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Cotton stomatal closure under varying temperature and vapor pressure deficit, correlation with the hydraulic conductance trait

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Abstract

Background: Cotton (*Gossypium hirsutum* L.) is often grown in locations characterized by high atmospheric evaporative demand. It has been hypothesized that plants which resist hydraulic flow under this condition will limit water use and conserve soil water. Therefore, in a series of controlled environment experiments ten cotton cultivars were exposed to two different temperature and vapor pressure deficit (VPD) conditions (i.e., 38 °C, > 3 kPa and 32 °C, 1～1.5 kPa) as well as a progressive soil drying. Then, individual differences in shoot hydraulic conductance (K_{shoot}) was measured using a hydraulic conductance flow meter (HCFM). Physiological parameters were reported included leaf area, dry leaf weight, stomatal conductance (g_s), and water use efficiency coefficient (WUE_k).

Results: Differences were observed in K_{shoot} among cultivars under the 38 °C, > 3 kPa but not the 32 °C, 1～1.5 kPa environment. Under the 38 °C, > 3 kPa environment, correlations were found between K_{shoot}, stomatal conductance (g_s), VPD breakpoint, WUE_k, total leaf area, dry leaf weight, fraction transpirable soil water (FTSW) threshold, and slope of TR decline after FTSW threshold.

Conclusion: Results show that the ability of some cotton cultivars to restrict water loss under high evaporative demand through early stomatal closure is associated with the cultivars' K_{shoot}. The K_{shoot} is influential in the limitation of TR trait under high temperature and VPD.

Keywords: Cotton, FTSW threshold, Shoot hydraulic conductance, Temperature, Transpiration rate, VPD

Introduction

Plant hydraulic conductance (K_{plant}) is a measure of the efficiency of water movement through a particular plant organ such as roots, leaves, or shoots and is defined as the flow rate per unit pressure driving force (Sperry 2000; Judd et al. 2016). A variety of plant physiological properties including water potential, stomatal conductance (g_s, mol (H_2O)·m^{-2}·s^{-1}), and overall plant growth are related to K_{plant} (Cochard et al. 1997, 2000; Sperry 2000; Franks et al. 2007; Brodribb et al. 2010). Reduced K_{plant} under high atmospheric vapor pressure deficit (VPD) conditions could be used as an approach to decrease the rate of soil water use by restricting plant water loss (Sinclair et al. 2005; Choudhary et al. 2013). Investigation into potential differences among cotton cultivars' ability to limit hydraulic conductance under periods of high evaporative demand and temperature is therefore warranted.

The ability of crop plants to limit transpiration under high ambient VPD has been described extensively (Gholipoor et al. 2010; Shekoofa et al. 2013; Sinclair et al. 2017; Sheldon et al. 2021). This limited transpiration trait (TR_{lim}) is expressed as a VPD breakpoint, above

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which the TR reaches a plateau or begins to decrease in response to the increasing VPD. A recent study on transpiration response to the increasing VPD among different cotton cultivars demonstrated that at a high temperature (38 °C), some cotton cultivars lose their ability to limit transpiration under increasing VPD conditions (Shekoofa et al. 2020). The study suggested that the loss of the VPD breakpoint [i.e., limited transpiration (TR_{lim}) rate] at higher temperature may indicate an abrupt increase in K_{plant}. The loss of a VPD breakpoint with a relatively small increase in temperature suggests that TR_{lim} may be a temperature-sensitive process which appears to be highly associated with changes in K_{plant} (Pradhan et al. 2019).

Under high temperatures, cultivars without the ability to restrict hydraulic conductance would allow water to readily replenish leaves, resulting in open stomata and unrestricted transpiration with the increasing VPD (Pradhan et al. 2019; Li et al. 2020; Sadok and Sinclair 2010; Sarkar et al. 2022). In soybean, observations of the TR_{lim} trait and low leaf hydraulic conductance (K_{leaf}) [i.e., decrease in transpiration rate (TR)] at 32 °C do not necessarily predict expression of the trait at higher temperatures (Sarkar et al. 2022). By increasing temperature from 32 to 37 °C, the expression of TR_{lim} trait was reduced to ~ 43%, 10%, and 0% for slow, moderate, and high wilting soybean genotypes, respectively. This could be because the remaining genotypes which expressed TR_{lim} (i.e., VPD breakpoint) and low leaf hydraulic conductance at 32 °C started to increase TR under 37 °C to disperse excess temperature and heat.

In 2013, Choudhary et al. demonstrated that two sorghum genotypes differed significantly in K_{leaf}. Additionally, this reduction in K_{leaf} was associated with conservative water use by the whole plant during soil drying and under high atmospheric VPD. Across a range of species and environmental conditions, hydraulic conductance resistance levels have been linked to maximum rates of g_s (i.e. gas exchange) (Sack and Frole 2006; Brodribb et al. 2007). Therefore, it is of interest to investigate the hydraulic conductance of cotton cultivars under varying VPD and temperature environments, assessing the possible involvement of hydraulic conductance resistance during periods of high evaporative demand and progressive soil drying.

To work toward resolving the hydraulic conductance hypothesis for regulating the stomata closure, studying the response of cotton cultivars that respond differently to high VPD and temperature could be beneficial (Shekoofa et al. 2020). To date, most hydraulic conductance researches in row crops have focused on K_{leaf}. Therefore, this study objective was to assess the shoot hydraulic conductance (K_{shoot}) of ten cotton cultivars, under varying VPD and temperature to gain insights into possible connections to previously observed drought tolerance trait responses to water-deficit stress.

Materials and methods

Plant materials and experimental setup

During July and September 2020, a set of two experiments were performed under controlled environment conditions at the West Tennessee Research and Education Center, USA (WTREC). Ten cotton cultivars were selected based on their observed TR responses to high VPD and temperature (Shekoofa et al. 2020). Commercial cultivars selected include: 'Stoneville (ST) 4949 GLT' and 'ST 6182 GLT' (Stoneville Cottonseed, BASF Corp., Research Triangle Park, NC); 'DeltaPine (DP) 1612 B2XF' (Deltapine Cottonseed, Bayer CropScience, St. Louis, MO); and 'Phytogen (PHY) 330 W3FE', 'PHY 340 W3FE' and 'PHY 490 W3FE' (Phytogen Cottonseed, Corteva Agroscience, Indianapolis, IN). Breeding lines selected include: 'DP 393' (Bowman et al. 2006), 'Arkot 9704' (Bourland and Jones 2009), 'UA107' (Bourland and Jones 2018a), and 'Arkot 0705' (Bourland and Jones 2018b).

Growth chamber study

In each experiment, all cultivars were replicated four times using a randomized complete block design. An experimental unit consisted of a 3.7-L pot filled with a soil mix composed of fifty percent sand and fifty percent Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf). Each pot was planted with six seeds at a depth of 2.5 cm. Pots were thinned to one plant ten days after planting (DAP). Fifteen DAP, all pots were fertilized with Miracle-Gro Water Soluble All Purpose Plant Food (Marysville, OH) containing 24-8-16 ($N–P_2O_5–K_2O$). Plants were grown under greenhouse conditions (14 h of light/10 h of dark, average day/night temperature (33 ± 4) / (23 ± 3) °C, average humidity (58 ± 12)%). Air temperature and relative humidity were recorded every 5 min with a humidity/temperature digital data logger (Lascar Electronics, Erie, PA). Natural light was supplemented with 600 W high-pressure sodium lamps (P. L. Light Systems, Lincoln, ON, Canada) to maintain 600 to 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant level. Plants were maintained in a well-watered condition, receiving approximately 250 $mL \cdot d^{-1}$ (i.e., pot capacity) during the initial pre-treatment period, until they had developed 4~6 true leaves.

