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Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and tester background

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Summary

A pearl millet mapping population from a cross between ICMB841 and 863B was studied for DNA polymorphism to construct a genetic linkage map, and to map genomic regions associated with grain and stover yield, and aspects of drought tolerance. To identify genomic regions associated with these traits, mapping population testcrosses of 79 F_3 progenies were evaluated under post-flowering drought stress conditions over 2 years and in the background of two elite testers. A significant genotype × drought stress treatment interaction was evident in the expression of grain and stover yield in drought environments and in the background of testers over the 2 years. As a result of this, genomic regions associated with grain and stover yield and the aspects of drought tolerance were also affected: some regions were more affected by the changes in the environments (i.e. severity and duration of drought stress) while others were commonly identified across the drought stress environments and tester background used. In most instances, both harvest index and panicle harvest index co-mapped with grain yield suggesting that increased drought tolerance and yield of pearl millet that mapped to these regions was achieved by increased partitioning of dry matter from stover to the grains. Drought stress treatments, years and testers interactions on genomic regions associated with grain and stover yield of pearl millet are discussed, particularly, in reference to genetic improvement of drought tolerance of this crop using marker-assisted selection.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is primarily grown as a rainfed crop in the low rainfall zones of sub-Saharan Africa and the Indian subcontinent. Post-flowering drought stress is one of the most common and serious environmental constraints in these regions (van Oosterom et al., 1996), reducing mean yields and increasing the magnitude of the annual variation in harvests and the incidence of crop failure (Ceccarelli & Grando, 1996). As a result, improving the adaptation and/or tolerance of pearl millet to drought stress is an important objective in most pearl millet breeding programmes. Phenotypic selection for improved drought tolerance, or for yield under stress conditions, is widely accepted as difficult (Blum, 1988a). This is due to the tremendous variation in the timing and in the severity of drought stress in naturally occurring stress environments, and the high degree of interaction between the timing and intensity of the stress, and the crop growth stage and crop genotype. This results in a very high degree of environment and genotype × environment variation in drought prone environments, which severely limits progress in selection for better adaptation/tolerance to stress.

In the last decade, quantitative trait locus (QTL) mapping has become an important tool in understanding responses to drought in many crop plants (Ribaut et al., 1997; Tuinstra et al., 1997; Teulat et al., 2001; Yadav et al., 2002). QTL mapping provides a means to dissect complex phenotypic characters such as drought tolerance into their component traits (QTLs), and allows the identification of molecular markers linked to desirable QTLs, so that these can be directly used in marker-assisted selection (Tanksley et al., 1989; Lee, 1995; Schneider et al., 1997). Using this approach, we recently reported QTLs associated with aspects of drought tolerance and yield under post-flowering drought stress in testcrosses (TCs) of progeny from a mapping population based on a cross of two elite inbred pollinators that differ in their tolerance to such stress (Yadav et al., 2002).

However, QTL expression/detection in phenotyping experiments is subject to both genotype and environmental effects (Hayes et al., 1993; Teulat et al., 2001; Kebede et al., 2001; Yadav et al., 2003). Different genetic stocks used in developing mapping populations can have both similar as well as different sets of alleles associated with various specific traits (Dudley, 1993). In addition, line by tester interactions may lead to the identification of different sets of QTLs in different tester backgrounds (Lübberstedt et al., 1997; Austin et al., 2000; Ajmone-Marson et al., 2001).

The present study was designed to assess the magnitude of the effects of (1) normal annual variation in a managed phenotyping environment, (2) variation in the timing of the target stress, and (3) the choice of tester, on the identification and strength of QTLs associated with aspects of post-flowering drought stress. The genetic material used consisted of 79 F_2-mapped F_3 progenies from a population developed by crossing two elite hybrid seed parent maintainer lines, ICMB841 and 863B. These were evaluated in the background of two elite testers, with very different phenotypes, in two managed terminal stress environments that differed in time of onset of stress, over two different years.

Materials and methods

Plant materials

Two agronomically elite inbred seed parents, ICMB841 and 863B, were crossed to develop a segregating population for genetic linkage map construction and trait analysis. The two parents are known to produce hybrids that distinctly differ in their response to post-flowering drought stress (Bidinger, unpublished data). Parent 863B was bred from *Iniadi* landrace material from Togo and was selected for this study based on its combination of agronomic eliteness and superior combining ability for grain filling under terminal drought stress conditions. Parent ICMB841 (Singh et al., 1990) is the maintainer of the female parent of several high yielding hybrids that are widely grown in India, but lack tolerance to terminal drought stress. Single plants of the two parental lines were crossed to produce a single F_1 plant that was self-pollinated to produce a large number of F_2 seeds. One hundred and fifty-one of these were picked at random to raise plants for DNA isolation and RFLP and SSR genotyping to construct a genetic linkage map necessary for QTL analysis. A random subset of 79 F_2-derived F_3 progenies (each derived from an individual skeleton-mapped F_2 plant) was testcrossed to two different elite testers (H77/833-2 and PPMI 301) to produce two paired sets of mapping population testcrosses. The two testers are known to produce phenotypically distinct hybrids; H77/833-2 produces hybrids with a large number of tillers per plant, but with small panicles, while PPMI 301 produces hybrids with fewer tillers per plant, but with considerably larger panicles.

