exhibit lower g_0, as g_0 is negatively correlated with water use efficiency (WUE) (Christman et al., 2008; Galmés et al., 2011). Additionally, g_0 is positively correlated with daytime stomatal conductance (g_{day}) and photosynthesis (Christman et al., 2008). The reasons g_0 varies among species and genotypes are inconclusive, however there has been conjecture that nighttime transpiration is a mechanism for the uptake of soluble nutrients by mass flow (Snyder et al., 2003; Daley and Phillips, 2006; Caird et al., 2007; Cramer et al., 2008) and nutrient replenishment in the root zone (Barber, 1995).

However, in 2009 Christman et al. showed that higher nighttime transpiration due to g_0 did not lead to increases in growth from nutrient benefits in the commonly-studied model plant species, *Arabidopsis thaliana*. Other hypotheses for g_0's functional significance are related to xylem cavitation recovery (Snyder et al., 2003), prevention of excess cell turgor (Donovan et al., 2003), or the transport of carbohydrates to guard cells (Easlon and Richards, 2009). Nonetheless, *A. thaliana* has been shown to exhibit both genetic and environmental variation in g_0 (Christman et al., 2008).

g_0 makes a significant contribution to the rate of nighttime water loss (Christman et al., 2008, 2009). Depending on the species and growing environment, nighttime transpiration can contribute up to 30% of total daily transpiration (Christman et al., 2008). The g_0 values reported in many species, including *A. thaliana* are much larger than cuticular conductance values (Christman et al., 2008). In some C_3 and C_4 desert species, g_0 has been reported to be 40-75% of g_{day} under drought-stressed conditions (Ogle et al., 2012). The amount that g_0 contributes to total daily transpiration intensifies under high VPD (Howard and Donovan, 2007; Christman et al., 2009) and drought conditions (Ogle et al., 2012). Given the importance of g_0 under these conditions, commonly-used g_s models include g_0 as an independent parameter (e.g. Ball et al., 1987; Leuning, 1995; Medlyn et al., 2011; Barnard and Bauerle, 2013). Moreover, g_0 continues to be important as g_s is scaled to crown (Bowden and Bauerle, 2008; Bauerle and Bowden, 2011) and canopy (Bauerle et al., 2013) transpiration predictions.

Recently, the parameterization of quantitative genetics via quantitative trait loci (QTL) in ecophysiological models has emerged as a way to predict and understand the mechanistic basis of trait variation across multiple environments (Reymond et al., 2003; Yin et al., 2004; Hammer

et al., 2006; Collins et al., 2008). This technique offers the ability to *in silico* predict the phenotypic outcome from breeding with known QTL that describe trait variation (e.g. Tardieu, 2003), providing insight into how a genotype will respond to the environment. Genetically based descriptions of stomatal responses to environmental drivers are needed to advance leaf water flux estimates because at best, current g_s models use a combination of physiological and empirical parameters to predict how g_s will respond to climate constraints (Damour et al., 2010). QTL for g_0 have been discovered in *A. thaliana* (Fletcher et al., 2013) and understanding how these loci influence g_0 is important for parameterizing g_s models. Hence, the phenotypic effect of these *A. thaliana* g_0 QTL can add mechanistically based advances to existing g_s models – an important first step for replacing empirical approximations with functional genomics. Ultimately, incorporating genetic parameters into g_s models may allow for improved predictions of transpiration, biomass, yield, photosynthesis, and *in silico* simulation of diverse genotypes (Blanco et al., 2002; Hammer et al., 2006; Bertin et al., 2010).

The primary objective of this study was to measure and model transpiration for Kas-1 and Tsu-1, two *A. thaliana* parental lines with divergent water use efficiencies (McKay et al., 2008), as well as individuals from a recombinant inbred line (RIL) population created from a reciprocal cross of the parental accessions. We simulated transpiration with a three-dimensional spatially explicit plant process model, MAESTRA (Multi-Array Evaporation Stand Tree Radiation Assay) originally developed by Wang and Jarvis (Wang and Jarvis, 1990) and described in detail by Medlyn et al. (2004). In this work, we aim to (1) measure g_0 values of *A. thaliana* individuals that have a known genotype at the QTL of interest in order to advance an existing g_s model, (2) parameterize MAESTRA with measured g_0 values (in addition to other measured parameter values) to confirm that the model accurately predicts transpiration for Kas-1 and Tsu-1, (3) test

the accuracy of transpiration predictions for RILs by substituting measured Kas-1 and Tsu-1 g_0 values for RIL g_0 values, and (4) determine if a relationship exists between g_0 values under well-watered and drought conditions.

MATERIALS AND METHODS

We used three independent experiments to obtain physiological parameter values, stomatal conductance (g_s), and leaf and whole-plant gas exchange. These data sets provided us accurate parameter values for the following important parameters for estimating transpiration (> 5% parameter effect on transpiration estimates): g_0, stomatal sensitivity to the marginal water cost of carbon gain (g_1), maximum rubisco-limited rate of photosynthesis (V_{cmax}), maximum rate of electron transport (J_{max}), quantum yield of electron transport (α), and dark respiration (R_d) (Table 1).

Plant material

In all three studies, we examined two accessions of *A. thaliana,* Kas-1 (CS903) and Tsu-1 (CS1640) (hereafter referred to as Kas and Tsu), known to be divergent in water use efficiency (McKay et al., 2003; Juenger et al., 2010). Kas is native to Kashmir, India (34.5°N, 76°E) and is adapted to a dry and cold climate. Tsu is from Tsushima, Japan (34.41°N, 129.33°E) and is adapted to a much warmer and wetter climate (McKay et al., 2003, 2008; Christman et al., 2008).

