CSIRO PUBLISHING

Crop & Pasture Science, 2016, **67**, 834–846
http://dx.doi.org/10.1071/CP15338

Variation in drought-tolerance components and their interrelationships in the core collection of foxtail millet (*Setaria italica*) germplasm

Lakshmanan Krishnamurthy[A,E], *Hari Deo Upadhyaya*[A,B,C], *Junichi Kashiwagi*[D],
Ramamoorthy Purushothaman[A], *Sangam Lal Dwivedi*[A], *and Vincent Vadez*[A]

[A]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Telangana, India.
[B]Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA.
[C]UWA Institute of Agriculture, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.
[D]Crop Science Laboratory, Graduate School of Agriculture, Hokkaido University, Kita 9 Nishi 9, Kita-Ku, Sapporo, 060-8589, Japan.
[E]Corresponding author. Email: l.krishnamurthy@cgiar.org, lkm1949@gmail.com

Abstract. Foxtail millet (*Setaria italica* (L.) P.Beauv.) is an ancient cereal cultivated worldwide in arid and marginal lands. It is an ideal crop for the changing climate, with high photosynthetic efficiency. A trait-based selection for drought tolerance is sought for yield stability. The present work had segregated the drought yield as total water use (T), transpiration efficiency (TE) and harvest index (HI) and assessed the importance of these components and their association with drought tolerance. The core collection of foxtail millet germplasm ($n = 155$) was evaluated in mini-lysimeters under both terminal drought stress (DS) and well-watered (WW) environments. The contribution of T to grain yield under drought was minor but the contribution of TE was positive and of HI negative. Crop duration, T and TE positively influenced, and HI negatively influenced, shoot biomass production. Under drought, the core germplasm accessions varied in shoot biomass, grain yield, HI and T by >3-fold and in TE by 2-fold. Categorisation of the germplasm for TE had differentiated groups of accessions as high TE ($n = 17$) and low TE ($n = 22$). Among the three races of foxtail millet, *indica* was strong for T and TE, and *maxima* and *moharia* for HI, with useful exceptions.

Additional keywords: crop productivity, genetic variability, G × E interaction, small millet, subraces, water deficit.

Received 6 October 2015, accepted 5 April 2016, published online 17 August 2016

Introduction

Foxtail millet (*Setaria italica* (L.) P.Beauv.) is an important crop among cereals, ranking next to pearl millet (*Pennisetum glaucum*). It is ancient, with its domestication in China dating back to 11 500 to 9500 years before present (Yang *et al.* 2012). It is widely cultivated in Asia, Europe, North America, Australia and North Africa for grain or forage (Austin 2006) and remains the crop of choice for the arid areas, as in China (Liu *et al.* 2011). With a small diploid genome (400 Mb) (Bennetzen *et al.* 2012), C_4 photosynthetic pathway and a short duration, foxtail millet has been considered a model system to investigate many aspects of plant architecture, genome evolution and physiology of the bioenergy grasses such as switchgrass (*Panicum virgatum*), Napier grass (*Pennisetum purpureum*) and pearl millet (Doust *et al.* 2009; Wang *et al.* 2010; Bennetzen *et al.* 2012).

Foxtail millet is an ideal crop for the changing climate because of its unique combination of low water requirement, drought tolerance and high photosynthetic efficiency (Vetriventhan *et al.* 2012). It has long since been used in dryland agricultural production systems around the world (Qie *et al.* 2014). Poor agronomic practices (Ahanchede *et al.* 2004) and inadequate attention to breeding for crop improvement (Vetriventhan *et al.* 2012) remain the causes of low yield. The unique advantage of this crop species is that it can mature and yield acceptably well with a single, pre-sowing precipitation (Dwivedi *et al.* 2012). Foxtail millet is often mentioned as a relatively drought-tolerant crop, most likely on the basis of its cultivated environment, but systematic studies proving its drought tolerance are lacking, except for a few cross-species comparisons. Considerable genetic diversity has been found to exist for drought-induced oxidative-stress tolerance by using measurement of lipid peroxidation to assess membrane integrity under stress among several cultivars (Lata *et al.* 2011). The water use efficiency (WUE) of foxtail millet has also been found to be greater than of maize and sorghum (Zhang *et al.* 2007). Enhanced WUE has been closely associated with several morphological characteristics such as dense root system, thick cell walls, epidermal cell arrangements and a small leaf area, indicating the constitutional nature of drought tolerance (Li 1997). Foxtail millet has been estimated to produce about 2-fold greater biomass per unit water than either maize or wheat (Diao 2007; Li and Brutnell 2011).

Thus, its short lifespan and high WUE have been suggested as qualities to make it a suitable crop for cultivation in semi-arid, dry and marginal lands. It has also been demonstrated to produce higher seed yield and greater number of seeds per ear than pearl millet and proso millet (*Panicum miliaceum*) under both deficit irrigation and non-stress conditions through maintenance of high WUE and harvest index (HI) (Seghatoleslami *et al*. 2008). In addition, low water requirement has been demonstrated by obtaining acceptable grain yield and WUE under mild drought stress (DS) conditions through a method of providing limited irrigation to one half of the roots and watering the other half of the root system in next irrigation (Zooleh *et al*. 2011).

Drought tolerance is a complex phenomenon involving adaptation through many different strategies. As a basis for future drought-management interventions, it is important to understand the type of strategy that this crop employs. In the semi-arid and arid tropics, where the soil-water limitation mainly fixes a ceiling for the choice of the length of cropping period (Ludlow and Muchow 1990), foxtail millet often faces both intermittent and terminal drought through long gaps between two episodes of rain (Qie *et al*. 2014). Intermittent drought occurs when periods of rain are limited and erratic during the growing season and thus cause periods of stress (Schneider *et al*. 1997). Intermittent drought is common in semi-arid tropical regions and is expected to become more erratic because of global warming (Ludlow and Muchow 1990; Urrea *et al*. 2009). Successful crops in the rainy season are those with a capacity to use water when available and with the ability to seek water when it becomes limited. Thus, in water-limited environments, options for a continued water extraction are a deeper and more prolific root system to access additional water from the soil profile, with strategies to enhance transpiration efficiency (TE). Strategies to improve partitioning into the grains are equally important but need to be pursued as a next step.

Under water-limited environments, Passioura's equation (Passioura 1977) describing yield as the product of water used, TE and HI has been widely used to understand and target traits contributing to drought adaptation. All three components are integrative, close to yield in organisation but difficult to measure in field trials. However, a lysimetric approach has been found useful to evaluate all components on the same plants with an equal degree of precision (Vadez *et al*. 2008, 2011; Ratnakumar *et al*. 2009). The contribution of TE to total productivity is controversial because high TE has been shown to relate to low total water use (T) (Blum 2005) or otherwise (Peng and Krieg 1992). However, Vadez *et al*. (2014) found no or limited trade-off between T and TE in a large range of germplasm of peanut, pearl millet and sorghum. It is also possible that one of the components of the equation will be favoured by a specific condition having a greater bearing on yield, thereby masking the true contribution of the other components to yield. However, in this crop species, it is important to understand the interaction of these components on yield formation under drought and to highlight the extent of existing variation in these yield components. Reasonable success had been achieved in characterising the germplasm by using a lysimetric approach (Vadez *et al*. 2008, 2011; Ratnakumar *et al*. 2009) to assess precisely all of the components of Passioura's equation on the same plant and to test their relationships by using a large set of germplasm.

To understand the extent of variation in the germplasm, core collections (10% of the entire collection) were considered ideal because these are subsets representing the diversity of the entire collection of particular species. Upadhyaya *et al*. (2009) developed a core collection of foxtail millet with 155 accessions representing the entire collection of 1474 accessions by using data on taxonomic and qualitative traits. The objectives of the present study were: (*i*) to clarify the extent of contribution of the analytical yield components to shoot biomass and grain yield under DS; and (*ii*) to characterise the core collection of foxtail millet germplasm for variation in grain yield and the analytical yield components under DS and to identify a few highly contrasting accessions for use in further improvement of drought adaptation of foxtail millet.

Materials and methods

Soil and growth conditions of the lysimeters

Plants were grown in mini-lysimeters, which were polyvinyl chloride (PVC) tubes 25 cm in diameter and 2.0 m deep. A PVC plate was fitted to the bottom of the PVC cylinder to retain the filled soil. This plate was loosely rested on horizontal screws to allow drainage when excessively irrigated. The mini-lysimeters were placed in concrete pits under open field conditions, with a rainout shelter that could be moved over the top of the crop when there was rain (Fig. 1). Mini-lysimeters were filled with Alfisol collected from the ICRISAT farm and sieved to a particle size <1 cm. Cylinders were filled with soil in three increments of 40 kg air-dried soil to ensure that all cylinders were filled to the same level. The filling varied by 1–2 kg, i.e. <1–2% variation across cylinders. Hence, all cylinders had a similar bulk density close to 1.35 g cm^{-3}. After filling each 40-kg increment, the soil was watered to ensure uniform soil settling. A previous assessment of maximum water-holding capacity (field capacity of the Alfisol soil ~20%) was used to inform the filling of the profile with the required amount of water without drainage. Therefore, 8 L water was added to each 40-kg soil increment. After adding and watering 40 kg soil three times, an additional 5 kg dry soil was added to each cylinder and provided with 1 L water. At that stage, the cylinders were almost filled to the desired level, i.e. ~5 cm from the top.