Genetic linkage map construction

Genotyping of 151 F_2 individuals of the mapping progeny was conducted using RFLP and microsatellite markers. DNA isolation, restriction enzyme digestion, gel electrophoresis, Southern transfer, probe labelling, and filter hybridisation were essentially as described in Liu et al. (1994). Microsatellite genotyping was done as described by Qi et al. (2001). Pearl millet microsatellites were from John Innes Centre marker collection (Qi et al., 2001; Allouis et al., 2001). Linkage analyses were performed using MAPMAKER/EXP 3.0 (Lander et al., 1987). A LOD score of 2.0 was used as a standard for all two-point analyses and a LOD score of 3.0 for all three-point and multipoint analyses. Linkage groups were named according to common anchor markers with the map of Liu et al. (1994).

Trait evaluation

Crop management and trait evaluations were essentially as described in Yadav et al. (2002). Briefly, testcrosses of the 79 skeleton-mapped progenies and

two parents, replicated twice (162 entries), were evaluated under field conditions on the ICRISAT, Patancheru (India), research farm during the dry season (January to May) of 1998 and 1999. Individual field plots were 2-rows × 0.6 m × 4.0 m, sown in an alpha design with 18 blocks per replication with 9 entries per block, in 3 replications. Field evaluations in each year were conducted in three environments (separate, but adjacent experiments): a non-stress control environment, and two (early- and late-onset) post-flowering drought stress environments. The control environment was irrigated approximately weekly throughout the growing period. Irrigation was terminated in the stress environments, so that drought stress commenced at the mid-flowering stage in the early-onset stress environment, and at early grain filling stage (about 1 week later, in the late-onset stress environment). In both the early- and late-onset drought experiments, symptoms of drought stress started to appear approximately 10 days after the last irrigation. The stress environments were managed similarly each year in order to replicate the timing and severity of the stress as much as possible. Drought stress in both terminal drought stress environments developed progressively, without any interruption by rain, in both years.

Data were recorded on a plot basis for panicle mass (unthreshed), grain mass, stover mass, and a number of other agronomic traits such as flowering time, productive (with grain) panicle numbers, and 100-grain mass. Dry mass data were based on oven-dried samples, except for stover, for which dry mass was determined as the product of stover fresh mass and moisture percentage estimated from an oven-dried sub sample from each plot (Yadav et al., 2002). Grain yield, stover yield and above ground biomass yield (stover + panicle dry mass) were expressed on a square metre basis. Harvest index (HI = grain dry mass/biomass dry mass) and panicle harvest index (PNHI = grain dry mass/panicle dry mass) were calculated from the plot yield data. PNHI provides a direct estimate of the success of individual entries in setting and filling grains (Bidinger et al., 1987; Fusell et al., 1991).

Data analysis

Analysis of variance was performed according to the field design, using PROC GLM of SAS version 8 (SAS Institute, 1989) to determine the significance of all sources of variation (year, stress treatment, replication, block, tester, progeny and all interactions among the years, treatment, progeny and tester). All sources of variation were considered fixed, and least squares means (adjusted for replication and block effects) were estimated for all variables. Both replication and block effects were significant, so the least squares means for various main effects and interactions are reported, and used in the subsequent QTL mapping. Broad sense heritability of traits were calculated by dividing genotypic variance of traits with that of genotype plus genotype × environment interaction variances, and expressed in percentage.

QTL mapping was performed by the interval mapping method, using the QTL mapping software package MAPMAKER/QTL 1.1 (Lander & Botstein, 1989), with the additive model (Beavis et al., 1994; Yadav et al., 2002). QTL mapping was performed on genotype main effects and genotype interaction effects across years, stress treatments, and tester combinations using a subset of 42 markers mapping to seven linkage groups of pearl millet. Selection of markers included in QTL analysis was based on their distances from each other (see Figure 1) such that only a single marker was included in the subset from clusters where a number of markers mapped to a similar location or close to each other. A LOD threshold of 2.0 was used for considering a QTL significant. QTL results from different environments were compared on the basis of overlapping support intervals: a decrease in LOD score of 1.0, relative to the maximum LOD score, determined the end point of support interval for each QTL (Lander & Botstein, 1989).

Results

Construction of genetic linkage map

The genetic linkage map using 151 F_2 individuals consisted of 91 markers including 30 microsatellites (loci designated with prefix *Xpsmp*) (Figure 1). These markers covered all seven chromosomes, spanned 476 cM and were distributed over the pearl millet genome. Of these markers, six displayed dominant genetics (Psm567, Psm573, Psm202, Psmp2050, Psmp2229.1 and Psmp2229.2) whereas all the others were co-dominant. The genomic composition of the F_2 plant population inferred from all marker loci had an approximately normal distribution with an average of 51% of alleles coming from ICMB841. Chi-square tests of the frequencies of individual parental alleles for each of the co-dominant markers indicated that two genomic