In addition to these two parental lines, we investigated four near isogenic lines (NILs). The NILs have a homozygous Kas introgression in a Tsu background, and the introgressions span a g_0 QTL near the top of chromosome 1. NILs TK201.137.6.04 and TK201.137.6.05 ’s Kas introgression is estimated to span physical positions 505,086 to 5,273,972, and NILs KT116.63.15.01 and KT116.63.15.02 have a larger estimated introgression from positions 2,040,091 to 19,225,223. The NILs KT116.63.15.01 and KT116.63.15.02 also contain small heterozygous regions at both ends of the introgression (Fletcher et al., 2013). We found that

Table 1: Kas and Tsu values for the six most important MAESTRA parameters for estimating transpiration

Parameter	Definition	Units	Kas Value	Tsu Value	Source
g_0	Minimum value of g_s	mol m^{-2} s^{-1}	0.0396	0.0674	This study
g_1	Stomatal sensitivity to the marginal water cost of carbon gain	Dimensionless	9	9	Gutschick and Simonneau, 2002
V_{cmax}	Maximum rubisco-limited rate of photosynthesis	µmol m^{-2} s^{-1}	61.3	73.03	Easlon et al., 2013
J_{max}	Maximum rate of electron transport	µmol m^{-2} s^{-1}	96.43	122.307	Easlon et al., 2013
α	Quantum yield of electron transport	mol electrons mol^{-1} photons	0.304	0.304	This study
R_d	Dark respiration	µmol m^{-2} s^{-1}	1.47	1.276	This study

TK201.137.6.04 and TK201.137.6.05, as well as KT116.63.15.01 and KT116.63.15.02 were not significantly different from each other in their g_0 values, therefore we pooled them into two NIL categories for the remainder of the analyses: TK201.137.6 and KT116.63.15.

Recombinant inbred lines (RILs) created from a reciprocal cross between Tsu and Kas accessions were chosen from a population previously used to map QTL for g_0. We selected these RILs for their known allelic genotype of either Tsu or Kas at the marker associated with the g_0 QTL at the top of chromosome 1.

Experiment 1: Minimum stomatal conductance, leaf area, and biomass

Sowing, Stratification and Germination

Prior to planting, 152 6.35cm x 8.89cm black form pots were lined with polyester batting to prevent soil loss from the bottom of the pots. Pots were filled with Profile Porous Ceramic (PPC) Greens Grade dry soil (Profile Products LLC, Buffalo Grove, IL, USA) to 1 cm below the lip of the pot. All pots were placed in non-slatted flats and bottom-filled with water, left to soak overnight, and siphoned off twice to leach any salts from the soil. Kas, Tsu and two NILs (each NIL had two biological replicates) were randomly assigned and sown in 152 pots distributed across five flats. To avoid cross-contamination, one line at a time was sown into assigned pots, for a total of four to five seeds at the center of each pot.

Immediately after sowing, flats were filled with half-strength Hoagland's solution, covered with clear plastic domes to prevent excess evaporation, and stratified in a dark refrigerator at approximately 4°C for five days. Soil surfaces were misted to saturation twice-daily until germination. After cold-stratification, the flats were transferred to a growth chamber

and grown under 8:16 h (light:dark) photoperiod, with approximately 330 µmol m^{-2} s^{-1} PPFD at crown height. Temperatures were set to 23°C and 40% humidity during the light period, and 18°C and 60% humidity in the dark. Temperature and humidity gradually ascended to daytime conditions over the course of half an hour (mimicking sunrise), and likewise in the transition to dark conditions (sunset). Germination occurred two days after transfer to the growth chamber with clear plastic domes remaining on the flats for three days post-germination. Approximately one week after germination, plants were thinned to one per pot.

Plant care

The pots were flood irrigated every three to four days by filling the flats with water and allowing the pots to become saturated for 5-10 min before draining off the water. This allowed the plants to experience well-watered conditions without the risk of root hypoxia/anoxia. Once per week, half-strength Hoagland's solution was used. During the second portion of the experiment, a gradual dry-down was imposed on the plants, decreasing gravimetric water content by up to 10% each day. Mean container maximum water capacity was approximately 93% at the beginning of the dry-down and ended near 40% gravimetric water content.

Stomatal conductance measurements

To determine g_s, we simultaneously used three Decagon SC-1 Leaf Porometers (Decagon Devices, Inc. Pullman, Washington). The porometers were cross-calibrated and allowed to equilibrate to ambient temperature and humidity for at least 30 minutes prior to measurement. *A. thaliana* g_0 has been shown to remain consistent throughout the night (Christman et al., 2008), but nevertheless nocturnal and daytime g_s values were recorded for all replicates between four

and two hours pre-dawn and at solar noon. g_0 measurements were taken with the aid of photosynthetically inactive light emitting diode headlamps to avoid PAR-driven stomatal opening. Daytime g_s values were recorded inside the environmentally controlled growth chamber and all g_s values were obtained after approximately 30s using the SC-1's automatic mode. This allowed consistent measurements between plants and days, and ensured that stomatal environmental reaction times were not reached (Zeiger and Field, 1982). All g_s measurements were collected from similar age, non-damaged leaves ($n \approx 23$) over two days during both well-watered (W) and drought (D) conditions.

Plant leaf areas

A destructive harvest immediately followed the final day of g_s measurements. All replicates were harvested by removing the rosette from the roots with a razor blade at the base of the stem. We dissected leaves from the stems and laid them flat on a white sheet of paper for overhead photographing. Leaf areas were calculated from the photographs with ImageJ (Schneider et al., 2012).

Experiment 2: Whole crown gas exchange

Whole-crown gas exchange data were collected by Easlon et al. (Easlon et al., 2013) using Kas and Tsu accessions grown in a 1:1 mixture of fritted clay and potting mix (Sunshine Mix, Sun Gro Horticulture, Bellevue, WA, USA) in 164 mL Conetainer^{TM} pots (Stuewe and Sons, Corvallis, OR, USA). Plants were grown in a 12h photoperiod with 350 µmol m^{-2} s^{-1} PPFD, and temperatures set to 23/20°C in the light/dark. A LI-6400 (Li-Cor Inc., Lincoln, NE, USA) portable gas exchange unit fitted with a whole-plant cuvette was used to measure net

photosynthesis (A) versus internal CO_2 concentration (C_i) responses (A-C_i curves, where A is expressed in µmol m^{-2} s^{-1} and C_i is CO_2 concentration in mol fraction of CO_2) for Tsu and Kas accessions. Cuvette conditions were as follows: saturating PPFD (1000 µmol m^{-2} s^{-1}), varied CO_2 levels, and temperature and humidity were maintained at ambient growth chamber conditions (23°C and 60% RH). J_{max} and V_{cmax} values were calculated from the A-C_i responses with the Farquhar and von Caemmerer models (Caemmerer and Farquhar, 1981) using the PC software Photosyn Assistant (Dundee Scientific, Dundee, Scotland).