All cylinders at field capacity weighed 163–164 kg (11 kg bare cylinder system + ~126 kg soil + ~26.5 kg added water). The soil in the mini-lysimeters had been fertilised with di-ammonium phosphate and muriate of potash, both at a rate of 300 mg kg^{-1} soil. It was supplemented with sieved and sterilised farmyard manure at 1 : 25 soil (v/v) to manage micro-nutrient deficiency.

Space arrangement of the mini-lysimeters and weighing

The top of the cylinders was fitted with a metal collar and rings for lifting to weigh. Cylinders were weighed by lifting with a chain pulley block suspended through an S-type load cell (Mettler-Toledo, Geneva). The scale (200-kg capacity), with a precision of 20 g, allowed repeated-measurements.

Fig. 1. Mini-lysimetric facility sown with the core collection of foxtail millet germplasm before imposition of drought stress in 2008. The rainout shelter at the background was programmed to move above the crop whenever there was a rain.

The mini-lysimeters were separated from each other by ~5 cm, so that foxtail millet (two plants per cylinder—see below) was planted at a spacing of ~22 plants m^{-2}, roughly comparable to the per-plant spacing provided by typical field plantings of 60 cm row-to-row and 10 cm plant-to-plant (17 plants m^{-2}) at ICRISAT. This similar spacing enabled plant growth comparable to the field. The tubes were arranged in two trenches of 2 m depth and 1.8 m width. Each trench was separated by a 20-cm concrete wall. Possible border effects were contained by placing a row of potted plants on the south side of the outer row of cylinders.

Treatments used and traits assessed

The DS treatment was imposed so that the plants at 50% flowering phase experienced the stress; this phase is considered the most sensitive to drought. The DS treatment received no water from 28 days after sowing (DAS) in 2008 and 31 DAS in 2009 until harvest, whereas the well-watered (WW) treatment was irrigated regularly (see below). Ten seeds were planted in each cylinder on 4 July 2008 and 12 June 2009 to raise a crop in rainy season. Plants were gradually thinned to two seedlings per cylinder at 12 DAS. All plants were fully irrigated until 28 DAS in 2008 and 31 DAS in 2009. This involved cylinders receiving 500 mL twice a week for the first 2 weeks after sowing, and then 1 L on alternate days until the imposition of drought.

Drought was imposed by stopping irrigation after a complete saturation of the soil in the cylinders by repeated irrigation. Drought imposition started at 28 DAS in 2008 and 31 DAS in 2009, after saturating the cylinders with more than twice the quantity of water required to ensure complete soaking of the soil to field capacity. Later, the cylinders were covered with a 3-cm layer of low-density polyethylene beads to prevent soil evaporation. Previous tests had indicated that placement of these beads curbed >90% of the soil evaporation, so that the water loss is truly attributable to T alone. Weighing of the cylinders was first done at 30 DAS in 2008 and 33 DAS in 2009, then approximately every 10 days, primarily to compensate for the water loss from the WW treatment (see below) and to assess the rate of decline in soil water from the cylinders. This gave seven weighing events until harvest in 2008 and 2009. The first weighing at 30 or 33 DAS provided the field capacity weight of each cylinder. In 2008, cylinders with the same water treatment were distributed in a trench and two replications per day were weighed. However, in 2009, all cylinders were weighed in one day. The same weighing order was maintained so that the intervals between weighing were the same for all cylinders. To keep the soil of the WW cylinders sufficiently wet for optimum growth of plants and to avoid excessive water application, WW plants were compensated for the water loss by water addition up to 90% field capacity once every 10 days, after weighing. This prevented drainage at the bottom and a waterlogged soil environment.

Plant material

In 2008, 160 accessions of the core collection of foxtail millet germplasm including four checks known for their superior agronomic performance (ISe 375, ISe 376, ISe 1468, ISe 1541) were evaluated. In 2009, a subset of 50 accessions, selected proportionately to represent the whole range of drought reaction along with the checks, was evaluated.

In addition to the DS and WW sets of plants used above, a third set of plants was sown at the same time in an area adjacent to the trenches. Plants were grown in 25-cm pots filled with 11 kg of the same Alfisol, in three replications. Previous experiments with foxtail millet in these pots showed no signs of growth limitation to anthesis that might be attributed to pot size. The same crop management was followed and the plants were kept in a well-watered state until harvest. This set was harvested at the beginning of DS imposition each year, with the purpose of estimating shoot biomass production before stress imposition and deducting from the total biomass at the final harvest.

Calculation of water-use and transpiration efficiency

Plants were harvested at 91 DAS or 63 days after treatment imposition in 2008 and 128 DAS or 73 days after treatment imposition in 2009. Leaf and stem (including leaf sheath) weights were recorded after drying in a forced-air oven at 70°C for 3 days. Calculation of T during treatment time was as the initial cylinder weight minus the final cylinder weight at the end of treatment period, in addition to the total quantity of water applied across the whole treatment period. Total shoot biomass produced during the treatment period was estimated as the shoot biomass harvested at the end of the treatment period minus the biomass produced before DS imposition. TE was calculated as the shoot biomass produced during treatment imposition for T during treatment time.

Statistical analyses

The experiments were planted in a 40 × 4 α lattice (incomplete block design) with three replications in 2008 and in a 25 × 2 α lattice with three replications in 2009. The residual maximum likelihood method in Genstat was used to obtain the unbiased estimate of the variance components and the best linear unbiased predictors (BLUPS) for the different parameters measured within each treatment, considering genotypes as random effects and replications as fixed effects. The significance of genetic variability among accessions was assessed from the standard error of the estimate of genetic variance.

For the pooled analysis, homogeneity of variance was tested by Bartlett's test (Bartlett 1937) with year (E) fixed, and the genotype (G) × E interaction as random. The variance due to G × E interactions and their standard errors were determined. The significance of the fixed effect of the year or drought treatment was assessed by using the Wald statistic. All statistical analyses were carried out using Genstat Release 14.1 software (Genstat 2011).

Results

Drought effects

Drought stress significantly influenced all traits in both years, except time to panicle emergence in 2009 (Table 1). DS slightly reduced time to panicle emergence and maturity, and reduced total shoot biomass by 26% in 2008 and 28% in 2009, grain yield by 35% in 2008 and 43% in 2009, HI by 13% in 2008 and 20% in 2009 and T by 42% in 2008 and 35% in 2009. It increased TE by 22% in 2008 and 5% in 2009 (estimated from Table 2). In 2008, when all 160 accessions were considered, the drought × genotype interaction was significant for most characteristics except HI and TE (Table 1), whereas in 2009, when only 50 accessions were tested, this interaction was significant only for the grain yield and TE.

The number of days to panicle emergence for the accessions under DS was closely associated with that under WW treatment ($r^2 = 0.91$*** in 2008 and 0.89*** in 2009), and similarly, number of days to maturity ($r^2 = 0.70$*** in 2008 and 0.88*** in 2009), indicating that crop duration under DS was proportionately reduced in most accessions (data not shown). The association between shoot biomass under DS and under WW conditions was also close and significant ($r^2 = 0.67$*** in 2008 and 0.69*** in 2009), suggesting that biomass productivity under no stress can largely indicate shoot biomass performance under drought. Similar association was seen for T ($r^2 = 0.64$*** in 2008 and 0.56*** in 2009) and TE ($r^2 = 0.47$*** in 2008 and 0.17*** in 2009).

