Small temporal differences in water uptake among varieties of pearl millet (Pennisetum glaucum (L.) R. Br.) are critical for grain yield under terminal drought

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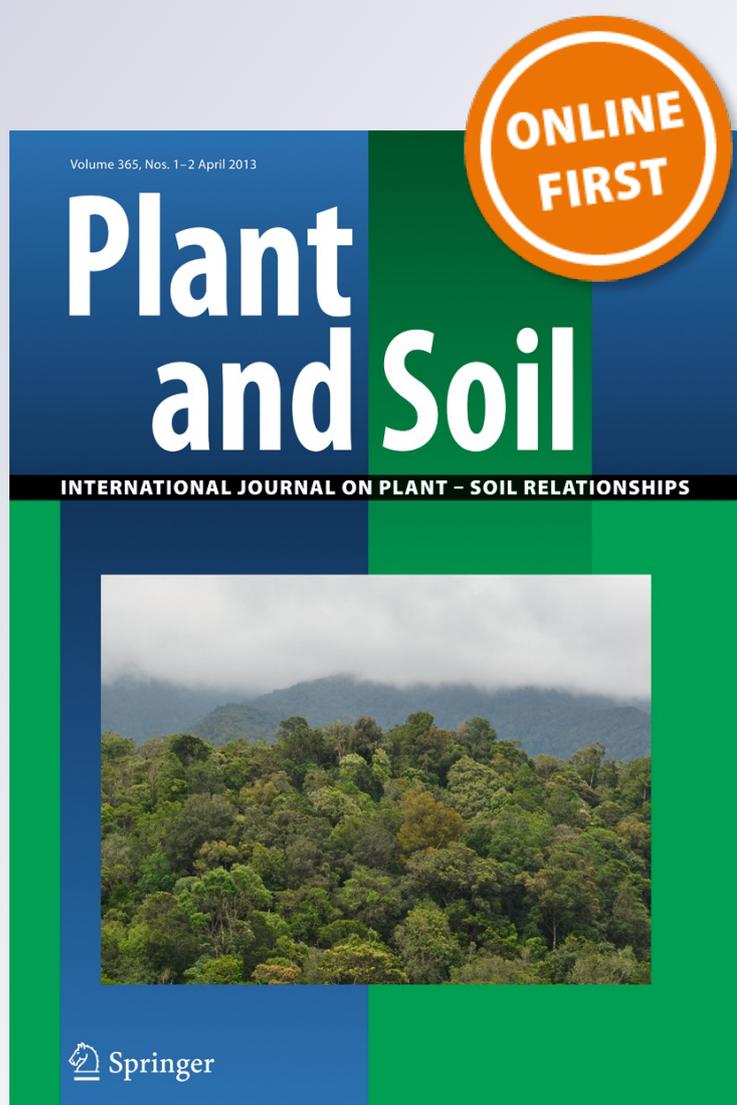Charles Tom Hash**

Abstract

Background and aim Intuitively, access to water from the soil at key phenological stages is important for adaptation to drought. This study aimed to assess the temporal pattern of water extraction under terminal drought stress.

Methods Pearl millet genotypes with varying levels of terminal drought tolerance were grown in a lysimetric system with a soil volume and plant spacing similar to field conditions. Water extraction was monitored until maturity under differing water regimes.

Results The yield did not differ among genotypes under well-watered (WW) conditions, and the water extraction profile of WW plants was similar across all genotypes. In contrast, the yield of sensitive genotypes was 30–100 % lower than that of tolerant lines under water stress (WS). The total volumes of water extracted by tolerant and sensitive genotypes were similar under WS; however, tolerant genotypes extracted less water prior to anthesis, and more water after anthesis. Grain yield was positively related to the amount of water extracted during week three after panicle emergence. Increased water extraction after anthesis benefitted the tillers more than the main culm and was correlated with higher staygreen scores.

Conclusion Increased water uptake after anthesis, which results from earlier water conservation during pre-anthesis, increases yield under terminal drought in pearl millet.

Keywords Roots · Water extraction · Lysimeter · Drought QTL · Water conservation

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Introduction

Drought is the most important abiotic stress that limits plant productivity and will only worsen in the future when constraints on water resources increase. Plant coping strategies under water deficit are limited and revolve around the need to use water in an efficient way, ensure that water is available for the grain filling period, and maximise water capture from the soil profile.

In previous reports, a major terminal drought tolerance quantitative trait locus (QTL) was identified and confirmed in two different genetic backgrounds (Yadav et al. 2002, 2004; Serraj et al. 2005). This major QTL was identified based on yield differences under terminal

drought, which was applied by stopping irrigation either at panicle emergence or at flowering, in repeated field trials. The phenotype used to detect this large QTL was the panicle harvest index, i.e., the ratio of the grain weight to the panicle weight, which reflects the success of seed set and seed fill. A higher grain yield under terminal drought was then a consequence of differences in seed number and size (Bidinger et al. 1987). However, this QTL spans a genomic region of approximately 30 cM, which is too large to be routinely used in an applied breeding program, and the underlying mechanisms of the QTL are still unclear. Therefore, dissecting this QTL into more easily transferable components is necessary for breeding. Obtaining an understanding of the mechanisms leading to improved seed fill and seed set that are conferred by this QTL is a critical first step.

In recent work, near isogenic lines (NILs) introgressed with this terminal drought tolerance QTL (NIL-QTLs) had a lower canopy conductance (Kholová et al. 2010a), which was also sensitive to a high vapour pressure deficit (Kholová et al. 2010b). These traits contribute to water conservation and cause genotypes to use the soil water at a slower pace, hence leaving water available for the grain filling period. Similar water conservation traits also appear to operate in terminal drought tolerant chickpeas (Zaman-Allah et al. 2011a), and indeed, these traits altered the pattern of water extraction in a way that left more water available for the grain filling period (Zaman-Allah et al. 2011b). In wheat, "water conservative" lines with low $\Delta^{13}C$ had an advantage when they were grown on stored soil moisture or in areas with limited rainfall (Condon and Richards 1993; Condon et al. 2002, 2004; Rebetzke et al. 2002).

Differences in root morphology may also be responsible for differences in terminal drought tolerance among different crops (Price and Tomos 1997; Tuberosa et al. 2002; Kashiwagi et al. 2006; Gaur et al. 2008). Grain yield under stress is indeed influenced by deep rooting in crops such as chickpea (Kashiwagi et al. 2006) and wheat (Ehdaie et al. 2012). In rice plants, lateral root branching was promoted under mild water stress and contributed to root plasticity and genotype performance (Kano et al. 2011). However, these preliminary studies relied on structural assessments of roots, which are destructive measurements. Measuring water uptake in vivo over the entire life of the crop would provide better information regarding the functional role of the root in water extraction compared with a one-time

root morphology assessment (Vadez et al. 2007, 2008). Indeed, effective use of water, which partially involves maximising plant water capture from the soil profile, is critical for drought adaptation (Blum 2009). For example, Manshadi et al. (2010) showed that a narrower root architecture in the wheat genotype SeriM82 increased water extraction and yield in water-limited environments. Drought-adapted water conserving wheat genotypes exhibited less root growth and water extraction; however, this was compensated for by a higher transpiration efficiency (Mori and Inagaki 2012). In maize, genetic variation in root architecture was also noted, although deeper rooting was related to lower water use efficiency (Hund et al. 2009). Thus, while roots are likely to play a role in the drought adaptation of different crops, the extent to which they contribute and their interactions with other traits that contribute to plant water use remain unclear. Additionally, it is not known whether this terminal drought tolerance QTL in pearl millet contributes to an increase in water uptake.

