Water saving traits co-map with a major terminal drought tolerance quantitative trait locus in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract Low transpiration rates in pearl millet under fully irrigated conditions decrease plant water use at vegetative stage and then increase the water availability during grain filling and finally the terminal drought tolerance. Hundred and thirteen recombinant inbred lines developed from a cross between H77/833-2 and PRLT2/89-33 (terminal drought-sensitive × tolerant genotype) were evaluated to map transpiration rate (Tr, a proxy for canopy conductance), organ weights, leaf area and thickness and to study their interactions. Transpiration rate was increased by two H77/833-2 and two PRLT2/89-33 alleles on linkage group (LG) 2, whose importance depended on the vapor pressure deficit. The two H77/833-2 and one PRLT2/89-33 alleles co-mapped to a previously identified major terminal drought tolerance quantitative trait locus (QTL), although in a much smaller genetic interval. The other Tr allele from H77/833-2 also enhanced biomass dry weight and co-located with a formerly identified stover and tillering QTL. Leaf characteristics were linked to two loci on LG7. Plant water use was increased and decreased by different loci combinations for Tr, tillering and leaf characteristics, whose respective importance depended on the environmental conditions. Therefore, different alleles influence plant water use and have close interactions with one another and with the environment, so that different ideotypes for plant water use exist or could be designed from specific allele combinations conferring particular physiological characteristics for specific adaptation to a range of terminal drought conditions.

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Introduction

Recent decades have seen an increased research interest in drought tolerance improvement of crops. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is considered a drought-tolerant crop per se, yet there exists considerable genotypic yield variation in drought stress environments (Bidinger and Hash 2004; Bidinger et al. 1987). Pearl millet is commonly grown on marginal lands of the semi-arid tropics where severe terminal droughts are the most yield-destructive factors (Mahalakshmi et al. 1987).

Breeders' efforts to identify drought-tolerant material and localize the genomic segments responsible for drought tolerance are generally based on yield performance in targeted environments, although genotype-by-environment (G × E) interaction effects on yield frequently hamper these selection efforts (e.g. Banziger and Cooper 2001; Tuberosa and Salvi 2007). G × E interactions likely reflect the relative importance of certain plant mechanisms and/or their interactions to specific environments, which can lead to specific adaptation to drought conditions. Further progress in drought tolerance breeding then depends on the identification of tolerance mechanisms and on the understanding of interactions of these mechanisms with each other and with the environment (Blum et al. 1988; Ludlow and Muchow 1990; Fussell et al. 1991). Several successful examples have been reviewed (Sinclair et al. 2004).

A major terminal drought tolerance quantitative trait locus (DT-QTL) was identified in two independent mapping populations on linkage group (LG) 2 explaining up to 32 % of pearl millet grain yield variability in severe terminal drought environments, i.e. those where crop growth depends almost entirely on moisture stored in the soil profile (Bidinger and Hash 2004; Yadav et al. 2002, 2004; Bidinger et al. 2007). A recent physiological dissection of mechanisms and traits underlying the DT-QTL has pointed to water-conserving mechanisms being associated with the DT-QTL (Kholová et al. 2010a, b). This current hypothesis—that these traits, mostly expressed under non-stressed conditions (vegetative developmental stage), allow water saving in the soil profile throughout the season and make it available for the grain filling period—is in agreement with the fact that the DT-QTL confers better grain filling and seed setting (Serraj et al. 2005; Vadez et al. 2012). However, trait

interaction with the environment is also critical (e.g. Tardieu et al. 2000; Reymond et al. 2003). Indeed, the lower transpiration rate of tolerant entries (Tr; g water transpired per cm^2 leaf area) (Kholová et al. 2010a) was also sensitive to the vapor pressure deficit (VPD) (Kholová et al. 2010b). Similar mechanisms have recently been characterized in sorghum (Gholipoor et al. 2010), groundnut (Devi et al. 2010) and chickpea (Zaman-Allah et al. 2011). Therefore, not only is a low transpiration rate important for water saving in pearl millet, but also how this trait responds to the environment. Mapping of these traits thus requires careful consideration of the environmental conditions in which they are assessed.

Although several pearl millet near-isogenic lines containing the DT-QTL (NIL-QTL) had lower Tr (Kholová et al. 2010a, b), not all NIL-QTL exhibited yield advantage under terminal drought conditions (Serraj et al. 2005), suggesting that recombination event(s) in that region might have "excluded" the beneficial fragments in some of the NIL-QTL. Studies in *A. thaliana* reported a genomic region responsible for drought avoidance co-mapping with a region contributing to a constitutively lowered Tr and simultaneously leading to an enhanced Transpiration efficiency (TE) (Masle et al. 2005; McKay et al. 2008). Following these examples, our current hypothesis is that only critical portions of the large DT-QTL, linked to specific mechanisms, matter for the terminal drought tolerance of pearl millet and these need to be accurately mapped to enhance the precision of marker-assisted introgression. In addition, Tr is only one component of plant water use, which likely interacts with other components of plant water use (tillering, leaf area, leaf thickness). Therefore we hypothesized that Tr would be the leading trait of the DT-QTL region, and that total plant water use could be finely regulated through interactions of genomic regions involved in plant water use, including Tr.

The overall objective of this study was to map QTL for Tr and their interactions with other traits related to plant water use. Specifically, this work intended to (1) assess whether putative Tr QTL co-map with the DT-QTL region, (2) identify other genomic regions related to plant water use (tillering, biomass components, leaf characteristics), (3) cross-compare the identified QTL of Tr- and growth-related traits with previous mapping studies in pearl millet, and (4) assess how individual or interactive loci determine total plant water use and

deduce linkages between traits, based on the genomic regions involved and their interaction with the environment, to design specific ideotypes.

Materials and methods

Plant material

The recombinant inbred line (RIL) population used in this study was based on the cross between terminal drought-tolerant inbred PRLT2/89-33 and terminal drought-sensitive inbred H77/833-2 (Hash et al. 1999). This parental inbred lines cross was advanced to the F6 generation RILs through the single seed descent method. DNA was isolated from F6 inbred lines for genotyping with simple sequence repeat (SSR) and Diversity Arrays Technology (DArT) markers (Supriya et al. 2011). Phenotypic evaluation was assessed on test-cross hybrids of these inbred parental lines, developed by crossing the inbred lines to the male sterile line tester 834A (Stegmeier et al. 1998). Use of testcross hybrids is desired mainly to restore heterotic vigor of inbred lines, because pearl millet is a highly cross-pollinated species and suffers severely from inbreeding depression (Jones et al. 1995).

Phenotyping and plant growing conditions

The RIL population of 113 testcross hybrids and two parental testcross genotypes were sown into 20-cm diameter plastic pots filled with 5 kg of Alfisol. Each pot was sown with several seeds in four separate hills, thinned to one plant per hill 1 week after sowing and to two homogenous plants per pot at 2 weeks after sowing. Adequate fertility was provided with 300 mg diammonium phosphate per kg soil at sowing and 200 mg urea per kg soil at 15 days after sowing (DAS). For logistical reasons, five experimental sets (replications) were sown sequentially on 24 November, 26 November, 28 November, 2 December and 4 December 2009. The maximum/minimum temperature and relative humidity percentage at day/night averaged 36/15 °C and 35/94 %, respectively, during the growth period but fluctuated (Supplementary Table 1 and Figure 1). Each experimental set contained one pot of each RIL testcross genotype and three pots of each parental testcross genotype; plants were maintained well-watered during the entire

duration of the experiment. Phenotyping was initiated on 18, 21, 23, 28 and 31 December for sets 1–5, so that plants had a similar age in all sets at the time of measurement (25–27 days) and all were at vegetative stage, i.e. the stage well prior to when the terminal drought would naturally occur and when water saving mechanisms were shown to operate (see Kholová et al. 2010a, b, c). Prior to experimentation, pots were watered to field capacity. The soil was covered with a plastic sheet and a 3-cm layer of plastic beads to limit soil evaporation. In each experimental set, six check pots containing no plants but treated similarly were kept to assess the extent of soil evaporation.

