

RESEARCH PAPER

# Restriction of transpiration rate under high vapour pressure deficit and non-limiting water conditions is important for terminal drought tolerance in cowpea

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## Keywords

Canopy temperature; growth; thermal imagery; transpiration.

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## ABSTRACT

Drought stress is a major constraint on cowpea productivity, since the crop is grown under warm conditions on sandy soils having low water-holding capacity. For enhanced performance of crops facing terminal drought stress, like cowpea, water-saving strategies are crucial. In this work, the growth and transpiration rate (TR) of 40 cowpea genotypes with contrasting response to terminal drought were measured under well-watered conditions across different vapour pressure deficits (VPD) to investigate whether tolerant and sensitive genotypes differ in their control of leaf water loss. A method is presented to indirectly assess TR through canopy temperature (CT) and the index of canopy conductance (Ig). Overall, plants developed larger leaf area under low than under high VPD, and there was a consistent trend of lower plant biomass in tolerant genotypes. Substantial differences were recorded among genotypes in TR response to VPD, with tolerant genotypes having significantly lower TR than sensitive ones, especially at times with the highest VPD. Genotypes differed in TR response to increasing VPD, with some tolerant genotypes exhibiting a clear VPD breakpoint at about 2.25 kPa, above which there was very little increase in TR. In contrast, sensitive genotypes presented a linear increase in TR as VPD increased, and the same pattern was found in some tolerant lines, but with a smaller slope. CT, estimated with thermal imagery, correlated well with TR and Ig and could therefore be used as proxy for TR. These results indicate that control of water loss discriminated between tolerant and sensitive genotypes and may, therefore, be a reliable indicator of terminal drought stress tolerance. The water-saving characteristics of some genotypes are hypothesised to leave more soil water for pod filling, which is crucial for terminal drought adaptation.

## INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important food legume and a major source of dietary protein and minerals for the rural and urban masses in the tropics and subtropics, where drought stress seriously affects its productivity (Singh & Awika 2010). Water deficits usually cause enormous decreases in yield of cowpea due to its production on soils with low water-holding capacity and in environments with low, unreliable and erratic rainfall (Ehlers & Hall 1997; Hall *et al.* 2003). Although cowpea is considered comparatively drought-tolerant and productive in areas of marginal soil fertility and water-limited conditions (Sanginga *et al.* 2000), there are significant differences in response to drought stress among genotypes (Diop *et al.* 2004; Muchero *et al.* 2008).

Efforts have been made to enhance the efficiency of selection for drought-tolerant cowpea genotypes based on yield and specific physiological traits, with often inconclusive results and insufficient differences between genotypes

(Ogbonnaya *et al.* 2003; Hamidou *et al.* 2007). A large genotype × environment interaction in cowpea is also common for yield, and precise and focused screening techniques for trait selection to assist breeding efforts (Anyia & Herzog 2004; Onwugbuta-Enyi 2004) are lacking. Unlike some other legume crops, such as soybean, groundnut and chickpea, where a dissection of traits related to the regulation of leaf water loss has been achieved (Fletcher *et al.* 2007; Sadok & Sinclair 2009; Devi *et al.* 2010; Zaman-Allah *et al.* 2011a), limited investigations have been carried out in cowpea. Hence, there are no comparable data in which several contrasting cowpea genotypes have been assessed for traits that are expected to play a key role in adaptation to terminal drought.

Cowpea is commonly exposed to terminal drought stress as a consequence of decreasing rainfall or plant available water and rising temperatures. Therefore, traits that control and limit water use at the vegetative stage are intuitively those that will lead to retaining water in the soil profile to

support plant growth later in the season, and especially for grain filling (Kholová *et al.* 2010a; Zaman-Allah *et al.* 2011b). One of these traits could be low canopy gas exchange during periods of high vapour pressure deficit (VPD) under non-limiting water conditions (Sinclair *et al.* 2005; Devi *et al.* 2010). In soybean, a transpiration increase at VPD >2.0 kPa was limited in certain genotypes, leading to a lower transpiration rate (TR) in drought-tolerant than in sensitive lines (Fletcher *et al.* 2007; Sadok & Sinclair 2009). In peanut, similar genotypic variation has also been demonstrated, with some genotypes exhibiting a breakpoint in VPD response at about 2.2 kPa, above which there was little or no further increase in TR, while others had a linear response in TR over the whole range of tested VPDs (Devi *et al.* 2010). Similar findings were reported in sorghum (Gholipoor *et al.* 2010) and pearl millet (Kholová *et al.* 2010b).

Other water-saving mechanisms could either be a lower rate of water loss per unit leaf area (*i.e.* TR,  $\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ ), regardless of VPD, or lower leaf area. Kholová *et al.* (2010b) found that pearl millet lines carrying a terminal drought tolerance quantitative trait locus (QTL) had a lower rate of water loss per unit leaf area under well-watered conditions. Genotypic differences in TR, in addition to leaf area differences, were also found in chickpea lines having contrasting terminal drought tolerance with a higher TR and lower leaf area in sensitive than in tolerant lines at the vegetative stage (Zaman-Allah *et al.* 2011a). Various studies on the response of transpiration and stomatal conductance to different levels of environmental stress have been achieved in cowpea, but always by combining atmospheric VPD and soil drying treatments (Bates & Hall 1982; Hall *et al.* 1997). Here, we simply assess whether water-saving mechanisms related to the leaf canopy operate even in the absence of any water stress in cowpea.

Measurement of TR under different VPDs involves weighing pots and can be time consuming if applied to a large number of entries. Therefore, methods are also needed to quickly and indirectly assess TR. For any given environmental conditions, canopy temperature (CT) is closely related to the rate of transpiration from the canopy surface, while canopy temperature depression (CTD), *i.e.* the difference between air and canopy temperature is an indication of the capacity of stomata to regulate leaf water loss according to environmental load (Isoda & Wang 2002). Therefore, measuring differences in CT could be an indirect way of assessing differences in TR and/or sensitivity of TR to VPD, which would provide a simple and relevant trait proxy to screen for the selection of plants having differences in TR (Gonzalez-Dugo *et al.* 2005; Bahar *et al.* 2008). Simultaneous measurements of TR and canopy temperature under different VPD conditions would also allow determination of conditions in which both traits are closely related. Canopy temperature could also be used as a proxy for TR and the index of canopy conductance (Ig) (Jones *et al.* 2002; Leinonen & Jones 2004); here, infrared thermography is used here to assess CT towards an indirect assessment of Ig and TR under well-watered conditions, as previously described (Merlot *et al.* 2002; Jones *et al.* 2009).

The overall goal of this work was both to test the hypothesis that drought-tolerant and sensitive cowpea genotypes have differences in traits related to the control of leaf water loss, and then to set the conditions (plant age, time of the day) in which TR could be indirectly assessed in a high throughput

mode using an infrared imaging system. Specifically, cowpea genotypes with contrasting terminal drought tolerance, based on several yield assessments in the field, were tested under fully irrigated conditions to (i) evaluate leaf canopy size and TR under natural VPD, (ii) assess the response of TR to increasing VPD, (iii) evaluate CT and Ig assessed by thermal imagery as an indirect measure of TR, and (iv) assess genotypic variations and relationships with TR.

## MATERIAL AND METHODS

### Plant material, experiments and growth conditions

Forty cowpea genotypes with contrasting response to terminal drought in the field (Table S1) were used (Nouhoun Belko, Ndiaga Cisse, Ndeye Ndack Diop, Samba Thiaw, Gerard Zombre, Satoru Muranaka, and Jeffery D. Ehlers, unpublished observation). The field assessments leading to this classification were carried out in well-managed experimental station fields in Senegal, Burkina Faso and California, and in seasons when VPD was high. Seeds were obtained from the Department of Botany and Plant Sciences, University of California Riverside, USA. Experiments were conducted at ICRISAT-India (17°30' N, 78°16' E, 549 m a.s.l.) between February and April 2010 and 2011.

