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Quantitative trait loci associated with constitutive traits controlling water use in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract Substantial genetic variation for drought adaption exists in pearl millet via traits controlling plant water use. Knowing genomic regions responsible for these traits is important. F_7 recombinant inbred lines were used to identify QTLs and allelic interactions for traits affecting plant water use, and we discuss their relevance for crop productivity in water-limited environments. Four QTLs contributed to increased transpiration rate under high VPD conditions, all with alleles from drought-sensitive parent ICMB 841. Of these four QTLs, a major QTL (35.7%) was mapped on LG 6. The alleles for 863B at this QTL decreased transpiration rate and this QTL co-mapped to a previously detected LG 6 QTL, with alleles from 863B, for grain mass and panicle harvest index across severe terminal drought stress environments. This evidence provided additional support for a link between water savings from lower transpiration rate under high VPD and drought tolerance. 863B alleles in this same genomic region also increased shoot weight, leaf area, and total transpiration under well-watered conditions. One unexpected outcome was a reduced transpiration under high VPD (15%) from the interaction of two alleles for high VPD transpiration (LG 6 (B), 40.7) and specific leaf weight and biomass (LG 7 (A), 35.3), (A, allele from ICMB 841, B, allele from 863B, marker position) . The LG 6 QTL appears to combine alleles for growth potential, beneficial for non-stress conditions, and for saving water under high evaporative demand,

beneficial for stressful conditions. Mapping QTLs for water-use traits, and assessing their interactions, offers considerable potential for improving pearl millet adaptation to specific stress conditions through physiology-informed marker-assisted selection.

Keywords Transpiration; Transpiration rate; Vapor pressure deficit (VPD); biomass; QTL interaction; *Pennisetum glaucum.*

Abbreviations QTL = quantitative trait loci, VPD = vapor pressure deficit, PVE = phenotypic variance explained, LG = linkage group, RIL = recombinant inbred lines, SSR = simple sequence repeats, DAP = diammonium phosphate and LOD = logarithm of odds

Introduction

Pearl millet is one of the most drought tolerant crops, which can provide significant grain and stover yield in spite of its frequent cultivation under severely water-limited conditions, where the other crops fail or suffer greater yield reductions. Extensive research has been conducted across environments to detect QTLs responsible for better than average grain and stover yield under terminal drought conditions (Yadav *et al*. 2002, 2004; Bidinger *et al*. 2005, 2007). However, grain and stover yields are complex traits with low heritabilities, and high QTL × QTL (Q × Q) and QTL × environment interactions (Q × E). Recent research on the dissection of physiological traits underlying yield-based drought tolerance QTLs (in both pearl millet and in sorghum) have shown that water conservation strategies are important

mechanistic components of at least some major grain yield drought tolerance QTLs, in particular low leaf conductance and leaf conductance sensitivity to high vapor pressure deficit (Kholova *et al*. 2010a).

Terminal-drought tolerance QTL donor parents PRLT2/89-33 and 863B have lower leaf conductance values than their terminal-drought sensitive H77/833-2 and ICMB 841 counterparts (Kholova *et al*. 2010a), which were previously used to develop two mapping populations and identify a major QTL contributing to a higher yield under terminal drought stress (Yadav *et al*. 2002, 2004; Bidinger *et al*. 2005, 2007). Drought-tolerance allele introgression lines in the background of H77/833-2 also had low leaf conductance, i.e. in the range of their donor parent (Kholova *et al*. 2010a), and also had leaf conductance sensitivity to high VPD when measured under fully-irrigated conditions (Kholova *et al*. 2010b). These mechanisms thus allow saving water in the soil profile during times that water is relatively plentiful, so that more soil water is available for the grain-filling period (Kholova *et al*. 2010b; Vadez *et al*. 2012) – provided that the soil has sufficient water-holding capacity to conserve at least a portion of the "saved" water at depths accessible to the plant root system. However, in some of the lightest-textured soils in which pearl millet is grown, this strategy would not be effective in reducing the risk of drought stress as any "conserved water" would likely be lost to deep drainage – on such soils a water-use strategy of "use-it-or-lose-it" appears to rule – with the additional forage biomass produced per unit of available soil water likely compensating for increased risk of grain production failure.

A recent mapping study (Kholova *et al*. 2012) has identified a QTL for transpiration rate that co-maps with the previously identified major terminal drought tolerant QTL on LG 2 (Yadav *et al*. 2002) at a smaller genetic interval than the original QTL. In addition this study mapped several simple traits, i.e. leaf area, organ weights and thickness, which revealed the genetics of several other aspects of plant

water use (Kholova *et al*. 2012). Here we expand this work to a second pearl millet RIL population in which the same terminal drought tolerance QTL was identified (Yadav *et al*. 2004; Bidinger *et al*. 2007). Earlier work has also shown a high degree of QTL × QTL interaction (two- and three-way interactions), interactions which also depended on the environment (Kholova *et al*. 2012). A given QTL allele can indeed have favorable, neutral or negative effects depending on the environment (including the genetic background) in which it is assessed (Chapman *et al*. 2003; Vargas *et al*. 2006). This complication can be resolved by estimating the QTL × QTL interactions for a given set of environmental conditions (and ideally in a common genetic background). Selection for enhanced trait expression based on these allelic interactions could be an effective approach for marker-assisted breeding.

One of our objectives was to narrow down the major terminal drought QTL previously detected on pearl millet LG 2 into smaller genetic intervals, and relate these to traits affecting plant water use, in order to increase the precision of the marker-assisted selection for superior combinations of productivity and yield stability under the erratic rainfall conditions in which this crop is most commonly grown. Specifically, the objectives were: 1) to identify the genomic regions associated with absolute transpiration under low and high VPD, leaf area and specific leaf weight (SLW), vegetative biomass and its component traits (leaf, stem, and root dry weight, tiller number); 2) to compare the QTLs identified with those from previous mapping studies of the cross ICMB 841 × 863B and with other mapping populations in order to understand the probable interrelations between these traits and previously identified yield-based QTLs for drought tolerance, and 3) to estimate the allelic interactions that control trait value, to design specific ideotypes, and to further improve the effectiveness of breeding programs.