Year effects

Based on the variance of 50 common genotypes, year of experimentation or season significantly influenced various

Table 1. Drought treatment and drought treatment × genotype variances for the phenology, yield and yield-component characters measured on foxtail millet germplasm accessions ($n = 160$) grown in 2008 and for the limited germplasm accessions ($n = 50$) grown in 2009 rainy seasons, ICRISAT, Patancheru, India

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., not significant ($P > 0.05$)

	Drought treatment		Drought treatment × genotype	
	Wald statistic	Significance level	σ^2_{g} (s.e.)	Significance level
		2008		
No. of days to panicle emergence	24.2	***	2.10 (0.770)	**
No. of days to maturity	3.62	*	8.65 (1.82)	***
Shoot biomass	223.3	***	38.6 (10.8)	***
Grain yield	230.3	***	5.06 (1.97)	**
Harvest index	81.0	***	1.18 (2.34)	n.s.
Total water use	647.2	***	3.53 (0.593)	***
Transpiration efficiency	289.8	***	0.036 (0.0214)	n.s.
		2009		
No. of days to panicle emergence	0.360	n.s.	2.12 (2.28)	n.s.
No. of days to maturity	4.79	*	0.420 (3.16)	n.s.
Shoot biomass	90.2	***	18.8 (11.1)	n.s.
Grain yield	73.4	***	6.38 (2.57)	**
Harvest index	46.2	***	7.63 (4.25)	n.s.
Total water use	244.5	***	1.09 (0.611)	n.s.
Transpiration efficiency	3.80	*	0.097 (0.032)	***

Table 2. Trial mean and variability for number of days to panicle emergence and maturity, total shoot biomass and grain yield (g cylinder^{-1}) produced, harvest index (%), mean water use (kg cylinder^{-1}), and transpiration efficiency (g kg^{-1}) in the core collection of foxtail millet germplasm ($n = 160$) in 2008 and part of the core collection ($n = 50$) in 2009 rainy seasons under both drought-stressed and well-watered conditions

	Trial mean	Range of predicted means	S. Ed.	σ^2_g (s.e.)	Heritability (h^2)
		2008, drought-stressed			
No. of days to panicle emergence	47.2	22.3–84.7	2.56	127.2 (14.7)	0.985
No. of days to maturity	74.2	54.6–124.7	3.30	123.6 (14.6)	0.973
Shoot biomass	45.3	15.5–67.0	6.48	132.0 (18.0)	0.896
Grain yield	12.7	6.2–17.7	2.41	6.62 (1.44)	0.677
Harvest index	29.9	5.4–62.5	5.06	117.2 (15.1)	0.932
Total water use	9.1	3.7–12.2	0.90	3.70 (0.47)	0.931
Transpiration efficiency	4.14	2.29–4.85	0.483	0.322 (0.060)	0.740
		2008, well-watered			
No. of days to panicle emergence	48.7	25.6–86.9	2.81	147.2 (17.1)	0.989
No. of days to maturity	75.1	54.6–124.5	3.66	139.1 (16.6)	0.980
Shoot biomass	61.0	23.1–98.5	12.6	399.5 (57.1)	0.910
Grain yield	19.5	11.0–26.9	4.38	18.4 (4.86)	0.697
Harvest index	34.2	8.7–54.3	5.35	78.8 (11.0)	0.919
Total water use	15.6	6.9–23.3	2.63	17.90 (2.54)	0.912
Transpiration efficiency	3.38	1.83–4.16	0.439	0.332 (0.055)	0.860
		2009, drought-stressed			
No. of days to panicle emergence	54.9	32.1–94.3	3.52	190.7 (40.2)	0.980
No. of days to maturity	84.8	65.0–119.6	5.82	162.5 (37.1)	0.935
Shoot biomass	35.7	18.8–50.4	4.64	82.9 (19.5)	0.918
Grain yield	7.8	3.2–11.8	1.84	4.58 (1.57)	0.737
Harvest index	23.2	3.3–44.5	4.41	96.8 (22.3)	0.938
Total water use	10.0	6.3–13.0	0.89	3.95 (0.90)	0.938
Transpiration efficiency	2.95	1.98–3.89	0.272	0.175 (0.046)	0.863
		2009, well-watered			
No. of days to panicle emergence	54.5	35.2–93.7	4.02	144.3 (31.2)	0.966
No. of days to maturity	86.6	71.6–113.9	4.51	91.4 (21.1)	0.930
Shoot biomass	49.5	21.0–72.5	9.04	232.6 (58.0)	0.886
Grain yield	13.7	4.3–22.0	3.21	18.5 (5.33)	0.813
Harvest index	28.8	5.5–50.1	4.61	79.0 (18.7)	0.916
Total water use	15.3	9.8–20.2	2.10	9.49 (2.56)	0.849
Transpiration efficiency	2.79	1.71–3.52	0.299	0.252 (0.063)	0.887

traits (Table 3). Overall, under DS, panicle emergence occurred later by 7 days and maturity later by 10 days, and total shoot biomass production was less by 21%, grain yield by 36% and HI by 23% in 2009 than 2008 (data not shown). In 2009, T was marginally higher and the TE substantially lower than in 2008. However, most of the characteristics were not affected by a year × genotype interaction in either DS or WW conditions (Table 3). Increased T, decreased TE and decreased productivity in 2009 is likely due to earlier planting, by 23 days, and the consequent exposure of the crop to greater vapour pressure deficits of 2.42 kPa during the vegetative and 2.07 kPa during the reproductive growth stages in 2009, compared with 1.54 and 1.42 kPa during 2008. The means of 50 common genotypes across years were associated with each other, with $r^2 > 0.83$ for time to panicle emergence and time to maturity, >0.68 for total shoot biomass, >0.31 for grain yield, >0.73 for HI, >0.45 for T and >0.39 for TE under both DS and WW (data not shown); this indicates close performance across years. Such an association for any of these traits was closer under DS condition. Absence of a year × genotype interaction allowed presentation of genotypic means for single years and inferences on genotypic variation. Large number of accessions ($n = 160$) were tested in 2008, and were used for categorising the genotypic response to DS.

Germplasm effects

Under DS in 2008, time to panicle emergence for accessions ranged from 22 to 85 days and time to maturity from 55 to 125 days (Table 2). Accessions varied in shoot biomass production, grain yield, HI and T by 3–4-fold and in TE by 2-fold. A similar range of variation in time to panicle emergence and maturity was seen under WW treatment. Compared with DS, the mean, minimum and maximum values of shoot biomass, grain yield and HI were greater but the extent of range remained about the same under WW treatment. The value of T was 1.5 times greater under WW than DS treatment. TE increased substantially under DS, although the extent of range remained the same. The broad-sense heritability was >0.9 for days to panicle emergence, days to maturity, and total shoot biomass production under both DS and WW treatments, whereas it was

Table 3. Year and year × genotype variances for the phenology, yield and yield component characters measured on some common foxtail millet germplasm accessions ($n = 50$) grown across 2008 and 2009 rainy seasons under both drought-stressed and well-watered conditions, ICRISAT, Patancheru, India

***$P < 0.001$; n.s., not significant ($P > 0.05$)

	Year		Year × genotype	
	Wald statistic	Significance level	σ^2_g (s.e.)	Significance level
Drought-stressed				
No. of days to panicle emergence	248.9	***	0.83 (1.22)	n.s.
No. of days to maturity	205.7	***	2.11 (2.85)	n.s.
Shoot biomass	87.6	***	7.15 (5.34)	n.s.
Grain yield	112.7	***	1.23 (1.23)	n.s.
Harvest index	86.4	***	0.17 (3.27)	n.s.
Total water use	31.9	***	0.01 (0.01)	n.s.
Transpiration efficiency	162.0	***	0.10 (0.044)	n.s.
Well-watered				
No. of days to panicle emergence	80.6	***	0.930 (2.22)	n.s.
No. of days to maturity	132.6	***	8.98 (4.51)	n.s.
Shoot biomass	46.8	***	1.70 (16.3)	n.s.
Grain yield	56.2	***	3.16 (3.26)	n.s.
Harvest index	44.6	***	0.69 (3.95)	n.s.
Total water use	0.03	n.s.	1.85 (1.23)	n.s.
Transpiration efficiency	89.3	***	0.060 (0.034)	n.s.

~0.7 for grain yield. It ranged from 0.92 to 0.94 for HI, from 0.85 to 0.94 for T and from 0.74 to 0.89 for TE.

Relationship of grain yield under drought with T, TE and HI

Simple and stepwise regression analyses showed that the relationship between grain yield and T was significant under WW treatment only ($r^2 = 0.46$ in 2008 and 0.32 in 2009), and not under DS (Fig. 2*a*, *b*). Similarly, grain yield was significantly related to TE under WW treatment ($r^2 = 0.37$ in 2008 and 0.10 in 2009) (data not shown), and although the relationship was significant under DS conditions, the regression coefficients were weak ($r^2 = 0.12$ in 2008 and 0.14 in 2009) (Fig. 2*c*, *d*). However, grain yield exhibited a significant and curvilinear relationship with HI under both WW ($r^2 = 0.32$ in 2008 and 0.30 in 2009) and DS conditions. Under DS, the regression coefficients were stronger ($R^2 = 0.48$ in 2008 and 0.58 in 2009) (Fig. 2*e*, *f*). Therefore, individually, only HI had a substantial bearing on yield and TE marginally explained the grain yield variation under both soil-water environments (Table 4). This was because the relationship between yield and HI was highly significant, and more so under DS than WW conditions (Table 4). The model based on yield components (T, TE and HI) explained >0.85 of the variation in yield across soil-water treatments and years. Two key components that explained the major variation in grain yield under DS were TE and HI, but under WW, these were T and HI.

As indicated by Fig. 2, for any given HI level, clearly there were substantial variations in yield that remained unexplained by HI (and for the other yield components). In previous work with sorghum, a sequence of associations was established with the residuals of grain yield, and the next level of components as TE and T in sorghum (Vadez *et al*. 2011). Similar efforts have shown the existence of a good relationship of TE with residuals of grain yield unexplained by HI. Subsequent relationships of T with residual of grain yield unexplained by both HI and TE were nil or poor. A change of order of the components in the regression sequence did not cause a major change in the proportion of variation explained by the components.