For assessment of plant biomass and TR, these 40 genotypes were grown under well-watered conditions and assessed both outdoors (Exp. 1) and in a glasshouse (Exp. 2), with the objective of measuring growth and TR responses under different VPD conditions. In a repeat experiment (Exp. 3) with 12 genotypes taken from the 40 mentioned above, the plants were grown only in a glasshouse under well-watered conditions. The primary purpose of Exp. 3 was a more detailed assessment of the TR response to naturally changing VPD outdoors (plants were transferred outdoors where higher VPD levels could be attained). The second purpose of Exp. 3 was to assess the TR response to VPD in a growth chamber, where light was maintained constant and temperature and relative humidity (RH) could be set to desired levels (see below). The third purpose of Exp. 3 was to set the conditions in which the genotypic variation in leaf TR could be indirectly assessed through CT imaging; therefore, during assessment of the TR response to naturally changing VPD outdoors, infrared images were taken (see details below) to measure CT (see below).

Pots of 20-cm diameter filled with 5 kg soil (Exp. 1 and 2) and of 12.5-cm diameter filled with 2.5 kg soil (Exp. 3, see below) were used for plant culture. The soil was a sandy clay-loam Alfisol collected from the ICRISAT farm and fertilised with di-ammonium phosphate (DAP) at a rate of 0.3  $\text{g}\cdot\text{kg}^{-1}$  soil. The day before sowing, the topsoil was treated with 2 g carbofuran to prevent damage from soil-borne pests. Each pot contained three seeds and was thinned to one seedling 10 days later. For each experiment, seven pots were prepared for each genotype  $\times$  environment condition. Plants were kept well watered at all times by increasing the frequency and amount of irrigation, applied by hand, during plant growth. The five most uniform plants of each genotype were chosen for the measurements. Experimental environments (glasshouse and outdoor conditions) were equipped with temperature and humidity sensors (Tinytag Ultra 2 TGU-4500; Gemini Dataloggers, Chichester, UK), which

recorded air temperature and RH every 15 min throughout the crop growth and measurement period. The sensors were covered with an upturned white plastic pot to prevent direct sunlight reaching the sensor. The day before measurements, pots were fully irrigated and allowed to drain to field capacity. Late in the evening, pots were bagged in plastic wrapped around the plant stem in Exp. 1 and 2. In Exp. 3, after drainage, the soil surface was covered with a plastic sheet and topped with 300 g plastic beads. Both methods prevented most soil evaporation during the assessment of plant TR. Five replicate plants were arranged in a randomised complete block design with one factor (genotype) in each environment.

#### Hypothesis testing: assessment of leaf TR under different VPD regimes

##### *Response of TR to natural change in VPD in glasshouse and outdoor conditions*

This was done in all three experiments, with 35-day-old plants in all cases. Plant TR was estimated gravimetrically from loss in pot weight. Pots were weighted to 0.01 g precision (PE 12; Mettler Toledo, Schweiz, Germany) between 07:00 and 17:00 h, at 2-h intervals (for evaluation of the 40 genotypes in Exp. 1 and 2) or 1-h intervals (for assessment of the selected 12 genotypes in Exp. 3). Plant time-transpiration ( $Tr$ , g  $H_2O$  plant<sup>-1</sup>·h<sup>-1</sup>) was calculated as:

$$Tr = (PW_n - PW_{n+1}) \quad (1)$$

where  $PW_n$  and  $PW_{n+1}$  are pot weights recorded at consecutive times.

In Exp. 1 and 2, plants were harvested after the final weighing, separated into their component parts, and dried to estimate dry mass. Leaf area was not measured and, therefore, transpiration data were converted into transpiration per unit leaf dry weight (Leaf DW) ( $TR$ , g  $H_2O$  g<sup>-1</sup>·leaf DW·h<sup>-1</sup>) in Exp. 1 and 2 to obtain a comparative estimate of TR among genotypes. Root, stem and leaf dry mass were recorded after placing tissues in an oven at 60 °C for 3 days. The atmospheric VPD was calculated using the air temperature and RH recorded by sensors positioned in the plant canopy during the experiments outdoors and in the glasshouse, such that:

$$VPD = ((100 - RH)/100) * VP_{sat} \quad (2)$$

where RH (%) is relative humidity and  $VP_{sat}$  is saturated vapour pressure (kPa) taken from a table of air temperature and RH data.

In Exp. 3, plants were transferred to the growth chamber at the end of assessment under naturally changing VPD outdoors, for later assessment of the TR response to VPD under controlled conditions (see below).

##### *Response of TR to increasing VPD in a growth chamber*

The response of plant TR to increasing VPD in Exp. 3 was performed in a controlled environment growth chamber (Convion CMP4030; Convion, Winnipeg, MB, Canada). At the end of the outdoor TR assessment, all five replicate plants per genotype were watered to field capacity then moved to the growth chamber, where they remained for 24 h for acclimation (day/night 28/23 °C and 70/80% RH). The growth

chamber was 2-m high and 3.3 m<sup>2</sup> in area, and the programmed air temperature and RH were 4.0–45.0 °C and 40–95%, respectively. The light source was a combination of fluorescent and incandescent lamps giving 500  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at the canopy level. The VPD was progressively increased from 0.55 to 4.30 kPa, with a 60-min exposure at each set VPD, including 5 min to change from one level to the next. To set VPD at these levels, the temperature (°C) was progressively increased, while the percentage RH (RH%) was progressively decreased, which mimics natural conditions. The °C/RH% conditions were: 23/80, 27/70, 29/60, 31/55, 34/55, 36/50, 38/50 and 40/45. The chamber was equipped with a 0.01 g precision balance (KERN 3600-2N; Kern & Sohn, Balingen, Germany) connected to a computer placed outside for automatic recording of pot weight every 10 min. Since there were 30 such balances available, 10 genotypes out of 12 were chosen and three replicates per genotype were run on 1 day and two on the following day. During assessment of the three replicates, plants of the two other replicates were kept in another growth chamber with similar day/night conditions (28/23 °C and 70/80% RH), and were shifted to the chamber containing the balance at the end on assessment of the first three replicates. The integrated computer–balance system (HEXATRIK Integrated Systems, Hyderabad, India) prevented any disturbance to plants that might occur during manual weighing outside the chamber. The TR of each plant was estimated from loss in pot weight, and leaf water loss as a function of time was calculated for each 1-h exposure to each VPD treatment. Leaf area per plant from Exp. 3 was determined by scanning individual leaflets with an area meter (LI-3100; LiCor, Lincoln, NE, USA). Transpiration data were converted to transpiration per unit leaf area (g  $H_2O$ ·cm<sup>-2</sup>·h<sup>-1</sup>) in Exp. 3. Specific leaf area (SLA, cm<sup>2</sup>·g<sup>-1</sup>) was calculated as the ratio between leaf area (LA) and leaf dry weight.

#### Method testing: estimating CT and index of canopy conductance with thermal imagery as a measure of TR

Canopy temperature (CT) was estimated from thermal images obtained with an infrared camera (IR FlexCam S; Fluke, Plymouth, MN, USA) with a sensitivity of 0.09 °C and an accuracy of  $\pm 2\%$ . The images were taken on well-watered plants from Exp. 3 during assessment of response of TR to naturally changing VPD outdoors, at low (0.80 kPa) and high (6.01 kPa) VPD. Analysis of the thermal images and estimation of canopy temperature were performed with the software SmartView 2.1.0.10 (Fluke). Measurements of CT were made on 35-day-old plants, and images were taken twice during the day, between 07:00–08:00 h (low VPD) and between 12:00–13:00 h (high VPD) to relate to TR measured gravimetrically at the same times during assessment of TR response to naturally changing VPD outside. SmartView 2.1.0.10 provides a histogram of the distribution of pixels related to temperature in the thermal image. The temperature distribution of the canopy should follow a normal distribution, and a temperature threshold was fixed beyond which pixels of higher temperature were considered as background, allowing estimation of the plant canopy temperature (Zaman-Allah *et al.* 2011a). Based on distribution of thermal image pixels compared



with target canopy temperatures, an average canopy temperature was calculated as:

$$T_{\text{Canopy}} = \text{Sum}((T_i * P_{xi}) / P_{xt}) \quad (3)$$

where  $P_{xi}$  is number of pixels for a given temperature  $T_i$ , and  $P_{xt}$  is total number of pixels for range of temperatures covering the whole canopy.