In each experiment, the evening before hydraulic conductance measurements, pots were over-watered until dripping, and aluminum foil was placed over the soil surface and around the plant stem to prevent soil evaporation. The next morning, plants were placed in a growth chamber (Conviron MTR30, Winnipeg, Manitoba, CA). Plants within each experiment were then exposed to a different environmental condition. In Experiment 1, conducted during July, 2020, plants were exposed to 38 °C and 50% humidity, VPD > 3 kPa (high temperature, high VPD). In Experiment 2, conducted during September, 2020, plants were exposed to 32 °C and 80% humidity, VPD 1 to 1.5 kPa (low temperature, low VPD). Light intensity for both experiments were 500 to 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air temperature and relative humidity inside the growth chamber were recorded as previously described and light intensity was measured using a photosynthetically active radiation (PAR) meter (Quantum PAR, Lifees, Ontario, CA).

Plants were allowed 45 min to acclimate to the condition. Then, all pots were individually weighed on a balance with a resolution of 0.1 g to establish a beginning weight. Plants were kept inside the growth chamber under the same environmental conditions for 90 more minutes after which a final weight was measured. Transpiration rate was calculated for each plant by subtracting the final pot weight after exposure to growth chamber conditions from the initial pot weight for each set of experiments.

After recording final weight, g_s and photosynthetic (Pn, $\mu mol\ (CO_2) \cdot m^{-2} \cdot s^{-1}$) rates were measured individually for each plant using a portable photosynthesis machine (LiCor 6400-XT, Li-Cor, Lincoln, NE). A 6 cm^2 of the leaf was confined in the instrument chamber and allowed to equilibrate to the chamber's environmental condition for 60 s before a measurement was taken. The leaf section within the LiCor 6400 chamber was exposed to 38 °C in Experiment 1 and 32 °C in 2. In both experiments, leaf sections were exposed to 2 000 $\mu mol\ (PAR) \cdot m^{-2} \cdot s^{-1}$ using a 6400-02B light source with a flow rate of 500 $\mu mol \cdot s^{-1}$. Carbon dioxide concentration was maintained at 400 $\mu mol\ (CO_2) \cdot mol^{-1}$ air. These settings were intended to replicate the temperature and VPD conditions the plant had experienced during the time in the growth chamber. Measurements for g_s and Pn were taken on two uppermost separated fully expanded leaves, with three measurements per leaf.

Since TR ($mmol\ (H_2O) \cdot m^{-2} \cdot s^{-1}$) and Pn were measured, it was possible to calculate the WUE_k (Pa) which is an instantaneous calculation of Pn and TR based on individual leaflets as well as the ambient VPD (kPa) (Shekoofa et al. 2015). This measurement gives insight to the ratio of plant productivity to water loss (Sinclair et al. 1984).

$$WUE_k = (Pn \times VPD)/TR \tag{1}$$

A hydraulic conductance flow meter (HCFM, Dynamax, Inc., Houston, TX) was calibrated (Tyree et al. 1995) and used to measure K_{shoot} within cotton plants. The HCFM measures hydraulic conductivity by perfusing water into an excised shoot or root while recording water flow (F, kg $(H_2O) \cdot s^{-1}$) and pressure (P, kPa). The hydraulic architecture of a whole shoot can be represented by a resistance diagram which plots the F into a plant shoot versus the applied P. The resulting slope between these two variables is K_{shoot} (Tyree et al. 1995). Each measurement required about 90 s. The ability to rapidly measure K_{shoot} with the HCFM allows for the screening of a large number of cultivars for their ability to limit hydraulic conductance during periods of high evaporative demands.

Immediately after measuring g_s and Pn using the Licor 6400 XT, the stem was cut 2 to 3-cm above the soil surface. The cut end of the stem was submerged in deionized water where it was cut again 1-cm above the original cut. The HCFM coupling was then attached to the cut stem under water to establish a water-tight seal. The plant with the attached HCFM coupling was then taken out of the water and placed horizontally on a flat surface. Degassed deionized water was then forced to flow through the stem system under steadily increasing pressure, ~ 5 $kPa \cdot s^{-1}$ until the supplying pressure reached 550 kPa (Fig. 1). The pressure was then released and a second measurement was taken, without the removal of the HCFM coupling between measurements (Vandeleur 2008). The second measurement was used to calculate K_{shoot}. A previous shoot pressurization is necessary to fully saturate vessel elements and avoid an overestimation of K_{shoot} (Tyree et al. 1995; Bogeat-Triboulot et al. 2002). Shoot hydraulic conductance was calculated from the slope of the linear section on the plot of F versus P (Bogeat-Triboulot et al. 2002).

$$K_{shoot} = \Delta F/\Delta P \tag{2}$$

The initial flow rate was rapid and not linear due to the compression of air bubbles within the HCFM machine as well as within the xylem of the cotton shoot. However, as pressure increases, the contribution of bubble compression to the overall flow rate of water declines. Therefore, Tyree et al. (1995) proposed the slope after 200 kPa is a good representation for the actual hydraulic conductance. In both experiments, the linear regression was calculated after P = 100 kPa, due to the liner relationship that began at this P.

After the LiCor 6400 XT and HCFM measurements were conducted, physiological measurements were taken on each plant included total leaf area using a leaf area meter (LI-3100, Li-Cor, Lincoln, NE) and dry leaf weight. Leaves were separated for each plant and placed in individual paper bags which were dried at 60 °C for 72 h before weighing.