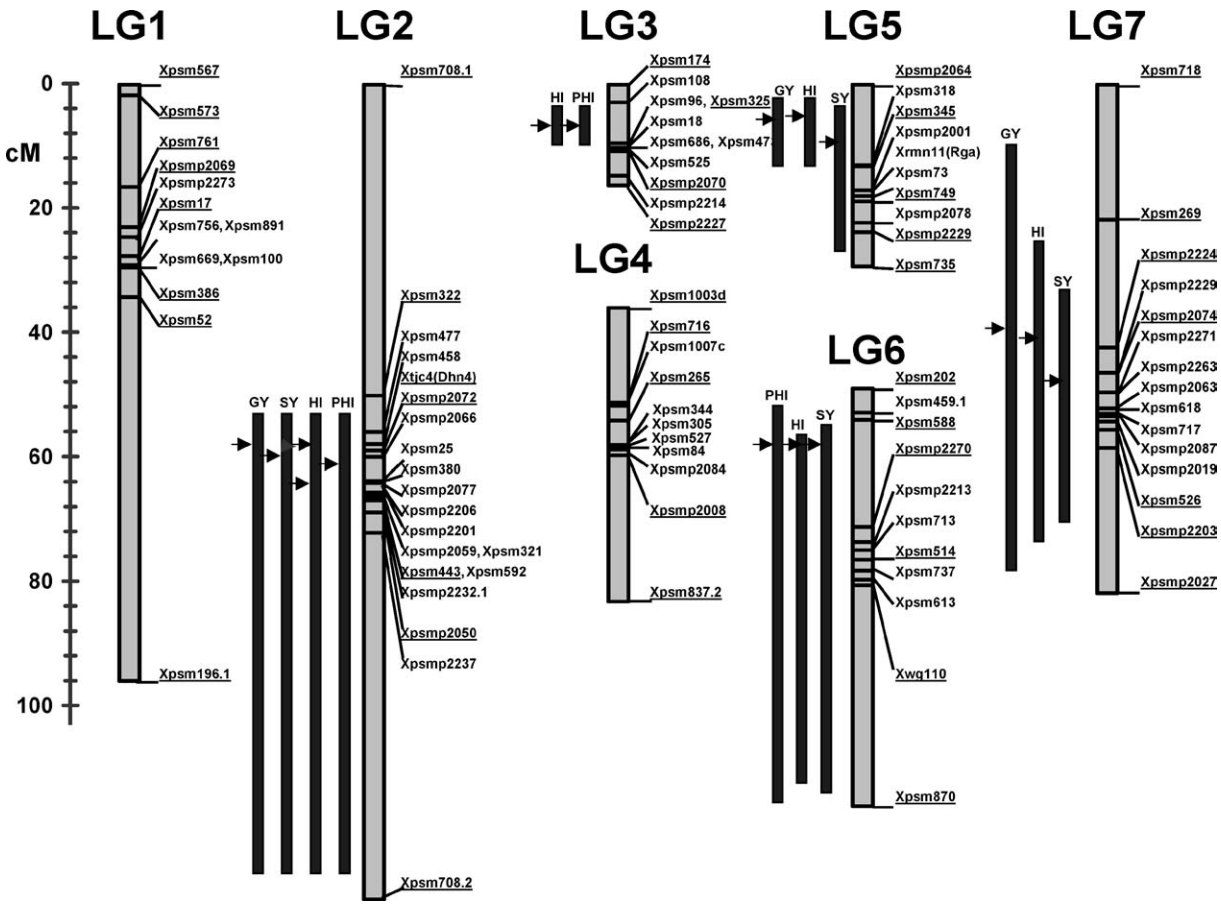

Figure 1. Genetic linkage map of seven pearl millet linkage groups based on the F_2 mapping population derived from ICMB841 × 863B. A scale of genetic distance in Haldane cM is provided for individual linkage groups. Markers used in QTL analysis are shown with an 'underline' on the map. Vertical bars shown on the left-hand side of the linkage groups indicate confidence interval of the QTLs for traits. QTL position on individual vertical bars is shown by an arrow. GY; Grain yield, SY; stover yield, HI; harvest index; PHI; panicle harvest index.

regions contained markers that displayed significant distorted segregation ($P = 0.01$) from the expected genotypic ratio of 1:2:1. These regions were located on linkage group 3 (*Xpsm459* to *Xpsm525*) and on linkage group 6 (*Xpsm325* to *Xwg110*). In both instances an excess of ICMB841 homozygotes was found. There were four large gaps (>30 cM between markers), due to the presence of extreme localization of recombination at the ends of LG 1, 2 and 6 (Figure 1). The genetic map length and the distribution of markers for this population was comparable to the consensus map of pearl millet (Devos et al., 2000) and to other maps published for this species (Busso et al., 1995; Jones et al., 1995; Yadav et al., 2002). The microsatellite loci were added subsequently to the RFLP markers being mapped due to their utility in subsequent marker-assisted selection. These also displayed a clustering of loci in the centromeric regions with very few loci mapping to the distal regions of the chromosomes.

Sources of variation: year, stress environment and tester effects

Despite attempts to manage the phenotyping experiments similarly in both years—planting date, fertilization, plant population, irrigation schedule, etc.—year was a significant source of variation for all variables reported (Table 1). Although yield reduction due to drought environments was quite similar (48.7% and 46.3%) for 1998 and 1999, respectively, mean grain yield in the year 1999 stress environments was 13% higher than in the year 1998 (Table 2). Year had a relatively small effect on partitioning between grain and stover (HI) but a highly significant effect on grain

Table 1. Mean squares of variables for year (1998 and 1999), stress treatment (early- and late-onset terminal stress), mapping population progeny, tester (H77/833-2 and PPMI 301) and their interactions. Data are from replicated evaluations of 158 testcrosses (79 mapped F_2-derived F_3 progenies × 2 testers) from a mapping population bred from the cross of ICMB841 × 863B. Evaluations were conducted in the dry season managed drought nursery at ICRISAT, Patancheru, India, in an 18 (blocks/rep) × 9 (entries/block) alpha design with three replications

