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Lower soil moisture threshold for transpiration decline under water deficit correlates with lower canopy conductance and higher transpiration efficiency in drought-tolerant cowpea

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Abstract. As water availability is critical for reproduction, terminal drought tolerance may involve water-saving traits. Experiments were undertaken under different vapour pressure deficit (VPD) and water regimes (water stress (WS) and well watered (WW)) to test genotypic differences and trait relationships in the fraction of transpirable soil water (FTSW) at which transpiration declines, canopy conductance (proxied by transpiration rate (TR, g H_2O cm^{-2} h^{-1})), canopy temperature depression (CTD, °C), transpiration efficiency (TE, g kg^{-1}) and growth parameters, using 15 contrasting cowpea (*Vigna unguiculata* (L.) Walp.) genotypes. Under WW conditions at the vegetative and early podding stages, plant mass and leaf area were larger under low VPD, and was generally lower in tolerant than in sensitive genotypes. Several tolerant lines had lower TR under WW conditions and restricted TR more than sensitive lines under high VPD. Under WS conditions, transpiration declined at a lower FTSW in tolerant than in sensitive lines. Tolerant lines also maintained higher TR and CTD under severe stress. TE was higher in tolerant genotypes under WS conditions. Significant relationships were found between TR, and TE, CTD and FTSW under different water regimes. In summary, traits that condition how genotypes manage limited water resources discriminated between tolerant and sensitive lines. Arguably, a lower canopy conductance limits plant growth and plant water use, and allows tolerant lines to behave like unstressed plants until the soil is drier and to maintain a higher TR under severe stress, as lower TR at high VPD leads to higher TE.

Additional keywords: canopy temperature depression, drought stress, fraction of transpirable soil water, *Vigna unguiculata*.

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Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.), a protein-rich grain legume is widely cultivated by resource-poor farmers in the semiarid tropics of Africa, Asia and Latin America, where it is immensely important for its central role in the diet and economy of millions people (Singh *et al*. 2003; Dadson *et al*. 2005; Muchero *et al*. 2009). Despite the crop's capacity to withstand water deficits, significant differences exist among cowpea genotypes for their response to terminal drought (i.e. water deficit stress occurring at the end of the growing season) (Mai-Kodomi *et al*. 1999*a*; Muchero *et al*. 2008). In Africa, cowpea is commonly grown in the Sudanian and Sahelian semiarid regions, where climate change is likely to make drought stresses even more severe in the future (Hall *et al*. 2003; Wittig *et al*. 2007; Vadez *et al*. 2012). Therefore, the identification of drought-tolerant cowpea cultivars adapted to these agro-ecological zones is needed (van Duivenbooden *et al*. 2002; Kholová *et al*. 2010*a*).

Extensive research has been carried out on the screening for mid- and late-season drought tolerance in cowpea, focussing on carbon isotope discrimination, the chlorophyll stability index, leaf gas exchange, relative turgidity, relative water content, water use efficiency and water potential (Hall *et al*. 1992; Cruz de Carvalho *et al*. 1998; Ashok *et al*. 1999; Singh and Matsui 2002; Ogbonnaya *et al*. 2003; Anyia and Herzog 2004; Hall 2004; Onwugbuta-Enyi 2004; Padi 2004; Slabbert *et al*. 2004; Souza *et al*. 2004; Hamidou *et al*. 2007). Nevertheless, only very few studies have used these indices to select parental genotypes in further genetic studies (Mai-Kodomi *et al*. 1999*b*; Muchero *et al*. 2009). We argue that despite the complexity of the drought response, simple hypotheses based on water needs can be developed to guide the selection of critical traits (Vadez *et al*.

2007). Here, we test one of these hypotheses, namely that water-saving traits are important for terminal drought adaptation, by comparing a range of contrasting lines.

Because water availability is critical for the reproduction and grain filling period, plant traits involved in a conservative use of soil water even if water is not limited are indeed likely to be relevant for yield improvement under limiting water (Vadez *et al.* 2012). This has been shown in chickpea (*Cicer arietinum* L.) (Zaman-Allah *et al.* 2011*a*, 2011*b*), groundnut (Ratnakumar *et al.* 2009) and pearl millet (*Pennisetum americanum* (L.) R. Br.) (Kholová *et al.* 2010*a*, 2010*b*). Recent findings showed that leaf area was lower in tolerant chickpea (Zaman-Allah *et al.* 2011*a*) and peanut (*Arachis hypogaea* L.) (Ratnakumar and Vadez 2011), and this logically limits plant water use. Significant variations in canopy conductance were also found among contrasting genotypes under nonlimited water conditions in cowpea (Hall and Schulze 1980), chickpea (Zaman-Allah *et al.* 2011*a*), soybean (*Glycine max* (L.) Merr.) (Purcell and Specht 2004; Fletcher *et al.* 2007; Sadok and Sinclair 2009), peanut (Bhatnagar-Mathur *et al.* 2007), *Sorghum* (Gholipoor *et al.* 2010) and pearl millet (Kholová *et al.* 2010*b*).

Another water-saving option is to have a different soil moisture threshold where transpiration begins to decline upon progressive exposure to water deficit. For instance, the transpiration decline occurred in wetter soil (a higher soil moisture threshold for transpiration decline) in tolerant chickpea genotypes than in sensitive ones (Zaman-Allah *et al.* 2011*a*). Genotypic differences for this trait were also found for a transpiration response to progressive water deficit stress in several other crops (Vadez and Sinclair 2001; Bhatnagar-Mathur *et al.* 2007; Hufstetler *et al.* 2007; Devi *et al.* 2010). This characteristic provides the opportunity to reduce water use but such information is not available for cowpea. Nevertheless, tolerant pearl millet had a lower fraction of transpirable soil water (FTSW) threshold for transpiration decline (Kholová *et al.* 2010*a*). This was interpreted to be a consequence of the lower canopy conductance and the lower plant transpiration of tolerant genotypes under well watered conditions, which helped maintain the relative transpiration of water stressed plants to a level similar to well watered plants until the soil was dryer. Whether these thresholds relate to the canopy conductance under well watered conditions is an important question to resolve. Whether these canopy conductance differences would also relate to genotypic differences in transpiration efficiency (TE), which is a major source of crop yield variation under drought stress (Condon *et al.* 2004; Sheshshayee *et al.* 2006; Krishnamurthy *et al.* 2007), is another one. None of these questions has been tested cowpea and they are addressed in here.

According to Gwathmey *et al.* (1992) and Gwathmey and Hall (1992), another important morphological trait that may contribute to drought adaptation of cowpea is a delayed leaf senescence under water stress, which would enhance plant survival after a mid-season drought and limit damage to the first flush of pods. Cultivars with delayed leaf senescence also have enhanced production of forage because their leaves remain green and attached to the plant until harvest. Moreover, delayed leave senescence can be easily measured by visual scoring using an appropriate scale as used by Muchero *et al.* (2008) to discriminate 15 cowpea genotypes that exhibit significant genetic variation for drought tolerance.

In summary, the overall objective of the present study was to assess whether cowpea genotypes contrasting for their response to terminal drought in the field differ in their response to progressive soil drying conditions. The specific objectives were to: (i) evaluate growth and canopy conductance in different atmospheric vapour pressure deficit (VPD) conditions, and test whether drought-tolerant lines differ from sensitive ones; (ii) compare whether tolerant and sensitive cowpeas differ in their growth response to progressive exposure to drought stress; (iii) determine whether there are variations in the soil moisture thresholds where transpiration declines across genotypes and environments; (iv) assess possible relationships between some of these water-saving traits.

Materials and methods

Plant growth and description of experiments

Experiments were simultaneously carried out under different VPDs by setting up experiments in a glasshouse and outdoor environments at International Crops Research Institute for the Semiarid Tropics (ICRISAT) Patancheru in India (17°30′N; 78°16′E; altitude 549 m) after the rainy season (i.e. between March and May) in 2010. The glasshouse was temperature-controlled with four desert coolers, and had transparent glass walls and windows. During the crop growing period, the VPD was lower in the glasshouse than outdoors, where the air temperature was higher and relative humidity lower. The air temperature, relative humidity and resulting VPD varied between 24°C and 40°C, 45% and 85%, and 0.55 kPa and 4.15 kPa, respectively in the glasshouse while varying between 25°C and 50°C, 20% and 70%, and 0.85 kPa and 7.45 kPa respectively outdoors (Fig. S1, available as Supplementary Material to this paper). Fifteen cowpea (*Vigna unuiculata* (L.) Walp.) genotypes, contrasting for their response to drought stress under field and controlled environment conditions (N Belko, N Cisse, DD Ndeye, G Zombre, JD Elhers, unpubl. data), were selected for this investigation (Table 1). The work leading to this classification was conducted in well managed experimental field stations in Senegal, Nigeria, Burkina Faso and California, and in controlled environments (a glasshouse and a growth chamber) in India, in seasons when the VPD was high. Seeds were obtained from the Department of Botany and Plant Sciences of the University of California, Riverside, CA, USA.

Plants were grown in plastic pots (20 cm diameter × 20 cm tall) filled with 5.5 kg of sandy clay loam Alfisol collected from the ICRISAT farm and fertilised with di-ammonium phosphate at the rate of 0.3 g kg^{-1} soil and with farmyard manure (1 : 50 v/v). The day before planting, the topsoil of each pot had 2 g carbofuran added to prevent seeds being damaged by soilborne pests. Each pot was sown with three seeds and thinned to one seedling 1 week later. For each environment (glasshouse and outdoor), 21 plants of each genotype were grown under well watered conditions until 30 days after sowing (DAS, the time when water treatment imposition started). Then, the 15 most uniform plants of each genotype were selected to design the experiments in both environments. A thermo-hygrograph sensor

Table 1. List of cowpea genotypes compared for their growth, transpiration rate, soil moisture thresholds, transpiration efficiency and canopy temperature depression in response to progressive soil drying
ISRA, Institut Senegalais de Recherche Agricole; IITA, International Institute of Tropical Agriculture; UC Davis, University of California at Davis; INERA, Institut National de l'Environnement et de Recherches Agricoles

Genotypes	Origin	Maturity
Drought-sensitive		
Bambey 21	ISRA	Early
IT82E-18	IITA	Early
IT83D-442	IITA	Medium
IT89KD-288	IITA	Medium
IT93K-93-10	IITA	Early
IT97K-556-6	IITA	Medium
UC-CB46	UC Davis	Early
Drought-tolerant		
IT84S-2049	IITA	Early
IT97K-207-15	IITA	Medium
IT97K-499-39	IITA	Early
IT98K-128-2	IITA	Medium
IT99K-124-5	IITA	Medium
KVx-61-1	INERA	Early
Mouride	ISRA	Medium
Suvita2	INERA	Medium

(Tinytag Ultra 2 TGU-4500 Gemini Dataloggers Ltd, Chichester, UK) was positioned within the plants' canopies in the glasshouse and outdoor environments for regular records of the air temperature and relative humidity throughout the crop growth and measurement period.