Experiment 3: Leaf-level gas exchange

Tsu, Kas, and individuals from the RIL population were sown in 3" pots containing Fafard 4P mix (Conrad Fafard Inc., Agawam, MA, USA), and stratified in the dark at 4°C for 5 d. The plants were transferred to a Conviron ATC60 growth chamber (Controlled Environments, Winnipeg, MB, Canada) set for 8:16 h (light:dark) days. Temperature and humidity were 23°C and 40% during the day, and 20°C and 50% at night. Plants were grown for approximately 6 weeks before gas exchange measurements. Leaf-level gas exchange data were collected with a CIRAS-2 portable gas exchange system fitted with a PLC(6) cuvette (PP Systems, Amesbury, MA, USA). Mean cuvette conditions were as follows for the light measurements: 397 ppm CO_2, 299 µmol m^{-2} s^{-1} PPFD, 32% RH, and 23°C. Each plant's measurements were averaged over 10 readings taken approximately every 10 seconds, post-equilibration. Prior to dark gas exchange measurements, plants were dark-adapted in the growth chamber for 20-28 hours. Dark gas exchange data were collected in a dark room (0 µmol m^{-2} s^{-1} PPFD) at 23°C. Cuvette environmental conditions for dark measurements were set to mimic those recorded in the light, with the exception of PPFD.

Reflectance, absorbance and transmittance

To estimate leaf reflectance, absorbance and transmittance, we used a SPAD meter (SPAD-502, Minolta Camera Co. Ltd., Japan). Conversion of SPAD readings to leaf reflectance, absorbance, and transmittance followed Bauerle et al. (2004).

Transpiration model description

We used a three-dimensional spatially explicit plant transpiration model, MAESTRA (Multi-Array Evaporation Stand Tree Radiation Assay, previously named MAESTRO) to estimate daily transpiration of two *A. thaliana* parental accessions and 14 RILs (described below) (Wang and Jarvis, 1990; Bauerle and Bowden, 2011). MAESTRA has been validated and applied in many previous studies, most of which are documented in a bibliography at the website *www.bio.mq.edu.au/maestra*. Using meteorological data, genotype-specific leaf-level physiological information and leaf and crown morphological parameters, MAESTRA computes whole crown estimates of transpiration and photosynthesis (Bauerle and Bowden, 2011). Photosynthesis is calculated from the Farquhar-von-Caemmerer biochemical submodel (Farquhar and von Caemmerer, 1982; Reynolds et al., 2009) coupled to the Ball-Berry-Leuning (BBL) stomatal conductance sub-model (Leuning, 1995) (Eq. 1):

$$g_{sw} = g_o + g_1 A h_s/(c_s - \Gamma) \qquad (1)$$

where g_{sw} is g_s to water, g_0 is minimum stomatal conductance, g_1 is stomatal sensitivity to the marginal water cost of carbon gain, A is net carbon assimilation rate, h_s is relative humidity, c_s is CO_2 mol fraction at the leaf surface, and Γ is the CO_2 compensation point.

MAESTRA inserts g_{sw} into the isothermal form of the Penman-Monteith equation to spatially calculate transpiration on a crown sub-volume basis, resulting in a whole-crown transpiration estimate (Medlyn et al., 2007) (Eq. 2):

$$\lambda E = \frac{mR_n + D_a g_h c_p M_a}{m + \gamma g_h / g_{sw}} \qquad (2)$$

where λ is the latent heat of water vapor (J mol $^{-1}$); E is transpiration per unit leaf area (mol m^{-2} s^{-1}); m is the slope of the curve relating saturation water vapor pressure to temperature (Pa K^{-1}); R_n is isothermal net radiation (W m^{-2}); D_a is vapor pressure deficit (kPa); g_h is total leaf conductance to heat (mol m $^{-2}$ s $^{-1}$); c_p is the specific heat of air (1010 J kg^{-1} K^{-1}); M_a is molecular mass of air (29 × 10– 3 kg mol $^{-1}$); γ is the psychometric constant (Pa K $^{-1}$) and g_{sw} is total leaf conductance to water vapor (mol m $^{-2}$ s $^{-1}$). MAESTRA accounts for crown structure and foliage distribution interactions with environmental drivers, calculating transpiration in three dimensions over an array of grid points within an individual crown (Emhart et al., 2007; Bauerle et al., 2009). Thus, the model predicts transpiration estimates by scaling up leaf level calculations.

Model parameterization and validation for well-watered Tsu and Kas

We parameterized *A. thaliana* accessions Kas and Tsu with measured values for parameters that were previously determined by Bauerle and Bowden (2011) to have a large influence on transpiration predictions (Table 1). A complete list of all parameter values, including Tsu and Kas morphology, are reported in Tables 2, 3, and 4.

MAESTRA transpiration estimates for Tsu and Kas were compared to measured transpiration values obtained from a separate leaf-level gas exchange experiment (Experiment 3). The mean measured day:night values for environmental conditions were used to parameterize the MAESTRA simulation: PAR (302:5 µmol m^{-2} s^{-1}), RH (0.3:0.6 %), T_{air} (28:24°C), and wind speed (0.5 m s^{-1}). We used 12 randomly-selected g_0 values for Kas and Tsu transpiration modeling to obtain an estimate of modeling error.

Model parameterization and validation for RILs

We modeled RIL transpiration for RILs containing either a Kas or Tsu allele at the QTL of interest. The model was separately parameterized with both Kas and Tsu default physiological parameters but with varying g_0 values. The mean g_0 values used were derived from the population of RILS with either a Kas or Tsu allele at the g_0 QTL. MAESTRA transpiration estimates were compared to measured gas exchange transpiration values for the RILs.