Materials and methods

Plant material

The genetic material consisted of two inbred parental lines contrasting for terminal drought tolerance, i.e. ICMB 841 (drought sensitive) and 863B (drought tolerant), and 101 recombinant inbred lines (RILs) of the F_7 generation of their cross. Testcrosses of F_2-derived F_3 progenies and F_2-derived F_4 progenies were used earlier to map QTLs for biomass, grain yield and their components under terminal drought-stressed and well-watered conditions (Yadav *et al*. 2002, 2004; Hash *et al*. 2003; Bidinger *et al*. 2007). ICMB 841 is a drought-sensitive seed parent maintainer line of northwestern Indian origin (Singh *et al*. 1990), but derived from a pearl millet forage hybrid seed parent maintainer from the USA, and contributed the female nuclear genome to several commercially successful hybrids grown by farmers in northwestern India. In contrast, parent 863B is drought tolerant with superior combining ability due to its *Iniadi* landrace origin from West Africa (Rai *et al*. 2008). Single plants of these two parental inbreds were crossed to produce a single F_1 plant that was selfed to produce F_2 seed, which was further advanced to the F_7 generation by modified single-seed decent. A non-random set of 101 F_7 lines, having SSR marker fingerprints that did not indicate the occurrence of outcrossing during their derivation, were selected for phenotyping and more detailed genotyping.

Phenotyping and plant growing conditions

Phenotyping was performed in 2010 and repeated in 2011 over a period of ten days during the February-March interval. The method was similar to that used to phenotype the same traits in another mapping population (Kholova *et al*. 2012), and the period was chosen so that plants would be exposed to both low and high VPD conditions under natural conditions at the time of the measurements. A

portable rain-out shelter was available to protect the pot-grown crop from unforeseen rain. The average day/night temperature during plant growth was 32/24˚C and relative humidity 37/70% during Feb 2010, average day/night temperature was 32/22˚C and relative humidity was 27/70% during Feb 2011.

Plants were grown in 20-cm diameter plastic pots filled with 5 kg of Alfisol. Each pot was sown with eight seeds, in four separate hills, two seeds per hill. Seedlings were thinned to maintain two homogenous plants per pot at 15 days after sowing (DAS). Nutrients were provided with DAP as basal dose at the rate of 300 mg per kg of soil and urea was applied at the rate of 200 mg per kg of soil at 15 DAS. Five replications of each entry were sown in a staggered manner (on 5th, 8th, 10th, 12th and 14th February 2010 and 3rd, 5th, 7th, 10th and 12th February 2011), in order to handle only one replication per day at the time of measurement. Growing of the plants and phenotyping was performed similarly in both years, under well-watered conditions. At 20 DAS, pots were well-watered and allowed to drain overnight to reach field capacity. The soil was covered with a polythene sheet and a 3-cm layer of plastic beads to limit evaporation of water from the soil surface. Pots were arranged spaciously with about 10-15 cm distance between pots. Six pots without plants were brought to field capacity in a similar way and kept as checks in every replication to assess the rate of soil evaporation. The following day, pots were weighed at 7:15 a.m., 10:15 a.m., and 2:15 p.m., in order to obtain estimates of transpiration of the plants under low (morning) and high VPD (early afternoon) conditions, and all of the replications (of the same age) were handled sequentially on consecutive days at those time points. Pot weighing of a given replication was repeated the following day in accordance with the staggered sowings so that all the experimental plants were at the same age (20-22 days of vegetative growth). The average low VPD was 1.87/1.38 kPa (between 7:15 a.m. and 10:15 a.m.) and the average high VPD was 3.56/3.76 kPa (between 10.15 a.m. and 2:15 p.m.) during 2010/2011. After the weighing at 2:15 p.m., plants were re-

watered to pot capacity and allowed to drain overnight, for a repeat transpiration assessment of this same replication the following day. After these two days of transpiration assessment, plants were harvested (after weighing at 2:15 p.m.) and leaf area was measured immediately (using LA meter, LI3000 model, Li-Cor, Lincoln, Nebraska). Transpiration rate was measured as transpiration (g) per unit leaf area (cm^2) per h. The other parameters were leaf dry weight, stem dry weight, shoot dry weight (leaf + stem dry weight), root dry weight, total biomass (shoot + root dry weight), tiller number and specific leaf weight (leaf dry weight/leaf unit area), tiller number was recorded only in one year, i.e. 2011. Of course, there was a high degree of relationship between these different traits. For instance, the dry weight components were all closely related and were considered as one group of traits. Others are either consequences of environmental factor affecting these traits or emerging consequences of other traits. For instance, the leaf area is in part a consequence of environmental conditions prevailing earlier when the leaves expanded and then the specific leaf area is a combination of leaf area expansion and dry mass accumulation, both of which are differently affected by environmental conditions (Tardieu *et al*. 1999). The transpiration values are a combination of the leaf area and of the transpiration rate, the latter being affected by how stomata respond to the evaporative demand, while the leaf area is also a factor of the degree of tillering of the lines.

Linkage map

A linkage map developed by colleagues of C.T. Hash (pers. comm.) was used in this study to map QTLs. The map consisted of 358 mapped loci distributed across seven linkage groups, developed by saturating the previous linkage map (Yadav *et al*. 2004). Gmendel software was used for grouping the markers into seven linkage groups (LGs) using LOD $\geq$3, and the ordering within LG was done using 1000 bootstrap iterations available in the same software. Additional markers were included in the map with the help of

"TRY" command from MAPMAKER/EXP 3.0 (Lander *et al*. 1987; Lincoln *et al*. 1992). The original pearl millet consensus map developed by Qi *et al*. (2004), the SSR map developed by Senthilvel *et al*. (2008), and the pearl millet SSR consensus map (Rajaram *et al*. 2013) were all used as reference maps to integrate the markers.

Data analysis

The replicated data of each year and across the two years was subjected to linear mixed model analysis to obtain best linear unbiased predictors (BLUPs) for each RIL, using ReML in Genstat (version 12). Frequency distributions of each trait and broad sense heritability (h^2) of each trait were also calculated using the same software [$h^2=\sigma^2G/(\sigma^2G + \sigma^2E)$ where σ^2G is genetic variance and σ^2E is error variance]. Composite interval mapping was performed with these BLUPs to detect the QTLs for each of the individual years and across the years, using the PLABQTL software (Utz and Melchinger, 1996). A threshold LOD (logarithm of odds) of 3, F to enter value of 8 (highly stringent input to declare QTLs when compared to default 2), with cofactors were the inputs used in the PLABQTL software to determine putative QTLs, and providing a value for the percentage variation explained (PVE) for any given trait. An additive model (A for individual QTLs and AA i.e additive × additive to detect epistatic effects) was considered to calculate the phenotypic variance of the detected QTLs. All QTLs with significant partial R^2 values from the model were considered significant. Positive sign of QTL additive effects indicates that the allele from drought-tolerant male parent 863B contributed positively to the trait and a negative sign indicates that the allele from drought-sensitive female parent ICMB 841 contributed positively to the trait. BLUPs of each individual year were fed together into the PLABQTL software to analyse the significance of genotype × year interactions based on F probability values. The Genotype Matrix mapping software (version 2.1) was used to assess the allelic interactions (Isobe *et al*. 2007) with a

default searching range set by the program. Allelic interactions were confined to two and three loci due to the limited size of the mapping population, although GMM also returns significant QTL output in cases of single loci. Single locus denotes the QTL position which is similar to PLABQTL output. The output of GMM includes symbol A which indicates that the allele from female parent ICMB 841 had positive additive effects and B indicates that the allele from male parent 863B had positive additive effects. GMM outputs provide significant QTLs. Then the F-value indicates the significance level of that particular QTL interaction combination. Number of lines indicates the number of RILs (out of 101 total) with that particular QTL combination. Therefore, important QTLs are those having a high F value and a large number of RILs having that combination. From the GMM output, a percentage change in the trait (either positive for an increase or negative for a decrease) can be calculated, from the comparison of the trait value with (true) or without (false) the allele combination.

