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Component traits of plant water use are modulated by vapour pressure deficit in pearl millet (*Pennisetum glaucum* (L.) R.Br.)

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Abstract. Traits influencing plant water use eventually define the fitness of genotypes for specific rainfall environments. We assessed the response of several water use traits to vapour pressure deficit (VPD) in pearl millet (*Pennisetum glaucum* (L.) R.Br.) genotypes known to differ in drought adaptation mechanisms: PRLT 2/89–33 (terminal drought-adapted parent), H 77/833–2 (terminal drought-sensitive parent) and four near-isogenic lines introgressed with a terminal drought tolerance quantitative trait locus (QTL) from PRLT 2/89–33 (ICMR01029, ICMR01031, ICMR02042, and ICMR02044). Plant water use traits at various levels of plant organisation were evaluated in seven experiments in plants exposed either transiently or over the long term to different VPD regimes: biomass components, transpiration (water usage per time unit) and transpiration rate (TR) upon transient VPD increase (g H_2O cm^{-2} h^{-1})), transpiration efficiency (g dry biomass per kg H_2O transpired), leaf expansion rate (cm per thermal time unit) and root anatomy (endodermis dimensions)). High VPD decreased biomass accumulation by reducing tillering, the leaf expansion rate and the duration of leaf expansion; decreased root endodermis cell size; and increased TR and the rate of TR increase upon gradual short-term VPD increases. Such changes may allow plants to increase their water transport capacity in a high VPD environment and are genotype-specific. Some variation in water use components was associated with terminal drought adaptation QTL. Knowledge of water use traits' plasticity in growth environments that varied in evaporative demand, and on their genetic determinacy, is necessary to develop trait-based breeding approaches to complex constraints.

Additional keywords: canopy development, endodermis, terminal drought adaptation, transpiration rate.

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Introduction

Terminal drought adaptation in pearl millet (*Pennisetum glaucum* (L.) R.Br.) confers better capacity to set seeds and fill grain in target cropping systems, and is a key breeding target (Bidinger *et al*. 1987; Yadav *et al*. 2002). Terminal drought (TD) adaptation is related to constitutive water-saving mechanisms during the vegetative stage, allowing more water availability for the post-anthesis period (Vadez *et al*. 2013*a*; Kholová and Vadez 2013). In this case, the water saving mechanisms of TD-adapted germplasm were linked to a low transpiration rate under low vapour pressure deficit (VPD) conditions (water transpired per unit of leaf area), which is a proxy for leaf conductivity (see figures 3–5 in Kholová *et al*. (2010*a*)) and was linked to the capacity to further limit the transpiration rate (TR) under high VPD under non stressed conditions (figures 4–6 in Kholová *et al*. 2010*b*). Similar mechanisms have been identified across crop species such as sorghum (*Sorghum bicolor* (L.) Moench.) (Gholipoor *et al*. 2010), chickpea (*Cicer arietinum* L.) (Zaman-Allah *et al*. 2011) and peanut (*Arachis hypogaea* L.) (Devi *et al*. 2010), and was recently hypothesised to be linked to increased transpiration efficiency (TE; biomass accumulation per unit of water transpired; (Vadez *et al*. 2014)). Three quantitative trait loci (QTLs) for TR under different VPD conditions were recently mapped (Kholová *et al*. 2012) in the interval of a validated major terminal drought tolerance QTL (Yadav *et al*. 2002).

Although limiting leaf conductivity is one way to save water, having a smaller leaf area is another component limiting water losses until anthesis (e.g. van Oosterom 2001; Hammer 2006; Borrell *et al*. 2014*a*; 2014*b*; Kholová *et al*. 2014). For instance,

maize (*Zea mays* L.) leaf expansion was sensitive to high VPD and there were genotypic differences in that response to VPD (Reymond *et al*. 2003; Sadok *et al*. 2007; Welcker *et al*. 2007, 2011). Genotypes having different sensitivities to high VPD would then develop canopies of different size under high VPD growth environments. Of course, a restriction in leaf expansion would also lead to variation in leaf thickness through altered dimensions of cells and cell structures, both of which are primarily related to photosynthetic capacity (e.g. Lambers 1998; Tardieu *et al*. 1999; Arredondo and Schnyder 2003). Leaf thickness is indeed an emerging consequence of leaf expansion rate and of mass accumulation over time, both of which are differently influenced by the environment (Tardieu *et al*. 1999). In fact, a previous QTL study in pearl millet revealed that leaf thickness was also involved in the determination of plant water use: genotypes with thicker leaves had higher transpiration rates and leaf thickness varied across VPD growth conditions (Kholová *et al*. 2012). This indirectly pointed to the existence of a similar phenomenon in pearl millet like the one found earlier in maize, where an interplay between leaf expansion and leaf thickness eventually had an influence on plant water use.

The response of transpiration rate to high VPD was tested earlier on plants that had been grown under fluctuating conditions within a glasshouse (Kholová *et al*. 2010*a*, 2010*b*) (i.e. either under the low VPD conditions of the rainy season or the high VPD conditions of the post-rainy and summer season), and no attention was paid to the influence growing conditions could have on the transient response of TR to VPD. Long-term growth conditions, especially with regards to VPD, could indeed influence TR's sensitivity to transient changes in VPD, as recently reported in maize, wheat and turfgrass (Yang *et al*. 2012; Sermons *et al*. 2012; Schoppach and Sadok 2013; Seversike *et al*. 2013; Schoppach *et al*. 2014). The environments where pearl millet is cultivated can experience high VPD conditions (>5 kPa). Therefore, an important question, beyond the possible effects of VPD on the plant and canopy development, is whether the TR response to transient VPD changes depends on the prior growth conditions. This is important because VPD conditions fluctuate largely in a typical rainy season in the semi-arid tropics (e.g. preceding or following rainfall events) and there could be genetic variation in how growth conditions eventually alters the TR response to transient VPD changes, such as those found in wheat (Schoppach and Sadok 2013).

Another question is then how the VPD conditions in the growth environment affect leaf area development, the pattern of plant water use over time and TE, and whether these putative differences could be linked to the hydraulic properties of plant organs. For instance, roots' hydraulic properties have been suggested to participate in plants' adaptation to variable environments (e.g. Steudle and Peterson 1998; Zimmermann and Steudle 1998; Bramley *et al*. 2009; Sadok *et al*. 2013; Schoppach *et al*. 2014; Vadez *et al*. 2014). Here, we also investigated the possibility that root anatomy could differ in response to growth environments and also within genotypes that are known to contrast in their transpiration response to high VPD. We were especially interested in the endodermis because of its known role in root hydraulic conductance (Zimmermann and Steudle 1998).

Therefore, processes determining plant water use are both transient and integrative, but they are both affected by VPD. TR is tightly and transiently regulated by key factors that determine stomata opening (light, water availability and VPD), and long-term growth conditions could also influence this plant response. By contrast, the leaf area at a given time integrates the processes that have ruled leaf development over time. Overall, we hypothesised that VPD would have both short- and long-term effects on plant water use, via effects on leaf development, on the control of stomata conductance or both, which may be related to root anatomical changes. The aim of this study was to compare how VPD affected these components in genotypes known to contrast for their transient TR response to increasing VPD conditions, and to assess how these differences could influence plant water use patterns and TE over time in plants grown under differing VPDs.

Materials and methods

Plant material

Two pearl millet (*Pennisetum glaucum* (L.) R.Br.) genotypes and four QTL introgression lines (near-isogenic lines or NILs) with similar phenology but contrasting tolerance to terminal drought stress were selected from previous field (Serraj *et al*. 2005) and lysimetric experiments (Vadez *et al*. 2013*a*): PRLT 2/89–33 (TD-adapted parent, referred to as 'PRLT') and H 77/833–2 (TD-sensitive parent, referred to as 'H77') and four NILs (i.e. TD-adaptation QTL holders) ICMR01029, ICMR01031, ICMR02042, ICMR02044. Details on NIL development have been described earlier (Hash *et al*. 1999; Kholová *et al*. 2010*b*). These NILs were selected to test whether or not the TD adaptation QTL also underlies plant growth responses to changes in VPD after this QTL was found to regulate transient TR responses to VPD (Kholová *et al*. 2010*b*). In these experiments, ICMR01029 and ICMR01031 had phenotypic responses similar to those of the TD-adapted PRLT, whereas ICMR02042 and ICMR02044 had phenotypic responses similar to the sensitive parent H77. This could relate to the fact that several QTLs for water-saving traits were found within the TD-adaptation QTL interval (Kholová *et al*. 2012). A crossing-over could have happened while introgressing large QTL with only a few flanking markers, possibly missing the important segments in some NILs.