Statistical analyses

All statistical analyses were completed with JMP (JMP Pro 10. SAS Institute Inc., Cary, NC. 1989-2013). One-way ANOVAs with Tukey's honestly significant difference (HSD) for multiple pairwise comparisons were used for determining the difference between W and D g_0 values for each genotype. Kruskal-Wallis rank sum tests with the Steel-Dwass method for comparisons of all pairs were performed on the non-parametric data. Specifically, this method of analysis was used for validation of Tsu and Kas measured versus predicted transpiration, and RIL modeling with substituted g_0 values.

Table 2: Complete list of MAESTRA physiological model parameters used in this study. If the parameter abbreviation is different in the MAESTRA model input file, our abbreviation is followed in parentheses by the abbreviation specifically used in the MAESTRA input file.

Parameter	Definition	Kas value	Tsu value	Units	Source
Photosynthesis:					
J_{max}	Maximum rate of electron transport	96.43	122.307	µmol m^{-2} s^{-1}	This study
THETA	Curvature of light response of electron transport	0.67	0.67	Dimensionless	Assumed in this study
EAVJ	Activation energy	54200	54200	KJ mol^{-1}	Bauerle and Bowden, 2011
EDVJ	Deactivation energy	220000	220000	J mol^{-1}	Bauerle and Bowden, 2011
DELSJ	Entropy	637	637	KJ mol^{-1}	Bauerle and Bowden, 2011
AJQ	Quantum yield of electron transport	0.304	0.304	mol electrons mol^{-1}	This study
V_{cmax}	Maximum Rubisco-limited rate of photosynthesis	61.3	73.03	µmol m^{-2} s^{-1}	This study
EAVC	Activation energy RuBP	48700	48700	J mol^{-1}	Bauerle and Bowden, 2011
Respiration:					
R_d	Dark respiration	1.47	1.276	µmol m^{-2} s^{-1}	This study
RTEMP	Temperature for R_d value specified	25	25	°C	This study
Q10F	Exponential coefficient of temperature response of foliage respiration	0.05	0.05	Dimensionless	Assumed in this study: not influential on transpiration estimates
DAYRESP	Fraction of dark respiration reduced in the light	0.6	0.6	Fraction	Assumed in this study: not influential on transpiration estimates

Table 2 continued:

Parameter	Definition	Kas value	Tsu value	Units	Source
Stomatal conductace:					
g_0 (G0)	Minimum value of g_s	0.0396	0.0674	mol m^{-2} s^{-1}	This study
g_1 (G1)	Stomatal sensitivity to the marginal water cost of carbon gain	9	9	Dimensionless	Gutschick and Simmoneau, 2002
GAMMA	CO_2 compensation point	4.06	3.09	µmol m^{-2} s^{-1}	This study
D0L	Stomatal sensitivity to VPD	1500	1500	Pa	Leuning, 1995
WLEAF	Leaf width	0.015	0.015	m	This study
NSIDES	Number of leaf sides with stomata	1	1	Dimensionless	This study
Reflectance and transmittance:					
ATAU	Leaf transmittance	12.177	12.177	% PAR, % NIR, % IR	This study
ARHO	Leaf reflectance	7.941	7.941	% PAR, % NIR, % IR	This study
RHOSOL	Soil reflectance	0.10 0.30 0.05	0.10 0.30 0.05	% PAR, % NIR, % IR	Default

Table 3: MAESTRA canopy structure model parameters

Parameter	Definition	Value	Units	Source
Crown shape:				
CSHAPE	Geometric shape of crown	ELIP	N/A	This study
NOLAY	Number of layers in crown	9	Layers	This study
Leaf incidence angle:				
ELP	Ratio of horizontal to vertical axis of an ellipsoid	2	Ratio	This study
NALPHA	Number of leaf angle classes	1	N/A	This study
AVGANG	Mean leaf inclination angle	15	Degrees	This study
Leaf area density distribution:				
JLEAF	Leaf area density	0	N/A	This study
RANDOM	Ratio of projected shoot area to projected leaf area	1	Ratio	This study
Canopy wind speed extinction:				
EXTWIND	Wind speed extinction coefficient	1	Dimensionless	This study
Crown spacing and dimensions:				
NOTREES	Total number of plants in plot	32	Plants	This study
XRAD and YRAD	Radius of canopy in X and Y directions	0.0325; 0.02	m	This study
HTCROWN	Height of live crown	0.04	m	This study
HTTRUNK	Height of leafless stem	0.01	m	This study
DIAM	Diameter of stem	0.001	m	This study
LAREA	Leaf area of crown	Kas: 0.004416; Tsu: 0.005311	m^2	This study
XMAX	Length of plot in X direction	0.6	m	This study
YMAX	Length of plot in Y direction	0.3	m	This study

Table 4: MAESTRA site-specific model parameters

Parameter	Definition	Value	Units	Source
Site characteristics:				
LAT	Latitude of plot	41 25 29.97	Deg, min, sec	This study
LATHEM	Latitudinal hemisphere of plot	N	N/A	This study
LONG	Longitude of plot	82 2 57.88	Deg, min, sec	This study
LONGHEM	Longitudinal hemisphere of plot	W	N/A	This study
TZLONG	Longitude of the meridian of the time zone	75	Degrees	This study
Plot details:				
BEARING	Bearing of X-axis from South	180	Degrees	This study
XSLOPE	Slope of plot in X direction	0	Degrees	This study
YSLOPE	Slope of plot in Y direction	0	Degrees	This study
Radiation calculations:				
PPLAY	Number of points per layer for radiation calculation	960	N/A	This study
NZEN	Number of zenith angles for diffuse transmittance calculation	7	N/A	This study
NAZ	Number of azimuth angles for diffuse transmittance calculation	11	N/A	This study

RESULTS

Figure 1 illustrates measured and predicted Kas and Tsu transpiration, where modeled versus measured transpiration estimates were not statistically different from one another (Kas: $p = 0.68$; Tsu: $p = 0.69$). Measured Tsu transpiration was 3.85 mmol m^{-2} s^{-1} (± 0.128 SEM) and MAESTRA predicted 3.622 mmol m^{-2} s^{-1} (± 0.225 SEM). Likewise, Kas measured and predicted transpiration was 2.86 mmol m^{-2} s^{-1} (± 0.067 SEM) and 2.90 mmol m^{-2} s^{-1} (± 0.171 SEM), respectively. Comparing Kas and Tsu accessions, Kas measured and simulated transpiration was 0.99 mmol m^{-2} s^{-1} and 0.722 mmol m^{-2} s^{-1} lower than Tsu.