The day before water treatment imposition, the pots were watered, allowed to drain to reach field capacity and then, late in the evening, the pots were bagged with a transparent plastic bag wrapped around the plant stem to prevent soil evaporation during the evaluation of plant transpiration. The 15 plants were divided in three sets of five plants: the first set was kept under well watered conditions (WW) and used for assessing the plants' transpiration rate (TR, in g water loss $cm^{-2} h^{-1}$, used as a simple proxy for canopy conductance) response to natural changes of atmospheric VPD during the course of an entire clear day before being harvested to measure the initial plant biomass (before the dry-down). The second set was maintained under WW conditions and the third set was gradually exposed to water stress (WS) (see below). The experimental layout was a randomised complete block design with treatment as the main factor and genotype as the subfactor randomised five times within each block.

Transpiration rate in response to VPD

The rate of water loss per unit of leaf area was assessed on WW plants from the first set (see above) under natural variations of VPD during the course of an entire sunny day in both glasshouse and outdoor conditions. Plant transpiration was measured gravimetrically from the losses in pot weight between consecutive weighings. Pots were weighed on a 0.01 g precision scale (PE 12, Mettler Toledo, Schweiz-GmbH, Germany) hourly between 0700 hours and 1700 hours (India Standard Time). At the end of the day, plants were harvested and the leaf area measured (LI-3100, Li-Cor, Lincoln, Nebraska, USA). Transpiration and leaf area data were used to estimate TR (i.e. leaf water loss per unit of leaf area). The plants' parts were dried in an oven at 60°C for 3 days and their dry masses were recorded. The specific leaf area (SLA, $cm^2 g^{-1}$) was calculated as the ratio between the leaf area and the leaf dry weight.

Plant exposure to progressive water deficit stress (dry-down)

After bagging, pots were weighed around 0900 hours at 31 DAS to obtain the initial pot weight and thereafter pots were weighed every day in the morning to calculate the daily plant transpiration. WW plants received daily rewatering up to 80% field capacity (i.e. bringing the pot weight to 200 g below the field capacity weight) every day. WS plants were exposed to stress by partially compensating plant water loss from transpiration; plants were allowed to lose no more than 70 g each day. Therefore, any transpiration in excess of 70 g was added back to the pots, as previously described by Vadez and Sinclair (2001), to allow a progressive development of water-deficit stress over approximately 2 weeks.

The transpiration values were normalised to facilitate comparison as previously described by Kholová *et al*. (2010*a*). First, the daily transpiration ratio for each plant was calculated as the ratio of the TR of each individual WS plant divided by the average TR for the five WW plants of that genotype. Second, the TR data were normalised by dividing each TR value over time by the average of the TR value for the first 3 days of the experiment when there was still no water limitation. This second normalisation aimed to remove variation resulting from differences in plant size among WS plants within a genotype. This gave the normalised transpiration ratio (NTR), which accounted for plant-to-plant variation in transpiration within each genotype. When the NTR of stressed plants fell below 0.10 (i.e. when the transpiration of WS plants was <10% of that of WW plants), all the plants were harvested and their different parts were dried in an oven at 60°C for 3 days and then their dry mass was measured. The genotypes all reached that stage within 2 days of each another.

After the final harvest, the daily FTSW (i.e. the amount of soil water available for transpiration) was back-calculated on each day of the experiment. First, the total transpirable soil water (TTSW) available to support plant transpiration in each pot was calculated as the difference between the initial and final pot weight, which was defined as the weight at the end of the experiment (Sinclair and Ludlow 1986). The FTSW values were calculated as:

$$FTSW = (Daily\ Pot_{\mathrm{weight}} - Final\ Pot_{\mathrm{weight}})\ TTSW^{-1}. \quad (1)$$

Since the plants were allowed to transpire no more than 70 g water per day, all the genotypes were exposed to similar stress intensities, at least from the viewpoint of the soil water content. Changes in NTR during the soil drying cycle were expressed as a function of FTSW, which was used as the indicator of stress intensity (Ritchie 1981).

Canopy temperature depression, transpiration efficiency and leaf scoring

The day before the end of the dry-down, leaf temperatures were recorded on five replicates plants for WW and WS treatments in both environments between 0800 and 0900 hours with an infrared thermometer (Fluke 574, Fluke Thermography, Annapolis Lane, Plymouth, MN, USA). Air temperature was recorded from a temperature and relative humidity recorder (Gemini Tiny Tag Ultra 2 TGU-4500 data logger), which was located at the crop canopy level. In each plant, temperatures were recorded on three leaves at the top of the canopy and averaged. The canopy temperature depression (CTD) was calculated as the difference between the air temperature and the leaf temperature ($CTD = T_{air} - T_{leaf}$).

Transpiration efficiency (TE, g biomass per kg water transpired) was calculated for all WW and WS plants in both environments as the ratio between the increase in plant biomass over the course of the dry-down and the total water transpired during the same time:

$$TE = (Final\ Harvest_{biomass} - Before\ Dry\text{-}down_{biomass})\ Total\ Water_{transpired}{}^{-1}, \quad (2)$$

where the biomass before the dry-down was the biomass of plants used to assess the TR response to VPD, which was harvested at the beginning of the dry-down. The final harvested biomass was that of WW and WS plants harvested at the end of the dry-down. The total water transpired was the sum of daily transpiration measured by daily weighing of pots during the dry-down.

Leaf senescence due to water deficit stress was scored at the end of the dry-down in both glasshouse and outdoor environments. The state of leaf senescence was rated on a scale from 1 to 5, with 1 = totally green and turgescent, 2 = green and slightly wilted, 3 = green-yellow and wilted, 4 = yellow-green and severely wilted, and 5 = completely yellow to brown and almost dead.

Statistical analysis

ANOVA was performed using the statistical program SAS (SAS Institute, Inc., Cary, NC, USA). One-way ANOVA was run to test the genotypic differences within each water treatment for plant growth parameters, TR, CTD, TE and visual scores. The Tukey–Kramer test was used for the analysis of differences between genotype means. The relationships between TE and TR, TE and the FTSW thresholds, TR and the FTSW threshold, and CTD and TR were also tested.

For the FTSW threshold analysis for each genotype, each NTR value was plotted against its corresponding FTSW value for each day of the experiment. A plateau regression procedure of the SAS program was used to estimate the specific FTSW threshold value where NTR initiated its decline (Ray and Sinclair 1997). This analysis provided an s.e. and 95% confidence interval for each threshold value for each genotype. A nonlinear regression analysis was done using GraphPad Prism (GraphPad 2.01, San Diego, CA, USA) to fit the exponential model presented by Muchow and Sinclair (1991) ($NTR = 1\ (1 + A \times \exp\ (B \times FTSW)^{-1})$. The regression result obtained using this equation was compared among genotypes based on the 95% confidence intervals of coefficients A and B. The plateau regression attempted to fit two linear segments where one segment was a plateau at $Y = 1$ and the second regression was a linear change in Y with respect to X. A key output from this analysis is the FTSW threshold for the two segments and the confidence intervals for this threshold. The averages of threshold values were compared across the genotypes using the Tukey–Kramer method of GENSTAT (GENSTAT ver. 12.1, VSN International Ltd, Hemel Hempstead, UK).

For the TR *vs* CTD relationship, the data were analysed with the split line regression option of GENSTAT (ver. 9.0), which provides a breakpoint value where the slope of the fitted regression significantly changes.

Results

Genotypic variation for plant growth under WW conditions at 30 DAS

Under glasshouse conditions, a majority of the tolerant lines had lower vigour than sensitive lines (Table 2). Higher root DW was found in five out of seven sensitive lines than in seven out of eight tolerant lines. All sensitive genotypes, except IT89KD-288, produced higher plant biomass than five tolerant lines. This was related more to differences in leaf DW (all but one sensitive genotypes had higher leaf DW than five out of eight tolerant ones) than in stem DW. The SLA varied between genotypes but did not discriminate tolerant from sensitive lines. Leaf area (cm^2 per plant) was the smallest in all drought-tolerant lines, except IT97K-499–39 and KVx-61–1, than in all drought-sensitive lines, except IT89KD-288 (Table 2).

Under outdoor conditions, growth parameters varied significantly among genotypes but did not clearly discriminate tolerant from sensitive lines, although the leaf area of five out of seven sensitive genotypes was higher than five out of eight tolerant ones (Table 2). In addition to the significant genotypic variations for all the growth parameters, there were highly significant differences between the glasshouse and outdoor environments for these growth attributes. Also, a significant effect of the interaction between genotype and environment (G × E) on the variation of the growth parameters was found, explaining a variance close to that of genotypic effect (Table 2).

In summary, the majority of the tolerant genotypes had low early vigour but their differences from the sensitive lines for growth parameters were not clearly expressed under high VPD conditions outdoors as compared with the glasshouse environment.

Response of leaf transpiration rate to changing atmospheric VPD

Under glasshouse conditions at 30 DAS, canopy conductance closely followed the diurnal pattern of atmospheric VPD, which ranged from 1.10 kPa to 4.08 kPa during the day. Canopy conductance was significantly lower in most of the tolerant genotypes (IT84S-2049, IT99K-124–5, Mouride, Suvita 2) than in most of the sensitive ones (Bambey 21, IT82E-18, IT89KD-288, UC-CB46). The largest differences between tolerant and sensitive lines for canopy conductance were recorded between 1100 hours and 1500 hours, when the VPD was above 3.5 kPa (the most representative genotypes are shown in Fig. 1*a*). TR, averaged for the whole day, was ~40% lower in tolerant than in sensitive lines (data not shown). The total water transpired per plant throughout the day was significantly

Table 2. Dry mass of plant parts (g per plant) of cowpeas grown under well watered conditions and harvested at 31 days after sowing, before the initiation of the dry-down, in glasshouse (top) and outdoor (bottom) environments

LA, leaf area (cm^2); SLA, specific leaf area ($cm^2\ g^{-1}$). Values shown are means with s.e. of five replicated plants per genotype. Lower case letters following means discriminate genotypes for each parameter based on Tukey's method at a significance level of 0.05. Outputs from the analysis of genotype, environment, and genotype × environment interaction effects on the different growth parameters are presented at the bottom of the table

