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Genotypic variation in whole-plant transpiration efficiency in sorghum only partly aligns with variation in stomatal conductance

Geetika Geetika[A,E], *Erik J. van Oosterom*[A], *Barbara George-Jaeggli*[B,C], *Miranda Y. Mortlock*[A], *Kurt S. Deifel*[A], *Greg McLean*[D] *and Graeme L. Hammer*[A]

[A]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, St Lucia, Qld 4072, Australia.
[B]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Hermitage Research Facility, Warwick, Qld 4370, Australia.
[C]Agri-Science Queensland, Department of Agriculture and Fisheries, Warwick, Qld 4370, Australia.
[D]Agri-Science Queensland, Department of Agriculture and Fisheries, Toowoomba, Qld 4350, Australia.
[E]Corresponding author. Email: geetika.geetika@uq.net.au

Abstract. Water scarcity can limit sorghum (*Sorghum bicolor* (L.) Moench) production in dryland agriculture, but increased whole-plant transpiration efficiency (TE_{wp}, biomass production per unit of water transpired) can enhance grain yield in such conditions. The objectives of this study were to quantify variation in TE_{wp} for 27 sorghum genotypes and explore the linkages of this variation to responses of the underpinning leaf-level processes to environmental conditions. Individual plants were grown in large lysimeters in two well-watered experiments. Whole-plant transpiration per unit of green leaf area (TGLA) was monitored continuously and stomatal conductance and maximum photosynthetic capacity were measured during sunny conditions on recently expanded leaves. Leaf chlorophyll measurements of the upper five leaves of the main shoot were conducted during early grain filling. TE_{wp} was determined at harvest. The results showed that diurnal patterns in TGLA were determined by vapour pressure deficit (VPD) and by the response of whole-plant conductance to radiation and VPD. Significant genotypic variation in the response of TGLA to VPD occurred and was related to genotypic differences in stomatal conductance. However, variation in TGLA explained only part of the variation in TE_{wp}, with some of the residual variation explained by leaf chlorophyll readings, which were a reflection of photosynthetic capacity. Genotypes with different genetic background often differed in TE_{wp}, TGLA and leaf chlorophyll, indicating potential differences in photosynthetic capacity among these groups. Observed differences in TE_{wp} and its component traits can affect adaptation to drought stress.

Additional keywords: photosynthesis, *Sorghum bicolor* (L.) Moench, vapour pressure deficit.

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a versatile crop that can be used for human consumption, animal feed or biofuel, and is recognised for its adaptation to dryland environments where water availability can be a major production constraint (Burke *et al.* 2015). Current changes in global climate are likely to increase the frequency of occurrence and severity of droughts, placing further strain on dryland cropping systems (Pachauri and Meyer 2014). However, given the pressing need to accelerate global food production, and the limited availability of new arable land and water resources (Food and Agricultural Organization of the United Nations 2009; Foley *et al.* 2011), dryland crop production systems assume a critical role. Increased productivity of crops that are adapted to dryland conditions, such as sorghum, is required and hence there is significant potential value in investigating traits associated with the efficient use of water.

Increased transpiration efficiency (TE), defined as total plant biomass produced (g) per unit of water transpired (kg) (Hammer *et al.* 1997), can produce 'more crop per drop' (Marris 2008) of water, and improve drought adaptation in sorghum (Hubick *et al.* 1990; Vadez *et al.* 2014). Since leaf-level TE is the ratio between instantaneous rates of CO_2 assimilation (photosynthesis) and water vapour flux through the stomata (Condon *et al.* 2002; Tuberosa 2012), TE can potentially be manipulated by enhancing photosynthetic rates or decreasing transpiration rates. However, the associations between these two processes via gas exchange that occurs at the stomata can cause trade-offs in terms of their effects on grain yield. Simulation studies for both sorghum (Sinclair *et al.* 2005) and maize (*Zea mays* L.) (Messina *et al.* 2015) have shown that restricting transpiration rates at high vapour pressure deficit (VPD) can reduce preanthesis water use, resulting in increased post-anthesis water availability and hence increased grain yield under postanthesis drought stress. The

associated reduction in CO_2 uptake through restricted stomatal conductance (*gs*), however, can cause a yield penalty in environments without drought stress where biomass accumulation is radiation-limited (Sinclair *et al*. 2005; Messina *et al*. 2015). Hence, changes in component traits that determine TE can result in complex genotype × environment × management interactions for grain yield.

In sorghum, genotypic variation in whole-plant TE has been linked to differences in biomass production, which is a proxy for photosynthetic capacity (P_c) (Xin *et al*. 2009; Vadez *et al*. 2011). However, Hammer *et al*. (1997) observed only a weak correlation of plant-level TE with biomass production and no correlation with transpiration rate per unit of leaf area, although leaf area was measured only at harvest. In contrast, Mortlock and Hammer (2000) reported that plant-level TE was negatively correlated with transpiration rate per unit of leaf area, and these results were consistent at the leaf level, where TE was negatively correlated with *gs*. This concurred with results for pearl millet (*Pennisetum glaucum* (L.) R.Br.) (Kholová *et al.* 2010), rice (*Oryza sativa* L.) (Impa *et al.* 2005) and maize (Ryan *et al.* 2016), which showed that genotypes with restricted transpiration rates tend to have high TE. Genotypic differences in the response of transpiration rates to VPD have also been reported for sorghum (Gholipoor *et al.* 2010). Thus the presence of such widespread variation in TE and its component traits of P_c and transpiration rate implies that different combinations of these leaf traits, rather than one single trait, could account for the observed variations in TE in sorghum.

Environmental effects on TE can be complex, making selection for TE difficult and hampering incorporation of this trait into breeding programmes. Thus greater focus needs to be placed on selection for component traits or combinations of traits associated with efficient water use in sorghum (Sinclair 2012). This requires comprehensive studies across diverse germplasm that link genotypic variation in TE to the underpinning components at the leaf level. For sorghum, such studies are scarce. Hence, the objectives of this study were to quantify the extent of genotypic variation in TE in a diverse range of sorghum germplasm and to determine any associations with the responses of the underpinning leaf-level processes to environmental conditions.

Materials and methods

Germplasm used

The experiments comprised of 27 sorghum (*Sorghum bicolor* (L.) Moench.) genotypes that included both inbred lines and hybrids (Table 1). Inbred lines represented a diverse range of

Table 1. Name, origin and stay-green characteristics of the sorghum genotypes used in the experiments
NIL, near isogenic line; ICRISAT, International Crops Research Institute for the Semi-Arid Topics.

Genotype	Pedigree	Stay-green characteristics
B010054 plus hybrids		
FF_B010054	B923171//FF_BTx3197/FF_SC170–6-4–4	Senescent; B923171 is stay-green sister line of B923296
A1*F_B010054/F4_R07528–91–1-1	F2_ms3*2_R986087–2-4–1//R986087–2-4–1/Karper 669	R986087–2-4–1 pedigree is FF_SC170–6-17/R931945–2-2
A1*F_B010054/F4_R08512–35	F2_ms3*2_R986087–2-4–1//R986087–2-4–1/IS22253	R986087–2-4–1 pedigree is FF_SC170–6-17/R931945–2-2
A1*F_B010054/F4_R08512–79	F2_ms3*2_R986087–2-4–1//R986087–2-4–1/IS22253	R986087–2-4–1 pedigree is FF_SC170–6-17/R931945–2-2
A1*F_B010054/F5_R07536–22–1-2	F2_ms3*2_R986087–2-4–1//R986087–2-4–1/FF_RTx2737	R986087–2-4–1 pedigree is FF_SC170–6-17/R931945–2-2
A1*F_B010054/F5_R08341–8	R993396//R993396/R011298	R011298 is 50% KS115
A1*F_B010054/F9_R04377–31	Elite line mixed parentage	–
Tx7000	–	–
RTx7000	Parent of NILs	Senescent male
NIL2219–3	Mostly Tx7000	Stay-green NIL (*Stg2*, Borrell *et al.* 2014*a*)
NIL2290–19	Mostly Tx7000	Stay-green NIL (*Stg3*, Borrell *et al.* 2014*a*)
NIL6078–1	Mostly Tx7000	Stay-green NIL (*Stg1*, Borrell *et al.* 2014*a*)
NIL6085–9	Mostly Tx7000	Stay-green NIL (*Stg4*, Borrell *et al.* 2014*a*)
RTAM422	Related to Tx7000	Senescent male
Tx642	–	–
BTx642 (B35)	Line highly related to SC35	Stay-green
FF_SC35–14E	Line highly related to B35	–
B923296	B872389–1-3/B886815–1-3 (B35 in genetics)	Elite stay-green female
R931945–2-2	R883373–3//B872389–2-3/R890562–1-2	Widely used stay-green male, from B35 and maybe QL12
B963676	–	–
B963676	B930083–1-4/B923223–1	Elite stay-green female next generation from B923000 series
A1*F_B963676/R931945–2-2	–	Elite hybrid of two stay-green lines
QL12/B963676	–	Hybrid of two stay-green lines
QL12		–
QL12	Related to Karper 669	Stay-green, yellow endosperm
Karper 669	Related to QL12	Stay-green, yellow endosperm male
SC170–6-8	–	–
SC170–6-8	Parent of R986087; related to Tx430 and Tx623	High yield potential conversion line
FF_RTx430	FF_RTx2536//FF_SC170–6/FF_SC110–14E	–
Tall 2dwarf	–	–
LR9198	Chinese germplasm line	–
Sureno	FF_SC423–14E/CS3541//E35–1/M62650	High-yielding line from ICRISAT has E35 stay green source
Check (commercial hybrid)	–	–
MR-Buster	Elite hybrid	–

germplasm that has been used as parents in the Australian sorghum prebreeding programme based at Warwick, Queensland. Genotypes were selected on the basis of available knowledge on their TE and their ability to retain green leaf area during grain filling (stay-green). Prior to experiments being conducted, genotypes were classified into eight groups (GENO-Groups) based on available pedigree information (Table 1). Some genotypes within the GENO-Groups were highly genetically related, such as RTx7000 and its four near-isogenic lines (NILs), which each contained quantitative trait loci associated with the stay-green trait from a donor parent. Genotypes within other GENO-Groups had a less direct genetic relationship, such as BTx642 and R931945–2-2 (Table 1). Most of the genotypes were 3dwarf sorghums, except for two tall 2dwarf sorghums, LR9198 and Sureno. The 2dwarf sorghums have one less major dwarfing gene and are between 20 and 50 cm taller than 3dwarf sorghums. Further, some of the hybrids had parents belonging to different groups (e.g. A1*F_B963676/R931945–2-2 and QL12/B963676). Nonetheless, the GENO-Groups provided the opportunity for enhanced data analysis and interpretation.

