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R.S. Yadav · C.T. Hash · F.R. Bidinger · G.P. Cavan
C.J. Howarth

Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought-stress conditions

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Abstract Drought stress during the reproductive stage is one of the most important environmental factors reducing the grain yield and yield stability of pearl millet. A QTL mapping approach has been used in this study to understand the genetic and physiological basis of drought tolerance in pearl millet and to provide a more-targeted approach to improving the drought tolerance and yield of this crop in water-limited environments. The aim was to identify specific genomic regions associated with the enhanced tolerance of pearl millet to drought stress during the flowering and grain-filling stages. Test-crosses of a set of mapping-population progenies, derived from a cross of two inbred pollinators that differed in their response to drought, were evaluated in a range of managed terminal drought-stress environments. A number of genomic regions were associated with drought tolerance in terms of both grain yield and its components. For example, a QTL associated with grain yield per se and for the drought tolerance of grain yield mapped on linkage group 2 and explained up to 23% of the phenotypic variation. Some of these QTLs were common across stress environments whereas others were specific to only a particular stress environment. All the QTLs that contributed to increased drought tolerance did so either through better than average maintenance (compared to non-stress environments) of harvest index, or harvest index and biomass productivity. It is concluded that there is considerable potential for marker-assisted backcross transfer of selected QTLs to the elite parent of the mapping population and for their general use in the improvement of pearl millet productivity in water-limited environments.

Keywords Pearl millet · Drought tolerance · Grain yield · Quantitative trait loci · Genetic mapping · Marker-assisted selection

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R.S. Yadav (✉) · G.P. Cavan · C.J. Howarth
Institute of Grassland and Environmental Research (IGER),
Plas Gogerddan, Aberystwyth, Ceredigion SY233 EB, Wales,
United Kingdom
e-mail: rattan.yadav@bbsrc.ac.uk
Fax: +44-1970-823242

C.T. Hash · F.R. Bidinger
International Crops Research Institute
for the Semi-Arid Tropics (ICRISAT),
Patancheru 502 324, Andhra Pradesh, India

Present address:
G.P. Cavan, 11 Albany Rd, Old Windsor, Berkshire SL4 2QD,
United Kingdom

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a staple food crop which is grown almost entirely under rainfed conditions on approximately 25 million ha of hot, drought-prone arid and semi-arid regions in Africa and south Asia (FAO and ICRISAT 1996). Inter- and intra-seasonal variation in rainfall in these regions is often the single most important environmental factor limiting pearl millet productivity (Mahalakshmi et al. 1987; van Oosterom et al. 1996). Although drought stress can occur any time during the crop cycle, terminal stress (flowering through grain filling) is more damaging to the productivity of the crop than stress at the vegetative or pre-flowering reproductive stages (Mahalakshmi et al. 1987). This is because pearl millet's asynchronous tillering behaviour and rapid growth rate allow it to recover rapidly from intermittent drought stress during the early stages of plant development, but provide no advantages under unrelieved terminal drought stress (Mahalakshmi et al. 1987). Improving the adaptation of pearl millet to terminal drought-stress environments is therefore a major objective for breeding programmes aimed at improving both the crop's productivity and its yield stability.

It has been suggested that the efficiency of breeding for stress environments could be enhanced if plant attributes that confer yield advantages in such environments could be identified and used as selection criteria (Blum 1988; Ludlow and Muchow 1990; Fussell et al. 1991).

However, traits that provide a consistent yield advantage across variable water-limited environments have been difficult to identify and use in breeding programs (Blum 1988; Ceccarelli and Grando 1996; Richards 1996; Turner 1997). Moreover, whole-plant response to drought stress is a complex process conditioned by a number of component responses that both interact and differ in their individual responses to the intensity and duration of water deficits, making it difficult to isolate individual traits with major effects on tolerance. In recent years, developments in molecular-marker technologies and their use in quantitative trait locus (QTL) analysis have provided effective new opportunities for the study of plant responses to the environment (Prioul et al. 1997; Ribaut et al. 1997; Tuinstra et al. 1997; Yadav et al. 1997; Frova et al. 1999; Quarrie et al. 1999). Molecular-marker technology provides opportunities not only to identify QTLs (and their functions) that determine complex phenotypes such as drought tolerance (Prioul et al. 1997), but also to improve greatly the efficiency of genetic improvement by facilitating the introgression of desirable traits through the use of linked markers (Tanksley 1993; Mohan et al. 1997).

