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Maize, sorghum, and pearl millet have highly contrasting species strategies to adapt to water stress and climate change-like conditions

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ABSTRACT

This study compared maize, sorghum and pearl-millet, leading C_4 cereals, for the transpiration rate (TR) response to increasing atmospheric and soil water stress. The TR response to transiently increasing VPD (0.9–4.1 kPa) and the transpiration and leaf area expansion response to progressive soil drying were measured in controlled conditions at early vegetative stage in 10–16 genotypes of each species grown in moderate or high vapor pressure deficit (VPD) conditions. Maize grown under moderate VPD conditions restricted TR under high VPD, but not sorghum and pearl millet. By contrast, when grown under high VPD, all species increased TR upon increasing VPD, suggesting a loss of TR responsiveness. Sorghum and pearl-millet grown under high VPD reduced leaf area, but not maize. Upon progressive soil drying, maize reduced transpiration at higher soil moisture than sorghum and pearl millet, especially under high VPD, and leaf area expansion declined at similar or lower soil moisture than transpiration in maize and sorghum. It is concluded that maize conserves water by restricting transpiration upon increasing VPD and under higher soil moisture than sorghum and millet, giving maize significantly higher TE, whereas sorghum and pearl millet rely mostly on reduced leaf area and somewhat on transpiration restriction.

1. Introduction

Maize, sorghum and pearl millet are the leading C_4 cereals of tropical and sub-tropical regions (below latitudes of 45°). Sorghum and pearl millet are the staple crop of large population in semi-arid regions of sub-Saharan Africa and Asia. Sorghum is cultivated on about 35 M ha across Africa and Asia, whereas pearl millet is cultivated on about 27 M ha across Africa and Asia, being sometimes the main subsistence mean for small-holder farming communities. These two crops are also highly valued for their crop residues to feed cattle, and in certain context the crop stover is regarded as equally or even more important that the grain. The C_4 plants represent about 3% of all plant species in the plant kingdom [1] but account for 30% of global terrestrial carbon fixation [2]. C_4 plants, by virtue of "Kranz" anatomy of leaves, have higher capacity to fix inorganic CO_2 and more efficient water utilization than C_3 plants [3]. Leading predictions of climate change, like the rise in temperature, inter-annual variation in total rainfall and distribution, may favor C_4 cereals [4]. However, these on-going environmental changes negatively impact yield even in C_4 crops. Maize breeding has undergone long-term yield and agronomic improvement for drought-prone regions [5–8] and is often and increasingly challenged by drought [9]. The rising demand for maize [10] has expanded its cultivation to marginal lands that has led to yield instability [11–15]. Pearl millet and sorghum are considered more resilient than maize, although there is very limited studies comparing these species [16,17]. Nevertheless, all three crops do face significant terminal drought due to cessation of rain towards the end of the rainy season in semi-arid tropics where the cropping period is limited. Therefore, despite their comparative advantage over non-C_4 crops, these three cereals also suffer water stress for which breeding efforts are needed.

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Breeding for drought tolerance can benefit from an integrated physiological approach to better focus on plant features contributing fitness to drought adaptation [18–20,8]. Indeed, breeding for drought adaptation also requires a comprehensive understanding of the nature of the constraint and of the possible plant traits that help adaptation [15,18,21,22,23,24]. Despite the advantage conferred by the "Kranz" anatomy with regard to water use efficiency, maize is considered to be more drought sensitive than sorghum and pearl millet, owing in part to its usually longer duration and larger crop canopy than sorghum and pearl millet. However, there has been no careful comparison whether other physiological mechanisms underlying drought adaptation differentiates maize from sorghum and pearl millet. Here, we tackle this by looking at three aspects of the plant response to either air or soil water limitations that can influence how genotypes respond to water limitation: (i) the transpiration rate (TR) response to an increase in vapor pressure deficit (VPD) under no soil water limitation; (ii) the transpiration response to soil drying, and: (iii) the leaf area expansion response to soil drying. We address both species differences and genotypic variation within species.

Limitation of TR under increasing atmospheric vapor pressure deficit (VPD) operates by a partial closure of stomata under high VPD conditions in the early afternoon hours and then contributes to water saving. Genotypic variability for this trait has been reported in soybean [25,26]; pearl millet [27]; sorghum [28,29]; peanut [30,31]; chickpea [32]; cowpea [33]; and maize [34]. Also, recent studies show that the transient TR response to high VPD can be influenced by the conditions of the growth environment such as temperature [35–37]. Here, we compared the TR response among species and among genotypes of pearl millet, sorghum and maize, and assessed whether the TR response to increases in VPD and the leaf area were affected by having plants grown under moderate and high VPD conditions.

The regulation of stomatal conductance and leaf expansion have been considered to be the main mechanisms by which plants respond to soil water deficits [38,39], and this is hypothesized to be possibly regulated by plant hydraulic conductance [40]. In other words, minimizing water loss can be achieved in response to water deficit conditions by declining either the transpiration per unit leaf area (NTR), or the leaf area expansion rate (LER), at higher levels of transpirable soil water (measured as the fraction of transpirable soil water, FTSW) in the root zone. Genetic variation was found for the FTSW threshold where a decline in transpiration and biomass growth begins (maize: [41,42]; sorghum: [41,42]; millet: [43]). It was observed that the soil–water threshold values where transpiration declines were usually higher than where leaf expansion declined (reviewed by [44]). However, the threshold values in most of the aforementioned studies addressed these two plant processes separately. Here, these responses are measured simultaneously, and they are compared among the three cereals species, taking also into consideration the environmental growth conditions.

Hence, the objective of the study was to compare three major C_4 cereals for traits that have been shown to be involved in the adaptation of crops to water deficit, specifically to: (i) compare the intra- and interspecific variation in the TR response to transient increases in VPD levels, or to progressive exposure to water deficit for plants grown in either moderate or high VPD conditions with no soil water limitation; (ii) compare the leaf expansion rate and transpiration in response to progressive exposure to water deficit.

2. Materials and methods

2.1. Plant material

Ten elite maize hybrids, ten pearl millet testcross hybrids from elite parents and sixteen sorghum elite breeding lines (for postrainy and rainy seasons) with different sensitivity to drought were selected for comparison (Table 1). Several experiments were carried out and several measurements made (described below in sections 2.2 to 2.5). A summary of experiments and measurements is given in Table 2.

2.2. Transpiration response to VPD

Plants were grown in glasshouse in plastic pots (diameter 22 cm at the brim and height 20 cm) filled with 5 kg of soil (composition 3 *Alfisol* : 1 Sand) and occupying a volume of about 4.5 L. The sol was collected from the ICRISAT farm, Patancheru, India, and was fertilized with diammonium phosphate at the rate of 0.30 g kg^{-1} soil, and muriate of potash at a rate of 0.30 g kg^{-1} soil. Farm manure was added also to the Alfisol-sand mix at a rate of 5% v/v. Sowing was done on Dec 06 2012 and April 1, 2013. Each pot was sown with three seeds and thinned to two seedlings per pot one week after sowing and then finally thinned to one plant per pot three weeks after sowing. Thermo-hygrograph sensors (Tinytag Ultra 2 TGU-4500 Gemini Datalogger Ltd, Chichester, UK), well protected from radiation, were positioned within the plants at canopy height in the glasshouse and recorded temperature and relative humidity (RH) % on an hourly basis. The VPD during midday hours ranged between 2.4–2.8 and 3.9-4.5 kPa for the December and April sowing, respectively, therefore exposing the plants to moderate and high VPD growth conditions respectively during their early vegetative period. For both experiments 7–8 plants were grown for each genotype under well-watered (WW) condition until 30 days after sowing (DAS), out of which only the five most uniform plants (replicates) were selected for the VPD response assessment. At 30 DAS, a standard protocol [43] (http://gems.icrisat.org/allinstruments/transpiration-response-to-increasing-vpd/) for the measurement of the transpiration response to increasing VPD was followed. Plants were watered abundantly close to saturation (between 1500–1700 hours) and then allowed to drain extra water overnight to reach field capacity. Then, early morning (0800-0900 h) the pots' soil surface was covered with a round shaped plastic sheet and a uniform layer (2–3 cm) of plastic beads to prevent soil evaporation while maintaining air circulation for root respiration. This reduced soil evaporation by about 90–95% so that transpiration was monitored from changes in pot weights. After covering the soil surface, plants were shifted to growth chambers (Conviron, Model PGW36, Controlled Environment Limited, Winnipeg Manitoba, Canada) with day/night temp and RH% of 32/26 °C and 60/80%, respectively, for one-day acclimatization in both the experiments. The photosynthetic photon flux density at canopy height in the growth chamber was about 670 μmol m^{-2} s^{-1} and 12 h of light. A possible caveat is that light intensity could have been limiting for the three crops tested and the conclusions of this paper need to be taken in that context. The next day, plants were exposed to an increasing ladder of VPD from 0.9 to 4.2 kPa by increasing temperature and decreasing RH% every hour from 0700 to 1600 h (India Standard Time). Plant transpiration was measured gravimetrically from the losses in pot weight between consecutive weighing. Pots were weighed every hour on a 0.01 g precision scale (KERN 3600-2 N, Kern & Sohn GmbH, Balingen, Germany). At the end of the day plants were harvested and the leaf area measured (LI-3100, Li-Cor, Lincoln, Nebraska, USA). Transpiration rate (TR) was calculated as transpiration (mg) per unit of leaf area (m^2) and per unit of time (s). Examples of the transpiration response types, some presenting a linear response across all VPD conditions, and those presented a two-segmented linear response with a breakpoint, in two genotypes of each crops are presented in Supplementary Fig. 1. Also, a temperature and humidity sensor (USB data loggers, Lascar Electronics) was mounted at canopy height inside each chamber to monitor air temperature and relative humidity in the chamber for every 5-min interval.