Experiment details

Two experiments were conducted during the summer of 2015–16 in an automated lysimetry platform located at the University of Queensland campus at Gatton (27°33′S,152°20′E) in south-east Queensland, Australia. The platform has been described in detail by Chenu *et al*. (2018). In brief, the lysimeters were set up inside an enclosure with a solarweave roof, meshed polyweave sides to allow airflow and gable fans to ensure additional airflow on hot days. Maximum daily radiation inside the enclosure was 25 MJ m^{-2} and average daily radiation was around 19 MJ m^{-2} in Experiment 1 (Exp1) and 14 MJ m^{-2} in Experiment 2 (Exp2). This was nearly two-thirds of the radiation outside the enclosure (data not shown). The platform itself consisted of 128 lysimeters with a capacity of ~51 L each. This was well above the threshold of 30 L, below which container size can affect root–shoot partitioning in sorghum (Yang *et al*. 2010), and ensured that the plants used in the experiments were representative of field-grown plants in terms of organ size. Each lysimeter was placed on its own load cell, which was mounted on a trolley that had two rows of four load cells each. Plant spacing was ~0.5 m within trolleys and 1 m between trolleys. Lysimeter weights were monitored continuously and every 10 min, the average of these weights was recorded in a comma-separated values (CSV) file. Watering was automated and once the weight of a lysimeter had dropped below a predetermined threshold value, 250 mL of water was applied into a 30 cm PVC access tube with a capacity of 750 mL that was embedded in the soil.

Prior to sowing, lyismeters were filled with a black vertisol soil from Dalby in south-east Queensland, located in the sorghum-growing area of north-eastern Australia (Hammer *et al*. 2014). Each lysimeter was filled to a weight of 49 kg, and ~42 g of Scotts Osmocote Plus (Scotts Australia) controlled release fertiliser (16% N, 3.5% P, 10% K) was applied to each lysimeter in six evenly distributed layers during filling. Lime and dolomite were added with the osmocote to prevent soil acidification. Each lysimeter was lined with a plastic bag to facilitate removal of soil at harvest. A few random soil samples were taken to estimate soil water content at sowing and hence the amount of dry soil in a lysimeter. Soil water content was calculated from the difference between wet weight and weight after drying in a dehydrator at 105°C for at least 3 days. To prevent waterlogging, lysimeters were rewatered to a weight that was slightly below the weight when the soil would be at the drained upper limit. The drained upper limit was estimated by sampling soil in the top half of three free-draining reference lysimeters that were filled with soil, then watered, covered and left to drain.

Experiments were sown on 2 October 2015 (Exp1) and 25 January 2016 (Exp2) and, on average, emerged 5 days later. In both experiments, five seeds were sown in each lysimeter and emerged seedlings were gradually thinned until one seedling was left per lysimeter at around 15 days after sowing. At this stage, the soil surface was covered with plastic sheets to minimise evaporation. Every fortnight, ~5 g of Thrive (Yates Australia) water soluble fertiliser (25% N, 5% P, 8.8% K) was added to the watering tubes to ensure that nutrients were not limiting plant growth. A solution of 0.3% $Ca(NO_3)_2$ was sprayed into the leaf whorl of each axis on a daily basis, and ~5 g of $Ca(NO_3)_2$ was added into each PVC access tube once every fortnight to facilitate sufficient uptake of Ca, which, in the presence of high levels of N and P, can be locked up and difficult for plants to access, especially in poorly aerated soils. The experiment design was a randomised complete block with two replications that each contained a control lysimeter without a plant to measure background evaporation. Plants were harvested on 15 December 2015, around early to mid-grain filling, in Exp1 and on 1 April 2016, around anthesis, in Exp2.

Environment conditions

Temperature, relative humidity and radiation were obtained from a weather station located in the centre of the enclosure. Six additional temperature probes were located throughout the enclosure. Records were logged every 10 min, along with the weights of the lysimeters. VPD was calculated following Rosenberg *et al*. (1983) from the average of the seven temperature records and the single record of relative humidity. Weather records were aggregated to either hourly or daily values, with daily average VPD taken as 75% of the difference between saturated vapour pressure at the daily maximum and minimum (dewpoint) temperatures (Tanner and Sinclair 1983).

Leaf area measurements

The number of fully expanded and senesced leaves was counted twice a week for the main shoot and all tillers of each plant. A leaf was considered fully expanded when its ligule was visible above the ligule of the previous leaf and senesced if less than 50% of its area was green. Once a leaf was fully expanded, its length from ligule to tip and width at the widest part were measured nondestructively. Individual leaf area was then estimated as the product of leaf length, leaf width and a shape factor. The shape factor was taken as the slope of the regression (forced through the origin) of leaf area measured with a LI-COR 3100 planimeter (LI-COR) vs the product of the observed length and width of individual leaves from a range of experiments. The

factor was 0.635 ($n = 181$, $R^2 = 0.98$) for flag leaves and 0.71 ($n = 1708$, $R^2 = 0.99$) for all other leaves.

For each day that leaves were counted, total leaf area per plant was calculated as the total area of all fully expanded leaves across all axes. The area of leaves that were still expanding was estimated following Muchow and Carberry (1990). Green leaf area per plant was estimated as the difference between total leaf area per plant and the area of senesced leaves. Daily green leaf area per plant was obtained through linear interpolation of the data calculated for days on which leaves were counted.

Transpiration rates

The transpiration of each plant throughout the experiments was calculated from the decline in weight of each lysimeter between successive records, adjusted for any water added during that period. Data were aggregated on both an hourly and daily basis from the day that thinning was completed and the soil was covered. Total water use per plant was the sum of the daily values, adjusted for water losses recorded in the control lysimeters (without a plant), and shoot fresh mass and root dry mass at harvest. To account for differences in plant size, transpiration rates (either hourly or daily) were divided by green leaf area for that day (transpiration per unit green leaf area, TGLA), where green leaf area was assumed to be constant within a day. Days with prolonged periods of missing records for transpiration or when plant size and water use were too small for accurate estimates of TGLA were excluded from further analysis. In total, 19 days between 18 November and 10 December 2015 were used for Exp1, and 21 days between 1 and 30 March 2016 were used for Exp2.

Biomass and TE

At harvest, plants were cut just below the base of the stem and the shoot fresh mass of each plant was taken. Roots were washed out from the soil and the dry mass of both plant parts was determined after drying in a fan-forced dehydrator at 60–70°C for at least 48 h. Whole-plant dry mass was calculated as the sum of shoot and root dry mass. Transpiration efficiency (g kg^{-1}) was calculated as the ratio of shoot dry mass to total transpiration (TE_{shoot}) and whole-plant dry mass to total transpiration (TE_{wp}). The ratio of root to whole plant dry mass was defined as the root weight ratio.

Leaf conductance

Leaf diffusive (stomatal) conductance to water vapour (gs_{leaf}) was measured with a porometer (Model SC-1, Decagon Devices Inc.). Compared with an infrared gas analyser, which measures both P_c and leaf conductance, the porometer is faster and uses ambient conditions, making it a more suitable and efficient tool to collect sufficient data to link gs_{leaf} to environmental conditions. Measurements were taken in Exp1 under cloudless conditions for each plant in Replicate 1, using the second last fully expanded leaf of the main shoot or the last fully expanded leaf if the other one was shaded. Two measurements of gs_{leaf} were taken per leaf, one on each side of the midrib. Measurements for all 27 plants, called a run, took ~1 h. In total, 36 runs were conducted over 9 days between 19 November 2015 and 11 December 2015. A complete diurnal set (0730 hours to 1700 hours) of nine runs was completed on each of two cloudless days (19 and 20 November 2015), whereas a set of three consecutive runs was completed on a day with a cloudless morning (11 December 2015). For each plant, the average of the two readings in a run was used for further analyses and the exact time of each reading was recorded.

Leaf chlorophyll measurements

Leaf chlorophyll measurements were taken with a SPAD-502 (Soil-Plant Analysis Development, Konica Minolta) on 9 and 10 December 2015 on the top five leaves of the main shoot of each plant in Exp1. Each measurement was the mean of six individual readings, three on each side of the midrib. As the interaction between genotype and leaf was not significant (data not shown), the average SPAD value across the 2 days and five leaves for each plant was used in subsequent analyses. SPAD values were transformed to associated specific leaf N (SLN, g m^{-2}) values, as detailed in the Supplementary Material to this paper, via the following equation ($R^2 = 0.90$, $P < 0.001$) (van Oosterom *et al*. 2010):

$$\ln(\mathrm{SLN}) = 0.041 \times \mathrm{SPAD} - 1.876 \; n = 75.$$

Average chlorophyll SPAD values for individual genotype × leaf position combinations ranged from 51.5 (SLN = 1.27 g m^{-2}) to 71.2 (SLN = 2.84 g m^{-2}), indicating that leaf N content was at a level that was not limiting to growth (van Oosterom *et al*. 2010).

Maximum carbon (C) assimilation rate

The maximum C assimilation rate (A_{max}) of all 27 genotypes was measured with a CIRAS-3 portable photosynthesis system (PP Systems). A_{max} was measured as the net C assimilation rate of a fully expanded sunlit leaf at constant high light intensity that was provided by a LED light source. Depending on ambient light conditions, either 1500 or 1800 µmol PAR quanta m^{-2} s^{-1} was selected and no significant differences in A_{max} were observed at these two light intensities. The CO_2 concentration in the reference air was kept at 390 ± 20 µmol mol^{-1}. The temperature inside the chamber was set to match the ambient temperature inside the solarweave enclosure. Measurements were taken in Exp1 on predominantly sunny days between 0900 hours and 1330 hours for each plant in Replicate 1. The second last fully expanded sunlit leaf of the main shoot was used, as it takes ~1week after full expansion for a leaf to reach its maximum SLN (van Oosterom *et al*. 2010) and hence A_{max} (Muchow and Sinclair 1994). For each run, one measurement per leaf was taken for each genotype and one or two runs could be completed on days with suitable weather.

Statistical analysis

ANOVA for TE_{wp}, TE_{shoot}, root weight ratio, gs_{leaf} and A_{max} was conducted in SAS Enterprise Guide 9.4 (SAS 2013) using the GLM procedure. In order to obtain estimates of maximum gs_{leaf}, the nine runs with the highest average gs_{leaf}, which all consisted of measurements taken between 1100 hours and 1500 hours, were used, with each run considered to be a replication. For A_{max}, an analysis of the average of each run indicated that A_{max} was constant until ~25 November 2015, a few days before anthesis in the first plants. Hence, data from eight runs until 26 November were used in the analyses. Data for which the CO_2 concentration in the reference air was outside the range of 370–410 µmol mol^{-1}

were discarded and missing data were filled in by using least square estimates. In the ANOVA, each run was considered a replication, and the error degrees of freedom were reduced by the number of filled-in data in order to determine the presence of genotypic differences in A_{max}. Regression analyses were carried out via the NLIN and REG procedures in SAS.

The effects of VPD and radiation on TGLA were analysed by using hourly diurnal (0600 hours–1800 hours) data for the 19 days between 18 November and 10 December 2015 in Exp1 that had complete diurnal data for TGLA and environmental conditions. Average hourly TGLA across all plants was calculated as the ratio between the total hourly transpiration rate and the total daily green leaf area across all plants. Hourly whole-plant *gs* (gs_{wp}) was calculated as the ratio between hourly TGLA and hourly VPD. The 19 days were classified into four groups based on their maximum hourly VPD (VPD_{max}): VPD_{max} < 2 kPa (2 days), VPD_{max} = 2–3 kPa (6 days); VPD_{max} = 3–4 kPa (7 days) and VPD_{max} > 4 kPa (4 days).