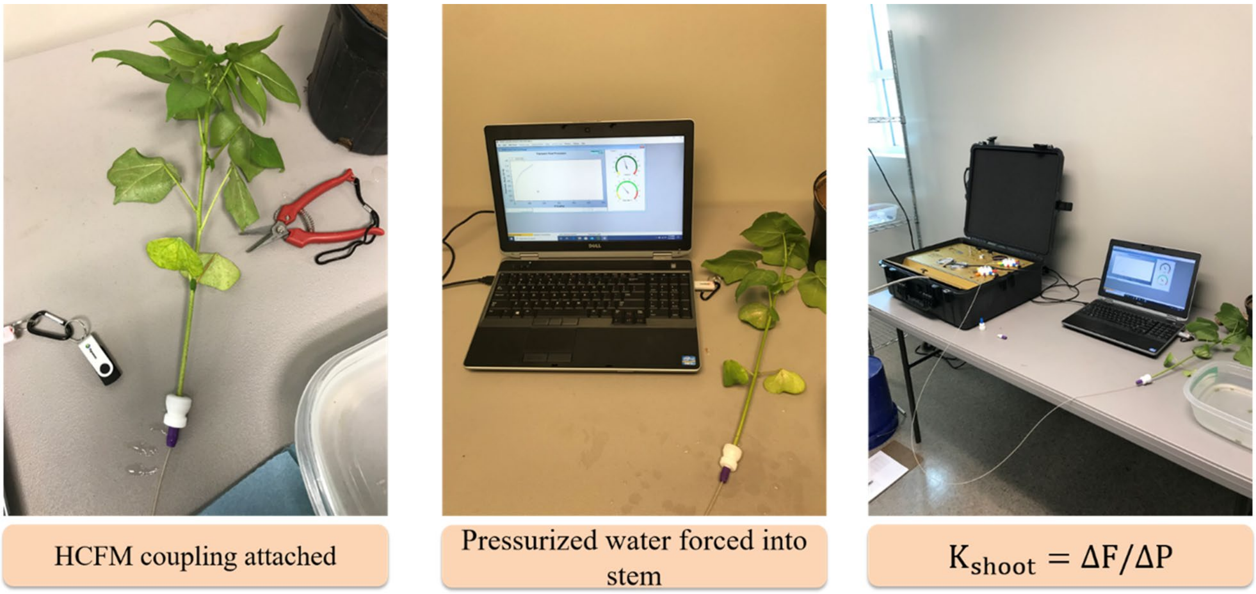

Fig. 1 Process of measuring shoot hydraulic conductance [K_{shoot} = change in water flow (F)/change in pressure (P) (kPa)] using a hydraulic conductance flow meter (HCFM)

Greenhouse study

A progressive soil drying experiment was carried out at West Tennessee Research and Education Center (WTREC) in Jackson, TN involving eight of the ten cotton cultivars tested in the growth chamber study during July and September 2020. Four seeds of each cultivar were sown as described previously in 3.7-L pots, thinned, and fertilized as described previously. Plants were grown in a greenhouse under natural light supplemented with 600 W high-pressure sodium lamps (P. L. Light Systems, Lincoln, ON, Canada) to maintain 600–650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant level, with a 15-h photoperiod. Air temperature and relative humidity were recorded as previously described. Temperature was regulated at an average of 35 ± 3 °C day/26 ± 4 °C night with $(58 \pm 14)\%$ humidity.

When plants developed 4 true leaves (approximately 28 to 30 DAP), the soil drying experiment was initiated. The pots were fully watered the evening before the experiment began. In the following morning, each pot was enclosed in two double-bagged 15-L (43 cm × 46 cm × 13.9 μm) plastic bags (Walmart, Bentonville, AR) with the bag opening bunched around the base of the stem and secured with a twist tie to prevent evaporation from the soil, following the method described by Shekoofa et al. (2013). An 8-mm-diameter × 80-mm-long tube was inserted adjacent to the plant stem to facilitate daily watering. Each pot was weighed after bagging and the weight was recorded as the initial pot weight. Then, pots were weighed daily between 11:00 and 13:00 CST to obtain gravimetric water loss through transpiration.

Daily TR of each plant was measured for three days by calculating the difference in weight of each pot on consecutive days. Plants were then assigned to one of two treatments: Water-deficit stressed (WDS) or well-watered (WW). Within each cultivar, four plants were assigned to the WDS treatment, and three were assigned to the WW treatment, which served as a reference for calculating the NTR.

Pots were weighed daily between 11:00 to 13:00 CST. After weighing, water was added to maintain the WW plants by replacing the amount of water lost from the pot to the 200-g deficit level. Soil water was allowed to gradually transpire at a rate of no greater than 100 $g \cdot d^{-1}$ from the WDS plants to simulate a prolonged development of water deficit stress. Water was only added to the WDS plants if daily water loss exceeded 100 g.

The transpiration data were analyzed by a procedure previously described by Shekoofa et al. (2013), in which daily TR data underwent two normalizations. The first normalization minimized the influence of large variations in daily TR, by dividing the TR of each WDS plant by the average daily TR for the WW plants within each cultivar. The daily TR was normalized again to account for differences in plant size. This second normalization was done for each plant by dividing the daily TR by the mean TR of that same plant during the first 3 days of the experiment when the soil was still near pot capacity. This describes the calculation of NTR and its value during the high-water content/wet phase of the dry-down cycle for each plant was, by definition, centered on a value of 1.0. Collection of data for each pot continued until the plant dried to a NTR ≤ 0.1, which was defined as the endpoint of transpirable soil water. The water content of each drying pot was based on the total transpirable soil water, which was the difference in weight between the initial and final weight of the pot. The fraction of transpirable soil water (FTSW) was calculated as the difference between daily weight and final weight, divided by the total transpirable water using the following equation (Ray and Sinclair 1998; Shekoofa et al. 2013).

$$\text{FTSW} = \left(\text{daily weight} - \text{final weight}\right)/(\text{initial weight} - \text{final weight}) \tag{3}$$

Statistical analysis

Growth chamber study

To compare the HCFM results among the cultivars, the F from each cultivar's replication was averaged and plotted against the average P increase from all measurements in each of the two experiments. Figure 2 represents specific cultivar's average change in water flow over the total average increase in pressure for each experiment.

Water flow and P increase were calculated and recorded using the HCFM computer program (HCFM-Gen3, Dynamax, Houston, TX). Shoot hydraulic conductance values were calculated through linear regression analyses by the HCFM computer program. Shoot hydraulic conductance was measured after P = 100 kPa. Physiological parameters were analyzed with a mixed model ANOVA and mean separation conducted with Tukey's HSD ($P = 0.05$) (JMP, version Pro 15, SAS Institute Inc., Cary, NC). Correlations were derived using bivariate analysis and linear analysis in JMP.

Greenhouse study

The daily NTR data were plotted against FTSW and fitted with a two-segment linear regression using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA). This software determined the FTSW threshold between the two segments where the NTR decrease was initiated. The FTSW value of the threshold where the two linear segments intersected was the critical statistic for comparing cultivars.

Results

Hydraulic conductance flow meter (HCFM)

When cultivars were exposed to an environment of high evaporative demand (Experiment 1), a wider range in flow response to increasing pressure was observed using the HCFM (Fig. 2a). In Experiment 2, cultivars were exposed to low evaporative demand and demonstrated a reduced range in flow response to increasing pressure (Fig. 2b). This difference in flow response range indicates that cultivars' flow rate behaves similarly under low evaporative demand. However, when exposed to a high evaporative demand (i.e., 38 °C, VPD > 3 kPa), a few cultivars