The TD-adapted genotype PRLT 2/89–33 derived from the International Crops Research Institute for the Semi-Arid Tropics Bold Seeded Early Composite, which is an elite breeding population based on *Iniadi* landrace germplasm from West Africa. PRLT 2/89–33 has low propensity to tiller and was shown to tightly control stomatal conductance, especially in VPD exceeding 2 kPa, which was shown to be a part of its water conservation mechanisms conferring it an advantage under terminal drought conditions (Kholová *et al*. 2010*a*, 2010*b*; Vadez *et al*. 2013*a*). Sensitive genotype H77 has a North Indian origin and is the heat-resistant parental genotype of many commercially used hybrids in this area. H77 was shown to lack a similar control of transpiration to that of the TD-adapted genotype (Kholová *et al*. 2010*a*, 2010*b*). This genotype is also characterised by profuse tillering (Yadav *et al*. 2002).

To develop QTL introgression lines in the background of H77, the latter was crossed to PRLT, followed by four

backcrosses with H77. At each backcross, the assessment for the presence or absence of the terminal drought tolerance QTL was made using flanking markers on pearl millet Linkage Group 2 (Hash *et al*. 1999). Work was carried out on test-cross hybrids of these genotypes, developed by crossing the inbred parental lines and QTL NILs to the male sterile line tester 843A to avoid inbreeding depression. Out of the selected NILs, ICMR01029 had the highest yield under terminal drought in the field (Serraj *et al*. 2005) and in lysimetric conditions (Vadez *et al*. 2013) and was also used as a tolerant parent for developing a fine mapping population in order to further dissect the mechanisms of drought-tolerant QTLs (Sehgal, Hash and Yadav, unpubl. data).

Plant growth conditions and experimental details

Seven experiments were carried out; the details are summarised in Table 1. Plants were grown individually in 15.2 cm and 25.4 cm diameter pots filled with 5 kg and 10 kg of soil mixture, respectively containing Alfisol, sand and manure (5 : 2 : 1) and kept well watered during all developmental stages. Smaller (15.2 cm diameter) pots were used for investigations at early growth stages. Seven experiments were carried out where, for overall analysis, experiments with an average daytime VPD <2.5 kPa were considered as low VPD regimes and those with an average daytime VPD >2.5 kPa were considered as high VPD regimes (details below).

Experiments 1a and 1b were meant to follow up the leaf area development and transpiration on a weekly basis during two seasons in which the VPD conditions varied inside the glasshouse. For each genotype, there were six and five separate sets that were harvested sequentially every week starting at 17 days after sowing (DAS) (Table 1).

The purpose of Experiment 2 was to assess the differences in plants' leaf area (destructively via a leaf area meter; LI-3100 Li-Cor, Lincoln, NE, USA) caused by growing plants under different VPD conditions and differences in root anatomical structure (Table 1). Experiments 3, 4, 5 and 6 were a suite

Table 1. Overview of water use experiments with pearl millet

With each experiment number, trait assessment window (number of days after sowing; DAS), the genotypes used, the growth system with the average vapour pressure deficit (VPD) conditions of growth (day–night) and season is shown. The traits measured were leaf area (LA), total transpiration (T), transpiration rate (TR; amount of water transpired per unit of LA per unit of time), biomass (plant organ dry weight), LER (leaf expansion rate; cm per unit of thermal time), root anatomy (endodermal cells parameters, stele diameter), VPD reaction (short-term response of TR to rising VPD), TE (transpiration efficiency; biomass accumulated per unit of water transpired). VPD_{max}, (maximum VPD)

Experiment no. (stage)	Genotypes	Conditions (day–night VPD_{max})	Traits measured	Season (duration)	No. replications
Experiment 1a (14–58 DAS,up to reproductive)	Parents (PRLT and H77) NILs (ICMR01029, ICMR01031, ICMR02042, ICMR02044)	Glasshouse (natural day cycle; VPD~ 4.5–0.5 kPa)	LA, T, TR, TE, biomass	Summer (22 April–27 June 2008)	5
Experiment 1b (14–51 DAS, up to reproductive)	Parents (PRLT and H77) NILs , ICMR02042, ICMR02044)	Glasshouse (natural day cycle; VPD~3.6–0.4 kPa)	LA, LER, TE, T, TR, biomass, root anatomy	Rainy (27 July–16 September 2008)	5
Experiment 2 (32 DAS, up to vegetative)	Parents (PRLT and H77)	Set 1: Glasshouse (natural day cycle; VPD~2.4–1.2 kPa) Set 2: Outdoors (natural day cycle; VPD ~4.4–1.3)	LA, VPD reaction, TR, T, TE, biomass, root anatomy	Post-rainy (7 January–2 February 2009)	6
Experiment 3 (7–16 DAS, up to vegetative)	Parents (PRLT and H77)	Chamber Set 1: VPD 1.13–0.79 kPa Set 2: VPD 2.55–0.95 kPa	LA, LER, TE, T, TR, biomass	Post-rainy (13–29 October 2008)	5
Experiment 4 (7–20 DAS, up to vegetative)	Parents (PRLT and H77) NILs(ICMR01029, ICMR01031, ICMR02042, ICMR02044)	Chamber Set 1: VPD 1.13–0.79 kPa Set 2: VPD 2.55–0.95 kPa	LA, LER, TE, T, TR, biomass, VPD reaction	Post-rainy (2–22 November 2012)	5
Experiment 5 (7–24 DAS, up to vegetative)	Parents (PRLT and H77) NILs (ICMR01029, ICMR02042)	Chamber Set 1: VPD 1.13–0.79 kPa Set 2: VPD 2.55–0.95 kPa	LA, LER, TE, T, TR, biomass, VPD reaction	Rainy (4–28 August 2010)	5–6
Experiment 6 (22 DAS, up to vegetative)	Parents (PRLT and H77) NILs (ICMR01029, ICMR01031, ICMR02042, ICMR02044)	Chamber Set 1: VPD 4.64–1.51 kPa Set 2: VPD 2.25–0.67 kPa	LA, TE, T, TR, biomass, VPD reaction	Post- rainy (18 December 2013–9 January 2014)	5–6

of experiments to assess the effect of VPD on plant growth and development. Plants were germinated in the glasshouse and transferred to growth chambers under different VPD conditions at 5 DAS (at the three-leaf stage). The two chambers had similar light intensity (800 µmol photon m^{-2} s^{-1}) and were set to a 12.5-h day cycle (Table 1).

Here, one caveat is that we did not have an absolute control of the growth conditions in the glasshouse. However, the environmental conditions across seasons varying in VPD were large enough to elicit clearly different growth conditions in the glasshouse.

Measurement and analysis of growth and development

Transpiration

In all experiments, plants were maintained at ~80% of field capacity of the soil moisture and transpiration (T) was monitored from ~100 degree-days (°day) (around five emerged leaves). Similar protocols were used previously for maintaining control plants (e.g. Kholová *et al*. 2010*a*). The day before starting the transpiration assessment, plants were watered abundantly and left to draining overnight to reach field capacity. Next day, morning pots were either wrapped at the base of the plant stem with a plastic bag (Experiment 1a and 1b), or the soil was covered with a 2-cm layer of plastic beads (Experiments 2, 3, 4, 5 and 6) to restrict soil evaporation. Both methods showed a reduction of 90% in soil evaporation so that transpiration was monitored from changes in pot weights and added water. The first pot weight was considered as the field capacity weight. Pots were then weighted every morning and rewatered up to field capacity weight. Transpiration was monitored gravimetrically throughout the experiment (daily in Experiments 1a, 1b, 4, 5; every few days in Experiment 2 and 3). At the end of Experiments 2, 3, 4, 5 and 6, TR was measured upon transient exposure to a regime of increasing VPD conditions in the growth chamber, using a 1-h timeframe for each VPD level. When the experiment finished, leaf area (LA) was estimated with a meter (LI-3100 Li-Cor) and TR was expressed as g H_2O cm^{-2} leaf h^{-1}. Subsequently, plant organs were separated, roots were washed, and all plant parts were dried (at 60°C) and weighed.