Results

Frequency distribution for the different traits is shown in Supplementary Figure 1. ReML-estimated BLUPs across years in the RIL population followed a normal distribution for low VPD transpiration and transpiration rate (Supplementary Fig. 1A, 1C), and total biomass (BDW, leaf + stem + root) (Supplementary Fig. 1F, 1H, 1I, 1J) but slightly skewed distributions for high VPD transpiration and transpiration rate (Supplementary Fig. 1B, 1D). 863B parent produced larger statured plants than ICMB 841 which got reflected in its larger shoot mass (25%) root mass (35%) and transpiration under low (29%) and high (15%) VPD conditions respectively (Table 1). Increase in transpiration of 863B was considerably less under high VPD conditions than that of ICMB 841. Indeed, under high VPD conditions the transpiration rate (g water loss cm^{-2} h^{-1}) of parent 863B was significantly (15.4%) lower than that of ICMB 841, whereas the transpiration rate of drought-tolerant 863B did not differ significantly (0.1%)

from drought-sensitive ICMB 841 under low VPD (Table1). The broad-sense heritability coefficients h^2 of most observed traits were high and ranged from 0.6-0.9 (Table 1); but medium in case of root dry weight (0.5), specific leaf weight (0.55) and tiller number (0.5).

QTL analysis for biomass and its components

The LG 2 QTL with favorable alleles from 863B was significant in 2010 and across both years, where it explained 14.5% of phenotypic variation for leaf dry weight. This QTL mapped towards the lower end of the drought tolerance QTL region of LG 2 (Yadav *et al*. 2002) (Fig. 1). Across years, a QTL on LG 6 with positive alleles from 863B parent explained a large proportion of phenotypic variation for leaf area (27.7% PVE, LOD 8.23) and leaf dry weight (26.9% PVE, LOD 8) (Table 2) (Fig. 2). Along with LG 6, QTLs for leaf dry weight were also found on both LG 2 and LG 7 (Table 2) (Fig. 3). The LG 7 QTL was observed consistently across years and with the favorable alleles from ICMB 841 (16.5% PVE, LOD 4.72 across years). This QTL co-mapped with a total biomass QTL and low VPD transpiration QTLs of LG 7 (Fig. 3). QTL detected in other linkage groups are displayed in Supplementary Figure 2. Specific leaf weight was mainly influenced by QTLs on LG 3 and LG 7 (Table 2) (Suppl. Fig. 2b; Fig.3). The LG 3 favorable alleles were contributed by 863B (14.4% PVE, LOD 4.76 across both years) while the LG 7 favorable alleles were inherited from ICMB 841 (13.1% PVE, LOD 3.08 in 2010, 11.7% PVE, LOD 4.47 in 2011, and 7.8% PVE, LOD 3.3 across years).

Three QTLs were identified for stem dry weight on LG 1, LG 2 and LG 5 (Table 2) (Suppl. Fig. 2a,c; Fig.1). Across years, none of these QTLs explained a large percentage of phenotypic variation (6.7% to 8.2%); although, the LG 1 QTL explained 16.9% of phenotypic variation in 2011 with the favorable allele from

ICMB 841. Tiller number was influenced by the regions of LG 1 and LG 4 (Table 2), although these QTLs were significant in 2011 only (Suppl. Fig. 2a,c). The LG 1 QTL explained a higher portion of phenotypic variation (15.7% PVE, LOD 5.26) than the LG 4 QTL (11.5% PVE, LOD 4.29), and the favorable alleles were inherited from ICMB 841 for both QTLs. The LG 4 QTL for tiller number overlapped with the QTL for high VPD transpiration rate (Suppl. Fig. 2c). Shoot weight (leaf + stem) was influenced by alleles on LG 2, LG 5, LG 6 and LG 7, (Table 2) with the most significant QTLs located on LG 5, LG 6 and LG 7 (Suppl. Fig.2d; Fig2&3). The LG 6 QTL accounted for the highest phenotypic variation (17.0% PVE, LOD 5.82 across years) with the favorable allele from 863B, followed by LG 7 QTL (12.7% PVE, 4.9 across years) with the favorable allele from ICMB 841 in 2010 and across both years. Two LG 5 QTLs (11.6% and 12.0% PVE across years) were detected; favorable alleles for the first QTL were contributed by 863B (11.6 %, LOD 3.95 across years) and was observed in 2011 and across both years, while favorable alleles for the second QTL on LG 5 were contributed by ICMB 841. The LG 5 QTL with favorable alleles from 863B co-mapped with the grain yield and harvest index QTL reported by Yadav *et al*. (2004) (Suppl. Fig. 2d). The LG 2 QTL with favorable 863B alleles co-mapped to the lower end of the major terminal drought tolerance QTL on LG 2 (Yadav *et al*. 2002, 2004; Bidinger *et al*. 2007), although it explained less phenotypic variation (5.8%, LOD 4.4 across years).

Root dry weight was influenced by QTLs on LG 2 and LG 7 (Table 2). Across both years, the LG 2 QTL explained the greatest phenotypic variation (19.5% PVE, LOD 5.56), with the favorable allele from 863B. The LG 7 QTL observed across both years explained 14.5% of phenotypic variation (LOD 4.01) with the favorable allele from ICMB 841 (Fig. 3). This QTL was also observed in 2010 and explained 11.4% of phenotypic variation. Total biomass was influenced by the alleles of LG 2 and LG 7 (Table 2). The LG 2 QTL with the favorable allele from 863B was found in 2010, across both years and explained a higher portion

of phenotypic variation (11.4% PVE, LOD 4.66) than the LG 7 QTL with the favorable allele from ICMB 841 (9.3% PVE, LOD 3.28).