Sources of variation	df	Grain yield ($\times 10^{-2}$)	Stover yield ($\times 10^{-2}$)	Harvest index	Panicle harvest index
Year*	1	2760.0***	10339.0***	1103.2***	6229.4***
Stress treatment*	1	18331.0***	4404.0***	10264.7***	8514.6***
Year × stress treatment*	1	741.8***	154.3**	740.2***	3525.2***
Rep (year × stress treatment)	8	115.3	42.7	234.8	264.7
Block (rep × year × stress treatment)	204	28.4	24.3	30.5	42.1
Progeny	78	32.0***	93.7***	113.6***	92.2***
Tester	1	1144.6***	10869.3***	13013.9***	10033.1***
Progeny × tester	78	14.2*	20.2*	14.3^{NS}	26.1^{NS}
Progeny × year	78	13.4^{NS}	26.9***	23.5***	26.0**
Tester × year	1	116.8***	1379.4***	711.6***	367.0***
Progeny × tester × year	78	12.8^{NS}	20.3*	12.3^{NS}	20.1^{NS}
Progeny × treatment	78	10.2*	14.8^{NS}	15.6***	32.5***
Tester × treatment	1	16.0^{NS}	403.4***	284.7***	0317.0***
Progeny × tester × treatment	78	11.5^{NS}	14.1^{NS}	13.3^{NS}	28.1**
Progeny × year × stress treatment	78	13.9**	15.7^{NS}	11.3^{NS}	22.6*
Tester × year × stress treatment	1	19.2^{NS}	13.2^{NS}	1.5^{NS}	60.0*
Progeny × tester × year × stress treatment	78	7.3^{NS}	10.2^{NS}	7.1^{NS}	12.4^{NS}
Error	881	9.9	14.1	10.6	16.9

F probability ***, <0.001; **, <0.01; *, <0.05.
*Tested against rep (year × treatment) mean square.

setting and filling (PNHI). For both HI and PNHI, values for 1999 were less than those for 1998, due to a larger decrease in both indices in the early stress environment of 1999 than in 1998 (Table 2).

Drought stress treatment effects were highly significant for all variables (Table 1); the early-onset stress treatment had a much larger effect on all variables than the late-onset treatment (Table 2). Greater reductions in grain yields and HI were expected as an earlier onset of stress affects grain yields to a greater degree than a later onset (Table 2). There was a significant difference between stress treatments in productive panicle number per square meter (20.5 in the early stress vs. 22.6 in the late stress; data not presented), indicating that a greater proportion of late tillers failed to make a panicle in the earlier stress. PNHI, which depends on both grain numbers set and grain filling, was also lower in the early stress (60.6%) than in the late stress (64.9%, Table 2). The major cause of lower PNHI in the early stress was a 23% lower mean 100-grain mass; differences in mean grain number per panicle between the two treatments were only 4% (data not presented). Both HI and PNHI were significantly and positively correlated to grain yield in early and late stress treatments of 1998 and 1999 (Table 3). On the other hand, stover yield was negatively correlated to harvest index in both stress treatments of 2 years. PNHI was also observed to be negatively correlated to stover yield but only in the late stress environments (Table 3).

The interactions of year and stress treatment were statistically significant for all traits evaluated, and the year × treatment MS was of at least an order of magnitude smaller than MS of the main effects of both year and treatment (Table 1). The exception to this was PNHI, for which year × treatment was highly significant, and accounted for 40–50% as much of the environmental variability as did the main effects (Table 1). This was due to a much larger effect of the 1999 early stress treatment on PNHI (57.0%) than any of the other year × treatment combinations (63.0–65.0%, Table 2). HI and PNHI appeared more sensitive to environmental interactions than did grain yield itself. This is because

Table 2. Means and ranges of grain yields, stover yield, harvest index and panicle harvest index recorded in the mapped progeny in the sources of variation. Sources of variation are year (1998 and 1999), stress treatment (early- and late-onset terminal stress), tester (H77/833-2 and PPMI 301) and their interactions. Data are from replicated evaluations of 158 progeny testcrosses (79 mapped F_2-derived F_3 progenies × 2 testers) from a mapping population bred from the cross of ICMB841 × 863B. Evaluations were conducted in the dry season in managed drought nursery at ICRISAT, Patancheru, India, in an 18 (blocks/rep) × 9 (entries/block) alpha design with three replications