The calculated canopy temperatures were used to estimate the index of canopy conductance ( $I_g$ ) as an indirect estimate of absolute stomatal conductance (Jones 1999).  $I_g$  was calculated using canopy and detached wet and dry leaf temperatures:

$$I_g = (T_{\text{Dry leaf}} - T_{\text{Canopy}}) / (T_{\text{Canopy}} - T_{\text{Wet leaf}}) \quad (4)$$

where  $T_{\text{Wet leaf}}$  and  $T_{\text{Dry leaf}}$  are temperatures of wet leaf and non-transpiring leaf surface, respectively.  $T_{\text{Wet leaf}}$  was measured on detached green leaves soaked in water for about 10 min and  $T_{\text{Dry leaf}}$  was measured on the same detached leaves previously dried in an oven at 60 °C for 48 h. These canopy temperatures were measured on six leaflets from six contrasting genotypes outdoors at the end of the experiments. The averages of  $T_{\text{Wet leaf}}$  and  $T_{\text{Dry leaf}}$  were calculated and applied in the  $I_g$  formula for all genotypes. CT and  $I_g$  data were then used in regressions against the TR data obtained above.

#### Statistical analysis

Analyses of variance (ANOVA) were performed with SAS (SAS Institute, Inc., Cary, NC, USA). One-way ANOVA was used to test genotype differences in plant growth parameters, TR, CT and  $I_g$ , and two-way ANOVA for testing genotype  $\times$  environment interactions. The Tukey–Kramer test was used for analysis of differences between genotype means. The relationships between TR, CT and  $I_g$  were also tested.

Analysis of TR response to VPD was performed using split-line regression in Genstat (Genstat 12.1; VSN International Ltd., Hemel Hempstead, UK), which provides a breakpoint value where the slope of the fitted regression changes, as well as values of the slopes. TR data of each genotype were plotted against each VPD treatment for further analysis and graphs using GraphPad Prism software (GraphPad 2.01, San Diego, CA, USA). TR of each plant was computed every 50 min of exposure to each stable VPD treatment, followed by a 10-min transition to the next VPD level. Regression analysis of TR response to increasing VPD was done using TR data of five replicate plants per genotype. First, for each genotype, an attempt was made to fit a two-segment linear regression to the data. The outputs of a successful regression fitting with the two-segment model were the coefficients defining two intersecting linear regressions

$$\begin{aligned} \text{If } \text{VPD} < X_0, \text{TR} &= S1(\text{VPD}) + C1(\text{Line1}) \\ \text{If } \text{VPD} \geq X_0, \text{TR} &= S2 + C2(\text{Line2}) \end{aligned} \quad (5)$$

where  $X_0$  is the breakpoint between the two linear segments, ( $S1$ ,  $C1$ ) and ( $S2$ ,  $C2$ ), the slope and constant of the first and the second line segments, respectively. GraphPad Prism also provides  $R^2$  of the regression equations for each genotype, including standard error (SE) and 95% confidence intervals

for the slopes and the breakpoint. The slopes of the two linear regressions ( $S1$  and  $S2$ ) were statistically compared ( $P < 0.05$ ). If the slopes differed, the genotypes were represented by a double-segment model; when the slopes were not significantly different, a simple linear regression model was applied to all data and is reported for the given genotype.

## RESULTS

### Genotype variation in growth under different environmental conditions

In Exp. 1 in outdoor conditions, growth varied substantially among cowpea genotypes. High dry mass was recorded in several drought-sensitive genotypes (IT90K-284-2, IT98K-317-2, K VX-403, K VX-525, Yacine, 58-53, IT89KD-288 and IT93K-93-10), while low dry mass was recorded in some drought-tolerant genotypes (IT84S-2049, IT95K-1090-2, IT97K-207-15, IT98K-128-2, IT98K-1111-1, K VX61-1, Mouride and Suvita 2), hence, there was no clear pattern distinguishing tolerant and sensitive genotypes on the basis of plant mass; means of tolerant and sensitive lines was similar (Table S2), as were  $t$ -test results.

In the glasshouse conditions (Exp. 2) the growth parameters also varied significantly among genotypes. Contrary to outdoors,  $t$ -tests showed that mean plant biomass and stem biomass of tolerant genotypes was significantly (10%) lower than in sensitive lines ( $P < 0.05$ ). Also, most (nine of 12 genotypes, IT84S-2049, IT93K-503-1, IT93K-693-2, IT85F-3139, IT98K-128-2, Mouride, Suvita 2, UC-CB27 and UCR-P-24) tolerant lines had lower plant dry mass ( $< 6 \text{ g plant}^{-1}$ ), whereas most (11 out of 15; IT90K-284-2, IT93K-93-10, IT98K-317-2, K VX-403, IAR8/7-4-5-3, IT82E-18, UC-CB46, Yacine, Prima, Apagbaala, and Bambe y 21) sensitive lines had higher aerial biomass ( $> 7 \text{ g plant}^{-1}$ ; Table S2). Also, growth was almost halved outdoors compared with the glasshouse environment. However, genotype and genotype  $\times$  environment ( $G \times E$ ) effects on leaf, stem and plant dry mass were highly significant, indicating that the growth decrease outdoors differed among lines (Table S2).

In Exp. 3 in 2011, the growth differences found in the glasshouse in 2010 were confirmed, with sensitive Bambe y 21, UC-CB46 and IT82E-18 having the highest biomass, and tolerant IT93K-503-1, IT93K-693-2 and Mouride having the lowest biomass. Moreover, five out of six sensitive lines had the highest leaf area, whereas five out of six tolerant lines had the lowest leaf area (Table 1). The other growth parameters did not discriminate tolerant from sensitive lines. Overall,  $t$ -tests failed to show significant differences between the grand mean of tolerant and sensitive groups for root, stem and plant dry mass, although these tests were significant at  $P < 0.1$  for leaf area and leaf dry weight.

### Response of TR to naturally changing atmospheric VPD

In outdoor conditions (Exp. 1), TR was assessed over an entire clear day with naturally changing VPD (1.5–7.5 kPa; Fig. S1A). There was genotypic variation for TR across genotypes at all except the last time of measurement, when VPD was  $< 1.5 \text{ kPa}$  (representative genotypes presented in Fig. 1A). TR was significantly higher in most sensitive genotypes (Apagbaala, Bambe y 21, IT89KD-288, IT93K-2046, IT97K-556-6,

**Table 1.** Growth attributes of cowpea genotypes from Exp. 3, grown under well-watered conditions in a glasshouse. The plant parts examined were root dry weight (root DW; g plant<sup>-1</sup>), stem dry weight (stem DW; g plant<sup>-1</sup>), leaf dry weight (leaf DW; g plant<sup>-1</sup>), plant dry weight (Plant DW; g plant<sup>-1</sup>), leaf area (LA; cm<sup>2</sup> plant<sup>-1</sup>) and specific leaf area (SLA; cm<sup>2</sup> g<sup>-1</sup>).