2.3. Canopy temperature assessment from thermal imagery in maize

The measurement of canopy temperature was done in maize using the protocol presented in Zaman-Allah et al. [32]. The canopy temperatures of the ten maize genotypes were measured in randomized individual field plots at the Pioneer Multi Crop Research Station,

Table 1
Information on ten genotypes of maize, sixteen genotypes of sorghum, and ten genotypes of pearl millet that were used in the experiments. All these genotypes were elite materials in their respective breeding programs.

Crop	Type	Characteristics	Drought response
Maize			
8315622	Hybrids	Tall, high yield	Moderately drought tolerant
18270413	Hybrids	Short, early maturity	Drought tolerant
783527	Hybrids	Medium tall, stable, girth ear	Drought tolerant
4695575	Hybrids	broad leaf, high yield, early maturity	Drought sensitive
22525674	Hybrids	medium yield, medium maturity	Drought tolerant
9424780	Hybrids	High yield, big cob, late maturity	Drought sensitive
14746185	Hybrids	Tall, high consistent yield, late flowering	Drought tolerant
30V92	Hybrids	High yield, response to high plant density, medium maturity	Drought tolerant
900 M Gold	Hybrids	Medium tall, high yield, late maturity	Drought sensitive
Public Check	Hybrids	Medium, high yield, late maturity	Drought sensitive
Sorghum			
296B	Breeding line	*Kharif* maintainer Line	Drought sensitive
BTx623	Breeding line	Template line for genome wide analysis, Hi-coverage BAC library, low canopy temp	Drought sensitive
E 36-1	Breeding line	Non-stay green check line	Drought tolerant
ICSR93024	Breeding line	–	
ICSV1	Breeding line	Postrainy sorghum, resistant to leaf diseases	–
ICSV700-P10	Breeding line	–	–
ICSV745	Breeding line	Dual purpose, midge resistant	–
ICSV93046-P1	Breeding line	–	–
IS18551	Breeding line	–	–
IS9830	Breeding line	–	–
M 35-1	Breeding line	Widely adapted postrainy sorghum	Drought tolerant
N13	Breeding line	Stay green donor	Drought tolerant
PB15220-1	Breeding line	Postrainy sorghum, high yielding	Drought sensitive
PB15881-3	Breeding line	Postrainy sorghum, high yielding	Drought sensitive
PVK 801-P23	Breeding line	Postrainy sorghum, high yielding	Drought sensitive
S35	Breeding line	Stay green	Post-flowering drought tolerant
Pearl millet			
841B	Single Cross	Medium tall, medium-early flowering	Drought sensitive
Pusa 322	Single Cross	841B x PPMI 301	–
863B	Single Cross	Medium tall, medium-early flowering	Drought tolerant
GB8735	Single Cross	Medium height, early flowering	Drought escaper
ICMP 451-P6	Single Cross	Tall, long panicle, bristles	Drought tolerant
H77/833-2	Single Cross	Short, many tillers, photoperiod-sensitive early flowering; seedling heat stress tolerant	Drought sensitive
ICMV-IS 92222	Single Cross	–	–
PT732B-P2	Single Cross	d_2 dwarf, photoperiod-sensitive late	–
PRLT	Single Cross	Medium tall, early flowering; seedling heat stress sensitive	Terminal drought tolerant
Tift 238D1-P158	Single Cross	Late flowering	–

Table 2
Overview of each experiment (either 'VPD', *i.e.* a transpiration response to increasing VPD conditions, or a 'dry-down' consisting in assessing the transpiration and leaf area expansion response to progressive soil drying) and treatment used (WW = well-watered, WS = water stress), average VPD (Vapor pressure deficit expressed in kilo Pascal; kPa) during growth, VPD max, (maximum VPD during the day), average temperature of growth condition (day/night) and number of genotypes included per crop shown in parenthesis. The traits measured were leaf area (LA), transpiration (T), transpiration rate (TR, *i.e.* the amount of water transpired per unit of LA), transpiration response to a transient increase in VPD (TR *vs.* VPD), total transpiration (TT), fraction of transpirable soil water (FTSW) for the initiation of the transpiration decline upon progressive soil drying, transpiration efficiency (TE; biomass accumulated per unit of water transpired), and leaf expansion rate (LER; cm per unit of thermal time) response to progressive soil drying.

ExpNo	Exp.	Treatment	VPD during growth	VPD (Max)	Temp (Max/Min)	Genotypes	Trait measured
1	VPD[a]	WW	Moderate	2.6 kPa	30/15 °C	Maize (10), Sorghum (16), PM (10),	T, TR, LA, TR *vs.* VPD
2	VPD	WW	High	4.2 kPa	36/20 °C	Maize (10), Sorghum (16), PM (10)	T, TR, LA, TR *vs.* VPD
3	Dry-down[b]	WW WS	Moderate	1.2 kPa	29/21 °C	Maize (10), Sorghum (10), PM (10)	TT, FTSW, TE, LER for maize (10) sorghum (10) and PM (2)
4	Dry-down	WW WS	High	4.3 kPa	38/26 °C	Maize (10), Sorghum (16), PM (10)	TT, FTSW, TE, LER for maize (2), sorghum (2) and PM (2)

[a] Transpiration response to VPD.
[b] Transpiration response to progressive soil drying (DD).

Hyderabad, India, in a trial that included maize only, and neither pearl millet nor sorghum genotypes. Canopy temperature was obtained from thermal images obtained with Precision Infrared Thermometer (Fluke 574 CF, Fluke Corporation, Germany) with a sensitivity of 0.09 °C and an accuracy of ± 2%. The images were taken outdoors at the highest atmospheric VPD (VPD between 1400–1500 h was 3.2 kPa; the average VPD at midday was 2.9 kPa). SmartView 2.1.0.10 software (Fluke Thermography Everett, WA, USA) was used for the analysis of the thermal images and the estimation of canopy temperatures. The index of canopy conductance (I_g) was used as an indirect estimation of the absolute canopy conductance ([27,45], modified). From the canopy temperature, I_g was estimated as:

$$Ig = (T_{dry}\text{-}T_{canopy}) / (T_{canopy}\text{-} T_{wet}) \quad (1)$$

Where T_{wet} is the temperature of a wet surface, T_{dry} is the temperature of a non-transpiring surface and T_{canopy} is the canopy temperature measured with the infrared camera. T_{wet} was measured on green leaves after soaking them with water for 5 min and T_{dry} was the temperature of dry leaves. These temperatures were measured under outdoor conditions after the end of the experiment, using green and dried leaves from extra plants of all hybrids, which were pooled to make the measurements.

2.4. Transpiration response to progressive soil drying condition

Maize, millet and sorghum plants were grown in a greenhouse facility (ICRISAT, India) with similar growth practices as the VPD experiment described above. The plants were grown in plastic pots (height 23 cm and diameter 27 cm) filled with 10 kg of red soil (*Alfisol*) and fertilized with di-ammonium phosphate at the rate of 0.30 g kg^{-1} soil. Each pot was sown with three seeds and two weeks later one seedling was thinned out and remaining two plants were allowed to grow in each pot. The maximum VPD during day hours was 1.24 and 4.31 kPa for moderate and high VPD conditions, respectively. For the moderate VPD condition, out of 15 sorghum genotypes only nine were grown due to seed shortage. For both VPD conditions 10 to 14 plants were grown for each genotype under well-water condition until 30 days after sowing (DAS), out of which only nine (3 control and 6 stress) representing the most uniform plants (reps) were selected for the measurement of transpiration response to progressive soil drying (dry-down). At 30 DAS plants were watered abundantly (between 1500–1700 h) and allowed to drain extra water overnight to reach field capacity. Then, next day early morning (0800–0900 h) one of the two plants in each pot was harvested (pre-treatment harvest) for initial biomass estimation and later TE measurement. Then the pots soil surface was covered as explained in the previous section. The pre-treatment harvest plants were kept for hot-air oven drying at 60 °C for 48–72 h. The dry-down experiment was performed using the protocol originally described by Sinclair and Ludlow [46] and standardized for Crop Physiology Laboratory, ICRISAT, India (http://gems.icrisat.org/allinstruments/controlled-imposition-of-water-stress/). Each pot was weighed after bagging and the weight was recorded as the initial pot weight. Thereafter, pots were weighed every day between 0830 and 1000 h. Three of the nine pots of each genotype were selected to be well-watered control plants. The well-watered plants of each genotype were maintained at 100 g below the initial pot weight by daily replacing the amount of water lost from the pot up to 100 g below the field capacity weight. This threshold corresponded approximately to 80% field capacity. The soils in the six water stress pots were allowed to dry. To control and limit the rate at which the soil dried, a maximum daily water loss, set at 100 g, was allowed. Any daily transpiration water loss exceeding 100 g was added back to the pot. The transpiration of all pots on each day was calculated as the difference in water loss between successive days, plus water added. To account for variable transpiration rates among plants within a genotype, a transpiration ratio for each pot on each day was calculated by dividing the transpiration for each drying pot by the average transpiration rate of the three well-watered pots. To smooth possible effects of plant size differences among plants, the transpiration ratios were again normalized for each pot against an average of their initial 3–4 values before plants experienced any stress, so that their normalized transpiration ratios were centered on 1.0 when the drying pot still was in the well-watered range. Dividing each pot transpiration ratio to the average transpiration ratio of first 3 or 4 days of the experiment gave the normalized transpiration ratio (NTR). The dry-down experiment continued until the NTR of a pot decreased to 0.1. After that, plants were harvested and kept for hot-air oven drying at 60 °C for 48–72 h. The total transpirable soil water (TTSW) content of each pot was the difference between the field capacity weight (first weighing) and weight of the pot when NTR fell below 0.1 (final weight). The FTSW (fraction of transpirable soil water) on each day of the drydown was back-calculated once TTSW was known, at the end of the drydown, and calculated as the difference between daily weight and final weight, divided by the total transpirable water.

The dry weight of the pre-treatment harvest and of the final harvest were taken after oven drying. Transpiration efficiency (TE) was calculated by dividing the increase in biomass during the dry-down experiment by the total water transpired during the same period of time. Plant biomass increase for each genotype was obtained by subtracting the genotypic mean of the pre-treatment biomass taken at the beginning of the dry-down from the biomass of each replicated plants harvested at the end of the dry-down experiment (final harvest). The total transpiration was obtained by adding all daily transpiration values.