Genotypes	Root DW (g)	Stem DW (g)	Leaf DW (g)	Plant DW (g)	LA (cm^2)	SLA ($cm^2\ g^{-1}$)
Glasshouse						
Drought-sensitive						
Bambey 21	1.73 ± 0.10c	3.36 ± 0.12bc	4.56 ± 0.16b	7.91 ± 0.11c	1265 ± 69.89a	278 ± 20.86c
IT82E-18	2.56 ± 0.31a	3.40 ± 0.21b	4.30 ± 0.23b	7.70 ± 0.21c	1320 ± 87.51a	307 ± 27.26b
IT83D-442	2.66 ± 0.12a	3.60 ± 0.23b	3.60 ± 0.16c	7.20 ± 0.16c	1096 ± 66.77b	305 ± 26.84b
IT89KD-288	1.21 ± 0.18d	2.40 ± 0.10c	2.41 ± 0.11d	4.82 ± 0.16f	499 ± 21.16f	207 ± 13.56d
IT93K-93-10	2.26 ± 0.09b	4.18 ± 0.21a	4.41 ± 0.15b	8.59 ± 0.25b	1166 ± 24.83ab	264 ± 16.31c
IT97K-556-6	2.00 ± 0.06b	4.45 ± 0.20a	4.19 ± 0.15b	8.64 ± 0.22b	1186 ± 59.29ab	284 ± 12.94c
UC-CB46	2.03 ± 0.07b	3.37 ± 0.10bc	3.87 ± 0.16c	7.25 ± 0.13c	1295 ± 59.33a	335 ± 19.03ab
Drought-tolerant						
IT84S-2049	1.12 ± 0.11d	1.68 ± 0.19e	1.57 ± 0.11e	3.25 ± 0.17g	569 ± 16.24e	364 ± 33.06a
IT97K-207-15	1.66 ± 0.13c	3.74 ± 0.18b	4.43 ± 0.18b	8.16 ± 0.19b	835 ± 19.86d	189 ± 18.83e
IT97K-499-39	2.16 ± 0.08b	4.23 ± 0.18a	5.29 ± 0.12a	9.51 ± 0.14a	1119 ± 38.84b	212 ± 10.41d
IT98K-128-2	1.66 ± 0.17c	3.48 ± 0.17b	2.83 ± 0.18d	6.31 ± 0.17d	867 ± 33.13d	307 ± 11.59b
IT99K-124-5	1.60 ± 0.16c	2.91 ± 0.18c	2.50 ± 0.20d	5.40 ± 0.34e	1016 ± 33.38c	409 ± 28.17a
KVx-61-1	1.73 ± 0.07c	3.83 ± 0.24b	3.61 ± 0.19c	7.44 ± 0.20c	1205 ± 30.46ab	334 ± 18.96ab
Mouride	1.01 ± 0.06d	2.22 ± 0.09d	2.63 ± 0.12d	4.84 ± 0.13f	588 ± 22.01e	224 ± 11.70d
Suvita2	1.08 ± 0.05d	2.65 ± 0.14c	2.62 ± 0.14d	5.27 ± 0 .12e	882 ± 47.61d	337 ± 20.35ab
Sensitive mean	2.06	3.54	3.91	7.44	1118	283
Tolerant mean	1.50	3.09	3.18	6.27	885	297
Outdoor						
Drought-sensitive						
Bambey 21	1.26 ± 0.05 cd	1.01 ± 0.04c	1.19 ± 0.06c	2.20 ± 0.06b	334 ± 18.55a	280 ± 24.08a
IT82E-18	1.53 ± 0.04c	1.11 ± 0.07b	1.40 ± 0.09b	2.52 ± 0.08b	388 ± 16.99a	278 ± 13.15a
IT83D-442	1.39 ± 0.05c	1.01 ± 0.06c	1.59 ± 0.06b	2.60 ± 0.07b	328 ± 29.72a	206 ± 21.46c
IT89KD-288	1.44 ± 0.07c	1.40 ± 0.05a	1.53 ± 0.04b	2.93 ± 0.05b	248 ± 23.84bc	162 ± 12.84d
IT93K-93-10	1.17 ± 0.10d	1.00 ± 0.06c	1.45 ± 0.08b	2.45 ± 0.20b	347 ± 27.53a	240 ± 12.69b
IT97K-556-6	0.99 ± 0.06e	1.11 ± 0.06b	1.17 ± 0.05c	2.28 ± 0.10b	229 ± 20.97c	197 ± 14.63c
UC-CB46	1.68 ± 0.07b	1.27 ± 0.06ab	2.17 ± 0.08a	3.43 ± 0.10a	367 ± 12.31a	170 ± 13.44d
Drought-tolerant						
IT84S-2049	0.73 ± 0.05f	0.76 ± 0.04d	0.76 ± 0.04e	1.53 ± 0.07d	215 ± 14.01c	283 ± 11.98a
IT97K-207-15	1.09 ± 0.10d	1.14 ± 0.06b	1.25 ± 0.11bc	2.39 ± 0.11b	250 ± 20.98bc	200 ± 12.88c
IT97K-499-39	1.84 ± 0.05a	1.30 ± 0.05a	1.61 ± 0.06b	2.91 ± 0.08b	362 ± 30.61a	224 ± 13.07b
IT98K-128-2	1.08 ± 0.03d	1.12 ± 0.04b	1.31 ± 0.09bc	2.43 ± 0.12b	296 ± 18.09b	227 ± 13.08b
IT99K-124-5	1.29 ± 0.09 cd	1.15 ± 0.07b	1.52 ± 0.06b	2.67 ± 0.06b	328 ± 21.72a	216 ± 11.79bc
KVx-61-1	1.10 ± 0.06d	1.32 ± 0.06a	1.46 ± 0.08b	2.78 ± 0.08b	249 ± 18.34bc	170 ± 10.42d
Mouride	0.92 ± 0.05e	1.03 ± 0.04c	0.99 ± 0.04d	2.02 ± 0.06c	282 ± 14.25b	285 ± 19.43a
Suvita2	1.27 ± 0.06 cd	1.33 ± 0.09a	1.52 ± 0.07b	2.85 ± 0.08b	348 ± 12.45a	230 ± 18.64b
Sensitive mean	1.35	1.13	1.50	2.63	320	219
Tolerant mean	1.17	1.14	1.30	2.45	288	229
Environment *F*-value	944.14	11175.20	11513.60	33483.70	14623.80	599.36
Environment Pr > *F*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Genotype *F*-value	122.90	113.51	242.58	470.39	200.44	55.49
Genotype Pr > *F*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Genotype × treatment *F*-value	47.78	94.55	161.03	334.36	121.45	49.67
Genotype × treatment Pr > *F*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

lower in five out of eight tolerant lines than in six out of seven sensitive lines in the glasshouse conditions (Fig. 2*a*).

Under outdoor conditions at 30 DAS, the results for the canopy conductance were similar to those under lower VPD conditions in the glasshouse: (i) canopy conductance was significantly lower in the most tolerant genotypes than in sensitive lines, (ii) the largest differences were recorded at VPD above 6.5 kPa (Fig. 1*b*), (iii) average TR for the whole day was 30% lower in tolerant lines than in sensitive lines (data not shown). The total water transpired per plant during the whole day was also significantly

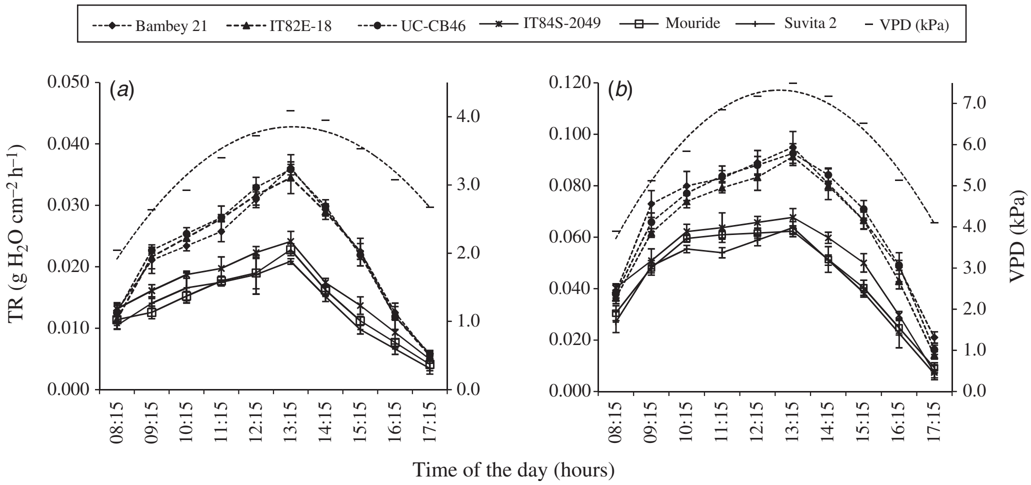

Fig. 1. Transpiration rate (TR, g H_2O cm^{-2} h^{-1}) under well watered conditions of cowpea genotypes contrasting for terminal drought tolerance (tolerant lines: IT84S-2049, Mouride, Suvita2 (solid lines); sensitive lines: Bambey 21, IT82E-18, UC-CB46 (dotted lines)) exposed to natural variation in the atmospheric vapour pressure deficit (VPD) cycle. Plants were grown in (*a*) a glasshouse and (*b*) outdoors, and tested at the vegetative stage over the course of an entire day. Values are the means ($\pm$ s.e.) of five plants per genotype. The polynomial dotted line fitting with the dashed points represents the VPD variation during the course of the day of the experiments.

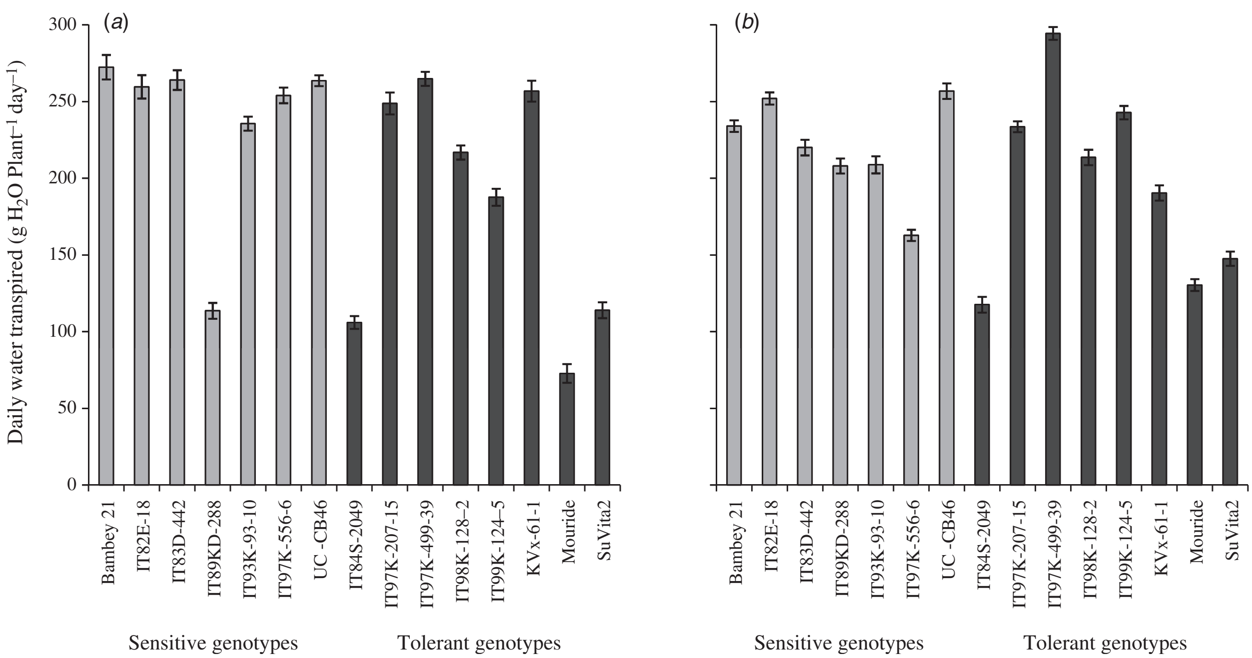

Fig. 2. Total water transpired (TR, g H_2O per plant per day] of drought-tolerant (dark grey bars) and sensitive genotypes (light grey bars) grown under non-limited water conditions. The amount of water loss was estimated on well watered plants over an entire day in (*a*) a glasshouse and (*b*) outdoor conditions at the vegetative stage. Values are the means ($\pm$ s.e.) of five plants for each genotype.

lower in four out of eight tolerant genotypes than in six out of seven sensitive genotypes under well watered conditions outdoors (Fig. 2*b*).