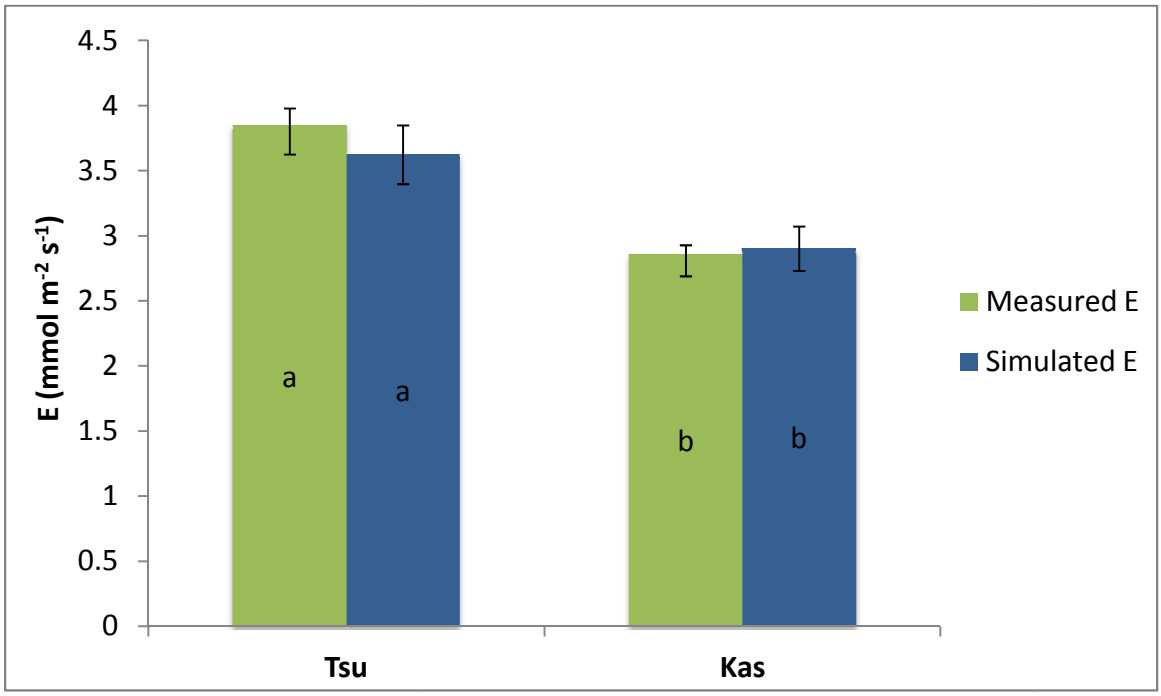


Figure 1: Measured vs. MAESTRA estimated transpiration (E) for Tsu and Kas accessions. Error bars represent standard error of mean (SEM). Bars not connected by the same letter are significantly different ($\alpha = 0.05$). The mean measured day:night values for environmental conditions were used to parameterize the MAESTRA simulation: photosynthetically active radiation (302:5 µmol m^{-2} s^{-1}), relative humidity (0.3:0.6 %), air temperature (28:24°C), and wind speed (0.5 m s^{-1}).

Next, we compared measured and simulated transpiration for RILs to test the accuracy of RIL g_0 values for predicting transpiration with all other parameters remaining constant at Kas or Tsu physiology. RILs were selected based on known genotypes at the g_0 QTL of interest: containing either a Kas or Tsu allele at the locus. All measured transpiration values were obtained from leaf-level gas exchange measurements. The mean RIL, Kas, and Tsu g_0 parameter values used in the model were as follows: 0.039 mol m^{-2} s^{-1} for RILs containing the Kas allele, 0.048 mol m^{-2} s^{-1} for RILs with the Tsu allele, and 0.039 mol m^{-2} s^{-1} and 0.067 mol m^{-2} s^{-1} for Kas and Tsu, respectively. The mean measured transpiration for the Kas-allele and Tsu-allele RILs was 3.13 mmol m^{-2} s^{-1} (± 0.022 SEM) and 3.22 mmol m^{-2} s^{-1} (± 0.03 SEM). Comparatively, MAESTRA-simulated transpiration, using RIL g_0 values with Tsu physiology yielded the following predictions for the RILs: 2.9 mmol m^{-2} s^{-1} for Kas-allele RILs, and 3.09 for Tsu-allele RILs. Simulated transpiration predictions for RILs using Kas default physiology parameters were 2.89 mmol m^{-2} s^{-1} and 3.08 mmol m^{-2} s^{-1} for Kas-allele and Tsu-allele RILs, respectively. Simulated transpiration values for Tsu-allele RILs predicted higher transpiration, which is in line with measured Tsu-allele RILs, and likewise for Kas-allele RILs. However, there is no significant difference between the simulated values (Figure 2).