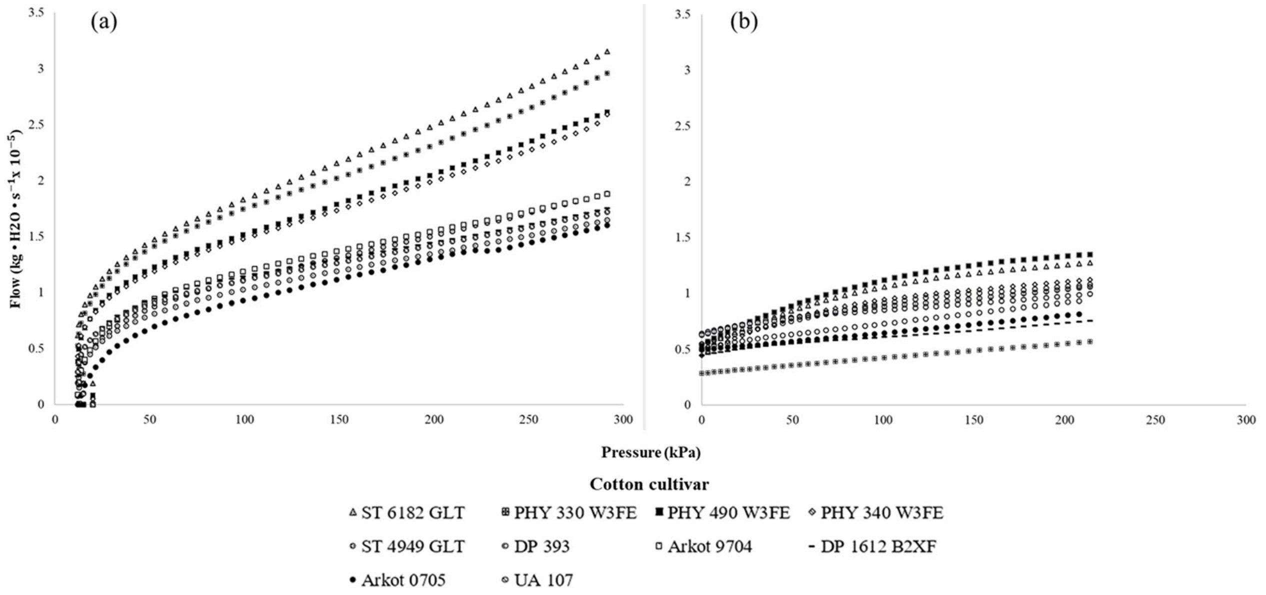

Fig. 2 Average water flow of each cotton cultivar over the total average increase in pressure from each measurement in **a** Experiment 1: [38 °C, vapor pressure deficit (VPD) > 3 kPa] and **b** Experiment 2: (32 °C, VPD = 1–1.5 kPa) in controlled environments

such as 'ST 6182 GLT', and 'PHY 330 W3FE'followed by 'PHY 490 W3FE', and 'Arkot 9704' had high and medium flow responses to increasing pressure, respectively, when compared to the other cultivars included in this study.

Shoot hydraulic conductance (K_{shoot}) among cultivars

In Experiment 1, the growth chamber study, (38 °C, VPD > 3 kPa) significant differences were observed for K_{shoot} among cotton cultivars (Table 1). Cultivar 'ST 6182 GLT' had the highest K_{shoot}, while 'DP 393 B2XF', 'Arkot 9704', 'DP 1612 B2XF', 'Arkot 0705', and 'UA 107' had lower K_{shoot} values ($P \leq 0.05$) (Table 1). The average K_{shoot} among cultivars ranged from 6.80×10^{-9} to 3.10×10^{-9} kg (H_2O)·s^{-1}·MPa^{-1} (Table 1).

In Experiment 2, under 32 °C, VPD = 1 to 1.5 kPa no significant differences were observed in K_{shoot} among all tested cotton cultivars at the 0.05 α level (Table 1). In Experiment 2, average K_{shoot} among cultivars ranged from 1.97×10^{-9} to 1.23×10^{-9} kg (H_2O)·s^{-1}·MPa^{-1} (Table 1).

Table 1 Shoot hydraulic conductance (K_{shoot}) (kg H_2O s^{-1} MPa^{-1}), standard deviation (SD), and R^2 response of cotton cultivars in Experiment 1: (38 °C, vapor pressure deficit (VPD) > 3 kPa) and Experiment 2: (32 °C, VPD = 1–1.5 kPa) in controlled environments

Cultivar	K_{shoot}	SD ($\times 10^{-11}$)	R^2
Experiment 1			
ST 6182 GLT	6.80×10^{-9} a	6.3	0.99
PHY 330 W3FE	6.23×10^{-9} ab	13.0	0.99
PHY 490 W3FE	5.62×10^{-9} ab	7.4	0.99
PHY 340 W3FE	5.43×10^{-9} ab	12.4	0.99
ST 4949 GLT	3.97×10^{-9} ab	2.1	0.99
Arkot 9704	3.55×10^{-9} b	1.5	0.99
Arkot 0705	3.37×10^{-9} b	7.7	0.99
DP 393	3.18×10^{-9} b	1.5	0.99
UA 107	3.16×10^{-9} b	1.6	0.99
DP 1612 B2XF	3.10×10^{-9} b	3.0	0.99
Experiment 2			
PHY 490 W3FE	$1.97 \times 10^{-9\ ns}$	16.4	0.97
DP 393	1.87×10^{-9}	0.8	0.9
ST 6182 GLT	1.83×10^{-9}	12.6	0.98
Arkot 0705	1.58×10^{-9}	0.4	0.99
PHY 340 W3FE	1.53×10^{-9}	4.6	0.99
Arkot 9704	1.53×10^{-9}	3.6	0.99
UA 107	1.37×10^{-9}	6.8	0.99
DP 1612 B2XF	1.28×10^{-9}	2.0	0.99
PHY 330 W3FE	1.25×10^{-9}	0.9	0.99
ST 4949 GLT	1.23×10^{-9}	1.7	0.99

Cultivars followed by the same letter within each experiment are not significantly different based on the 95% confidence interval

ns not significant ($P < 0.05$)

Shoot hydraulic conductance (K_{shoot}) and stomatal conductance (g_s)

In Experiments 1 and 2, significant differences were observed for the g_s rate among cultivars (Table 2). A negative correlation was found between K_{shoot} rates under high evaporative demand and the change in g_s among cultivars between Experiment 1 and 2 ($R^2 = 0.37$, $P = 0.05$) (Fig. 3a). This indicates that cultivars that demonstrated a greater reduction in g_s in response to a high VPD environment had lower K_{shoot} values in Experiment 1. A larger difference in g_s between high (Experiment 1) and low (Experiment 2) VPD environments suggests that a cultivar is better able to regulate g_s in response to the environmental changes.

Shoot hydraulic conductance (K_{shoot}) and VPD breakpoint (i.e., TR_{lim})

When plotting K_{shoot} values under high VPD in Experiment 1 against a cultivars VPD breakpoint from Shekoofa et al. (2020), a positive correlation was established between K_{shoot} and VPD breakpoints ($R^2 = 0.39$, $P = 0.05$) (Fig. 3b). This finding indicates that cultivars that limited their TR response earlier under increasing VPD levels had lower K_{shoot} values under a high evaporative demand environment.

Shoot hydraulic conductance (K_{shoot}) and water use efficiency coefficient WUE_k

In Experiment 1, a negative correlation was found between K_{shoot} and WUE_k ($R^2 = 0.25$, $P = 0.14$) (Fig. 3c).