Leaf expansion measurements

In Experiments 1b, 3 and 4, an increase in the length of all leaves on the main stem (Experiment 1b) and the fifth to ninth leaves (Experiments 3 and 4) was measured at 0800 hours (in Experiments 1b and 4) and at 0800 hours and 2000 hours (in Experiment 3). Lengths were measured with a ruler from the last emerged node, carefully correcting for the stem growth below that node by subtracting the length of the nodes below that had fully developed. In Experiment 1b, there were 4 or 5 sets of plants that were harvested weekly for destructive LA assessment. In Experiment 5, the whole LA was nondestructively assessed three times during plant development using individual leaf measurements of length and width (predicted *v.* observed LA: $R^2 = 0.90$; $P < 0.001$); the total LA was then the sum of the leaf area of individual leaves, calculated by the formula: $LA = length \times width \times 0.69$, where 0.69 is a shape coefficient derived from our previous experiments on pearl millet (the range of this coefficient is usually reported to be 0.74–0.64; e.g. van Oosterom *et al*. 2001). At harvest, total plant LA was measured destructively with an LA meter (LI-3100, Li-Cor). For expressing leaf expansion, the time scale in °day was used (according to Singh *et al*. 1998), with minimal, optimal and maximum cardinal temperatures of 10°C, 30°C and 45°C respectively. Consequently, the leaf expansion rate (LER, cm °day^{-1}) was calculated considering the window of maximum daily linear leaf length increase (typically a 3-day interval on the linear part of the curve).

Root anatomical analysis

Initially, free-hand sections from Experiment 1b were observed under a light microscope (model Olympus BX51) and clear differences in endodermal cells dimensions were seen. The endodermis is known to affect root radial water flow resistance and therefore has an important role in regulating of overall water absorption in cereals like maize (Zimmermann and Steudle 1998) and wheat (Bramley *et al*. 2009). Therefore, we focussed on the analysis of root endodermal cell dimensions in Experiment 1b (genotypes H77, PRLT, ICMR01029 and ICMR02042) and Experiment 2 (H77 and PRLT) grown in low or high VPD conditions (inside and outside the glasshouse). At the time of harvest, the roots were carefully washed and the roots from the second whorl from the top of the crown root bundle were conserved in a formalin: ethanol: acetate: distilled water solution (5 : 50 : 5 : 40) (O'Brien and McCully 1981) until analysis. The standardisation experiment, where root tissue maturation was observed from the tip to 10 cm distance in 1 cm intervals, showed a strong subberinisation or lignification of the endodermis in segments above 5–6 cm from the root tip (data not shown), suggesting that possible restrictions for water absorption may happen in further segments of these roots. Therefore, the transversal free-hand root sections were made close to the root tip (3–4 cm) where, in cereals, the water absorption capacity of the root is supposed to be larger than in the lignified root parts (Greacen *et al*. 1976; Bramley *et al*. 2009). These segments were stained with Sudan Red 7B (p.n: 46290, Sigma) and HCl-Phloroglucinol (p.n.: P3502, Sigma-Aldrich) according to Brundrett *et al*. (1991) and Johansen (1940). The remaining parts of these segments were embedded into wax (according to Jensen (1962)) and longitudinal sections were processed using an automated microtome (model RM2155, Leica Microsystems). These sections were stained with Ehrlich's hematoxylin (Ehrlich *et al*. 1910). The anatomical structure was observed using a light microscope (Olympus BX51) with camera (Nikon DS-U1) for any differences between genotypes. Finally, cells of the root endodermis from a minimum of five replicates from each genotype and treatment, were evaluated for transversal length and width, longitudinal length and stele diameter using Lucia Image software (ver. 4.8, Laboratory Imaging, Prague, The Czech Republic).

Statistical analysis

The data were first divided in two groups on the basis of VPD conditions during growth (i.e. low VPD regimes (VPD <2.5) and high VPD regimes (VPD >2.5)) and these were compared. The 2.5 kPa value was chosen based on previous observations that the TR response to VPD in pearl millet changed after crossing this threshold (Kholová *et al*. 2010*b*; similar ranges of thresholds in

the TR response to VPD have been described in, for example sorghum (Gholipoor *et al*. 2010), chickpea (Zaman-Allah *et al*. 2011) and peanut (Devi *et al*. 2010)). The choice of this threshold was further justified by ANOVA outputs (Table 2; see below) showing significant differences in plant growth characteristics between these two data categories (VPD <2.5 kPa *v.* VPD >2.5kPa). Finally, this was a simple way to group experiments according to main and intended VPD-related differences in growth conditions. For the comparison of main trends across very diverse experiments, it was also necessary to normalise the data to ensure a uniform data distribution and magnitude of the variation within each experiment. To do so, the repetition-wise data point of each parameter was divided by the grand mean of this parameter in each experiment, and these normalised values were used in the ANOVA at $P \leq 0.05$. In this way, regardless of parameter and experiment, the genotypic variation in the parameters measured was represented by a distribution of normalised values below and above 1, with a mean value of 1 (expressed as % in Table 2). This dataset was subjected to (i) one-way ANOVA to analyse the differences within each treatment and genotype, (ii) one-way ANOVA with genotypes as blocks to compare the effect of VPD conditions and (iii) one-way ANOVA with the VPD treatments as blocks to compare the genotypes across conditions. In the experiments conducted in fluctuating greenhouse conditions, we had no intention of controlling for possible interactions between temperature and VPD effect, as was done previously (Schoppach and Sadok 2013), as our purpose was primarily to assess the general effect of long-term growth conditions (with VPD and T possibly interacting) on the transient TR response to VPD.

Absolute values for the leaf development traits, the TR response to increasing VPD, and root anatomical traits are presented in Tables 3, 4, and 5. Differences between genotypes and VPD effects were analysed using the statistics program package CoStat ver. 6.204 (CoHort Software, Monterey, CA, USA). For absolute value analysis (Tables 3, 4, and 5), one-way ANOVA analysis within genotypes and treatment followed by the Tukey–Kramer test was performed separately for each experiment at the $P \leq 0.05$ level of significance. The relationships among the parameters in the dataset were analysed by correlation analysis of the normalised means from Table 2 and are summarised in Table 6. The split-line linear regression analysis for evaluation of the TR response to VPD was done replicate-wise within each genotype × VPD combination using GENSTAT (i.e. slopes1 (slope of the TR-VPD regression in VPD <2.5 kPa); slope2 (slope of the TR–VPD regression in VPD >2.5 kPa); the point of significant change in slope is the 'breakpoint'). To compare slopes and breakpoints across experiments, the GENSTAT (version 12, Oxford, UK) outputs were normalised similarly to as other traits shown above, and subjected to ANOVA (Table 2) and correlation analysis (Table 6), alongside other traits.

Results

Effect of VPD growth regime on biomass accumulation and biomass partitioning

The analysis of normalised values across experiments showed inherent differences in biomass accumulation characteristics among genotypes. Across all experiments TD-adapted PRLT had smaller total dry mass (TDM). Also, PRLT had significantly thinner leaves and a lower proportion of tillers (tiller dry mass (Till DM) to TDM ratio; Table 2). Almost all NILs (except ICMR01031) had a TillDM : TDM close to that of TD-sensitive H77 (Table 2). There were genotypic differences in the LA : TDM ratio (Table 2) and TD-adapted PRLT had a proportionally larger LA : TDM ratio than TD-sensitive H77, although this ratio was intermediate in NILs and there were only marginal differences in the root-to-shoot biomass ratio (data not shown).

High atmospheric evaporative demand decreased the growth of all plant organs (Table 2). VPD values above 2.5 kPa during growth decreased tillering in TD-sensitive H77 significantly but not in TD-adapted PRLT and the NILs. Under elevated VPD, the leaf thickness (specific leaf weight, SLW); leaf dry weight per unit of leaf area ($g\,cm^{-2}$)) of parental genotypes was almost unchanged; however, NILs with a SLW similar to H77 under low VPD tended to have thinner leaves (lower SLW) and similar to TD-adapted PRLT under high VPD. Interestingly, VPD treatment did not significantly affect the ratio of LA to TDM (Table 2).

Effect of VPD growth regime on canopy development

As shown above, high VPD conditions during growth decreased leaf biomass accumulation and LA development compared with plants grown in lower VPD regimes (Table 2). TD-adapted PRLT had generally lower LER compared with TD-sensitive H77 but the leaf expansion of TD-adapted PRLT lasted usually longer than that of H77 (Experiments 1b, 3, 4 and 5; Table 3). The longer duration of leaf expansion in PRLT was especially apparent under low VPD conditions (the maximum difference shown in Fig. 1*b*). For this reason, PRLT had similar or longer leaves than TD-sensitive H77 depending on VPD during growth (Fig. 1). Across experiments, the LER of NILs was not statistically different from that of parental lines (except for ICMR01031, whose LER was significantly lower than that of TD-sensitive H77, Table 2). A similar trend was observed on all the remaining leaves (data not shown).