	Genotype	root DW (g)	stem DW (g)	leaf DW (g)	plant DW (g)	leaf area (cm <sup>2</sup> )	SLA (cm <sup>2</sup> g <sup>-1</sup> )
drought sensitive	Bambey 21	1.93 ± 0.04a	4.72 ± 0.07a	6.80 ± 0.07a	11.53 ± 0.04a	1240 ± 8.99ab	182 ± 2.59b
	IT82E-18	1.59 ± 0.04b	4.53 ± 0.06a	5.61 ± 0.04b	10.14 ± 0.02b	1346 ± 7.96a	240 ± 2.68a
	IT83D-442	2.21 ± 0.08a	3.32 ± 0.05c	4.91 ± 0.04c	8.22 ± 0.04d	1129 ± 6.59b	230 ± 2.85a
	IT89KD-288	1.26 ± 0.03c	3.85 ± 0.04c	4.49 ± 0.04c	8.34 ± 0.04d	844 ± 7.10 e	188 ± 3.12b
	KVX-525	1.76 ± 0.06b	3.81 ± 0.03c	5.32 ± 0.05b	9.13 ± 0.02c	1148 ± 6.67b	216 ± 2.26ab
	UC-CB46	1.89 ± 0.04a	4.51 ± 0.06a	6.41 ± 0.05a	10.92 ± 0.05a	1120 ± 5.37b	175 ± 1.31b
Sensitive Grand mean	1.77	4.12	5.59	9.71	1138	205	
drought tolerant	IT93K-503-1	1.49 ± 0.04c	4.35 ± 0.03b	4.79 ± 0.05c	9.14 ± 0.03c	1048 ± 6.99c	219 ± 2.39ab
	IT93K-693-2	1.19 ± 0.04c	2.48 ± 0.05d	4.21 ± 0.03c	6.70 ± 0.06e	773 ± 12.29f	184 ± 3.55b
	IT97K-499-39	1.86 ± 0.04a	4.11 ± 0.04b	5.51 ± 0.05b	9.62 ± 0.04b	977 ± 6.19 d	177 ± 2.71b
	IT98K-428-3	1.94 ± 0.04a	4.59 ± 0.04a	5.56 ± 0.04b	10.15 ± 0.03b	1034 ± 4.74c	186 ± 1.57b
	Mouride	1.73 ± 0.04b	4.20 ± 0.06b	4.51 ± 0.03c	8.71 ± 0.04d	1145 ± 9.91b	254 ± 2.11a
	Suvita 2	1.39 ± 0.03c	4.12 ± 0.03b	5.26 ± 0.04b	9.38 ± 0.03b	1081 ± 6.70c	205 ± 2.14ab
tolerant grand mean	1.60	3.98	4.97	8.95	1009	204	

Values shown with SE are means of five replicate plants per genotype. For each parameter, genotype means followed with the same letter are not significantly different. *T*-tests were performed to compare grand means of the sensitive and tolerant group and did not reveal any significant difference.

KVX-525, UC-CB46, IT82E-18, IT83D-442, IT84S-2246) than in most tolerant ones (IT93K-503-1, IT93K-693-2, Mouride, Suvita 2, IT85F-3139, UC-CB27, 58-57, UCR-P-24, IT84S-2049, IT98K-428-3) under both low and high VPD (Fig. 2A and B). Although genotypic differences in TR were significant under both low and high VPD, the differences in TR were larger under the highest VPD (Fig. 2A and B).

In the glasshouse (Exp. 2; 0.5–4.0 kPa; Fig. S1B) similar results were obtained (Figs 1B, 2C and D), with TR being significantly lower in most tolerant genotypes (IT93K-503-1, IT93K-693-2, Mouride, Suvita 2, UC-CB27, KVX61-1, Danila) than in most sensitive ones (Bambey 21, IT89KD-288, KVX-525, UC-CB46, IT82E-18, IT83D-442, IT84S-2246). In addition, the discrimination for TR between tolerant and sensitive lines appeared clearer under glasshouse conditions than outdoors (Fig. 2C and D).

Twelve genotypes with contrasting TR were selected from Exp. 1 and 2, grown in the glasshouse and transferred outdoors to measure TR over the course of an entire day (Exp. 3; VPD 1.35–5.95 kPa; Fig. S2A). No genotype difference in TR occurred until VPD was above 2.5 kPa (Fig. 1C). Above VPD 3.5 kPa, the increase in TR was significantly lower in tolerant genotypes (IT93K-503-1, IT93K-693-2, Mouride, Suvita 2) than in sensitive ones (Bambey 21, IT89KD-288, KVX-525, UC-CB46). The other genotypes had intermediate values. The largest difference in TR between genotypes was at the time of highest VPD (5.95 kPa), around 13:00 h (Fig. 1C). As a consequence of their lower TR (Fig. 1C) and lower leaf area (Table 1) tolerant genotypes also had lower total transpiration (Table S3).

#### Patterns of TR under increasing VPD in the growth chamber

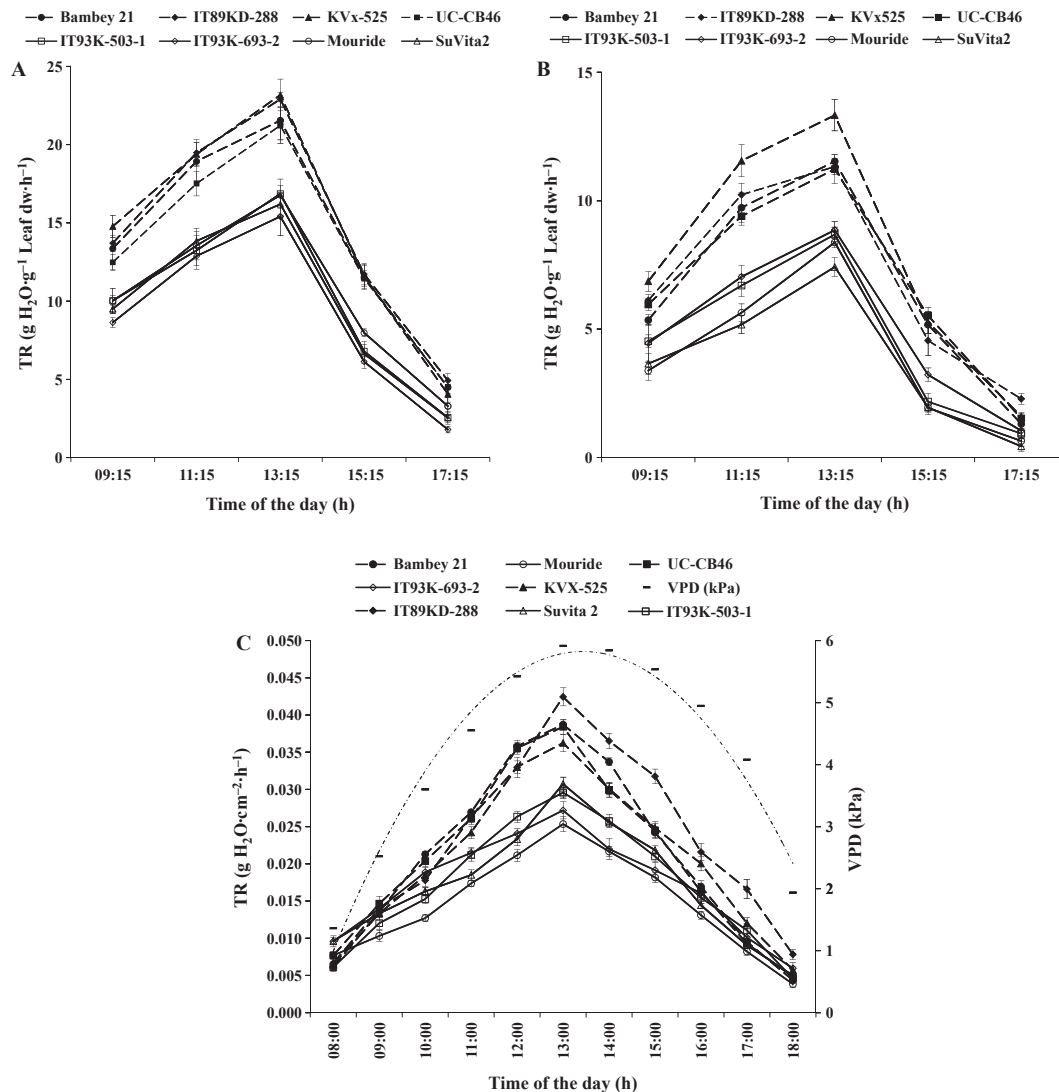
During TR assessment, the VPD increased regularly from 0.35 to 4.50 kPa (Fig. S2B). TR of all tested genotypes increased with increasing VPD, with clear differences between genotypes in the TR response. Three genotypes, IT93K-693-2,

Mouride, and Suvita 2 (all of which are tolerant to drought), fitted a two-segment linear model with VPD breakpoints of 1.81, 2.09 and 2.92 kPa, respectively (Table 2). In these tolerant genotypes, the slope of TR significantly decreased beyond their respective VPD breakpoint, indicating an effective slowing of TR as VPD increased (Fig. 3A and B). By contrast, seven genotypes, including five sensitive lines, had a linear increase in TR over the whole range of tested VPDs with no breakpoint (Table 2). The drought-tolerant genotype IT93K-503-1 had a lower slope resulting in a lower TR than sensitive genotypes UC-CB46, KVX-525 or IT83D-442 (Table 2, Fig. 3C and D). Moreover, the average TR over the whole range of VPD tested in the growth chamber was higher in sensitive (Bambey 21, IT83D-442, KVX-525, UC-CB46) than in tolerant (IT93K-503-1, IT93K-693-2, Mouride, Suvita 2; data not shown) lines.