2.5. Leaf area expansion under progressive soil drying

Non-destructive leaf area expansion during the progressive soil drying study was monitored between 25 and 45 DAS in well-watered (3 replicates) and water-stress (6 replicates) conditions, in the dry-down experiment described above and beginning 5 days before starting the dry-down as initial measurements were difficult and required time to practice. Increase in length and width of all leaves on the main stem was measured with a centimeter scale every morning at the same time to ensure equal time interval between measurements. In the high VPD experiment, measurements were taken on only two contrasting genotypes of each crop in all replications. In the moderate VPD experiment, measurements were taken on ten genotypes of maize, ten genotype of sorghum, and two genotypes of pearl millet. Leaf area (LA) was non-destructively assessed using length and width measurements, calculated by the formula: LA = leaf length*width*0.71; where 0.71 is a shape coefficient [47]. The total LA was then the sum of the leaf area of all individual leaves. At the time of harvest, the total plant leaf area (LA) was measured with a leaf area meter (Model LI-3100 Li-Cor, Lincoln, NE). For expressing leaf expansion, the time scale in thermal units was used [48], with minimal, optimal and maximum cardinal temperatures of 10 °C, 30 °C and 45 °C, respectively. Consequently, the leaf expansion rate (LER) was calculated considering the window of maximum daily linear leaf length increase (typically a 2-day interval on the linear part of the curve). Consequently, the leaf expansion rate [cm $°day^{-1}$] of each individual leaf was calculated considering the linear parts of the leaf expansion curves.

2.6. Statistical analysis

Initially TR *vs*. VPD data were combined for a two-segment linear regression analysis (Prism 5.0, GraphPad, Software Inc.). If a difference in slope ($p < 0.05$) was not obtained in the two-segment linear regression, then all the data for that genotype were represented by a single linear regression. For those genotypes found to be represented by a two-segments linear model, the regression analysis generated the VPD breakpoint which is a junction between the two linear segments. The breakpoint found in moderate and high VPD experiment ranged around 3 kPa (2.76 to 3.42 kPa; Supplementary Table 1), therefore, the cut-off value of 3 kPa was taken to compare the slopes before and after the breakpoint. A central objective of this work was to compare slopes before and after the breakpoint, if any, in plants that had been previously grown under low and high VPD conditions. To do that it was necessary to have slopes measured within comparable VPD intervals across experiments. The analysis of the transpiration response to increasing VPD was then re-done with replicated data points of the transpiration response to VPD below 3.0 kPa and above 3.0 kPa from each genotype, analyzed separately for TR *vs*. VPD as linear regression. Slope values below and above 3.0 kPa and mean data for leaf area (cm^2) and transpiration (g day^{-1}) and transpiration rate (mg H_2O cm^{-2} s^{-1}) of each genotype were then taken as replication within species (crop) for the crop-by-VPD analysis of variance (ANOVA) using GenStat (version 14.1; www.genstat.co.uk). To test the genotypic differences within species two-way ANOVA carried between VPD and genotype for each species. The mean value for slope of transpiration response to VPD below 3.0 kPa and above 3.0 kPa in moderate and high VPD growth conditions were compared using Tukey-Kramer test at $P = 0.05$. Standard error (SE) values were taken for graphical representation of slope above and below 3 kPa in low and high VPD growth condition.

For the dry-down data, two analyses were performed separately for each genotype based on the six pots subjected to drying; firstly, data from the dry-down were analyzed by plotting the NTR *vs.* FTSW and secondly, the leaf expansion rate (LER) under progressive dry-down were analyzed by plotting the NLER *vs.* FTSW. For each genotype a two-segment linear regression was used to describe the data from the six pots and identify the breakpoint, defined as "FTSW threshold (X_T) for linear decline in TR" and "FTSW threshold (X_L) for linear decline in LER". Regression analyses were conducted using Prism 5.0 (GraphPad Software Inc.). The linear segment for the well-watered control plants was defined to be a plateau with the slope equal to zero. FTSW threshold (X_T) and mean data of total water transpired (g), dry biomass (g) and TE (g dry weight accumulated per kg of water transpired) for each genotype were subjected to analysis of variance to test for crop-by-VPD interaction by using GenStat (version 14.1; www.genstat.co.uk). To test the genotypic differences within species for total water transpired and TE two-way ANOVA carried between VPD of growth condition and genotype for each species. The mean value for FTSW threshold for transpiration (X_T) and leaf area expansion (X_L) were compared using Tukey-Kramer test at $P = 0.05$.

3. Results

3.1. Transpiration response to increasing VPD conditions

3.1.1. Analysis of variance for the slope of the transpiration response to VPD, leaf area, and transpiration

The three C_4 species were different ($P < 0.05$) for the slopes of the transpiration rate (TR) response to increasing VPD (TR *vs*. VPD) above 3 kPa ($P < 0.05$) but not in the TR *vs*. VPD slope below 3 kPa (Table 3). VPD in the plant growth environment had indeed a strong effect on the slopes of the transpiration response to increasing VPD below 3 kPa, these being much higher in plants grown under moderate VPD than in plants grown under high VPD ($P < 0.001$). Similarly, the slope of the transpiration response above 3 kPa was mildly higher ($P < 0.05$) in plants grown under moderate VPD than in plants grown under high VPD (Table 3; Fig. 1ab). In addition, there was a Crop × VPD interaction ($P < 0.01$) for TR *vs*. VPD slope above 3 kPa, showing that the transpiration response of the species above 3 kPa depended on the VPD conditions during growth. Specifically, the slope above 3 kPa was higher in high VPD grown maize than in moderate VPD grown maize, whereas it was the contrary for sorghum and pearl millet, *i.e.* the slope above 3 kPa was lower in high VPD grown sorghum and pearl millet. By contrast, there was no Crop x VPD interaction ($P > 0.05$) for TR *vs*. VPD slope below 3 kPa.

Comparing the TR *vs*. VPD slopes below and above 3 kPa separately for plants grown under moderate VPD and for high VPD conditions also revealed important specie differences (Fig. 1ab). In plants grown under moderate VPD conditions, the TR *vs*. VPD slopes below 3 kPa did not differ between plant species. By contrast, TR *vs*. VPD slope above 3 kPa were higher in sorghum and pearl millet than in maize (Fig. 1a). There was also a dramatic decline in the TR *vs*. VPD slope of maize above 3 kPa ($P < 0.0001$), whereas there was no significant decline in pearl millet ($P = 0.4498$) and sorghum ($P = 0.0675$). In plants grown under high VPD conditions, all three species had higher TR *vs*. VPD slopes above 3 kPa than below 3 kPa. The TR *vs*. VPD slopes above 3 kPa did not differ significantly between species (Fig. 1b).

The sorghum and pearl millet were also different ($P < 0.001$) in how the leaf area changed between growth conditions (moderate or high VPD). Sorghum and pearl millet species had a significant reduction in leaf area under high VPD conditions ($P < 0.001$), but not maize. The leaf area decrease was 36% and 39% for sorghum and pearl millet (Fig. 1c). Interestingly, the species also differed in how transpiration (g day^{-1}) changed between the growth conditions: transpiration of maize did not differ between the moderate and high VPD growth conditions. By contrast, high VPD grown sorghum and pearl millet had lower transpiration when grown under high VPD than under moderate VPD (Fig. 1d).

In summary, high VPD had a depressive effect on the leaf area and on the TR *vs*. VPD slopes, and while maize had the most dramatic decrease in the TR *vs*. VPD slope above 3 kPa for plants grown under moderate VPD, sorghum and pearl millet appeared to be those with the largest leaf area decrease when grown under high VPD.

3.1.2. Genotypic difference in maize, sorghum and pearl millet for the slope of transpiration vs. VPD response

There were genotypic differences in all three crops for most traits.

Maize: The slope values for TR *vs* VPD below 3.0 kPa ranged from 8 to 16 mg m^{-2} s^{-1} kPa^{-1} for plants grown under moderate VPD, and ranged from 1.0 to 4.9 mg m^{-2} s^{-1} kPa^{-1} for plants grown under high VPD (Fig. 2a & b; Table 4). The genetic variation observed in the slope of the TR response to VPD above 3.0 kPa VPD ranged from -6.5 to 10.4 mg m^{-2} s^{-1} kPa^{-1} for plants grown under moderate VPD conditions, and from 2.6 to 8.4 mg m^{-2} s^{-1} kPa^{-1} for plants grown under high VPD conditions. Although not significant for all genotypes Table 4), the TR *vs* VPD slopes decreased above 3 kPa in most maize genotypes

Table 3

Crop × VPD analysis of variance for crops (maize, sorghum and pearl millet) and VPD (moderate and high conditions during growth) conducted for the slope of the transpiration response to VPD below 3.0 kPa, the slope of the transpiration response to VPD above 3.0 kPa, leaf area, and transpiration rate in plants grown under moderate and high VPD conditions. ANOVA represents F values and LSD represents least significant difference. *, **, *** Significant at $p < 0.05$, 0.01 and 0.001 respectively.