Association of phenology and shoot biomass with other yield components

Under DS, total shoot biomass exhibited a positive curvilinear response with maximum productivity at 60 DAS, and HI a negative curvilinear response, to days to panicle emergence (Fig. 3). Consequently, grain yield had a sparse and negative curvilinear response to days to panicle emergence. However, this negative effect and association became nil when a few ($n = 7$) long-duration accessions were not included in the regression. Also under WW treatment, a very similar response was seen for total shoot biomass ($r^2 = 0.60$***), HI ($r^2 = 0.67$***) and grain yield ($r^2 = 0.32$***) to days to panicle emergence (data not shown). The association of total shoot biomass production with the grain yield was sparse and positive under DS in 2008 only ($r^2 = 0.09$**) and not in 2009 ($r^2 = 0.03$n.s.). Under WW conditions, this association was close and explained more variation ($r^2 = 0.54$*** in 2008 and 0.36*** in 2009). Total shoot biomass was negatively associated with HI, explaining 0.43 of the variation in HI under DS in 2008 and 0.36 in 2009, and 0.45 under WW in 2008 and 0.17 in 2009. Total shoot biomass was closely and positively associated with T (data not shown). Likewise, total shoot biomass was closely and positively associated with TE under both DS and WW conditions, and TE explained most variation ($r^2 = 0.81$) under DS condition in 2008 (Fig. 4). Comparatively, TE explained the least variation ($r^2 = 0.50$) under DS condition in 2009.

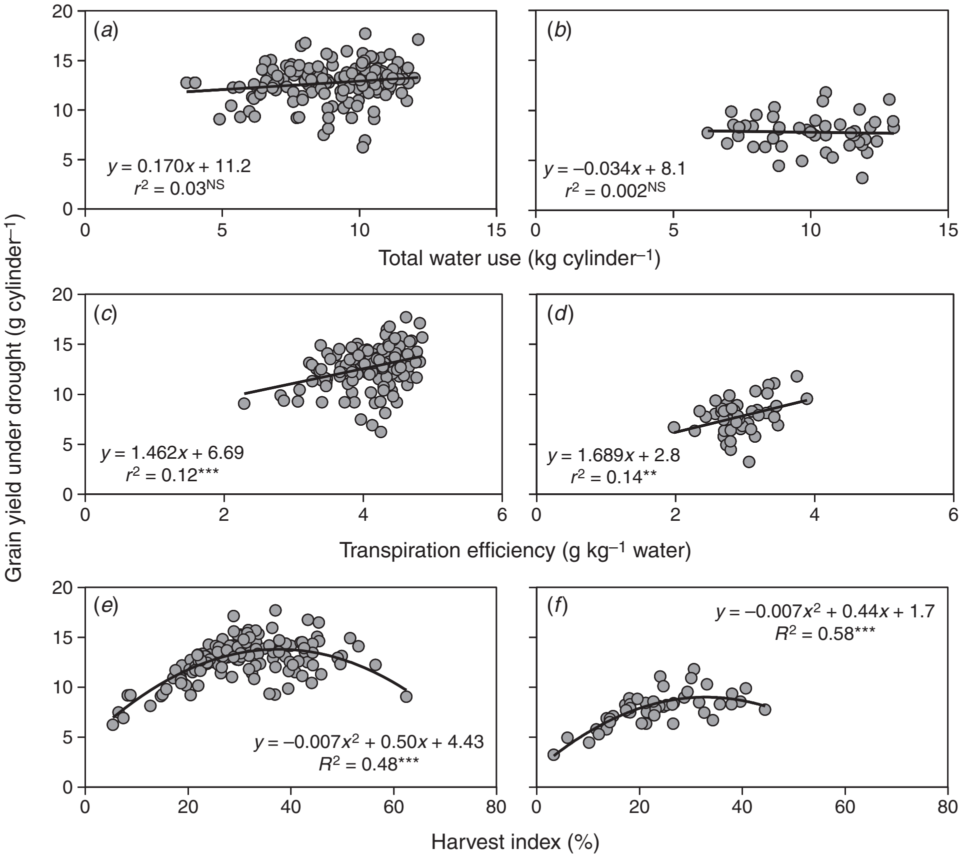

Fig. 2. Association of total water use in (*a*) 2008 and (*b*) 2009, transpiration efficiency in (*c*) 2008 and (*d*) 2009, and harvest index in (*e*) 2008 and (*f*) 2009 with grain yield per cylinder of foxtail millet accessions under drought-stressed condition.

Table 4. Variability of grain yield explained by total water use (T), transpiration efficiency (TE) and harvest index (HI), individually or in various combinations of the core collection in 2008 and a subset of the core collection in 2009 under drought-stressed and well-watered conditions

$^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant ($P > 0.05$)

Yield components	Drought-stressed 2008	Drought-stressed 2009	Well-watered 2008	Well-watered 2009
T	0.03n.s.	0.00n.s.	0.46***	0.32***
TE	0.12***	0.14**	0.37***	0.10*
HI	0.48***	0.58***	0.32***	0.30***
T, TE	0.13***	0.22***	0.51***	0.46***
TE, HI	0.74***	0.74***	0.53***	0.61***
T, HI	0.55***	0.76***	0.76***	0.88***
T, TE, HI, HI^2	0.85***	0.90***	0.95***	0.95***

Interrelationship of analytical yield components

Under DS, T was closely and positively associated with TE, T was closely and negatively associated with HI, and TE was sparsely and negatively associated with HI (Fig. 5). Similar trends were noticed under WW condition.

Drought-response groups

A pooled analysis using the performance of 50 accessions in both the years showed no significant year × genotype effect for shoot biomass and TE. Therefore, individual accession means of TE in 2008 were used for segregating the groups into various response groups. In addition, the close linear relationship of T with TE, and the fact that TE explained more grain yield variation under DS, permitted the use of TE for further drought-tolerant selection along with HI. The accessions tested in 2008, comprising the whole core germplasm set including four controls ($n = 160$) and the 2-fold range of variation, were clustered into representative groups of TE by using the BLUPs of accessions under DS by the standard error of difference. There were 17 accessions with TE greater than the overall mean by 1-fold standard error of difference, and 22 accessions with TE less than the overall mean by 1-fold standard error of difference; these are listed as high TE and low TE accessions (Table 5). The rest were grouped as moderate TE accessions.

Drought response of races and subraces

Among the three races (*indica*, *maxima* and *moharia*) of foxtail millet (further divided into subraces) broadly, there were no large differences in grain yield (Fig. 6). In general, though, there were broad differences among the races in growing duration and shoot biomass productivity, and this reflected on T, TE and HI. Race *indica* ($n = 103$) had accessions that were

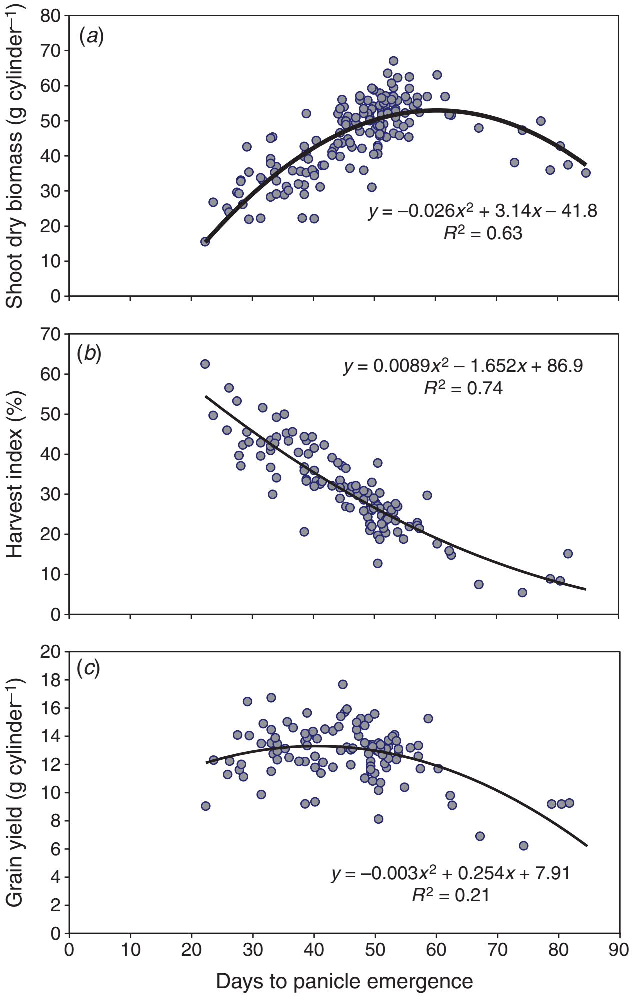

Fig. 3. Association of days to panicle emergence with total production of shoot biomass, harvest index and grain yield of foxtail millet accessions under drought-stressed conditions in cylinders during the 2008 rainy season.

characterised as medium to longer in duration, moderate to high in shoot biomass productivity (data not shown), moderate to high in T, moderate to high in TE and moderate to low in HI (Fig. 6). However, there were apparent exceptions such as accessions ISe 1134, –1136 and ISe 1137 from Syrian Arab Republic, which had opposite characteristics. Subraces *indica erecta* and *indica nana* had accessions that were similar among themselves but subrace *indica glabra* had accessions that varied extensively (data not shown).