An additional difficulty in genetically improving drought tolerance is the lack of a clear measure of drought tolerance or sensitivity. Although a number of (mainly short-term) physiological parameters have been proposed as indicators of tolerance to drought stress (for reviews see Ludlow and Muchow 1990; Turner 1997), it has not been possible to relate these clearly to differences in grain yield under stress conditions (Blum 1988; Bidinger and Witcombe 1989). For most plant breeders, tolerance has a meaning only if it confers greater yield or stability under stress. Grain yield itself has limitations as an index for stress tolerance, as grain yield in a particular drought environment is influenced not only by genetic differences in drought tolerance, but also by differences in time to flowering (drought escape) and differences in yield potential (Fischer and Maurer 1978; Bidinger et al. 1987a). In terminal drought stress, the combined effects of phenology and yield potential can account for as much as 50% of the variation in pearl millet grain yield (Bidinger et al. 1987a). Because of these effects, the most-effective means of improving pearl millet's grain yield in terminal stress environments should be to incorporate specific traits (or responses to stress) that improve the tolerance of terminal stress into otherwise high yielding genotypes of appropriate crop duration (Bidinger et al. 1987b; Fussell et al. 1991).

Grain yield is not itself a simple trait but is conditioned by a number of morphological and physiological processes. Sensitivity to post-flowering drought stress in pearl millet is often characterized by reduced grain filling and reduced numbers of grains per panicle, which jointly reduce grain yield. Although genetic variation in the expression of these traits in terminal drought-stress environments exists in the available pearl millet germplasm (Bidinger et al. 1987b), the inheritance of such variation and the interactions of its various components in determining grain yield in variable water-limited environments are not well understood. The objectives of this study were: (1) to understand better the genetic architecture of grain yield, and its component traits, among lines drawn from a cross of contrasting parents, in a range of terminal stress environments, and (2) to identify individual genomic regions associated with responses or traits associated with reduced sensitivity (i.e. increased tolerance) to this range of stresses. The long-term objectives of the research to which this study contributes, are to enhance the efficacy and cost-effectiveness of breeding programmes to improve grain yield and the grain yield stability of pearl millet in drought-prone environments.

Material and methods

Plant materials

In order to produce a segregating population for genetic map construction and subsequent QTL analysis, two early maturing inbred lines, H 77/833–2 and PRLT 2/89–33, were used as parents. H 77/833–2, referred to hereafter as H 77, is the male parent of a number of thermotolerant, extra-early, high-tillering and high-yielding pearl millet hybrids, including HHB 67 (843A × H 77/833–2; Kapoor et al. 1989), which is widely cultivated in the Thar desert margins of northwestern India. The second parent, PRLT 2/89–33 (referred to hereafter as PRLT), is an inbred derived from the ICRISAT Bold Seeded Early Composite (BSEC). BSEC is an elite breeding population based predominantly on Iniadi landrace germplasm from West Africa (Witcombe and Soman 1992; Andrews and Anand Kumar 1996). The Iniadi landrace materials differ from northwestern Indian germplasm, such as H 77, in many plant characteristics. Iniadi germplasm has fewer basal and nodal tillers, larger seeds, thicker stems and panicles, and broader leaves.

The two parental inbred lines were crossed and a single F_1 plant was self-pollinated to produce F_2 seed. Leaf-tissue samples were collected from each of 150 individual F_2 plants for DNA isolation and subsequent RFLP genotyping and genetic map construction. A subset of 92 F_3 progenies (each derived from an individual skeleton-mapped F_2 plant) were crossed to a common male-sterile line tester (843A; Stegmeier et al. 1998) to produce testcross hybrids for phenotyping the mapped progenies for grain yield and its component traits under terminal drought stress. Testcross hybrids were also produced with the two parental inbred lines, and these were used as control entries in the phenotyping experiments. The mapped progenies were phenotyped as testcross hybrids, rather than using their derived inbred progenies (e.g. F_3 or F_4 as in Jones et al. 1995) for several reasons:

(1) to restore heterotic vigour to inbred mapping progenies that might otherwise be too weak for effective screening under stress conditions (pearl millet is highly cross-pollinated in nature and suffers considerably from inbreeding depression);
(2) to use the dominantly inherited early flowering of the tester to reduce variation in flowering time among the test units, in order to focus the mapping on specific drought-tolerance traits rather than traits or responses associated with drought escape; and finally;
(3) to have test units that approximate the genetic structure of the F_1 hybrids grown by farmers rather than F_3 or F_4 inbred lines.