	Slope ≤ 3.0 kPa			Slope > 3.0 kPa			Leaf area (cm^2)			Transpiration (g)			Transpiration rate (mg H_2O cm^{-2} s^{-1})		
VPD	Moderate	High	Mean	Moderate	High	Mean	Moderate	High	Mean	Moderate	High	Mean	Moderate	High	Mean
Maize	11.06	2.8	**6.76**	2.18	5.15	**4.5**	2453	1873	**2161**	205	191	**198**	34.76	36.51	**35.63**
Sorghum	10.6	1.95	**6.6**	7.2	2.9	**5.81**	2029	1303	**1298**	161	126	**143.5**	32.56	36.88	**34.72**
Pearl millet	9.78	0.92	**5.18**	8.51	4.31	**5.89**	1722	1050	**1664**	129	115	**122**	36.61	41.78	**39.19**
Mean	**10.56**	**1.95**		**6.59**	**4.35**		**1970**	**1309**		**165**	**144**		**34.64**	**38.39**	
Crop	1.62ns			3.12*			52.78***			65.25***			78.39***		
VPD	170.57***			5.43*			122.43***			268.15***			294.71***		
Crop x VPD	0.06ns			5.66**			0.43ns			13.74***			15.10***		
LSD (Crop)	2.55			3.64			221.3			9.64			5.67		
LSD (VPD)	1.35			1.92			116.6			5.51			3.69		

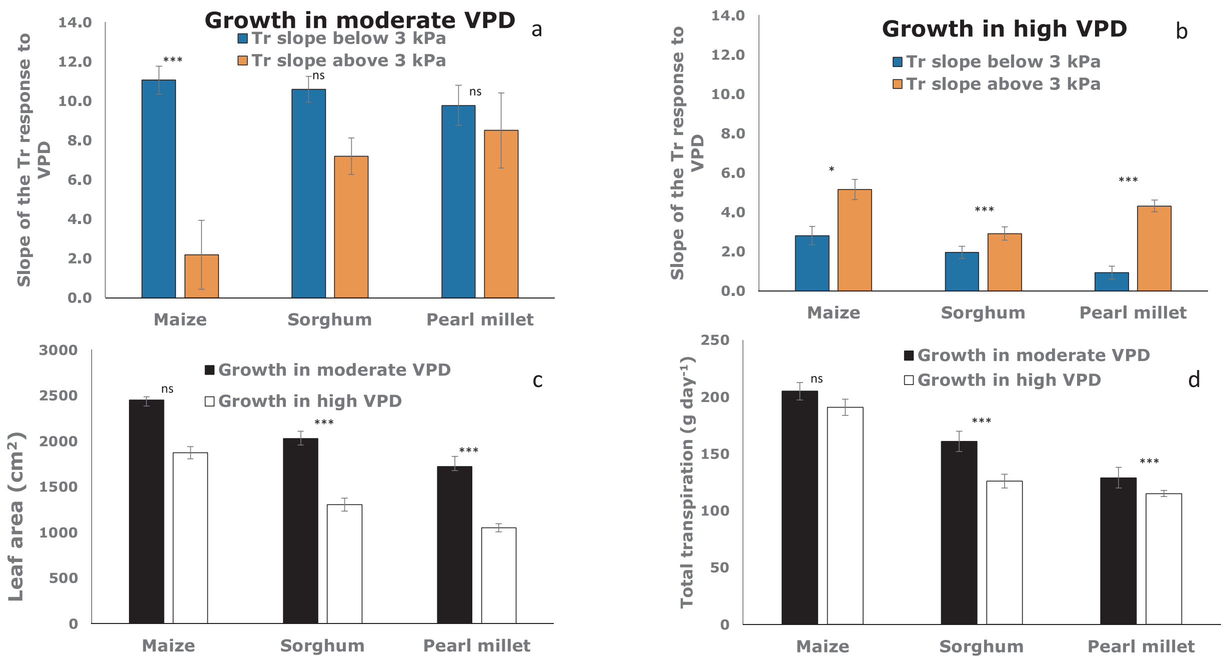

Fig. 1. Transpiration (mg H_2O cm^{-2} s^{-1} kPa^{-1}) *vs.* VPD slope above and below 3 kPa for plants grown under moderate VPD (a), plants grown under high VPD (b), leaf area (cm^2) of plants grown under moderate and high VPD conditions (c), and transpiration (g day^{-1}) (d) in ten genotypes of maize, sixteen genotypes of sorghum and ten genotypes of pearl millet. Bars are SE, calculated by taking each genotype as a replication within a specie. Differences in slopes below and above 3 kPa within species were tested by t-using mean slope values of each genotype (a and b). Differences in leaf area and transpiration were tested by two-way Anova (Table 5). Ns, non-significant.

grown under moderate VPD conditions. By contrast, in plants grown under high VPD conditions the slope of the TR *vs* VPD above 3 kPa increased in most maize genotypes (Fig. 2b; Table 4). Hybrids 783527, 18270413, 22525674, 14746185 and 30V92 showed 4 to 8-fold restriction in TR slope above 3.0 kPa for plants grown under moderate VPD conditions and could save water under high VPD compared to hybrid 8315622 and the public check, which both showed no significant restriction in TR above 3.0 kPa (Fig. 2a).

Sorghum: The slope values for TR *vs* VPD below 3.0 kPa ranged from 6.5 to 16.7 mg m^{-2} s^{-1} kPa^{-1} for plants grown under moderate VPD conditions and ranged from 0.5 to 4.0 mg m^{-2} s^{-1} kPa^{-1} for plants grown under high VPD (Table 4). The slope value for TR *vs* VPD above 3.0 kPa ranged from 2.5 to 13.3 mg m^{-2} s^{-1} kPa^{-1} in moderate VPD condition and from 1.6 to 6.3 mg m^{-2} s^{-1} kPa^{-1} for plants grown under high VPD condition. Different from maize, sorghum genotypes either increased, decreased, or kept unchanged the TR *vs.* VPD slopes above 3 kPa in plants grown under moderate VPD conditions (Table 4; Fig. 2a). By contrast, in plants grown under high VPD conditions the slope of the TR *vs* VPD above 3 kPa increased in most sorghum genotypes (Fig. 2b; Table 4). Genotypes IS18551, N13 and IS9830 showed a 4-5-fold restriction in TR *vs* VPD slope above 3.0 kPa for plants grown under moderate VPD conditions. Genotypes PB15220-1, PB15881-3, PVK 801-P23, ICSV93046-P1, ICSR93024 and ICSV1 showed the highest slope values above 3.0 kPa for plants grown under both the VPD conditions (Fig. 2a & b).

Pearl Millet: The slope values for TR *vs* VPD below 3.0 kPa ranged from 4.0 to 15.3 mg m^{-2} s^{-1} kPa^{-1} for plants grown under moderate VPD conditions and ranged from 0.2 to 3.5 mg m^{-2} s^{-1} kPa^{-1} for plants grown under high VPD conditions (Fig. 2a & b). The slope values for TR *vs.* VPD above 3.0 kPa ranged from −1.7 to 16.2 mg m^{-2} s^{-1} kPa^{-1} for plants grown under moderate VPD conditions and ranged from 1.8 to 4.8 mg m^{-2} s^{-1} kPa^{-1} above 3.0 kPa for plants grown under high VPD

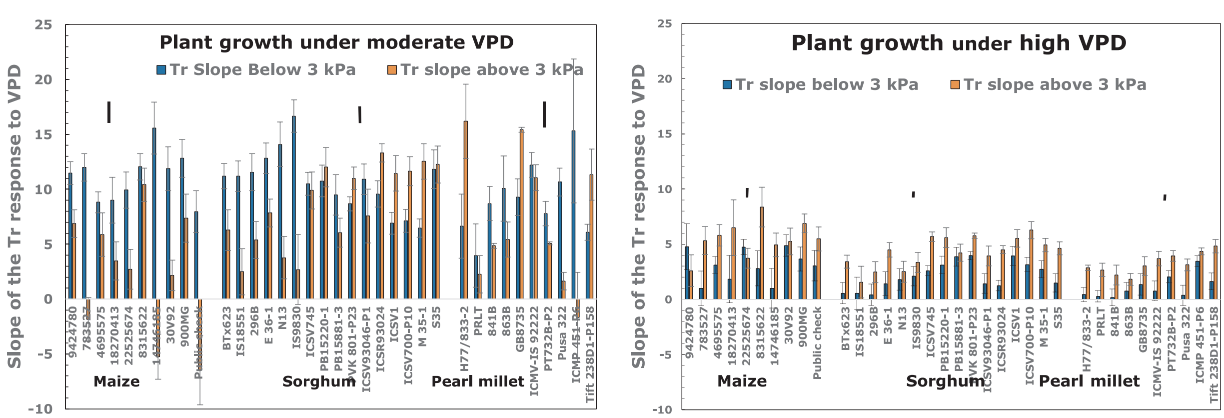

Fig. 2. Genotypic variation in the transpiration *vs.* VPD slope (mg H_2O cm^{-2} s^{-1} kPa^{-1}) measured upon increasing VPD in the course of the day, below 3 kPa and above 3 kPa, and plotted separately for plants grown under moderate VPD (a) and under high VPD conditions (b) in ten genotypes of maize, sixteen genotypes of sorghum and ten genotypes of pearl millet. Bars are SE of five replicated plants in each genotype. Bars are SED's, provided for each specie and growth condition to allow comparison of slope in each genotype.

Table 4
Genotypic variation for the slope of the transpiration response to increasing VPD conditions below 3 kPa and above 3 kPa. Measurements were made in ten genotypes of maize, sixteen genotypes of sorghum and ten genotypes of pearl millet grown under moderate VPD and high VPD conditions. Five replicated plants of each genotype were used.