Table 2 Stomatal conductance (g_s, mol (H_2O)·m^{-2}·s^{-1}) ratios and standard deviation (SD) for cultivars from Experiment (Exp.) 1 (38 °C, vapor pressure deficit (VPD) > 3 kPa) and Exp. 2: (32 °C, VPD = 1～1.5 kPa) in controlled environments

Cultivar	Stomatal conductance (SD) (g_s)		
	Exp. 1 High VPD (SD)	Exp. 2 Low VPD (SD)	Exp. 1–Exp. 2
DP 1612 B2XF	0.29 (0.11) a	0.45 (0.07) a	− 0.16
DP 393	0.22 (0.08) ab	0.48 (0.10) a	− 0.26
UA 107	0.19 (0.08) abc	0.51 (0.18) a	− 0.32
ST 6182 GLT	0.17 (0.07) abcd	0.23 (0.02) b	− 0.06
PHY 330 W3FE	0.15 (0.07) bcd	0.42 (0.07) a	− 0.27
PHY 490 W3FE	0.13 (0.09) bcd	0.23 (0.08) b	− 0.10
Arkot 9704	0.12 (0.05) bcd	0.57 (0.14) a	− 0.45
ST 4949 GLT	0.10 (0.05) bcd	0.53 (0.07) a	− 0.43
Arkot 0705	0.07 (0.05) cd	0.54 (0.09) a	− 0.47
PHY 340 W3FE	0.06 (0.12) d	0.21 (0.10) b	− 0.15

Cultivars followed by the same letter within each experiment are not significantly different based on the 95% confidence interval

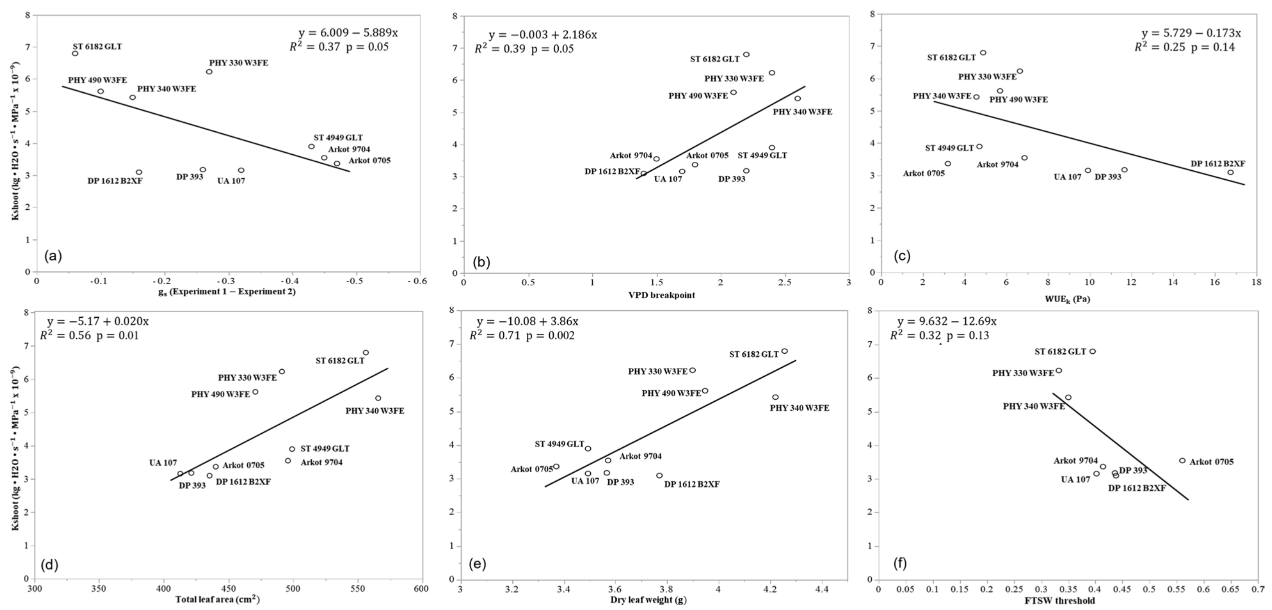

Fig. 3 Graphs of values of cotton cultivars' average shoot hydraulic conductance (K_{shoot}) under a high evaporative demand [38 °C, vapor pressure deficit (VPD) > 3 kPa] in a controlled environment versus various drought tolerance parameters. Correlations include: K_{shoot} and difference in stomatal conductance (g_s, mol (H_2O)·m^{-2}·s^{-1}) between a low evaporative demand (32 °C, VPD = 1∼1.5 kPa) and a high evaporative demand environment in controlled environments (**a**); vapor pressure deficit (VPD) breakpoint (**b**); water use efficiency coefficient (WUE_k, Pa) (**c**); total leaf area (cm^2) (**d**); leaf dry weight (**g**) and (**e**); and fraction of transpirable soil water (FTSW) threshold under progressive soil drying (**f**). $R^2 = 0.37$, $P = 0.05$ (**a**); $R^2 = 0.39$, $P = 0.05$ (**b**); $R^2 = 0.25$, $P = 0.14$ (**c**); $R^2 = 0.56$, $P = 0.01$ (**d**); $R^2 = 0.71$, $P = 0.002$ (**e**); $R^2 = 0.32$, $P = 0.13$ (**f**)

This correlation indicates that under high evaporative demand, cultivars which limited K_{shoot} had greater WUE_k.

Shoot hydraulic conductance (K_{shoot}) and leaf characteristics

In Experiments 1 and 2, differences were observed among cultivars for total leaf area (cm^2) and dry leaf weight (g) (Table 3). In Experiment 1, a positive correlation was found between K_{shoot} and total leaf area (Fig. 3d) and between K_{shoot} and leaf dry weight (Fig. 3e) ($R^2 = 0.56$, 0.71, $P = 0.01$, 0.002, respectively), indicating that under high evaporative demand, cultivars with a larger leaf area and leaf dry mass will likely exhibit a higher K_{shoot}.

Shoot hydraulic conductance (K_{shoot}) and fraction of transpirable soil water (FTSW) threshold

Cultivars' K_{shoot} values from Experiment 1 were plotted against the FTSW results from the progressive soil drying experiment in the greenhouse. A negative correlation was found between K_{shoot} and FTSW threshold among cultivars ($R^2 = 0.32$, $P = 0.13$) (Fig. 3f). This finding indicates that cultivars that limited TR earlier in the soil drying process, had lower K_{shoot} under a high evaporative demand.

Shoot hydraulic conductance (K_{shoot}) and normalized transpiration rate (NTR)

A positive correlation among cultivars was found between K_{shoot} values from Experiment 1 and the slope of NTR decrease after the FTSW threshold from the soil drying experiment ($R^2 = 0.55$, $P = 0.03$) (Fig. 4a).

To allow for a more gradual NTR reduction, NTR must be limited earlier in the soil drying cycle. In support of this, a negative correlation was found between the NTR slope and FTSW threshold among cotton cultivars in the soil drying experiment ($R^2 = 0.84$, $P = 0.001$) (Fig. 4b), indicating a more negative NTR slope is associated with a lower FTSW threshold. Thus, the later in the soil drying cycle a plant begins to decrease NTR, the steeper the slope of NTR decrease will be.