QTL analysis for transpiration rate (Tr) under low VPD and high VPD

Transpiration rate under low VPD conditions was influenced only by one QTL on LG 3, and this QTL was detected across both years, explaining 12.9% of phenotypic variation (LOD 3.02), with the positive alleles contributed by 863B. By contrast, four QTLs were detected for transpiration rate under high VPD, located on LG 1, LG 2, LG 4 and LG 6, (Table 2) with the positive alleles contributed by ICMB 841 for all the QTLs. Among the four QTLs, the highest phenotypic variation (35.7% PVE, LOD 7.27 across both years) was observed with the LG 6 QTL and the remaining three were minor QTLs explaining less phenotypic variation (5.8 to 9.2%). This LG 6 QTL was also detected in 2010 (26.5% PVE, LOD 7.73). The LG 2 QTL was detected in 2010 (9.9% PVE, LOD 5.19), and across years (5.8% PVE, LOD 3.27), and this QTL co-mapped with the lower end of the major terminal drought tolerance QTL region (Fig. 1) reported earlier on LG 2 (Yadav *et al*. 2002, 2004; Bidinger *et al*. 2007).

QTL analysis for transpiration under low and high VPD

Low VPD transpiration (g h^{-1}) (7:15 a.m. – 10:15 a.m.) was influenced by QTLs on LG 6 and LG 7. The LG 6 QTL (LOD 6.35) explained 24.5% of phenotypic variation across both years and was also detected in each year, with the positive allele from 863B (Table 2). This LG 6 QTL co-mapped with the leaf area and shoot weight QTLs with favorable alleles also coming from tolerant 863B (Fig. 2). A QTL for low VPD transpiration was also detected (2010) on LG 7 with the favorable allele being contributed by drought-sensitive ICMB 841 (24.7% PVE, LOD 6.17).

High VPD transpiration was influenced by QTLs on LG 1, LG 2, LG 5, LG 6 and LG 7 (Table 2), explaining a large percentage of phenotypic variation (95% PVE in total) compared to low VPD transpiration. Positive alleles for both of the QTLs on LG 1 (30% PVE, LOD 7.19) and LG 7 (14.8% PVE, LOD 3.72) were both contributed by ICMB 841. The LG 1 QTL was detected in 2011 and across both years. The LG 7 QTL was detected in 2010 and across both years. In contrast, positive alleles for the QTLs on LG 6 (21.7% PVE, LOD 7.0), LG 5 (17.2% PVE, LOD 4.3), and LG 1 (11.4% PVE, LOD 3.31) were contributed by 863B. Similar to the low VPD transpiration QTL, the high VPD transpiration QTL on LG 6 (21.7% PVE, LOD 7 across years), was detected consistently in 2010 (16.3% PVE, LOD 3.79,) and 2011 (19.9% PVE, LOD 4.73), co-mapped with leaf area and shoot weight QTLs (Fig. 2) with the positive allele from 863B, and also co-mapped with grain mass and panicle harvest index QTLs detected under severe water stress and across stress environments with favorable alleles from 863B (Bidinger *et al*. 2007). The LG 1 QTL detected in 2011 (27.3% PVE, LOD 5.47) and across years (11.4% PVE, 3.31 LOD) with favorable alleles from 863B co-mapped with one of the secondary QTLs for grain yield (suppl. Fig. 2a), HI and PHI reported earlier (Bidinger *et al*. 2007).

Identification of epistatic QTLs affecting transpiration, biomass and its components

Transpiration and biomass being polygenic traits, possible interactions between loci across different LGs were examined. The present study identified allelic interactions that contributed to either a positive or a negative effect on the phenotypic value of the trait when compared to the effects of single loci evaluated in isolation. The QTL locus (single locus) detected using the Genotype Matrix Mapping (GMM) analysis was essentially the same in many cases as that identified by the CIM analysis of PLABQTL, even though the two approaches use different algorithms. Symbols A and B are used in the text below, where A indicates that homozygosity of

the allele of the drought-sensitive parent ICMB 841 was associated with positive additive effects for the trait and B indicates that homozygosity of the allele of the drought-tolerant parent 863B was associated with positive additive effects for the trait, and the number represents the chromosome position of the QTL. The mention of a "positive" (increase in trait value) or a "negative" (decrease in trait value) effect on the trait by the presence of the homozygous allele had no meaning in terms of "beneficial" or "detrimental" effect on the drought response. A minimum number of ten recombinant inbred lines involved in these allelic interactions were considered to estimate the epistatic effects. All observed significant allelic interactions were included and the interactions described in the text below are highlighted in bold letters (Table 3) (Fig. 4).

Leaf area (13.4% increase) and leaf dry weight (12.4% increase) were commonly influenced by the LG 6, 51.3(B) allele. One of the significant interactions detected for the trait leaf area, namely that between LG 6, 52.3(B) and LG 1, 42.7(B), increased leaf area (14.6% increase), and both of the favorably interacting alleles were contributed by drought-tolerant parent 863B (Table 3). In case of leaf dry weight, only one allele from 863B (LG 6, 51.3(B) QTL) increased this trait and no significant two- and three-loci combinations were represented by enough individuals to provide confidence that the interactions were real. Specific leaf weight was decreased by the presence of ICMB 841 alleles at a LG 3 locus, 115.4 (A, 6.3% decrease). Two loci combinations were significant for this trait, with the combination LG 3, 115.4(A) + LG 2, 73.3(A) showed a decrease in SLW (7.7%) when alleles from ICMB 841 were homozygous at both loci, whereas the interaction of ICMB 841 alleles at LG 7, 42.9(A) with 863B alleles at LG 3, 115.4(B) led to a 9.2% increase in SLW. Two sets of three locus combinations, all involving homozygous ICMB 841 alleles,

led to an increase in SLW of 10.7 to 11.3%. The combination of LG 6, 26.8(B) + LG 1, 54.4(A) alleles increased tiller number (20.4%). Total biomass was increased by the combination of the three loci LG7, 93.7(A)+LG 4, 0.0(A)+LG 2, 0.0(B) (17.6% increase) and a two locus combination involving 863B alleles on LG 7, 40.7(B) + ICMB 841 alleles on LG 6, 40.7(A) decreased biomass (21.1%) (Fig. 4).