Pots were weighed three times at 7:00 a.m., 10:30 a.m. and 2:30 p.m. The weighing took typically ten minutes and the pots were weighed following the same sequence, so that the time interval between pot weighing was the same for all pots. These timings were chosen to assess plant transpiration (T) during a period with low evaporative demand in the morning hours (average VPD 1.57 kPa) and during a period with high evaporative demand in the early afternoon hours (average VPD 3.53 kPa), following previous results in similar experiments (Kholová et al. 2010b). After the third weighing, plants were re-watered to pot capacity, left to drain overnight and the same procedure was repeated the following day using the same set of plants (except for set 5, which was assessed only once). After the last weighing on the 2nd day of observation in a given set, the plants were harvested and leaf area (LA) was measured immediately (using LA meter, LI3000 model, Li-Cor, Lincoln, Nebraska, US) and later used for calculation of Tr (g water cm^{-2} leaf area h^{-1}). Other parameters were also measured: root dry weight (RDW, measured in sets 3–5), stem dry weight (StDW), leaf dry weight (LDW), shoot dry weight (ShDW = StDW + LDW), biomass dry weight (BDW = RDW + ShDW; measured in sets 3–5), specific leaf weight (SLW = LDW/LA). The hourly temperature and humidity were recorded in two spots of the experimental set to calculate the average VPD within the time interval of observation. Spacing between the pots was wide so that the leaf area index was below one, avoiding mutual leaf shading.

Genotyping and linkage map development

The linkage map consisting of 321 markers (258 DArT and 63 SSR) was used to identify the QTL. The details

of genotyping and map construction are available in Supriya et al. (2011). The F6 generation of that population is characterized by increased homozygosity accompanied by increase in segregation distortion, which is common in pearl millet but also in other crops (see Supriya et al. 2011). In short, about 35 % of the markers showed segregation distortion, with about 10/25 % in favor of H77/833-2/PRLT2/89-33, respectively. Most of the markers showing distortion in favor of PRLT2/89-33 were concentrated on LG1 and LG6. More details on the segregation distortion can be found in Supriya et al. (2011). While segregation distortion can cause an overestimation of the recombination frequency between markers, some argue that segregation distortion has little effect on marker order and map length (Hackett and Broadfoot 2003). This phenomenon was not overly important in two of the linkage groups (LG2 and LG7) in which many of the QTL reported here were identified. Therefore, while we are aware that segregation distortion could be an issue for QTL detection in many crops, we assumed that much of the conclusions that are drawn in this paper, in large part from information in LG2 and LG7, would remain valid, as previously argued (Semagn et al. 2006). The earlier Qi et al. (2004) consensus linkage map using SSR and restriction fragment length polymorphism (RFLP) information (liu et al. 1994) allowed us to cross-compare our results to previous mapping studies (Yadav et al. 2002, 2003, 2004, Nepolean et al. 2006 and Bidinger et al. 2007).

Statistical analysis

Since some traits such as the transpiration rate (Tr) depended on VPD at the time of the experiment (Kholová et al. 2010b), and others like the leaf area could be influenced by VPD conditions during plant growth (Kholová et al. 2010c), the analysis was performed both on individual experimental sets and on the best unbiased linear predicted values (BLUPs; calculated using SAS version 9.2) which were generated for every trait and used in mapping analysis. The variation within and between sets was assessed by the set average, standard deviation (SD), minimum and maximum trait values. Differences between parental genotypes across experimental sets were further evaluated using a block ANOVA design with blocks defining particular observations in time. Simple

correlations were analyzed between the BLUPs for each trait (CoHort software 6.204, Monterey, CA, USA).

The composite interval mapping approach (CIM) was used to detect QTL using PLABQTL, where QTL are initially identified by simple interval mapping (SIM) and then used as co-variants for CIM, with a F-to-enter value of 8. The additive model was engaged in detecting the QTL effect for any individual loci and the additive × additive model (A × A) was employed to detect interacting loci (Utz and Melchinger 1996). A threshold of 2.5 was used and 1000 bootstrap runs were performed using the same software.

The genotype matrix mapping (GMM software, version 2.1) approach was used to assess putative interactions between loci (Isobe et al. 2007). In our case the number of interacting loci was limited to two and three because of the modest RIL population size. Nevertheless, the GMM approach uses a different method of QTL identification (GMM uses the F-measures algorithm of QTL detection) from PLABQTL software (CIM), and the single QTL estimation cannot be rigorously compared between these two methods.

Principal component analysis (PCA) was used to visualize the relationships between the investigated traits in multidimensional space using R software (version 2.11.1). PCA output was further used to map the common genetic background (pleiotropy) of all three major components, where each major component clubbed together related traits (using PLABQTL). For this PCA analysis, only the BLUP means were used. The broad-sense heritability (h^2) was calculated as $h^2 = \sigma^2{}_G/(\sigma^2{}_G + \sigma^2{}_E)$ where is $\sigma^2 G$ the genetic variance and $\sigma^2 E$ is the error variance with GenSTAT (version 12).

Results

Individual QTL determining transpiration rate (Tr)

Tr at low VPD (morning hours) was about half the Tr at high VPD (noon hours) but, as expected, there was also variability between sets within a particular VPD regime (details in Supplementary Table 1). The Tr of drought-tolerant PRLT2/89-33 was among the lowest values of the Tr distribution (morning–noon Tr; 0.011–0.025 g cm^{-2} h^{-1}), and was 40–45 % lower at low–high VPD than sensitive H77/833-2 which

tended to the opposite end of the Tr distribution (morning–noon Tr; 0.016–0.035 g cm^{-2} h^{-1}), with several transgressive segregants on both sides of the distribution (Supplementary Table 2). There was a highly significant genotypic effect for Tr under both low and high VPD.

Three QTL for the transpiration rate mapped in the major DT-QTL interval on LG2. (1) One QTL under low VPD conditions only, around 260 cM (between 258 and 264 cM) and hereafter referred to as LG2, 260 for simplicity, where '260' is used as a short name for the full confidence interval. As expected, the terminal drought-sensitive H77/833-2 provided the allele for the LG2, 260 QTL for Tr under low VPD, which was responsible for up to 26 % of the Tr variation. (2) One QTL explaining up to 16 % of Tr variation under low VPD, located at LG2, 322, with a positive effect from H77/833-2 (Supplementary Table 3, Fig. 1b). This QTL controlled Tr under both low and high VPD conditions, although the QTL accounted only for a maximum of 13 % of the phenotypic variations explained (PVE) in high VPD conditions. Interestingly, the VPD condition in which this QTL was identified under low VPD was relatively higher (2.19 kPa) compared to the average morning VPD of the other experimental sets. (3) One QTL explaining up to 14 % of Tr variation under low VPD only, located on LG2, 315, with a positive effect from PRLT2/89-33 (Supplementary Table 3, Fig. 1b). A fourth QTL for Tr, explaining up to 25 % of the Tr variation, was found on LG2, 10, away from the major DT-QTL region, under low VPD conditions only, with a positive allele contributed by the PRLT2/89-33 parent. Interestingly, the very same genome position was also found controlling biomass accumulation and its components (LG2, 10; see below). Additionally, a few minor loci affecting Tr were located on LG3 and LG7 (Supplementary Table 3).

In sum, the transpiration rate Tr was strongly linked to several major QTL regions across the range of VPD conditions, in particular three different regions co-mapping with the DT-QTL, two of them with a positive allele from the drought-sensitive parent and one of these specific to high VPD conditions, but also another region not reported before with positive loci being contributed by the PRLT2/89-33 allele, suggesting the complexity of the transpiration rate trait, but also its rather simple genetic determination (Supplementary Table 3, Fig. 1).

Individual QTL determining biomass and its components

Variation in biomass and components was found across both the RIL population and experimental sets (Supplementary Table 2). PRLT2/89-33 developed about 60 % larger LA and thinner leaves at this early developmental stage than H77/833-2 (Supplementary Table 2), in agreement with the earlier report (Kholová et al. 2010c).

Biomass accumulation was influenced by multiple genomic regions across LG1, LG2, LG4, LG6 and LG7 (Supplementary Table 4, Fig. 1, Supplementary Fig. 2). A major QTL explaining up to 22 % variability in the BDW and its components (LDW, SDW, RDW, StDW and ShDW) was located on LG2, 10, with a positive effect from drought-sensitive H77/833-2. Another QTL explaining more than 10 % of the variation was found on LG7, 110, with positive allele from terminal drought-tolerant PRLT2/89-33. Several smaller QTL were found on LG1, explaining usually less than 10 % of the variability. A few other alleles were also identified on LG1, 54, LG4, 100 and LG6, 20–50.

Interestingly, the positive PRLT2/89-33 allele for BDW on LG7, 110 also explained 29 % of the LA variations and 21 % of the LDW variations. Another locus on LG7, 75 explained up to 15 % of the variation in the SLW with positive allele from H77/833-2, and up to 12 % LA variation with positive allele from PRLT2/89-33. This locus, distinct from LG7, 110, thus appeared to play a specific role in balancing leaf area and leaf thickening (high SLW indicating thick leaves). The positive PRLT2/89-33 allele on LG 2, 10 discussed above also explained up to 18 % of the SLW variation (Supplementary Table 4; Fig. 1). None of the QTL interactions detected for biomass traits using the A × A model in PLABQTL software was further considered because these interactions could not explain the higher proportion of variation compared to the single detected QTL.