To analyse the response of TGLA to VPD for individual genotypes, hourly data from 0600 hours to 1400 hours for the three groups of days with VPD_{max} > 2 kPa were used, with the exception of the data for 1300 hours and 1400 hours for the group with VPD_{max} = 3–4 kPa, as TGLA started to decline slightly earlier in the day in that instance (Fig. 3). This resulted in 25 data points when using average hourly TGLA values across the two replications (the ratio between the total hourly transpiration rate and the total daily green leaf area). For each genotype, both linear and bilinear regressions were fitted to the data via the REG and NLIN procedures in SAS respectively. If the bilinear model significantly improved the fit, the bilinear model was used for that genotype; otherwise, the linear model was used. To determine if genotypes with a bilinear relationship differed significantly for their VPD breakpoint (BP), bilinear relationships were fitted for those genotypes using a common BP, and the BP with the lowest *F*-ratio compared with the original model with genotype-specific BPs was determined. Similar analyses were conducted for the slope of the regression below the BP. In cases where a common parameter value across all genotypes could not be used, an analysis was conducted to determine if at least some genotypes could be grouped by common parameter values. This grouping was guided by a cluster analysis for the estimated (from the bilinear relationship) TGLA at VPDs of 2 kPa and 5 kPa, using Ward's minimum variance cluster analysis with standardised data in the CLUSTER and TREE procedures of SAS. Genotypes were grouped together if an *F*-test indicated that the error mean square (EMS) of the combined model was not significantly greater than the EMS using the original model with genotype-specific parameter estimates, where EMS was calculated as the ratio between the total error sum of squares and the total degrees of freedom across all regressions. Similar covariance analyses were performed for the genotypes with a linear relationship between TGLA and VPD.

Results

Genotype and environment effects on TE

There was no significant experiment effect on TE_{shoot} (Table 2), but the effect on root weight ratio was highly significant (P < 0.0001), with Exp2 recording a higher average root weight ratio (0.22) than Exp1 (0.19). Consequently, the average TE_{wp} in Exp2 (7.80 g kg^{-1}) was significantly
(P < 0.0001) greater than in Exp1 (7.42 g kg^{-1}).

The genotype effects on TE_{shoot} and root weight ratio were highly significant (P < 0.0001) across the two experiments, which resulted in highly significant genotypic differences in TE_{wp} (P < 0.0001). Nonetheless, inclusion of roots had only a small effect on the ranking of genotypes for TE. The association between TE_{wp} and TE_{shoot} was highly significant for both Exp1 (R^2 = 0.85, P < 0.0001) and Exp2 (R^2 = 0.86, P < 0.0001), and so was the Spearman rank correlation (r = 0.93, P < 0.0001 for Exp1; r = 0.86, P < 0.0001 for Exp2). Genotype × experiment interactions were significant (P < 0.05) but small compared with the main effects (Table 2).

The eight GENO-Groups explained 75% of the genotypic differences in TE_{wp} (Table 2). Genotypes belonging to the Tx7000 and QL12 groups (Table 1) consistently had low TE_{wp} (Fig. 1) and these group averages were significantly lower than those of any of the other groups (Table 3). In addition, BTx642, the related genotype FF_SC35–14E and

Table 2. ANOVA of shoot transpiration efficiency (TE_{shoot}), root weight ratio and whole-plant transpiration efficiency (TE_{wp}) across the two experiments

E, experiment; G, genotype; ns, not significant; *, P < 0.05; ***, P < 0.001

Source	Degrees of freedom	TE_{shoot}		Root weight ratio		TE_{wp}	
		Sum of squares	Significance	Sum of squares	Significance	Sum of squares	Significance
E	1	0.24	ns	0.0183	***	3.97	***
G	26	52.61	***	0.1081	***	51.07	***
Group	7	37.87	***	0.0435	***	38.57	***
G (group)	19	14.73	***	0.0647	***	12.50	***
G × E	26	8.20	*	0.0466	*	10.22	*
Group × E	7	3.62	*	0.0242	**	5.77	***
G(group) × E	19	4.58	ns	0.0224	ns	4.45	ns
Replication(E)	2	3.90	***	0.0015	ns	5.73	***
Error	52	8.97	–	0.0496	–	9.94	–

B923296, which has BTx642 in its pedigree (Table 1), also had below average TE_{wp} (Fig. 1). The other groups on average did not differ significantly in TE_{wp} (Table 3), as genotypes belonging to these groups all had medium to high TE_{wp}. Sureno, a tall 2dwarf genotype, had the highest average TE_{wp} (Fig. 1).

Table 3. Shoot transpiration efficiency (TE_{shoot}), root weight ratio and whole-plant transpiration efficiency (TE_{wp}) across the two experiments
Means followed by a different letter differ at $P < 0.05$, according to Tukey's test for pairwise comparisons.

Group	Number of genotypes	TE_{shoot} (g kg^{-1})	Root weight ratio	TE_{wp} (g kg^{-1})
Tall 2dwarf	2	6.99a	0.185b	8.55a
B010054	7	6.57a	0.189b	8.10a
B963676	3	6.51a	0.183b	7.98ab
SC170–6-8	2	6.46ab	0.209b	8.18ab
Check	1	6.46ab	0.184b	7.93ab
Tx642	4	5.95b	0.218ab	7.61b
Tx7000	6	5.46c	0.208b	6.89c
QL12	2	4.89d	0.259a	6.60c

Environmental effects on plant-level transpiration rates

In order to analyse environmental effects on transpiration rates, all analyses in this section used average data across genotypes. Average daily TGLA responded linearly to daily VPD ($R^2 = 0.77$, $n = 62$, $P < 0.0001$; Fig. 2) for the observed range of data, and the relationship was independent of experiment (time of year) and days within the experiment (development stage of plants). Exp1 had greater daily VPD and therefore higher TGLA (Fig. 2) and, consequently, a lower TE_{wp} than Exp2.

Environmental effects on TGLA were analysed in more detail by using hourly data across the 19 days in Exp1 that had complete diurnal records and by dissecting TGLA into whole-plant conductance (gs_{wp}, TGLA ÷ VPD) and VPD. Hourly values for VPD, total radiation, TGLA and gs_{wp}, averaged across days for each of the four groups of days with contrasting VPD_{max}, are presented in Fig. 3. The four days with VPD_{max} > 4 kPa

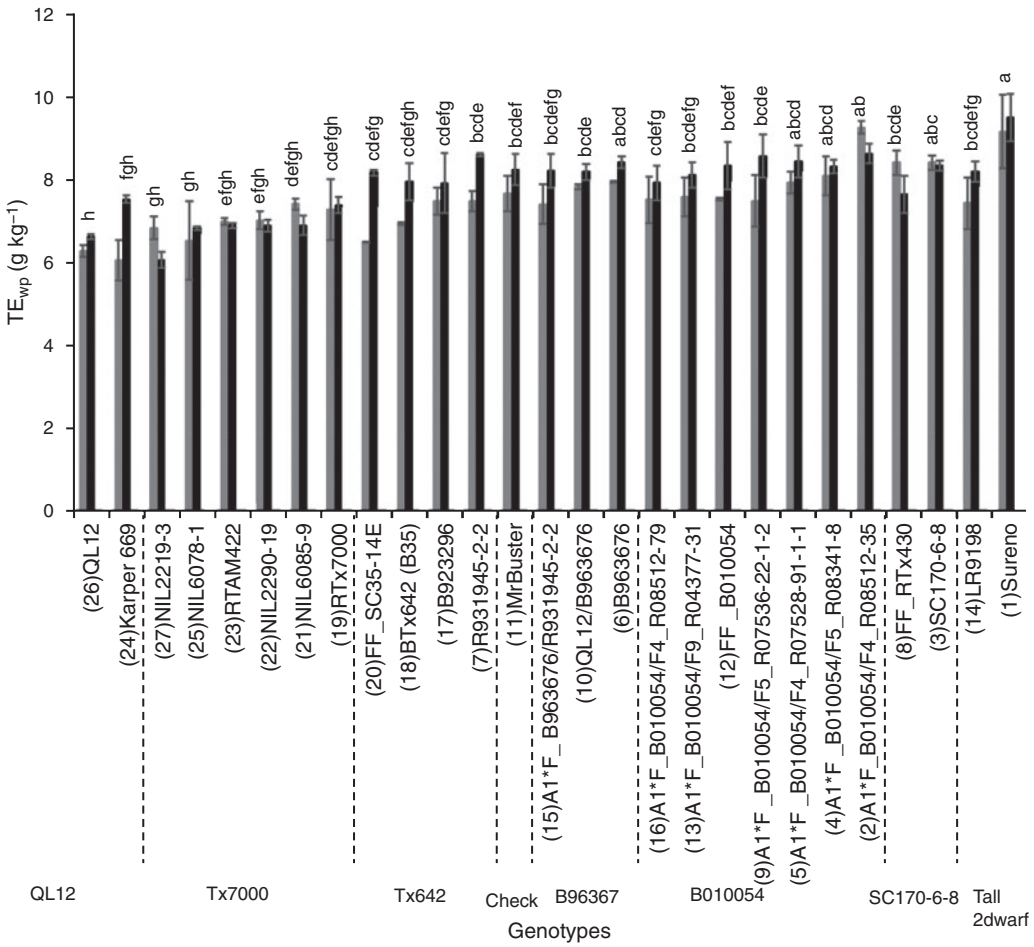

Fig. 1. Genotypic variation in whole-plant transpiration efficiency (TE_{wp}, g kg^{-1}) in Experiment 1 (Exp1) (grey bars) and Experiment 2 (Exp2) (black bars) under well-watered conditions. Sorghum genotypes are grouped according to GENO-Group (Table 1). GENO-Groups are sorted in ascending order for average TE_{wp} across Exp1 and Exp2 (Table 3) and genotypes are similarly sorted within each GENO-Group. Bars show means ($n = 2$) ± s.e. for individual genotypes. Genotype means with the same letter are not significantly ($P < 0.05$) different for TE_{wp} averaged across the two experiments, using Tukey's test for pairwise comparisons. Values in brackets preceding the genotype name represent the ranking of genotypes for average TE_{wp} across the two experiments with (1) the highest and (27) the lowest average TE_{wp}. GENO-Group names are listed along the bottom of the figure.

represented cloudless days with high radiation and high TGLA. Days with VPD_{max} = 3–4 kPa had only marginally lower radiation levels, but the lower VPD resulted in lower TGLA. Days with VPD_{max} = 2–3 kPa had more intermittent cloud cover, as indicated by the lower midday radiation. Diurnal differences for TGLA among these three groups generally followed the diurnal differences in VPD and radiation, but diurnal patterns for gs_{wp} were similar, except for the period between 1100 hours and 1500 hours. (Fig. 3*d*). For the 2 days with VPD_{max} < 2 kPa, hourly radiation, on average, did not exceed 1 MJ m^{-2} h^{-1}, and TGLA and gs_{wp} were low.