Race *maxima* and subrace *compacta* ($n = 20$) had accessions that were characterised as short to medium in duration, low to moderate in shoot biomass productivity (data not shown), low to moderate in T, low to moderate in TE and moderate to high in HI (Fig. 6). On the other hand, the other two subraces, *assamense* ($n = 2$) and *spongiosa* ($n = 4$), had accessions that were opposite in nature and with more desirable characteristics for drought tolerance. Race *moharia* had accessions that were characterised as short to medium in duration, low to moderate in shoot biomass productivity (data not shown), T and TE, and moderate to high in HI (Fig. 6). Between the two subraces, *aristata* accessions had greater shoot biomass, T and TE and lower HI than *glabra*. Among the whole core collection, accessions belonging to subrace *glabra* of race *moharia* were the smallest producers of shoot biomass (T + TE) with the highest HI. In summary, there are vast choices available across the races for various combinations of analytical components for various soil water environments and breeding purposes.

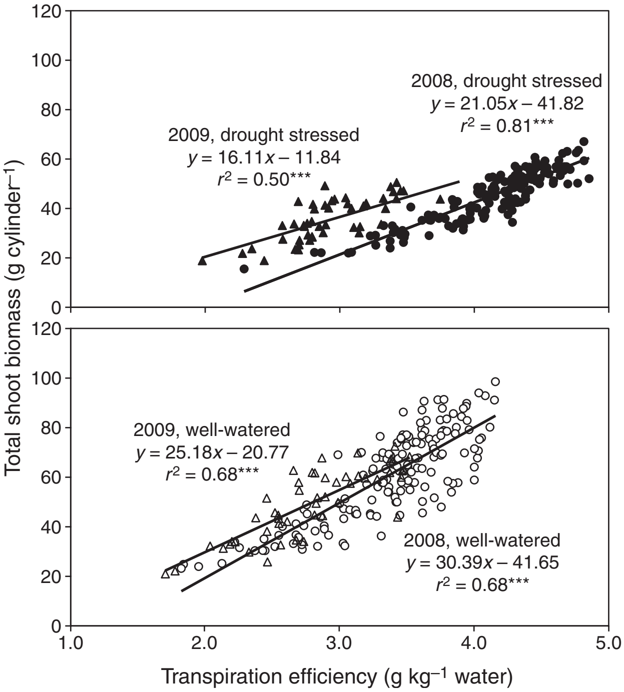

Fig. 4. Association between transpiration efficiency and total shoot biomass production in foxtail millet germplasm under drought-stressed and well-watered conditions in cylinders during the 2008 and 2009 rainy seasons.

Discussion

There is a wide consensus that the reproductive growth stage is the most sensitive to water deficit (Doorenbos and Kassam 1979; O'Toole 1982; Zhang *et al*. 2012). It is also recognised that DS at the reproductive stage is the most prevalent problem in drought-prone rainfed agriculture, simply because in most rainfed ecosystems, the crop season's rains diminish towards flowering and harvest time. Therefore, irrespective of biomass production up to flowering, continuance of water uptake and T into the reproductive growth stage is crucial for reproductive success and the desired levels of drought tolerance (Merah 2001; Kato *et al*. 2008). An effective means of achieving reproductive success under DS is soil-moisture capture by deep root systems where deep soil moisture is available (Kirkegaard *et al*. 2007; Blum 2009; Vadez *et al*. 2011; Wasson *et al*. 2012). In addition, continued water uptake can partly repress ABA production in the shoot or the root under DS (Westgate *et al*. 1996), which might impede with reproductive processes (Davies and Jones 1991).

This study had provided clear evidence that the root system of foxtail millet is likely to be shallow and that the amount of soil water utilised was intrinsically poor. In the present study,

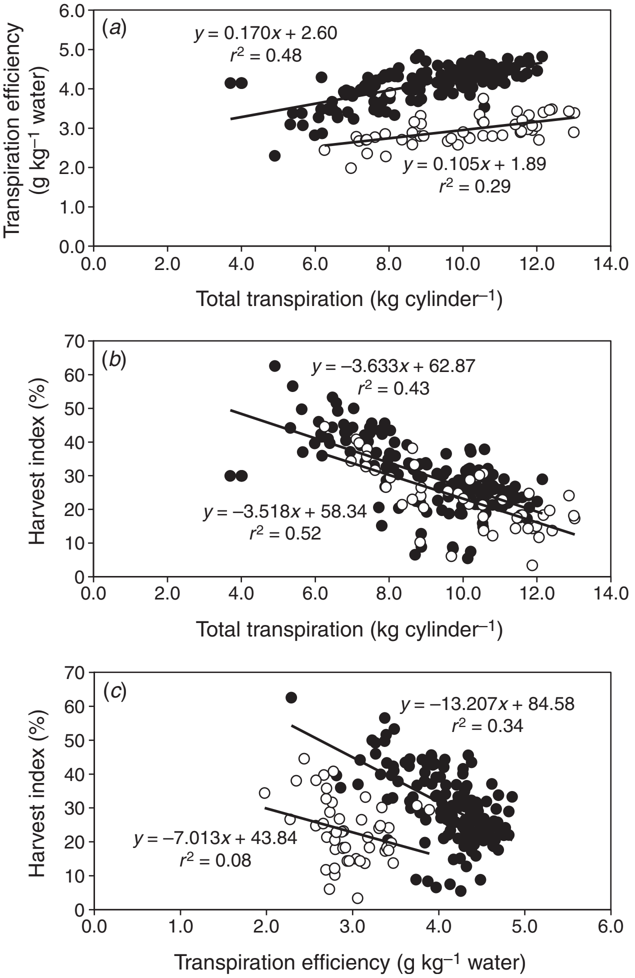

Fig. 5. Associations of (*a*) total water use with transpiration efficiency, (*b*) total water use with harvest index, and (*c*) transpiration efficiency with harvest index of foxtail millet accessions under drought stress in cylinders during the 2008 and 2009 rainy seasons.

the major amount of shoot biomass, 84–86% across years under DS and 88–90% under WW conditions, was accrued at the phase post-DS imposition (after 28 or 31 DAS) or at the reproductive phase, indicating that early growth, early leaf area development and consequent water uptake are low. A close dependence of shoot biomass on T observed in this study (Fig. 2) and recorded in other crop species (Condon *et al*. 2002; Fereres and Soriano 2007; Steduto *et al*. 2007; Blum 2009; Kashiwagi *et al*. 2015) supports that majority of the soil water from the cylinders was used during the post-anthesis period. However, the average T recorded under DS was a mere 10 kg, whereas the total available soil water in the whole depth of the cylinder was ~16.5 kg. Even under the WW condition, the highest transpiring accession never used more than 23.3 kg despite the cylinders receiving average additional water of ~10 kg (range 0.5–18.4 kg) at the reproductive stage. Even sorghum under DS, a crop species well adapted to drought, has been shown to transpire 13.9 kg under a similar DS cylinder-culture system (Vadez *et al*. 2011). In addition, T was seen to be dependent on the growing duration, which in turn would limit rooting depth. Thus, a ~30% drought-induced reduction in shoot biomass, shorter duration and meagre extraction of water under drought indicate that the root system of foxtail millet is relatively shallow and shoot growth is conservative. In addition, the total shoot biomass produced was suboptimal for cereals, and the average estimates equate to 3800 kg ha^{-1} under DS and 5880 kg ha^{-1} under WW condition. The shoot biomass productivity of accessions continued to increase linearly up to 60 days to panicle emergence, and the accessions that reached panicle emergence after this suffered relative shoot biomass losses. Shoot biomass productivity was able to explain a maximum of 54% of grain yield variation under WW condition but only 9% under DS, indicating that grain yield did not increase with the shoot biomass in most cases under DS. Therefore, an overall effect was that the partitioning (HI) was reduced and this reduction was ~35% relative to the mean performance under the WW environment.