RFLP assay and map construction

Procedures for DNA isolation, restriction enzyme digestion, gel electrophoresis, Southern transfer, probe labeling and filter hybridization were essentially as described in Liu et al. (1994). Linkage analysis was carried out using Mapmaker (Lander and

Table 1 Details of three experiments containing paired irrigated control and terminal drought-stress environments conducted at Patancheru, India, during the hot, dry summer seasons of 1997 and 1998

Experiment	Year, location	Rainfall (mm)	Treatment	Stress Environment	Mean flowering time, DAE	Final irrigation, DAE	Harvest, DAE
1	1997, drought nursery	50 DAE: 3.0 mm, 52 DAE: 48.6 mm, 53 DAE: 5.2 mm, 62 DAE: 8.6 mm	Control		37.3	74	80
			Late stress	1	36.9	43	78
			Early stress	2	37.2	37	75
2	1998, drought nursery	39 DAE: 1.4 mm	Control		34.8	56	68
			Early stress	3	34.4	34	63
3	1998, rain out shelter	0 mm	Control		33.1	56	70
			Early stress	4	34.0	33	66

Botstein 1989). The RFLP map of this cross currently comprises 50 markers distributed over the seven linkage groups of pearl millet, with an average spacing of approximately 7 cM. The genetic map length (approximately 352 Haldane cM) and the order of markers obtained for this population was comparable to the consensus map of pearl millet (K.M. Devos, personal communication) and the most extensive map published to-date for this species (Devos et al. 2000).

Crop management and data recorded

The testcrosses of the 92 mapping population F_3 lines and their two parents were evaluated in three separate field experiments at the ICRISAT-Patancheru research farm in India (17.53°N, 78.27°E) during the dry seasons (January to May) of 1997 and 1998 (Table 1). Dry seasons at ICRISAT-Patancheru are generally rain-free with high mean air temperatures and large vapour pressure deficits, which provide an excellent opportunity to expose plants to a controlled, but severe, terminal drought stress, by managing the timing of irrigation (Bidinger et al. 1987a). All experiments reported in this study consisted of paired stress and control treatments (Table 1). One experiment was conducted in a rainout shelter.

All three experiments were conducted on shallow sandy loam (alfisol) soils of 0.6-m to 0.8-m depth (to gravelly subsoil material). Crop nutritional requirements were met by banding 40 kg of N and 18 kg of P ha^{-1} into the ridges before sowing and side dressing an additional 45 kg of N ha^{-1} 3–4 weeks after sowing. Plant-available water was sufficient for about 7–9 days transpiration of a full-crop canopy before afternoon wilting became visible. All experiments were sprinkler-irrigated weekly until the final irrigation of the early onset stress treatment, which was given by furrows in stress environments 2 and 3 or by drip line in stress environment 4. The final irrigations in the stress treatments were done to fill the entire soil profile (e.g. by allowing the water to stand in the furrows for 4 h). Drought stress in the early onset treatment (stress environments 2, 3 and 4) was initiated by withholding irrigation from 50% flowering (Table 1). Drought stress in the late-onset treatment (stress environment 1) was initiated during early grain filling by providing one additional furrow irrigation 1 week after 50% flowering. The irrigated control treatments of each experiment were furrow-irrigated weekly thereafter until crop maturity. Weeds were controlled by a combination of cultivation and one-hand weeding. There was no significant pest or disease incidence.

Testcrosses were evaluated for the expression of yield and yield-component traits in both terminal stress and irrigated control environments (using three replications in randomized complete block designs for each environment) in all experiments. In experiment 1 testcrosses were evaluated in plots of two rows×4 m, and plants in the central 3-m portion of these 2-row plots were used for recording pre- and post-harvest data. In experiments 2 and 3 testcrosses were evaluated in plots of one row×4 m, due to space constraints in the rainout shelter. Pre- and post-harvest data were recorded from the central 3 m of each single-row plot. Inter-row spacing was maintained at 0.6 m and plots, initially over-sown, were thinned within 2 weeks of seedling-emergence to a uniform stand of approximately 8 plants m^{-2} in experiment 1 and approximately 12 plants m^{-2} in experiments 2 and 3.

Flowering time (FT) was recorded as days from seedling emergence to stigma emergence in 50% of the main shoots in a plot. At harvest, data were recorded for the harvested area on plant numbers, effective (with grain) panicle numbers, stover fresh mass, panicle mass and grain mass (all on a plot basis), and on 100-grain mass (HGM). All dry weights were determined from oven-dry samples, except for stover, which was determined as the product of stover fresh mass and moisture percentage estimated from an oven-dried sub-sample from each plot. Data on grain yield (GY), stover yield (SY), total above ground biomass yield (BMY), panicle numbers (PN) and plant numbers were expressed per square metre. Numbers of grains per panicle (PGN) were derived from these primary data [=(100×GY)/(PN×HGM)]. The harvest index (HI) was calculated for each plot as the ratio of GY and BMY. A drought-tolerance index was estimated for each of these traits as the trait expression in the stress environment relative to that in the control, calculated by dividing each testcross entry trait mean (over replications) in a particular stress environment by its corresponding mean in the paired irrigated control environment.