Transpiration rate under low VPD was increased by 863B alleles (LG 3, 67.2(B), 10.9%) and two similar combinations of alleles, i.e. LG 3, 68.5(B) +LG 1, 81.3(A) and LG 3, 75.4(B) +LG 1, 81.3(A) also showed large increasing effects (22.7% and 21.1%), but were not present in enough individuals for these interaction effects to be considered reliable. Transpiration rate under high VPD was decreased by 863B alleles of the LG 6 QTL (e.g., LG 6, 42.5(B), LG 6, 51.3(B), LG 6, 30.1(B), with 11.7%, 11.2% and 11.5% decrease, respectively). There was one two locus combination of alleles i.e., LG 6, 42.5(A)+LG 4, 193.0(A), which provided an increasing effect (12.1%), while all the other three-loci interactions increased the transpiration rate under low VPD between 14.9% and 16.3%, for combinations in which alleles for all three loci were contributed by ICMB 841 (A) (Table 3).

Transpiration under low VPD was increased by the alleles of single loci (LG 6, 51.3(B) and LG 6, 52.3(B), 9.7 % and 9.4%, respectively) and by several two- and three-loci combinations that were represented by too few individuals for estimations of their effects to be considered reliable, i.e. one combination of two loci, LG 7, 35.3(B)+LG 5, 44.8(B) (16.7% increase) and one combination of three loci, LG 7, 146.6(A)+LG 6, 49.7(B)+LG 5, 9.1(B) (19.9% increase) increased transpiration under low VPD. Similar to transpiration under low VPD, the transpiration under high VPD was also linked to the alleles of LG 6, 51.3(A, 4.7% decrease). Combinations of two and three loci alleles, LG 7, 35.3(B)+LG 6, 40.7 (A), and LG 7, 39.4(B) +LG 6, 49.7(A) +LG 4, 101.5(B) decreased high VPD transpiration (15.6% and 18.6% decreases) . However, here

too the number of lines involved in these interactions were limited to less than ten, so estimates of their effects cannot be considered reliable.

The LG 6 QTL region was the most common locus influencing leaf area, leaf dry weight, shoot weight and transpiration, which also indicates the influence of leaf area component on transpiration. It was also involved in many of the allelic interactions either increasing or decreasing these traits.

Discussion

QTLs associated with biomass and its component traits

Grain yield of pearl millet under terminal drought is in part related to the yield potential under no water limitation (Bidinger *et al*. 1987) and therefore any attempt to breed drought adapted pearl millet material should consider having yield potential as part of the breeding target. In this study, most of the traits related to biomass (shoot weight, leaf area, leaf dry weight) were strongly linked to QTLs on LG 6 and LG 5, in both cases with the positive effects contributed by drought-tolerant parent 863B. The LG 5 QTL co-mapped with grain yield and HI QTLs across stress environments (Yadav *et al*. 2004), suggesting the involvement of this QTL in determining yield (suppl. Fig. 2d) and also supporting the concept that yield under stress is related in part to its yield potential (irrigated treatment) in pearl millet (Bidinger *et al* 1987). Yadav *et al*. (2004) have also reported that selection of this genomic region would be beneficial in all environments as it was associated with the genotype main effects for gain yield. Similarly, co-location of dry matter accumulation QTLs with the same or adjacent QTL regions of grain yield and its component traits was also previously reported in wheat (Liang *et al*. 2009). High root dry weight was also associated with one allele from 863B parent (LG 2, 19.5% PVE across years) and one from ICMB 841 parent allele (LG 7, 14.5% PVE across the years). This LG 2 QTL was mapped to the lower end of a large terminal drought

tolerant QTL region (spanning around 30 cM) reported earlier (Yadav *et al*. 2002, 2004; Bidinger *et al*. 2007). This LG 2 locus was narrowed down to a small genetic interval of 2.5 cM which gives us a precise marker link to target root dry weight and possibly help in marker-assisted development of large root ideotypes. Various biomass components (shoot dry weight, leaf and stem dry weight) also mapped to the lower end of this terminal drought tolerance region (Fig. 1) and were narrowed down to smaller genetic intervals than those previously identified, thereby generating precise marker trait association, although these genomic regions were specifically associated with one environment (2010). Therefore, it appears quite clearly that drought tolerant 863B contributed to a number of alleles, notably on LG 5, LG 6 and LG 2 (in this case co-mapping with a terminal drought tolerance QTL), which contributed to plant overall vigor and possibly leading to higher yield potential, and which would give advantage to genotypes containing these alleles at least in environment facing no or mild stress.

QTLs associated with transpiration rate

Environments where pearl millet is commonly grown are characterized by high evaporative demand. Water use efficiency is inversely related to evaporative demand so that maintaining photosynthetic activity under high evaporative demand has a high water cost. Therefore, alleles contributing to a reduced transpiration rate under high VPD are intuitively favorable for pearl millet, especially so for water limited conditions that require vegetative stage water savings to sustain reproduction and grain filling (Vadez *et al*. 2013). Alleles associated with high VPD transpiration rate for all four high VPD transpiration rate QTLs were all contributed by drought-sensitive parent ICMB 841 (Table 2). Involvement of ICMB 841 alleles in increasing high VPD transpiration rates was also clearly evident from multiple loci interactions (Table 3). High VPD transpiration rate was strongly linked to the LG 6 QTL (35.7% PVE across years, with alleles from ICMB 841 conferring increased high VPD transpiration rate), although ICMB 841 had lower absolute

transpiration than drought-tolerant parent 863B. The higher absolute transpiration of 863B was driven by leaf area QTLs at this locus (LG 6, Tables 1 & 2) but the transpiration restriction (lower transpiration rate) of this locus at high VPD by 863B parental alleles provided a mechanism to save water under high VPD conditions as discussed previously (Kholova *et al*. 2010a), i.e. at times when fixing carbon comes at a high water cost. These alleles controlling water saving mechanisms would be quite interesting in environments where the rainfall is limited and plant growth is purely dependent on available soil moisture and growth takes place under high VPD conditions, as discussed by Kholova *et al*. (2012). Being also alleles for plant vigor and possibly yield potential, as well as alleles for water saving under high VPD when fixing carbon is water-costly, these alleles offer a win-win situation both for water limited and unlimited environments.