Effect of drought exposure on plant growth and TE

Under glasshouse conditions at the end of the dry-down experiment under WW conditions, the root, stem, leaf and plant biomasses of tolerant genotypes (IT84S-2049, Mouride, Suvita 2, KVx-61–1) remained lower than those of the sensitive ones (IT82E-18, IT83D-422, IT93K-93-10, IT97K-556-6). The same applied to a lesser extent in the WS treatment (Table 3). Biomass increase, total water transpired and TE under WW conditions did not discriminate tolerant from sensitive lines (Table 4). In the WS treatment, the total

Table 3. Dry mass of plant parts of cowpeas subjected to water stress (WS) and well watered (WW) conditions and harvested at the end of the dry-down experiments in glasshouse (top) and outdoor (bottom) environments

Values shown with s.e. are the means of five replicated plants per genotype. Lower case letters following means discriminate genotypes for each parameter based on Tukey's method at a significance level of 0.05. Outputs from the analysis of genotype, water treatment and genotype × treatment interaction effects on the growth parameters are presented at the bottom of the table

Genotypes	Root DW (g)		Stem DW (g)		Leaf DW (g)		Plant DW (g)	
	WW	WS	WW	WS	WW	WS	WW	WS
Glasshouse								
Drought-sensitive								
Bambey 21	3.18 ± 0.11d	2.30 ± 0.18b	6.86 ± 0.10c	4.49 ± 0.14b	4.64 ± 0.19e	4.61 ± 0.16b	11.50 ± 0.13f	9.10 ± 0.17c
IT82E-18	4.15 ± 0.16b	3.11 ± 0.18a	6.65 ± 0.12c	4.61 ± 0.20b	4.74 ± 0.18e	4.64 ± 0.11b	11.39 ± 0.15f	9.25 ± 0.19c
IT83D-442	5.19 ± 0.18a	3.50 ± 0.21a	7.26 ± 0.17b	4.50 ± 0.15b	6.44 ± 0.14b	4.59 ± 0.15b	13.70 ± 0.22c	9.10 ± 0.20c
IT89KD-288	4.64 ± 0.19b	3.22 ± 0.11a	7.16 ± 0.17b	4.35 ± 0.21b	5.21 ± 0.11d	4.16 ± 0.16bc	12.37 ± 0.16e	8.51 ± 0.23d
IT93K-93-10	4.18 ± 0.17b	3.73 ± 0.17a	8.69 ± 0.12a	6.43 ± 0.17a	6.43 ± 0.18b	4.82 ± 0.10b	15.11 ± 0.17b	11.26 ± 0.20a
IT97K-556-6	3.61 ± 0.13c	2.65 ± 0.10b	8.69 ± 0.13a	5.06 ± 0.12ab	7.34 ± 0.15a	5.29 ± 0.15ab	16.03 ± 0.12a	10.35 ± 0.10b
UC-CB46	2.34 ± 0.13e	2.17 ± 0.10b	5.77 ± 0.24d	4.92 ± 0.15ab	4.83 ± 0.13e	3.76 ± 0.18c	10.59 ± 0.30g	8.68 ± 0.18d
Drought-tolerant								
IT84S-2049	3.19 ± 0.15d	3.16 ± 0.15a	3.42 ± 0.15e	3.39 ± 0.17c	3.51 ± 0.19g	3.40 ± 0.17c	6.93 ± 0.16 i	6.79 ± 0.14e
IT97K-207-15	2.70 ± 0.17e	2.50 ± 0.15b	7.44 ± 0.14b	4.82 ± 0.18ab	5.40 ± 0.14d	4.55 ± 0.13b	12.83 ± 0.11d	9.37 ± 0.12c
IT97K-499-39	4.18 ± 0.18b	2.76 ± 0.18b	8.53 ± 0.17a	4.66 ± 0.18b	7.33 ± 0.15a	5.79 ± 0.12a	15.86 ± 0.22a	10.44 ± 0.20b
IT98K-128-2	3.47 ± 0.18c	2.78 ± 0.16b	7.16 ± 0.10b	4.36 ± 0.15b	5.92 ± 0.18c	3.85 ± 0.10c	13.08 ± 0.18d	8.22 ± 0.12d
IT99K-124-5	3.57 ± 0.18c	2.62 ± 0.16b	5.66 ± 0.11d	4.49 ± 0.16b	5.49 ± 0.19d	3.82 ± 0.13c	11.15 ± 0.14f	8.32 ± 0.19d
KVx-61-1	2.87 ± 0.19e	2.51 ± 0.18b	6.61 ± 0.14c	4.49 ± 0.18b	4.72 ± 0.11e	4.12 ± 01.0bc	11.33 ± 0.18f	8.62 ± 0.22d
Mouride	3.71 ± 0.15c	3.10 ± 0.14a	5.79 ± 0.12d	4.39 ± 0.18b	3.89 ± 0.17f	3.78 ± 0.11c	9.57 ± 0.17 h	8.28 ± 0.16d
Suvita2	2.41 ± 0.21e	2.39 ± 0.16b	5.57 ± 0.10d	4.60 ± 0.19b	4.76 ± 0.18e	4.70 ± 0.16b	10.33 ± 0.24g	9.30 ± 0.17c
Sensitive mean	3.90	2.96	7.30	4.91	5.66	4.55	12.96	9.46
Tolerant mean	3.26	2.73	6.27	4.40	5.13	4.25	11.39	8.67
	F-value	*P* > *F*	*F*-value	*P* > *F*	*F*-value	*P* > *F*	*F* -value	*P* > *F*
Genotype	248.17	<0.0001	544.22	<0.0001	556.54	<0.0001	1165.44	<0.0001
Treatment	1329.36	<0.0001	10213.80	<0.0001	2956.41	<0.0001	14515.10	<0.0001
Genotype × treatment	48.17	<0.0001	147.52	<0.0001	116.20	<0.0001	238.48	<0.0001
Outdoor								
Drought sensitive								
Bambey 21	1.88 ± 0.14b	1.66 ± 0.16b	1.87 ± 0.15b	1.33 ± 0.16ab	1.96 ± 0.13c	1.46 ± 0.15 cd	3.83 ± 0.17b	2.79 ± 0.17b
IT82E-18	2.12 ± 0.10ab	1.85 ± 0.17ab	1.79 ± 0.16b	1.50 ± 0.18ab	2.19 ± 0.14b	1.87 ± 0.12b	3.98 ± 0.18b	3.38 ± 0.09a
IT83D-442	2.63 ± 0.16a	2.30 ± 0.16a	1.61 ± 0.18c	1.29 ± 0.10b	2.11 ± 0.16b	1.89 ± 0.16b	3.72 ± 0.11c	3.15 ± 0.16b
IT89KD-288	2.44 ± 0.12a	1.78 ± 0.14b	1.72 ± 0.16c	1.60 ± 0.17a	1.88 ± 0.15c	1.79 ± 0.17b	3.60 ± 0.15c	3.39 ± 0.13a
IT93K-93-10	1.99 ± 0.17b	1.72 ± 0.15b	1.73 ± 0.19c	1.14 ± 0.15bc	2.21 ± 0.15b	1.59 ± 0.13c	3.94 ± 0.14b	2.73 ± 0.17b
IT97K-556-6	1.80 ± 0.14b	1.47 ± 0.13b	1.51 ± 0.16c	1.26 ± 0.18b	1.88 ± 0.15c	1.46 ± 0.14 cd	3.39 ± 0.10c	2.72 ± 0.10b
UC-CB46	2.51 ± 0.14a	1.91 ± 0.17ab	2.22 ± 0.17a	1.56 ± 0.15ab	2.80 ± 0.10a	2.31 ± 0.14a	4.88 ± 0.15a	3.89 ± 0.14a
Drought-tolerant								
IT84S-2049	1.54 ± 0.12b	1.27 ± 0.18c	1.17 ± 0.13d	1.07 ± 0.10c	1.53 ± 0.13d	1.21 ± 0.14d	2.71 ± 0.12d	2.28 ± 0.08c
IT97K-207-15	1.78 ± 0.13b	1.49 ± 0.18b	1.54 ± 0.13c	1.35 ± 0.12ab	1.95 ± 0.20c	1.61 ± 0.14c	3.49 ± 0.10c	2.96 ± 0.19b
IT97K-499-39	2.69 ± 0.15a	2.14 ± 0.13a	1.83 ± 0.14b	1.56 ± 0.12ab	2.68 ± 0.17a	1.83 ± 0.17b	4.52 ± 0.14ab	3.39 ± 0.16a
IT98K-128-2	1.47 ± 0.12b	1.24 ± 0.14c	1.61 ± 0.10c	1.32 ± 0.11b	1.90 ± 0.13c	1.53 ± 0.13c	3.51 ± 0.10c	2.85 ± 0.11b
IT99K-124-5	1.98 ± 0.11b	1.76 ± 0.13b	1.87 ± 0.16b	1.45 ± 0.15ab	2.28 ± 0.15b	1.65 ± 0.13c	4.15 ± 0.11b	3.10 ± 0.14b
KVx-61-1	1.84 ± 0.18b	1.31 ± 0.18bc	1.66 ± 0.12c	1.48 ± 0.15ab	1.86 ± 0.13c	1.64 ± 0.16c	3.51 ± 0.14c	3.12 ± 0.14b
Mouride	1.66 ± 0.13b	1.46 ± 0.12b	1.95 ± 0.16b	1.69 ± 0.18a	2.19 ± 0.16b	1.78 ± 0.15b	4.13 ± 0.19b	3.47 ± 0.15a
Suvita2	2.03 ± 0.14ab	1.53 ± 0.18b	1.90 ± 0.13b	1.80 ± 0.16a	2.09 ± 0.18b	1.93 ± 0.11b	3.99 ± 0.17b	3.73 ± 0.15a
Sensitive mean	2.20	1.81	1.78	1.38	2.15	1.77	3.91	3.15
Tolerant mean	1.87	1.52	1.69	1.47	2.06	1.65	3.75	3.11
	F-value	*P* > *F*	*F*-value	*P* > *F*	*F*-value	*P* > *F*	*F*-value	*P* > *F*
Genotype	308.35	<0.0001	51.11	<0.0001	251.84	<0.0001	298.91	<0.0001
Treatment	1335.75	<0.0001	3997.19	<0.0001	1986.66	<0.0001	2645.69	<0.0001
Genotype × treatment	16.41	<0.0001	12.23	<0.0001	33.40	<0.0001	44.44	<0.0001

water transpired was higher in six out of eight tolerant lines than in five out of seven sensitive lines (Table 4). By contrast, at the end of the dry-down treatment, all genotypes had extracted a similar amount of water from the soil (TTSW, data not shown). TE values did not discriminate tolerant from sensitive lines.