High VPD during growth significantly decreased LER and the duration of leaf expansion in TD-adapted PRLT and ICMR02044 (Fig. 1, Table 2). By contrast, LER and duration of leaf expansion was not affected by high VPD in TD-sensitive H77 (example in Fig. 1; Tables 2 and 3). The duration of leaf expansion in NILs was similar to that in TD-sensitive H77 (Fig. 1*a*).

There was a significant positive relationship between LER and TE ($r^2 = 0.81$), between LER and TDM ($r^2 = 0.76$), and between LER and TDM components, which pointed to an expected link between the growth rate and biomass accumulation. By contrast, there was a negative relationship between LER and the sum of transpiration ($r^2 = 0.70$).

Effect of VPD growth conditions on TR and the TR response to a transient increase in VPD

TR ($g\,H_2O\,cm^{-2}\,h^{-1}$) and the TR response to short-term increasing VPD (i.e. slopes and the breakpoint in the TR–VPD relationship) were assessed in plants that were grown under different VPD conditions (Experiments 2, 4, 5 and 6). Across

Table 2. Comparison of pearl millet plant characteristics obtained at the end of each experiment

LA (leaf area, cm^2), leaf weight, stem weight, root weight, TDM (total dry mass; g per plant), T (total transpiration), TillDM (tiller dry matter), SLW (specific leaf weight; $g\ cm^{-2}$), LER (leaf expansion rate ($cm\ °day^{-1}$)), TE (transpiration efficiency), TR (transpiration rate average), VPD, vapour pressure deficit; Slope 1 (slope of the TR–VPD regression before breakpoint), Slope 2 (slope of TR–VPD regression after breakpoint), breakpoint (point of significant change in slopes). To compare these parameters across diverse experiments, the original values have been normalised in relation to the grand average value of the characteristics within particular experiment and multiplied by 100 (%). In this way, 100 represents the value corresponding to the mean of given experiment and lower or higher values represents the proportional (%) decrease or increase in given parameter. The ratio of tillers and leaves with TDM (%) illustrates the proportion of these organs to TDM. The letters with the means of normalised values indicate the results of ANOVA followed by the Tukey–Kramer test, which was run for particular trait: (i) within the genotypes and conditions (first 12 rows), (ii) within the genotypes (following six rows) and (iii) within the conditions (last two rows). Different letters indicate significant differences at $P \leq 0.05$ in separate groups

Genotype	VPD	TDM	TillDM : TDM	Leaf weight	LA	Stem weight	Root weight	LA : TDM	SLW	LER	TR	Slope 1	Slope 2	Breakpoint	T	TE
ICMR01029	>2.5 kPa	89cde	104.4ab	90.3abc	101ab	81.9de	87.3bc	108.3ab	90.3d	99.6ab	93.6cd	108.8bc	73de	96.2a	115.3a	73.8b
ICMR01031	>2.5 kPa	75.5e	79.5bc	77.2c	77.7b	72.6e	69.9c	97.4ab	96.2abcd	93.1b	120.5a	148.6ab	93abcd	109.5a	110.6ab	67.4b
ICMR02042	>2.5 kPa	89cde	95.6abc	87.1bc	93.5ab	87.6cde	90.4bc	100.8ab	92.7cd	99ab	108.4ab	122.9bc	118.2ab	97.7a	113.7a	76.2b
ICMR02044	>2.5 kPa	107.6abc	99.7abc	108.8ab	109.5ab	108.5abcd	106.3abc	96.7ab	96.6abcd	96b	98.8bc	98.3c	109.2abc	93.6a	118.8a	86.8b
H77	>2.5 kPa	92bcde	87.5bc	91.3abc	89.4ab	94.8abcde	94.5bc	93.2b	102ab	101.9a	119.5a	157.5a	124.6a	100.8a	111.2a	84.4b
PRLT	>2.5 kPa	82.1de	77.9c	88.4bc	90.9ab	73.9e	83.8bc	111.3ab	94.8bcd	93.4b	98.2bc	112.8bc	74.1de	94a	106.8ab	73.1b
ICMR01029	<2.5 kPa	114.4ab	113.1a	113a	112.4a	119.9ab	108.7abc	96.6ab	99abcd	103.6a	83.1de	104.6c	50.4e	94.7a	88.5bc	121.1a
ICMR01031	<2.5 kPa	113.7abc	102.2ab	109ab	109.1ab	117.4abc	110abc	98.2ab	95.8abcd	100.1ab	88.5cde	98.2c	63.9de	98.6a	87.4bc	124.6a
ICMR02042	<2.5 kPa	115.4a	109.4a	111.5a	104.2ab	119ab	115.1ab	88.1b	105.3a	103.8a	96.1bc	104.1c	90.6bcd	102a	89.8bc	125a
ICMR02044	<2.5 kPa	118.8a	100.6abc	114a	104.3ab	124.1a	136a	81.6b	105a	104.8a	88.8cde	101.4c	79.6cde	104.1a	88.1bc	127.4a
H77	<2.5 kPa	113.2abc	111.2 a	108.5ab	103.3ab	115abc	105.8abc	90.9b	100.5abc	103.8a	99bc	111bc	90.5bcd	93.5a	88bc	126.4a
PRLT	<2.5 kPa	101.2abcd	96.6abc	102.8ab	111.4a	93.4bcde	104.6abc	115.3a	92.8cd	103.2a	78.9e	99.2c	58.3de	86.6a	84.3c	122.9a
ICMR01029	Total	102ab	109.4a	101.9ab	106.9a	101.3a	98ab	103.9ab	94.7bc	101.3ab	88.2c	106.7a	61.7b	95.5a	101.2a	98.6a
ICMR01031	Total	95.3b	94.6ab	93.7b	93.9a	95.8ab	90.7b	97.6ab	96abc	95.7b	102.7ab	120.6a	76.8ab	104.1a	98.6a	97a
ICMR02042	Total	103.1ab	103.7a	100.2ab	99.1a	104.4a	103.6ab	95.9b	99.4abc	101ab	101.9ab	113.2a	103.9a	99.9a	100.9a	102.3a
ICMR02044	Total	113.4a	100.3ab	111.5a	106.8a	116.6a	121.2a	92.6b	100.9ab	99.1ab	93.5bc	99.9a	93.7ab	98.9a	102.5a	108.4a
H77	Total	103.7ab	101ab	100.7ab	97.1a	106a	100.8ab	92.3b	101.1a	102.7a	108.2a	134.9a	108a	97.1a	98.6a	107.2a
PRLT	Total	92b	88.9b	95.8b	101.7a	84.1b	94.4b	112.9a	93.8c	97.6b	88.2c	106a	66.2b	90.3a	95.8a	97.1a
Total	>2.5 kPa	89.2b	91.2b	90.5b	93.7b	86.6b	88.7b	101.6a	95.4b	97.6b	106.5a	125.4a	99a	97.9a	112.7a	76.9b
Total	<2.5 kPa	112.8a	106a	109.8a	107.5a	114.8a	113.4a	97.3a	99.7a	103.4a	89.1b	103.5b	72.8b	95.8a	87.7b	124.6a

Table 3. Leaf expansion rates (LER (cm °day^{-1})) estimated from the seventh and eighth leaves (~200-300 °day) of terminal drought-tolerant (PRLT) and terminal drought-sensitive (H77) pearl millet parents, and terminal drought quantitative trait locus-introgressed near isogenic lines (ICMR01029, ICMR01031, ICMR02042 and ICMR02044)

The experiments were conducted in glasshouse conditions (Experiment 1b) and growth chamber environments with various vapour pressure deficit (VPD) regimes (Experiments 3, 4 and 5). Different letters behind the means show the results of ANOVA followed by a Tukey–Kramer test, where different letters indicate statistically significant differences within a particular experiment at $P \leq 0.05$. These original values were further normalised (details are given in the Materials and Methods) and cross-compared across experiments (Table 2)