In this work, we first wanted to understand whether contrasting genotypes differ in the total amount of water extracted from the soil profile, and second, we wanted to explore whether the timing of water uptake is an important factor. Our first focus follows recent work in sorghum showing differences in total water extraction under terminal drought among germplasm entries (Vadez et al. 2011a) and among staygreen QTL-introgressed sorghum lines (Vadez et al. 2011b). Our second focus follows recent work in chickpea, in which greater water extraction during the grain filling period (rather than total water uptake) discriminated terminal drought-tolerant from terminal drought-sensitive lines (Zaman-Allah et al. 2011b). Here, the pattern of water use was assessed in NIL-QTLs to explore possible variations leading to differences in grain yield. This work was done in a lysimetric system, i.e., a set of long, large tubes that mimic field conditions with regard to soil profile, water availability and aerial plant spacing (Vadez et al. 2008). Yield evaluations in this system agree with field assessments (Zaman-Allah et al. 2011b; Vadez et al. 2011a, b) and are combined to achieve a precise assessment of the components of a water-based framework ($Y = WU \times TE \times HI$) (Passioura 1977), where Y, WU, TE, and HI represent yield, water uptake, transpiration efficiency and harvest index, respectively. Few reports have assessed plant water use starting from an early vegetative stage and continuing until maturity using large containers (Payne et al. 1991,

1992). Additionally, the staygreen phenotype, which is hypothesised to be a consequence of increased water extraction during grain filling, was measured here as an attempt to transfer this knowledge to field applications. Therefore, the current approach creates a framework in which several traits that potentially contribute to drought adaptation are combined, using NIL-QTLs, in an attempt to more effectively bridge physiology, genetics, and breeding (Cattivelli et al. 2008).

The main objective of this work was to assess the temporal pattern of water extraction in pearl millet and test possible relationships with yield differences under terminal drought. The specific objectives were to (i) assess seed yield differences under terminal drought and fully irrigated conditions, (ii) compare the timing of water extraction and the total amount of water extracted by these lines, and (iii) assess how putative differences in the temporal water extraction patterns correlate with seed yield and particularly with tiller seed yield, seed size, and staygreen scores. The work was performed using contrasting pearl millet test-cross hybrids, including NILs for a terminal drought tolerance QTL, in a lysimetric system that allows plant water extraction to be monitored.

Materials and methods

Plant material

Two pearl millet (*Pennisetum glaucum* [L.] R. Br.) genotypes with contrasting tolerance to drought stress (PRLT 2/89-33 [tolerant] and H 77/833-2 [sensitive])and six QTL-introgression lines (ICMH 01029, ICMH 01031, ICMH 02040, ICMH 01046, ICMH 02042, ICMH 02044) were tested (details on their development are available in Kholová et al. 2010a). The work was performed using test-cross hybrids of these genotypes, which were developed by crossing the inbred parental lines and QTL-NILs to the male-sterile line tester 843A (Yadav et al. 2004). ICMH 01029, ICMH 02040, ICMH 01046, and ICMH 01031 were selected for their superior combined seed yield across a range of terminal drought environments (Serraj et al. 2005). ICMH 01029, ICMH 01031, ICMH 02042, and ICMH 02044 also exhibited contrasting water conservation traits at various vegetative stages under non-stress conditions (Kholová et al. 2010a, b).

Growth conditions in the lysimeters and water in the soil profile

Plants were grown outdoors in lysimeters, i.e., PVC tubes 25 cm in diameter and 2.0 m long that were filled with a sandy clay loam Alfisol at a bulk density of approximately 1.35 g cm^{-3}, which is a standard value for Alfisols. The Alfisol was collected from the ICRISAT farm and sieved to particles smaller than 1 cm. Although at first filling the original structure of the soil was disturbed, a normal soil structure was likely to have been established over the lifetimes of the different crops that preceded this one. Details regarding soil filling and fertilisation are described in an earlier paper (Vadez et al. 2011a). Plant spacing and soil volume available for water extraction in the lysimetric system were similar to those in field conditions; however, the system did not reproduce root-to-root competition.

The cylinders could be lifted and weighed with a block-chained pulley and an S-type load cell with a 200-kg capacity (Mettler-Toledo, Geneva, Switzerland) and an accuracy of 20 g. The lysimeters were separated from one another by a distance of approximately 5 cm; thus, the plant density was approximately 10 plants m^{-2}, which is similar to typical pearl millet field plantings at ICRISAT. This allowed an accurate assessment of the water extraction pattern of a crop cultivated under conditions similar to those in the field.

Prior to their use with the pearl millet crop, the lysimeters were used to grow a crop of foxtail millet followed by a crop of sorghum. While harvesting the previous crops, only the main root stock of the plants was removed, and the soil was tilled superficially with sickles. Therefore, the soil profile in which the pearl millet was cultivated was mostly undisturbed during the previous croppings except for minimal surface tillage.

Water extraction measurements and treatments used

Seeds were sown in four hills in each cylinder on February 7th, 2009. During the cropping period, the maximum and minimum temperatures ranged from 29.4–40.7 °C to 15–27.5 °C, respectively, and the maximum and minimum relative humidity ranged from 50–91 to 14–52 %, respectively. The average vapour pressure deficits during the cropping and transpiration monitoring

periods were 2.55 kPa and 2.69 kPa, respectively. The plants were thinned to two seedlings per cylinder at 14 days after sowing (DAS) and later thinned to one plant per cylinder at 21 DAS. The crop was top dressed with 1.38 g N plant^{-1} (as urea) at 28 DAS. All the plants were fully irrigated until 28 DAS. Each cylinder received 500 mL of water twice a week until 14 DAS and 500 mL on alternate days thereafter until 28 DAS. At 26 DAS, the cylinders were covered with a 2-cm layer of low-density polyethylene beads to prevent soil evaporation. No correction was made for biomass increase between weighings as this was negligible compared to plant water use. Weighings were performed at 29, 34, 39, 46, 54, 60, and 77 DAS.

Three water treatments were used: a well-watered control (WW), which was watered each week, an early water stress (ES) treatment imposed by applying a final irrigation of 2 L plant^{-1} at 34 DAS (at panicle emergence), and a late water stress (LS) treatment imposed by applying a final irrigation of 2 L plant^{-1} at 41 DAS (2 days after the mean flowering date). A randomised block design was used with treatment as the main factor and genotype as the sub-factor, which was randomised within each main factor. Well-watered plants were watered every week, and re-watering was performed up to 2 kg below the field capacity weight (obtained on 29 DAS) to avoid water drainage. At 68 DAS, the WW plants received an additional irrigation with the same amount of water applied after the 60 DAS weighing.