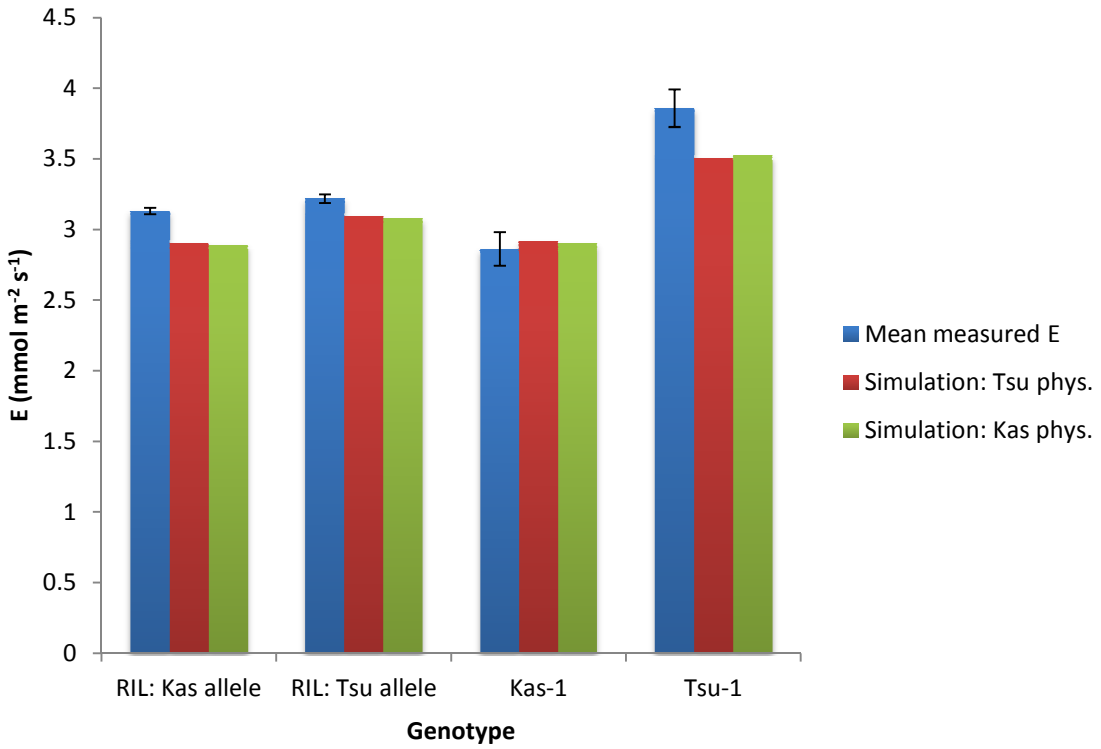
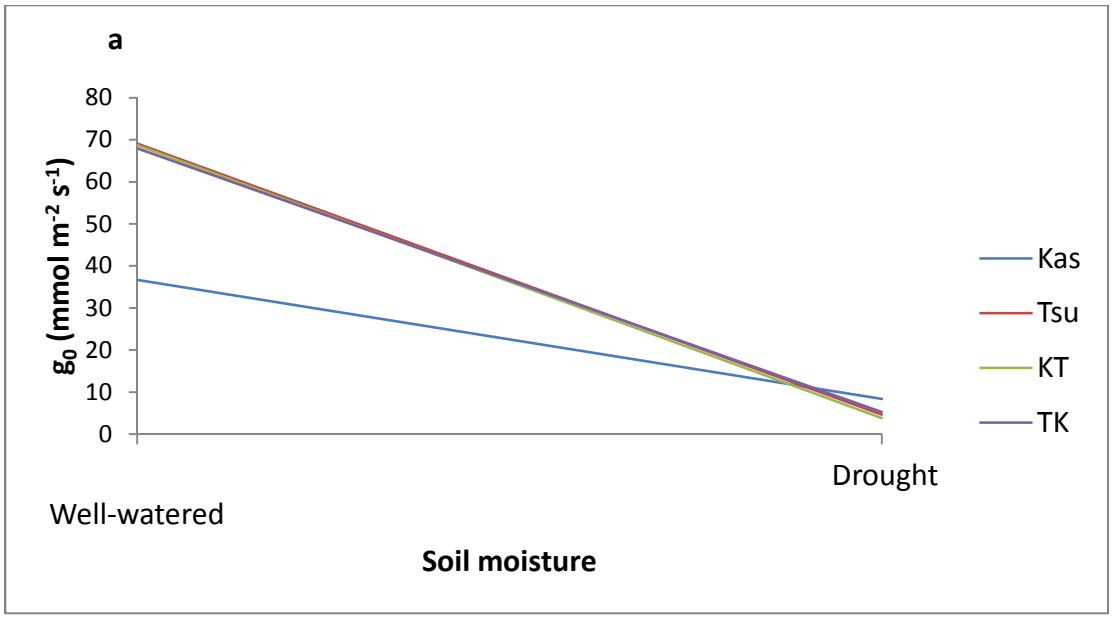


Figure 2: Measured and MAESTRA-simulated transpiration (E) for RILs, Kas and Tsu using mean measured RIL, Kas, and Tsu minimum stomatal conductance (g_0) values for simulated E. "RIL: Kas allele" bars represent RILs containing the Kas allele at the g_0 QTL of interest, and likewise for Tsu alleles with "RIL: Tsu allele" bars. Mean measured transpiration for Kas, Tsu, and RILs was determined via leaf-level gas exchange. Simulated transpiration values represent MAESTRA model transpiration estimates produced by varying g_0 with mean genotype values, while keeping all other parameters constant at Tsu or Kas physiology (Phys.). Each bar represents the mean measured or simulated transpiration. Error bars for measured transpiration represent standard error of the mean values. Kas-allele and Tsu-allele RIL simulated transpiration values are not significantly different from one another, however, as expected, Tsu-allele RIL transpiration estimates and measured values are larger than Kas-allele simulated and measured transpiration.

We examined the relationship between W and D g_0 values for Kas, Tsu, and the NILs by plotting their norms of reaction between environments. For each genotype, we determined the mean g_0 value for W and D conditions, and plotted their phenotypes across the two environments. Our results show that Kas has a narrower range of g_0 values than Tsu or the NILs. Conversely, TSU and the NILs experienced a steeper decline in g_0 when transitioning from W to D conditions. We found Tsu and the NILs experienced a similarly sharp decline in g_0 from the W to the D conditions, where pairwise comparisons of Tsu and NILs for the difference between W and D g_0 all had $p > 0.96$. Tsu, KT116.63.15, and TK201.137.6 had a mean W to D g_0 difference of 64.37, 64.88, and 62.68 mmol m^{-2} s^{-1}, respectively. Relative to Tsu and the NILs, Kas had a significantly lower difference between W and D g_0 of 28.24 mmol m^{-2} s^{-1}, with pairwise comparisons of Kas to other genotypes all significantly different ($p < 0.0001$). In other words, Kas maintained a more static g_0 value throughout the course of the dry down, relative to Tsu and the NILs. Interestingly, although Kas had a lower mean g_0 in the W environment, it maintained a higher mean g_0 than the other genotypes during D (Figure 3). Multiple regression analysis showed genotype, environment, and the genotype X environment interaction terms to be highly significant ($p < 0.0001$) for predicting g_0. A plot of actual versus predicted g_0 values had an R^2 of 0.63 and $p < 0.0001$.