Discussion

Regulation of plant water use is critical in developing plants that are better adapted to soil drying and high VPD conditions (Li et al. 2016). Plants may regulate water use under these conditions through a limitation of K_{shoot} (Choudhary et al. 2013). Therefore, it is of importance to investigate potential differences in cultivars' ability to limit K_{shoot} under water-deficit stress conditions. In this study, it was found that cotton cultivars demonstrated differences in K_{shoot} response to soil drying and high

Table 3 Leaf area (cm^2) and leaf dry weight (g) and standard deviation (SD) of cotton cultivars from Experiment 1: (38 °C, vapor pressure deficit (VPD) > 3 kPa) and Experiment 2: (32 °C, VPD = 1~1.5 kPa) in controlled environments

Cultivar	Leaf area (SD) (cm^2)	Leaf dry weight (SD) (g)
Experiment 1		
ST 6182 GLT	556.3 (92.9) a	4.25 (0.24) a
PHY 340 W3FE	565.8 (21.5) a	4.22 (0.17) ab
ST 4949 GLT	499.2 (36.4) ab	3.49 (0.15) c
PHY 330 W3FE	491.4 (38.5) ab	3.89 (0.30) abc
PHY 490 W3FE	470.8 (57.9) ab	3.94 (0.44) abc
Arkot 9704	496.0 (46.6) ab	3.57 (0.27) bc
Arkot 0705	440.2 (31.7) b	3.37 (0.14) c
DP 1612 B2XF	435.4 (19.7) b	3.77 (0.32) abc
DP 393	421.3 (26.8) b	3.56 (0.26) bc
UA 107	412.8 (24.3) b	3.49 (0.31) c
Experiment 2		
PHY 490 W3FE	304.6 (44.2) a	2.67 (0.62) a
ST 6182 GLT	277.0 (34.9) a	2.57 (0.15) a
PHY 340 W3FE	275.5 (19.9) a	2.47 (0.30) a
Arkot 9704	145.5 (61.1) b	1.17 (0.56) b
Arkot 0705	141.9 (32.8) b	0.95 (0.13) b
ST 4949 GLT	139.1 (25.5) b	0.92 (0.09) b
UA 107	133.1 (47.4) b	0.92 (0.35) b
DP 393	125.6 (31.8) b	0.82 (0.25) b
DP 1612 B2XF	113.7 (22.6) b	0.87 (0.25) b
PHY 330 W3FE	104.7 (43.5) b	0.70 (0.036) b

Cultivars followed by the same letter within each measurement are not significantly different based on the 95% confidence interval

VPD conditions and that a cultivars' ability to limit K_{shoot} under water-deficit stress conditions correlated with a group of drought tolerance parameters.

When plotting K_{shoot} values under high VPD in Experiment 1 against a cultivars VPD breakpoint from Shekoofa et al. (2020), the K_{shoot} response to high VPD among cultivars followed a trend for the TR_{lim} trait reported by Shekoofa et al. (2020). Shekoofa et al. (2020) tested the TR of the same cultivars included in this study under increasing VPD levels at low (32 °C) and high (38 °C) temperature rates. They indicated that all cultivars responded similarly under 32 °C by demonstrating the TR_{lim} trait and reducing TR as VPD increased past a certain point (1.4~2.6 kPa, VPD) (Shekoofa et al. 2020). In our study, cultivars responded similarly under 32 °C by demonstrating similar K_{shoot} rates.

However, Shekoofa et al. (2020) reported that when exposed to increasing VPD levels under 38 °C, cultivars 'ST 6182 GLT', 'Arkot 9704', and 'Arkot 0705' lost their ability to limit transpiration and demonstrated a liner relationship of TR to increasing VPD. In our study, cultivars differed significantly in their K_{shoot} response at 38 °C. Cultivar 'ST 6182 GLT' had the highest K_{shoot} at 38 °C, suggesting that the loss of TR_{lim} is associated with an increase in K_{shoot} at high temperatures. This result is consistent with the hypothesis that a low VPD breakpoint results from a low hydraulic conductance in the plant, which is expected to result in a reduced ability to move water as rapidly through the plant to the stomata (Choudhary et al. 2014).

It has been hypothesized that a plant with lower K_{shoot} would transport less water through the shoot to supply leaves. With a limited water supply, a plant under high evaporative demand would be required to partially close stomata to conserve leaf water potential and avoid leaf desiccation (Bunce 2006; Choudhary et al. 2013; Sinclair et al. 2017). The relationship between K_{shoot} and g_s

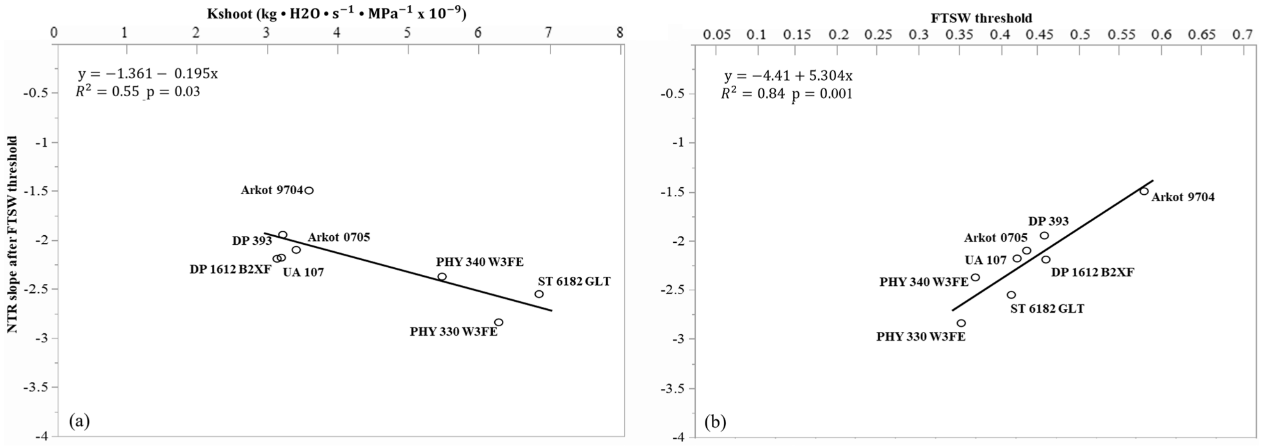

Fig. 4 Graphs of values of shoot hydraulic conductance (K_{shoot}) under a high evaporative demand [38 °C, vapor pressure deficit (VPD) > 3 kPa] in a controlled environment (**a**) and the fraction of transpirable soil water (FTSW) threshold under progressive soil drying (**b**) versus the normalized transpiration rate (NTR) slope after FTSW threshold. $R^2 = 0.55$, $P = 0.03$ (**a**). $R^2 = 0.84$, $P = 0.001$ (**b**)

measured in this study supports this hypothesis and suggest that a low K_{shoot} acts as a "hydraulic regulator", effectively limiting stomatal activity under high evaporative demand.