Table 4. Biomass increase (g per plant), total water transpired (g per plant) and transpiration efficiency (g biomass kg^{-1} water transpired) of cowpea genotypes subjected to well watered (WW) (control) and progressive and controlled drought stress (WS) during the dry-down experiments in the glasshouse and outdoor environments

Values shown are the means with s.e. of five replicated plants for each genotype. Genotype means followed with the same letter are not significantly different based on Tukey's test at a significance level of 0.05. Outputs from the analysis of genotype, environment and genotype × environment interaction effects on the different growth parameters are presented at the bottom of the table

Genotypes	Biomass increased (g)		Total water transpired (g)		Transpiration efficiency (g kg^{-1})	
	WW	WS	WW	WS	WW	WS
Glasshouse						
Drought-sensitive						
Bambey 21	3.59 ± 0.11d	1.18 ± 0.09e	1684 ± 20.90e	843 ± 14.39b	2.13 ± 0.07b	1.40 ± 0.11e
IT82E-18	3.68 ± 0.31d	1.55 ± 0.09d	2073 ± 13.48c	647 ± 21.28d	2.38 ± 0.16b	1.80 ± 0.17d
IT83D-442	6.50 ± 0.23b	1.90 ± 0.15c	2234 ± 11.12b	749 ± 27.89c	2.91 ± 0.10ab	2.54 ± 0.22b
IT89KD-288	7.55 ± 0.24a	2.70 ± 0.29b	2183 ± 28.91c	967 ± 25.34ab	3.46 ± 0.12a	2.79 ± 0.08b
IT93K-93-10	6.52 ± 0.20b	2.67 ± 0.22b	2166 ± 14.54c	737 ± 12.56c	3.01 ± 0.11ab	2.62 ± 0.32b
IT97K-556-6	7.39 ± 0.31a	1.71 ± 0.17d	2359 ± 34.12a	713 ± 23.45c	3.13 ± 0.13a	2.40 ± 0.27c
UC-CB46	3.35 ± 0.21d	1.43 ± 0.07d	1878 ± 29.50d	751 ± 15.25c	1.78 ± 0.11d	1.91 ± 0.08d
Drought-tolerant						
IT84S-2049	3.57 ± 0.28d	3.66 ± 0.09a	1524 ± 26.60e	1177 ± 20.69a	2.34 ± 0.19b	3.11 ± 0.08a
IT97K-207-15	4.67 ± 0.19c	1.21 ± 0.18e	2363 ± 27.83a	850 ± 13.04b	1.98 ± 0.08c	1.42 ± 0.22e
IT97K-499-39	6.34 ± 0.29b	0.93 ± 0.07e	2210 ± 23.87b	771 ± 19.91c	2.87 ± 0.13ab	1.20 ± 0.09e
IT98K-128-2	6.77 ± 0.21b	1.90 ± 0.22c	2429 ± 17.13a	730 ± 15.03c	2.79 ± 0.10ab	2.61 ± 0.27b
IT99K-124-5	5.75 ± 0.40c	2.91 ± 0.22b	2126 ± 28.50c	885 ± 17.94b	2.70 ± 0.18ab	3.30 ± 0.29a
KVx-61-1	3.89 ± 0.18d	1.18 ± 0.24e	2051 ± 25.37c	855 ± 19.33b	1.90 ± 0.09c	1.37 ± 0.26e
Mouride	4.73 ± 0.11c	3.44 ± 0.09a	1537 ± 36.76e	1108 ± 15.34a	3.08 ± 0.08a	3.10 ± 0.05a
Suvita2	5.00 ± 0.23c	2.08 ± 0.08c	1515 ± 23.33e	1005 ± 19.41ab	3.30 ± 0.17a	2.07 ± 0.04c
Sensitive mean	5.51	1.88	2083	772	2.69	2.21
Tolerant mean	5.09	2.16	1969	923	2.62	2.27
Outdoor						
Drought-sensitive						
Bambey 21	1.63 ± 0.12b	0.59 ± 0.07c	1516 ± 17.48c	717 ± 14.34b	1.08 ± 0.08bc	0.82 ± 0.01d
IT82E-18	1.46 ± 0.06b	0.56 ± 0.08c	1924 ± 19.73a	707 ± 15.18b	0.76 ± 0.04d	0.79 ± 0.12d
IT83D-442	1.12 ± 0.07c	0.56 ± 0.03c	1382 ± 18.75d	630 ± 16.26c	0.81 ± 0.05c	0.88 ± 0.05d
IT89KD-288	0.67 ± 0.07d	0.45 ± 0.04d	1449 ± 14.16d	560 ± 18.80d	0.46 ± 0.04f	0.81 ± 0.07d
IT93K-93-10	1.49 ± 0.08b	0.27 ± 0.04f	1485 ± 25.47c	546 ± 23.92d	1.00 ± 0.05bc	0.50 ± 0.06g
IT97K-556-6	1.11 ± 0.05c	0.44 ± 0.04d	1549 ± 28.95c	603 ± 15.79c	0.71 ± 0.04d	0.73 ± 0.04e
UC - CB46	1.45 ± 0.06b	0.46 ± 0.05d	1742 ± 26.80b	644 ± 15.85c	0.83 ± 0.03c	0.71 ± 0.06e
IT84S-2049	1.28 ± 0.08c	0.85 ± 0.05b	1142 ± 13.70e	894 ± 13.35a	1.12 ± 0.07b	1.55 ± 0.03ab
IT97K-207-15	1.10 ± 0.10c	0.57 ± 0.03c	1367 ± 19.78d	564 ± 14.02d	0.81 ± 0.07c	1.01 ± 0.04c
IT97K-499-39	1.61 ± 0.11b	0.48 ± 0.05d	1338 ± 16.91d	707 ± 14.90b	1.20 ± 0.10b	0.68 ± 0.03e
IT98K-128-2	1.08 ± 0.05c	0.43 ± 0.05d	1363 ± 11.33d	608 ± 14.97c	0.80 ± 0.04c	0.70 ± 0.03e
IT99K-124-5	1.48 ± 0.10b	0.43 ± 0.03d	1189 ± 12.18e	628 ± 18.28c	1.24 ± 0.08b	0.69 ± 0.02e
KVx-61-1	0.73 ± 0.05d	0.34 ± 0.04e	1328 ± 23.24d	556 ± 15.77d	0.55 ± 0.03e	0.61 ± 0.04f
Mouride	2.11 ± 0.13a	1.45 ± 0.06a	1013 ± 17.99f	824 ± 19.88a	2.08 ± 0.11a	1.75 ± 0.08a
SuVita2	1.05 ± 0.10c	0.98 ± 0.09b	1055 ± 16.33f	811 ± 17.12a	1.99 ± 0.09a	1.21 ± 0.10b
Sensitive mean	1.28	0.48	1578	629	0.81	0.75
Tolerant mean	1.31	0.69	1224	699	1.10	0.95
Environment *F*-value	17759.30	5644.42	70707.80	7838.01	10031.50	4163.08
Environment Pr > *F*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Genotype *F*-value	150.32	204.50	2613.57	830.49	138.50	106.57
Genotype Pr > *F*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Genotype × environment *F*-value	198.43	134.25	1153.12	156.20	96.97	95.95
Genotype × environment Pr > *F*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Under outdoor conditions, in the WW treatment, all tolerant lines had lower total water transpired than five out of seven sensitive lines. TE was also higher in five out of eight tolerant lines than in five out of seven sensitive lines. In the WS treatment, there was genotypic variation for the biomass increase, total water uptake and TE, but no discrimination between tolerant and sensitive lines (Table 4).

Overall, at the end of the dry-down, the most tolerant lines showed lower biomass than the sensitive ones, especially under WW and, to some extent, under WS stress conditions in the low

VPD conditions of the glasshouse. Cowpea accumulated more biomass under low VPD than under high VPD conditions and, as expected, TE was lower under high VPD as compared with the low VPD conditions, for both water treatments. However, several drought-tolerant lines (Mouride, Suvita 2 and IT84S-2049) maintained TE at higher level as compared with all the sensitive lines under high VPD conditions outdoors and especially in the WS treatment.

Response of leaf gas exchange to progressive soil drying

In the glasshouse, the FTSW thresholds for transpiration decline were lower in six out of eight tolerant than in five out of seven sensitive lines. The FTSW thresholds varied between 0.44 and 0.70 (Table 5) with the lowest thresholds recorded in the tolerant genotypes (Mouride, IT84S-2049, Suvita 2) and the highest threshold shown by the sensitive lines (Bambey 21, IT83D-442, IT93K-93–10). A typical transpiration response discriminating tolerant from sensitive lines is presented in Fig. 3*a*, *b*. In outdoor conditions, similar results were obtained, with six out of eight tolerant lines having lower FTSW thresholds than six out of seven sensitive ones (Table 5; Fig. 3*c*, *d*).

Genotypic differences in CTD in response to drought

Under glasshouse conditions in the WW treatment, the CTD did not discriminate tolerant from sensitive lines at the end of the dry-down (45 DAS); Fig. 4*a*). In contrast, under WS conditions, CTD varied among genotypes and was lower in sensitive lines (an average of –0.03°C) than in tolerant lines (an average of 1.39°C) (Fig. 4*b*). Only one sensitive and one tolerant line differed from this. Under outdoor conditions, similar results were obtained. In the WW treatment, there was no clear CTD discrimination between tolerant and sensitive genotypes (Fig. 4*c*). Under WS conditions, the CTD was lower in sensitive lines (an average of –0.74°C) than in tolerant lines (an average of 1.82°C) (Fig. 4*d*).

Scoring for greenness under water deficit

Leaf senescence caused by drought stress varied across genotypes under both glasshouse and outdoor conditions (Table 6), and several cowpea genotypes preserved stem and leaf greenness more than others (Fig. S2). Tolerant Mouride, Suvita 2, IT84S-2049 and IT97K-499–39 remained greener (lower scores) than sensitive Bambey 21, IT82E-18, IT97K-556-6 and UC-CB46 (higher scores). There was a close agreement between the two environments for visually rated leaf damage.