Flowering time (d) was recorded for each individual plant. The staygreen phenotype was scored 3 weeks after panicle emergence in the LS treatment group using a 3-point scale (1, all leaves are wilted or showing senescence; 2, some of the leaves are wilting or showing senescence; 3, most/all leaves are green and turgid). Water extraction to support transpiration was calculated from cylinder weight differences and water additions. Six (WW and LS) and five (ES) transpiration values were obtained. Pre-anthesis water extraction was calculated as the sum of the first two transpiration values (until 39 DAS). Post-anthesis water extraction was calculated as the sum of the transpiration values after 39 DAS. At harvest, the main culm and tillers were harvested separately, and for each part, the masses of leaves and stems including sheaths, panicles, and grains were recorded after drying for 3 days in a forced air oven set at 70 °C. The number of grains per plant and the 100-grain weight were then determined without separating the main culm from the tillers. The panicle harvest index (PNHI) was calculated by dividing the grain yield by the total panicle biomass. Transpiration efficiency (TE) was calculated by subtracting the biomass at 29 DAS from the total aboveground biomass and dividing the result by the total amount of transpiration from 29 DAS until maturity. The biomass at 29 DAS was measured using an extra set of plants grown under WW conditions and ranged from 17 to 23 g across the genotypes. Roots were not extracted in this trial; thus, the TE assessment was based only on shoot biomass and was slightly underestimated. In earlier studies, we hypothesised that omitting the roots was not likely to alter the genotypic ranking (Vadez et al. 2011a, b).

Data management and statistical analysis

The crop preceding pearl millet in the lysimeters (Vadez et al. 2011a, b) underwent different watering regimes. Watering prior to sowing the pearl millet crop did not restore a full soil profile in all cylinders. Therefore, the soil moisture content differed significantly among the cylinders when pearl millet was initially planted. The cylinder weight ranged from 156 to 164 kg, whereas tubes at field capacity normally weigh between 163 and 165 kg. This caused significant linear relationships between the total seed yield and the initial cylinder weight in each of the two WS treatments (R^2=0.21 in the LS; R^2=0.48 in the ES). However, in each WS treatment, linear regressions were run on separate groups of lysimeters with cylinder weights either below or above a median weight (159.4 kg in LS and 160.5 kg in ES). These relationships were non-significant in the LS group (R^2=0.04 and R^2=0.04 for cylinders below and above the median weight, respectively), and were significant but relatively weak in the ES group (R^2=0.07 and R^2=0.12 for cylinders below and above the median weight, respectively). Therefore, the cylinders were separated into those with an unfilled profile (weight below the median) and those with a full profile (weight above the median), and four WS treatments were considered in the data analysis: ES with either a full profile (FP) or an unfilled profile (UP) and LS with either an FP or a UP. In fact, what appeared initially to be an experimental caveat provided us with an interesting opportunity to test crop responses under a wide range of stress conditions.

For the statistical analysis, regressions were analysed using Pearson correlation coefficients. One-way ANOVAs were performed to assess genotypic effects, which were fixed effects, within each of the watering regimes (including the WW treatment). Subsequently, a two-way ANOVA was used to assess treatment (T), genotypic (G) and genotype-by-treatment interactions (GxT) considering each of the four WS treatments individually and excluding the WW treatment. To perform the multi-linear regression analysis, we used the multi-linear additive model in STATA (Stata Corp., College Station, TX, USA) with yield as an additive function of water uptake (WU), transpiration efficiency (TE), harvest index (HI), and WU at different time points during the crop cycle. A similar model was used in which the PNHI was considered an additive function of WU at different time points during the crop cycle.

Results

Agronomic traits under well-watered conditions

The genotypes did not differ significantly with respect to flowering time, total water use, or water use in either the pre- or post-anthesis period. Notably, ICMH01046 had the lowest dry mass, and ICMH01031 and ICMH02044 had the highest dry mass (Table 1). Yield differed among the varieties, with ICMH02044 and ICMH01046 producing the largest and smallest yields, respectively. PRLT-2/89-33 had fewer tillers and more grains per panicle than the other genotypes. Only the NILs ICMH01031 and ICMH01046 had significantly fewer tillers than the recurrent parent H77/833-2. However, the QTL did not influence the number of grains under WW conditions (Table 1). TE was significantly lower in PRLT-2/89-33 than in most other genotypes. PRLT-2/89-33 and the recurrent parent H77/833-2 had the highest and lowest 100-seed weights, respectively, while the NILs had intermediate values (Table 1).

Agronomic traits under water stress conditions

The grain yield differed significantly among the genotypes under three out of the four water stress treatments (data not shown). Overall, there was a highly significant G effect on yield, and the yields of H77/833-2, ICMH01031, and PRLT-2/89-33 were approximately 30 % lower across all treatments than

Table 1 Means and one-way ANOVAs for agronomic and water use traits under well-watered (WW) conditions

	Flowe-ring time	Tiller number	Total grain yield	Total dry weight	Total WU	Pre-anthesis WU	Post-anthesis WU	TE	Tiller grain yield	100-seed weight	Seed number panicle^{-1}	PNHI
01029	35	8.1	22.7	92.8	28.2	6.1	22.2	2.58	15.9	0.77	1229	0.66
01031	35	7.3	26.6	98.0	30.2	6.2	24.0	2.50	19.8	0.71	1274	0.71
01040	35	8.0	22.6	83.9	26.8	5.7	21.1	2.50	16.5	0.70	1045	0.69
01046	35	7.8	20.5	77.7	27.1	5.4	21.6	2.48	16.2	0.58	1105	0.65
02042	36	8.5	27.0	93.1	30.1	6.2	23.9	2.35	19.7	0.68	1148	0.71
02044	36	9.1	28.6	99.6	31.6	5.9	25.6	2.56	20.4	0.69	1205	0.73
H77	37	10.0	25.6	93.5	30.1	5.9	24.2	2.44	19.1	0.52	1352	0.72
PRLT	36	6.5	25.4	83.9	27.6	6.2	21.4	2.18	13.0	0.84	2524	0.69
Mean	36	8.2	24.9	90.3	29.5	6.0	23.0	2.45	17.6	0.69	1360	0.69
LSD	ns	1.9*	5.3*	14.1*	ns	ns	ns	0.24*	4.8*	0.12***	390***	0.05**