Experiment 1b		Experiment 3		Experiment 4		Experiment 5	
3.6 kPa VPD	LER	1.13 kPa VPD	LER	VPD 1.13 kPa	LER	VPD 1.13 kPa	LER
PRLT	0.61a	PRLT	0.981a	H77	0.704a	ICMR01029	0.673a
H77	0.591a	H77	0.865b	ICMR1029	0.646abc	ICMR02042	0.67a
ICMR01029	0.627a	–	–	ICMR01031	0.626abc	H77	0.663a
ICMR01031	0.588a	–	–	ICMR02042	0.651abc	PRLT	0.596b
ICMR02042	0.607a	–	–	ICMR02044	0.655ab	–	–
ICMR02044	0.597a	–	–	PRLT	0.65abc	–	–
		VPD 2.55 kPa	LER	VPD 2.55 kPa	LER	VPD 2.55 kPa	LER
–	–	PRLT	0.73c	H77	0.629abc	ICMR01029	0.661a
–	–	H77	0.949ab	ICMR01029	0.578bc	ICMR02042	0.651ab
–	–	–	–	ICMR01031	0.555c	H77	0.651ab
–	–	–	–	ICMR02042	0.602bc	PRLT	0.616ab
–	–	–	–	ICMR 2044	0.585bc	–	–
–	–	–	–	PRLT	0.599bc	–	–

growth conditions, the TD-adapted PRLT had always a lower TR at each step of the VPD ladder compared with the TD-sensitive H77 (Fig. 2, Table 2). However, plants grown in high VPD regimes always had higher TR values than plants grown in low VPD conditions (Fig. 2, Table 2). Across and within the VPD growth conditions, the TR of NIL ICMR01029 was consistently similar to that of TD-adapted PRLT and lower than that in H77, whereas the TR values of ICMR02042 were close to those of TD-sensitive H77 (Fig. 2*b*; Tables 2, 4).

The values of the slopes characterising the TR response to a transient increase in VPD from all experiments are summarised in Table 4; the normalised data and related statistics are summarised in Table 2, and a graphical example is provided in Fig. 2. First, plant exposure to high VPD growth regimes significantly increased the value of the slope of the TR response to a transient increase in VPD, especially after the breakpoint (Slope 2; Table 2, Fig. 2*a*). Second, across low and high VPD growth regimes, the TD-adapted PRLT and the NIL 01029 had a lower Slope 2 than TD-sensitive H77 and NIL 02042. Generally, larger genotypic differences in slope values were found when plants were grown under high VPD conditions than when plants were grown in low VPD regimes (Table 2).

The occurrence of statistically significant breakpoints in the VPD–TR regressions was more frequent in plants grown under lower VPD regimes. The position of the breakpoints tended to occur at different VPD values in plants cultivated under different VPD regimes (Table 4). Interestingly, even TD-sensitive H77, reported previously to be a 'transpiration unrestricted' genotype, showed a breakpoint in the TR–VPD regression (Fig. 2).

The absolute TR values were strongly correlated with the characteristics of the TR response to a transient VPD increase (i.e. Slopes 1 and 2 of the split-linear regression and breakpoint value; Table 6), indicating that higher TR absolute values would likely lead to a higher rate of TR increase upon increasing VPD.

Effect of VPD growth regime on root anatomy

Microscopic observations of the endodermal cell sizes revealed striking genotypic differences (Table 5, Fig. 3). The TD-adapted genotype (PRLT) had smaller endodermal cells (smaller transversal length and width, and longitudinal length; absolute values in Table 5, summary in Table 2) than the TD-sensitive genotype (H77). Both NILs (ICMR01029 and ICMR02042) also developed smaller endodermal cells than the sensitive parent, H77. In particular, ICMR01029 showed differences from H77 in all cell dimension measurements (Table 5). By contrast, there were no significant differences in stele diameter between genotypes measured in this particular experiment.

Endodermal cells of PRLT plants grown under high VPD conditions were smaller than those in plants grown under low VPD conditions, and smaller again than those in H77 (Table 5, Experiment 2). Endodermal cells of H77 remained similar across VPD conditions during growth. Therefore, parental genotypes had different cell sizes; even more so when grown under high VPD conditions. Importantly, there was a strong positive link between the characteristics of transpiration response to VPD (Slope 1 and Slope 2) and the parameters expressing the structure of the endodermis root cells (transversal width and area), indicating a link between the mechanisms controlling TR and the root cell anatomical characteristics (Fig. 3, Table 6).

Additionally, in high VPD growth conditions, PRLT (tolerant) developed stele with smaller diameters (Table 5, Experiment 2). In addition, as the standardisation experiments suggested, the differences in endodermis cell dimensions were maintained in older parts of lignified roots (data not shown).

Dynamics of transpiration

Water use patterns varied with genotypes and growth conditions. In Experiments 1a and 1b (4.5 and 3.6 kPa in the glasshouse) where plants were observed up to late developmental stages

Table 4. Overview of main parameters from analysis of the transpiration rate (TR) response to instantaneously rising vapour pressure deficit (VPD) for pearl millet plants from Experiments 3, 4, 5 and 6 grown in different environments with different VPD regimes

For each genotype × environment, the mean slope of the linear regression of VPD against TR in low VPD (Slope 1, before the breakpoint) and high VPD (Slope 2, after the breakpoint) is shown along with the mean VPD value where the regression changed significantly (the breakpoint). Different letters beside the breakpoint indicate the significant differences based on a comparison of the 95% confidence intervals within a particular experiment. These original values were further normalised (see the details in the Materials and Methods) and cross-compared across experiments (Table 2)

Experiment	Conditions	Genotype	Breakpoint (kPa)	Slope 1	Slope 2
2	2.4 kPa (glasshouse)	PRLT	2.419a	0.0175	0.0067
		H77	2.592a	0.0182	0.0084
	4.4 kPa (outdoors)	PRLT	2.111a	0.0171	0.0095
		H77	–	0.0184	0.0184
4	1.33 kPa (growth chamber)	H77	2.768a	0.0034	0.0049
		ICMR01029	2.010a	0.0020	0.0035
		ICMR01031	2.680a	0.0027	0.0039
		ICMR02042	2.610a	0.0026	0.0043
		ICMR02044	3.246a	0.0028	0.0039
		PRLT	2.713a	0.0017	0.0034
	2.55 kPa (growth chamber)	H77	2.74a	0.0050	0.0045
		ICMR01029	2.928a	0.0034	0.0026
		ICMR01031	2.290a	0.0051	0.0036
		ICMR02042	2.790a	0.0045	0.0049
		ICMR02044	–	0.0038	0.0038
		PRLT	–	0.0028	0.0028
5	1.13 kPa (growth chamber)	PRLT	1.146b	0.0042	0.0027
		H77	2.330ab	0.0037	0.0043
		ICMR01029	–	0.0027	0.0027
		ICMR02042	–	0.0031	0.0031
	2.55 kPa (growth chamber)	PRLT	3.158a	0.0037	0.0007
		H77	–	0.0037	0.0037
		ICMR01029	–	0.0021	0.0021
		ICMR02042	–	0.0028	0.0028
6	2.25 kPa (growth chamber)	PRLT	2.033ab	0.0078	0.0004
		H77	2.081ab	0.0080	0.0006
		ICMR01029	2.133ab	0.0067	–0.0008
		ICMR01031	1.885ab	0.0141	0.0004
		ICMR02042	2.325ab	0.0121	0.0010
		ICMR02044	2.100ab	0.0121	0.0005
	4.95 kPa (growth chamber)	PRLT	1.824ab	0.0080	0.0006
		H77	2.656ab	0.0097	0.0045
		ICMR01029	2.900a	0.0060	0.0004
		ICMR01031	1.603b	0.0093	0.0016
		ICMR02042	2.456ab	0.0170	0.0018
		ICMR02044	2.856a	0.0110	0.0014

Table 5. Measurements of endodermal cells and stele dimensions of terminal drought(TD)-tolerant (PRLT) and TD-sensitive (H77) parents and TD quantitative trait locus-introgressed near-isogenic lines (ICMR01029, ICMR02042) of pearl millet. The roots from two experiments were analysed (Experiments 1b and 2)

Different letters beside the means show the results of ANOVA followed by a Tukey–Kramer test, where different letters indicate statistically significant differences within a particular experiment at $P \leq 0.05$. These original values were further normalised (details are given in the Materials and Methods) and cross-compared across experiments (Table 2)