VPD	TR *vs.* VPD Slope below 3.0 kPa		TR *vs.* VPD Slope above 3.0 kP	
	Moderate	High	Moderate	High
Maize				
9424780	11.48c	4.77	6.87	2.61a
783527	12.01cd	1.00a	−1.85	5.35d
4695575	8.82ab	3.13b	5.87	5.81de
18270413	9.0ab	1.84ab	3.48	6.52e
22525674	9.93b	4.75d	2.71	3.74b
8315622	12.05cd	2.81b	10.42	8.39f
14746185	15.58e	1.02a	−5.25	4.95c
30V92	11.88c	4.88d	2.16	5.27d
900 MG	12.82d	3.66c	7.38	6.9e
Public check	7.96a	3.05b	−6.48	5.49d
P (0.05)	***	***	ns	***
Sorghum				
BTx623	11.2de	0.57a	6.29c	3.43c
IS18551	11.19de	0.55a	2.49a	1.57a
296B	11.56e	0.43a	5.38c	2.48b
E 36-1	12.83f	1.42b	7.85d	4.49d
N13	14.1g	1.81bc	3.77b	2.52b
IS9830	16.66h	2.11bcd	2.69a	3.35c
ICSV745	10.49d	2.61cd	9.9e	5.7e
PB15220-1	10.75d	3.11d	12.02	5.61e
PB15881-3	9.5c	3.87de	6.04c	4.23d
PVK 801-P23	8.69b	3.97e	11f	5.79e
ICSV93046-P1	10.92	1.44b	7.57d	3.95cd
ICSR93024	9.58c	1.24b	13.33h	4.52d
ICSV1	6.91a	3.94e	11.45fg	5.54e
ICSV700-P10	7.14a	3.16d	11.65fg	6.29f
M 35-1	6.48a	2.75cd	12.55gh	4.94de
S35	11.84e	1.5b	12.26gh	4.64d
P (0.05)	***	***	***	***
Pearl Millet				
H77/833-2	6.66b	0.44a	16.19	2.87bc
PRLT	3.96a	0.28a	2.25b	2.68bc
841B	8.71cd	0.19a	4.86c	2.21a
863B	10.07d	0.76ab	5.43d	1.83a
GB8735	9.28d	1.36b	15.43f	3.04b
ICMV-IS 92222	12.22e	0.77ab	11.06e	3.7c
PT732B-P2	7.8c	2.05bc	5.08cd	3.96cd
Pusa 322	10.66d	0.37a	1.63b	3.14b
ICMP 451-P6	15.32f	3.47d	−1.68a	4.36cd
Tift 238D1-P158	6.08b	1.64bc	11.33e	4.84d
P (0.05)	***	***	***	***

conditions. Here also, different from maize and similar to sorghum, the TR *vs*. VPD slopes above 3 kPa in plants grown under moderate VPD conditions either increased, decreased, or remained unchanged (Table 4; Fig. 2a). As for sorghum and maize, in plants grown under high VPD, the slope of the TR *vs* VPD above 3 kPa increased in most pearl millet genotypes (Table 4; Fig. 2b). Genotypes 841B, 863B, ICMP-451-P6 and Pusa 322 showed a 2 to 6-fold transpiration restriction for plants grown under moderate VPD conditions compared to GB8735, H77/833-2, and Tift 238D1-P158, which showed an increase in slope above 3.0 kPa.

3.1.3. Genotypic difference in maize, sorghum and pearl millet for the leaf area and transpiration

Leaf area of maize, sorghum and pearl millet genotypes ranged between 2173-2547 cm^2, 1254-2526 cm^2 and 1467-2553 cm^2 when grown in moderate VPD, and ranged between 1351-2211 cm^2, 424-1504 cm^2 and 893-1438 cm^2 when grown in high VPD condition, respectively (Table 5). However, this reduction caused by high VPD conditions was significant for sorghum and pearl millet but was not significant for maize (Table 5). This was also shown by the relative closeness of leaf area values to the 1:1 line for maize (Fig. 3a). The analysis also showed an absence of significant genotype-by-VPD interactions in sorghum and pearl millet, indicating that all genotypes were affected by the VPD conditions.

The total transpiration in maize, sorghum and pearl millet ranged between 142–232, 116–239 and 90–170 g per day in plants grown under moderate VPD conditions and 136–217, 72–149 and 107–135 g per day in plants grown under high VPD conditions, respectively (Table 5; Fig. 3b). Similarly, it was observed that the mean values showed reduction in total transpiration, by 7%, 30% and 21% in maize, sorghum and pearl millet, respectively. Here also this reduction was significant for sorghum and pearl millet but was not significant for maize (Table 5; Fig. 3b).

In summary, pearl millet and sorghum differed from maize in that they dramatically decreased leaf area and transpiration under high VPD conditions, while maize didn't.

3.1.4. Relationships between measured traits in moderate VPD and high VPD

The regression between leaf area and transpiration by species was significant ($P < 0.05$) for plants grown under moderate VPD conditions (Fig. 4a) but not for plants grown under high VPD conditions (Fig. 4b). For plants grown under moderate VPD conditions (Fig. 4a) leaf area explained 82% of the total transpiration in maize but only 46% and 38% in sorghum and pearl millet, respectively.

3.2. Canopy temperature assessment from thermal imagery in maize

The canopy temperature assessed by thermal imagery ranged from 35.8 to 41.1 °C among the ten maize hybrids (Fig. 5). These measurements were made on maize only, taking the opportunity of a field trial carried out at the Pioneer Multi Crop Research Center and where the maize hybrids used in this work were tested. Hybrids 783527, 18270413, 22525674, 14746185 and 30V92 showed a comparatively higher canopy temperature over the other maize hybrids. These five genotypes also showed higher restriction in TR slope above 3.0 kPa in plants grown under moderate VPD conditions (Fig. 2).

3.3. Transpiration response to progressive soil drying condition

A non-linear two segment regression graph was plotted for NTR *vs.* FTSW value to obtain FTSW threshold (X_T) representing the initiation of a significant decline in transpiration of stressed plant compared to controlled plants in response to soil water stress. Averaged total water transpired, biomass accumulation, TE and FSTW threshold (X_T) at which transpiration started to decline are presented in Table 6.

3.3.1. Analysis of variance for transpiration, TE, biomass, and the TR response to soil drying

Maize, sorghum and pearl millet crop species were significantly different ($P < 0.001$) for total water transpired, plant biomass, TE and TR response to soil drying when grown in moderate and high VPD conditions (Table 6). In particular, the FTSW thresholds for the transpiration decline upon soil drying were higher in maize than in sorghum and pearl millet for plants grown under high VPD conditions. VPD conditions in the growth environment significantly increased ($P < 0.001$) total water transpired and biomass but did not change ($P > 0.05$) the TE and the FTSW thresholds for the transpiration decline upon soil drying. There was also a significant ($P < 0.01$) crop × VPD interaction effect for total water transpired ($P < 0.001$), plant biomass ($P < 0.01$) and TR response to soil drying ($P < 0.001$) but not ($P > 0.05$) for TE. Both TE and FTSW threshold for the transpiration decline were not significantly different for plants grown under moderate and high VPD conditions.

Total water transpired during the dry-down for plants grown under high VPD was highest for maize (3857 g $plant^{-1}$) followed by sorghum

Table 5
Genotypic variation for leaf area measured at the end of the VPD response experiment (1 and 2 – see Table 2), total transpiration measured during the VPD response experiment (with no soil moisture limitation), and genotypic variation for the total transpiration and transpiration efficiency (TE) under progressive soil drying, measured in the dry-down experiments (3 and 4 – see Table 2) over a 15 days period at vegetative stage (V_5-V_9) in ten genotypes of maize, sixteen genotypes of sorghum and ten genotypes of pearl millet grown under high and moderate VPD conditions.

	Transpiration response to (VPD)				Soil-Moisture Stress (Dry-Down)			
	Leaf area (cm^2)		Transpiration ($g\ day^{-1}$)		Total Water transpired (g)		TE ($g\ DW\ kg^{-1}\ H_2O$)	
Growth VPD	Moderate	High	Moderate	High	Moderate	High	Moderate	High
Maize								
9424780	2489	1857	197.9	217	1516	3113	8.7	8.9
783527	2547	1767	231.6	192.2	1637	4261	6.5	6.2
4695575	2454	1784	204	173.9	1532	3837	7.6	7.7
18270413	2342	1818	198.8	196.9	1589	3893	7	6.4
22525674	2448	1885	210.1	185.2	1606	4118	8.2	7.7
8315622	2173	1796	185.8	164.2	1530	3816	7.1	9.3
14746185	2328	2211	207.8	197.1	1531	4165	6.6	8.3
30V92	2356	1685	202	190.2	1627	3626	7.1	7.3
900 MG	2265	1351	217.6	135.6	1638	3961	6.9	7.9
Public check	2406	1859	141.6	198.3	1615	3774	7.3	8.4
F (Genotype)	42.13***		3.84*		5.08**		5.19***	
F (VPD)	0.77ns		0.71ns		457.2***		0.22ns	
F (Geno x VPD)	0.64ns		0.75ns		1.3ns		0.69ns	
LSD (Genotype)	397.24		43.98		442.4		1.01	
LSD (VPD)	524.3		19.67		571.1		1.51	
Sorghum								
BTx623	1673	424	186	72	1390	3295	4.7	6.8
IS18551	1840	650	150.2	95.5	1268	3014	7.5	7
296B	1254	751	203.5	102.8	1284	4118	4.1	6.7
E 36-1	1360	1504	199.2	97.2	1220	2697	6.7	6.9
N13	1874	1031	239.3	133.6	1349	3042	7	6.6
IS9830	1704	1064	149.8	136	1286	3067	7.9	6.6
ICSV745	1575	1163	173.4	77	1237	4192	5.7	7.5
PB15220-1	1541	736	115.8	126.9	1307	3296	7.3	6.5
PB15881-3	1660	1105	163.6	102.4	1285	3363	10	7.3
PVK 801-P23	1692	952	127.3	102.4	1487	3548	7.6	7.6
ICSV93046-P1	1764	1265	178.5	102.9		2498		8.2
ICSR93024	1884	1248	144.7	127.8		3267		5.9
ICSV1	2254	1391	123.4	130.6		3121		7.2
ICSV700-P10	1955	1134	122.5	149		3781		6.9
M 35-1	1993	1104	141.6	147.2		3283		7.8
S35	2526	1274	197.9	137.4		3867		5.4
F (Genotype)	2.35**		4.71***		6.32***		5.02***	
F (VPD)	150***		59.43***		186.75***		2.23ns	
F (Geno x VPD)	1.44ns		1.53ns		0.94ns		1.70ns	
LSD (Genotype)	351.4		27.65		659.9		0.67	
LSD (VPD)	497		39.25		930		0.9	
Pearl Millet								
H77/833-2	1715	1163	89.8	116.8	1590	2970	6.3	6
PRLT	2210	1289	131	125.4	1809	2917	6.9	6.9
841B	1726	1199	102.5	112.6	1881	3501	5.9	6.6
863B	2248	1338	130.5	125.9	1791	3384	6.6	6.3
GB8735	1583	893	161.4	106.9	1842	3044	6.2	6.3
ICMV-IS 92222	2226	1291	169.6	116.3	2001	3713	6.3	7.1
PT732B-P2	2553	1193	94.3	128.8	1864	3098	5.2	5.6
Pusa 322	2002	1236	149.7	128.6	2097	3533	6.2	8.6
ICMP 451-P6	1837	1438	150.1	134.7	1767	3301	5.8	6.1
Tift 238D1-P158	1467	1275	106.2	119.7	1593	3469	5.1	5
F (Genotype)	4.42***		7.83***		0.87ns		5.55***	
F (VPD)	42.57***		13.60***		149.45***		3.22ns	
F (Geno x VPD)	1.45ns		0.78ns		0.41ns		1.75ns	
LSD (Genotype)	411.5		25.25		239.2		0.39	
LSD (VPD)	581.9		35.71		754.6		1.24	