The diurnal association between TGLA and VPD across days with contrasting VPD_{max} (Fig. 4*a*) was more complex than was suggested by the daily data in Fig. 2. This complexity could largely be explained by the response of gs_{wp} to both radiation and VPD. Between 0600 hours and 1100 hours, gs_{wp} increased asymptotically with radiation, until a maximum gs_{wp} was reached when radiation exceeded 1.5–2.0 MJ m^{-2} h^{-1} (Fig. 4*b*). The relationship was common across the three groups of days with VPD_{max} > 2 kPa, for which this threshold was reached between 0800 hours and 0900 hours (Fig. 3*b*). In contrast, days with VPD_{max} < 2 kPa never reached this threshold (Fig. 3*b*), indicating that gs_{wp} was always radiation-limited. Consequently, hourly TGLA for these days was linearly related with hourly radiation throughout the day (R^2 = 0.94, n = 13). For the three groups with VPD_{max} > 2 kPa, TGLA increased linearly with VPD, with the intercept indicating that TGLA was 0 g m^{-2} h^{-1} at a VPD of 0.2 kPa (Fig. 4*a*). Between 1100 hours and 1400 hours, gs_{wp} declined once VPD exceeded a threshold of 2.5 kPa (Fig. 4*c*), which was reached at ~1000 hours on days with VPD_{max} > 4 kPa and 1100 hours on days with VPD_{max} = 3–4 kPa (Fig. 3*a*). This decline in gs_{wp} partially offset the increase in TGLA with increasing VPD, resulting in a BP in the response of TGLA to VPD at 2.5 kPa (Fig. 4*a*). Between 1500 hours and 1800 hours, when radiation dropped below the threshold of 1.5–2.0 MJ m^{-2} h^{-1}, gs_{wp} again became radiation limited, with the response of gs_{wp} to declining radiation being independent of VPD_{max} (Fig. 4*d*).

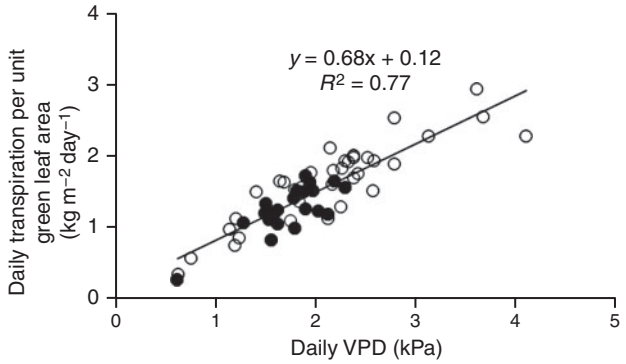

Fig. 2. Relationship between daily transpiration per unit of green leaf area (TGLA, kg m^{-2} day^{-1}), averaged across 54 well-watered sorghum plants, and daily vapour pressure deficit (VPD) (kPa) for Experiment 1 (Exp1, ○) and Experiment 2 (Exp2, ●).

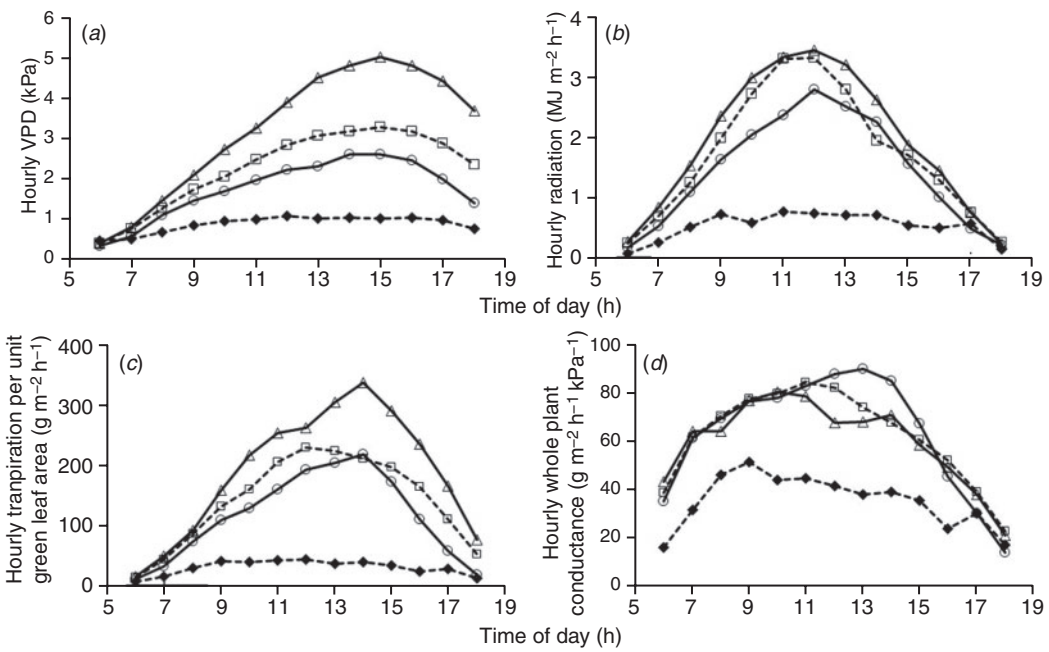

Fig. 3. Diurnal patterns for (*a*) hourly vapour pressure deficit (VPD) (kPa), (*b*) hourly radiation (MJ m^{-2} h^{-1}), (*c*) hourly transpiration per unit of green leaf area (TGLA, g m^{-2} h^{-1}) and (*d*) hourly whole-plant stomatal conductance (gs_{wp} (TGLA ÷ VPD), g m^{-2} h^{-1} kPa^{-1}), averaged across 27 sorghum genotypes (in *c* and *d*) for days with maximum hourly VPD < 2 kPa (♦- - - ♦, 2 days), between 2 and 3 kPa (○—○, 6 days), between 3 and 4 kPa (□—□, 7 days), and >4 kPa (Δ—Δ, 4 days). Data are from Experiment 1.

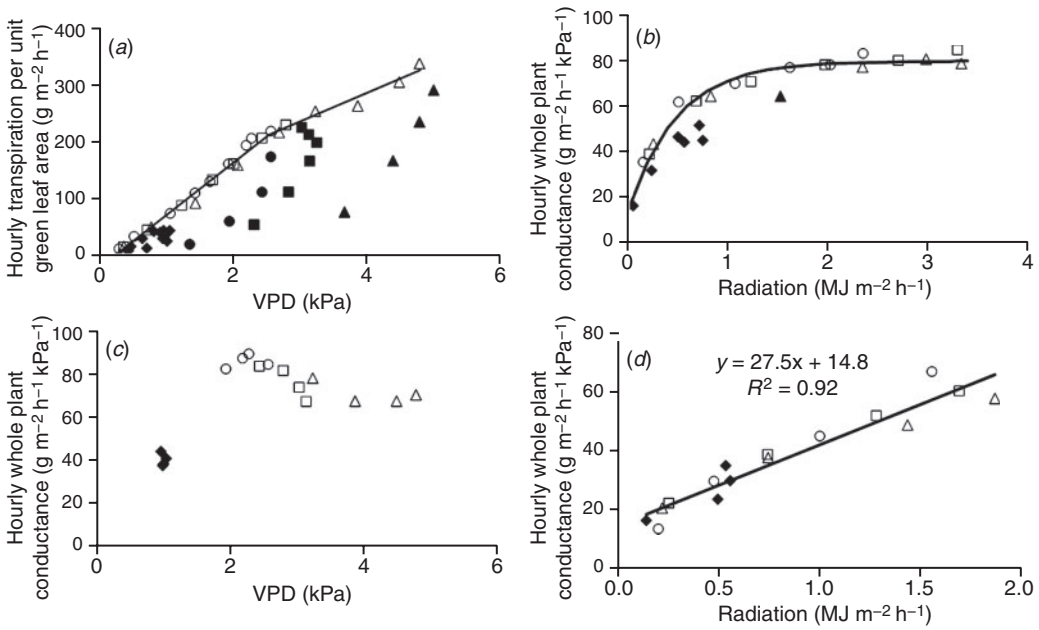

Fig. 4. Response of hourly transpiration and whole-plant stomatal conductance (gs_{wp}) to diurnal changes in vapour pressure deficit (VPD) and radiation averaged across all 27 sorghum genotypes measured on 19 days with varying maximum VPD in Experiment 1. (*a*) Hourly transpiration per unit of green leaf area (TGLA, g m^{-2} h^{-1}) vs hourly VPD, (*b*) hourly gs_{wp} (TGLA ÷ VPD, g m^{-2} h^{-1} kPa^{-1}) from 0600 hours to 1100 hours vs hourly radiation (RAD, MJ m^{-2} h^{-1}), (*c*) hourly gs_{wp} from 1100 hours to 1400 hours vs hourly VPD (kPa), and (*d*) hourly gs_{wp} from 1500 hours to 1800 hours vs hourly radiation (MJ m^{-2} h^{-1}). Data were grouped by days with maximum hourly VPD < 2 kPa (closed and filled diamonds), between 2 and 3 kPa (closed and filled circles), between 3 and 4 kPa (closed and filled squares) and >4 kPa (closed and filled triangles). Closed symbols in (*a*) have been excluded from the regression and represent data for days with maximum hourly VPD < 2kPa and data from 1500 hours onwards for days with maximum hourly VPD > 2 kPa (plus 1300 hours and 1400 hours for days with maximum VPD between 3 and 4 kPa). Closed symbols in (*b–d*) represent data for days with maximum hourly VPD < 2kPa, plus one additional outlier in (*b*). Closed symbols are excluded from the regression in (*b*) but were included in the regression in (*d*). Regression for (*a*): TGLA = –21.8 + 92.3 × VPD if VPD < 2.53 kPa; TGLA = –85.5 + 49.9 × VPD if VPD > 2.53 kPa. Regression for (*b*): gs_{wp} = 12.6 + (66.7 × (1 – exp(–2.03 × RAD))).

Genotypic differences in the response of TGLA to VPD

Average daily TGLA increased from 405 g m^{-2} day^{-1} for days with VPD_{max} < 2 kPa to 1547 g m^{-2} day^{-1} for days with VPD_{max} = 2–3 kPa, 1919 g m^{-2} day^{-1} for days with VPD_{max} = 3–4 kPa and 2530 g m^{-2} day^{-1} for days with VPD_{max} > 4 kPa. Genotypic differences in daily TGLA were consistent across these contrasting groups, as the daily TGLA of the 27 genotypes on days with VPD_{max} > 4 kPa was significantly (P < 0.0001) correlated with that in each of the other three groups, ranging from R^2 = 0.71 (VPD_{max} = 3–4 kPa) to R^2 = 0.74 (VPD_{max} <2 kPa) and R^2 = 0.78 (VPD_{max} = 2–3 kPa).

Establishing the relationship between TGLA and VPD in Fig. 4*a* (open symbols) for each individual genotype indicated that for 10 of the 27 genotypes, the bilinear regression did not converge. For six of these, this was associated with the absence of a clear BP, as the EMS of the linear relationship did not differ significantly from that of the bilinear relationship. In total, for 10 of the 27 genotypes no significant BP could be detected (Table 4).

The 17 genotypes with a significant BP differed significantly in their response of TGLA to VPD because the *F*-ratio between the EMS of a common model and the EMS across the 17 individual regressions was highly significant (P < 0.0001). However, the 17 genotypes did not differ significantly in their BP. A common BP of 2.49 kPa gave the best fit and the *F*-ratio of the EMS of this model compared with the individual regressions was not significant (F(374 : 357) = 1.01). In contrast, the genotypes differed significantly in their response of TGLA to VPD below the BP, as the fit of the model with the common slope giving the lowest EMS was still significantly worse than the fit of the individual regressions (F(374 : 357) = 1.31, P < 0.01). Based on their response of TGLA to VPD, the 17 genotypes with a bilinear relationship could be classified into five groups and the 10 genotypes with a linear response could be classified into three groups (Table 4). Four of these eight groups contained at least five genotypes, two groups had two genotypes and two groups contained only a single genotype. For each group with multiple genotypes, *F*-tests indicated no significant (P > 0.10) differences in parameter values among the genotypes within that group. The reduction in parameterisation from 27 genotypes to eight groups increased the error sum of squares by 34% (Table 4).