Experiment	Genotype	Radial length (μm)	Radial width (μm)	Area (μm^2)	Cell length (μm)	Stele diameter (μm)
Exp. 1b: 4.5kPa	PRLT	17.5c	14.2c	459.5c	17.9c	440a
	H77	21.2a	16.4a	736.1a	31.5a	464.3a
	ICMR01029	18.3bc	14.8bc	643b	24.2b	437.3a
	ICMR02042	19.1b	15.1b	698ab	28.9a	461.8a
	LSD	0.8	0.6	64.1	3.7	23.1
Exp. 2: 4.4 and 2.4 kPa	PRLT (low VPD)	18.5c	15.6b	699.9b	24.8a	550.4b
	H77 (low VPD)	19.6b	15.8b	746.7b	25.3a	447.5c
	PRLT (high VPD)	17.4d	15.3b	644.4c	21b	580.6a
	H77 (high VPD)	20.6a	17.7a	883.4a	25.3a	560.3ab
	LSD	0.7	0.6	45.1	3.1	22.7

Table 6. Correlation analysis of investigated traits of pearl millet

Leaf area (LA); leaf weight (W_{leaf}); stem weight (W_{stem}); root weight (W_{root}); TDM (total dry mass); T (total transpiration); TillDM (tiller dry matter); SLW (specific leaf weight; g cm^{-2}); TE (transpiration efficiency); TR (transpiration rate average); Slope 1, Slope 2 and breakpoint (i.e. characteristics of TR response to instantaneously increasing VPD); L_{trans} (transversal length), W_{trans} (transversal width), A_{trans} (transversal area) and L_{long} (longitudinal length) (i.e. characteristics of endodermal cell size); and stele diameter. The correlation analysis was performed using normalised values from Table 2. The Pearson's correlation coefficient was considered at $P < 0.05$: *, $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$; ns, nonsignificant correlation. For the root cell parameters, Pearson's correlation coefficient and the *P*-level up to $P < 0.1$ are shown (fewer points were available for correlation)

Trait	W_{stem}	W_{Leaf}	W_{Root}	TDM	T	TE	TR	SLW	TillDM : TDM	LA : TDM	LER	Slope 1	Slope 2	Breakpoint	L_{Trans}	W_{trans}	A_{trans}	L_{long}	Stele diameter
LA	0.73**	0.87***	0.73**	0.82**	ns	0.74**	–0.88**	ns	0.79**	ns	0.61*	–0.86**	ns	–0.61*	ns	ns	ns	ns	ns
W_{Stem}	–	0.95***	0.91***	0.98***	–0.65*	0.86**	ns	0.66*	0.78**	–0.74**	0.74**	ns	ns	ns	ns	ns	ns	ns	–0.73 (0.1)
W_{leaf}	–	–	0.91***	0.98***	–0.68*	0.88***	–0.70*	ns	0.78**	ns	0.70*	–0.73**	ns	ns	ns	ns	ns	ns	ns
W_{root}	–	–	–	0.92***	–0.65*	0.84***	–0.61*	0.62*	0.64*	–0.57*	0.76**	–0.62*	ns	ns	ns	ns	ns	ns	ns
TDM	–	–	–	–	–0.70*	0.90***	–0.63*	0.57*	0.81*	–0.64*	0.76**	–0.66*	ns	ns	ns	ns	ns	ns	–0.76 (0.078)
T	–	–	–	–	–	–0.93***	0.67*	ns	ns	ns	-0.70*	ns	0.63*	ns	ns	ns	ns	ns	ns
TE	–	–	–	–	–	–	–0.68*	ns	0.70*	–0.65*	0.81**	–0.59*	ns	ns	ns	ns	ns	ns	ns
TR	–	–	–	–	–	–	–	ns	–0.59*	ns	ns	0.81***	0.59*	0.66*	ns	0.77 (0.075)	0.75 (0.084)	ns	ns
SLW	–	–	–	–	–	–	–	–	ns	–0.80**	ns	ns	ns	ns	ns	ns	ns	ns	ns
TillDM : TDM	–	–	–	–	–	–	–	–	–	ns	0.74**	–0.61*	ns	ns	ns	ns	ns	ns	–0.79 (0.062)
LA : TDM	–	–	–	–	–	–	–	–	–	–	–0.59*	ns	ns	ns	–0.83*	ns	ns	ns	ns
LER	–	–	–	–	–	–	–	–	–	–	–	ns	ns	ns	ns	ns	ns	0.73 (0.1)	ns
Slope 1	–	–	–	–	–	–	–	–	–	–	–	–	0.60*	ns	0.77 (0.072)	0.93**	0.79 (0.08)	ns	ns
Slope 2	–	–	–	–	–	–	–	–	–	–	–	–	–	ns	0.76 (0.081)	0.79 (0.062)	0.87*	ns	ns
Breakpoint	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ns	ns	0.73 (0.1)	ns	ns
L_{trans}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.91*	0.87*	0.85*	ns
W_{trans}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.89*	0.86*	ns
A_{trans}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.86**	ns
L_{long}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ns

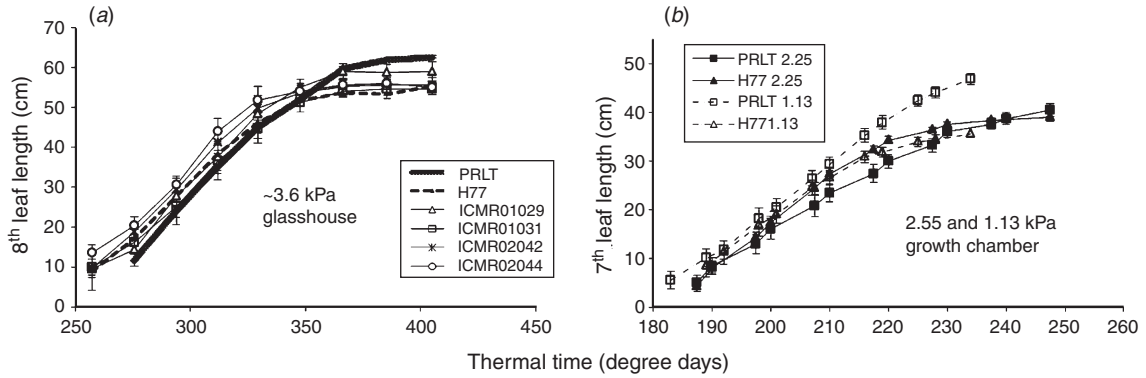

Fig. 1. Selected examples of leaf elongation of pearl millet genotypes grown in various ambient conditions: (*a*) in a glasshouse in Experiment 1b (eighth leaves) and (*b*) growth chamber with two vapour pressure deficit regimes in Experiment 3 (seventh leaves). In these experiments, terminal drought (TD)-adapted (PRLT) and TD-sensitive (H77) genotypes, and TD-adaptation quantitative trait locus-introgressed near-isogenic lines (ICMR01029, ICMR01031, ICMR02042, ICMR02044) were used. At each point in time, s.e. bars ($n = 5–6$) are shown. The growth rates of leaves across all experiments are summarised in Table 3 and analysed in Table 2.

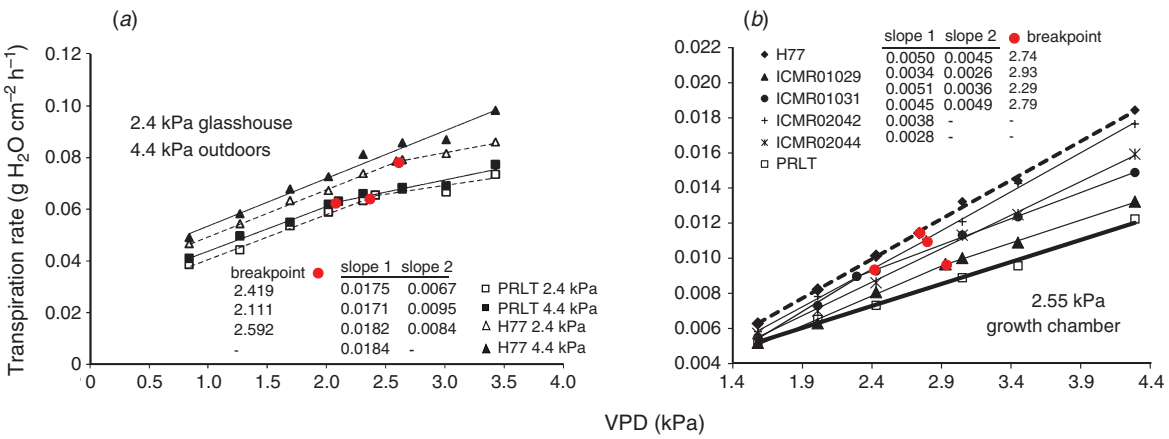

Fig. 2. Examples of genotypic variability in the short-term transpiration response (TR) of pearl millet to hourly increments in vapour pressure deficit (VPD) in (*a*) Experiment 2 and (*b*) Experiment 4 in high VPD conditions. In these experiments, terminal drought (TD)-adapted (PRLT) and TD-sensitive (H77) genotypes, and TD-adapted quantitative trait locus- introgressed near-isogenic lines (ICMR01029, ICMR01031, ICMR02042, ICMR02044) were used. The TR–VPD relationship was fitted with one or two linear segments, and the estimated slopes of the rise in TR with VPD are shown in the legend along with the position of the 'breakpoint' (i.e. the significant change in the TR–VPD relationship; here, indicated as a grey dot). Further details on the TR response to VPD in these and other experiments are shown in Table 4 and analysed in Table 2.