#### Canopy temperature and Ig, and their relationship with TR

Canopy temperatures were measured under both low (08:00 h) and high (13:00 h) VPD conditions outdoors. Figure 4A and B indicates that the separation of leaf canopy area from the background area is based on a Gaussian distribution in temperature of the canopy. Under low VPD (0.80 kPa) conditions, CT varied significantly and ranged from 24.84 ± 0.16 °C for IT83D-422 to 26.51 ± 0.18 °C for Mouride (Fig. 5A). Tolerant genotypes had on average higher CT (average 26.18 °C) than sensitive ones (average 25.39 °C). Under high VPD (6.01 kPa), the CT ranged from 32.37 ± 0.28 °C for IT83D-422 to 39.58 ± 0.25 °C for Mouride. Similarly, sensitive genotypes were significantly cooler than tolerant ones (33.93 °C *versus* 36.90 °C), with larger differences between tolerant and sensitive lines at this higher VPD (Fig. 5B).

The CTs under high VPD were used to calculate the Ig, as an indirect estimate of stomatal conductance. The Ig varied across genotypes and ranged between 0.26 ± 0.07 for



**Fig. 1.** Evolution of the transpiration rate as a function of time of day in drought-tolerant (IT93K-503-1, IT93K-693-2, Mouride, SuVita 2) and sensitive (Bamby 21, IT89KD-288, KVx-525, UC-CB46) cowpea genotypes assessed under fully irrigated conditions outdoors (A, Exp. 1) and in a glasshouse (B, Exp. 2). The genotypes represent the largest variations among 40 genotypes tested. C represents the evolution of the transpiration rate as a function of time of day, measured in the same genotypes in Exp.3 in outdoor conditions (C, Exp. 3). All data were collected from well-watered plants. Plants were exposed to natural change of VPD over the course of an entire clear day. Values are means  $\pm$  SE of five replicate plants for each genotype. Where no error bars are visible, they are smaller than the data point.

Mouride and  $3.48 \pm 0.15$  for IT83D-422 (Fig. 5C). Tolerant lines had a significantly lower average Ig (0.80) than sensitive lines (2.04). Thus, TR was highly significantly and negatively related to the CT (Fig. 6A;  $R^2 = 0.85$ ), while TR was highly significantly and positively related to Ig (Fig. 6B;  $R^2 = 0.86$ ).

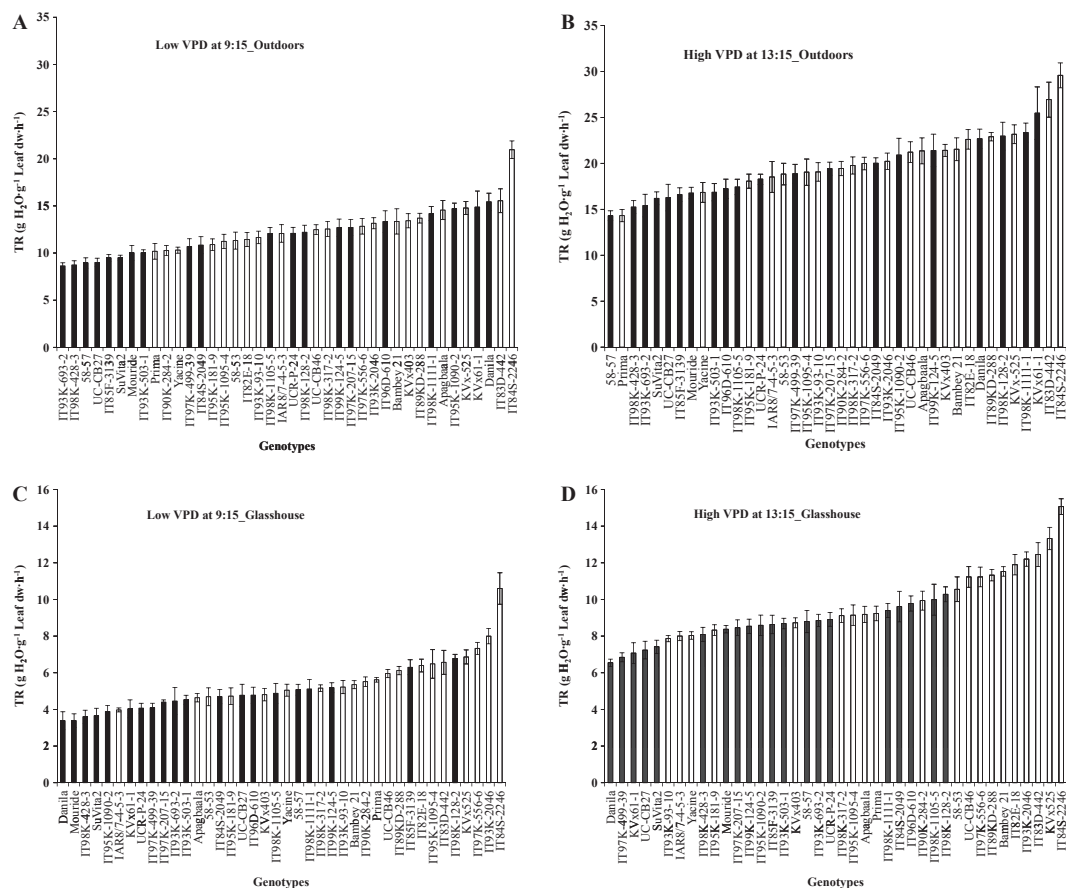
## DISCUSSION

From these 2 years of experiments conducted in both natural and semi-controlled environments, three results were obtained that showed the importance of water-saving traits. These results were all obtained under non-limiting water conditions and helped to better understanding the terminal drought tolerance strategies of the cowpea tolerant genotypes,

which, on average, had: (i) lower biomass than sensitive lines; (ii) lower transpiration rates at both low and high VPD; and (iii) TR in several tolerant genotypes was sensitive to VPD. The canopy temperature and index of canopy conductance were closely related to transpiration rate (Table 3), opening the possibility to indirectly and rapidly assess genotype differences in TR *via* measurement of canopy temperature.

### Genotype differences in plant growth under non-limiting water conditions

Under well-watered conditions, several tolerant cowpea genotypes showed lower growth than many sensitive ones, in agreement with previous reports in chickpea (Zaman-Allah *et al.* 2011a), pearl millet (Kholová *et al.* 2010a) and wheat