Traits include flowering time (days after sowing), tiller number per plant, total grain yield (g plant^{-1}), total dry weight (g plant^{-1}), total water uptake (WU) (kg plant^{-1}), water uptake in the pre-anthesis and post anthesis period (kg plant^{-1}), transpiration efficiency (TE, g kg^{-1}), tiller grain yield (g kg^{-1}), 100-seed weight (g plant^{-1}), seed number per panicle and panicle harvest index (PNHI) in pearl millet entries with varying levels of terminal drought tolerance . *ns* not significant

those of ICMH01029, ICMH01040, and ICMH01046 (Table 2). However, the yields of H77/833-2, ICMH01031, and PRLT-2/89-33 were up to 100 % higher under specific treatments, especially in an unfilled profile (data not shown). The total dry mass differed significantly among the genotypes under the ES treatment in an unfilled profile and across all stress treatments. Differences in flowering time were small, although there were significant differences among genotypes under the LS treatment in an unfilled profile and across stress treatments in a filled profile. Differences in tiller number among genotypes were also small and were mostly related to the lower tiller number of PRLT-2/89-33 (Table 2). Genotype-by-treatment (GxT) interaction effects were not significant for flowering time, tiller number, grain yield or total dry mass (Table 2). The yield differences between plants exposed to LS vs. ES treatments were smaller (10.75 vs. 9.25 g plant^{-1}) than the differences between plants grown in a full profile vs. an unfilled profile (12.0 vs. 8.0 g plant^{-1}), showing that the amount of water in the soil profile was a more important factor than the timing of water stress imposition.

The total water extracted from the soil profile differed significantly between the LS and ES treatments in an unfilled profile. There was also a small but significant G effect on water uptake across the treatments ($P<0.02$) (Table 3). However, this was mainly due to the low water uptake of the tolerant parent PRLT-2/89-33. In contrast, none of the NILs differed from the recurrent parent with respect to total water uptake, indicating that the QTL had no effect on the overall water capture of the recurrent parent (Table 3). However, pre-anthesis water use differed significantly among genotypes and across treatments, with H77/833-2 exhibiting significantly higher pre-anthesis water use compared with all other genotypes except ICMH01031 and ICMH02042. In contrast, the post-anthesis water uptake was significantly lower in H77/833-2 compared with ICMH01029 and ICMH01046 (Table 3). Genotypes with the highest yields exhibited lower water uptake under WS conditions before anthesis and higher water uptake compared with sensitive H77/833-2 during the post-anthesis period—this was especially the case for ICMH01029 at 3 weeks after flag leaf appearance

Table 2 Flowering time (days after sowing), tiller number per plant, total grain yield (g plant^{-1}), and total dry mass (g plant^{-1}) of eight pearl millet genotypes across all water stress treatments (Genotype) or under four water stress treatments across all genotypes (Treatment)

The water stress treatments were a combination of a late stress (LS) and early stress (ES) applied in lysimeters with either an initial full soil moisture profile (FP) or an initial unfilled soil moisture profile (UP). Some of the pearl millet genotypes contained a terminal drought tolerance QTL. Two-way ANOVA was used to determine genotype (G), treatment (T), and genotype-by-treatment interaction (GxT) effects as well as least significant differences (LSDs)

	Flowering time (days after sowing)	Tiller number plant^{-1}	Total grain yield (g plant^{-1})	Total dry mass (g plant^{-1})
Genotype				
01029	37	6.2	11.5	55.5
01031	36	5.8	8.5	48.3
01040	37	6.8	10.8	52.0
01046	36	6.9	11.8	51.8
02042	37	6.3	10.0	52.7
02044	38	6.3	9.2	50.7
H77	38	7.5	8.5	52.8
PRLT	38	5.2	8.7	47.7
LSD	0.9	1.1	1.8	4.3
Treatment				
LS-FP	36	5.9	12.9	54.8
LS-UP	35	5.3	8.5	49.1
ES-FP	37	7.2	11.1	52.7
ES-UP	36	7.2	7.5	49.7
LSD	0.7	0.8	1.3	3.0
	F-Probability	F-Probability	F-Probability	F-Probability
T	0.002	0.001	0.001	0.001
G	0.001	0.005	0.001	0.01
GxT	ns	ns	ns	ns

Table 3 Total water uptake (WU) (kg plant^{-1}), transpiration efficiency (TE, in g kg^{-1} WU), and water uptake during pre- and post- anthesis (kg plant^{-1}) of eight pearl millet genotypes across all water stress treatments (Genotype) or under four water stress treatments across all genotypes (Treatment)

	Total WU (kg plant^{-1})	TE (g kg^{-1} WU)	Pre-anthesis WU (kg plant^{-1})	Post-anthesis WU (kg plant^{-1})
Genotype				
01029	12.2	2.89	5.8	6.4
01031	11.4	2.37	5.9	5.5
01040	11.5	2.90	5.5	6.0
01046	11.8	2.95	5.6	6.2
02042	11.6	2.71	6.0	5.6
02044	11.7	2.70	5.8	5.9
H77	11.8	2.89	6.3	5.5
PRLT	11.2	2.19	5.4	5.8
LSD	0.6	0.35	0.5	0.7
Treatment				
LS-FP	14.3	2.46	5.7	8.6
LS-UP	12.3	2.38	6.0	6.3
ES-FP	11.5	2.88	5.5	5.9
ES-UP	10.2	2.91	5.8	4.4
LSD	0.4	0.25	0.3	0.5
	F-Probability	F-Probability	F-Probability	F-Probability
T	0.001	0.001	0.026	0.001
G	0.02	0.001	0.02	0.04
GxT	0.05	ns	ns	0.05

The water stress treatments were a combination of late stress (LS) and early stress (ES) applied in lysimeters with either an initial full soil moisture profile (FP) or an initial unfilled soil moisture profile (UP). The pearl millet genotypes contrasted for a terminal drought tolerance QTL. Two-way ANOVA was used to determine genotype (G), treatment (T), and genotype-by-treatment interaction (GxT) effects as well as least significant differences (LSDs)

(Fig. 1a). In contrast, under fully irrigated conditions, there were no differences in the timing of water uptake except during the 2 weeks before maturity when the water uses of H77/833-2 and ICMH02042 were higher than those of ICMH01040 and ICMH01046 (Fig. 1b). Transpiration efficiency did not differ between H77/833-2 and the NILs regardless of treatment (Table 3); however, PRLT-2/89-33 had a lower TE than several of the other genotypes.