Table 5. Fraction of transpirable soil water (FTSW) threshold values for the 15 cowpea genotypes grown under progressive soil drying in glasshouse and outdoor conditions

FTSW thresholds were calculated using the two-segment plateau regression procedure ± s. e. and 95% confidence interval (CI). Data are the means of five replicates plants for each genotype. FTSW Threshold values identified with the same letter are not statistically different from each other based on Tukey's test at a significance level of 0.05

Genotypes	FTSW threshold	Approximate s.e.	95% CI
Glasshouse			
Drought-sensitive			
Bambey21	0.6319c	0.0341	0.6036–0.6702
IT82E-18	0.6234c	0.0365	0.6062–0.6727
IT83D-442	0.6788d	0.0553	0.6458–0.7160
IT89KD-288	0.6201c	0.0326	0.5048–0.6555
IT93K-93-10	0.6972d	0.0289	0.6492–0.7452
IT97K-556-6	0.6217c	0.0417	0.5780–0.6654
UC-CB46	0.6275c	0.0462	0.6047–0.6702
Drought-tolerant			
IT84S-2049	0.4730a	0.0133	0.4466–0.4999
IT97K-207-15	0.5274b	0.0495	0.4882–0.5667
IT97K-499-39	0.5679b	0.0307	0.5362–0.6095
IT98K-128-2	0.5923b	0.0312	0.5797–0.6349
IT99K-124-5	0.5247b	0.0303	0.4840–0.5655
KVx-61-1	0.5904b	0.0355	0.5692–0.6215
Mouride	0.4449a	0.0232	0.4186–0.4715
Suvita2	0.4765a	0.0240	0.4368–0.5032
Outdoor			
Drought-sensitive			
Bambey21	0.6886c	0.0191	0.6503–0.7168
IT82E-18	0.7129d	0.0307	0.6813–0.7544
IT83D-442	0.6613c	0.0128	0.6358–0.6869
IT89KD-288	0.6650c	0.0102	0.6447–0.6853
IT93K-93-10	0.6864c	0.0128	0.6608–0.7121
IT97K-556-6	0.7227d	0.0140	0.6956–0.7518
UC-CB46	0.6724c	0.0160	0.6403–0.7045
Drought-tolerant			
IT84S-2049	0.4920a	0.0125	0.4670–0.5271
IT97K-207-15	0.6596c	0.0145	0.6310–0.6890
IT97K-499-39	0.6092b	0.0148	0.5896–0.6388
IT98K-128-2	0.6180b	0.0113	0.5953–0.6307
IT99K-124-5	0.6353b	0.0071	0.6012–0.6694
KVx-61-1	0.5978b	0.0121	0.5736–0.6219
Mouride	0.4821a	0.0056	0.4310–0.5033
Suvita2	0.4817a	0.0094	0.4630–0.5104

Discussion

Several traits related to plant growth and patterns of soil water use under WW and WS conditions discriminated terminal drought-tolerant from sensitive genotypes and were able to do so in the glasshouse and outdoor environments.

Plant growth under WW and drought stress conditions

At 30 DAS under WW conditions, most of the tolerant genotypes had lower growth than sensitive lines under low VPD conditions in the glasshouse. These growth differences were not clearly expressed under high VPD conditions outdoors, where growth was depressed, probably because of a depressive VPD effect on leaf expansion (Tardieu *et al*. 2000). These early growth differences were explained by two different mechanisms: (i) lower leaf area in the tolerant lines, and (ii) lower TR. These present results are consistent with previous studies in chickpea (Zaman-Allah *et al*. 2011*a*) and pearl millet (Kholová *et al*. 2010*a*). We suggest that under situations of terminal drought, high early vigour and development of large leaf areas could lead to rapid water depletion and leave plants facing water scarcity while completing their cycle. Therefore, lower early growth caused by decreased leaf area and lower canopy conductance under WW conditions, as found in some tolerant lines, could be an important adaptive response against late

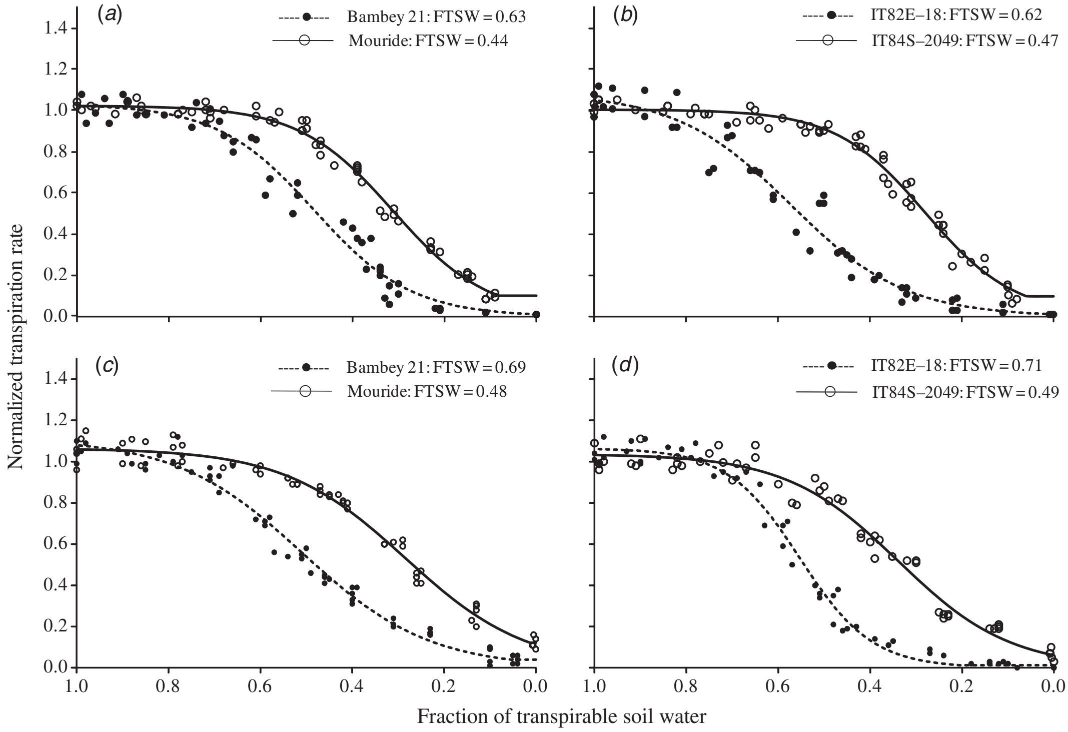

Fig. 3. Normalised transpiration rate (NTR) *vs* fraction of transpirable soil water (FTSW) of tolerant (Mouride and IT84S-2049; open symbols and solid lines) and sensitive (Bambey and IT82E-18; closed symbols and dotted lines) cowpea genotypes exposed to progressive soil drying under (*a*, *b*) glasshouse and (*c*, *d*) outdoor conditions. (*a*, *c*) Mouride and Bambey; (*b*, *d*) IT82E-18 and IT84S-2049. Values indicate the transpiration data of five replicated plants for each genotype at each FTSW level. The FTSW thresholds where transpiration initiated its decline were calculated with a plateau regression procedure. The regression lines of the relationships between NTR and FTSW were drawn by fitting NTR to FTSW data above and below the respective threshold for transpiration decline in each genotype.

season drought stress, as previous hypothesised (Hammer 2006). Our findings in cowpea, added to the earlier work on chickpea, pearl millet or *Sorghum* facing similar stress, clearly indicate that limiting plant growth is a common mechanism across crops facing terminal drought stress. Of course, limiting plant growth would limit potential yield in years or locations where the stress is mild.

At the end of the dry-down experiment, the biomass increase under WS was higher in the tolerant than in the sensitive lines. This was related to the higher soil moisture thresholds where transpiration declined in sensitive genotypes. Similar findings have been reported in peanut (Bhatnagar-Mathur *et al*. 2007; Devi *et al*. 2009), maize (*Zea mays* L.) (Ray *et al*. 2002) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) (Kholová *et al*. 2010*a*). This could also relate to the fact that although TE decreased considerably under high VPD conditions across all genotypes, this decrease was relatively less in drought-tolerant genotypes. Similar result were obtained in wheat (*Triticum aestivum* L.), where tolerant lines maintained higher growth, and increased biomass, water extraction and TE than sensitive lines under water stress (Condon *et al*. 2004).

Genotypic differences in the TR response to natural changes in VPD

Tolerant lines had lower canopy conductance than sensitive lines, and these results were consistently observed under both low VPD (glasshouse) and high VPD (outdoors). The largest differences between tolerant and sensitive lines were recorded around midday, when the VPD was above 3.5 kPa and 6.5 kPa in the glasshouse and outdoors, respectively. The TR computed for the whole day of the experiment was ~40% and 30% lower in tolerant lines than in sensitive lines under low VPD and high VPD conditions, respectively. These lower TR values led to, overall, lower total water transpired per plant per day in the majority of tolerant genotypes than in the sensitive lines under WW conditions in both environments. These results are in agreement with similar findings of lower canopy conductance in terminal drought-tolerant lines of pearl millet where both mechanisms were found: (i) a low canopy conductance at low VPD; (ii) a further restriction of canopy conductance at high VPD (Kholová *et al*. 2010*a*). Terminal drought-tolerant chickpea also had lower TR than sensitive lines, but tolerant and sensitive lines had a response in TR to VPD (Zaman-Allah *et al*. 2011*a*). In previous work on pearl millet, we interpreted that the rapid changes in canopy conductance upon an increase in VPD could only be mediated by hydraulic signals. Our results are, as far as we know, the first evidence of possible hydraulic limitations to transpiration under high VPD in cowpea (Fig. 1), and genotypic differences associated with it that open the possibility of exploiting that feature towards breeding for drought adaptation.