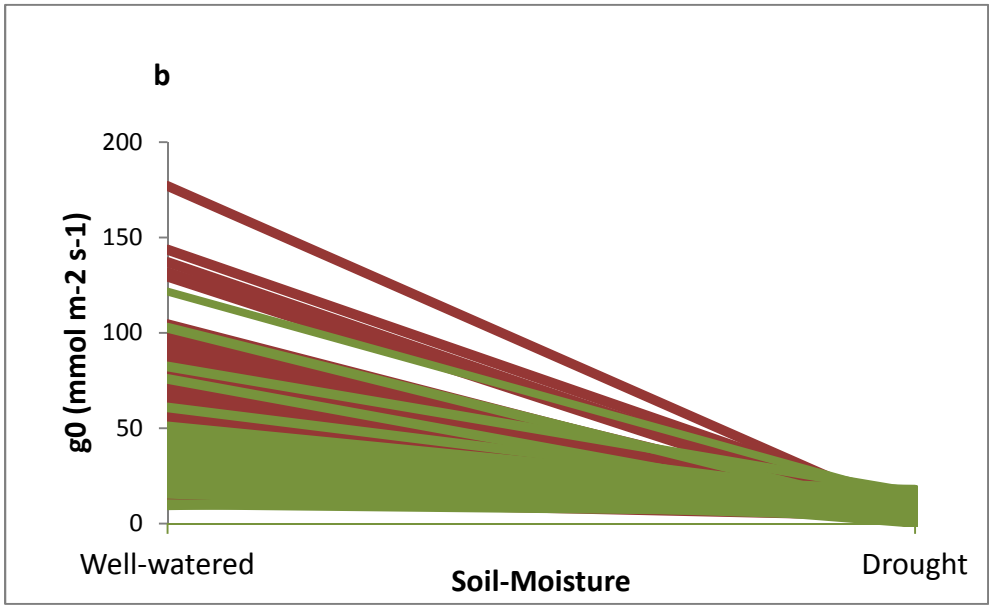


Figure 3: a.) Mean minimum stomatal conductance (g_0) norm of reaction plot for Kas, Tsu, and NIL genotypes under well-watered (W) and drought (D) soil moisture conditions. The lines for NILs KT116.63.15 and TK20.137.6 are represented by the labels "KT" and "TK," respectively. Tsu and the NILs experienced a significantly sharper decline in g_0 between W and D conditions than Kas ($p < 0.0001$). Tsu and the NILs' decline in g_0 from W to D were not significantly different ($p > 0.96$). b.) Minimum stomatal conductance (g_0) norm of reaction plot for Kas and Tsu individuals under well-watered (W) and drought (D) soil moisture conditions, where Kas is represented by green lines and Tsu by red. On average, Tsu has steeper declines in g_0 between W and D treatments.

DISCUSSION

In addition to g_{day}, many plants in diverse environments experience significant g_0 (Snyder et al., 2003; Caird et al., 2007; Christman et al., 2009; Ogle et al., 2012; Barnard and Bauerle, 2013). Values of g_0 during the nighttime are much larger than cuticular conductance alone, demonstrating that the additional water loss occurs via the stomata (Ogle et al., 2012). Under drought conditions, g_0 has been observed to be as high as 75% of g_{day} (Ogle et al., 2012), but the mechanism regulating g_0 is still unidentified. Some have hypothesized that different mechanisms control day- and nighttime conductance (Ogle et al., 2012; Barnard and Bauerle, 2013). Others attribute g_0 responses to the same regulating mechanisms as g_{day} (e.g., decreasing rhizospheric water availability or higher atmospheric VPD) (Caird et al., 2007; Christman et al., 2008).

At night or under low light conditions, g_0 can, by definition, be the primary contributor to total g_s (Barnard and Bauerle, 2013). Moreover, the magnitude of g_0 can have a large influence on an individual's daily transpirational water loss (Christman et al., 2008). Thus, g_0's influence on g_s predictions can be significant (Barnard and Bauerle, 2013). This is because g_0 comprises an additive portion of the Ball et al. (1987) family of g_s equations (e.g. Leuning, 1995), so it constantly influences transpiration estimates, increasing its percent contribution as light levels diminish (Barnard and Bauerle, 2013). We confirmed the parameter's effect: by varying only the g_0 parameter, the MAESTRA model predicted significantly different transpiration estimates, with all other parameters held constant (e.g. Fig. 2, this study). Similar to Barnard and Bauerle (2013), we suggest that g_0 is an important and easily-measured parameter that can improve g_s and transpiration model estimates.

Currently, in hot and moderately dry environments, g_s can be used as a predictor of crop yield (Lu et al., 1998), because g_s is highly correlated with photosynthesis and yield (Radin et al., 1994). One reason for g_s's correlation with yield is that genotypes with high g_s allow more CO_2 gas exchange for photosynthesis and experience cooler canopy temperatures in hot environments, allowing photosynthesis to function at a more optimal temperature (Lu et al., 1994, 1998; Radin et al., 1994). A further advantage of selecting for elevated g_s is that there is some evidence that differences in g_s between high- and low-yielding lines are under genetic control (Radin et al., 1994; Percy et al., 1996). Genotypes with elevated g_s and yield may in fact have a greater capacity to uptake available soil water via increased root area (Mitchell et al., 1996) or osmotic adjustment (Blum, 2005), and therefore, can maintain transpiration during mild water stress conditions (Blum, 2009). However, elevated g_s is a disadvantage when soil water deficits are more consistent throughout a growing season, or when all individuals in an area have a similar capacity for soil moisture uptake (Donovan et al., 2007). Increased WUE is one strategy that many plants employ to avoid drought: decreasing g_s allows plants to extend their water supply longer (McKay et al., 2008). In high soil moisture-stress conditions, plants respond by lowering their g_s, where less WUE plants exhaust available water faster, lose turgor, and eventually die if they do not succeed in a drought escape strategy (Donovan et al., 2007). McKay et al. (2008) found Kas to have lower internal CO_2 and g_s, but higher survival than Tsu, indicative of the Kas increased WUE strategy for drought survival. Our findings echo this for Kas and Tsu accessions, with Kas demonstrating a significantly lower difference between W and D g_0 than Tsu or the NILs. This indicates that g_0 is negatively correlated with WUE, and highly sensitive to drought in *A. thaliana*. Our data show that in general, genotypes with relatively low W g_0 tend to maintain higher g_0 during dry rhizospheric conditions than genotypes that

commence a drought with high g_0 values. This result is consistent with and indicative of known Kas and Tsu WUE strategies.