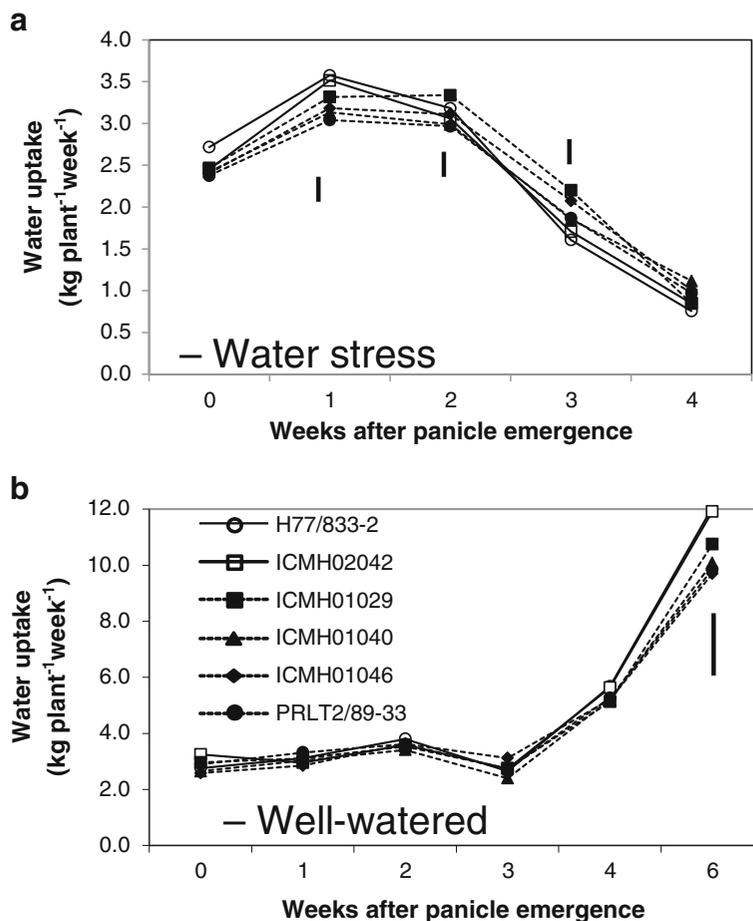Relationships between water uptake patterns and yield components

The water uptake differences in week three after panicle emergence were regressed against the grain yield data, and a clear positive relationship emerged in both the LS (Fig. 2a) and ES treatments (Fig. 2b). These regressions revealed similar trends in water uptake at 4 weeks after sowing and post-anthesis water uptake (data not shown).

Because tillers flower later than the main culm, water uptake differences during the grain filling period might be more critical for the tillers than for the main culm. Indeed, the relationship between water uptake at 3 weeks after panicle emergence and grain yield in the tillers was highly significant ($R^2=0.74$; Fig. 3a). There was also a significant relationship between water uptake at 3 weeks after panicle emergence and the grain yield in the main head ($R^2=0.61$; Fig. 3b).

These water uptake differences had notable effects on the tiller grain yields of the NILs, which were 50-100 % higher in ICMH01029, ICMH01040, and ICMH01046 compared with H77/833-2 and ICMH02044 (Table 4). The lower tiller grain yield of PRLT-2/89-33 was related to its lower tillering ability. The 100-seed weight of the NILs also differed significantly among genotypes exposed to LS treatment in an unfilled profile, ES treatment in a filled profile, and WS treatment in all cases (data not shown). The 100-seed weight of H77/833-2 was approximately 50 % lower than that of the NILs. The seed number per panicle varied significantly among genotypes: ICMH01029 and ICMH01046 had a higher seed number per panicle compared with H77/833-2 ($P<0.1$) and ICMH01031 ($P<0.05$). ICMH01042 also had a lower seed number compared with ICMH01029 ($P<0.05$). Hence, the panicle harvest index (PNHI) of

Fig. 1 Pattern of water extraction from the time of panicle emergence until maturity in six pearl millet germplasm entries including the recurrent parent H77/833-2 and lines introgressed with a terminal drought tolerance QTL from the donor (PRLT2/89-33) into the background of recurrent parent. Water uptake (kg plant^{-1} week^{-1}) was measured under water stress (WS, a) and well watered (WW, b) conditions. *Vertical bars* indicate LSD at $P<0.05$. The absence of bars above specific dates indicates that genotypic differences were not significant. The timing of intervals indicates the number of weeks after panicle emergence, and the water uptake data assigned to each date corresponds to the time period (usually 1 week) preceding that date. The data are the means of 20 lysimeters exposed to early water stress and late water stress and include cylinders with filled and un-filled profiles

ICMH01029, ICMH01040, and ICMH01046 was higher than that of H77/833-2 across all water stress treatments (Table 4).

Relationships between water uptake and staygreen score

Staygreen scores were recorded for plants exposed to LS treatment, and these scores were tightly correlated with water uptake during week three after panicle emergence in both filled ($R^2=0.79$) and unfilled profiles ($R^2=0.76$) (excluding values for PRLT-2/89-33 on the right side of the regression) (Fig. 4).

Interactions between yield, water use, transpiration efficiency and harvest index

A multi-linear additive model indicated a predominant role for the harvest index (HI) under all watering regimes (Table 5). Under WW conditions, the magnitude of the HI effect was slightly above that of the water uptake (WU) effect, whereas under the LS and ES treatments, HI had the most prominent effect.

Other multi-linear regressions were performed in which water uptake amounts during each of the weeks after panicle emergence were included in the additive model. Under WW conditions, the temporal water uptake pattern did not significantly affect the total water use coefficient (data not shown). In contrast, under the late stress treatment, the magnitude of the total water use coefficient was small compared with the TE and HI coefficients (Table 6). In agreement with the results reported above (Fig. 1–Suppl Fig. 1), the amount of water uptake in weeks three and four after panicle emergence had a strong effect on yield that was similar in magnitude to the effect of total water use. Similar results were obtained with the early stress treatment (data not shown). The effects of temporal patterns of water use on the panicle harvest index (PNHI) were tested in the late stress treatment,

Fig. 2 Relationships between the total grain yield at maturity and the water extracted (kg plant^{-1}) during the third week after panicle emergence under late stress (LS, a, open and closed circles) and early stress (ES, b, open and closed squares) conditions. Lysimeters with an initial full soil profile are represented by *closed symbols*, while those with an initial unfilled profile are represented by *open symbols*
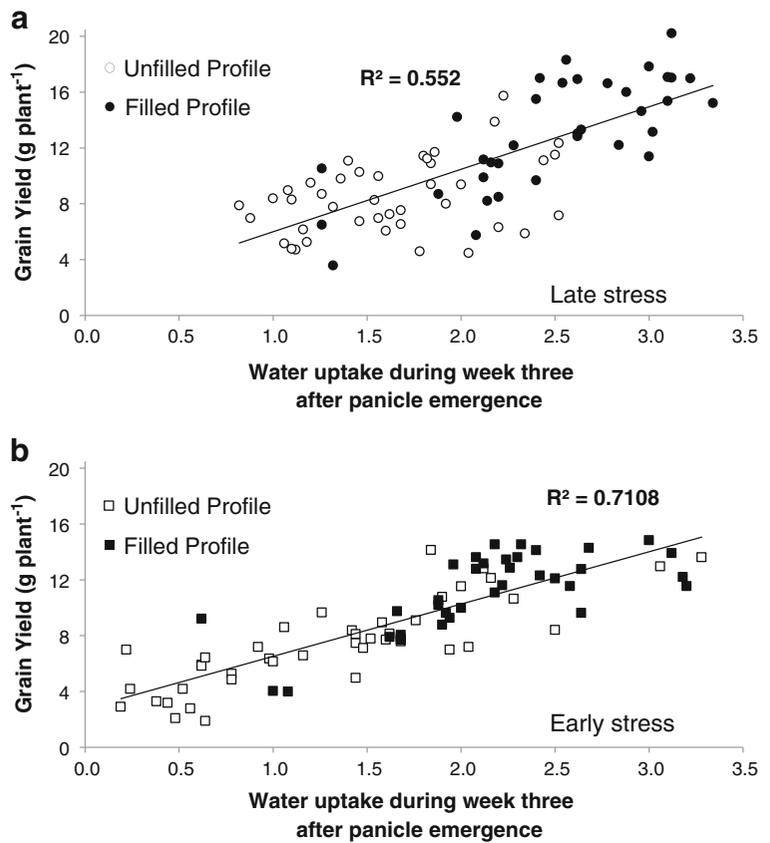
and it was found that water uptake had a very strong negative effect on PNHI in week two after panicle emergence. However, water uptake had very strong positive effects on PNHI in weeks three and four after panicle emergence. Similar results were obtained with the early stress treatment (data not shown). Additionally, similar coefficient values were obtained when the harvest index was used as a dependent variable in place of PNHI (data not shown), indicating strong interactions between the coefficients of the water-based framework (WU, TE and HI).