	Grain yield (g m^{-2})		Stover yield (g m^{-2})		Harvest index (%)		Panicle harvest index (%)	
	Source mean	Range in progeny	Source mean	Range in progeny	Source mean	Range in progeny	Source mean	Range in progeny
Early stress (ES)	159.5	134–188	255.7	212–296	30.8	25.6–36.0	60.6	54.6–66.0
Late stress (LS)	222.3	176–258	286.4	230–339	35.5	29.9–41.6	64.9	58.3–69.1
PPMI 301 (PPMI)	182.9	148–227	295.4	236–338	30.5	25.6–36.1	60.4	54.5–65.8
H77/833-2 (H77)	198.7	155–235	246.7	197–299	35.8	30.1–41.5	65.1	57.7–69.8
1998 × ES	153.7	123–186	235.1	166–297	32.2	24.4–37.3	63.8	55.8–69.4
1998 × LS	203.7	146–264	260.0	197–353	35.7	29.1–43.6	65.3	56.7–70.6
1999 × ES	165.4	128–203	276.3	233–333	29.4	24.4–35.6	57.4	49.8–64.1
1999 × LS	240.5	204–294	312.8	245–382	35.4	29.6–42.2	64.4	57.1–70.5
1998 × PPMI	173.3	130–248	263.2	200–350	31.9	25.3–39.6	62.7	57.5–70.2
1998 × H77	184.0	130–237	231.9	173–328	35.9	28.6–42.9	66.5	58.4–71.6
1999 × PPMI	192.5	146–228	327.5	258–395	29.1	23.8–36.5	58.1	49.0–65.5
1999 × H77	213.4	160–250	261.6	204–320	35.7	30.0–42.7	63.7	53.6–69.7
ES × PPMI	152.6	122–192	275.3	224–324	28.5	23.8–33.7	58.7	52.5–65.6
ES × H77	166.5	124–205	236.2	178–296	33.1	25.7–38.5	62.5	51.7–68.9
LS × PPMI	213.3	158–262	315.4	230–382	32.4	25.2–38.5	62.1	52.4–67.9
LS × H77	230.9	183–276	257.4	190–317	38.6	32.6–44.7	67.6	60.1–73.3
1998 × ES × PPMI	148.2	99–200	246.9	180–309	30.5	22.8–37.8	62.5	52.5–72.4
1998 × ES × H77	159.1	96–209	223.3	153–315	33.9	24.2–40.6	65.1	48.8–72.2
1998 × LS × PPMI	198.4	127–296	279.5	219–397	33.2	24.2–41.5	62.9	51.4–72.6
1998 × LS × H77	208.9	142–291	240.5	171–364	38.1	30.1–46.9	67.8	58.4–75.7
1999 × ES × PPMI	156.9	110–206	303.8	237–359	26.5	21.2–34.7	54.9	45.4–62.2
1999 × ES × H77	173.8	119–227	248.9	189–308	32.3	23.9–39.5	60.0	48.6–68.2
1999 × LS × PPMI	228.1	182–287	351.2	256–481	31.7	25.7–38.3	61.4	51.2–68.8
1999 × LS × H77	252.9	201–315	274.3	195–381	39.1	31.9–46.7	67.4	58.5–73.2
Overall mean	190.8	156–219	271.0	219–314	33.2	28.7–38.8	62.8	57.9–67.1
Broad-sense heritability	49.1		59.3		66.1		52.7	

grain yield under stress is also significantly affected by differences in potential (non-stress) productivity and drought escape (flowering time) that were unaffected by stress treatment.

The tester used had an overwhelming effect on all variables; MS for tester was an order of magnitude greater than that of virtually any other genotype (G) or genotype × environmental (G × E) source of variation assessed in the trial (Table 1). The two testers are known to produce hybrids that differ in phenotype, productivity and tolerance to terminal drought. In the absence of stress H77/833-2 hybrids produced a greater biomass (891 vs. 802 g m^{-2}), grain (364 vs. 351 g m^{-2}) and stover (400 vs. 329 g m^{-2}) yields than the PPMI 301 hybrids. In the stress treatments, however, the H77/833-2 hybrids produced a greater biomass and stover yield than the PPMI301 hybrids (Table 2), but lower grain yields (199 g vs. 183 g m^{-2}, Table 2). The only variable

Table 3. Phenotypic correlation coefficients of four agronomic traits in early and late stress environments of the years 1998 and 1999

	Grain yield	Harvest index	Panicle harvest index
Harvest index			
1998 early stress	0.64***		
1998 late stress	0.82***		
1999 early stress	0.91***		
1999 late stress	0.64***		
Panicle harvest index			
1998 early stress	0.60***	0.59***	
1998 late stress	0.71***	0.83***	
1999 early stress	0.88***	0.89***	
1999 late stress	0.67***	0.87***	
Stover yield			
1998 early stress	0.17^{ns}	−0.60***	0.08^{ns}
1998 late stress	−0.16^{ns}	−0.66***	−0.36***
1999 early stress	−0.18^{ns}	−0.53***	−0.19^{ns}
1999 late stress	−0.03^{ns}	−0.76***	−0.46***

F probability ***, <0.001; **, <0.01; *, <0.05, ns; non-significant.

for which the hybrids on the two testers did not differ in the irrigated control was PNHI (data not shown), which indicates there were no constitutive differences in the ability of their hybrids to set and fill grains in the absence of drought stress. However, in both stress environments, the PPMI 301 hybrids maintained a significantly greater PNHI, inconsistent with its known poor tolerance of terminal drought stress, than did the H77/833-2 hybrids (Tables 1 and 2). The strong effects of the testers themselves thus present an excellent opportunity to assess tester and tester × stress severity effects on QTL identification.

Tester interactions with both year and treatment were significant for all variables except for the tester × stress treatment interaction for grain yield (Table 1). The testers × year interactions were due to a greater difference between the two sets of hybrids for all variables in 1999 than in 1998 (Table 2). This pattern was repeated in the case of the tester × treatment interactions, in which the differences between the two sets of hybrids in the late stress exceeded those in the early stress (Table 2). Both sets of interactions appeared to be a consequence of the larger effect of tester in the 1999 late stress treatment, than in any of the other year × treatment combinations (Table 2). For example, differences in mean biomass for the hybrids based on the two testers were 74 g m^{-2} in the 1999 late stress treatment, compared to between 17 and 50 g m^{-2} for the other three combinations of year and treatment (Table 2). These sorts of G × E interactions are expected in managed stress nursery field trials (Bidinger et al., 1987; Yadav et al., 2002) but less is known of their effects on QTL identification.