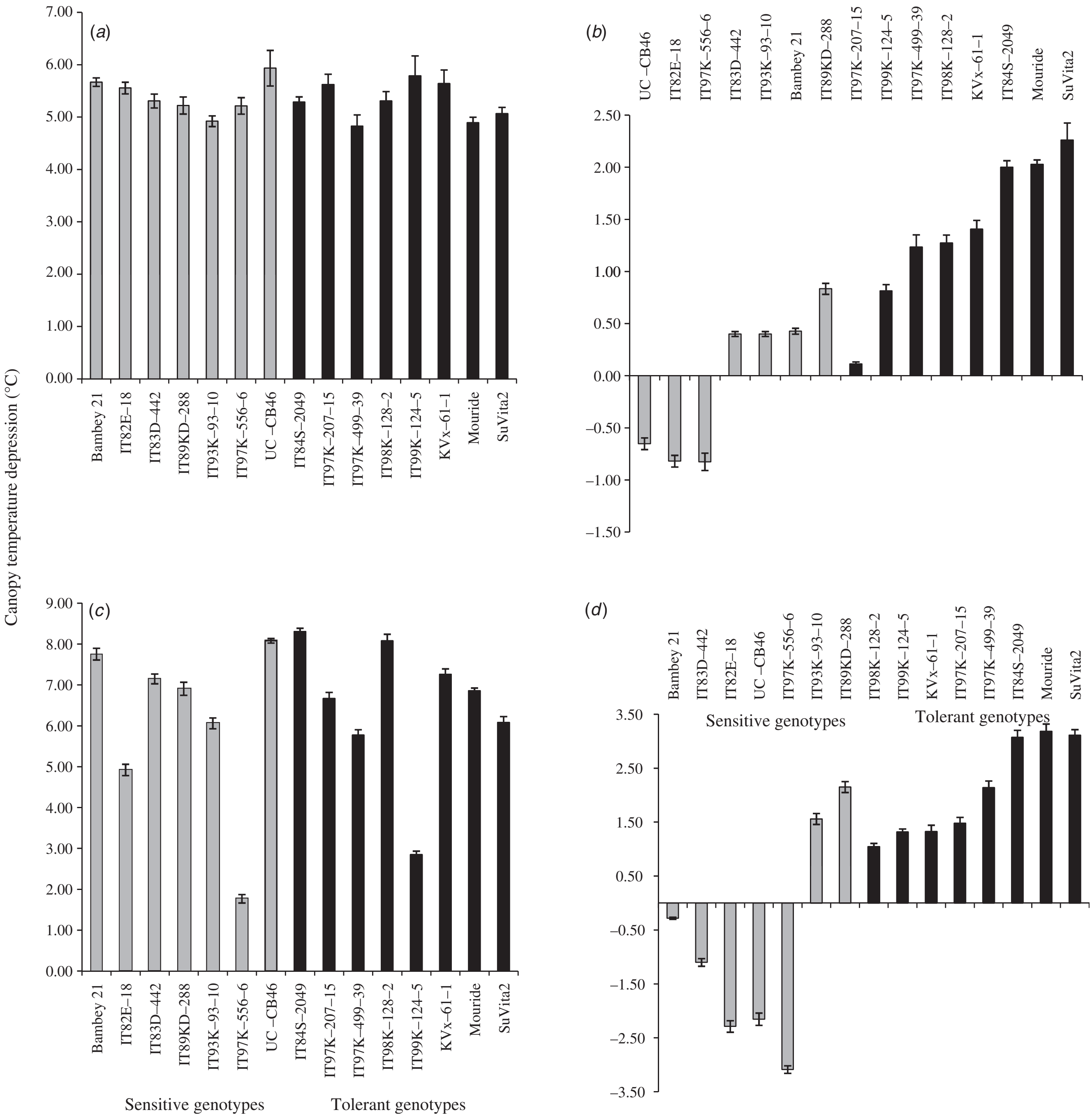

Fig. 4. Differences in canopy temperature depression among contrasting cowpea genotypes (tolerant, black bars; sensitive, grey bars) grown under (*a*, *c*) well watered (WW) and (*b*, *d*) water stress conditions grown (*a*, *b*) in a glasshouse and (*c*, *d*) outdoors. Measurements of leaf temperature were done on the three most recent fully expanded leaves at the end of the dry-down between 0800 and 0900 hours. Values are the means (± s.e.) of five replicates plants per treatment and genotype.

Therefore, in genotypes that are likely to restrict TR, especially at high VPD, there is scope for saving water that would then be available and essential for grain filling late in the season (Sinclair *et al*. 2005; Gholipoor *et al*. 2010; Kholová *et al*. 2010*a*, 2010*b*; Zaman-Allah *et al*. 2011*a*, 2011*b*). It was argued that a lower canopy conductance would lead to higher TE (Sinclair *et al*. 2005). There was indeed a close relationship between a lower TR and a higher TE under high VPD conditions and both water treatments ($R^2 = 0.40$ and 0.76 under WW and WS respectively; Fig. 5*c*, *d*), but this relationship was weak or nonsignificant under low VPD conditions (Fig. 5*a*, *b*). Our interpretation is that, in agreement with the theory, plants that are capable of suppressing

Table 6. Visual scores for the greenness of contrasting cowpea genotypes rated under drought stress conditions outdoors and in the glasshouse

This rating was done at the end of the dry-down experiment (45 days after sowing). Score values are the means (± s.e.) of five replicated plants per genotype. Average scores of sensitive and tolerant lines are presented at the bottom of the table. Threshold values identified with the same letter are not statistically different from each other

Genotypes	Glasshouse	Outdoor
Drought-sensitive		
Bambey 21	2.0 ± 0.0b	3.2 ± 0.4a
IT82E-18	3.6 ± 0.5a	4.0 ± 0.0a
IT83D-442	1.6 ± 0.5b	2.0 ± 0.0b
IT89KD-288	1.4 ± 0.5b	3.2 ± 0.5a
IT93K-93-10	1.2 ± 0.4b	1.4 ± 0.5c
IT97K-556-6	3.2 ± 0.4a	4.4 ± 0.5a
UC-CB46	3.6 ± 0.5a	3.6 ± 0.5a
Drought-tolerant		
IT84S-2049	1.8 ± 0.4b	1.6 ± 0.5b
IT97K-207-15	1.8 ± 0.4b	1.4 ± 0.5c
IT97K-499-39	1.2 ± 0.4b	1.2 ± 0.4c
IT98K-128-2	1.0 ± 0.0c	3.0 ± 0.5a
IT99K-124-5	1.4 ± 0.5b	2.2 ± 0.4b
KVx-61-1	1.6 ± 0.5b	1.8 ± 0.4b
Mouride	1.0 ± 0.0c	1.6 ± 0.5b
Suvita2	1.0 ± 0.0c	1.4 ± 0.5c
Sensitive mean	2.37	3.11
Tolerant mean	1.35	1.78

transpiration at high VPD would have an increased TE; logically, the capacity to limit transpiration at high VPD would be more beneficial in environments where high VPD conditions are more common, like the outdoor conditions in our experiment here. It should be mentioned that a lower TR could also lead to yield penalties, for example under mild stress or unlimited water supply (Sinclair and Muchow 2001; Cho *et al*. 2003; Richards *et al*. 2007; Sinclair *et al*. 2010), and could be the reason for the lower biomass of tolerant lines seen here. Thus, both traits as described above are important to consider for the breeding of crops with enhanced terminal drought-tolerance for regions with high VPD and low water supply.

Variation in FTSW threshold and TE under drought conditions

One of the key findings of this investigation was that the FTSW threshold for transpiration was lower in most tolerant lines than in most sensitive lines in both the glasshouse and outdoors. Therefore, upon progressive exposure to water deficit, transpiration declined in relatively dryer soil (lower FTSW) in the tolerant lines than in the sensitive ones in both low and high VPD conditions. The basis for the calculation of the FTSW threshold is the TTSW, which is the amount of water that can be extracted to support transpiration from the same volume of soil. This trait did not vary between cowpea genotypes, which also agrees with our findings in other crop species. There is often confusion between TTSW and the total water transpired, which is the sum of TTSW and the water added to the WS plants in the course of the dry-down. The water added, of course, varies between genotypes and reflects growth differences between genotypes, and the very purpose of using a WW control is to normalise these differences. The differences in the FTSW thresholds where transpiration declines were in agreement with data obtained in peanut (Bhatnagar-Mathur *et al*. 2007; Devi *et al*. 2009), soybean (Vadez and Sinclair 2001; Hufstetler *et al*. 2007), maize (Ray *et al*. 2002) and pearl millet (Kholová *et al*. 2010*a*). However, these results were different from those obtained in chickpea, where sensitive lines had a decline in transpiration in dryer soils than tolerant lines (Zaman-Allah *et al*. 2011*a*). Sinclair and colleagues (2010) showed that a higher FTSW threshold would contribute to an increase in grain yield in soybean. Our finding of large genotypic contrast for the FTSW thresholds in cowpea opens the possibility of using that trait in breeding. Here, the FTSW thresholds for the decline in transpiration with soil drying were similar across VPD conditions. These results agreed with those reported in maize hybrids (Ray *et al*. 2002), although they differ from earlier assumptions by Denmead and Shaw (1962), who held that the FTSW threshold for the decline of transpiration upon imposition of water deficit should increase if the imposition of water deficit took place in conditions of higher evaporative demand.

Relationships among TR, CTD, TE and the FTSW thresholds for transpiration decline

Since the largest TR differences between tolerant and sensitive lines were achieved at the time of day when the VPD was the highest, the first question was whether these large TR differences could lead to differences in TE, as hypothesised above. TE and TR were indeed closely related but the relationships were significant only in outdoor conditions, where the VPD was high (Fig. 5). The interpretation is that the low TR at high VPD was caused by partial stomata closure under high VPD, as has been shown in other crops (Kholová *et al*. 2010*b*; Devi *et al*. 2010). Therefore, the effective VPD for transpiration in these plants is shifted to a lower value, leading to a higher TE according to the definition of TE (Tanner and Sinclair 1983). Also, a recent report indicates that soybeans with transpiration sensitivity to high VPD reduced stomatal conductance under high VPD, but this was not accompanied by a proportional decrease in photosynthetic activity. This led to differences in intrinsic water use efficiency (Gilbert *et al.* 2011).

A second question was whether the differences in FTSW thresholds for the transpiration decline were related to the lower TR under WW conditions. There was indeed a tight positive relationship between the FSTW thresholds for transpiration decline and TR under both low and high VPD conditions ($R^2 = 0.66$ and 0.71 respectively; Fig. 6*a, b*). Our interpretation is that a lower TR, which leads, in part, to a lower absolute transpiration (Fig. 2), makes drought-stressed plants function like WW ones until the soil has become dryer, as has been previously found and discussed (Kholová *et al*. 2010*a*). This then leads to having a lower FTSW threshold where transpiration drops upon progressive exposure to water deficit stress.

Since TR and the FTSW thresholds are related, as are TR and TE, the third question was then whether these FTSW threshold differences could be related to TE. Under low VPD conditions,