Ideotyping

In view of the earlier two sections, an ideal pearl millet ideotype would be one having a good yield potential, yet capable of restricting transpiration rate under high vapor pressure deficit, both to achieve a higher water use efficiency but also to save water for later crop stages. In other words, what is needed is a high overall transpiration (that would represent vigor) but a restricted rate under high VPD. Seven QTLs were detected for transpiration under high VPD conditions (Table 2). Of these, five QTLs (on LG 1 (two), LG 2, LG 5, and LG 6) had positive effects from 863B parent alleles. Only two QTLs (on LG 6 and LG 7) were detected for total transpiration under low VPD conditions. The large number of QTLs controlling transpiration under high VPD, and the large portions of the phenotypic variation explained by each (11 to 30%), indicated the complex genetic nature and its close interaction with high VPD conditions. As expected, several of these QTL were also proxying for plant vigor QTLs. The 863B parental alleles of the first LG 1 QTL co-mapped with primary stress tolerance QTLs (favorable alleles from 863B) for grain mass and panicle harvest index across the moisture environments, showing significant interaction with

environment (Q × E), as reported by Bidinger *et al*. (2007). Similarly, the high VPD transpiration QTL on LG 6 co-mapped to a panicle harvest index QTL under severe stress and across environments (Bidinger *et al*. 2007) providing additional evidence of common genetics of transpiration under high evaporative demand and tolerance to water stress (high panicle harvest index is a proxy for tolerance, as it is an indicator of better seed setting and better seed filling under terminal stress). Therefore, selection of these LG 6 alleles that were mapped consistently in both the environments (2010 & 2011) for transpiration and cluster of biomass components would be good targets for the improvement of genotypes. Further, these traits could be parameterized into crop simulation modeling to fine tune the degree of vigor and of transpiration restriction under high VPD that are needed for specific environments (Kholova et al., 2014). The high VPD transpiration and transpiration rates were traits with high heritability (0.62 and 0.75 respectively), making them traits of choice for breeding selection. In addition, not all QTL alleles contributing to high total transpiration under high VPD were contributed by 863B. For instance those for the third LG 1 locus for high VPD transpiration in Table 2 (30% PVE) were contributed by drought-sensitive parent ICMB 841. This opens the possibility of finding transgressive segregants for total transpiration under high VPD conditions.

A potentially exciting finding was the allelic interactions between the ICMB 841 alleles co-located for specific leaf weight and total biomass on LG 7, 35.3(A) and the 863B allele for transpiration on LG 6, 40.7(B), which resulted in decreased transpiration under high VPD (15%). It should first be noted that few markers associated with the LG 7 specific leaf weight QTL were identical in two mapping populations (H77/833-2 × PRLT2/89-33 and ICMB 841 × 863B, Kholova *et al*. 2012), in which positive effects were provided by alleles from drought-sensitive parents H77/833-2 and ICMB 841. Specific leaf weight is highly variable and depends on leaf expansion and dry mass accumulation, both of them being dependent on

the environmental conditions (Tardieu *et al*. 1999). In spite of the unclear relationships between specific leaf weight and transpiration in pearl millet, it was observed in rice that leaf thickness was inversely related to transpiration rate (Giuliani *et al*. 2013). Assuming leaf thickness is a consequence of restricted leaf expansion (Tardieu *et al*. 1999), itself being a consequence of hydraulic restriction limiting the expansion of leaves (Tardieu *et al*. 2014), we speculate that the alleles from ICMB 841 may contribute to some hydraulic limitations that could be causing the limitation of transpiration under high VPD as hypothesized earlier (Vadez *et al*. 2014).

Conclusion

In this work we found a number of loci, namely on LG2, 5, and 6 which contributed to higher plant vigor and overall transpiration with most of the alleles contributed by drought tolerant 863B. A number of these loci also contributed to reduce the transpiration rate under high VPD, which has two important virtues: (i) saving water for later crop stages; (ii) possibly increasing water use efficiency. Specific allele interactions also contributed to reduce the transpiration rate under high VPD. The LG 6 QTL appeared to be a locus for leaf growth (greater leaf mass and leaf area) and also for lower transpiration rate. As in a previous study (Kholova *et al*. 2012), allelic interactions with specific combinations of QTLs affecting a number of traits affect plant vigor and water use give us the opportunity to manipulate these loci to "tailor" recombinants having pre-set water use and therefore pre-set potential to fit specific drought conditions. Our favored target ideotype would be the one combining the 863B alleles on LG6 and on LG2, which would both increase vigor and reduce transpiration rate under high VPD, with the ICMB 841 alleles for specific leaf weight on LG7, which in association with the latter loci would further decrease the transpiration rate under high VPD.

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Conflicts of interest

The authors have declared no conflicts of interest.

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Table 1. Evaluation of traits among parents and RIL population.

Trait	ICMB 841	863B	min	max	Grand mean	Heritability	SE(±)	% of 863B against ICMB 841
Shoot dry weight (g)	5.11	6.79	4.91	8.83	6.81	0.79	0.638	24.74
Leaf area (cm^2)	833.1	1185.7	783.5	1374.5	1108.2	0.69	100.7	29.74
Leaf dry weight (g)	3.45	4.52	3.11	5.86	4.61	0.77	0.434	23.67
Specific leaf weight (g cm^2)	3.93	3.85	3.49	5.28	4.25	0.55	0.358	-2.08
Tiller number	3	3	2	4	3	0.50	0.246	0.00
Root dry weight (g)	1.273	1.966	1.19	3.66	2.12	0.53	0.368	35.25
Total biomass (g)	7.107	9.297	6.64	12.45	9.81	0.60	1.043	23.56
Low VPD transpiration rate ($g\ cm^{-2}\ h^{-1}$)	0.022	0.022	0.018	0.03	0.024	0.80	0.002	0.00
High VPD transpiration rate ($g\ cm^{-2}\ h^{-1}$)	0.051	0.044	0.037	0.062	0.047	0.75	0.004	-15.91
Low VPD transpiration (g h^{-1})	19.086	26.884	17.963	34.93	26.083	0.90	2.276	29.01
High VPD transpiration (g h^{-1})	43.819	51.293	40.008	59.098	51.828	0.62	4.03	14.57

Mean value of the evaluated quantitative traits across two years, in the RIL parents ICMB 841 and 863B, and the maximum, minimum and mean trait BLUPs observed among 101 lines of their RIL population.

Table 2. QTLs detected for the transpiration and biomass related traits under well watered conditions.

Trait	LG	Locus interval	2010			2011			Across the two years			
			LOD	R^2	Additive effect	LOD	R^2	Additive effect	LOD	R^2	Additive effect	Q x E
Leaf dry weight (g)	2	PgPb11641-PgPb9870	4.41	10.7	0.296				3.37	14.5	0.364	-
	7	PgPb9471-PgPb6243	3.99	7.15	-0.259	4.7	9.4	-0.27	4.72	16.5	-0.235	-
	6	Xpsmp2270-PgPb11761	5	24.7	0.445	5.52	10.1	0.251	8	26.9	0.452	-
Leaf area (cm^2)	2	PgPb9747-Xpsmp2232	3.73	7.6	53.804				3.71	5.2	27.052	<0.01
	5	PgPb7186-Xipes0217				3.15	18.8	76.442	3.09	14.6	51.448	<0.01
	6	Xpsmp2270-PgPb11761	7.95	16.2	54.737	3.79	20.7	73.436	8.23	27.7	68.928	<0.01
Specific leaf weight (g cm^{-2})	3	Xipes0213-PgPb7379	3.87	14.7	0.141							-
	3	PgPb11235-Xipes0142							4.76	14.4	0.143	-
	7	PgPb9471-PgPb6243	3.08	13.1	-0.111	4.47	11.7	-0.597	3.33	7.8	-0.123	-
Stem dry weight (g)	1	PgPb11162-PgPb12275				4.07	16.9	-0.182	3.18	7.5	-0.107	-
	2	PgPb11641-PgPb9870	3.24	11.6	0.136				2.68	6.7	0.089	-
	5	PgPb7186-Xipes0217				3.53	12.0	0.156	3.27	8.2	0.098	<0.01
Tiller number	1	PgPb8955-PgPb7517				5.26	15.7	-0.236				