The presence of a BP was partly related to the different GENO-Groups identified in Table 1. Most of the B010054 and Tx7000 genotypes had a BP, as had both tall genotypes and two of the three B963676 genotypes. In total, 15 of the 18 genotypes across these four groups had a BP. In contrast, the check genotype, both SC170–6-8 genotypes and three of the four Tx642 genotypes did not have a BP, such that six of the seven

Table 4. Parameter estimates for the linear or bilinear response of transpiration per unit of green leaf area (TGLA) to vapour pressure deficit (VPD), including Slope 1 below the breakpoint (BP) VPD, the position of the BP VPD, Slope 2 beyond the BP, the error sum of squares (ESS), degrees of freedom (df) and the error mean squares (EMS)

Derived parameters include the threshold VPD at which TGLA = 0 g m^{-2} h^{-1} and the estimated TGLA at VPD = 2 kPa or 5 kPa. The top part of the table provides parameter estimates for individual genotypes; the bottom part provides the same for genotypes grouped with common parameter estimates. Genotypes are ordered by response type (bilinear and linear) and then by the estimated TGLA at VPD = 5 kPa. Groups are classified into low, medium and high TGLA, based on the TGLA at VPD = 2 and 5 kPa. BL, bilinear; LIN, linear

Genotype name	Genotype ID	Group	Slope 1 (g m^{-2} h^{-1} kPa^{-1})	VPD BP (kPa)	Slope 2 (g m^{-2} h^{-1} kPa^{-1})	VPD threshold (kPa)	TGLA at VPD = 2 kPa (g m^{-2} h^{-1})	TGLA at VPD = 5 kPa (g m^{-2} h^{-1})	ESS	df	EMS
Parameter estimates for individual genotypes											
LR9198	15	BL1	75.1	2.49	42.1	0.20	135	277	1354	21	64
A1*F_B010054/F4_R08512–35	2	BL2	109.7	2.43	16.4	0.26	190	280	8445	21	402
NIL6085–9	20	BL3	87.3	2.51	36.8	0.25	153	289	1372	21	65
Sureno	27	BL3	90.7	2.46	37.9	0.28	156	294	2026	21	96
FF_SC35–14E	13	BL3	76.3	2.60	45.2	0.14	142	297	1825	21	87
NIL2290–19	18	BL3	90.3	2.43	38.8	0.22	161	300	1457	21	69
NIL6078–1	19	BL3	86.7	2.63	44.2	0.27	150	309	1609	21	77
NIL2219–3	17	BL4	101.2	2.73	25.0	0.16	187	317	2480	21	118
A1*F_B010054/F5_R08341–8	5	BL4	100.7	2.46	45.0	0.24	178	338	5229	21	249
RTAM422	24	BL4	86.7	2.79	52.1	0.15	160	344	1979	21	94
A1*F_B010054/F4_R08512–79	3	BL4	104.6	2.49	50.4	0.26	182	360	3488	21	166
FF_B010054	11	BL4	103.6	2.48	55.5	0.26	180	369	4379	21	209
A1*F_B010054/F5_R07536–22–1-2	4	BL5	122.1	2.37	44.4	0.23	216	378	3483	21	166
A1*F_B010054/F4_R07528–91–1-1	1	BL5	108.0	3.05	37.5	0.20	195	381	1981	21	94
QL12/B963676	22	BL5	109.4	2.42	60.6	0.23	193	396	2872	21	137
A1*F_B963676/R931945–2-2	7	BL5	113.0	2.43	59.9	0.22	202	404	3925	21	187
Karper 669	14	BL5	110.0	3.23	58.7	0.26	192	431	3555	21	169
SC170–6-8	26	LIN1	52.8	–	–	0.04	104	262	5193	23	226
FF_RTx430	12	LIN1	62.6	–	–	0.07	121	309	3676	23	160
RTx7000	25	LIN2	72.2	–	–	0.03	142	359	8891	23	387
MR-Buster	16	LIN2	76.8	–	–	0.13	144	374	9731	23	423
B963676	9	LIN3	87.7	–	–	0.20	158	421	12 747	23	554
BTx642 (B35)	10	LIN3	87.3	–	–	0.16	161	423	4577	23	199
R931945–2-2	23	LIN3	83.8	–	–	0.06	163	414	4253	23	185
A1*F_B010054/F9_R04377–31	6	LIN3	88.2	–	–	–0.01	177	442	24 640	23	1071
B923296	8	LIN3	91.2	–	–	0.04	179	452	9569	23	416
QL12	21	LIN3	89.1	–	–	–0.11	188	455	6117	23	266
Total									14 0854	587	240
Parameter estimates for groups											
Low TGLA											
12, 26	–	LIN1	57.7	–	–	0.06	112	285	14 213	48	296
15	–	BL1	75.1	2.49	42.1	0.20	135	277	1354	22	62
13, 18, 19, 20, 27	–	BL3	86.5	2.49	41.0	0.23	153	298	11809	122	97
Medium TGLA	–	–	–								
16, 25	–	LIN2	74.5	–	–	0.08	143	366	19 027	48	396
2	–	BL2	108.4	2.49	14.3	0.26	189	278	8503	22	386
3, 5, 11, 17, 24	–	BL4	100.2	2.49	47.8	0.22	178	347	27294	122	224
High TGLA											
6, 8, 9, 10, 21, 23	–	LIN3	87.9	–	–	0.06	171	434	80 794	148	546
1, 4, 7, 14, 22	–	BL5	113.0	2.49	58.5	0.23	200	402	25 884	122	212
Total									188 878	654	289

genotypes in these groups did not have a BP. One of the two QL12 genotypes also had a BP.

The reduction in TGLA at high VPD that was associated with the presence of a BP could potentially affect TE. To determine any such effect, TE_{wp} in Exp1 was plotted against the response of TGLA to VPD at low VPD (Slope 1 in Table 4), using average data for each of the eight GENO-Groups from Table 1. For the three GENO-Groups for which most genotypes did not have a BP, there was a negative trend between TE_{wp} and Slope 1 (open symbols in Fig. 5), such that groups with high TGLA tended to have low TE_{wp}. The QL12 group fitted the same relationship. However, for three of the four groups for which most genotypes had a BP, the observed TE_{wp} was located well above this relationship, indicating that the presence of a BP may have had a positive effect on TE_{wp}. The main exception was the Tx7000 group, which fitted the same relationship as the B010054, Tx642 and check groups, even though most Tx7000 genotypes had a BP.

Based on the response of TGLA to VPD, the genotype groups in Table 4 could be classified into those with low (Linear (LIN) 1, Bilinear (BL) 1, BL3), medium (LIN2, BL2, BL4) and high (LIN3, BL5) TGLA. Group BL2 (A1*F_B010054/F4_R08512–35) had the most distinct behaviour, as it combined high TGLA at low VPD with a low TGLA at high VPD. The threshold VPD below which there was no transpiration was ~0.20–0.25kPa for genotype groups with a bilinear response

(Table 4). The generally lower threshold for groups with a linear response (0.06–0.08 kPa) was likely to be an artefact of fitting a linear relationship through observations that had a nonsignificant tendency for a BP. There were some consistent genetic effects on TGLA at high VPD. SC170–6-8 and the related genotype FF_Tx430 both had low TGLA, whereas R931945–2-2, B963676, QL12, their hybrids (A1*F_B963676/R931945–2-2 and QL12/B963676) and Karper 669 (related to QL12) all had high TGLA. The six genotypes belonging to the RTx7000 group (Table 1) all had low or medium TGLA, whereas FF_B010054 and all but one of its hybrids had either high or medium TGLA. However, FF_SC35–14E had low TGLA (BL3), whereas the closely related BTx642 had high TGLA (LIN3).

Relationship of plant-level transpiration rates with gs_{leaf}

Across all 36 runs for gs_{leaf}, the stomatal water vapour flux, defined as the product of gs_{leaf} and ambient VPD, was highly correlated with the average hourly plant-level TGLA that most closely coincided with the period it took to conduct each run

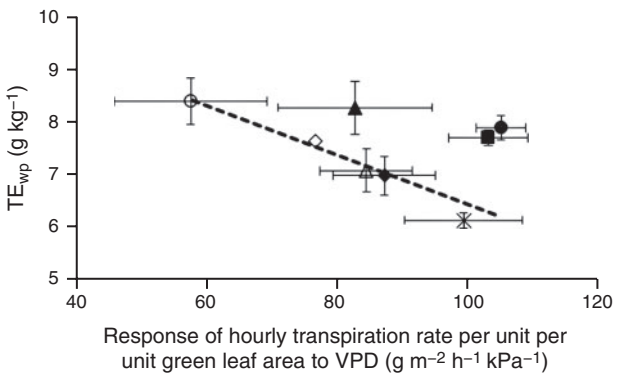

Fig. 5. Whole-plant transpiration efficiency (TE_{wp}, g kg^{-1}) in Experiment 1 vs the response of hourly transpiration rate per unit of green leaf area (TGLA) to vapour pressure deficit (Slope 1 in Table 4), averaged for the eight sorghum GENO-Groups in Table 1: B010054 (●), B963676 (■), Tx642 (Δ), check (◇), SC170–6-8 (○), QL12 (*), Tx7000 (♦), and tall 2dwarf (▲). Open symbols represent groups for which more than half of the genotypes had a linear response; closed symbols represent groups for which more than half of the genotypes had a bilinear response; * indicates a group with equal numbers. Bars represent s.e. The dotted line represents the linear relationship between TE_{wp} and TGLA for the three groups for which more than half of the genotypes had a linear response (open symbols).

Table 5. ANOVA for leaf-level stomatal conductance, using nine runs with the highest average conductance as replications

All data were collected between 1100 hours and 1500 hours across five different days (three runs each on of 19 and 20 November 2015; one run each on of 30 November 2015, and 8 and 11 December 2015). *, $P < 0.05$; ***, $P < 0.0001$; TGLA, transpiration per unit of green leaf area

Source	Degrees of freedom	Sum of squares	*F*-value	Significance
Genotype	26	943 076	3.76	***
TGLA-Group	2	259 453	15.15	***
Genotype(TGLA-Group)	24	683 623	3.33	***
Replication	8	153047	2.23	*
Error	208	1 781 222	–	–

(Fig. 6). A curvilinear relationship fitted the observed data well ($R^2 = 0.97$, $n = 36$, $P < 0.0001$), indicating that the relationship approached linearity at higher transpiration rates.