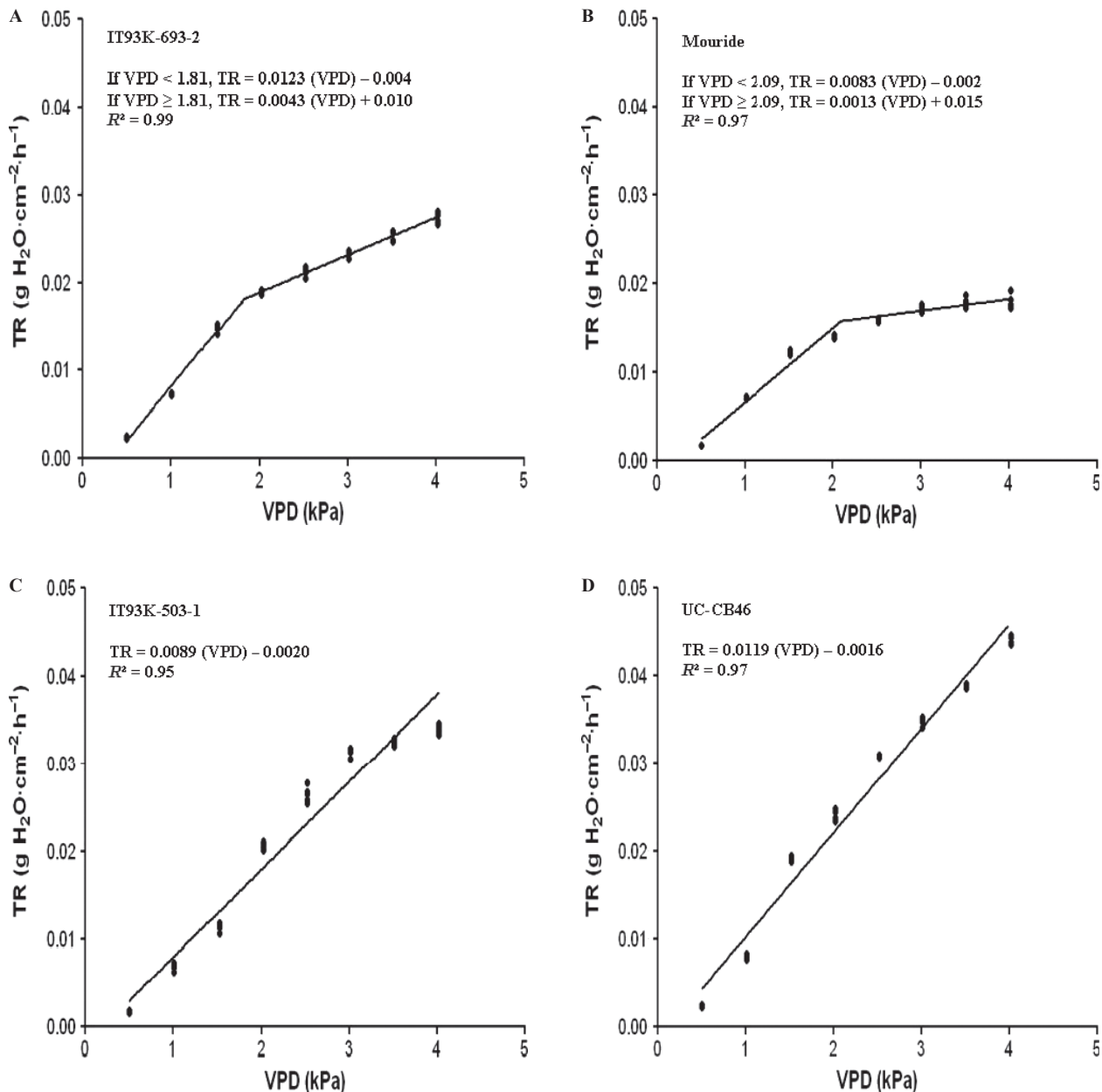


**Fig. 2.** Range of variation in transpiration rates (TR;  $\text{g H}_2\text{O g}^{-1}\text{ leaf DW h}^{-1}$ ) among tolerant (black bars) and sensitive (white bars) cowpea genotypes. TR were measured on well-watered plants, grown outdoors (Exp. 1) with low (A, morning) and high (B, midday) VPD, and on well-watered plants grown in the glasshouse (Exp. 2) at low (C, morning) and high (D, midday) VPD. Values are means  $\pm$  SE of five plants for each genotype.

**Table 2.** Outputs from analysis of the two-segment linear regression and single linear regression models for drought-tolerant (in bold) and sensitive (normal) cowpea genotypes. Three tolerant lines fitted the double-linear model and are displayed in ascending order on the basis of their breakpoint value. Five sensitive and two tolerant lines fitted the single linear model and are listed in ascending order based on their slope value.

Genotype	slope 1 $\pm$ SE	slope 2 $\pm$ SE	breakpoint $X_0 \pm$ SE	confidence interval of $X_0$	$R^2$
segmented regression with a VPD breakpoint					
<b>IT93K-693-2</b>	0.0123 $\pm$ 0.0004	0.0043 $\pm$ 0.0002	1.81 $\pm$ 0.05	1.71–1.92	0.99
<b>Mouride</b>	0.0083 $\pm$ 0.0003	0.0013 $\pm$ 0.0003	2.09 $\pm$ 0.08	1.94–2.25	0.97
<b>Suvita 2</b>	0.0094 $\pm$ 0.0004	0.0053 $\pm$ 0.0008	2.92 $\pm$ 0.19	2.54–3.31	0.98
linear regression without VPD breakpoint					
Genotype	slope $\pm$ SE	CI of slope	Y-intercept	X-intercept	$R^2$
<b>IT93K-503-1</b>	0.0089 $\pm$ 0.0004	0.0083–0.0095	–0.0020	0.2019	0.95
IT82E-18	0.0091 $\pm$ 0.0005	0.0087–0.0097	0.0010	–0.1278	0.88
Bambey21	0.0095 $\pm$ 0.0003	0.0089–0.0099	0.0008	–0.0845	0.97
KVX-525	0.0112 $\pm$ 0.0002	0.0108–0.0116	–0.0009	0.0884	0.98
IT83D-442	0.0116 $\pm$ 0.0005	0.0106–0.0126	–0.0024	0.2063	0.94
UC-CB46	0.0119 $\pm$ 0.0003	0.0113–0.0124	–0.0016	0.1325	0.97
<b>IT98K-428-3</b>	0.0127 $\pm$ 0.0004	0.0118–0.0135	–0.0029	0.2321	0.96

The table includes the values of slopes  $\pm$  SE (TR;  $\text{g H}_2\text{O cm}^{-2}\text{ h}^{-1}$ ), 95% confidence interval (CI) of slopes, breakpoint  $\pm$  SE (kPa), 95% confidence limit of breakpoint, Y-intercept when  $X = 0.0$ , X-intercept when  $Y = 0.0$ , and  $R^2$  of the regressions.



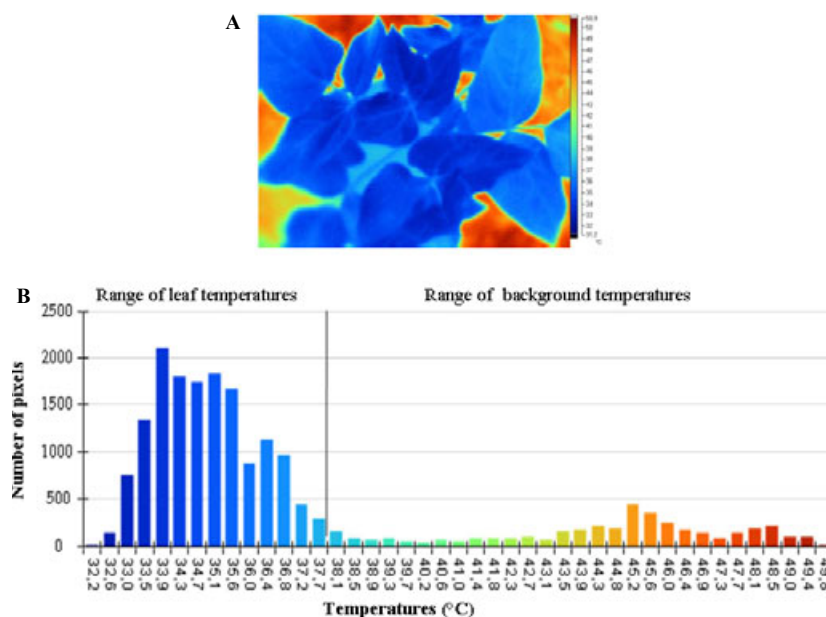
**Fig. 3.** Transpiration rates (TR;  $\text{g H}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) of four selected cowpea genotypes from Exp. 3 in response to increasing VPD (kPa). TRs were measured on well-watered plants grown in the glasshouse, which were temporarily transferred to a growth chamber with control over temperature and relative humidity. There, plants were exposed to increasing VPD, set by modifying temperature and humidity. TR data are the mean of five replicate plants, computed hourly at each of the eight VPD levels ( $n = 40$ ). Data were used to draw a segmental or a single linear regression for each genotype. Data in panels (A) and (B) fitted a double linear regression, whereas data in panels (C) and (D) fitted a single linear regression. The slopes, breakpoint and  $R^2$  of regressions are displayed in the figures.

(Condon *et al.* 2004) at the vegetative stage. Accelerated early plant growth and/or larger leaf areas could lead to more rapid water depletion, and insufficient residual soil moisture for the plant to complete its life cycle. Therefore, in situations of terminal drought, genotypes having conservative use of water would produce seeds for the next generation (Taiz & Zeiger 2002; Passioura & Angus 2010). Lower early growth and/or leaf area development under well-watered conditions could be considered an important adaptive response against late-season drought stress.