	4	PgPb7039-PgPb12538				4.29	11.5	-0.188				
Shoot weight (g)	2	PgPb9747-Xpsmp2232	4.06	12.4	0.336	2.79	3.6	0.429	4.4	5.8	0.181	-
	5	PgPb7186-Xipes0217				4.92	6.8	0.273	3.95	11.6	0.279	-
	6	Xpsmp2270-PgPb11761	3.75	10.5	0.465				5.82	17.0	0.333	-
	5	Xipes0157-PgPb9241	2.88	9.1	-0.25				4.66	12.0	-0.238	-
	7	PgPb7664-PgPb7406	5.53	19.2	-0.271				4.9	12.7	-0.372	<0.01
Root dry weight (g)	2	PgPb9747-Xpsmp2232	4.6	8.1	0.229							-
	2	Xipes0163-Xipes0162				4	3.5	0.109	5.56	19.5	0.196	<0.01
	7	PgPb8626-Xpsmp2040				5	8.8	-0.159				<0.01
	7	PgPb9471-PgPb6243	5.66	11.4	-0.241				4.01	14.5	-0.182	<0.01
Total biomass dry weight (g)	2	Xipes0163-Xipes0162	2.83	7.3	0.403				4.66	11.4	0.339	<0.01
	7	PgPb9471-PgPb6243	6.06	11.6	-0.619							
	7	PgPb8626-Xpsmp2040				3.8	14.0	-0.484				-
	7	Xipes0015-Xipes0153							3.28	9.3	-0.278	-
Low VPD transpiration rate (g cm^{-2} h^{-1})	3	PgPb13135-PgPb10076							3.02	12.9	0.00124	<0.01
High VPD transpiration rate (g cm^{-2} h^{-1})	1	PgPb7814-Xipes0042							4.1	9.2	-0.0013	<0.01
	2	PgPb9747-Xpsmp2232	5.19	9.9	-0.00192				3.27	5.8	-0.00107	

	4	Xipes0076-PgPb6358	4.5	16.7	-0.00254				4.4	7.3	-0.0012	<0.01
	6	Xpsmp2270-PgPb11761	7.73	26.5	-0.00313				7.27	35.7	-0.00307	<0.01
	6	Xpsmp3038-PgPb10674				4.14	16.6	-0.00284				-
Low VPD transpiration (g h^{-1})	6	Xpsmp2275-Xpsmp2270	3.7	11.4	0.884	5.27	18.9	1.923	6.35	24.5	2.312	<0.01
	7	PgPb9471-Xipes0198	6.17	24.7	-1.559							-
High VPD transpiration (g h^{-1})	1	PgPb12664-PgPb6112				5.47	27.3	2.214	3.31	11.4	1.607	-
	1	PgPb9927-PgPb10531	4.99	20.4	2.165							-
	2	PgPb7736-PgPb12542	3.79	12.3	2.085							-
	5	PgPb9755-Xipes0175							4.3	17.2	1.222	-
	6	Xpsmp2270-PgPb11761	3.79	16.3	2.039	4.73	19.9	1.69	7	21.7	1.355	-
	1	PgPb11162-PgPb12275				6.24	22.5	-2.454	7.19	30.0	-2.227	-
	7	PgPb10822-PgPb11890	3.43	13.68	-2.266				3.72	14.8	-1.044	-

Quantitative trait loci (QTLs) detected for various traits, and their interactions with the environment (Q × E), detected via composite interval mapping analysis using PLABQTL with BLUPs from each year (2010 and 2011) and across the years for 101 RILs derived from cross ICMB 841 × 863B. Additive effect is positive when allele is contributed by 863B. The r-square represented the percentage of the phenotypic variation explained by the QTL.

Table 3. Summary of QTL interactions on the investigated traits at two and three loci.

Trait name	F	No. of lines	Locus(allele)			% change in trait value	QTL position (LG, cM)		
Shoot dry weight	21.83	49	PgPb8635(B)			10.65	6, 51.3		
	40.57	5	Xicmp3027(B) Xipes0214(A)			-32.603	5, 77.7	5, 44.8	
Leaf area	36.91	49	Pgpb8635(B)			13.41	6, 51.3		
	40.08	16	Xpsmp2270(B)	Xpsmp2273(B)		16.03	6, 52.3	1, 50.8	
	40.94	**24**	**Xpsmp2270(B)**	**PgPb6149(B)**		**14.65**	**6, 52.3**	**1, 42.7**	
	49.79	11	Xpsmp2270(B)	PgPb8532(B)	Xpsmp2273(B)	18.78	6, 52.3	5, 52.0	1, 50.8
Leaf dry weight	26.4	49	PgPb8635(B)			12.44	6, 51.3		
Specific leaf weight	15.02	61	PgPb11235(A)			-6.29	3, 115.4		
	22.87	**39**	**PgPb11235(A)**	**PgPb10959(A)**		**-7.71**	**3, 115.4**	**2, 73.3**	
	34.83	**19**	**Xipes0198(A)**	**PgPb11235(B)**		**9.15**	**7, 42.9**	**3, 115.4**	
	45.3	14	PgPb5850(A)	Xipes0175(A)	PgPb6246(A)	11.28	6, 50.4	5, 44.8	1, 10.8
	46.06	16	Xipes0175(A)	PgPb6246(A)	PgPb7517(A)	10.7	5, 44.8	4, 193.0	1, 10.8
Tiller number	8.39	48	PgPb12538(B)			-13.24	4, 154.8		