Individual QTL determining transpiration

The RIL population segregated widely for transpiration-related traits. Despite its 60 % higher leaf area, PRLT2/89-33 had only 25–20 % higher transpiration than H77/833-2 at this developmental stage under low
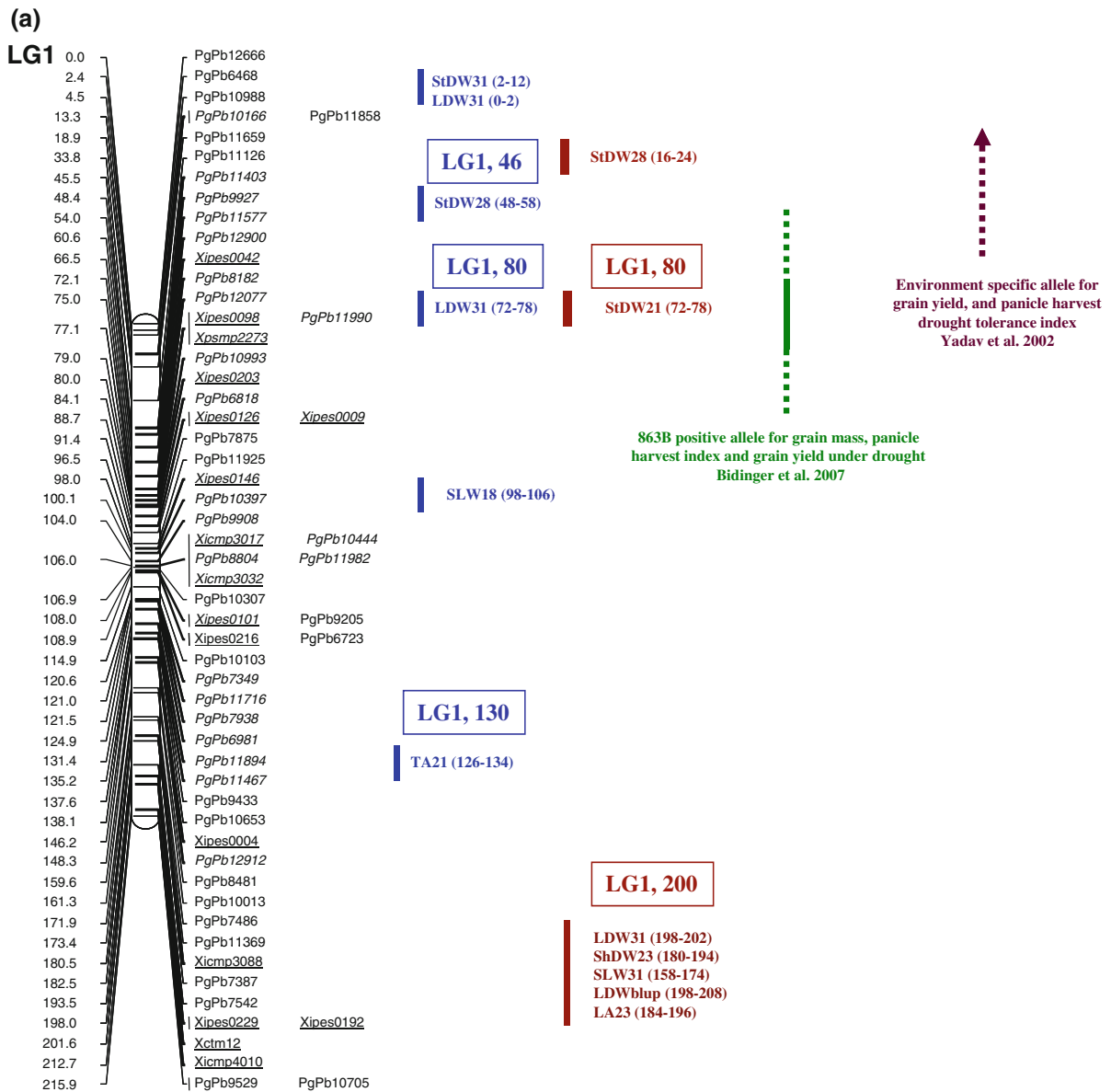
(a)

Fig. 1 Visualization of approximate QTL positions within the linkage groups of major importance (LG; chromosomes 1, 2, and 7, **a–c**) consisting of SSR and DArT markers and their positions in cM. QTL were detected for all investigated traits, viz. leaf dry weight (LDW), root dry weight (RDW), shoot dry weight (ShDW), stem dry weight (StDW), biomass dry weight (BDW), leaf area (LA) and specific leaf weight (SLW) and principal components (PCA 1, 2, 3). Transpiration rate (Tr) and absolute transpiration (T) are presented with suffix M standing for measurements under low VPD (morning hours) and with high VPD (Supplementary Table 2), which was related to a 40–45 % lower Tr at low–high VPD in PRLT2/89-33 than in H77/833-2.

suffix A representing measurements under high VPD (noon hours). QTL are shown for particular replications (suffix of trait name indicates day of measurement in December 2009) and for replication-based BLUP means (indicated as *blup* suffix of the trait). The content of the *brackets* behind the trait name stands for the 95 % confidence interval of the particular QTL. *Red or blue type* indicates that the positive effect comes from PRLT (drought-tolerant) or H77 (drought-sensitive) allele, respectively. Approximate positions of previously detected QTL are also visualized on the sides of the chromosomes. (Color figure online)

A QTL explaining 9 and 5 % of the transpiration variation under high and low VPD was found on LG7, 110, with positive allele from PRLT2/89-33. Under low
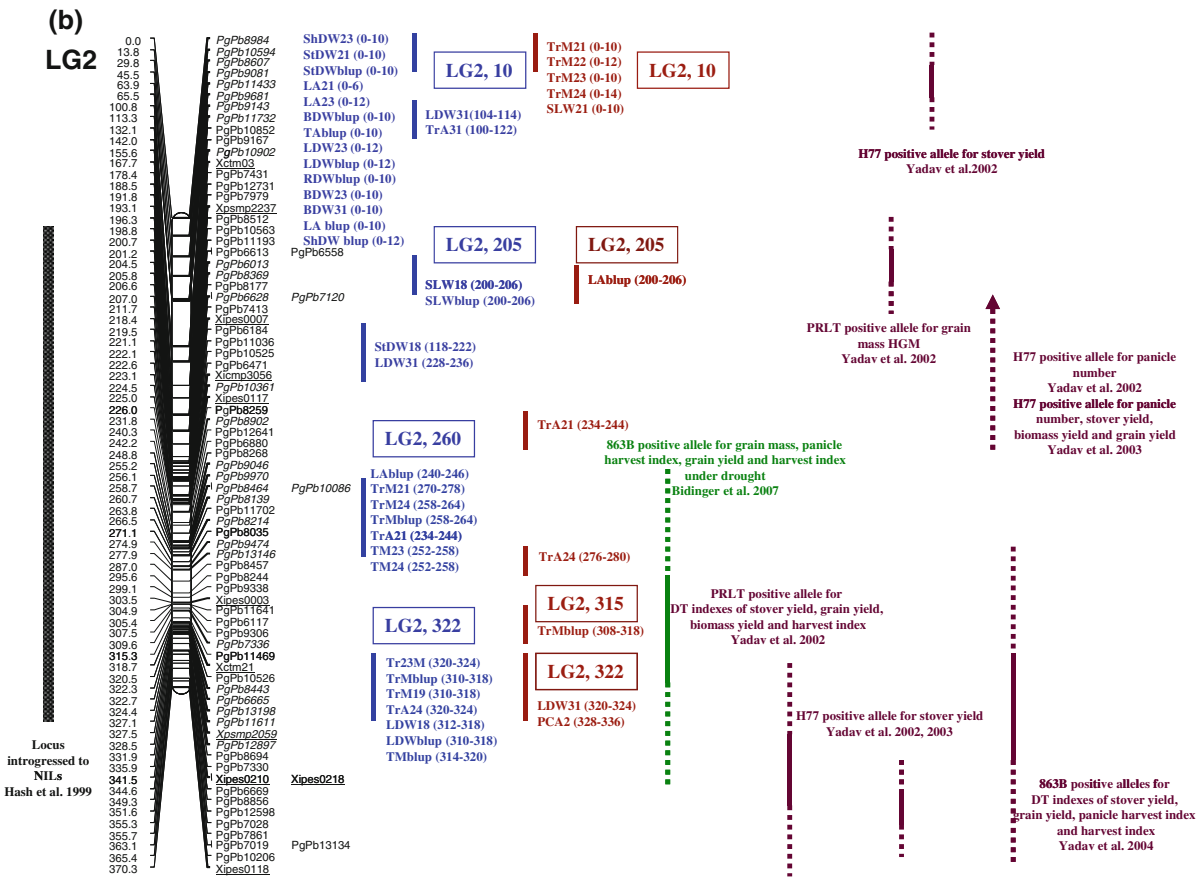
(b)