Discussion

This work showed yield differences under terminal stress conditions and a lack of yield differences under well-watered conditions in the lysimetric system. This confirmed the results obtained with these genotypes in earlier field trials (Serraj et al. 2005) and therefore validated the use of the lysimetric platform for obtaining relevant agronomic assessments under controlled conditions. The highest yielding genotypes under terminal drought stress did not extract more water from the soil profile but rather exhibited a pattern of water extraction that favoured increased water uptake during the post-anthesis period and less water uptake around and before anthesis. Higher water uptake at 3 and 4 weeks after panicle emergence then led to higher grain yields, especially under the early stress treatment, and enhanced tiller yield slightly more than the main head yield. A very important result obtained during this study was that the increased water extraction at 3 weeks after panicle emergence was significantly correlated with higher staygreen scores, which can be recorded in the field and used as a simple proxy for measurement of water extraction during grain filling under terminal drought.

Yield and evaluation of Passioura's equation components in the lysimetric system

The results presented here provide confirmation that lysimeters are suitable for obtaining relevant agronomic assessments under various environmental conditions.

Fig. 3 Relationships be-
tween the grain yield of the
tillers (**a**) and the main head
(**b**) and the water extracted
(kg plant^{-1}) during the third
week after panicle emer-
gence in the late stress (*tri-
angle*) and early stress
(*square*) conditions. Lysim-
eters with an initial full soil
profile are indicated by
closed symbols, while those
with an initial unfilled pro-
file are represented by *open
symbols*. The data are the
means of five replicate
plants per genotype and wa-
ter treatment combination.
The large circles (open and
closed representing unfilled
and filled profiles, respec-
tively) represent the data
points for PRLT2-98/33, a
low-tillering line that was
excluded from the regres-
sion analysis involving tiller
grain yield
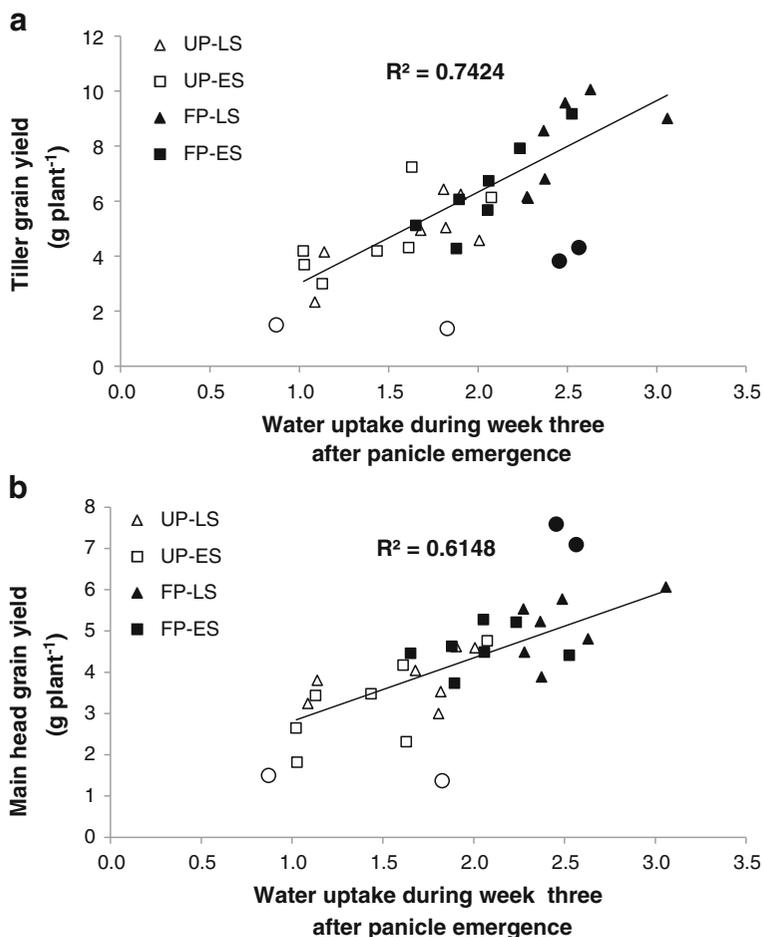
This is important because the system was used here to assess possible relationships between temporal patterns of water use and yield. These results confirm other recent assessments using this system. For example, in a trial examining twenty chickpea genotypes that were selected based on their highly divergent phenotypes under termi-nal drought stress during 3 years of field assessments (Krishnamurthy et al. 2010), eleven out of twelve tolerant lines were also tolerant in the lysimeters, and seven out of eight sensitive lines were also sensitive in the lysimeters (Zaman-Allah et al. 2011a). Similar results were obtained in peanut (Ratnakumar and Vadez 2011) and sorghum (Vadez et al. 2011b). The reason for the similarity be-tween lysimetric and field assessments is partially due to the similarity in plant spacing, soil availability for water exploration, and general growing conditions as the lysi-metric facility is outdoors. Of course, plants are grown individually in the tubes, and the system cannot mimic potential root-to-root competition. Therefore, this system

provides an opportunity to measure yield and other com-ponents and relate these to *in vivo* assessments of water uptake and phenotypes that are normally measured in the field.