Relevance of WUE for breeding

Selection for high WUE under limited water supply had been argued to result in a genetic shift towards plant traits that limit crop water use, such as early flowering and smaller leaf area (as reviewed by Blum 2009). There is a continuing debate on the usefulness of selection for high WUE, because it raises the question to what extent the often-observed high yield–low WUE association can be uncoupled (Araus *et al*. 2002; Morison *et al*. 2008; Blum 2009). Under non-limiting soil-water environments, opportunistic water use at lower WUE is expected to lead to higher yields than conservative water use. However, under higher DS, it is often hypothesised that those cultivars with higher WUE should be better yielding; consequently, selection for this trait is recommended for dry environments. Indeed, wheat lines with low $\Delta^{13}C$ (and high WUE) selected in a breeding program for dry environments were shown to increase aboveground biomass and kernel weight (Rebetzke *et al*. 2002). But this was once again explained as a unique occurrence specific to the stored soil moisture growing condition of that study (Blum 2009). Notwithstanding the relevance of WUE to water-limited environments, the ease of use and the avenues for selection of this trait are improved with the isolation of a gene (*ERECTA*) that regulates TE (Masle *et al*. 2005). This regulatory gene had been known for its effects on inflorescence development and as a major contributor to a locus for carbon isotope discrimination in *Arabidopsis*. Importantly in this study, TE explained a major portion of the variation in T under DS (49% in 2008 and 29% in 2009), and explained >10% of the variation in grain yield under DS, confirming that such a negative interaction between T and WUE does not exist in this set of (foxtail millet) germplasm. Thus, TE can readily be used as a selection criterion to co-select T and to improve the drought tolerance. This also shows that foxtail millet, with a suboptimal growth duration, leaf area development and shoot biomass productivity, can accrue benefits through high TE to its grain productivity.

Harvest index is the integration of success in reproduction and thus in grain yield that involves assimilate partitioning towards grain filling. Reproductive success depends on number of panicles (tillers) per plant, size of the panicles,

Table 5. Number of days to panicle emergence and maturity, total shoot biomass and grain yield (GY), harvest index, and mean total water use and transpiration efficiency (TE) of the foxtail millet germplasm accessions of the high and low TE response groups in the 2008 season under drought stress

C, Check entry. High TE group: greater than the overall mean by 1-fold S. Ed.; low TE group: less than the overall mean by 1-fold S. Ed.

Reaction group or accession	No. of days to:		Total shoot biomass (g cylinder^{-1})	Total GY	Harvest index (%)	Mean total water use (kg cylinder^{-1})	Mean TE (g kg^{-1})
	Panicle emergence	Maturity					
			High TE				
ISe 200	53.2	77.2	59.4	12.2	18.9	10.62	4.63
ISe 238	55.5	81.3	55.7	13.3	23.1	10.22	4.71
ISe 289	53.2	83.9	67.0	17.1	28.9	12.15	4.82
ISe 480	53.5	79.8	53.3	10.9	18.6	9.97	4.64
ISe 525	55.1	77.5	56.8	13.9	25.7	10.24	4.69
ISe 783	44.7	68.9	49.8	15.3	33.7	9.00	4.70
ISe 796	52.2	76.2	63.5	14.2	24.1	11.78	4.78
ISe 840	52.8	79.1	55.2	13.6	25.4	10.44	4.67
ISe 869	53.8	82.3	62.2	12.7	20.5	11.80	4.66
ISe 1251	38.9	64.8	52.0	15.6	33.2	8.81	4.85
ISe 1454	58.7	84.5	56.8	15.2	29.7	10.51	4.67
ISe 1468 (C)	44.7	70.2	56.0	17.7	37.0	10.22	4.62
ISe 1511	60.3	86.1	63.0	11.7	17.6	11.55	4.69
ISe 1664	52.9	78.2	56.0	14.0	26.0	10.59	4.64
ISe 1805	49.3	75.3	50.2	11.6	22.4	8.73	4.77
ISe 1881	49.6	76.6	59.2	13.2	22.0	10.46	4.82
ISe 1892	55.8	83.3	62.5	13.2	21.9	11.22	4.76
			Low TE				
ISe 719	28.2	60.0	33.0	12.5	39.8	8.15	3.33
ISe 827	29.5	55.5	21.9	9.3	36.9	5.67	3.07
ISe 828	38.2	71.5	22.2	10.4	44.1	5.33	3.09
ISe 1119	31.7	60.0	33.0	14.9	55.5	6.58	3.39
ISe 1136	38.9	62.2	35.1	13.9	43.3	7.76	3.61
ISe 1137	41.2	63.2	31.1	11.4	32.5	7.92	3.41
ISe 1151	25.9	56.5	25.0	11.3	45.9	6.10	3.27
ISe 1163	31.4	65.1	22.1	9.8	39.5	5.99	2.81
ISe 1187	35.3	64.8	28.8	13.1	49.9	7.05	3.23
ISe 1201	26.2	60.9	23.9	12.2	56.5	5.40	3.38
ISe 1227	27.5	55.5	29.6	14.1	53.2	6.47	3.49
ISe 1234	28.5	56.2	26.2	11.1	42.2	6.16	3.47
ISe 1254	22.3	55.2	15.5	9.0	62.5	4.91	2.29
ISe 1258	31.0	87.1	29.1	12.9	49.2	6.61	3.27
ISe 1286	23.6	54.6	26.7	12.3	49.7	5.64	3.38
ISe 1302	38.6	72.1	31.6	9.2	20.5	7.73	3.41
ISe 1312	27.8	54.9	29.2	11.6	39.6	6.40	3.45
ISe 1460	49.6	73.1	31.0	10.8	32.9	7.60	3.47
ISe 1474	40.2	61.9	22.0	9.3	35.9	6.19	2.86
ISe 1547	36.0	61.3	30.2	12.5	43.3	6.89	3.40
ISe 1593	41.8	67.3	37.3	14.5	42.3	7.82	3.66
ISe 1629	50.6	78.8	40.5	13.5	37.8	10.59	3.53

seedset and seed (size) filling, and overall the quantum of water availability at the developmental stages of these components to the plant. DS reduced the partitioning to the grains (HI) by ~35% relative to the mean HI under WW conditions. Such a reduction occurred in almost all accessions with a few exceptions (data not shown), indicating not only shoot biomass productivity but also seedset and seed filling to be adversely affected. Under DS, HI was inversely associated with T ($r^2 = 37\%$ in 2008 and 46% in 2009) and TE ($r^2 = 28\%$ in 2008 and 7% in 2009). Here, T is closely linked with crop duration, and therefore, the longer duration accessions seemingly suffered more reduction in HI than the early ones (Fig. 3*b*). A negative relationship of WUE with HI has been considered an outcome of selection for high yields with an unconscious selection pressure on HI (Rizza *et al*. 2012) and is expected to reduce grain yield either through reduction in T or through WUE. This study also reveals that, under DS, the TE individually accounts for more grain yield than T, and TE increases linearly with T (Fig. 5*a*), confirming the importance of TE over T. However, the inverse relationship of TE with HI means the need for a parallel selection of TE and HI to enhance the grain yield under DS.

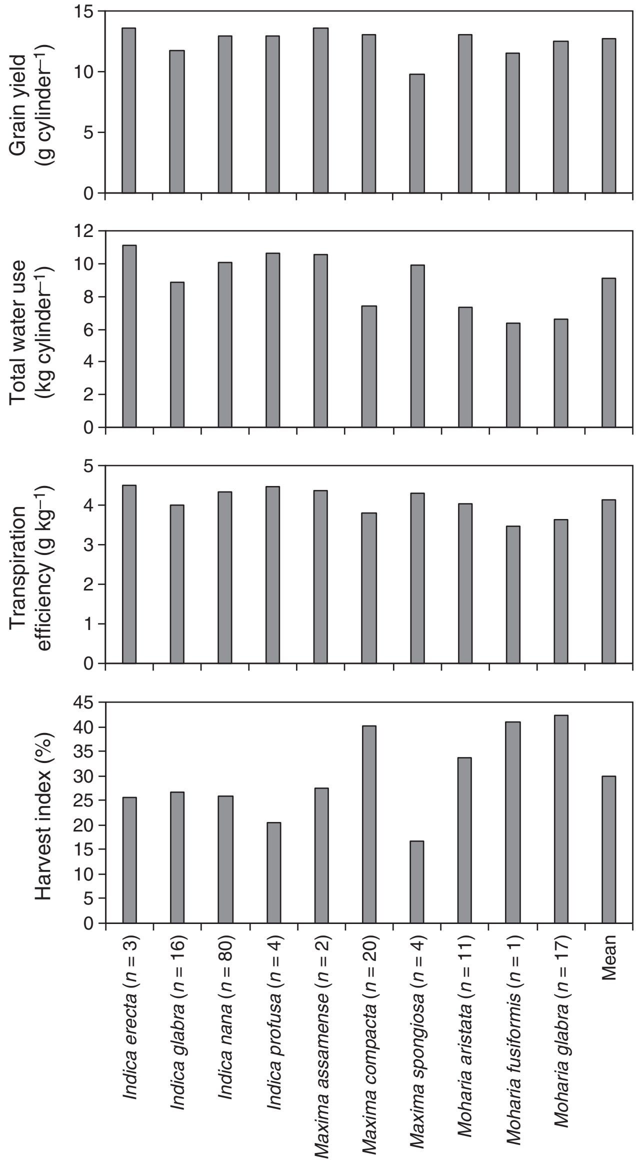

Fig. 6. Mean grain yield and yield component performances of races and subraces of the core collection of foxtail millet under drought-stressed condition as observed in the 2008 rainy season.