LG2

Position	Marker
0.0	PgPb8984
13.8	PgPb10594
29.8	PgPb8607
45.9	PgPb9081
63.9	PgPb11433
65.5	PgPb9681
100.8	PgPb9143
113.3	PgPb11732
132.1	PgPb10852
142.0	PgPb9167
155.6	PgPb10902
167.7	Xctm03
178.4	PgPb7431
188.5	PgPb12731
191.8	PgPb7979
193.1	Xpsmp2237
196.3	PgPb8512
198.8	PgPb10563
200.7	PgPb11193
201.2	PgPb6613
204.5	PgPb6013
205.8	PgPb8369
206.6	PgPb8177
207.0	PgPb6628
211.7	PgPb7413
218.4	Xipes0007
219.5	PgPb6184
221.1	PgPb11036
222.1	PgPb10525
222.6	PgPb6471
223.1	Xicmp3056
224.5	PgPb10361
225.0	Xipes0117
226.0	PgPb8259
231.8	PgPb8902
240.3	PgPb12641
242.2	PgPb6880
248.8	PgPb8268
255.2	PgPb9046
256.1	PgPb6970
258.7	PgPb8464
260.7	PgPb8139
263.8	PgPb11702
266.5	PgPb8214
271.1	PgPb8035
274.9	PgPb9474
277.9	PgPb13146
287.0	PgPb8457
295.6	PgPb8244
299.1	PgPb9338
303.5	Xipes0003
304.9	PgPb11641
305.4	PgPb6117
307.5	PgPb9306
309.6	PgPb7336
315.3	PgPb11469
318.7	Xctm21
320.5	PgPb10526
322.3	PgPb8443
322.7	PgPb6665
324.4	PgPb13198
327.1	PgPb11611
327.5	Xpsmp2059
328.5	PgPb12897
331.9	PgPb8694
335.9	PgPb7330
341.5	Xipes0210
344.6	PgPb6669
349.3	PgPb8856
351.6	PgPb12598
355.3	PgPb7028
355.7	PgPb7861
363.1	PgPb7019
365.4	PgPb10206
370.3	Xipes0118

PgPb6558
PgPb7120
PgPb10086
Xipes0218
PgPb13134

Locus introgressed to NILs Hash et al. 1999

ShDW23 (0-10)
StDW21 (0-10)
StDWblup (0-10)
LA21 (0-6)
LA23 (0-12)
BDWblup (0-10)
TAblup (0-10)
LDW23 (0-12)
LDWblup (0-12)
RDWblup (0-10)
BDW23 (0-10)
BDW31 (0-10)
LA blup (0-10)
ShDW blup (0-12)

LG2, 10

TrM21 (0-10)
TrM22 (0-12)
TrM23 (0-10)
TrM24 (0-14)
SLW21 (0-10)

LG2, 10

LDW31(104-114)
TrA31 (100-122)

H77 positive allele for stover yield Yadav et al. 2002

SLW18 (200-206)
SLWblup (200-206)

LG2, 205

LABlup (200-206)

LG2, 205

PRLT positive allele for grain mass HGM Yadav et al. 2002

StDW18 (118-222)
LDW31 (228-236)

TrA21 (234-244)

H77 positive allele for panicle number Yadav et al. 2002
H77 positive allele for panicle number, stover yield, biomass yield and grain yield Yadav et al. 2003

LG2, 260

LABlup (240-246)
TrM21 (270-278)
TrM24 (258-264)
TrMblup (258-264)
TrA21 (234-244)
TM23 (252-258)
TM24 (252-258)

863B positive allele for grain mass, panicle harvest index, grain yield and harvest index under drought Bidinger et al. 2007

TrA24 (276-280)

PRLT positive allele for DT indexes of stover yield, grain yield, biomass yield and harvest index Yadav et al. 2002

LG2, 315

TrMblup (308-318)

LG2, 322

Tr23M (320-324)
TrMblup (310-318)
TrM19 (310-318)
TrA24 (320-324)
LDW18 (312-318)
LDWblup (310-318)
TMblup (314-320)

LG2, 322

LDW31 (320-324)
PCA2 (328-336)

H77 positive allele for stover yield Yadav et al. 2002, 2003

863B positive alleles for DT indexes of stover yield, grain yield, panicle harvest index and harvest index Yadav et al. 2004

Fig. 1 continued

VPD conditions there was a significant positive effect of loci LG2, 260 (up to 10 %) and LG2, 322 (up to 8 %) on transpiration, both from sensitive parent H77/833-2, co-located in loci controlling Tr. Under high VPD conditions, only the locus LG2, 10 explained up to 18 % of the variation. Again, any detected A × A QTL interactions could not explain the higher proportion of variation than the single detected QTL. In sum, the total amounts of water transpired (T) in low and high VPD conditions were controlled by loci that were also linked to Tr and biomass components, and to a QTL on LG3, 25, explaining 13 % of the variation, with positive allele from H77/833-2 (Supplementary Fig. 2; Supplementary Table 3 and 4).

Analysis of trait relationships and mapping of PCA

A simple correlation analysis (Supplementary Table 5) and a principal component analysis (PCA; Fig. 2 and Supplementary Table 6) were performed to decipher the relationships between parameters. As expected, simple correlation analysis showed that the majority of investigated traits were interrelated. The purpose of the PCA was to group individual parameters in a more comprehensive manner. Three principal components (PC) explained 86 % of the variability. PC1 (60 %) had strong positive loading from all shoot biomass traits and transpiration (T), and relatively strong negative loading from Tr and SLW, which agrees well with the strong negative correlations between these traits (Supplementary Table 5). PC2 (20 %) had strong negative loading from Tr, SLW, and to some extent T under low VPD. Finally, PC3 (6 %) had a strong negative loading for root dry weight (RDW) and a strong positive loading from transpiration under low VPD (TM) (Supplementary Table 6). Interestingly, both analyses showed that Tr was significantly, though less strongly, related to traits corresponding to the biomass and/or absolute
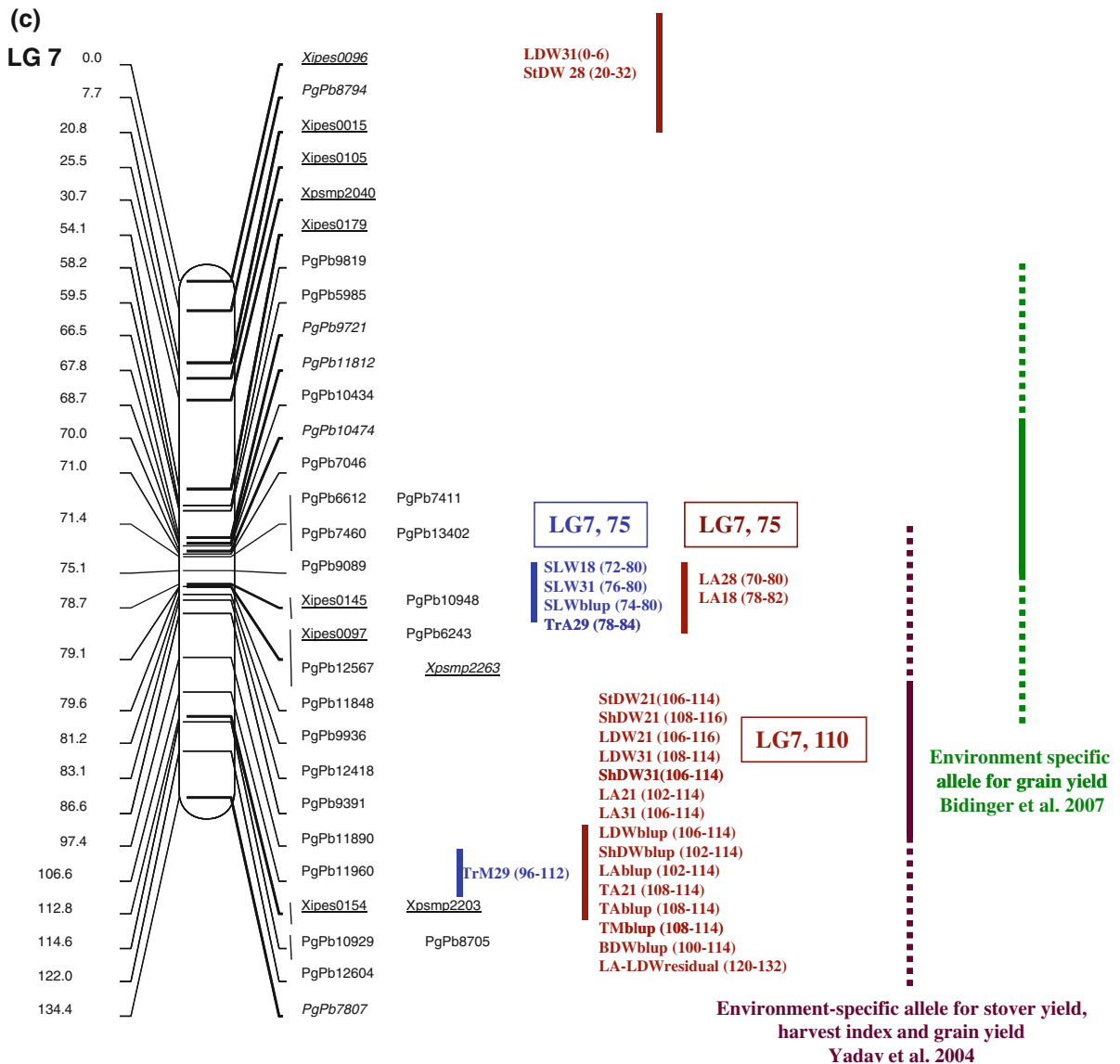
(c)