(Fig. 4*a*, *b*), TD-adapted PRLT used higher or similar amounts of water than TD-sensitive H77 during early development up to ~400–500°days (~25–30 DAS or around booting). From this point onwards, the total transpiration of H77 became higher than that in PRLT (this interval also coincided with the beginning of profuse tiller development; Fig. 4*a*, *b*; Experiments 1a, 1b and 4). These water use patterns were similar to those previously reported in field-like conditions (Vadez *et al*. 2013*a*). Higher VPD regimes during growth shortened the duration of the phase where PRLT had higher transpiration (data not shown). This also agrees well with the decreased LER and duration of expansive growth in PRLT under high VPD described above.

Effect of VPD growth regime on TE

There was no significant genotypic variation in TE across experiments (Table 2). As expected, there was a considerable decrease in TE under high VPD (Table 2). In addition, many of the water-use-related parameters were significantly correlated with TE (Table 6). As expected, there was a negative correlation of TE with total transpiration (T) and a positive correlation of TE with TDM. There was also a positive correlation of LER with TE and a negative relation between TE and TR, and between Slope 1 of the TR response to VPD (Table 6).

Discussion

VPD conditions during cultivation affect plant growth and related processes

In our study, cultivation of plants in high VPD decreased plant growth and related processes. High VPD inhibited the LER and the duration of leaf expansion. High VPD during growth restricted biomass accumulation and its partitioning into stems

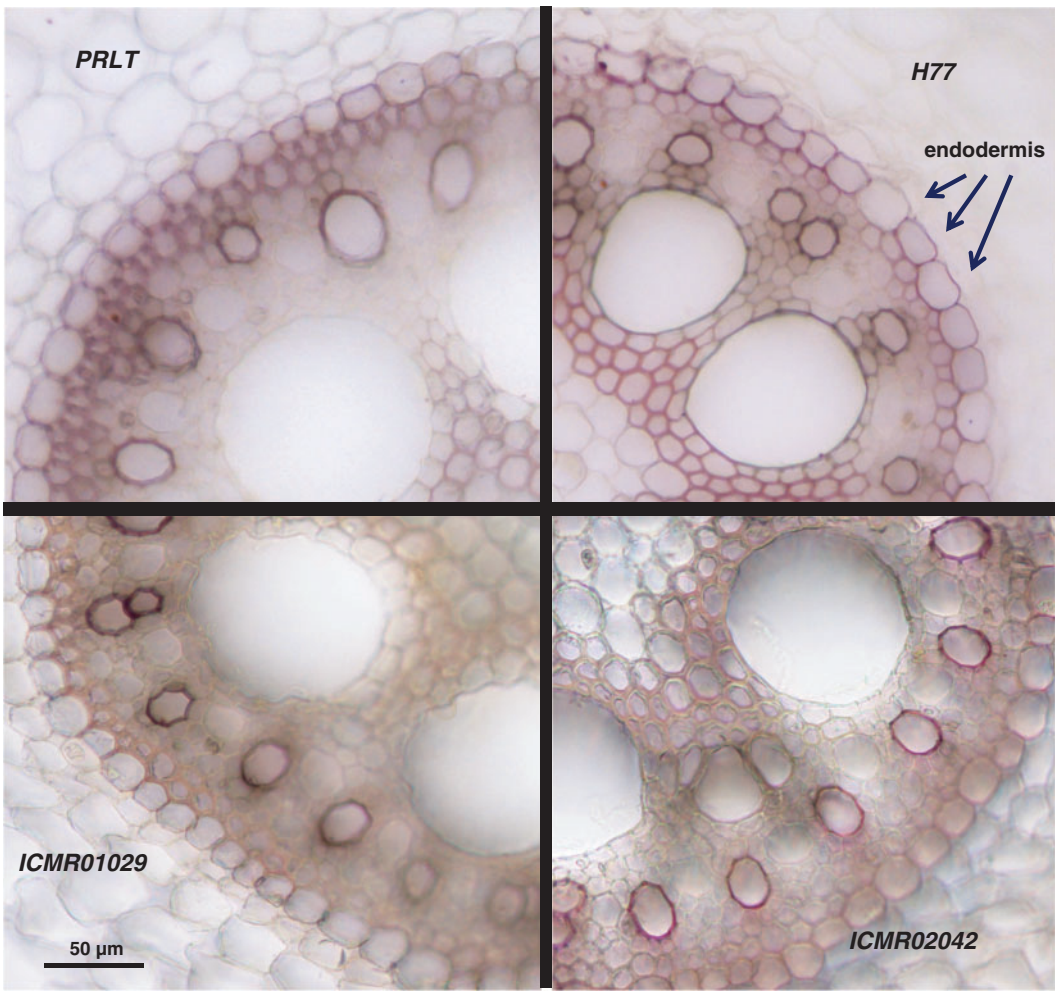

Fig. 3. Visualisation of the anatomical structure of pearl millet root segments stained with Sudan Red VIIB/ HCl-floroglucinol in terminal drought (TD)-tolerant (PRLT) and TD-sensitive (H77) genotypes, and TD quantitative trait locus-introgressed near-isogenic lines (ICMR01029, ICMR02042) from Experiments 1. Sections were cut freehand 3–4 cm from the root tip and observed under a light microscope (Olympus BX51) at 20 × 10 magnification. Endodermal cell characteristics from all observations are analysed in Table 2.

via a limited production of tillers and high VPD restricted transpiration.

The observed decrease in LER in high VPD growth regimes could reflect altered plant water acquisition properties, which are hypothesised to play a significant role in controlling leaf expansion (Caldeira *et al*. 2014). Similar responses of LER to VPD, though short-term, have been documented (e.g. Reymond *et al*. 2003; Sadok *et al*. 2007; Welcker *et al*. 2007, 2011). Comparatively less attention has been paid to the mechanisms and variability in duration of leaf expansion, which were the major contributors to the genotypic differences in the final leaf length in our work. As a consequence, plants developing shorter leaves under high VPD accumulated less biomass. By decreasing LER, high VPD would have indeed reduced the canopy size and then light capture capacity, resulting in a biomass reduction. Similar results were found in other crop species such as, pea (*Pisum sativum* L.)(Sakalauskiene *et al*. 2008), spinach (*Spinacia oleracea* L.) (Iwabuchi *et al*. 1996) and tomato (*Solanum lycopersicum* L.)(Lorenzo *et al*. 2002). However, because high VPD was partially driven by higher temperature, the plants grown in this regime might also have had a reduced carbohydrate supply : demand ratio as a consequence of shortened developmental phases (i.e. the same number of thermal time units accumulated during fewer days thus limiting the light-days available for photosynthesis; Kirnak *et al*. 2001) but also as a consequence of higher energetic demand (e.g. increased respiration) and decreased photosynthetic activity by the cells (Sakalauskiene *et al*. 2008). Decreased tiller production in high VPD growth regimes would then be an indirect consequence of this altered carbohydrate supply–demand balance, as shown earlier in sorghum (Kim *et al*. 2010*a*, 2010*b*; Alam *et al*. 2014).