The system also allows for the assessment of TE over the long term; therefore, comparisons can be made to previous studies in which long-term assess-ments were performed. For example, TEs in Payne et al. (1992) were approximately 5 and 8 g kg^{-1} under WW and WS conditions, respectively (approximately 600 and 200 g of biomass transpired approximately 120 and 25 kg of water under WW and WS conditions, respectively). Using Bierhuizen and Slatyer's equation (1965), which is akin to TE=k/VPD, 'k' coefficient values of 7.5 and 12 Pa under WW and WS were estimated from Payne's experiment using 1.5 kPa as the average VPD. In comparison, the mean TE in our experiment were 2.45 and 2.70 under WW and WS, respectively, yielding 'k' values of 6.1 and 6.7 Pa

Table 4 Tiller grain yield (g plant^{-1}), 100-seed weight (g per 100 seeds), seed number per panicle and panicle harvest index (PNHI) of eight pearl millet genotypes across all water stress treatments (Genotype) or under four water stress treatments across all genotypes (Treatment)

	Tiller grain yield (g plant^{-1})	100-seed weight (g)	Grain number panicle^{-1}	Panicle harvest index (PNHI)
Genotype				
01029	6.8	0.43	989	0.51
01031	5.1	0.45	692	0.44
01040	6.7	0.48	790	0.52
01046	8.0	0.51	937	0.53
02042	5.8	0.40	731	0.48
02044	4.8	0.38	788	0.47
H77	4.2	0.31	782	0.44
PRLT	2.7	0.48	1387	0.48
LSD	1.4	0.08	221	0.06
Treatment				
LS-FP	7.6	0.49	960	0.55
LS-UP	4.4	0.40	794	0.44
ES-FP	6.1	0.49	941	0.53
ES-UP	4.3	0.35	864	0.43
LSD	1.0	0.05	–	0.04
	F-Probability	F-Probability	F-Probability	F-Probability
T	0.001	0.001	ns	0.001
G	0.001	0.001	0.001	0.007
GxT	ns	ns	ns	ns

The water stress treatments were a combination of late stress (LS) and early stress (ES) applied in lysimeters with either an initial full soil moisture profile (FP) or an initial unfilled soil moisture profile (UP). The pearl millet genotypes contrasted for a terminal drought tolerance QTL. Two-way ANOVA was used to determine genotype (G), treatment (T), and genotype-by-treatment interaction (GxT) effects as well as least significant differences (LSDs)

under WW and WS, respectively. The slightly lower values in our experiment are related to the omission of roots from the biomass calculation; in contrast, roots were included in Payne's experiment and accounted for approximately one third of the biomass. The larger differences in the coefficients under WS could be related to differences in the VPD during WS and indicate that future studies should include distinct VPD assessments for each treatment. Regardless of this, the lysimetric approach provided a more precise assessment of plant water use than experiments performed in the field where transpiration was inferred from neutron probe data (Azam-Ali et al. 1984). A key finding was that the QTL introgression lines did not vary with respect to total water extraction. It must be noted that individual plant evaluations in the lysimetric system are no substitute for field data, which provide a "real" assessment of plant populations.
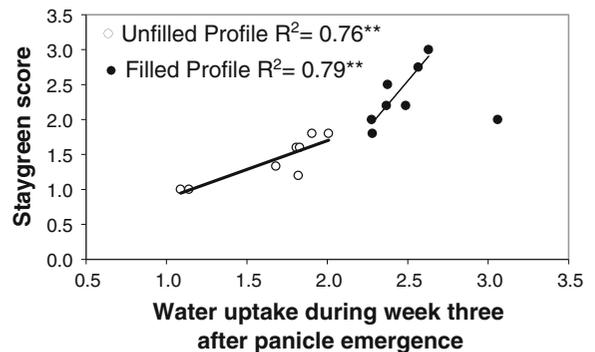

Fig. 4 Relationships between the staygreen scores (1, most of the leaves are wilted and senescing; 2, approximately half of the leaves are wilted and senescing; 3, almost all the leaves are green and turgid) and the water extracted (kg plant^{-1}) during the third week after panicle emergence under the late stress treatment. Lysimeters with an initial full soil profile are represented by *closed symbols* while those with an initial unfilled profile are represented by *open symbols*. The data point obtained from the filled lysimeter on the right hand side of the graph was excluded from the regression analysis

Table 5 Multi-linear additive model regressions between the total grain yield and the total water uptake (WU), transpiration efficiency (TE), and harvest index (HI) under the well-watered (WW), late stress (LS), and early stress (ES) treatments

	Coefficient	Standard error	t	P > t
Grain yield – WW (R²=0.98)				
WU	0.00094	0.00002	42.6	0.001
TE	9.34	0.33	27.9	0.001
HI	98.9	1.8	55.2	0.001
Constant	−58.8	1.3	−44.7	0.001
Grain yield – LS (R²=0.98)				
WU	0.00074	0.00003	23.7	0.001
TE	2.69	0.09	29.9	0.001
HI	53.3	0.7	72.1	0.001
Constant	−20.7	0.6	−32.5	0.001
Grain yield – ES (R²=0.99)				
WU	0.00079	0.00003	26.5	0.001
TE	1.58	0.08	19.7	0.001
HI	50.4	0.7	74.0	0.001
Constant	−15.4	0.6	−28.4	0.001

't' and P > t are the coefficient and the probability that the parameter exerts a significant effect on the dependent variable, respectively

In summary, the lysimetric system proved to be a critical tool for obtaining accurate assessments of the different components of the Passioura equation while also allowing for highly relevant agronomic assessments.

Table 6 Upper section: multi-linear regression between the total grain yield under the late stress treatment and the following explanatory variables: total water uptake (WU), transpiration efficiency (TE), harvest index (HI), and water uptake at weeks 0, 1, 2, 3, and 4 after panicle initiation (WU-Wk0, WU-Wk1, WU-Wk2, WU-Wk3, WU-Wk4)

	Coefficient	Standard error	t	P > t
Grain yield (R²=0.98)				
WU	0.00041	0.00009	4.7	0.001
TE	2.57	0.09	28.6	0.001
HI	51.6	1.0	52.1	0.001
WU-Wk0	0.00047	0.00012	4.03	0.001
WU-Wk1	0.00017	0.00008	2.01	0.05
WU-Wk2	0.00015	0.00013	1.19	Ns
WU-Wk3	0.00036	0.00017	2.16	0.03
WU-Wk4	0.00060	0.00013	4.63	0.001
Constant	−19.2	0.7	−25.87	0.001
Panicle harvest index (R²=0.53)				
WU-Wk0	0.000011	0.000011	0.98	Ns
WU-Wk1	2.8 10-6	9.0 10-6	0.31	Ns
WU-Wk2	−0.000100	0.000014	−7.31	0.001
WU-Wk3	0.000063	0.000016	3.87	0.001
WU-Wk4	0.000055	0.000013	4.36	0.001
Constant	0.61	0.07	8.74	0.001

Lower section: multi-linear regression between the panicle harvest index (PNHI, i.e., the ratio of total grain weight to total panicle weight) under the late stress treatment and water uptake at weeks 0, 1, 2, 3, and 4 after panicle emergence. 't' and P > t are the coefficient and the probability that the parameter exerts a significant effect on the dependent variable, respectively

ns non-significant

Differences in water uptake patterns are critical
for achieving higher yields under drought conditions