Fig. 1 continued

values of transpiration under high VPD while the Tr-TM relationship was non-significant. Simultaneously, all biomass traits and transpiration were positively related (Fig. 2, Supplementary Table 5).

To assess the pleiotropy of putative QTL regions, the coordinate values of RIL lines on each of the three main principal vectors and of each PC cluster were used in a CIM analysis. A single QTL for PC2 on LG2, 322 (14 %) was found, with a positive allele from drought-tolerant parent PRLT2/89-33 (Supplementary Table 4). Considering the that strongest loadings of

this PC2 were Tr and SLW, we concluded that both these traits are probably cross-regulated from this genome portion.

Interaction of QTL affecting transpiration rate (Tr)

GMM analysis showed that the interaction of two and three loci could in many cases increase the PVE compared to the single-locus PVE (Table 1). Although the RIL population exhibited some level of segregation distortion, preferring male alleles
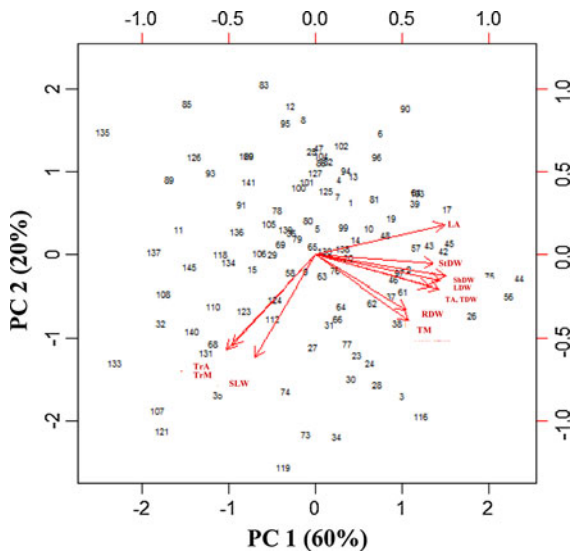
Fig. 2 Graphical output of principal component analysis (PCA). In the bi-plot, the values of traits vectors based on two major principal components (PC1—x axis; PC2—y axis) are presented as *red arrows*, i.e. transpiration rate (Tr), absolute transpiration (T), leaf dry weight (LDW), root dry weight (RDW), shoot dry weight (ShDW), stem dry weight (StDW), biomass dry weight (BDW), leaf area (LA) and specific leaf weight (SLW). Traits with suffix M were measured during 7:30–10:30 a.m. and traits with suffix A were measured during 10:30 a.m.–2:30 p.m. The numbers represent RIL numbers and their positions show the particular RIL trait loadings with regards to PC1 and PC2. (Color figure online)

(Supriya et al. 2011), a majority of the interaction estimates were supported by a reasonable number of RILs allowing reliable analysis (Table 1). The major loci found by GMM were identical to those found using the CIM approach, although GMM output could not be rigorously compared to CIM results from PLABQTL. In the following text, symbols A, B, H stand for alleles originated from the drought-sensitive parent (H77/833-2), the drought-tolerant parent (PRLT2/89-33) and their heterozygous alleles combination (AB), respectively. Only the strongest QTL or QTL interactions are discussed.

The major loci for Tr identified through CIM (namely LG2, 260 and LG2, 315) had large interactions with other loci and consequently explained more variation than the sum of their single locus effects (Table 1). Tr under low VPD was increased by the combination of alleles on LG2, 315 (B)–LG2, 261 (A) (positive effect 26 %) and/or from the LG7, 75 (A)–LG2, 216 (B)–LG1, 115 (A) (positive effect 29 %) (Fig. 3a). Similarly, Tr under high VPD was

increased by the interaction of LG7, 75 (A)–LG2, 315 (B)–LG2, 258 (A) (positive effect 23 %). Surprisingly, the allele on LG2, 322 (A), though identified as a single strong QTL enhancing Tr in the CIM analysis, did not interact with other loci of similar effect on Tr [note: LG2, 315 (B) and LG2, 322 (A) appear to be two distinct antagonistic QTL].

Interaction of QTL affecting biomass and biomass components

Total biomass (BDW) was positively influenced by the single effect of LG2, 0 (A, 20 %), a locus that was also identified in the CIM analysis. No allele combinations could explain a higher proportion of the variation than this single region on LG2, 0 (A) (Table 1).

Biomass increases were mostly explained by a higher leaf biomass (LDW), which was linked to a single locus on LG7, 113 (B, 11 %) and in particular to the allele combination of LG7, 107 (B)–LG5, 13 (B)–LG2, 0 (A) (17 %). Leaf area was also strongly influenced by this LG7 region (around 110, B, 15 %) but also by a different locus on LG7, 75 (A). The combination of alleles on LG7, 71 (A)–LG2, 205 (A) and on LG7, 107 (A)–LG2, 323 (A)–LG2, 260 (A) decreased LA (–19 %). By contrast, the combination of alleles on LG7, 71 (B)–LG7, 113 (B)–LG2, 0 (A), and on LG7, 71 (B)–LG2, 0 (A) increased LA, explaining 21 and 22 % of the variations. Finally, most of the SLW variation was also dependent on three-allele combinations on LG7, 75 (A)–LG1, 296 (A)–LG1, 115 (A) and LG7, 75 (A)–LG1, 296 (A)–LG1, 131 (A) explaining 17 and 19 % of SLW variation. The remaining biomass part, root dry weight, was strongly influenced by the combinations of alleles on LG7, 81(B)–LG4, 18 (B)–LG2, 346 (A), explaining 36 % of the RDW variation, where PVE estimates were based on 10 RILs carrying that marker combination (Table 1).

Interaction of QTL affecting transpiration

The percentage variation in absolute transpiration also increased with allele combinations, although many of these loci were not identified by the CIM algorithm (Supplementary Table 3 and Table 1). The most effective allele combination positively influencing transpiration in low VPD conditions (TM) was: LG5, 10 (B)–LG2, 319 (A)–LG1, 75 (B) (17 %) (Fig. 3b).