(3341 g $plant^{-1}$) and pearl millet (3393 g $plant^{-1}$). Dry biomass accumulation per plant for plants grown under high VPD was highest for maize (29.4 g) followed by sorghum (22.6 g) and pearl millet (21.3 g) (Table 6). For plants grown under moderate VPD conditions, pearl millet (1824 g $plant^{-1}$) transpired significantly more than maize (1583 g $plant^{-1}$) and sorghum (1312 g $plant^{-1}$). However, the biomass accumulation at the end of dry-down for maize (11.6 g) and pearl millet (11.0 g) were higher than for sorghum (9.0 g). As a consequence, maize had significantly higher TE than sorghum and pearl millet for plants grown under high VPD conditions and significantly higher TE than pearl millet for plants grown under moderate VPD conditions. Therefore, for maize the water conserved by restricting transpiration under high VPD condition was associated with greater sustained biomass accumulation during the water stress than for sorghum and pearl millet.

3.3.2. Genotypic difference in maize, sorghum and pearl millet for TE, and the FTSW threshold for TR decline upon soil drying

Maize: TE showed significant genotypic differences but showed no

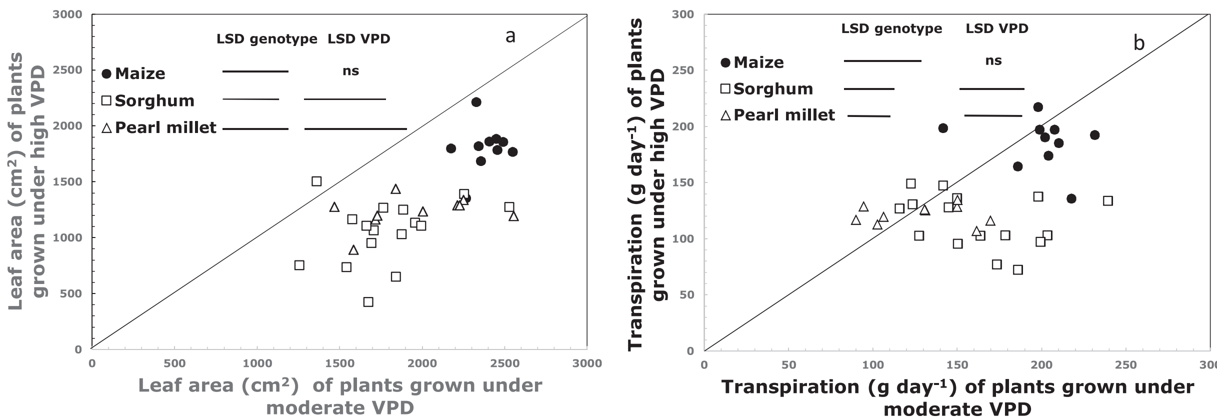

Fig. 3. Relationship between (a) leaf area and (b) transpiration values obtained from plants grown under low and high VPD, and genotypic variation for (a) leaf area (cm^2) and (b) transpiration in ten genotypes of maize, sixteen genotypes of sorghum and ten genotypes of pearl millet for plants grown under moderate and high VPD conditions. Line across the graphs represents the 1:1 line. LSD bars indicate VPD and genotypic effect within each specie, coming from a two-way Anova (see Table 5 for details). ns, non-significant.

significant VPD effect, nor genotype-by-VPD interactions (Table 5). TE for plants grown under high VPD conditions ranged from 6.4 to 9.3, whereas the variation for plants grown under moderate VPD conditions from 7.0 to 8.7 g DW kg^{-1} (g dry weight accumulation per kg of water transpired). Genotype 4695575 had the lowest TE and genotype 783527 had comparatively the highest TE in both VPD growth conditions. Genotype 18270413 recorded the highest TE in high VPD conditions (9.3 g kg^{-1}). The FTSW threshold showed significant genotypic differences and ranged between 0.5 (900 M Gold) to 0.64 (9424780) for plants grown under high VPD and 0.37 (783527 and 4695575) to 0.59 (Public check) for plants grown under moderate VPD. Genotype 783527 had the lowest FTSW threshold among all genotypes (Table 7).

Sorghum: TE showed significant genotypic differences but showed no significant VPD effect, nor genotype-by-VPD interactions (Table 5). TE ranged between 4.1 and 10 g kg^{-1} for plants grown under high VPD conditions (Table 5), much larger than for plants grown in moderate VPD conditions (6.6-7.6 g kg^{-1}). The FTSW threshold showed significant genotypic differences and ranged from 0.21 (ICSV1) to 0.57 (ICSR93024) for plants grown under high VPD and 0.32 (ICSV745) to 0.54 (IS9830) for plants grown under moderate VPD growth conditions (Table 7).

Pearl Millet: TE showed significant genotypic differences but showed no significant VPD effect, nor genotype-by-VPD interactions (Table 5) and ranged between 5.0–8.6 g kg^{-1} for plants grown under high VPD conditions and from 5.1 to 6.9 g kg^{-1} for plants grown under moderate VPD conditions. The genotype ICMV-IS-92222 (8.6 g kg-1) and GB8735 (7.1 g kg^{-1}) showed the highest TE for plants grown under high VPD conditions, whereas Pusa 322 (6.6 g kg-1) had the highest TE for plants grown under moderate VPD conditions. Tift238D1-P158 and ICMP 451-P6 had the lowest TE for plants grown under both VPD conditions. The FTSW threshold showed significant genotypic differences and ranged from 0.32 (Pusa 322) to 0.57 (PT732B-P2) for plants grown under high VPD and 0.48 (863B) to 0.57 (Tift238D1-P158) for plants grown under moderate VPD conditions (Table 7).

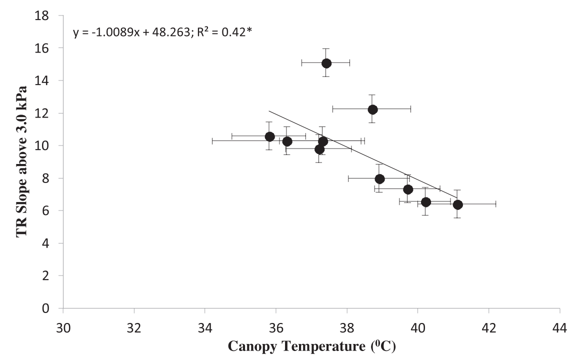

Fig. 5. Relationship between variation of canopy temperature (ºC) captured by Infra-red cameras (Fluke Thermography Everett, WA, USA) at the highest atmospheric VPD of the day (VPD between 1400–1500 h) and the slope of the transpiration response to VPD at VPD values above 3 kPa . SmartView 2.1.0.10 software was used for the analysis of the thermal images and the estimation of canopy temperatures in maize hybrids contrasting for VPD sensitivity grown outdoors under well watered conditions and the measurements were made at 56 DAS.

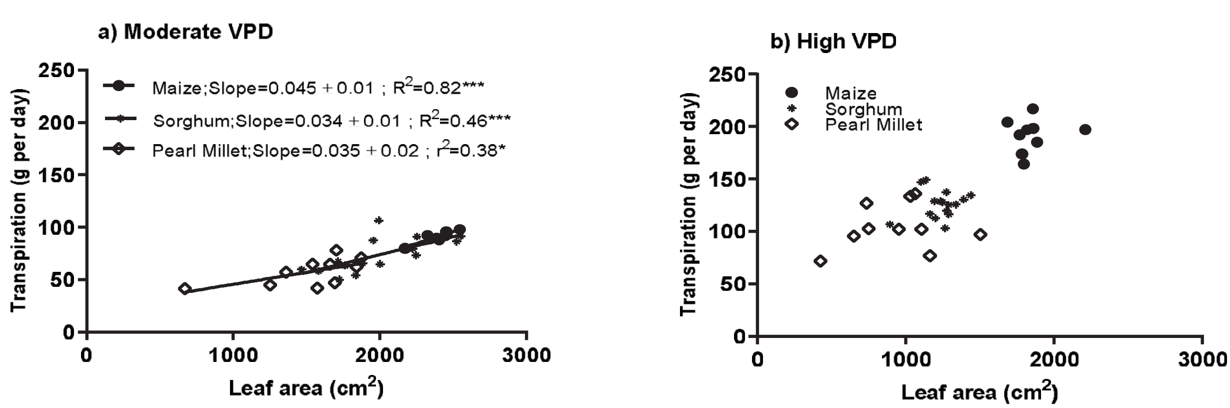

Fig. 4. Regression relationship between leaf area (cm^2) and transpiration (g per day) in eight genotypes of maize, fifteen genotypes of sorghum and eight genotypes of pearl millet grown under moderate (a) and high (b) VPD conditions.

Table 6
Crop × VPD analysis of variance for crops (maize, sorghum and pearl millet) and VPD (moderate and high) conducted for average magnitude of total water transpiration, dry biomass, TE and FTSW threshold (X_T) in plants grown under moderate and high VPD conditions. ANOVA represents F value and LSD represents least significant difference. *, **, *** Significant at $p < 0.05$, 0.01 and 0.001 respectively.