Data analysis

Analyses of variance were performed using GENSTAT (GENSTAT 5 Committee 1993) to determine the significance of variation among testcrosses for all the traits measured in each of the seven irrigated control and stress treatments. Combined analyses of variance across each pair (or triplet in experiment 1) of post-flowering drought stress and irrigated control treatments were also conducted to determine interactions between moisture treatment and genotype in individual experiments. QTL mapping was performed on both the testcross entry means and on the drought-tolerance index obtained for each trait from each of the four stress environments (Cowen 1988; Soller and Beckmann 1990) using the method of interval mapping (Lander and Botstein 1989). The additive genetic model implemented in the software package MAPMAKER-QTL was considered appropriate for QTL analysis of the testcross experimental units used (Cowen 1988; Beavis et al. 1994; Schön et al. 1994). A LOD threshold of 2.0 was used for considering a QTL significant. Results from different stress 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 points of the support interval for each QTL (Lander and Botstein 1989). Additive genetic effects attributed to individual QTLs, and the percentage of phenotypic variation explained by each QTL, were also estimated using this software. In environments where more than one QTL was detected

for a particular trait, a combined model was fitted to include each of the individually significant QTLs. Secondary peaks identified when the major QTL had been fixed were considered significant if their inclusion made the whole-genome model 100-times more likely (i.e. increased the overall LOD by at least 2.0). For these combined models, the software provided estimates of the the total phenotypic variance and total LOD score explained by the model.

Results and discussion

Expression of grain yield and grain yield component traits

In all experiments, terminal drought reduced GY and BMY below that of the irrigated controls (Table 2). Mean GY reduction (measured as the average percentage grain yield in the stress treatment relative to the irrigated control) ranged from 27.5% in environment 1 to 61.1% in stress environment 4. Differences in drought-stress intensities in these four stress environments were not only due to differences in the timing of the onset of drought stress but also due to climatic differences between the 2 years of study and edaphic differences between the fields used. In 1998 (environments 3 and 4), higher temperature conditions were encountered both before flowering (mean maximum daily temperature between seedling emergence and flowering was 32°C in 1997 and 35°C in 1998) and between flowering and harvest (mean maximum daily temperature was 35°C in 1997 and 39°C in 1998) as compared to 1997 (environments 1 and 2). Crop development was thus accelerated in 1998 with the mean 50% flowering time of the testcrosses being 3 days earlier in 1998 than 1997 and harvest occurring over a week earlier in 1998 than in 1997 (Table 1). Drought stress in 1997 was also interrupted by unexpected rain and hail, which both relieved stress for several days and caused some damage to leaves, compared to an uninterrupted stress in 1998 (Table 1). In the two experiments conducted in 1998, the reduction in GY was far greater in environment 4 because the soil was shallower and more compacted under the rainout shelter than in the adjacent field used for environment 3.

The components of yield were also affected differently by drought. For example, SY, BMY and PN were reduced more in environment 2 (early onset stress treatment) than in environment 1 (where stress began a week later). Conversely, HI and HGM were less affected by drought in environment 2 as compared to environment 1. The most drought-sensitive component of grain yield in all experiments was HGM although PN and PGN were also reduced by drought. In addition, reductions in SY, BMY and HI were also evident in each of these four terminal drought-stress environments (Table 2).

The major factor affecting differences in grain yield in all stress environments was the total above-ground crop growth (BMY). This was primarily a consequence of differences in crop growth rate as differences in FT among lines were small and explained no more than 22%

Table 2 Mean performance of pearl millet mapping-population testcross progenies for grain and biomass yield component traits in three experiments containing paired irrigated control and terminal drought-stress environments, conducted at Patancheru, India, during the hot, dry summer seasons of 1997 and 1998