Genetic variation

Large variation existed for all components of yield and drought response in the core collection of foxtail millet, with germplasm accessions performing better than the check entries ISe 375, ISe 376 and ISe 1541 but trailing ISe 1468. These checks were chosen based on their agronomic performance over years rather than drought-tolerance performance because no such drought-tolerant checks are available (Upadhyaya *et al.* 2009, 2011). The performance of these check entries indicated some critical details about adaptation to this region. Overall, the 2009 crop can be characterised as having lower production of shoot biomass, much lower production of grain yield, similar T, and lower TE and HI than the 2008 crop. However, the relative performance of these checks was stable across years. Shoot biomass productivity of all of the accessions was close to the overall means of the respective soil-water treatments. The grain yields of ISe 375 and ISe 376 were close to the means of the relevant soil-water treatment, whereas grain yield of ISe 1468 was high and that of ISe 1541 was substantially low under DS and marginally low under WW conditions. The T and the TE of these checks were comparable to the overall soil-water treatment means. The HI of ISe 1541 caused the reduction in grain yield and was due to the longer duration (92 DAS) to maturity. However, this longer duration did not enhance soil-water use. These comparisons further strengthen the conclusion of this paper that drought-tolerance screenings need to target TE and HI simultaneously as selection criteria for the best drought-tolerant foxtail millet. The highest yielding accession under DS in 2008 was ISe 1468, which was greater than the overall mean by 13% in T, 12% in TE and 24% in HI.

The 10 highest grain yields under drought were achieved by accessions (ISe 2, 49, 96, 289, 1454, 1468, 1704, 1774, 1788, 1859) that had an average superiority in T by 17%, in TE by 9% and in HI by 4% above the overall mean.

Race-dependent variation useful for breeding

Broadly, the differences in mean grain yield of the 10 subraces belonging to the races *indica*, *maxima* and *moharia* were minor except for the clearly poor-yielding *maxima spongiosa*. However, large variations were available among the accessions within each subrace for higher grain yield, offering diverse choices for selection. The races and subraces varied markedly for the yield components that seemed characteristic of a particular subrace or race. Races *indica* and *maxima* in general were characterised by a high T and TE, except for the subraces *indica glabra* and *maxima compacta*, whereas the race *moharia* was poor in T and TE. On the other hand, HI was poor in races *indica* and *maxima*, except for subraces *indica glabra* and *maxima compacta*, and was high in the race *moharia*.

The major growth and developmental characteristics of various races were typical to the region of their origin, with differing strategies of adaptation. The origin of the race *indica* is largely South East Asia (predominantly tropical), race *maxima* North West Asia (temperate) and *moharia* Middle East Asia (dry arid areas). However, useful variations are available within races that might be of great help in crop-improvement efforts. The race *indica* has the best potential for shoot-biomass production and is more suited to the short-duration environments of Indian growing regions, whereas the lower tillering *maxima compacta* with heavy earheads is more suited to the temperate regions, which promote longer duration. However, the earliness, profuse tillering and high partitioning of race *moharia* can help in fodder productivity.

Advances in hybrid development and molecular-marker studies in China include identification of various male-sterile lines of foxtail millet having contrasting genes for photo- or thermo-sensitive nuclear system (Yuan *et al.* 2008), lines with gene-interaction male sterility and lines with cytoplasmic male sterility (Liu *et al.* 2014), and cytoplasmic-nuclear male-sterile type (as reviewed by Wang *et al.* 2013). However, these may not serve the needs of other regions because adaptation strategies may vary across tropical and Mediterranean environments.

High TE and HI lines identified in this work may help in breeding for well-focused, drought-tolerant cultivars and hybrids of foxtail millet.

Conclusions

Wide genotypic variation existed for drought response in the core collection of foxtail millet. In general, foxtail millet has poor biomass productivity and water use due to a short growth duration, with normal TE and a poor HI. The TE contributed positively and HI negatively to grain yield under DS, maintaining less variation in grain yield across accessions. Growth duration, T and TE were closely linked to each other, permitting selection for any one of the characteristics for successful identification of drought tolerance. DS equally reduced shoot biomass and reproductive traits; therefore, selection for both these should be necessary to ensure identification of better drought adaptation. Foxtail millet races *indica* and *maxima* possessed higher T and TE, whereas *moharia* possessed greater HI; however, presence of considerable intra-racial variations would permit choices for selection within each race.

Acknowledgements

This work was supported by the Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung/Deutsche Gesellschaft für Technische Zusammenarbeit (BMZ/GTZ) project 'Sustainable conservation and utilisation of genetic resources of two underutilised crops—finger millet and foxtail millet—to enhance productivity, nutrition and income in Africa and Asia' funded by the Federal Ministry for Economic Cooperation and Development (BMZ), Germany. Expert technical assistance by Mr N. Jangaiah and Mr J. Shankaraiah, (ICRISAT) is also greatly appreciated.

References

Ahanchede A, Hamon SP, Darmency H (2004) Why no tetraploid cultivar of foxtail millet? *Genetic Resources and Crop Evolution* **51**, 227–230. doi:10.1023/B:GRES.0000024020.91764.8d

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C_3 cereals: What should we breed for? *Annals of Botany* **89**, 925–940. doi:10.1093/aob/mcf049

Austin DF (2006) Foxtail millets (*Setaria:* Poaceae)—abandoned food in two hemispheres. *Economic Botany* **60**, 143–158. doi:10.1663/0013-0001(2006)60[143:FMSPFI]2.0.CO;2

Bartlett MS (1937) Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences* **160**, 268–282. doi:10.1098/rspa.1937.0109

Bennetzen JL, Schmutz J, Wang H, Percifield R, Hawkins J, Pontaroli AC, Estep M, Feng L, Vaughn JN, Grimwood J, Jenkins J, Barry K, Lindquist E, Hellsten U, Deshpande S, Wang X, Wu X, Mitros T, Triplett J, Yang X, Ye C-Y, Mauro-Herrera M, Wang L, Li P, Sharma M, Sharma R, Ronald PC, Panaud O, Kellogg EA, Brutnell TP, Doust AN, Tuskan GA, Rokhsar D, Devos KM (2012) Reference genome sequence of the model plant *Setaria*. *Nature Biotechnology* **30**, 555–561. doi:10.1038/nbt.2196

Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* **56**, 1159–1168. doi:10.1071/AR05069

Blum A (2009) Effective use of water (EUW) and not water-use efficiency is the target of crop yield improvement under drought stress. *Field Crops Research* **112**, 119–123. doi:10.1016/j.fcr.2009.03.009

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. *Crop Science* **42**, 122–131. doi:10.2135/cropsci2002.0122

Davies WJ, Jones HG (1991) 'Abscisic acid physiology and biochemistry.' (BIOS Scientific Publishers: Oxford, UK)

Diao X (2007) Foxtail millet production and future development direction in China. In 'Reports on minor grain development in China'. (Eds Y Chai, SH Wan) pp. 32–43. (China Agricultural Science and Technology Press: Beijing)

Doorenbos J, Kassam AH (1979) Yield response to water. Irrigation and Drainage Paper No. 33. FAO, Rome.

Doust AN, Kellogg EA, Devos KM, Bennetzen JL (2009) Foxtail millet: A sequence-driven grass model system. *Plant Physiology* **149**, 137–141. doi:10.1104/pp.108.129627

Dwivedi S, Upadhyaya HD, Senthilvel S, Hash CT, Fukunaga K, Diao X, Santra D, Baltensperger D, Prasad M (2012) Millets: Genetic and genomic resources. *Plant Breeding Reviews* **35**, 246–375.