The positive correlation between K_{shoot} and VPD breakpoint measured in this study agree with the results of Choudhary et al. (2014), who found a positive correlation between plant hydraulic conductance (K_{plant}) and VPD breakpoint among 12 maize hybrids ($R^2 = 0.30$). Choudhary et al. (2014) considered this R^2 value to be low and proposed that K_{plant} does not focus on a specific segment of the hydraulic pathway and does not segregate between root and shoot limitations. Similarities between R^2 reported by Chaudhary et al. (2014) and the present study suggests that segregation of root hydraulic conductance (K_{root}) is necessary to develop a better understanding of K_{plant} relationship with VPD levels. Overall, the positive correlation in this study further supports the hypothesis that low K_{shoot} lessens the ability to transport water through the plant to the stomata under high evaporative demand, which causes a low VPD breakpoint (Choudhary et al. 2014).

The negative correlation between K_{shoot} and WUE_k measured in this study is consistent with Sinclair et al. (2008) which observed a low K_{leaf} to be associated with higher water use efficiency, limited TR, and increased water conservation in a "slow wilting" soybean cultivar. The observed "slow wilting" phenotype is thought to be indicative of several desirable drought-tolerance traits (Purdom et al. 2021). These results suggest the limitation of K_{shoot} under high evaporative demand could be directly related to stomatal associated traits under high VPD and water deficit stress conditions. The restriction in K_{shoot} observed in this study will limit TR, conserving soil water earlier during plants growth for extended use throughout a growing season. Because stomatal regulation in cotton can be genotype-specific (Devi and Reddy 2018), a comparison of traits associated with water relations across genotypes is important.

In our study, the positive correlations were reported between K_{shoot} and leaf area as well as K_{shoot} and leaf dry weight (Fig. 3d, e). Leaves are intricately connected to a plant's vascular system and represent between 30% to 90% of the hydraulic resistance of the whole plant (Brodribb and Holbrook 2006), as more leaf area is amassed, a greater amount of water must be supplied to the leaves to avoid desiccation under high evaporative stress (Brodribb 2009). Therefore, from a drought tolerance perspective it would be advantageous for a cultivar to have limited leaf area. When high evaporative demand occurs, the plant will then not be required to transport as much water to support leaf functions and will conserve soil water (George-Jaeggli et al. 2017).

The relationship between K_{shoot} and FTSW threshold measured in this study differs from the results acquired in Choudhary and Sinclair (2013). Choudhary and Sinclair (2013) hypothesized that low K_{plant} means that decreases in TR will occur at a higher FTSW in the soil drying cycle. However, their results showed the opposite; a significant positive correlation between K_{plant} and the FTSW threshold was measured, suggesting low plant conductance was associated with a low FTSW threshold. They suggested the measured positive correlation may be a result of conductance measurements being made on well-watered plants under no imposed stresses. In our study, K_{shoot} measurements were made on plants subjected to high evaporative stress (Experiment 1), which is likely the reason a negative correlation was measured. Li et al. (2016) conducted a study that measured changes in hydraulic conductivity of the soil–plant system (K_{total}) with decreasing soil volumetric water content. Their results showed a reduction in K_{total} was influential in the response of TR to soil drying, suggesting a more restrictive hydraulic conductivity in response to soil drying would impact FTSW threshold as a consequence of a limited TR.

The slope of NTR decrease after the FTSW threshold is the rate at which NTR decreases after the initial decrease in NTR in response to soil drying. A more negative NTR slope indicates a higher rate of NTR reduction after FTSW threshold. A plant which decreases NTR earlier in the soil drying cycle can conserve soil moisture for longer use throughout the soil drying process (Shekoofa et al. 2013; Shekoofa and Sinclair 2018; Devi and Reddy 2018). The soil water conservation, a consequence of limited K_{shoot}, would allow for a more gradual reduction in NTR through the soil drying process. The gradual reduction in NTR maximizes water use efficiency, allowing a plant to maintain physiological functions for a longer period under soil drying. This results in a less rapid decline in TR as soil drying progresses (Devi et al., 2009). On the contrary, a cultivar with no limitation of K_{shoot} under high evaporative stress will have maximum NTR, and rapid depletion of soil water leading to a lower FTSW threshold and a steeper transpiration decline (Jørgensen et al. 2010).

Conclusion

This study provides information to researchers and breeders on the K_{shoot} response of a variety of cotton cultivars under high evaporative demand. These experiments highlight the importance of K_{shoot} in influencing the limitation of TR under high temperature and evaporative demand. Correlations of K_{shoot} under high evaporative demand with a variety of drought tolerance parameters such as g_s, WUE_k, FTSW threshold, VPD breakpoint, and leaf area indicate that K_{shoot} is associated with drought tolerance in plants. Differences in K_{shoot} response to high evaporative demand among cotton cultivars reveal that genotypic differences can lead to diverse hydraulic conductance within plant shoots. These findings suggest that those cultivars with low sensitivity in K_{shoot} have restricted shoot water flow which can be used as an effective measurement to screen cultivars for drought tolerance traits under high evaporative demand and soil drying environments. Further experimentation is needed to investigate root hydraulic conductance.

Abbreviations

FTSW: Fraction of transpirable soil water; g_s: Stomatal conductance; K_{leaf}: Leaf hydraulic conductance; K_{plant}: Plant hydraulic conductance; K_{shoot}: Shoot hydraulic conductance; NTR: Normalized transpiration rate; Pn: Photosynthesis rate; TR: Transpiration rate; VPD: Vapor pressure deficit; TR_{lim}: Limited transpiration trait.

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Author contributions

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Availability of data and materials

All the related data and files are presented.

Declarations

Ethics approval and consent to participate

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Competing interests

The authors declare no conflicts of interest.

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References

Bogeat-Triboulot MB, Martin R, Chatelet D, et al. Hydraulic conductance of root and shoot measured with the transient and dynamic modes of the high-pressure flowmeter. Ann For Sci. 2002;59(4):389–96. https://doi.org/10.1051/forest:2002010.

Bowman DT, Gutierrez OA, Percy RG, et al. Pedigrees of upland and pima cotton cultivars released between 1970 and 2005. Miss Agric For Exp Station Tech Bull. 2006;1155(57):66.

Brodribb TJ. Xylem hydraulic physiology: the functional backbone of terrestrial plant productivity. Plant Sci. 2009;177(4):245–51. https://doi.org/10.1016/j.plantsci.2009.06.001.

Brodribb TJ, Holbrook NM. Declining hydraulic efficiency as transpiring leaves desiccate: two types of response. Plant Cell Environ. 2006;29(12):2205–15. https://doi.org/10.1111/j.1365-3040.2006.01594.x.

Brodribb TJ, Feild TS, Jordan GJ. Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiol. 2007;144(4):1890–8. https://doi.org/10.1104/pp.107.101352.

Brodribb TJ, Bowman DJ, Nichols S, et al. Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. New Phytol. 2010;188(2):533–42. https://doi.org/10.1111/j.1469-8137.2010.03393.x.

Bunce JA. How do leaf hydraulics limit stomatal conductance at high water vapour pressure deficits? Plant Cell Environ. 2006;8:1644–50. https://doi.org/10.1111/j.1365-3040.2006.01541.x.

Choudhary S, Sinclair TR, Prasad PVV. Hydraulic conductance of intact plants of two contrasting sorghum lines, SC15 and SC1205. Funct Plant Biol. 2013;40(7):730–8. https://doi.org/10.1071/FP12338.