VPD during growth affects how plants respond to transient changes in VPD

Our data demonstrated that plants grown under high VPD developed in a way that they were able to transport more

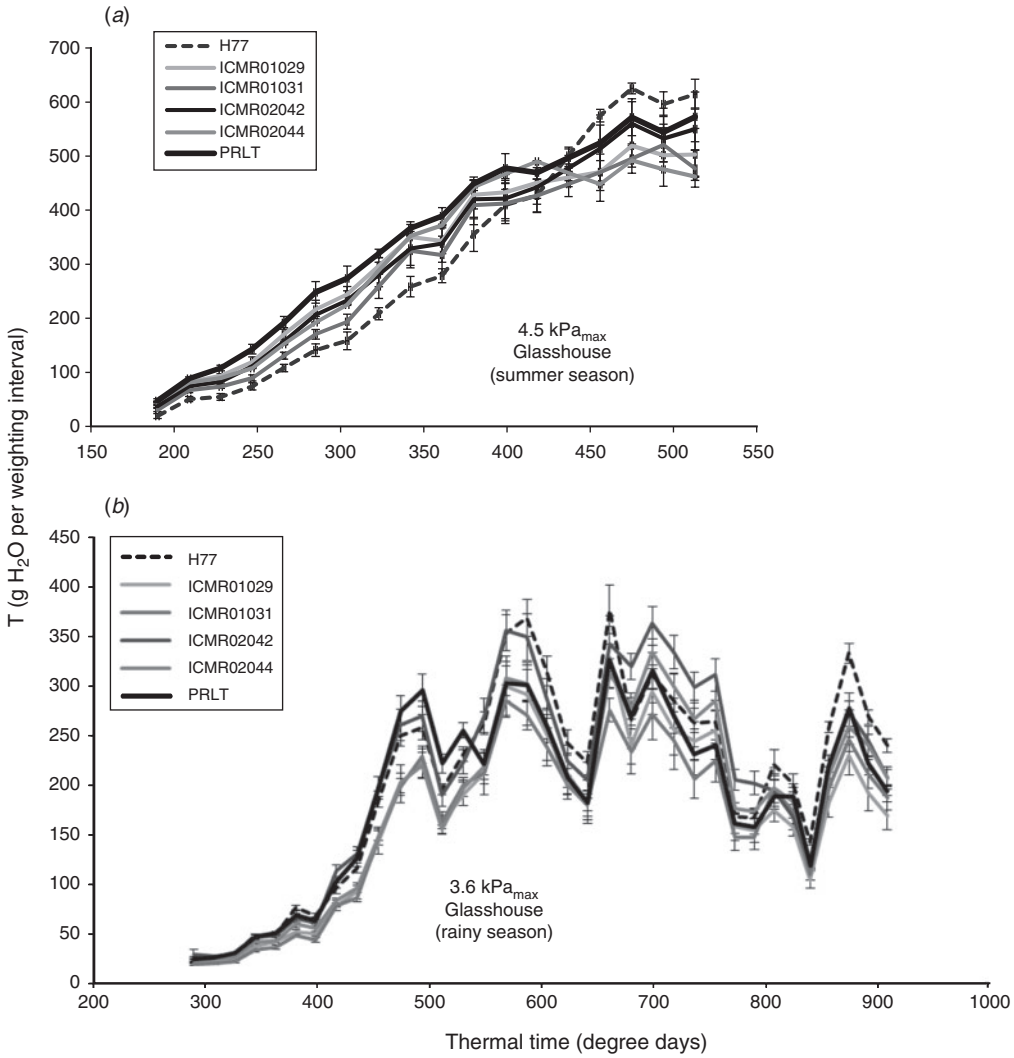

Fig. 4. Selected examples of water use patterns of terminal drought (TD)-adapted (PRLT) and TD-sensitive (H77) genotypes, and TD-adapted quantitative trait locus-introgressed near-isogenic lines (ICMR01029, ICMR01031, ICMR02042, ICMR02044). Genotypes were followed during their growth in the glasshouse in Experiments 1a and 1b during (*a*) summer and (*b*) the rainy season.

water through their tissues. In particular, the plants cultivated under high VPD conditions had a higher TR and higher rates of TR increase with increasing VPD, and there were differences in the magnitude of the genotypic response to the long-term VPD conditions of cultivation. This is in line with the work of Sermons *et al*. (2012), Seversike *et al*. (2013), Schoppach and Sadok (2013), and Yang *et al*. (2012), who showed that turfgrass, soybean, wheat and maize plants that had developed in lower VPD conditions had more effective TR regulation, and that these differences in TR regulation eventually altered plant canopy development and drought responses.

The VPD conditions of cultivation also altered the root anatomical structure of the genotypes, specifically the endodermis, which is hypothesised to be the root cell layer with high resistance to water flow (Steudle and Peterson 1998; Zimmermann and Steudle 1998; Bramley *et al*. 2009). The differences in endodermal cell dimensions (transversal width and area) were indeed positively correlated with characteristics expressing plants' ability to channel water (TR slopes). This link agrees with the previous hypothesis that the water flow through plant tissues is determined by the structure of these tissues (e.g. Rieger and Litvin 1999; Sperry *et al*. 2002; Bouchabke *et al*. 2006), particularly the roots, which also determines the conservative or nonconservative plant water use (i.e. a limited TR mechanism; Comstock 2002; Bramley *et al*. 2009; Sadok *et al*. 2012, 2013; Schoppach *et al*. 2014). Therefore, the correlations between some of the endodermis characteristics and TR features suggest a possible role of the root anatomical structure in the whole-plant capacity to channel water. We may speculate that the larger endodermal root cells found in TD-insensitive genotypes enabled higher radial water flux into the root vascular tissues and thus stimulated higher canopy conductivity and nonconservative water use behaviour (similarly in Schoppach *et al*. 2014). Of course, a caveat here was

that the VPD conditions were not fully controlled and additional work would be needed to confirm the VPD effects on the root anatomical characteristics during growth.

Effect of altered plant growth and related processes on water use over time and TE

TD-adapted PRLT and ICMR01029 had higher water usage during crop establishment compared with the TD-sensitive parent, followed by a period of comparatively lower water use from ~20 DAS to 25 DAS (~ 400–500 °day) up until the booting stage (similar to previous field-like observations in Vadez *et al*. 2013*a*). This phase of relatively higher initial water use in TD-adapted genotypes could be shorter if plants had been grown under high VPD conditions. Such behaviour was the result of interplay between the components of canopy growth and the canopy conductivity response to the prevalent VPD during growth, and is an important factor determining crop production in water-limited cropping systems (Chenu *et al*. 2011; Chauhan *et al*. 2013; Vadez *et al*. 2013*b*, 2014; Kholová *et al*. 2014).

A negative relationship between TE and the slope of the first part of segmental VPD–TR relationship was found (i.e. conservative water use led to higher TE), which agrees with a previous hypothesis (Vadez *et al*. 2014) that the genotype-specific capacity to restrict transpiration under high VPD reduced the mean daily effective VPD and then increased TE (Vadez *et al*. 2014). Limited TR could be a consequence of the hydraulic features of plant tissues (Sinclair *et al*. 2005; Vadez *et al*. 2014). In addition, there was a strong positive correlation between LER and TE, indicating that TE was also driven by leaf expansion processes. We may speculate that plants exhibiting higher LER have established a larger sink that would drive up the source (photosynthesis) and eventually enhance TE (Condon *et al*. 2002; Tardieu *et al*. 2013).

Water use component traits and their response to transient and long-term VPD changes are genetically determined

The comparison of NILs with the parental lines in this study suggests that TD-adaptation QTL was involved in modulating the responses of plants to the VPD conditions during growth. Though it might be difficult to distinguish a causal phenotype (QTL-determined) from the consequential phenotype (the result of genetic background interactions with QTL), in our case, the causal phenotype appeared to be the altered TR and TR responsiveness to short-term VPD exposure (as shown before in Kholová *et al*. 2010*b*, 2012), as well as the TR and TR responsiveness to the VPD growth regime. Specifically, the lower TR of NILs (determined by QTLs, similar to TD-adapted PRLT) in combination with limited leaf expansion (LER, not determined by QTL; similar to TD-sensitive H77) probably resulted in variability in the NILs' leaf thickness (SLW), which responded differently to changes in VPD growth regime compared with both parental lines (similarly in Tardieu *et al*. 1999).

Conclusion

Plant growth conditions affected the water use component traits. The long-term effects of growth conditions on plant growth and development (biomass accumulation and distribution, tillering, LER, duration of leaf expansion and root anatomy), and the consequent effects on the TR response to transient increases in VPD influenced the pattern of plant water use and productivity of water use (TE). Our work demonstrates the importance of understanding and evaluating component traits rather than complex traits (e.g. water use or TE). The large variability in the water use and TE components of NILs suggests that the TD-adaptive QTL influenced the plant processes linked to the developmental response to VPD. The environment-specific genotypic plasticity needs to be considered if trait-based breeding approaches are to be successfully realised.

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