Fereres E, Soriano MA (2007) Deficit irrigation for reducing agricultural water use. *Journal of Experimental Botany* **58**, 147–159. doi:10.1093/jxb/erl165

GENSTAT (2011) 'GENSTAT software for windows Release 14.1.' (VSN International Ltd: Hemel Hempstead, UK) Available at: http://www.vsni.co.uk

Kashiwagi J, Krishnamurthy L, Purushothaman R, Upadhyaya HD, Gaur PM, Gowda CLL, Ito O, Varshney RK (2015) Scope for improvement of yield under drought through the root traits in chickpea (*Cicer arietinum* L.). *Field Crops Research* **170**, 47–54. doi:10.1016/j.fcr.2014.10.003

Kato Y, Kamoshita A, Yamagishi J (2008) Preflowering abortion reduces spikelet number in upland rice (*Oryza sativa* L.) under water stress. *Crop Science* **48**, 2389–2395. doi:10.2135/cropsci2007.11.0627

Kirkegaard JA, Lilley JM, Howe GN, Graham JM (2007) Impact of subsoil water use on wheat yield. *Australian Journal of Agricultural Research* **58**, 303–315. doi:10.1071/AR06285

Lata C, Jha S, Dixit V, Sreenivasulu N, Prasad M (2011) Differential antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars [*Setaria italica* (L.)]. *Protoplasma* **248**, 817–828. doi:10.1007/s00709-010-0257-y

Li YM (1997) Drought-resistant mechanism and genetic expression of foxtail millet. In 'Foxtail millet breeding'. (Ed. YM Li) pp. 433–434. (China Agricultural Press: Beijing)

Li P, Brutnell TP (2011) *Setaria viridis* and *Setaria italica*, model genetic systems for the Panicoid grasses. *Journal of Experimental Botany* **62**, 3031–3037. doi:10.1093/jxb/err096

Liu Z, Bai G, Zhang D, Zhu C, Xia X, Cheng R, Shi Z (2011) Genetic diversity and population structure of elite foxtail millet [*Setaria italic* (L.) P. Beauv.] germplasm in China. *Crop Science* **51**, 1655–1663. doi:10.2135/cropsci2010.11.0643

Liu Z, Zhang T, Li C, Bai G (2014) Genetic diversity and classification of cytoplasm of Chinese elite foxtail millet [*Setaria italica* (L.) P. Beauv.] germplasm. *Crop Science* **54**, 659–666. doi:10.2135/cropsci2012.11.0646

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. *Advances in Agronomy* **43**, 107–153. doi:10.1016/S0065-2113(08)60477-0

Masle J, Gilmore SR, Farquhar GD (2005) The *ERECTA* gene regulates plant transpiration efficiency in *Arabidopsis. Nature* **436**, 866–870. doi:10.1038/nature03835

Merah O (2001) Potential importance of water status traits for durum wheat improvement under Mediterranean conditions. *The Journal of Agricultural Science* **137**, 139–145. doi:10.1017/S0021859601001253

Morison JI, Baker NR, Mullineaux PM, Davies WJ (2008) Improving water use in crop production. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **363**, 639–658. doi:10.1098/rstb.2007.2175

O'Toole JC (1982) Adaptation of rice to drought-prone environments. In 'Drought resistance in crops with emphasis on rice'. pp. 195–213. (International Rice Research Institute: Los Baños, Philippines)

Passioura JB (1977) Grain yield, harvest index and water use of wheat. *Journal of the Australian Institute of Agricultural Science* **43**, 117–120.

Peng S, Krieg DR (1992) Gas exchange traits and their relationship to water use efficiency. *Crop Science* **32**, 386–391. doi:10.2135/cropsci1992.0011183X003200020022x

Qie L, Jia G, Zhang W, Schnable J, Shang Z, Li W, Liu B, Li M, Chai Y, Zhi H, Diao X (2014) Mapping of quantitative trait locus (QTLs) that contribute to germination and early seedling drought tolerance in the interspecific cross *Setaria italica* × *Setaria viridis*. *PLoS One* **9**, e101868. doi:10.1371/journal.pone.0101868

Ratnakumar P, Vadez V, Nigam SN, Krishnamurthy L (2009) Assessment of transpiration efficiency in peanut (*Arachis hypogaea* L.) under drought by lysimetric system. *Plant Biology* **11**, 124–130. doi:10.1111/j.1438-8677.2009.00260.x

Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2002) Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. *Crop Science* **42**, 739–745. doi:10.2135/cropsci2002.0739

Rizza F, Ghashghaie J, Meyer S, Matteu L, Mastrangelo AM, Badeck FW (2012) Constitutive differences in water use efficiency between two durum wheat cultivars. *Field Crops Research* **125**, 49–60. doi:10.1016/j.fcr.2011.09.001

Schneider KA, Rosales-Serna R, Ibarra-Perez F, Cazares- Enriquez B, Acosta-Gallegos JA, Ramirez-Vallejo P, Wassimi N, Kelly JD (1997) Improving common bean performance under drought stress. *Crop Science* **37**, 43–50. doi:10.2135/cropsci1997.0011183X003700010007x

Seghatoleslami MJ, Kafi M, Majidi E (2008) Effect of drought stress at different growth stages on yield and water use efficiency of five proso millet (*Panicum miliaceum* L.) genotypes. *Pakistan Journal of Botany* **40**, 1427–1432.

Steduto P, Hsiao TC, Fereres E (2007) On the conservative behavior of biomass water productivity. *Irrigation Science* **25**, 189–207. doi:10.1007/s00271-007-0064-1

Upadhyaya HD, Pundir RPS, Gowda CLL, Reddy VG, Singh S (2009) Establishing a core collection of foxtail millet to enhance the utilization of germplasm of an underutilized crop. *Plant Genetic Resources; Characterization and Utilization* **7**, 177–184. doi:10.1017/S1479262108178042

Upadhyaya HD, Ravishankar CR, Narasimhudu Y, Sarma NDRK, Singh SK, Varshney SK, Reddy VG, Singh S, Parzies HK, Dwivedi SL, Nadaf HL, Sahrawat KL, Gowda CLL (2011) Identification of trait-specific germplasm and developing a mini core collection for efficient use of foxtail millet genetic resources in crop improvement. *Field Crops Research* **124**, 459–467. doi:10.1016/j.fcr.2011.08.004

Urrea CA, Yonts CD, Lyon DJ, Koehler AE (2009) Selection for drought tolerance in dry bean derived from the Mesoamerican gene pool in Western Nebraska. *Crop Science* **49**, 2005–2010. doi:10.2135/cropsci2008.12.0694

Vadez V, Rao S, Kholova J, Krishnamurthy L, Kashiwagi J, Ratnakumar P, Sharma KK, Bhatnagar-Mathur P, Basu PS (2008) Roots research for legume tolerance to drought: quo vadis? *Journal of Food Legumes* **21**, 77–85.

Vadez V, Krishnamurthy L, Hash CT, Upadhyaya HD, Borrell AK (2011) Yield, transpiration efficiency, and water-use variations and their interrelationships in the sorghum reference collection. *Crop & Pasture Science* **62**, 645–655. doi:10.1071/CP11007

Vadez V, Kholova J, Medina S, Aparna K, Anderberg H (2014) Transpiration efficiency: new insights into an old story. *Journal of Experimental Botany* **65**, 6141–6153. doi:10.1093/jxb/eru040

Vetriventhan M, Upadhyaya HD, Anandakumar CR, Senthilvel S, Parzies HK, Bharathi A, Varshney RK, Gowda CLL (2012) Assessing genetic diversity, allelic richness and genetic relationship among races in ICRISAT foxtail millet core collection. *Plant Genetic Resources* **10**, 214–223. doi:10.1017/S1479262112000287

Wang C, Chen J, Zhi H, Yang L, Li W, Wang Y, Li H, Zhao B, Chen M, Diao X (2010) Population genetics of foxtail millet and its wild ancestor. *BMC Genetics* **11**, 90. doi:10.1186/1471-2156-11-90

Wang J, Wang Z, Yang H, Yuan F, Guo E, Tian G, Yuanhuai AN, Li H, Wang Y, Diao X, Guo P (2013) Genetic analysis and preliminary mapping of a highly male-sterile gene in foxtail millet (*Setaria italica* L. Beauv.) using SSR markers. *Journal of Integrative Agriculture* **12**, 2143–2148. doi:10.1016/S2095-3119(13)60392-5

Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SV, Rebetzke GJ, Kirkegaard JA, Christopher J, Watt M (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany* **63**, 3485–3498. doi:10.1093/jxb/ers111

Westgate ME, Passioura JB, Munns R (1996) Water status and ABA content of floral organs in drought-stressed wheat. *Australian Journal of Plant Physiology* **23**, 763–772. doi:10.1071/PP9960763

Yang X, Wan Z, Perry L, Lu H, Wang Q, Zhao C, Li J, Xie F, Yu J, Cui T, Wang T, Li M, Ge Q (2012) Early millet use in northern China. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 3726–3730. doi:10.1073/pnas.1115430109

Yuan A, Hou A, Zhang F, Guo Y (2008) Inheritance and effects of the photoperiod sensitivity in foxtail millet (*Setaria italica* P. Beauv). *Hereditas* **145**, 147–153. doi:10.1111/j.0018-0661.2008.02060.x

Zhang JP, Liu TS, Zheng J, Jin Z, Zhu Y, Guo JF, Wang GY (2007) Cloning and characterization of a putative 12-oxophytodienoic acid reductase cDNA induced by osmotic stress in roots of foxtail millet. *DNA Sequence* **18**, 138–144. doi:10.1080/10425170601060764

Zhang W, Zhi H, Liu B, Xie J, Li J, Li W, Jia G, Wang Y, Li H, Chai Y, Li Y, Diao X (2012) Screening of indexes for drought tolerance test at booting stage in foxtail millet. *Plant Genetic Resources* **13**, 765–772.

Zooleh HH, Jahansooz MR, Yunusa I, Hosseini SMB, Chaichi MR, Jafari AA (2011) Effect of alternate irrigation on root-divided foxtail millet (*Setaria italica*). *Australian Journal of Crop Science* **5**, 205–213.