Depending on the timing and severity of drought, g_0's influence on nighttime transpiration can have important implications for plant success and survival. We observed that genotypes with relatively high W g_0 transpired water faster, and therefore reduced g_s more rapidly than genotypes with lower initial g_0 in response to drought. Specifically, Tsu and the NILs experienced the sharpest decline in g_0 over the course of the gradual dry down, while simultaneously using the most water. On average, Kas individuals lost 73g (combined transpiration and evaporative water loss) of water compared to Tsu's 100g over the course of 11 days.

Due to the influence and correlation of g_0 with WUE, we have found this easy-to-measure parameter to function as somewhat of a proxy for the WUE response to drought stress, and therefore it may be a good predictor of turgor and gas exchange maintenance during drought. To extend the use of g_{day} as a yield predictor, g_0 has promise as a crop breeding selection tool for both moderate and high water-stress environments. Sinclair (2011) outlines the idea of a multi-tier selection scheme to work around the difficulty, time, and expense of phenotyping the many hundreds of genotypes and replicates often required for improving crop performance. This selection scheme works by first employing a broad screen for an easily measurable trait, and working towards more specific screens over time (Sinclair, 2011). Many secondary traits (traits that are correlated with a primary trait of interest) are easier and/or faster to measure (Lafitte et al., 2003), and therefore good candidates for an initial broad screen. We propose that g_0 can be used as a secondary trait for WUE and drought response.

For over a decade, there has been a call to utilize an interdisciplinary approach for improving crop yields (Yin et al., 2003; Sinclair, 2011). Plant physiological modeling is a useful technology for linking phenotypic selection techniques with molecular methods for breeding selection (Hammer et al., 2006). Combining the fields of genetics and plant physiology with the use of dynamic simulation models is a promising way to improve on ideotype breeding (Yin et al., 2003). If successful, it allows for the creation of 'virtual genotypes' with combinations of alleles similar to real plants (Hammer et al., 2006). This can be accomplished by substituting model parameters with identified genetic coefficients from QTL analysis (Yin et al., 2003; Hammer et al., 2006). DNA markers that are linked with physiologically significant QTL can be used as a substitute for phenotypic measurement and used in marker-assisted selection (MAS) of crop species (Collard et al., 2005; Masuka et al., 2012), and physiological modeling can aid this process (Yin et al., 2003). We believe that based on g_0's influence on transpiration and WUE, as well as its importance for accurate transpiration estimates, g_0 provides a link between genetics and plant physiological modeling: a parameter for predicting WUE under soil moisture stress.

The mechanism underlying genetic variation in g_0 has yet to be fully described or understood (Barnard and Bauerle, 2013), but here we examined one locus in the *A. thaliana* genome that has been correlated with g_0 variation between genotypes. This locus does not solely control g_0 variation, but we have found it to be one potential piece of the puzzle, along with other as yet unknown genetic loci and observed genotype by environment interactions (e.g. VPD, soil moisture). This is just one step in the long line of molecular work needed to arrive at a gene network that would fully describe the g_0 mechanism. However, isolating QTL responsible for some aspect of phenotypic control can already be thought of as a "meta-mechanism" (Tardieu,

2003): this knowledge can allow us to better predict how a genotype will respond to a given rhizospheric water content.

FUTURE RESEARCH

If alleles at specific marker positions associated with g_0 are identified, and their effects are quantified, incorporating genetic information into models is a technique that can be used to predict transpiration responses of novel genotypes in defined soil moisture conditions. g_0 is an important parameter both in its large effect on transpiration estimates and in its ability to be examined as a proxy for drought response/WUE. Using g_0 for modeling drought response may end up explaining a huge portion of the variation in transpiration estimates during drought, but only improved validation techniques will demonstrate this with more certainty.

QTL for drought response – or any other trait – are complicated, and their responses can vary dramatically, depending on the environment and genotype by environment interaction. Expression differences of genes in an individual are very difficult to predict (Sinclair, 2011). QTL and genetic markers can point toward *possible* genotypic responses, but are not necessarily definitive (Sinclair, 2011). Although much more information will be needed in the future to be able to accurately predict individual responses in varying environments (Yin et al., 2003; Sinclair, 2011), we assert that g_0 is a parameter that can guide selection of high and low WUE genotypes.

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LIST OF ABBREVIATIONS

A	Photosynthesis, net carbon assimilation
BBL	Ball-Berry-Leuning stomatal conductance sub-model
C_i	Internal CO_2 concentration
D	Drought treatment
E	Transpiration
g_0	Minimum stomatal conductance
g_1	Stomatal sensitivity to the marginal cost of water gain
g_{day}	Daytime stomatal conductance
g_s	Stomatal conductance
J_{max}	Maximum rate of electron transport
MAESTRA	Multi-Array Evaporation Stand Tree Radiation Assay model
MAS	Marker-assisted selection
NIL	Near isogenic line
PAR	Photosynthetically active radiation
PPFD	Photosynthetic photon flux density
QTL	Quantitative trait loci/locus
R_d	Dark respiration
RIL	Recombinant inbred line
V_{cmax}	Maximum rubisco-limited rate of photosynthesis
VPD	Vapor pressure deficit
W	Well-watered treatment
WUE	Water use efficiency