Table 1 Genotype matrix mapping (GMM) mapping analysis of BLUP means of investigated traits; i.e. leaf dry weight (LDW), root dry weight (RDW), shoot dry weight (ShDW), stem dry weight (StDW), biomass dry weight (BDW), leaf area (LA) and specific leaf weight (SLW). Transpiration rate (Tr) and absolute transpiration (T) are presented with suffix M which stands for measurements under low VPD (morning) and with suffix A representing measurements under high VPD (noon hours)

| Trait name	No. of lines	F	Number of QTL	Locus (allele)	PVE	Peak QTL position (LG, cM)	Probable CIM QTL corresponding interval (LG, interval)
TrM	17	10.46	1	P13113(A)	11.38	6, 32	6, 40
	42	12.55	1	P12608(A)	9.98	4, 97	4, 100
	69	15.63	1	P8443(A)	11.17	2, 322	2, 322
	63	16.68	1	P8464(A)	13.19	2, 258	2, 260
	67	14.24	1	P6013(A)	11.20	2, 204	2, 205
	19	12.20	1	P8984(B)	11.62	2, 0	2, 10
	40	14.08	1	P10103(A)	10.82	1, 115	X
	10	41.76	2	P11469(B) P8139(A)	24.60	2, 315-2, 261	2, 315-2, 260
	10	64.81	3	P9089(A) P9529(B) P10103(A)	29.27	7, 75-2, 216-1, 115	7, 75-X-X
TrA	35	7.86	1	P7046(A)	6.30	7, 71	7, 75
	43	7.62	1	P10653(A)	5.58	1, 138	1, 130
	40	8.07	1	P10103(A)	6.26	1, 115	X
	10	26.15	2	P11469(B) P8139(A)	15.50	2, 315-2, 261	2, 315-2, 260
	7	50.85	3	P9089(A) P11469(B) P8464(A)	22.85	7, 75-2, 315-2, 258	7, 75-2, 315-2, 260
TM	56	13.84	1	Xipes0154(B)	7.33	7, 113	7, 110
	58	13.07	1	P9391(B)	7.76	7, 87	7, 75
	16	24.56	2	P7046(A) P12228(A)	−12.44	7, 71-5, 17	7, 75-X
	18	32.38	3	P9391(B) Xctm21(A) P9306(A)	13.15	7, 87-2, 319-2, 308	7, 75-2, 322-2, 315
	7	33.25	3	Xipes0105(B) Xctm21(A) P10902(A)	15.47	2, 26-2, 319-2, 156	X-2, 322-X
	12	32.97	3	Xipes0015(B) P6931(B) Xctm21(A)	15.34	7, 21-6, 37-2, 319	X-6, 40-2, 322
	12	32.70	3	P11397(B) P7861(B) P12641(A)	15.04	6, 88-2, 356-2, 240	X-X-2, 260
	13	33.41	3	P11628(B) Xctm21(A) P12077(B)	15.31	5, 42-2, 319-1, 75	X-2, 322-1, 80
	9	32.68	3	P13090(B) Xctm21(A) P12077(B)	16.99	5, 10-2, 319-1, 75	X-2, 322-1, 80
TA	74	19.91	1	P8984(A)	13.49	2, 0	2, 10
	21	28.86	2	P12604(A) P11369(A)	−12.24	7, 122-1, 173	7, 110-1, 200
	29	26.17	2	P11960(B) P8182(B)	12.12	7, 107-1, 72	7, 110-1, 80
	12	36.11	3	P12604(A) P11502(A) P11369(A)	−16.63	7, 122-5, 23-7, 173	7, 110-X-1, 200
	21	35.13	3	P9391(B) P13090(B) P11990(B)	13.35	7, 87-5, 10-1, 77	7, 75-X-1, 80
	23	35.60	3	P9391(B) P8984(A) P10013(B)	14.48	7, 87-2, 0-1, 161	7, 75-2, 10-X
	22	33.99	3	Xipes0145(B) P8984(A) P11369(B)	14.51	7, 113-2, 0-7, 173	7, 110-2, 10-1, 200
	10	34.97	3	P12608(A) P7542(A) P11403(B)	−17.86	4, 98-1, 194-1, 46	4, 100-1, 200-1, 52
LA	47	29.29	1	P11960(B)	15.01	7, 107	7, 110
	20	49.44	2	P6612(A) P6013(A)	−19.45	7, 71-2, 205	7, 75-2, 205
	36	52.28	2	P7046(B) P8984(A)	21.77	7, 71-2, 0	7, 75-2, 10
	27	53.35	3	Xipes0154(B) P7046(B) P8984(A)	21.05	7, 113-7-71-2, 0	7, 110-7, 75-2, 10
	22	54.38	3	P11960(A) P6665(A) P11702(A)	−18.85	7, 107-2, 323-2, 264	7, 110-2, 322-2, 260
LDW	56	26.62	1	Xipes0154(B)	10.78	7, 113	7, 110
	41	43.81	2	Xipes0154(B) P8984(A)	14.14	7, 113-2, 0	7, 110-2, 10
	22	51.81	3	P11960(B) P12052(B) P8984(A)	17.16	7, 107-5, 13-2, 0	7, 110-X-2, 10
SLW	67	15.91	1	P6013(A)	7.13	2, 205	2, 205
	18	35.34	2	P9089(A) P9529(B)	11.51	7, 75-1, 216	7, 75-X

Table 1 continued

Trait name	No. of lines	F	Number of QTL	Locus (allele)	PVE	Peak QTL position (LG, cM)	Probable CIM QTL corresponding interval (LG, interval)
	19	35.77	2	P9089(A) Xicmp4010(B)	11.20	7, 75-1, 216	7, 75-1, 200
	9	55.23	3	P9089(A) P8244(A) P10103(A)	17.05	7, 75-1, 296-1, 115	7, 75-X-1, 100
	7	55.87	3	P9089(A) P11894(A) P10103(A)	19.05	7, 75-1, 296-1, 131	7, 75-X-X
RDW	74	14.25	1	P8984(A)	22.20	2, 0	2, 10
	22	19.59	2	Xpsmp2203(B) P10184(B)	22.36	7, 113-4, 0	7, 110-X
	3	19.93	2	P6932(B) Xipes0101(H)	53.65	5, 3-1, 108	X-1, 80
	11	20.21	2	P10768(A) P8984(B)	−24.26	4, 106-2, 0	4, 100-2, 10
	23	20.33	2	P7958(B) P7330(A)	24.02	4, 96-2, 336	4, 100-2, 322
	27	19.49	2	P7958(B) P8694(A)	22.52	4, 96-2, 20	4, 100-2, 10
	10	38.16	3	P9936(B) P11711(B) Xipes0218(A)	36.37	7, 81-4, 18-2, 346	7, 75-X-X
BDW	74	26.16	1	P8984(A)	20.25	2, 0	2, 10
	50	35.22	2	P9721(B) P8984(A)	17.67	7, 67-2, 0	7, 75-2, 10
	16	40.68	3	Xpsmp2203(B) P8694(A) Xctm12(B)	22.75	7, 113-2, 332-1, 202	7, 110-2, 322-1, 200
	36	43.07	3	P9721(B) P7494(B) P8984(A)	18.97	7, 67-5, 13-2, 0	7, 75-X-2, 10
	19	41.46	3	P8694(A) P10594(A) Xctm12(B)	20.54	2, 332-2, 14-1, 202	2, 322-2, 10-1, 200
LDW	56	26.62	1	Xipes0154(B)	10.78	7, 113	7, 110
	41	43.81	2	Xipes0154(B) P8984(A)	14.14	7, 113-2, 0	7, 110
	22	51.81	3	P11960(B) P12052(B) P8984(A)	17.16	7, 107-5, 13-2, 0	7, 110-X-2, 10
StDW	74	24.83	1	P8984(A)	20.95	2, 0	2, 10
	21	31.08	2	Xipes0154(A) P9529(B)	−17.61	7, 113-1, 216	7, 110-X
	20	31.72	2	P6478(A) P7387(A)	−17.51	4, 98-1, 183	4, 100-1, 200
	27	33.29	2	P10110(A) P8268(B)	−16.66	4, 95-2, 249	4, 100-2, 260
	15	31.08	2	P10110(A) P8984(B)	−20.74	4, 95-2, 0	4, 100-2, 10
	31	30.94	2	P6665(A) P8268(B)	−15.51	4, 95-2, 323	4, 100-2, 322
	21	55.58	3	P12608(A) P6665(A) P8268(B)	−21.85	4, 98-2, 323-2, 249	4, 100-2, 322-2, 260
ShDW	56	24.56	1	Xipes0154(B)	10.90	7, 113	7, 110
	74	24.68	1	P8984(A)	15.78	2, 0	2, 10
	41	45.25	2	Xipes0154(B) P8984(A)	15.15	7, 113-2, 0	7, 110-2, 10
	22	55.19	3	P11960(B) P12052(B) P8984(A)	18.30	7, 107-5, 13-2, 0	7, 110-X-2, 10

F represents the significance level for a particular QTL or QTL combination. The column 'No. of lines' shows the number of lines carrying this particular marker combination in the RIL population. The column 'Locus (allele)' presents the QTL peak marker along with the origin of alleles; A, B, H stand for alleles originated from H77 (drought-sensitive) and PRLT (drought-tolerant) and H means heterozygous locus (A and B present in genome). For any particular QTL or their combination, the percentage of variation explained (PVE) is shown (positive values signify that the QTL combination increases the trait value and vice versa). The table shows the exact QTL peak marker position with regards to linkage group (LG) and the interval identified by CIM to which this marker probably belongs. DArT markers with prefix PgPb in Fig. 1 are abbreviated with prefix P in this table

In contrast, transpiration at high VPD regime (TA) was strongly and positively influenced by a single effect QTL on LG2, 0 (A, 13 %), this loci being the same as that influencing biomass in the CIM analysis (Supplementary Table 3 and Table 1, Fig. 3c). Under high VPD also, the following combination of three alleles from loci on LG7, 122 (A)–LG5, 23 (A)–LG7, 173 (A) (17 %) and LG4, 98 (A)–LG1, 194 (A)–LG1, 46 (B) (18 %) had a negative effect on TA. In sum, a majority of strong allele combinations participating in transpiration regulation combined "biomass" QTL effects with the effects of loci playing a part in Tr regulation (Supplementary Tables 3 and 4, Fig. 1).