Sources of variation: progeny and progeny × environment and × tester interactions

Mapping population progeny was also a highly significant source of variation for all variables reported (Table 1). Of particular interest for the purposes of this paper is the magnitude of progeny × environment (year and stress treatment), progeny × tester and progeny × tester × environment interactions. If such interactions were significant, different genomic regions would be identified associated with traits in different year, tester and stress treatment combinations. Progeny × year interactions were significant for all variables except grain yield in the stress treatments (Table 1). A similar pattern of progeny × year interaction also occurred in the absence of stress (data not reported). Therefore, despite an attempt to manage the evaluations in the same manner both years, there were still environmental differences between the 2 years that differentially affected partitioning of dry mass between grain and stover yields among the hybrids of different progenies. In contrast to the case of progeny × year interactions, there were no significant progeny × stress treatment interactions for biomass, grain or stover yields, although there were significant interactions for HI and PNHI (Table 1). Significance of the three-way interaction of progeny, year and treatment varied with the individual variable (Table 1). In general, however, the MS for all of the interactions of progeny with environment were relatively small, compared to the MS for progenies themselves, and especially to MS for tester (Table 1).

Progeny × tester interactions were also significant for all variables (Table 1), indicating a potential for tester effects on QTL identification. The significance of progeny × tester × environment interactions varied with both the variable concerned and with the component of environment. In general, interactions of progeny × tester × year were more likely to be significant than were interactions of progeny × tester × treatment (Table 1). However, as in the case of progeny × environment interactions, the MS for progeny × tester × environment interactions were almost without exception smaller than the progeny MS and much smaller

than the tester MS. Broad sense heritability of traits ranged from 49.1% for grain yield to 66.1% for harvest index (Table 2).

Genomic regions associated with traits mean across stress environments and testers background

QTL analysis conducted on grand means of traits across stress environments, years and tester background identified three genomic regions associated with grain yield, four associated with stover yield, five associated with HI and three associated with PNHI (Table 4). These genomic regions had different genetic effects on trait values, and explained phenotypic variation in traits ranging from 12.6% to 40.2%. The total R^2 explained by the genomic regions identified for individual traits were not additive (Table 4), indicating that the genetic effects associated with individual genomic regions were not independent of each other. Smaller population size used in the study would also have contributed to the inflated genetic effects observed for individual genomic regions in this study.

All three genomic regions found to be associated with grain yield co-mapped with HI and stover yield, and the parental allele contributing to increase in HI also contributed to increase in grain yield but reduced stover yield. Parental allele effects observed for these traits were in agreement with the traits correlation observed (Table 3). Most but not all genomic regions found to be associated with HI and PNHI in pooled environment means mapped to common positions and similar parental alleles had increasing effects to these two related indices.

Genomic regions associated with genotype effects and genotype interaction effects of traits in environments

Since there were significant genotype interactions in stress environments of years and tester used (Table 2), QTLs were also analysed separately for trait genotype main effects (G effects) and for trait genotype interaction effects in individual stress environments of 2 years and testers. This analysis was necessary to identify if similar or different genomic regions would be identified in different environments and to dissect the environmental interaction effects present, if any, to the genomic regions associated with G main effects of traits. Genotype main effect and genotype interaction effects (GE) of traits were calculated such that G effects reflected the cumulative genotype effect across environments while genotype interaction effects were the result of individual genotype interactions with a given environment (Yan et al., 1999; Zhu, 1998; Yadav et al., 2003).

Of the three genomic regions identified for grain yield on grand means, two (on LG 2 and 5) were found to be associated with G main effects of grain yield, while the third one (on LG 7) was associated with GE interaction effects only. The genomic region identified for grain yield on LG 7 was specific to the late stress environment of the year 1998 in the genetic background of both testers (Table 3). By definition, the genomic regions identified with G main effects of traits will be effective in all environments (Yan et al., 1999; Yadav et al., 2003) but those identified with GE interaction effects and not with G main effects will be effective in those particular environments only.

Both genomic regions associated with the G main effects of grain yield (on LG 2 and 5) were unaffected by variations in stress environments across years and testers used except in the early stress environment of 1998 in H77/833-2 tester background and the late stress environment of the year 1999 in PPMI 301 tester background (Table 4). In these two stress environments, interaction effects on these genomic regions were of crossover type (in comparison to their G main effects), and ICMB841 parental allele, instead of the 863B parental allele, contributed to increased grain yield. A new genomic region associated with grain yield was also identified on LG 1 (Table 4), which was neither identified for grand means across environments nor for G main effects of grain yield. This genomic region was specifically associated with grain yield in the late stress environment of the year 1999 and in the PPMI tester background only. Interestingly a similar interaction in these genomic regions was also observed for harvest index and panicle harvest index (Table 4) and the ICMB841 parental allele was associated with increased HI and PNHI in these stress environments (see below). Stover yield QTLs identified on LG 2, 5 and 7 were unaffected by GE interaction. Only one genomic region associated with stover yield (mapping on LG 6, Table 4) was significantly affected by GE interactions and this interaction was evident in both the early as well as in late-onset stress environments of the year 1998 only.