In these experiments, HI was most closely associated
with yield under LS and ES treatments. Aside from
HI, total water extraction had a direct and significant
effect on yield, although this effect was much stronger
under ES compared with LS treatment (R^2=0.12 un-
der LS treatment and R^2=0.48 under ES treatment).
This was also reflected by the high magnitude of the
WU component under LS and ES treatments in the
multi-linear analysis (Table 5). However, the high
magnitude of the WU component was mainly related
to higher water uptake in weeks three and four after
panicle emergence (Fig. 2), which indicated the rela-
tive importance of temporal patterns of water use vs.
total water uptake. The slopes of the regressions be-
tween grain yield and water extracted (LS, Fig. 2a; ES,
Fig. 2b) were 4.5 g kg^{-1} and 3.7 g kg^{-1} for LS and ES,
respectively, which can be extrapolated to 45 g mm^{-1}
and 37 g mm^{-1} water at the sowing density used in our
experiments (10 plants m^{-2}). These values are close to
those previously reported for wheat: each millimetre
of water extracted during grain filling led to a yield
increase of 55 kg ha^{-1} (Manschadi et al. 2006) or 59
kg ha^{-1} (Kirkegaard et al. 2007). Additionally, the
multi-linear analysis showed that the panicle harvest
index was negatively related to water uptake early in
the cycle and positively related to water uptake late in
the cycle (Table 6), indicating that strong interactions
occur between the components of the Passioura equa-
tion, and as suggested in previous reports, these in-
teractions require close temporal scrutiny while using
this water-based framework (Condon and Richards
1993).

An important finding was that higher water extrac-
tion in week three after panicle emergence was also
closely related to staygreen score. It is interesting that
among the main mechanistic hypotheses proposed
thus far to explain the expression of staygreen, most
have been related to plant nitrogen status (e.g., Borrell
et al. 2001; Thomas and Howarth 2000) while only a
few have been related to water status (Tuinstra et al.
1998). However, several reports have shown that
staygreen was likely to be associated with the mainte-
nance of root growth (Hatlitligil et al. 1984; MacKay
and Barber 1986), which could be another means of
making water available. Therefore, the expression of
staygreen is related to the maintenance of water

uptake. Scoring of the staygreen phenotype then offers
a simple proxy for measuring water uptake differences
in the field and is now used to phenotype pearl millet
genotypes under terminal drought conditions. Of
course, other confounding factors such as the geno-
type, the size of the panicle, or the number of grains
could also affect the staygreen score. The increased
water extraction late in the grain filling period benefit-
ted the tillers more than the main head. The quality
and precision of yield data could be improved by
measuring tiller yield rather than total grain yield.
This has also some important implications because
germplasms vary with respect to the rate at which
tillers flower. Intuitively, genotypes in which the tillers
flower soon after the main head would have an advan-
tage over genotypes in which these tillers flower later
with respect to their level of terminal drought stress
tolerance. In summary, the lysimetric system proved to
be a critical tool for elucidating the mechanisms of
terminal drought adaptation by illustrating the critical
importance of increased water extraction during grain
filling while also allowing for a highly relevant agro-
nomic evaluation and providing effective scoring sys-
tems (e.g., staygreen scores) for breeding programs.

These results, which illustrate the importance of
temporal water use patterns, are in close agreement
with earlier results indicating that terminal drought-
tolerant chickpea genotypes also exhibited increased
water extraction during the grain filling period
(Zaman-Allah et al. 2011a). In fact, converging evi-
dence from different crops indicates that "shifting"
water use from the vegetative to the reproductive stage
may increase yield under water-limited conditions,
and there are several different ways to achieve this.
Lower canopy conductance and transpiration sensitiv-
ity to a high vapour pressure deficit were reported
previously in pearl millet germplasm entries that
achieved high yields under drought conditions
(Kholová et al. 2010a, b). Therefore, lower water use
around and before anthesis is likely to be related to
water conservation traits operating at the vegetative
stage in the tolerant genotypes. Similar results were
previously reported in chickpea and cowpea, where
lower canopy conductance and decreased vegetative
biomass at the vegetative stage, both of which con-
tribute to water conservation, were used to distinguish
tolerant from sensitive genotypes in a series of pot
experiments (Zaman-Allah et al. 2011b; Belko et al.
2012) and altered the pattern of water uptake, leading

to seed yield differences in the lysimeters (Zaman-Allah et al. 2011a). Previous work in wheat also uncovered genotypes with a decreased rate of water use (e.g., Condon et al. 2004) or a small plant size (e.g., Fischer and Wood 1979), which are adaptations to conditions in which the crop depends mostly on stored soil moisture (e.g., Condon et al. 2004). ICMH01046, which had a decreased biomass under WW conditions, is similar to these examples, and it also exhibited reduced tillering under WW conditions. The reduction in tillering also contributes to a decrease in plant size and agrees with a recent report indicating that under certain planting densities and moderate temperature conditions, a faster leaf appearance rate could increase the vigour of the main culm and lead to reduced tillering in sorghum, which would then result in a decrease in leaf area at anthesis and less water use before anthesis (van Oosterom et al. 2011). Therefore, in various crops, adaptation to end-of-season drought is associated with water conservation traits during pre-anthesis including lower canopy conductance and/or small plant size. The exact mechanism by which small plant size is achieved may vary; however, tillering seems to play a role in this mechanism.

Water uptake differences between genotypes at 3 and 4 weeks after panicle emergence accounted for a maximum of 1.0–1.5 L plant^{-1}. Because the planting density in the lysimeters was approximately 10 plants m^{-2}, these water uptake differences corresponded to approximately 10–15 mm. This is small but critical, as previously shown in wheat (Manschadi et al. 2006). In fact, similar findings were obtained in chickpeas that were tolerant to terminal drought; in these plants, differences in temporal patterns of water uptake were estimated to be approximately 25 mm (Zaman-Allah et al. 2011a). In contrast, the lack of significant differences in total water uptake between H77/833-2 and the tolerant introgression lines indicates that the terminal drought tolerance QTL was not likely to have an effect on the water extraction capacity of the root system. Although this could have been caused by a constraint on root development, this possibility is unlikely because earlier work in sorghum (Vadez et al. 2011a, b) reported a water extraction of over 15 L in the same tubes and a pearl millet germplasm extracted over 13 L (unpublished). This re-emphasises the importance of temporal patterns of water use for increasing grain yield under terminal drought stress. It appears that these characteristics are shared among crops that face similar constraints. Therefore,

the current study showed that yield increases cannot be accomplished by simply maximising plant water extraction (earlier referred to as "efficient use of water, EUW", Blum 2009). Here, the total amount of water extracted by all genotypes was similar, and yield differences arose when this limited water resource was managed in a way that maximised post-anthesis water use. Thus, the concept of "efficient water use" requires some consideration of the dynamics of water use.

Conclusion

Temporal patterns of water use, rather than total water extraction, were essential for explaining the terminal drought tolerance of pearl millet genotypes containing a terminal drought tolerance QTL. Therefore, the terminal drought QTL did not affect the water extraction capacity of the root system. Increased water uptake during the post anthesis period led to a higher yield, especially with respect to tillers, and also led to the expression of a staygreen phenotype. The differential patterns of water uptake were thought to be consequences of the water saving traits reported earlier. The lysimetric setup was able to provide a highly relevant agronomic assessment, and its capacity to achieve a direct, precise and dynamic measurement of the components of a water-based framework (Y = WU × TE × HI) revealed the high level of interactions between these components.

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