Genotypic differences in maximum gs_{leaf} were highly significant ($P < 0.0001$, Tables 5 and 6) and ranged from 381 mmol m^{-2} s^{-1} (SC170–6-8) to 611 mmol m^{-2} s^{-1} (R931945–2-2, Fig. 7). In order to link plant-level TGLA to gs_{leaf}, the average

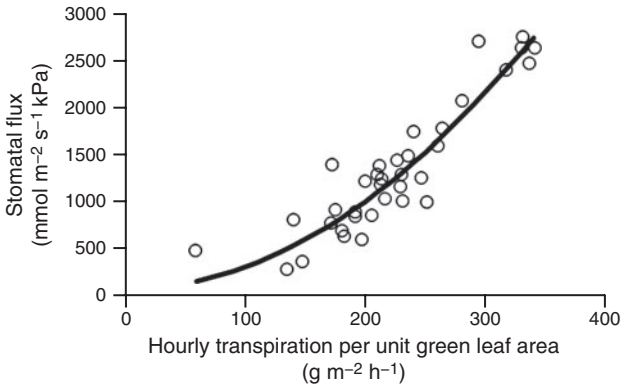

Fig. 6. Hourly transpiration per unit of green leaf area (TGLA) vs stomatal flux, which is the product of leaf-level stomatal conductance and vapour pressure deficit. Each data point is the mean of measurements across 27 sorghum genotypes, which took 1 h to complete. Conductance measurements were taken in cloudless conditions at different times of the day over 7 days between 19 November 2015 and 11 December 2015 in Experiment 1. Regression equation ($R^2 = 0.97$, $n = 36$, $P < 0.0001$)

$$flux = 12.23 \times \frac{\exp(0.00733 \times (TGLA - 251.4))}{1 + \exp(0.00733 \times (TGLA - 251.4))} \times TGLA$$

Table 6. Leaf-level stomatal conductance (*gs*), using nine runs with the highest average conductance as replications, and specific leaf N (SLN) using the average SLN of the top five main shoot leaves, which were measured on 9 and 10 December 2015 in Experiment 1

All *gs* data were collected between 1100 hours and 1500 hours across five different days (three runs each on of 19 and 20 November 2015; one run each on 30 November 2015, and 8 and 11 December 2015). Genotypes were classified into three (low, medium and high) groups for transpiration per unit of green leaf area (TGLA) based on Table 4. Means followed by a different letter differ at $P < 0.05$, according to Tukey's test for pairwise comparisons; letters in bold differ at $P < 0.0001$. LIN, linear; BL, bilinear

Group	Number of genotypes	*gs* mmol m^{-2} sec^{-1}	SLN g m^{-2}
Means for TGLA groups			
Low (LIN1, BL1, BL3)	8	463**a**	–
Medium (LIN2, BL2, BL4)	8	504b	–
High (LIN3, BL5)	11	542**c**	–
Means for GENO-Groups			
B010054	7	–	2.35a
QL12	2	–	2.28ab
Tx642	4	–	2.26ab
Check	1	–	2.24abc
B963676	3	–	2.18bc
SC170–6-8	2	–	2.04cd
Tx7000	6	–	1.95d
Tall 2dwarf	2	–	1.76e

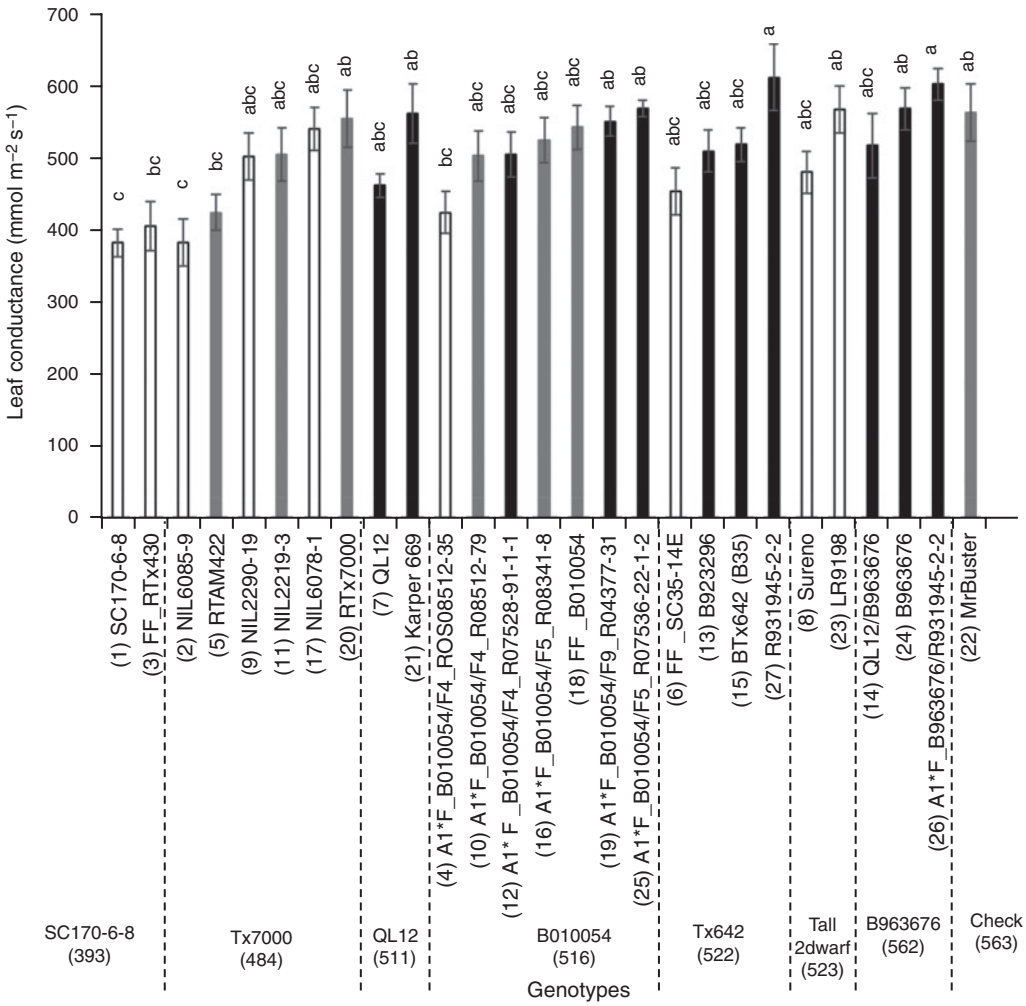

Fig. 7. Genotypic variation in leaf stomatal conductance (gs_{leaf}) plus s.e. across nine runs for sorghum genotypes with low (white), medium (grey) and high (black) transpiration per unit of green leaf area (TGLA) based on Table 4. Genotypes are grouped according to GENO-Group (Table 1). GENO-Groups are sorted in ascending order for average gs_{leaf} and genotypes are similarly sorted within each GENO-Group. Means with the same letter are not significantly ($P < 0.05$) different according to Tukey's test for pairwise comparisons. Values in brackets preceding the genotype name represent the ranking of genotypes for gs_{leaf} with (1) the lowest and (27) the highest gs_{leaf}. GENO-Group names plus the average gs_{leaf} (in brackets) are listed along the bottom of the figure. Data are from Experiment 1.

gs_{leaf} of genotypes with low, medium or high TGLA (Table 4) was calculated. Genotypes with low TGLA on average had significantly lower gs_{leaf} than those with high TGLA (463 vs 542 mmol m^{-2} s^{-1}, $P < 0.0001$; Table 6). The genotypes with medium TGLA on average had intermediate gs_{leaf} (504 mmol m^{-2} s^{-1}) and the difference with both the other two groups was significant at $P < 0.05$ (Table 6). Across genotypes, SC170–6-8, FF_Tx430 and NIL6085–9 combined low gs_{leaf} with low TGLA, whereas B963676, R931945–2-2, and their hybrid combined high gs_{leaf} with high TGLA (Fig. 7).

Role of transpiration, SLN and P_c in determining genotypic differences in TE

Daily TGLA per genotype, averaged for the 4 days with VPD_{max} > 4 kPa, was only poorly negatively associated with TE_{wp} (Fig. 8). As genotypic differences in daily TGLA were well conserved across days with contrasting VPD environments, a similar weak relationship was observed for TGLA on days with lower VPD_{max}. This implies that genotypes with higher TE_{wp} for a given TGLA may have greater P_c than those with low TE_{wp}. As TE at the leaf level is the ratio between photosynthetic rate and transpiration rate, the product of TE_{wp} and TGLA in Fig. 8 provides an estimate of P_c. Across genotypes, P_c was significantly positively associated with SLN (Fig. 9). This was particularly the case if the two tall genotypes were excluded from the regression ($R^2 = 0.52$; $n = 25$), as the increased stem N demand of these genotypes may have caused a dilution of SLN compared with the shorter 3dwarf genotypes (van Oosterom *et al*. 2010). GENO-Groups explained most of the observed genotypic variation in SLN (Table 7). Genotypes located in the top half of the data envelope in Fig. 8, such as those in the B010054,

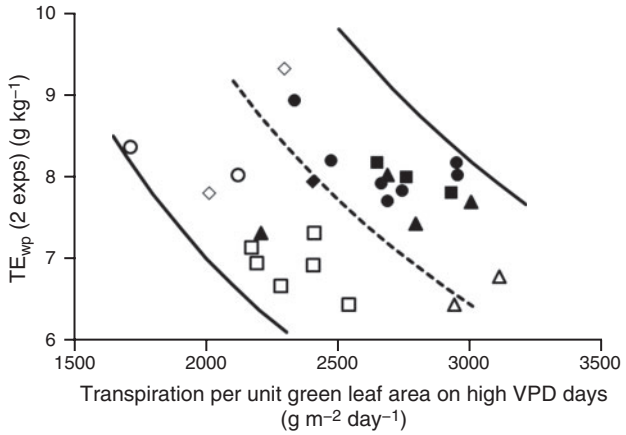

Fig. 8. Whole-plant transpiration efficiency (TE_{wp}, g kg^{-1}), averaged across two experiments, vs daily transpiration per unit of green leaf area (TGLA, g m^{-2} day^{-1}), averaged across 4 days with maximum vapour pressure deficit (VPD) > 4 kPa in Experiment 1 for 27 sorghum genotypes, classified into groups based on common genetics according to Table 1: B010054 (●), B963676 (■), Tx642 (▲), check (♦), SC170–6-8 (○), QL12 (Δ), Tx7000 (□), and tall 2dwarf (◇). The solid lines represent the upper and lower boundaries of the data envelop and the dotted line represents the midway point. All lines represent different levels of photosynthetic capacity, which is defined as the product of TE_{wp} and TGLA.

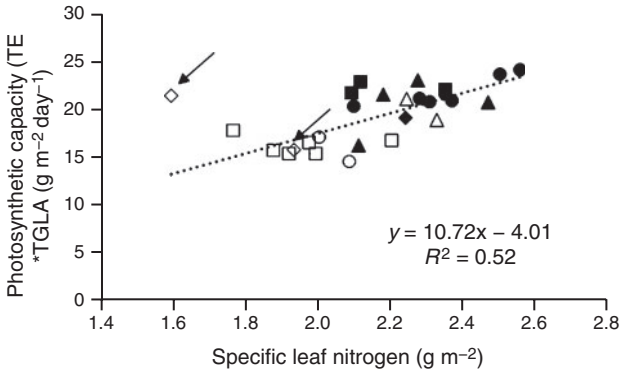

Fig. 9. Photosynthetic capacity, estimated from the product of whole-plant transpiration efficiency (TE_{wp}) and daily transpiration per unit green leaf area (TGLA) vs specific leaf N (SLN, g m^{-2}) for 27 genotypes, classified into groups based on common genetics according to Table 1: B010054 (●), B963676 (■), Tx642 (▲), check (♦), SC170–6-8 (○), QL12 (Δ), Tx7000 (□) and tall 2dwarf (◇). The two tall 2dwarf genotypes (indicated by the arrows) have been excluded from the regression, as taller stature tends to reduce SLN (van Oosterom *et al.* 2010). Data for TE_{wp} and TGLA are the same as in Fig. 8; SLN was the average SLN of the top five leaves of the main shoot of each plant, based on leaf chlorophyll measurements taken on 9 and 10 December 2015.