All five genomic regions associated with harvest index on grand means were also found to be associated with G main effects of harvest index (Table 4). Of these, two (on LG 3 and 6) were free of environmental interactions in all environments, while the other three (mapping to LG 2, 5 and 7) exhibited significant

Table 4. Genomic regions for grain yield, stover yield, harvest index, panicle harvest index identified with pooled drought stress environment means, genotype main effects, and genotype interaction effects (with years, stress treatments and tester background interaction). Markers flanking the peak of the LOD score of traits are shown. Phenotypic variation (R^2) explained by individual QTL along with the parental allele contributing to increased trait values at the QTL are provided. Parental alleles from parent ICMB841 are shown as 841 and that from 863B as 863

		G main effects		Late stress treatment (tester H77/833-2 × year interactions)				Late stress treatment (tester PPMI 301 × year interactions)				Early stress treatment (tester H77/833-2 × year interactions)				Early stress treatment (tester PPMI 301 × year interactions)				Pooled mean	
				1998		1999		1998		1999		1998		1999		1998		1999			
Trait *Flanking markers*	LG	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD	Parent	LOD (R^2)	Parent
Grain yield (g m^2)																					
Xpsm761–	1									2.74	863										
Xpsmp2069										(15.2)											
Xpsm322–	2	6.07	863							2.28	841	2.93	841							5.71	863
Xpsm2050		(33.2)								(15.3)		(36.8)								(32.1)	
Xpsmp2064–	5	2.39	863									3.70	841							2.25	863
Xpsm345		(16.9)										(20.1)								(15.6)	
Xpsmp2074–	7			2.2	863			2.14	863											2.21	863
Xpsmp2027				(13.0)				(18.0)												(13.3)	
Total																				9.64	
																				(45.4)	
Stover yield (g m^2)																					
Xpsm322–	2	2.82	841																	2.84	841
Xpsm2050		(30.0)																		(31.6)	
Xpsmp2064–	5	4.36	841																	4.00	841
Xpsm345		(22.7)																		(21.0)	
Xpsm514–	6	2.86	841					2.45	841			2.29	863							2.70	841
Xwg110		(15.5)						(14.1)				(24.0)								(14.8)	
Xpsmp2074–	7	6.83	841																	6.34	841
Xpsmp2027		(40.8)																		(40.2)	
Total																				13.4	
																				(61.7)	
Harvest index (%)																					
Xpsm322–	2	5.35	863			2.91	863					5.54	841							4.88	863
Xpsm2050		(30.4)				(18.4)						(50.4)								(27.5)	

Table 4. Continued

		G main effects		Late stress treatment (tester H77/833-2 × year interactions)				Late stress treatment (tester PPMI 301 × year interactions)				Early stress treatment (tester H77/833-2 × year interactions)				Early stress treatment (tester PPMI 301 × year interactions)				Pooled mean	
				1998		1999		1998		1999		1998		1999		1998		1999			
Trait *Flanking markers*	LG	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD (R^2)	Parent	LOD	Parent	LOD (R^2)	Parent
Xpsm325–	3	2.17	841																	2.26	841
Xpsmp2070		(12.1)																		(12.6)	
Xpsmp2064–	5	3.79	863									2.76	841							3.69	863
Xpsm345		(21.9)										(15.4)								(21.1)	
Xpsm514–	6	3.15	863																	2.89	863
Xwg110		(17.4)																		(16.1)	
Xpsmp2074–	7	3.92	863	3.26	863			2.00	863											4.53	863
Xpsmp2027		(21.7)		(19.3)				(16.6)												(24.6)	
Total																				18.9	
																				(71.2)	
Panicle harvest index (%)																					
Xpsm573–	1													2.13	863						
Xpsm761														(13.8)							
Dhn4–	2	4.30	863			3.79	863					4.53	841							4.70	863
Xpsm443		(23.6)				(38.4)						(40.1)								(28.4)	
Xpsm325–	3	4.17	841																	3.87	841
Xpsmp2070		(22.3)																		(20.8)	
Xpsmp2064–	5					4.93	863					4.39	841								
Xpsm345						(25.6)							(22.9)								
Xpsm514–	6	2.43	863	3.03	863															2.55	863
Xpsm870		(14.4)		(35.6)																(14.8)	
Xpsmp2074–	7			4.54	863																
Xpsm526				(26.6)																	
Total																				13.5	
																				(72.0)	

interactions in one or the other specific environment. Interestingly, all genomic regions (except on LG 1) mapping to G main effects and/or genotype interaction effects of grain yield co-mapped with genomic regions identified for HI and parental allele associated with increased HI was also found to be associated with increased grain yield but reduced stover yield (Table 4).

All three genomic regions associated with panicle harvest index on grand means were found to be associated with G main effects of panicle harvest index in this study. In addition, three additional environmental-specific genomic regions (on LG 1, 5 and 7) were also found that were associated with environmental interaction effects of PNHI in different stress environments of years and in the genetic background of two testers (Table 4). The environment-specific interactions observed on LG 5 in the late-onset stress environment of 1999, and that of early stress environments of 1998, were opposite to each other and opposite parental alleles had an increasing effect on PNHI in these environments. As a result, this genomic region was not identified to be associated with PNHI on grand mean. One more genomic region (on LG 7) for PNHI was identified that was associated with PNHI in late stress environment of 1998 in the H77/833-2 tester background. Again this genomic region was not identified when QTLs for PNHI were analysed on grand means of PNHI across environments (Table 4). The reasons for this are not very clear from the mapping results but as on LG 7, there probably were opposing interactions in this region in other environments, which were small and hence not detected at the prescribed LOD threshold of 2.0.