#### Differences in transpiration rate and response to different VPD regimes

A key finding of this investigation was the low TR of many tolerant lines (15 of the 20 lines), and the high TR of many sensitive lines, *i.e.* the ten lines with highest TR were all sensitive lines (Fig. 2D). These genotypic differences in TR response were consistent at both low and high VPD at the vegetative stage. The findings of genotypic differences for TR in cowpea agree with recent results reported in pearl millet





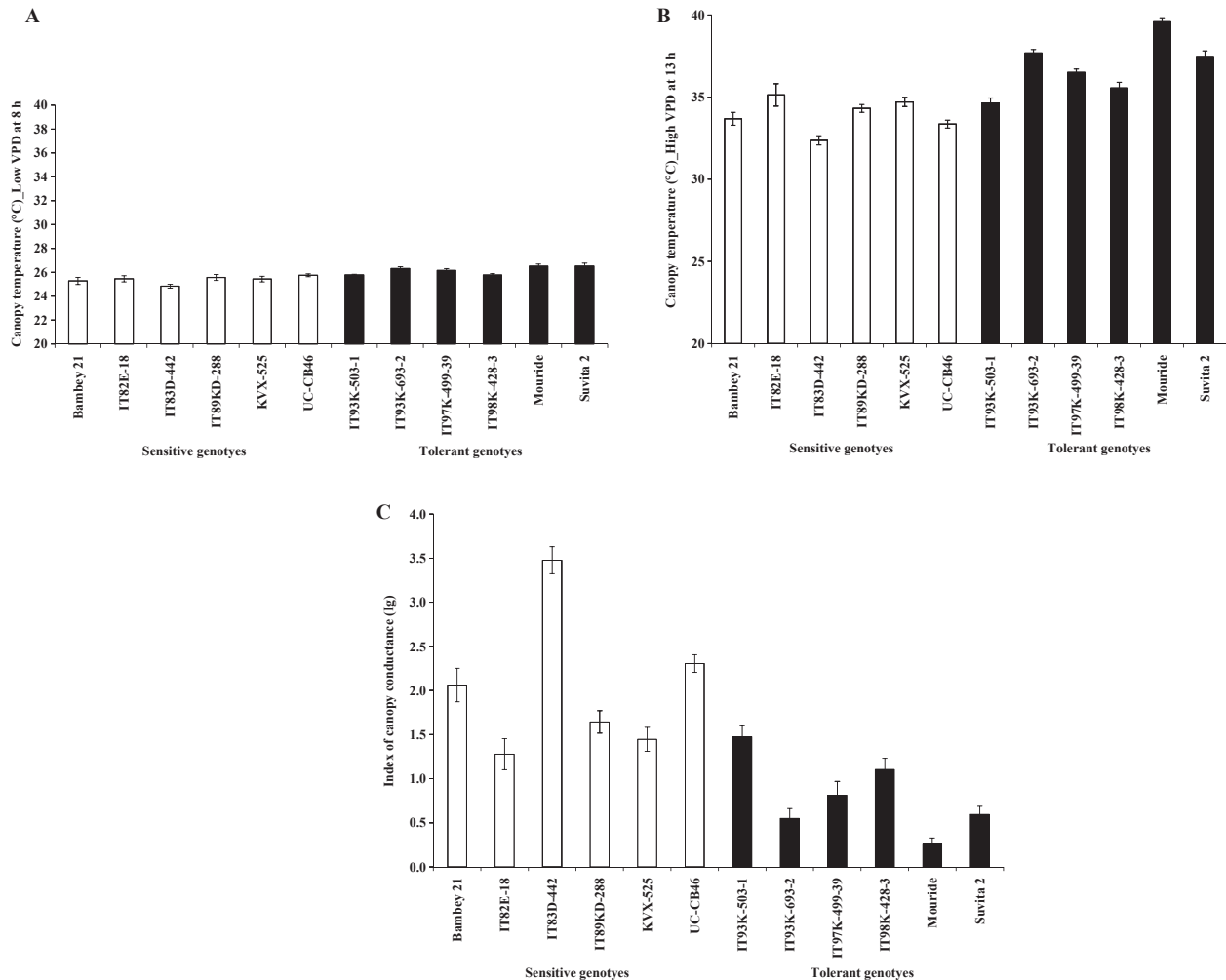
**Fig. 4.** Thermal image of cowpea genotype showing leaves, background and temperature scale (A) and distribution of number of pixels over the range of canopy and background temperatures (B). These thermal images were taken from plants of Exp. 3 at the time TR response to naturally changing VPD was assessed.

(Kholová *et al.* 2010b) and chickpea (Zaman-Allah *et al.* 2011a). High leaf–air VPD imposes high leaf–air gradients and drives water out of the leaves at a faster rate, leading to more rapid depletion of soil moisture, especially when water is available (Vadez *et al.* 2012). Hence, there is scope for water saving in lines that restrict transpiration at high VPD. The discrimination in transpiration between genotypes was more pronounced during the day with the highest VPD, suggesting differences in TR response to VPD across genotypes. This sensitivity of TR to high VPD in tolerant genotypes was confirmed in growth chamber Exp. 2, with three tolerant genotypes giving a two-segment linear model with an average VPD breakpoint of about 2.25 kPa. By contrast, all sensitive lines presented a linear increase in TR over the whole range of tested VPDs. These findings agree with previous results in soybean (Fletcher *et al.* 2007; Sadok & Sinclair 2009), peanut (Devi *et al.* 2010), pearl millet (Kholová *et al.* 2010a) and sorghum (Gholipour *et al.* 2010). The only difference was with recent chickpea results, where the TR response to VPD did not discriminate tolerant from sensitive lines (Zaman-Allah *et al.* 2011a). These results indicate clearly that in cowpea, two distinct water-saving mechanisms operate under well-watered conditions: (i) a low TR in tolerant lines acting across VPD conditions; and (ii) a high sensitivity to VPD in tolerant lines that further limit TR when VPD is >2–3 kPa. Both traits would contribute to save water in the soil layers that would then be available and essential for later grain filling (Zaman-Allah *et al.* 2011a).

The reasons for the rapid change in TR with a VPD increase are unclear and would require rapid control of stomatal conductance. Hydraulic signals are more likely to mediate such a rapid VPD response (Zwieniecki *et al.* 2001; Sperry *et al.* 2002; Sinclair *et al.* 2008), at least the case in the growth chamber, where other environmental parameters that could have affected the response (*e.g.* light) were held constant. Indeed, VPD outdoors was related to time of day, and it is not clear whether other factors could have played a role in the TR response, especially after reaching peak TR values.

For instance, TR decreased after reaching the peak VPD, although VPD remained at the same level at 14:00 h, which could be explained as photo-inhibition from cumulative hours of sunlight. Also, TR values at the end of the day were lower than in the morning at similar VPD, which could be due to decreasing light levels later on. Therefore, using outdoor conditions to characterise the response to an increase in VPD should probably focus only on the ascending portion of the TR response curve. Nevertheless, among the genotypes with a lower TR under natural conditions, only IT93K-503-1 did not show the ‘broken stick’ response of TR to VPD in the growth chamber. However the slope of the TR increase of IT93K-503-1 was among the lowest in the growth chamber and agreed with the low TR of this genotype under high VPD in outdoor conditions. Indeed, in outdoor conditions, the TR response was linear until the highest VPD at 13:00 h in all genotypes, but the slope of TR increase of tolerant lines were lower than that of sensitive lines, and consequently TR of tolerant lines was well below that of sensitive lines at high VPD. Under outdoor conditions, measurement of TR under high VPD as a response to changes in VPD provides a simple and convenient method to characterise the response observed in the growth chamber.

A limiting maximum TR associated with an early, lower growth rate could have drawbacks, resulting in yield reductions, especially when soil water supply is plentiful and there is no other limitation to yield (Sinclair & Muchow 2001; Richards *et al.* 2007; Sadok & Sinclair 2010; Sinclair *et al.* 2010). For example, among 18 soybean genotypes with similar maturity tested in the field, the most drought-tolerant, slow-wilting line, which exhibited a breakpoint in its TR response to increasing VPD, had the second-lowest yield under non-limiting water conditions (Cho *et al.* 2003). Also, biomass in outdoor conditions was lower than in the glasshouse, and was affected by large genotype  $\times$  environment interactions. These results imply that TR does not respond in the same way to VPD in all genotypes, leading to biomass accumulation differences under changing VPD conditions.