	Total water transpired (g)			Biomass (g plant-1)			TE (g DW kg^{-1} H_2O transpired)			FTSW threshold (X_T) for transpiration		
	Moderate	High	Mean	Moderate	High	Mean	Moderate	High	Mean	Moderate	High	Mean
Maize	1583	3857	**2856**	11.57	29.43	**21.83**	7.31	8.14	**7.72**	0.47	0.56	**0.52**
Sorghum	1312	3341	**2367**	9.02	22.6	**15.64**	6.88	6.86	**6.87**	0.43	0.37	**0.39**
Pearl millet	1824	3393	**2659**	11.02	21.29	**17.13**	6.03	6.44	**6.23**	0.5	0.44	**0.46**
Mean	**1546**	**3485**		**10.4**	**24.27**		**6.7**	**7.12**		**0.46**	**0.45**	
Crop	9.2***			15.0***			9.46***			12.08***		
VPD	701.8***			322.3***			2.14ns			1.07ns		
Crop x VPD	10.8***			7.9**			0.79ns			8.11***		
LSD (Crop)	281.8			3.13			1.03			0.08		
LSD (VPD)	146.7			1.63			0.53			0.04		

Table 7
Genotypic variation for the FTSW threshold for the decline in transpiration (X_T) and for the leaf area expansion rate (X_L) decline under progressive soil drying conditions in ten genotypes of maize, sixteen genotypes of sorghum, and ten genotypes of pearl millet, grown under moderate and high VPD conditions. Confidence intervals for these FTSW thresholds and the R2 square values for the determination of the thresholds are also provided. Five replicated plants per genotype were used.

	FTSW Threshold				Confidence interval ($p < 0.05$)				R^2			
VPD growth condition	Moderate		High		Moderate		High		Moderate		High	
	X_T	X_L	X_T	X_L	X_T	X_L	X_T	X_L	X_T	X_L	X_T	X_L
Maize												
783527	0.37a	0.5d	0.5a	–	0.34-0.40	0.41-0.58	0.45-0.55	–	0.93	0.93	0.89	–
4695575	0.37a	0.37b	0.55b	–	0.34-0.39	0.43-0.47	0.50-0.60	–	0.97	0.98	0.91	–
8315622	0.55cd	0.55de	0.55b	–	0.52-0.58	0.48-0.61	0.51-0.58	–	0.97	0.96	0.95	–
9424780	0.46b	0.34a	0.64d	0.23a	0.42-0.48	0.28-0.40	0.58-0.68	0.15-0.29	0.95	0.85	0.93	0.73
14746185	0.52c	0.67f	0.61c	–	0.48-0.55	0.62-0.73	0.51-0.71	–	0.96	0.96	0.78	–
18270413	0.46b	0.45c	0.61c	–	0.41-0.51	0.38-0.52	0.55-0.66	–	0.89	0.91	0.89	–
22525674	0.53c	0.56de	0.49a	0.58b	0.48-0.57	0.49-0.64	0.45-0.53	0.38-0.78	0.92	0.96	0.92	0.52
30V92	0.5bc	0.48cd	0.6c	–	0.47-0.53	0.43-0.53	0.55-0.65	–	0.96	0.96	0.92	–
900 MGold	0.34a	0.46cd	0.5a		0.32-0.37	0.43-0.59	0.46-0.54	–	0.96	0.84	0.92	–
Public Check	0.59d	0.51d	0.56bc		0.54-0.62	0.44-0.59	0.49-0.60	–	0.95	0.96	0.91	–
P (0.05)	***	***	**	***								
Sorghum												
E 36-1	0.45c	0.49e	0.39cd	–	0.42-0.47	0.39-0.57	0.32-0.45	–	0.96	0.86	0.8	–
ICSV1	0.41bc	0.51e	0.21a	–	0.38-0.44	0.32-0.70	0.17-0.25	–	0.94	0.51	0.81	–
ICSV700-P10	0.37b	0.18a	0.4cd	–	0.35-0.40	0.07-0.17	0.37-0.43	–	0.95	0.58	0.95	–
ICSV745	0.32a	0.53e	0.31b	–	0.29-0.34	0.37-0.69	0.27-0.35	–	0.89	0.54	0.9	–
IS18551	0.45c	0.18a	0.47e	–	0.43-0.48	0.16-0.20	0.40-0.54	–	0.94	0.9	0.86	–
IS9830	0.54e	0.36c	0.47e	–	0.52-0.57	0.30-0.43	0.3-0.51	–	0.96	0.9	0.94	–
M 35-1	0.4bc	0.21ab	0.37cd	0.32	0.37-0.43	0.19-0.24	0.35-0.40	0.22-0.39	0.95	0.96	0.95	0.62
N13	0.51d	0.23ab	0.42de	–	0.48-0.53	0.17-0.30	0.38-0.46	–	0.96	0.77	0.94	–
PB15220-1	0.48cd	0.35c	0.22a	–	0.46-0.51	0.28-0.42	0.18-0.25	–	0.95	0.79	0.89	–
S35	0.39b	0.38cd	0.41cde	0.37	0.37-0.41	0.33-0.41	0.36-0.46	0.33-0.44	0.95	0.89	0.85	0.8
296B	–	–	0.37cd	–	–	–	0.25-0.50	–	–	–	0.46	–
BTx623	–	–	0.2a	0.33	–	–	0.15-0.25	0.11-0.57	–	–	0.86	0.41
PB15881-3	–	–	0.29b	–	–	–	0.25-0.33	–	–	–	0.91	–
PVK 801-P23	–	–	0.43de	–	–	–	0.40-0.47	–	–	–	0.92	–
ICSV93046-P1	–	–	0.34c	–	–	–	0.31-0.36	–	–	–	0.93	–
ICSR93024	–	–	0.57	–	–	–	0.51-0.62	–	–	–	0.93	–
P (0.05)	***	***	***	ns								–
Pearl Millet												–
H77/833-2	0.29a	0.38a	0.26a	0.39a	0.22-0.36	0.32-0.44	0.23-0.28	0.28-0.44	0.81	0.65	0.91	0.68
PRLT	0.42b	0.54b	0.38c	0.62b	0.38-0.45	0.52-0.62	0.36-0.40	0.55-0.68	0.96	0.78	0.98	0.75
841B	0.51cd	–	0.35bc	–	0.48-0.53	–	0.32-0.38	–	0.97	–	0.93	–
Pusa 322	0.55cde	–	0.32b	–	0.52-0.59	–	0.24-0.40	–	0.96	–	0.57	–
863B	0.48c	–	0.5de	–	0.45-0.51	–	0.44-0.55	–	0.95	–	0.86	–
GB8735	0.55de	–	0.45d	–	0.51-0.59	–	0.41-0.49	–	0.96	–	0.9	–
ICMP 451-P6	0.56de	–	0.52e	–	0.48-0.64	–	0.47-0.57	–	0.86	–	0.92	–
ICMV-IS-92222	0.56def	–	0.5de	–	0.51-0.59	–	0.45-0.54	–	0.93	–	0.92	–
PT732B-P2	0.51cd	–	0.57ef	–	0.46-0.56	–	0.49-0.64	–	0.89	–	0.87	–
Tift238D1-P158	0.57f	–	0.56ef	–	0.48-0.65	–	0.50-0.62	–	0.76	–	0.93	–
P (0.05)	***	***	***	***								

3.4. Leaf area expansion rate (LER) under progressive soil drying

In maize, the FTSW threshold for decline in LER for plants grown under moderate VPD conditions ranged from 0.34 to 0.67. Only two genotypes were evaluated for the LER response to soil drying in plants grown under high VPD and showed very contrasting thresholds (9424780 = 0.23 and 22525674 = 0.58; Table 7). On an average, the FTSW thresholds for LER decline were 0.49 and 0.42 for plants grown under moderate and high VPD, respectively (Table 7). In comparison to the FTSW threshold values for transpiration decline (0.48 and 0.56 for plants grown under moderate and high VPD, respectively), these values were similar for plants grown under moderate VPD conditions and lower for the FTSW threshold for LER decline than for the FTSW threshold for TR decline for plants grown under high VPD conditions. This result indicates that, as far as the comparison holds with only two genotypes measured in plants grown under high VPD conditions, the LER would remain at its maximum beyond the FTSW point where transpiration starts to decline in the case of maize.

The FTSW threshold for decline in LER in sorghum ranged from 0.18 to 0.53 for plants grown under moderate VPD condition. Only two genotypes were measured for LER for plants grown in high VPD conditions (M35-1 = 0.32 and BTx623 = 0.33). On an average, the FTSW thresholds for LER decline were 0.32 and 0.31 for plants grown under moderate and high VPD, respectively (Table 7). The FTSW threshold values for transpiration decline were 0.44 and 0.36 for plants grown under moderate and high VPD, respectively, and these values were higher than the FTSW threshold for LER decline in plants grown under moderate VPD conditions, but similar for plants grown under high VPD conditions. Sorghum genotype M35-1 showed leaf expansion continuation till FTSW 0.32 in high VPD and 0.21 in moderate VPD, whereas transpiration started to decline at FTSW 0.44 in high VPD and 0.40 in moderate VPD, respectively.

The FTSW threshold for decline in LER in pearl millet for genotype H77/833-2 was 0.38 and for PRLT was 0.54 for plants grown under moderate VPD condition. However, when grown under high VPD condition H77/833-2 was at 0.39 and PRLT at 0.62. The FTSW threshold for LER did not change further when grown under high VPD condition in H77. However, PRLT LER threshold declined at still at higher soil moisture compared to the moderate VPD condition. The FTSW threshold for LER was at higher soil moisture compared FTSW threshold for transpiration (Table 6) in both the genotypes under both VPD growth condition.

In summary, while LER in maize and sorghum appeared to decline at similar or lower FTSW values than transpiration upon soil drying, *i.e.* leaf expansion continued unaffected by water stress beyond the point where transpiration started declining upon progressive drying, pearl millet's LER appeared more sensitive to soil drying than transpiration.