Choudhary S, Sinclair TR, Messina CD, et al. Hydraulic conductance of maize hybrids differing in transpiration response to vapor pressure deficit. Crop Sci. 2014;54:1147–52. https://doi.org/10.2135/cropsci2013.05.0303.

Cochard H, Peiffer M, Le Gall K, et al. Developmental control of xylem hydraulic resistances and vulnerability to embolism in *Fraxinus excelsior* L.: impacts on water relations. J Exp Bot. 1997;48(3):655–63. https://doi.org/10.1093/jxb/48.3.655.

Cochard H, Martin R, Gross P, et al. Temperature effects on hydraulic conductance and water relations of *Quercus robur* L. J Exp Bot. 2000;51(348):1255–9.

Devi MJ, Reddy VR. Transpiration response of cotton to vapor pressure deficit and its relationship with stomatal traits. Front Plant Sci. 2018;9:1572. https://doi.org/10.3389/fpls.2018.01572.

Devi MJ, Sinclair TR, Vadez V, et al. Peanut genotypic variation in transpiration efficiency and decreased transpiration during progressive soil drying. Field Crop Res. 2009;114(2):280–5. https://doi.org/10.1016/j.fcr.2009.08.012.

Franks PJ, Drake PL, Froend RH. Anisohydric but isohydrodynamic: seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance. Plant Cell Environ. 2007;30(1):19–30. https://doi.org/10.1111/j.1365-3040.2006.01600.x.

George-Jaeggli B, Mortlock MY, Borrell AK. Bigger is not always better: reducing leaf area helps stay-green sorghum use soil water more slowly. Environ Exp Bot. 2017;138:119–29. https://doi.org/10.1016/j.envexpbot.2017.03.002.

Gholipoor M, Prasad PV, Mutava RN, et al. Genetic variability of transpiration response to vapor pressure deficit among sorghum genotypes. Field Crop Res. 2010;119(1):85–90. https://doi.org/10.1016/j.fcr.2010.06.018.

Jørgensen ST, Liu F, Ouédraogo M, et al. Drought responses of two Bambara groundnut (*Vigna subterranea* L. Verdc.) landraces collected from a dry and a humid area of Africa. J Agron Crop Sci. 2010;196(6):412–22. https://doi.org/10.1111/j.1439-037X.2010.00435.x.

Judd LA, Jackson BE, Fonteno WC, et al. Measuring root hydraulic parameters of container-grown herbaceous and woody plants using the hydraulic conductance flow meter. Hortic Sci. 2016;51(2):192–6. https://doi.org/10.21273/HORTSCI.51.2.192.

Li X, Sinclair TR, Bagherzadi L. Hydraulic conductivity changes in soybean plant-soil system with decreasing soil volumetric water content. J Crop Improv. 2016;30(6):713–23. https://doi.org/10.1080/15427528.2016.1231729.

Li X, Smith R, Choat B, et al. Drought resistance of cotton (*Gossypium hirsutum*) is promoted by early stomatal closure and leaf shedding. Funct Plant Biol. 2020;47(2):91–8. https://doi.org/10.1071/FP19093.

Pradhan D, Shekoofa A, Sinclair TR. Temperature effect on peanut (*Arachis hypogaea* L.) transpiration response to vapor pressure deficit and its recovery. J Crop Improv. 2019;33(2):177–86. https://doi.org/10.1080/15427528.2018.1552900.

Purdom S, Shekoofa A, McClure A, et al. Genotype identification for a water saving trait: exploring early stomatal closure under soil drying among mid-South soybean. Agron J. 2021;114:545–54. https://doi.org/10.1002/agj2.20918.

Ray JD, Sinclair TR. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. J Exp Bot. 1998;49(325):1381–6. https://doi.org/10.1093/jxb/49.325.1381.

Sack L, Frole K. Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. Ecology. 2006;87(2):483–91. https://doi.org/10.1890/05-0710.

Sadok W, Sinclair TR. Transpiration response of "slow-wilting" and commercial soybean (*Glycine max* (L.) Merr.) genotypes to three aquaporin inhibitors. J Exp Bot. 2010;61(3):821–9. https://doi.org/10.1093/jxb/erp350.

Sarkar S, Shekoofa A, McClure A, et al. Phenotyping and quantitative trait locus analysis for the limited transpiration trait in an upper-mid south soybean recombinant inbred line population ("Jackson" × "KS4895"): high throughput aquaporin inhibitor screening. Front Plant Sci. 2022;12:779834. https://doi.org/10.3389/fpls.2021.779834.

Shekoofa A, Sinclair TR. Aquaporin activity to improve crop drought tolerance. Cells. 2018;7(9):123. https://doi.org/10.3390/cells7090123.

Shekoofa A, Devi J, Sinclair T, et al. Divergence in drought-resistance traits among parents of recombinant peanut inbred lines. Crop Sci. 2013;53(6):2569–76. https://doi.org/10.2135/cropsci2013.03.0153.

Shekoofa A, Rosas-Anderson P, Sinclair TR, et al. Measurement of limited-transpiration trait under high vapor pressure deficit for peanut in chambers and in field. Agron J. 2015;107(3):1019–24. https://doi.org/10.2134/agronj14.0570.

Shekoofa A, Safikhan S, Snider JL, et al. Variation in stomatal conductance responses of cotton cultivars to high vapour pressure deficit under controlled and rainfed environments. J Agron Crop Sci. 2020;207(2):332–43. https://doi.org/10.1111/jac.12440.

Sheldon K, Shekoofa A, Walker E, et al. Physiological screening for drought-tolerance traits among hemp (*Cannabis sativa* L.) cultivars in controlled environments and in field. J Crop Improv. 2021;35(6):816–31. https://doi.org/10.1080/15427528.2021.1883175.

Sinclair TR, Tanner CB, Bennett JM. Water-use efficiency in crop production. Bioscience. 1984;34:36–40. https://doi.org/10.2307/1309424.

Sinclair TR, Hammer GL, van Oosterom EJ. Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Funct Plant Biol. 2005;32(10):945–52. https://doi.org/10.1071/FP05047.

Sinclair TR, Zwieniecki MA, Holbrook NM. Low leaf hydraulic conductance associated with drought tolerance in soybean. Physiol Plant. 2008;132(4):446–51. https://doi.org/10.1111/j.1399-3054.2007.01028.x.

Sinclair TR, Devi J, Shekoofa A, et al. Limited-transpiration response to high vapor pressure deficit in crop species. Plant Sci. 2017;260:109–18. https://doi.org/10.1016/j.plantsci.2017.04.007.

Sperry JS. Hydraulic constraints on plant gas exchange. Agric For Meteorol. 2000;104(1):13–23. https://doi.org/10.1016/S0168-1923(00)00144-1.

Tyree MT, Patiño S, Bennink J, et al. Dynamic measurements of roots hydraulic conductance using a high-pressure flowmeter in the laboratory and field. J Exp Bot. 1995;46(1):83–94. https://doi.org/10.1093/jxb/46.1.83.

Vandeleur R. Grapevine root hydraulics: the role of aquaporins. Adelaide, Australia: University of Adelaide. 2008. https://hdl.handle.net/2440/57505. Accessed 11 Apr 2022.