Most genomic regions (except on LG 6) in which HI mapped in individual environments co-mapped with PNHI in those environments and alleles from the same parent were found to be associated with increased performance of these two indices. Genomic regions that were found to be associated with interaction responses of grain yield also co-mapped with interaction responses of HI and PNHI and similar parental alleles were associated with increased yield as well as the two indices in these environments. This indicated that most processes associated with two indices—HI and PNHI—were common under terminal drought stress conditions.

Discussion and conclusion

In this study, compared to the variation observed for progeny genotypes, those for years, stress treatments, and testers used were overwhelmingly large. On the other hand, the interaction effects of these factors with progeny genotypes, although significant, were not that large. Due to this, environmental and tester interaction effects were found for only a few genomic regions associated with traits reported in this study. In most cases, observed environmental interactions (GE effects) mapped to similar genomic regions were found to be associated with genotype main effects of traits. In some cases, new genomic regions associated with the traits in specific environments were also detected. The nature of interactions detected in genomic regions associated with G main effects of traits was either crossover type or non-crossover type. Non-cross type interactions were generally of the type where genomic regions showed quantitative differences in LOD score (signifying differences in genetic effects in environments), while crossover type of interactions were where opposite parental alleles contributed to increased trait values in different environments. Due to crossover type interactions, not every genomic region identified to be associated with G and/or GE interaction effects of traits in individual environments was identified on trait means using pooled means across environments. Different parental alleles contributing to trait performances in opposite direction in different environments (years, stress-onset and intensity) and/or genetic backgrounds (testers) lead to cancellation of effects of some genomic regions and hence were not detected using grand means. Opposite parental alleles contributing to traits a in environments have been reported in some earlier studies (Yan et al., 1999; Yadav et al., 2003) and this does raise a concern that some potentially useful QTLs may go undetected when the analysis is conducted on trait means across environments and genetic backgrounds.

Of the four genomic regions identified to be associated with grain yield, two (on LG 1 and 7) were environment-specific indicating that they will contribute to increased yield only in those particular environments. Environment-specific QTL for grain yield mapping to LG 7 was specific to the late-onset stress environments of 1998 in both tester backgrounds. Drought stress in the late stress environments of 1998 was more severe than that of the late-onset stress environment of 1999. This was evident from the larger yield reduction in these environments (43.2 and 38.5% for testers H77/833-2 and PPMI, respectively, compared to 39.9 and 31.2% in the year 1999). It was clear that the genomic region identified on LG 7 was associated with grain yield when drought-induced yield losses were

severe in late-onset stress environments. Similarly, the environment-specific interaction mapping to LG 1 was also associated with differences in drought intensities as the late-onset stress environment of 1999 (but in the PPMI tester background only) observed minimum yield reduction (28.8%) compared to 38.5 to 43.3% in other test environments reported in this study.

Despite interactions, a number of genomic regions found to be associated with G and/or genotype interaction responses of grain yield co-mapped with either HI or with both HI and PNHI. A similar relationships between grain yield and harvest index have been previously reported in pearl millet (Bidinger et al., 1987; Yadav et al., 2002), maize (Ribaut et al., 1997), barley (Teulat et al., 2001), wheat (Blum, 1988b). Genomic regions associated with stover yield also co-mapped with the regions associated with grain yield, HI and PNHI but parental alleles associated with increased effects on these traits have reducing effects on stover yield. This further clarified that these genomic regions contributed to increased grain yield by their effects on increased partitioning of assimilates from stover to the filling grains under stress conditions.

Interestingly, despite similar trait heritability, not every genomic region associated with HI, PNHI and grain yield co-mapped with grain and stover yield in this study. This indicates that by carefully selecting parental alleles associated with increased harvest index and grain yield, it could be possible to improve simultaneously both grain as well as stover yield under terminal drought stress conditions. It is generally considered that increased HI (i.e. partitioning of dry matter from stover to grains) may prove counterproductive as it may lead to lower stover yield and weaker stems that would be more prone to lodging under field conditions (Bidinger et al., 1977; Passioura, 1977; Slafer & Araus, 1998). However, given the opportunity to select for genomic regions associated with HI but not grain yield as detected in this study, simultaneous improvement of grain yield and stover yield could be achieved in pearl millet. Of five genomic regions found to be associated with HI, three consistently co-mapped with grain yield. On the other hand, two identified on LG 3 and LG 6 did not co-map with grain yield. For genomic regions where harvest index in stress environments co-mapped with grain yield in stress environments, selection of parental alleles increasing harvest index would contribute to increased grain yield in those stress environments. However, for genomic regions where HI mapped independently from grain yield, selection of parental allele associated with reduced harvest index would lead to increased stover yield.

Different genotypic responses to the timing and duration of drought stress are well known in crop plants (Blum, 1988b; Ceccarelli & Grando, 1996; Passioura, 1996). Tester genotypes used can also lead to different responses in different tester backgrounds (Lübberstedt et al., 1997; Austin et al., 2000; Ajmone-Marson et al., 2001). Although genotype × stress environment interactions obtained in this study were in accordance with earlier observations, some genomic regions were free of genotype × environment interactions while others were more affected by these. Some genomic regions reported in this study were associated with increased grain yield in a range of stress environments while others were more specific to a particular environment. Keeping in mind the unpredictable nature of drought occurrence (both in terms of timing and duration) accumulating both types—the QTLs that contribute to increased grain yield in a range of stress environments and those specific to particular set of stress environment conditions—would be a worthwhile strategy to increase yield and yield stability in water-limited environments. We are currently following such a strategy to improve the genetic potential of yield of drought-sensitive pearl millet lines in water-limited environments using several marker-assisted selection approaches.

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