4. Discussion

4.1. VPD conditions during growth affect plant transpiration response to transient increase in VPD

It is postulated that a restricted transpiration under elevated VPD may contribute to early season water conservation and, as a consequence, improve yield under drought. Limiting transpiration when VPD is the highest can increase daily transpiration efficiency and then increase the proportion of water that is used during reproductive stages of crop development, which is critical for yield determination under water stress [8,49–51]. The restriction in transpiration under high VPD was previously reported in maize [34], sorghum [52,42] and pearl millet [43], reviewed [24,53], and used for simulation studies [20,29,54]. However, there are an increasing number of reports showing that consistent identification of this trait is difficult and likely interacts with the growth environment of the crop prior to the transient exposure to increasing VPD [35–37,55–58], especially in C_4 species where surrogate measurements of transpiration rate have been of limited use [59]. Results of the present experiments showed that the growth conditions affected the response of transpiration to a transient increase in VPD range from 0.9 to 4.1 kPa in a controlled environment. The genotypes of all three C_4 species grown under moderate VPD conditions had lower slope values above 3.0 kPa than below 3.0 kPa indicating that there was, as expected, a transpiration restriction at VPD above 3.0 kPa. In contrast, plants grown under high VPD showed an average of two-fold higher slope value above 3.0 kPa VPD (Fig. 1). The higher slope above 3.0 kPa indicate a shift in the TR restriction or a loss of TR responsiveness due to the high VPD conditions. Our interpretation is that plants having developed under high VPD conditions may have developed an hydraulic architecture allowing high water fluxes from the root to the leaves. It could be interpreted that under transient low VPD conditions the evaporative demand would have been too low to fulfil the transpirational water flux capacity of plants having developed under high VPD. By contrast, transpiration would increase when reaching the transient VPD conditions corresponding to the growth environment under which the plants developed, leading to the surge in transpiration that was observed when VPD increased. The mechanistic explanation of the altered transpiration behavior in high VPD is beyond the scope of this study and our hypothesis is that it may be controlled by hydraulic traits.

4.2. Transpiration restriction in plants grown under moderate and high VPD

Although three C_4 species showed genetic variation in transpiration restriction when grown under moderate VPD conditions, the decrease in slope value above 3.0 kPa was large in the case of maize while the decrease in slope above 3 kPa was non-significant for pearl millet and sorghum (Table 4). The contrary happened for plants grown under high VPD conditions, and here all three species had higher slope values above 3 kPa. This may suggest that maize would conserve water better than sorghum and pearl millet during high VPD hours of the day happening during periods of otherwise moderate VPD conditions. By contrast, when grown under high VPD conditions maize, sorghum and pearl millet would no longer restrict transpiration under high VPD episodes. An additional interpretation in the case of maize is that when grown under high VPD conditions maize could adjust to favor heat dissipation in the high VPD conditions, which is in part achieved by increasing transpiration. Restriction in transpiration would also restrict the evaporative cooling of leaves and increase the canopy temperature under higher atmospheric VPD [60]. Therefore, the five genotypes 783527, 18270413, 22525674, 14746185 and 30V92 of maize which had slope value below 3.5 mg H_2O cm^{-2} s^{-1} kPa^{-1} for plants grown under high VPD conditions, and showed a restriction in transpiration at high VPD, also showed comparatively higher canopy temperature (Fig. 5). A close relationship with canopy conductance and leaf temperature has also been reported in other crops [32,61–63]. These results suggest that the capacity to restrict transpiration, or a lower increase in transpiration, under high VPD would come at the cost of being more sensitive to temperature stress.

4.3. Effect of moderate and high VPD growth condition on leaf area

Maize was different from sorghum and pearl millet, and showed non-significant differences in leaf area decrease when grown under high VPD growth conditions, whereas leaf area dramatically decreased in sorghum and pearl millet grown under high VPD. Fig. 4 also showed that leaf area explained 82% of the total transpiration in maize but only 46% and 38% in sorghum and pearl millet when plants grown under moderate VPD conditions. Reduction of the total leaf area in pearl millet and sorghum under high VPD growth conditions could be a water conservation strategy. By contrast, a larger transpiring leaf surface means higher potential transpiration capacity, which is beneficial for fixing carbon only when the water supply is sufficient and VPD is favorable (moderate). This must have been the case of maize, having probably the longest history of breeding, well documented from 1970's [5,64,8,50], where breeding for large and stable leaf area across environments must have been a key trait exploited in the breeding programs to maximize light interception and increase potential yield. In a comparison of C_4 cereals yield potential across varying temperature and radiation of different sowing dates, maize grain yield was found more stable and fairly high across all sowing dates than sorghum and pearl millet [16]. This may have happened because maize benefitted from high cumulative radiation interception (RI) from a higher canopy size. Efforts have also been made to understand the maize adaptation by reducing the leaf growth in maize MON 87460 (transgenic-CspB). The reduced leaf growth in transgenic led to greater reduction in water use compared with the wild type, by reduced sap flow and higher residual soil water content, and then led to higher yield under terminal drought [52]. In summary, an apparent distinction for high evaporative stress tolerance in these three cereals was that maize genotypes used in this study mainly relied on water conservation from restricting transpiration under high VPD during pre-flowering stages [20,65] whereas sorghum and pearl millet rather reduced canopy size under high VPD to cut off water use.

4.4. FTSW threshold for transpiration decline upon progressive soil drying

Maize also differed from sorghum and pearl millet. The FTSW threshold for sorghum and pearl millet were not significantly different in plants grown under high VPD or moderate VPD conditions. By contrast, the FTSW thresholds for maize were significantly higher for plants grown under high VPD conditions than in plants grown under moderate VPD conditions. This could be explained by the higher evaporative demand leading to restriction in the supply of water in the soil-root-leaf continuum happening at higher soil moisture level. Common to all three species were the large genotypic differences in these thresholds, both in plants grown under low and moderate VPD conditions. Numerous studies have shown that transpiration rate does not begin to decline from well-watered rates until the soil–water content reaches FTSW values of between 0.3 and 0.4, and that this response is consistent across species and treatments [44,66,67]. The results of this study dismiss these earlier claims and show a large genotypic variation. Our interpretation is that fewer genotypes were tested in these earlier studies (for the difficulty to do these measurements) and the context in which we did this work, with access to labor, allowed us to test a fairly large set of genotypes, and then really test if there was or not genotypic variation. Apart from comparing different C_4 cereals, this study tested whether the two VPD conditions in which plants were grown altered the FTSW threshold for transpiration decline. The results for maize, showing differences in the thresholds across VPD conditions, are different from an earlier report showing no effect of the VPD conditions on the FTSW thresholds when evaluated under four different VPD conditions that ranged from 1.1 to 3.6 kPa [68]. In their study all plants were grown under similar conditions until 26 DAS, and the exposure to the different VPD conditions occurred only during the period of the dry-down, whereas in the present study the plants were grown and tested in separate environments. Our results are in agreement with an increasing number of reports showing that the environmental conditions during plant development affect transpiration response to change to VPD [69]. Additional work would be needed to assess whether the hydraulic features of the crops are altered during growth under varying VPD's.

The three C_4 species showed a larger range of genetic variation for total transpiration, TE and FTSW threshold for transpiration decline under progressive water stress in plants grown under high VPD than in plants grown under moderate VPD conditions. A few genotypes in maize (783527 and 1827043), sorghum (IS9830, PB15220-1, 296B and ICSV93046-P1), and pearl millet (GB8735 and ICMV-IS-92222) showed consistently high TE across both VPD growth conditions and also demonstrated comparative early FTSW threshold. These data suggest a link between higher TE and the capacity for a transpiration decline at a higher soil moisture (higher FTSW thresholds), as suggested earlier [51]. This strategy would contribute to water conservation by an early decline in transpiration upon soil drying.

4.5. FTSW threshold for leaf area expansion rate decline upon progressive soil drying in maize and sorghum

Leaf measurements were taken during progressive soil drying as leaf development is reported to be sensitive to soil drying and termination of leaf growth occurs before the decline in transpiration [44]. However, since most of the aforementioned studies considered transpiration and leaf expansion response to VPD separately, no information is known whether these two plant processes were regulated interactively or independently under VPD variations. Here, the thresholds for LER and transpiration decline were similar in the case of maize, while the LER thresholds were lower than the transpiration decline thresholds for sorghum. Two types of genetic behavior with regards to how productive functions respond to progressive soil drying were earlier hypothesized [17]: a "conservative" strategy, where the plants react to drought stress by reducing leaf expansion and close their stomata when FTSW is still relatively high and a "productive" strategy, whereby the crop keeps expanding and transpiring despite increasing soil water deficit. In our study a third type of response occurred in some maize and sorghum genotypes, where leaf expansion continued to low FTSW values at time when transpiration had already started declining. More work would be needed to further confirm these findings and understand whether both process' (*i.e.* "volumetric" increase from the expansive processes, and "mass" increases from transpiration and photosynthesis) responses to soil drying share a common regulation. A major challenge in doing this is in being able to monitor leaf development, especially when leaf rolling makes measurements difficult. Furthermore, large numbers of genes with different actions may be involved in control of leaf growth under fluctuating environmental conditions, and the controlling processes themselves are still poorly understood [70].

5. Conclusion

This study showed major differences between maize and sorghum and pearl millet in their strategy to adapt to water stress and climate change-like conditions. Maize restricted water losses mostly by restricting transpiration under high VPD, in plants grown under moderate VPD conditions, whereas sorghum and pearl millet restricted water losses by decreases in their leaf area when grown under high VPD. Upon progressive exposure to water stress, maize also showed a decline in transpiration at higher soil moisture than sorghum and pearl millet, especially in plants grown under high VPD conditions. Both these features in maize likely explained its higher transpiration efficiency compared to sorghum and pearl millet. High VPD during growth also profoundly affected how plants developed and responded to transient changes in atmospheric conditions. Crops appeared to "lose" their capacity to restrict transpiration under high VPD. Collectively, these results highlight specific strategies adopted by these three C_4 species to cope with water limitation and climate change. In all cases, there was genotypic variation available in each crop specie, for each of the traits that was measured, then opening the opportunity to apply these strategies to the other species not having it.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2019.110297.

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