Fig. 3 QTL interactions from the GMM analysis for the transpiration rate in the morning under low VPD conditions (TrM) and in the afternoon under high VPD conditions (TrA) (**a**), for the transpiration in the morning (TM) (**b**), and for the transpiration in the afternoon (TA) (**c**)
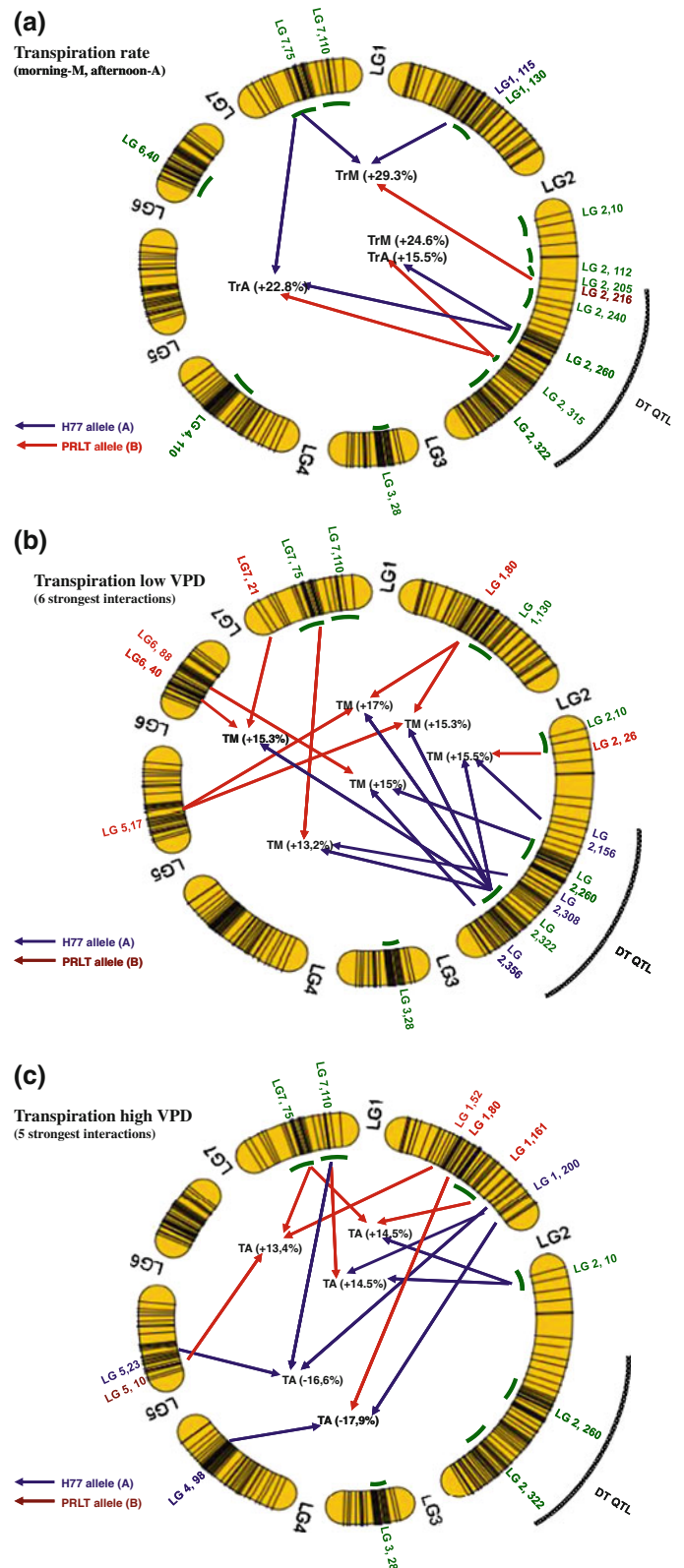
Heritability analysis

The broad-sense heritability coefficient h^2 of Tr was high (0.84 and 0.80 in low and high VPD, respectively). The heritability of other investigated traits was 0.70 (TM), 0.74 (TA), 0.46 (StDW), 0.53 (ShDW), 0.27 (RDW), 0.45 (BDW), 0.50 (LDW), 0.63 (LA) and 0.44 (SLW).

Discussion

Several major loci were related to transpiration rate (a proxy for leaf conductance), leaf characteristics (area, thickness) and biomass. Transpiration rate (Tr) was linked to four major loci on LG2, 10, LG2, 260, LG2, 315 and LG2, 322, three of them co-mapping with a previously identified major terminal drought-tolerance QTL interval on LG2. The relative importance of these regions for Tr determination was dependent on current vapor pressure deficit. The locations of these major QTL were consistent with previous studies on similar plant material. The majority of QTL identified by GMM were consistent with those detected by PLA-BQTL and showed that the combined action of some strong loci and/or loci of negligible individual effect (e.g. for biomass components) could explain a far higher portion of trait variation than single locus effects. The PCA clearly indicated that, depending on PC dimension, Tr and transpiration vectors had either same or opposite direction. The genetic analysis then also pointed out that transpiration depended mostly on loci interactions involving Tr-related loci and biomass-related loci. The heritability of the traits was high and the loci linked to them were usually very small, which opens the possibility to breed, for example by marker-assisted recurrent selection (MARS), lines having specific allele combinations leading to set levels of plant water use, towards adaptation to specific drought conditions.

Transpiration rate (Tr)

The major QTL for the traits investigated were obtained consistently, thought not always across experimental sets. Such a situation was expected because both the environmental conditions in which the plants developed and in which Tr was assessed varied, and these have been reported to greatly affect water use traits, in particular leaf area and Tr (Kholová et al. 2010c). In this report, it was shown, for instance, that leaf area development of PRLT2/89-33 was decreased under high VPD conditions. In the case of Tr, QTL explaining large percentage of the Tr variation were found in the region on LG2 previously identified as the major QTL determining drought tolerance (DT-QTL) in two populations (Yadav et al. 2002, 2004; Bidinger et al. 2007). In this region, two major positive alleles from the drought-sensitive parent H77/833-2 (LG2, 260 and LG2, 322) spanned a genetic interval of 6 and 8 cM, which is much smaller than the interval of the DT-QTL between markers *Xpsmp2237*, 193 cM and *Xpsmp2059*, 328 cM. This is in agreement with the lower Tr under fully-irrigated conditions of NILs introgressed with the DT-QTL (Kholová et al. 2010a, b). However, two other strong regions controlling Tr, both with positive alleles contributed by drought-tolerant PRLT2/89-33, were identified on LG2 (LG2, 10 and LG2, 315). The LG2, 10 locus with allele from H77/833-2 also enhanced biomass, probably through tillering, whereas the allele from PRLT2/89-33 at this locus might be related to leaf expansion and thickening processes. The LG2, 315 allele from PRLT2/89-33 was also an important region interacting with other loci influencing Tr.