**Fig. 5.** Variations in canopy temperature and index of canopy conductance among tolerant (black bars) and sensitive (white bars) cowpea genotypes evaluated in Exp. 3. The plants were grown in a glasshouse under well-watered conditions, and transferred outdoors for assessing TR response to naturally changing VPD (Fig. 1C). The canopy temperature images were then taken at times of day with low VPD (0.80 kPa) (A) and high VPD (6.01 kPa) (B) outdoors. The index of canopy conductance was estimated using canopy temperature data measured under high VPD (C) Values are means  $\pm$  SE of five replicate plants for each genotype. Where no error bars are visible they are smaller than the data point.

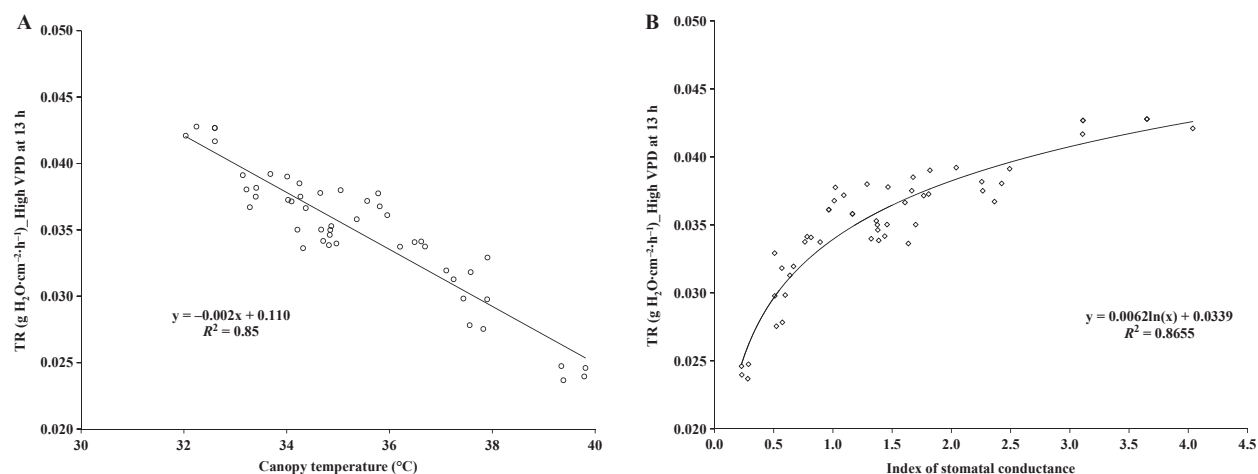
Therefore, the traits described above are important for breeding of crops with enhanced terminal drought tolerance, but further work is needed to assess their window of fitness to defined water availability.

#### Canopy temperature as a proxy for estimating canopy conductance

Under low VPD (0.80 kPa around 08:00 h), but more so at high VPD (6.01 kPa around 13:00 h), tolerant genotypes were on average warmer than sensitive ones. The difference in canopy temperature between the two groups of genotypes was ca. 1 °C at low VPD and ca. 3 °C at high VPD. These observations agree well with the differences in TR described above, especially at high VPD (Fig. 2D), and with the lower TR of the majority of tolerant genotypes. The index of canopy conductance (Ig), calculated using canopy temperatures and presented as an indirect estimate of can-

opy conductance, was significantly lower in tolerant (0.80) than in sensitive lines (2.04). Also, significant relationships were found between canopy temperature and TR and between canopy temperatures and Ig in well-watered cowpea plants subjected to high air-leaf VPD differences. These results agree with other studies using thermography for physiological and genetic screening in several crops in non-limiting water environments (Ayeneh *et al.* 2002; Merlot *et al.* 2002; Jones *et al.* 2009; Munns *et al.* 2010). Here, the canopy temperature and Ig could be used as a reliable proxy to screen for TR among cowpea germplasm, using the high VPD period of the day as the most suitable measurement period.

The existence of cowpea germplasm with differences in VPD response and canopy temperature opens possibilities for further physiological and genetic studies, since contrasting genotypes used here are also parents of available recombinant inbred populations (Mouride/Bambey 21 and IT93K-503-



**Fig. 6.** Relationships between leaf TR and canopy temperature (A) and between leaf TR and index of canopy conductance (B) in 12 contrasting cowpea genotypes grown in the glasshouse under well-watered conditions and transferred outdoors for assessment of the TR response to naturally changing VPD (6.01 kPa). The data used for these regression analyses are replicated data, obtained under high VPD. For each genotype, four replicates data points ( $n = 48$ ) were used to draw the single linear and the polynomial regressions.

**Table 3.** Summary of traits that discriminated tolerant and sensitive cowpea genotypes and that could contribute to terminal drought tolerance through conservative water use mechanisms.

	Genotype	plant biomass	leaf area	leaf TR	TR response to increasing VPD	canopy temperature	index of canopy conductance
Drought sensitive	Bambey 21	High	High	High	Linear	Cooler	High
	IT82E-18	High	High	Low	Linear	Cooler	High
	IT83D-442	Low	High	High	Linear	Cooler	High
	IT89KD-288	Low	Low	High	–	Cooler	High
	KVX-525	High	High	High	Linear	Cooler	Lower
	UC-CB46	High	High	High	Linear	Cooler	High
Drought tolerant	IT93K-503-1	Low	Low	Low	Linear	Warmer	Lower
	IT93K-693-2	Low	Low	Low	Breakpoint	Warmer	Lower
	IT97K-499-39	High	Low	High	–	Cooler	Lower
	IT98K-428-3	High	Low	High	Linear	Cooler	Lower
	Mouride	Low	High	Low	Breakpoint	Warmer	Lower
	Suvita 2	Low	Low	Low	Breakpoint	Warmer	Lower

Rating was made on the basis of outdoor and growth chamber experimental data collected at the vegetative stage on 12 selected genotypes under non-limited water conditions in Exp. 3.

The Low/High, Cooler/Warmer or Linear/Breakpoint rankings among genotypes were made based on results presented in different tables and figures.

1/UC-CB46)). Thus, genetic variation in response to changing VPD could be exploited and targeted to specific stress environments.

## CONCLUSION

Cowpea genotypes with contrasting response to terminal drought stress in the field also had contrasted control of leaf water loss under non-limiting water conditions. Tolerant genotypes generally had: (i) lower vegetative biomass 5 weeks after sowing, (ii) lower transpiration rate, and (iii) restricted TR in response to increasing VPD. Then, the lower TR, especially at high VPD, could be proxied as: (iv) a warmer canopy temperature and (v) a lower index of canopy conductance (Table 3). It is hypothesised that these water-saving characteristics of some cowpea genotypes under non-limited water conditions would contribute to making water

available for the grain filling stage. Genotypes having such water conservation traits would be especially desirable in low humidity environments with high atmospheric VPD, limited available water, and where water deficits commonly develop later in the growing season.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in this online version of the article:

**Figure S1.** (A, B) Distribution of air temperature (°C), relative humidity (%) and VPD (kPa) during the assessment of the transpiration rates under well-watered conditions of forty cowpea genotypes in response to natural change of atmospheric vapor pressure deficit (VPD) outdoors (A: Exp. 1) and in a glass house (B: Exp. 2).

**Figure S2.** (A, B) Evolution of air temperature (°C), relative humidity (%) and VPD (kPa) during the assessment of

the transpiration rates under well-watered conditions of twelve contrasting cowpea genotypes (Exp. 3) in response to (A) natural change of VPD outdoors and (B) increasing VPD in a controlled environment growth chamber.

**Table S1.** List of cowpea genotypes studied across experiments.

**Table S2.** Growth attributes of cowpea genotypes from Exp. 1.

**Table S3.** Amount of water transpired as a function of time of day in drought-tolerant and sensitive cowpea genotypes assessed under fully irrigated conditions outdoors (Exp. 3).

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