Table 7. ANOVA for specific leaf N (SLN) using the average SLN of the top five main shoot leaves, measured on 9 and 10 December 2015 in Experiment 1

***, $P < 0.0001$; ns = not significant ($P > 0.10$)

Source	Degrees of freedom	Sum of squares	*F*-value	Significance
Genotype	26	2.672	10.69	***
GENO-Group	7	1.841	27.35	***
Genotype(GENO-Group)	19	0.831	4.55	***
Replication	2	0.017	1.74	ns
Error	26	2.939	–	–

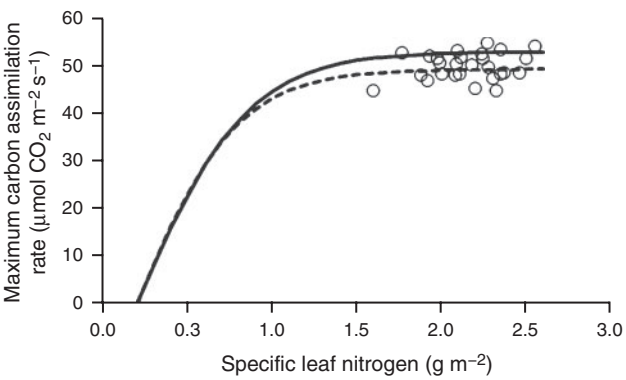

Fig. 10. Maximum carbon assimilation rate (A_{max}) in Experiment 1 vs specific leaf N (SLN, g m^{-2}). The lines represent relationships reported by Muchow and Sinclair (1994) for sorghum grown in Katherine, Australia (——) and Gainesville, FL, USA (- - -). SLN data are the same as in Fig. 9.

B963676 and BTx642 GENO-Groups, generally had above-average P_c and SLN (Fig. 9, Table 6). In contrast, genotypes located in the bottom half of Fig. 8 generally belonged to the SC170–6-8 and Tx7000 GENO-Groups, and had below average P_c and SLN (Fig. 9, Table 6). However, SC170–6-8 and the related genotype FF_RTx430 both had low TGLA, resulting in above-average TE_{wp}, whereas the low TE_{wp} of RTx7000, its four NILs and TAM422 was the result of a higher TGLA. The low TE_{wp} of QL12 and Karper 669 was associated with average P_c but very high TGLA (Fig. 8).

Despite this positive association between SLN and P_c, increased SLN did not result in higher A_{max} (Fig. 10). This was because SLN was, in general, well above the critical value of ~1.0–1.2 g m^{-2}, below which A_{max} is adversely affected by SLN (Muchow and Sinclair 1994). Values for A_{max} ranged from 44.4 µmol CO_2 m^{-2} s^{-1} for QL12 to 54.4 µmol CO_2 m^{-2} s^{-1} for B923296, which was consistent with values reported by Muchow and Sinclair (1994) for field-grown sorghum in the United States and Australia (Fig. 10). Although genotypic differences in A_{max} were significant at $P = 0.05$, A_{max} was significantly positively associated with maximum gs_{leaf} ($R^2 = 0.33$, $P < 0.01$).

The minor genotype × experiment interaction that was observed for TE_{wp} (Table 2) could partly be explained by gs_{leaf}. Across genotypes, the difference in TE_{wp} between Exp1 and Exp2 was significantly associated with gs_{leaf} ($R^2 = 0.36$, $n = 27$, $P = 0.0010$), particularly if the genotype with a large difference in TE_{wp} (FF_SC35–14E, Fig. 1) was excluded from the regression ($R^2 = 0.55$, $n = 26$, $P < 0.0001$). Genotypes with high gs_{leaf} tended to have the greatest decline in TE_{wp} under the high VPD conditions of Exp1 compared with Exp2.

Discussion

Our results showed significant genotypic variation in TE_{wp} for sorghum under fully irrigated conditions, which was only partly linked to genotypic differences in the response of plant-level transpiration rates to VPD. Plant-level transpiration rates were closely associated with gs_{leaf}. Hence, differences in TE_{wp} were an emergent consequence of differences in the underpinning physiological mechanisms that operated at the organ level. Genotypes with a common genetic background generally had similar combinations of transpiration rates and P_c. This could facilitate the identification of germplasm with attributes that increase TE_{wp} under specific environmental conditions.

VPD and radiation could explain most of the environmental effects on transpiration rates (TGLA) under fully irrigated conditions

The close association between daily TGLA and VPD (Fig. 2) has been well documented for a range of crops, including sorghum (Gholipoor *et al*. 2010), pearl millet (Kholová *et al*. 2010), maize (Gholipoor *et al*. 2013; Sunita *et al*. 2014) and soybean (*Glycine max* (L.) Merr.) (Sadok and Sinclair 2009). The observation that the relationship was robust across both experiments and days within each experiment (Fig. 2) indicates that the time of year and development stage of the crop, respectively, had no substantial effect on the response of daily TGLA to VPD. However, the observed plant-level TGLA is a function of gs_{wp} and VPD, and our results indicate that gs_{wp} can be limited by both low radiation and high VPD (Fig. 4).

The effects of radiation and VPD on gs_{wp} allowed the development of a framework that could explain most of the observed environmental effects on diurnal TGLA patterns (Fig. 4). On overcast days with low radiation and $VPD_{max} < 2$ kPa, TGLA was predominantly radiation-limited, as radiation never exceeded the threshold of 1.5–2.0 MJ m^{-2} h^{-1} (Figs 3 and 4). This radiation threshold was similar to the threshold of ~1000 μmol m^{-2} s^{-1} (or ~1.6 MJ m^{-2} h^{-1}) reported for gs_{leaf} in wheat (*Triticum aestivum* L.) (Yu *et al*. 2004). For days with $VPD_{max} >$ 2 kPa, the radiation threshold was reached early in the morning. This resulted in fully open stomata that could maintain the CO_2 concentration inside the stomatal cavity at a ratio that was constant to the ambient CO_2 concentration, in order to optimise tradeoffs between photosynthesis and conductance (Wong *et al*. 1979). Consequently, the observed TGLA increased linearly with VPD until a threshold of ~2.5 kPa, beyond which the slope of the relationship was reduced (Fig. 4*a*, *c*). This decline was probably caused by decreased *gs* in response to high VPD (Fig. 3*d*), which partly offset the effect of increased VPD on TGLA and resulted in a slower rate of response of TGLA to increasing VPD above the threshold compared with VPD below the threshold (Table 4, Fig. 4*a*). Even under fully irrigated conditions, stomata may close to maintain constant leaf water potential once the plant reaches maximum hydraulic conductivity. Limitations to plant hydraulic conductivity have been related to either leaf (Brodribb and Jordan 2008) or root hydraulic conductance (Sadok and Sinclair 2010). The threshold VPD of 2.49 kPa (Table 4) was slightly higher than the value of 2.26 kPa reported for sorghum (Gholipoor *et al*. 2010), 2.4 kPa for pearl millet (Kholová *et al*. 2016) and 2.1–2.2 kPa for maize (Gholipoor *et al*. 2013; Sunita *et al*. 2014). These differences might be related to the larger pot size and thus the likely greater root volume, leading to increased root hydraulic capacity in our study. The threshold VPD may also be affected by temperature, either via direct temperature effects on hydraulic limitations (Yang *et al*. 2012) or indirectly via the effects of temperature on photosynthetic rate, which, in turn, can affect *gs* (Duursma *et al*. 2014).

In the afternoon, the common relationship between gs_{wp} and radiation across days with contrasting VPD (Fig. 4*d*) indicated that gs_{wp} became radiation-limited once radiation dropped below the threshold of 1.5–2.0 MJ m^{-2} h^{-1}. However, gs_{wp} tended to be lower in the afternoon than in the morning for similar radiation levels (Fig. 4*b*, *d*), indicating that stomata remained more closed in the afternoon (Fig. 3*c*). This was not just a consequence of stomatal closure in response to VPD levels beyond the VPD threshold, as this response was also observed on days with low VPD_{max} (Fig. 3*d*). Such diurnal changes to *gs* have been observed before, although the exact drivers for this are still unknown (de Dios *et al*. 2012; Matthews *et al*. 2017).

Associations between plant-level transpiration and gs_{leaf} facilitates the development of phenotyping methods

Significant genotypic differences in the response of TGLA to VPD were observed (Table 4). This probably represented differences in stomatal properties, because leaf-level stomatal flux ($gs_{leaf} \times$ VPD) was positively correlated with TGLA (Fig. 6). In addition, genotypes with low, medium or high TGLA showed significant differences in gs_{leaf}, with genotypes with high TGLA having the greatest gs_{leaf} (Table 6). This concurs with observations in sorghum that genotypic differences in transpiration rates per unit of leaf area are linked to the density of abaxial stomata (Muchow and Sinclair 1989; Borrell *et al*. 2014*a*). Moreover, among the four stay-green NILs, the one with the greatest TGLA (NIL2219–3, Table 4) was also the one with the highest stomatal index (Borrell *et al*. 2014*a*). The close relationship between plant-level TGLA and organ-level gs_{leaf} (Fig. 6) indicates that under field conditions, where plant or canopy transpiration rates are hard to measure, estimates of transpiration rates could be obtained by using a porometer, provided that the measurements are done during the middle of the day under clear skies and provided that any effects of VPD and radiation on gs_{leaf} are taken into account. This is consistent with observations by Rebetzke *et al*. (2003) that field measurements of gs_{leaf} in wheat showed the most significant genetic differences if the measurements were conducted during the middle of the day. Dissection of gs_{leaf} into the physiological mechanisms that underpin its response to environmental conditions could allow the development of phenotyping methods that can be used to select for its genetic control (Rebetzke *et al*. 2003). Our results (Fig. 6) indicate this can potentially translate to the identification of germplasm with desirable whole-plant TGLA.

Control of TE_{wp} by both TGLA and photosynthetic properties has implications for crop adaptation

Significant genotypic differences in TE_{wp} were observed (Tables 2 and 3). The average TE_{wp} across the two

experiments was 7.7 g kg^{-1}, with a range of 6.4–9.3 g kg^{-1}. This was comparable to the range reported in earlier studies (Hammer *et al*. 1997; Mortlock and Hammer 2000; Xin *et al*. 2009; Vadez *et al*. 2011), indicating that the 27 genotypes adequately sampled the known variation in TE_{wp} in sorghum. Previous reports have associated such variation in sorghum with differences in P_c and biomass production (Hammer *et al*. 1997; Xin *et al*. 2009; Vadez *et al*. 2011), transpiration rates (Mortlock and Hammer 2000) and leakage of CO_2 from the bundle sheath (Henderson *et al*. 1998). Photosynthesis and transpiration are intrinsically linked through stomatal gas exchange, but the slower diffusion rate of CO_2 through stomata compared with water vapour (von Caemmerer and Farquhar 1981) means that increased conductance is likely to reduce TE, as has been observed in wheat (Li *et al*. 2017). Consistent with this, genotypes with high gs_{leaf} tended to have a greater decline in TE_{wp} under the high VPD conditions of Exp1 compared with Exp2 than genotypes with low gs_{leaf}, such that gs_{leaf} under high VPD conditions could explain some of the genotype × environment interactions for TE_{wp}. This reflects observations that under high VPD, when water use is least efficient, high TGLA can reduce TE (Mortlock and Hammer 2000; Sinclair *et al*. 2005; Ratnakumar *et al*. 2009). Low gs_{leaf} and the associated low TGLA can thus minimise any adverse effects of high VPD on TE_{wp}.