Interestingly, all four QTL on LG2 affected Tr under low VPD, whereas only LG2, 322 from sensitive H77/833-2 had a strong effect on Tr under high VPD. In view of our previous results showing a close involvement of the DT-QTL in a reduction of Tr under high VPD in lines introgressed with the DT-QTL but cultivated under low VPD conditions in the glasshouse (Kholová et al. 2010b), we could have expected to find more QTL for Tr under high VPD. Here, the plants were cultivated outdoors, under higher VPD conditions than the glasshouse. Our recent work also shows that the Tr differences between parental lines are smaller when the plants developed under high VPD conditions (Kholová et al. 2010c), and this is related to a lower leaf area development of PRLT2/89-33. Such results indicate the existence of different, but interlinked, physiological mechanisms to regulate Tr, in which the environmental conditions play an important role (as in Kholová et al. 2010c). From our data we interpret that two loci (LG2, 322 and, to a lesser extent, LG2, 260) may increase Tr synergistically across a wide VPD range while others may modulate this "Tr

tuning" when the environment changes. For example, there may be an antagonistic influence of the PRLT2/89-33 alleles on LG2, 10 and LG2, 315 on Tr under low VPD that may neutralize the effects of LG2, 260 and LG2, 322. This observation is in complete agreement with Yadav et al. (2002), where the very same regions were described and suggested to counteract each other's effect on grain yield and panicle harvest drought tolerance indexes depending on the onset of drought and its severity. Therefore, from the physiological point of view, the balance of several interacting mechanisms determine Tr, depending on the prevailing environmental conditions: (1) mechanisms determined by LG2, 260, LG2, 322 (H77/833-2 allele increasing Tr); (2) mechanisms related to leaf area and perhaps leaf thickening influenced by the presence of the PRLT2/89-33 positive allele on LG2, 315, and their interaction with loci on LG7, 75 (allele influencing leaf thickening from H77/833-2) and LG2, 10 (PRLT2/89-33 allele influencing leaf mass, area, and thickening). This simple genetic determination of Tr, an important trait contributing to terminal drought tolerance of pearl millet, opens the possibility of recombining different sets of alleles towards the desired Tr level for fitness under specific drought situations.

Leaf characteristics

Leaf area and leaf thickness are other critical factors influencing a plant's transpiration and one of the objectives of the study was to compare their genetic regulation to that of Tr. Leaf weight and leaf area were influenced by a major region on LG7, 110 (positive allele from PRLT2/89-33) and a smaller region on LG2, 10 (positive allele from H77/833-2). The LA QTL on LG7 from PRLT2/89-33 agrees with our recent results of a higher leaf area in PRLT2/89-33 than in H77/833-2 under low VPD conditions (Kholová et al. 2010c). This QTL is also in line with a stover yield QTL found in the same genomic region on LG7 (Yadav et al. 2002; Bidinger et al. 2007). This stover yield QTL was found under low VPD only, which also agrees with an enhanced leaf area development under low VPD in drought-tolerant genotypes (PRLT2/89-33; Kholová et al. 2010c). In synthesis, we suggest that the action of the LG7, 110 regions may be specific to leaf expansion and is probably prevalent under low VPD conditions of growth.

In contrast, SLW was positively influenced by the PRLT2/89-33 allele LG2, 10, the H77/833-2 alleles on LG2, 204 and LG7, 75, and a few minor alleles on LG1. Therefore it appears that leaf thickening processes (proxied by SLW) might be mostly related to the H77/833-2 positive alleles on LG2, 204 and LG7, 75, which are also distinct from those affecting LA and LDW. This is in line with observations on *A. thaliana* showing independent genetic control of meristematic cell division and proliferation (Tsuge et al. 1996; Tsukaya 2005). The allele from PRLT2/89-33 at the LG2, 10 loci increased SLW, whereas the H77/833-2 allele at that locus increased stem weight/tillering (as discussed further). We hypothesized that this locus pre-determines the allocation of carbon assimilates towards leaves or stems. A similar principle was recently emphasized for sorghum where the "tillering" versus "leafy" phenotype was discussed as a result of internal plant competition for carbon sink (Kim et al. 2010a, b). The plants directing their carbon sources towards leaf mass were also hypothesized to be more capable of withstanding harsh drought conditions, whereas genotypes investing in tillering were hypothesized to succeed in environments where water is plentiful (Hammer et al. 1996; Kim et al. 2010a), which fits our case.

Interestingly, the H77/833-2 allele LG7, 75 interacted closely with loci LG2, 260 (H77/833-2)–LG2, 315 (PRLT2/89-33) and increased Tr under high VPD. Therefore it appears that a plant possessing thick leaves and both Tr-enhancing mechanisms on LG2, 260 and LG2, 315 loci would have higher Tr values. This fact fits with the PCA showing Tr and SLW being closely related and mapping results of PC2 where a common QTL was found for both these trait combinations (LG2, 320).

Biomass: total biomass, root, stem and shoot

The phenotypic expression of biomass and its components relied largely on the presence of one allele from H77/833-2 on the LG2, 10 locus and one allele from PRLT2/89-33 on LG7, 110 (Supplementary Table 4). The presence of LG2, 10 alleles from H77/833-2 also explained 12 % of root biomass variation and 19 % of stem weight variation. These results agree with previous reports (Bidinger and Hash 2004; Bidinger et al. 2007; Yadav et al. 2002, 2003, 2004). A region close to LG2, 10 was previously identified to

contribute to increased biomass and stover yield under optimal conditions (Yadav et al. 2002). This positive H77/833-2 allele was also responsible for an increased number of panicles, likely related to the higher tillering habit of this genotype (Yadav et al. 2002). This region on LG2 also increased tillering in other work (Poncet et al. 2002; Nepolean 2002). The GMM analysis of biomass and its components confirmed these results by showing a single locus on LG2, 0 increasing total biomass and stem dry weight, whereas no combination of effects explained a notably larger percentage of the variation for these traits.

Absolute transpiration

Water-saving mechanisms under non-stress conditions are hypothesized to keep water in the soil profile to sustain grain filling and are therefore critical for the terminal drought tolerance of crops (Vadez et al. 2012). Therefore, the ultimate purpose of this study was to dissect the genetic regulation of absolute transpiration. The GMM approach strengthened the idea (Isobe et al. 2007; Ravi et al. 2010) that synergistic action of several QTL can add up effects on complex quantitative traits, like absolute transpiration here. However, these previous studies limited themselves to listing the possible effects of multiple loci interaction and highlighting the need for consideration of these interactions when employing markers in breeding programs, but did not explain the important physiological implications of specific trait combinations for adapting to variable drought scenarios.

CIM showed that water used depended on several loci—in most cases similar to those determining Tr (Supplementary Table 3, Fig. 1), biomass accumulation and its partitioning (LG2, 10, LG7, 110), and also on independent QTL on LG3 (LG3, 28). However, none of these loci had large individual effect on water used, except LG2, 10. The outcome of the GMM analysis validated the hypothesis that a larger proportion of the variation in a plant's transpiration could be explained by loci interaction (Fig. 3b, c). Interestingly, under low VPD, all interactions involved loci related to Tr with contribution from the drought-sensitive H77/833-2 allele, and all interactions increased plant transpiration. In contrast, under high VPD conditions, none of the interaction involved Tr loci but rather leaf and tillering characteristics loci, with both the tolerant and sensitive alleles being

involved in positive and negative interaction effects on water used. The plant's transpiration could be tuned up by alleles enhancing biomass accumulation (LG2, 10 and possibly a few minor QTL on LG1, LG4, LG5, and LG6) and tuned up or down by mechanisms decreasing Tr, depending on allele presence (LG2, 260; LG2, 315), themselves closely interacting with LA- and SLW-related loci on LG7. For instance, ideotypes with thicker leaves, lower LA and high biomass (high tillering) had higher transpiration. This knowledge considerably deepens the understanding of G × E interactions and opens the possibility of designing plant ideotypes with desired levels of transpiration, thus suited for specific target environments. For example, in environments where water is intermittently available through the season, the focus would likely be on ideotypes capable of maximizing water uptake. In environments where incoming rainfall is limited and water supply restricted to soil moisture (terminal drought situation), the focus would be on ideotypes capable of using water slowly and leaving some for the grain-filling period (e.g. low transpiration). This latter ideotype would consist of genotypes having alleles responsible for low tillering phenotype, higher LA and thin leaves (lower SLW). A similar concept was previously presented for sorghum using simulation modeling (Hammer et al. 1996; Hammer 2006; Kim et al. 2010a, 2010b).

Conclusion

Four QTL for Tr were found, two from H77/833-2 (drought-sensitive genotype) and two PRLT2/89-33 (drought-tolerant genotype) alleles on linkage group (LG) 2, the importance of each region being dependent on the environmental conditions at the time of measurement. Three of these Tr QTL mapped to a region previously identified as the major terminal drought-tolerance QTL on LG2, explained the large variation of the Tr phenotypic variance, and spanned small genome portions. Two QTL were specific to low VPD conditions. Absolute transpiration was closely linked to interaction of Tr loci from sensitive H77/833-2, with plant biomass and leaf characteristics (from LG7) under low VPD, whereas transpiration under high VPD conditions resulted from interaction of loci for biomass and leaf characteristics. This study revealed the genetic basis of different traits

influencing plant water use under non-stressed conditions, their genetic basis, and their interactions with the environment (G × E), and opens the possibility of engineering successful ideotypes with set levels of water use from specific combinations of alleles for location-specific requirements.

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