	8.05	45	PgPb11162(B)			-10.94	1, 15.1		
	21.96	**14**	**PgPb10674(B)**	**PgPb12612(A)**		**20.37**	**6, 26.8**	**1, 54.4**	
Root dry weight	16.75	64	PgPb6243(A)			15.05	7, 40.7		
	16.63	20	PgPb11641(B)			15.29	2, 55.4		
	17.35	40	Xipes0017(B)			12.14	1, 111.2		
Total biomass	17.42	21	PgPb6832(B)			9.79	2, 48		
	17.72	29	Xpsmp2270(B)			8	6, 52.3		
	19.02	49	PgPb8635(B)			9.24	6, 51.3		
	33.5	12	PgPb12567(B)	PgPb10299(A)		-20.87	7, 38.8	6, 40.7	
	34.15	11	PgPb9471(B)	PgPb10299(A)		-21.48	7, 39.4	6, 40.7	
	35.31	**12**	**PgPb6243(B)**	**PgPb10299(A)**		**-21.12**	**7, 40.7**	**6, 40.7**	
	48.36	12	Xipes0153(A)	PgPb10727(A)	PgPb6832(B)	16.72	7, 129.8	7, 78.2	2, 48.0
	49.66	**11**	**PgPb8626(A)**	**PgPb10483(A)**	**Xipes0163(B)**	**17.59**	**7, 93.7**	**4, 0.0**	**2, 0.0**
Low VPD transpiration rate	11.89	22	Xipes0166(B)			10.93	3, 67.2		
	30.48	**6**	**PgPb10076(B)**	**PgPb7814(A)**		**22.67**	**3, 68.5**	**1, 81.3**	
	31.53	**8**	**PgPb9688(B)**	**PgPb7814(A)**		**21.25**	**3, 75.4**	**1, 81.3**	
High VPD transpiration rate	24.7	37	Xipes0207(B)			-11.53	6, 30.1		
	25.61	49	PgPb8635(B)			-11.17	6, 51.3		

	26.66	32	Xicmp3058(B)			-11.73	6, 42.5		
	35.68	**21**	**Xicmp3058(A)**	**PgPb6246(A)**		**12.08**	**6, 42.5**	**4, 193**	
	48.42	11	PgPb8664(A)	PgPb6383(A)	PgPb6967(A)	14.87	6, 46.3	6, 1.4	4, 171.1
	48.6	11	PgPb10715(A)	PgPb6827(A)	PgPb11431(A)	15.71	6, 46.3	4, 52.1	1, 85.1
	49.62	12	PgPb8664(A)	PgPb6383(A)	PgPb11249(A)	15.1	6, 46.3	6, 1.4	4, 169.6
	50.98	11	PgPb8635(A)	PgPb6827(A)	PgPb11431(A)	16.26	6, 51.3	4, 52.1	1, 85.1
Low VPD transpiration	13.32	33	Xpsmp2213(B)			9.42	6, 52.3		
	14.55	49	PgPb8635(B)			9.68	6, 51.3		
	34.08	**6**	**Xctm08(B)**	**Xipes0214(B)**		**16.69**	**7, 35.3**	**5, 44.8**	
	43.83	**6**	**Xicmp3092(A)**	**PgPb10713(B)**	**Xpsmp2276(B)**	**19.89**	**7, 146.6**	**6, 49.7**	**5, 9.1**
High VPD transpiration	13.17	36	PgPb8635(A)			-4.67	6, 51.3		
	39.43	**7**	**Xpsmp2087(B)**	**PgPb10299(A)**		**-15.58**	**7, 35.3**	**6, 40.7**	
	58.47	**5**	**PgPb9471(B)**	**PgPb10264(A)**	**PgPb9351(B)**	**-18.57**	**7, 39.4**	**6, 49.7**	**4, 101.5**

Genotype matrix mapping (GMM) analysis of the traits using BLUPs across the years i.e. leaf area, low and high VPD transpiration, transpiration rate, total biomass, leaf dry weight, root dry weight, tiller number. Symbol A indicates that the allele from female parent ICMB 841 had positive additive effects and B indicates that the allele from male parent 863B had positive additive effects. F indicates the significance level of that particular QTL interaction combination, considered with either one, two, or three loci. Number of lines indicates the number of RILs (out of 101 total) with that particular QTL combination. The percentage change in trait values comes from the comparison of trait value with and without the allele combination from the GMM output, the sign being positive if the combination increases the trait value and negative otherwise. QTL position column indicates the linkage group (LG) and the position of the QTL on that particular LG. Bold type observed in the table were the interactions explained in the text.

Figure legends

Figure 1. QTLs detected via the PLABQTL CIM (composite interval mapping) approach were positioned on pearl millet linkage groups LG 2 (Fig. 1). Symbols following the traits indicate the significance of the QTL in each year (2010 or 2011) and across the years. Solid line indicates the positive allele was from drought-tolerant parent 863B and broken line indicates that the positive allele was from drought-sensitive parent ICMB 841. Very approximate positions of previously mapped QTLs are included.

Figure 2. QTLs detected via the PLABQTL CIM (composite interval mapping) approach were positioned on pearl millet linkage groups LG 6. Symbols following the traits indicate the significance of the QTL in each year (2010 or 2011) and across the years. Solid line indicates the positive allele was from drought-tolerant parent 863B and broken line indicates that the positive allele was from drought-sensitive parent ICMB 841. Very approximate positions of previously mapped QTLs are included.

Figure 3. QTLs detected via the PLABQTL CIM (composite interval mapping) approach were positioned on pearl millet linkage groups LG 7. Symbols following the traits indicate the significance of the QTL in each year (2010 or 2011) and across the years. Solid line indicates the positive allele was from drought-tolerant parent 863B and broken line indicates that the positive allele was from drought-sensitive parent ICMB 841. Very approximate positions of previously mapped QTLs are included.

Figure 4. QTL interactions from the GMM analysis for the traits, low and high VPD transpiration, transpiration rate, total biomass and root dry weight. Solid arrow indicates the positive allele was from drought-tolerant parent 863B and broken arrow indicates that the positive allele was from drought-sensitive parent ICMB 841.

Supplementary figure 1. Histograms showing the distribution of the 101 RILs derived from the cross ICMB 841 × 863B, for the following traits: low VPD and high VPD transpiration, transpiration rate, shoot weight, total biomass, leaf area, leaf dry weight, stem dry weight, specific leaf weight, root dry weight and tiller number.

Supplementary figure 2. QTLs detected via the PLABQTL CIM (composite interval mapping) approach were positioned on LG 1 (Suppl. Fig. 2a), LG 3 (Suppl. Fig. 2b), LG 4 (Suppl. Fig. 2c), LG 5 (Suppl. Fig. 2d). Symbols following the traits indicate significance of the QTL detected in each year (2010 or 2011) and across the years. Solid line indicates the positive allele is from drought-tolerant parent 863B and broken line indicates the positive allele is from drought-sensitive parent ICMB 841. Very approximate positions of the previously mapped QTLs are indicated.