In addition, TE_{wp} can be increased by changes in the biochemistry of photosynthesis that would increase photosynthetic rates but are not driven by *gs*. Within this context, the observed differences in TE_{wp} for genotypes with similar TGLA (Fig. 8) indicated potential genotypic differences in P_c, which were significantly positively associated with leaf chlorophyll (SPAD) and hence SLN (Fig. 9). Increased leaf chlorophyll could potentially increase Rubisco carboxylation capacity and electron transport capacity, as observed for rice when SLN increased from 1.0 to 1.4 g m^{-2} (Gu *et al*. 2012). This could increase TE_{wp}. However, higher SLN did not translate into increased A_{max} (Fig. 10) because in the absence of N deficiency, SLN was at luxury levels where A_{max} was not adversely affected by a decline in SLN (Muchow and Sinclair 1994). The observation that genotypic differences in TE_{wp} were associated with contrasting combinations of TGLA and P_c that were consistent across GENO-Groups indicates that these differences in TE_{wp} were likely to be partly associated with differences in the underpinning crop physiological processes and genetics.

The low TE_{wp} of QL12 and the related genotype Karper 669 (Table 3, Fig. 1) was associated with high TGLA (Table 4) and average P_c (Fig. 8). High conductance is generally associated with low TE because of the slower diffusion rate of CO_2 compared with water vapour (von Caemmerer and Farquhar 1981); a low TE for QL12 has been reported previously (Mortlock and Hammer 2000; Kulathunga 2013). The combination of high TGLA, average P_c and hence low TE_{wp} is not conducive to water saving and is therefore best suited to well-watered conditions where crop production is radiation-limited (Sinclair *et al*. 2005; Hammer *et al*. 2010).

Genotypes SC170–6-8 and FF_RTx430 both had above-average TE_{wp} (Table 3, Fig. 1). This was associated with low TGLA (Table 4) and low P_c (Fig. 8), which were a consequence of low gs_{leaf} (Fig. 7). In fact, gs_{leaf} was sufficiently low that despite the absence of a BP in the response of TGLA to VPD in these genotypes (LIN1, Table 4), TGLA at high VPD was as low as that of low-TGLA genotypes with a BP (BL1 and BL3 in Table 4). The low P_c was also reflected in low SLN (Table 6). The high TE_{wp} of SC170–6-8 was consistent with the high TE of SC170–6-17 and BTx623, which are both related to SC170–6-8 (Xin *et al*. 2008). At high VPD, when water use is inefficient, low gs_{leaf}, and hence low TGLA, can delay onset of drought stress in water-limited environments and thus increase grain yield (Turner 2004; Sinclair *et al*. 2005; Vadez *et al*. 2014; Messina *et al*. 2015). However, there is likely to be a yield penalty under well-watered conditions when crop production is radiation-limited and restricted gas exchange can limit photosynthetic rates (Sinclair *et al*. 2005; Messina *et al*. 2015). Thus the combination of low gs_{leaf} and above-average TE_{wp} provides a specific adaptation to drought-stressed environments with high VPD.

The TE_{wp} of RTx7000 and its related genotypes was similar to that of QL12 and Karper 669 across the two experiments (Table 3, Fig. 8), despite RTx7000 genotypes having lower TGLA (Table 4, Fig. 8). This lower TGLA of RTx7000 genotypes resulted in slightly higher TE_{wp} than that in QL12 under the higher VPD conditions in Exp1 (Fig. 5) but slightly lower TE_{wp} in Exp2, consistent with the effect of TGLA on the genotype × environment interaction for TE_{wp}. These results imply a low P_c, consistent with the generally below-average gs_{leaf} (Fig. 7) and low SLN (Table 6) of these genotypes. The low TE_{wp} of RTx7000 confirms the findings from previous experiments (Hammer *et al*. 1997; Balota *et al*. 2008; Kulathunga 2013). The observation that the four NILs generally had similar TE_{wp} and TGLA to RTx7000, despite each carrying a *Stg* quantitative trait locus (Table 1, Borrell *et al*. 2014*a*) indicates that expression of stay-green associated with these quantitative trait loci is not substantially related to TE_{wp} and TGLA. Instead, the improved stay-green and grain yield of these NILs under drought stress compared with RTx7000 is associated with smaller canopy size, which reduces preanthesis water use (George-Jaeggli *et al*. 2017) and increases postanthesis water availability under end-of-season drought stress (Borrell *et al*. 2014*a*; 2014*b*). Conversely, the relatively large leaf area of RTx7000 would partly offset the low P_c, which could account for its generally high grain yield under optimum growing conditions (e.g. Mahama *et al*. 2014). The below-average TGLA of these genotypes can provide an important mechanism for drought adaptation, similar to that of SC170–6-8 and FF_RTx430. However, the significantly higher grain yield of *Stg* NILs compared with RTx7000 under drought stress (Borrell *et al*. 2014*b*) highlights that low TGLA will only be useful under drought stress if it is not offset by large canopy size.

B963676 and B010054 germplasm and, to a lesser extent, genotypes associated with BTx642 (except FF_SC35–14E) generally had above-average TE_{wp} (Table 3, Fig. 8). The high TE_{wp} of B963676 has been reported previously (Kulathunga 2013). Combined with above-average TGLA, the above-average TE_{wp} implies high P_c, consistent with their above-average gs_{leaf} (Fig. 7). This combination of traits is well adapted to optimum growing conditions, where high P_c can maximise growth but where any associated increase in demand for water, combined

with high TGLA and high gs_{leaf}, are unlikely to cause drought stress.

However, the wide range in observed TE_{wp} makes it unlikely that these differences were solely a consequence of differences in gs_{leaf}. For example, the high TE_{wp} of Sureno may have been associated with its taller stature. Tall sorghum can have greater radiation use efficiency than short sorghum, although this effect does depend on the genetic context (Hammer *et al*. 2010; George-Jaeggli *et al*. 2013). Especially if we compare tall 2dwarf and short 3dwarf sorghums, which differ in height because of the effects of major dwarfing genes, TE_{wp} could also be affected by hormonal regulation, which can affect sink strength, leading to differences in biomass accumulation via C retention, independent of differences in SLN or potential leaf photosynthetic rates. C retention, for example, is affected by respiration. Dark respiration has been reported to increase with temperature from an average of ~14 μmol CO_2 kg^{-1} s^{-1} at 15°C to 50 μmol CO_2 kg^{-1} s^{-1} at 35°C, with significant genotypic differences in this response of dark respiration to temperature (Gerik and Eastin 1985). In addition, TE_{wp} can be affected by leakage of CO_2 from the bundle sheath (Henderson *et al*. 1998), although these authors did not observe significant genotypic differences for this trait in a limited set of sorghum germplasm.

Another explanation for the inability of differences in A_{max} to explain the range of observed TE_{wp} is that A_{max} is only measured on one small part of a well-lit leaf at single time points. The so-called 'patchiness' of stomatal responses across the surface of a leaf has been reported (Cardon *et al*. 1994), although the importance of this phenomenon in our study was unclear, as point measurements of stomatal flux ($gs_{leaf} \times$ VPD) were highly correlated with TGLA (Fig. 6). Nonetheless, uncertainty with regard to the extent to which differences in photosynthetic activity throughout the day, or by leaves further down in the canopy, contribute to whole-plant photosynthesis could lead to a misalignment between A_{max} and TE_{wp}.

Implications for breeding

Efficient use of water is an important selection criterion in the quest to enhance the adaptation of sorghum to dryland cropping systems and ensure the resilience of new sorghum genotypes to climate change. Simulation studies in sorghum (Sinclair *et al*. 2005) have shown that increased TE could be an emergent consequence of limiting maximum TGLA at high VPD. As a component trait of TE, TGLA is likely to be under simpler genetic control than TE itself and would thus exhibit fewer genotype × environment interactions. Nonetheless, the reported genotype × environment interactions for TE in sorghum are generally either nonsignificant or small compared with genotype and environmental effects (Mortlock and Hammer 2000; Sinclair *et al*. 2005; Xin *et al*. 2009; Vadez *et al*. 2011). Environmental factors generally affect component traits like TGLA, with the effects on TE being an emergent consequence of these relationships. Hence, selection for TGLA potentially provides better insights into environmental effects on grain yield and consequences on genotype × environment interactions for grain yield than selection for TE itself (Sinclair 2012). Although TGLA is ideally observed in automated lysimetry platforms (Vadez *et al*. 2015; Chenu *et al*. 2018), the highly significant cross-scale association between TGLA and gs_{leaf} (Fig. 6) indicates that porometers could be used in field conditions where TGLA is not easily measureable. Alternatively, infrared imaging can capture the genotypic differences in canopy temperature that are associated with differences in gs_{leaf} (Jerbi *et al*. 2015). Particularly if it is integrated with crop modelling and molecular biology, selection for TGLA could provide valuable insights into the implications of genotypic differences in TGLA for specific adaptation (Sinclair *et al*. 2005; Messina *et al*. 2011, 2015; Sinclair 2012).

The observation that genotypes with a common genetic background grouped together when their TE_{wp} was plotted against TGLA (Fig. 8) indicates potential genetic variation in P_c that could be exploited through selection. Differences in TE_{wp} at similar TGLA indicate that these differences in TE_{wp} were independent of gas exchange, implying an association with processes that determine either the maximum CO_2 assimilation rate (such as Rubisco activity, chloroplast electron transport rate or rates of substrate regeneration) or the slope of the response of the CO_2 assimilation rate to intercellular CO_2 (potentially mediated by plasma membrane aquaporins, carbonic anhydrase or phosphoenolpyruvate carboxylase activity) (von Caemmerer and Furbank 2016). Candidate genes for many of these processes, including genes related to C metabolism and access of CO_2 in the mesophyll cells, have been identified for C_4 photosynthesis (von Caemmerer and Furbank 2016). However, even if allelic variability for these traits exists across the germplasm used in this experiment, the effects of any such potential variability on grain yield depends largely on environmental conditions (von Caemmerer and Furbank 2016). Hence, photosynthetic simulation models that integrate across biological scales (Wu et al. 2019) provide a suitable tool to explore these effects in crop design studies (Hammer *et al*. 2014). This highlights the importance of the integrated, cross-disciplinary approach to trait selection advocated by Condon *et al*. (2004) and Chenu *et al*. (2018) in order to maximise the potential benefits of trait selection in terms of specific adaptations to environmental conditions.

Measurement of TE can be done relatively easily by using sealed containers and measuring total plant water use and dry mass (Xin *et al*. 2008). This provides an integrated measure of TE that covers the duration of the experiment but provides limited quantitative insights into the responses to environmental conditions (Sinclair 2012). Acquisition of such insights requires continual measurements of TGLA and environmental conditions over time, which requires automated weighing, watering and leaf area measurements if done on a large scale for phenotyping mapping populations (Vadez *et al*. 2015; Chenu *et al*. 2018). If such automated facilities are unavailable, large-scale screening of TE can also provide powerful phenotypic data on TE, particularly if recurrent checks and partially replicated row–column designs are used to account for any temporal and spatial variability in environmental conditions that could affect TE across and within experimental runs (Chenu *et al*. 2018).

Conflicts of interest

The authors declare no conflicts of interest.

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