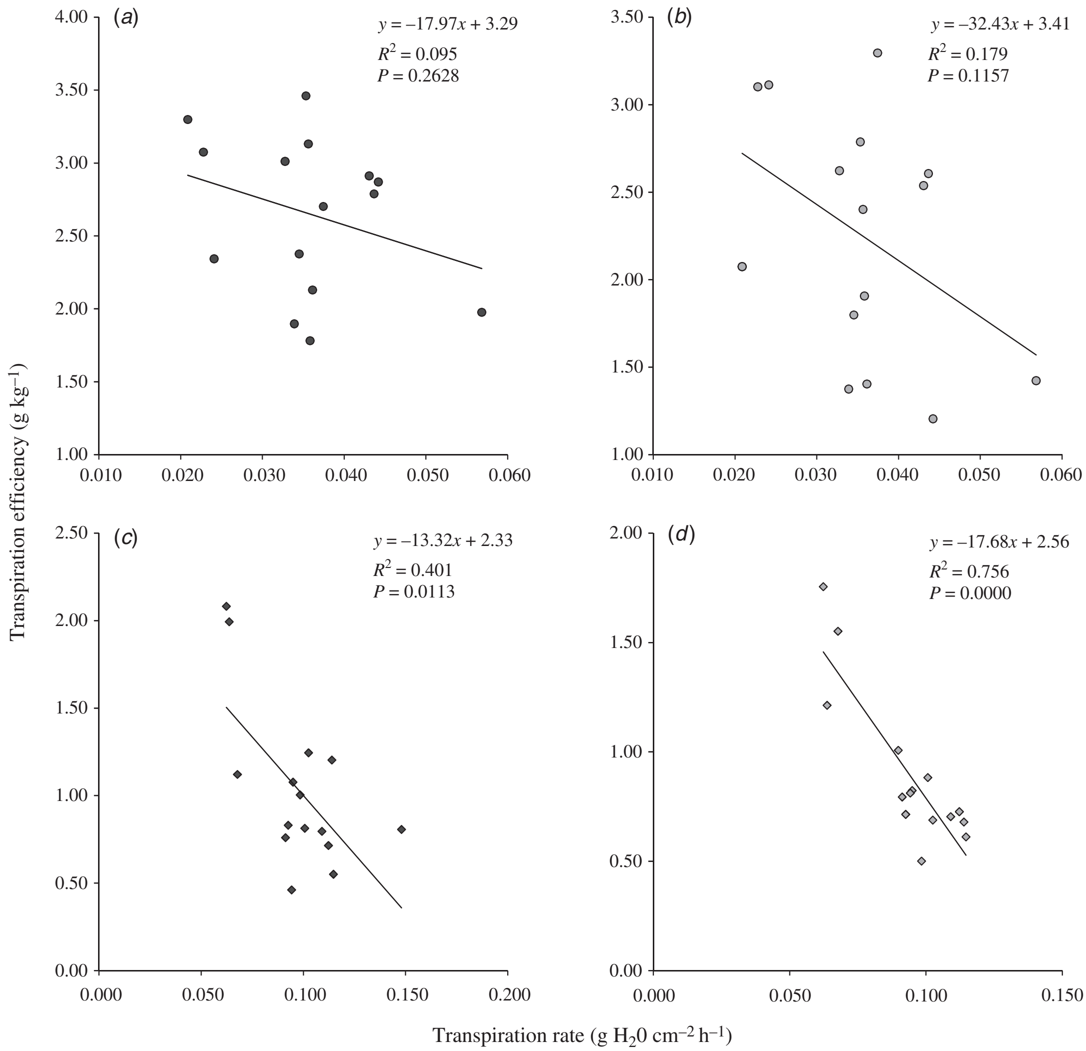

Fig. 5. Relationship between transpiration rate (TR, g H_2O cm^{-2} h^{-1}) and transpiration efficiency (TE, g biomass per kg water transpired) of 15 tolerant and sensitive cowpea genotypes. TR was measured at the highest VPD under well watered (WW) conditions at the vegetative stage, and TE was estimated under both WW and water stress (WS) conditions (*a*, *b*) in the glasshouse and (*c*, *d*) outdoors. (*a*, *c*) WW treatment; (*b*, *d*) WS treatment. Data points are themeans of five replicated plants per genotype and treatment.

the relationship between TE and the threshold for transpiration decline was not significant (Fig. 6*c*). By contrast, under high VPD conditions there was a negative trend between the thresholds and TE (Fig. 6*d*). This agreed with the fact that no difference were observed in TE among genotypes under low VPD conditions; under high VPD conditions, there were substantial TE differences among genotypes. These results were different from those found in peanut (Devi *et al*. 2009), although the polynomial relationship in the 0.2–0.6 FTSW range in this study was relatively poor ($R^2 = 0.39$). By contrast, the results presented here are in agreement with more recent results, which also show a strong negative relationship ($R^2 = 0.88$) between the FTSW thresholds and TE (Devi *et al*. 2011).

Our overall interpretation on these three questions is that the lower TR of tolerant lines during the time of day when the VPD was the highest, which was related to a partial closure of stomata, had two consequences. Firstly, the lower TR of tolerant plants at high VPD led to an increasing TE level, especially in conditions with high VPD such as outdoors in our experiment. This is what we found here in the negative relationships between TE and TR in outdoor conditions (Fig. 5*c*, *d*). Secondly, a lower TR saved water and allowed these plants, when exposed to stress, to function like fully irrigated plants for a larger part of the drying cycle. This is our interpretation of the positive relationships between the FTSW threshold for transpiration decline and TR (Fig. 6*a*, *b*), where plants with low TR have low FTSW values for transpiration decline. It is also illustrated by Fig. 3, in which the NTR of tolerant lines remains at a value of 1 until lower FTSW values are reached (i.e. for a longer time during the drying period). These two consequences are the causal factors behind the relationship between the FTSW thresholds for the transpiration decline and TE at high VPD (Fig. 6*d*). Therefore, the FTSW threshold

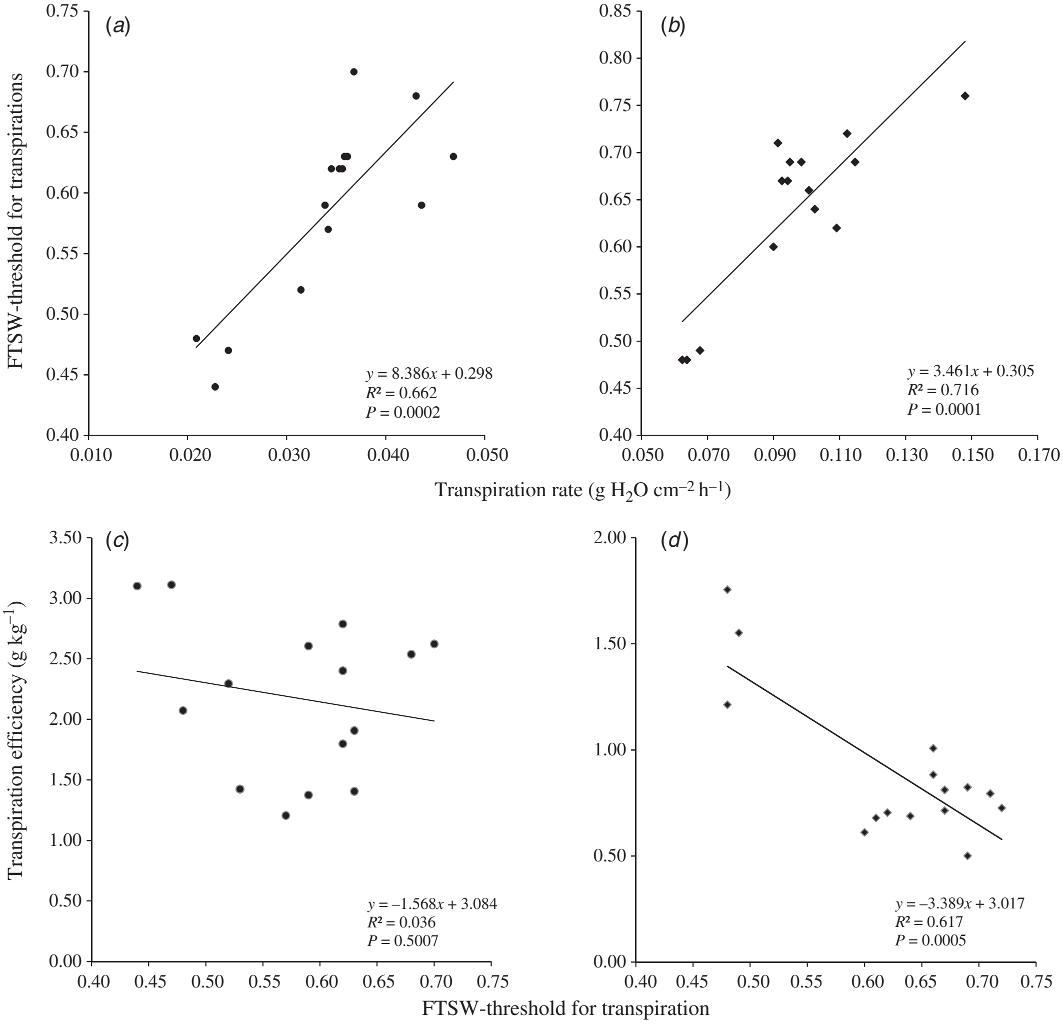

Fig. 6. Relationship between TR and the FTSW threshold for transpiration decline ((*a*) glasshouse; (*b*) outdoors), and between the FTSW threshold and transpiration efficiency ((*c*) glasshouse; (*d*) outdoors) of 15 contrasting cowpea genotypes. Data points are the means of five replicated plants per genotype.

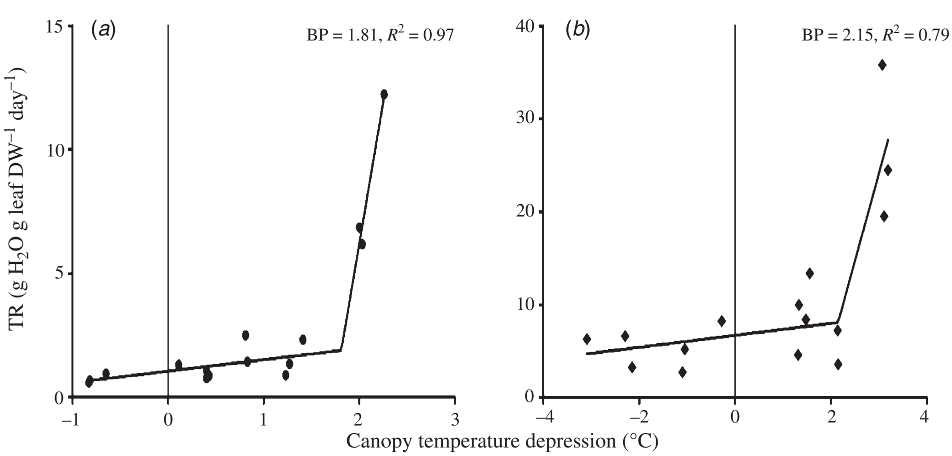

Fig. 7. Relationship between canopy temperature depression (CTD) and transpiration rate (TR) of 15 contrasting cowpea genotypes under drought stress conditions. CTD and TR were measured at the end of the dry-down experiment (*a*) in a glasshouse and (*b*) outdoors. Data points are the means of five replicated plants per genotype.

becomes a very powerful tool for selecting plants that have the capacity to restrict transpiration at high VPD, leading to increasing TE.

At the end of the experiment under WS conditions, tolerant genotypes showed higher CTD than sensitive lines, which indicated that at these late stages of stress, tolerant lines were likely to maintain transpiration activity; this was closely related to the lower leaf senescence scores in these lines. CTD was also closely and positively related to TR in a broken stick regression that described this relationship under both low and high VPD (Fig. 7). Therefore, the measurement of canopy temperature could become an easy way to assess TR in cowpea.

Conclusion

For enhancing crops' terminal drought tolerance, water availability during reproduction and grain filling is crucial (Vadez *et al*. 2007; Zaman-Allah *et al*. 2011*a*). Lower early vigor, lower TR under WW conditions during the vegetative stage, lower leaf area development, sustained transpiration until the soil was relatively drier and lower canopy conductance under high VPD conditions appeared to be the main features discriminating tolerant from sensitive genotypes. Also, significant and close relationships were found between TR and: (i) TE under WW and WS treatments outdoors, (ii) CTD under water stress conditions in both environments, and (iii) FTSW thresholds for transpiration decline. These results support the importance of TR regulation in explaining the differences in adaptation between tolerant and sensitive lines, especially under high VPD where lower TR, explained by partial stomata closure, contributes to water-saving and increases water use efficiency.

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