Trait	Experiment 1			Experiment 2		Experiment 3		
	Irrigated control	Stress environment 1	% Reduction under stress	Stress environment 2	% Reduction under stress	Irrigated control	Stress environment 3	% Reduction under stress	Irrigated control	Stress environment 4	% Reduction under stress
Grain yield (GY), g m^{-2}	300	220	27.5	210	32.4	340	240	28.9	400	160	61.1
Stover yield (SY), g m^{-2}	270	250	7.5	210	21.1	360	280	22.7	430	190	55.2
Biomass yield (BMY), g m^{-2}	670	570	14.9	500	25.7	800	620	25.4	950	440	62.5
Harvest index (HI), %	45.1	38.6	14.5	40.9	9.4	43.2	39.4	8.7	42.3	35.0	17.3
100-grain mass, (HGM) g	1.05	0.82	21.9	0.87	17.1	0.94	0.73	22.3	0.97	0.61	37.1
Panicle number, (PN) m^{-2}	24.5	23.8	3.1	20.8	15.3	24.0	23.7	1.2	29.5	23.3	20.9
Panicle grain number, (PGN)	1,220	1,170	3.5	1,180	3.1	1,580	1,450	8.1	1,440	1,120	22.3

Table 3 Correlation coefficients for pearl millet grain and stover yield-determining component traits measured in the four stress environments of three experiments at Patancheru, India, during the hot, dry summer seasons of 1997 and 1998. Critical values for these correlation coefficients are 0.20 and 0.26 for $P<0.05$ and $P<0.01$ respectively

Trait[a]	GY	HGM	PGN	PN	SY	BMY	HI	Environment[b]
FT	0.02	–0.19	0.48	–0.37	0.74	0.47	–0.51	1
	0.12	–0.09	0.61	–0.47	0.40	0.11	–0.01	2
	–0.14	–0.27	0.43	–0.35	0.71	0.48	–0.77	3
	–0.13	–0.34	0.26	–0.32	0.64	0.32	–0.63	4
GY		0.08	0.24	0.45	0.43	0.77	0.45	1
		0.21	0.16	0.58	0.44	0.87	0.64	2
		0.40	0.22	0.46	0.40	0.78	0.54	3
		0.61	0.59	0.31	0.51	0.89	0.80	4
HGM			–0.20	–0.29	–0.22	–0.17	0.37	1
			–0.25	–0.10	–0.02	0.11	0.25	2
			–0.25	–0.04	–0.01	0.18	0.40	3
			0.17	0.02	0.10	0.41	0.69	4
PGN				–0.57	0.22	0.14	0.19	1
				–0.56	0.27	0.14	0.10	2
				–0.53	0.18	0.21	0.09	3
				–0.42	0.47	0.57	0.41	4
PN					0.23	0.52	–0.04	1
					0.12	0.58	0.33	2
					0.18	0.39	0.18	3
					0.08	0.30	0.24	4
SY						0.85	–0.52	1
						0.78	–0.34	2
						0.86	–0.52	3
						0.83	–0.04	4
BMY							–0.21	1
							0.17	2
							–0.09	3
							0.44	4

[a] FT=Flowering time; GY=Grain yield; HGM=Hundred grain mass; PGN=Panicle grain number; PN=Panicle number m^{-2}; SY=Stover yield; BMY=Biomass yield; HI=Harvest index

[b] 1=1997 early stress environment; 2=1997 late stress environment; 3=1998 field stress environment; 4=1998 rainout shelter stress environment

of the differences in BMY (Table 3). Some of the differences in BMY could be explained by differences in tillering (indicated by PN) among lines; hence PN was also significantly correlated with GY in all stress environments (Table 3). The major differences among correlations between GY and its various components between the stress and control environments were in the increased importance of HGM and HI under stress. Whilst differences in GY were not related to differences in HGM in the irrigated control environments (data not shown), under drought the strengths of the correlations between GY and HGM were directly proportional to the severity of the stress, increasing from 0.076 ($P>0.47$) in stress environment 1 to 0.613 ($P<0.000001$) in stress environment 4 (Table 3). This clearly indicates the increasing importance of the ability to fill grains (probably using translocated stem reserves) as the severity of stress increased and post-flowering assimilation decreased. Similarly, the strength of correlations of GY with HI also increased as the stress environments became more severe (Table 3). The increased importance of a high HI in the terminal stress environments almost certainly reflects increased differences in the post-flowering growth (i.e. grain filling) of the testcrosses, as their pre-flowering growth was little affected by the stresses imposed. In the most-severe stress environment (environment 4) HI was related also to PGN suggesting that in this stress environment grain set, as well as grain filling, was a factor in grain dry matter accumulation. Similar relationships between GY and HGM have been reported in pearl millet by Bidinger et al. (1987b) and Fussell et al. (1991) in the evaluations of advanced breeding materials in terminal drought-stress environments.

In none of the experiments reported in this study was GY in any of the four stress environments significantly correlated ($P<0.05$) to FT (Table 3) or to GY measured in the paired irrigated control environment (data not shown). This indicated that expression of differences in testcross grain yields under drought was influenced little by differences among testcrosses in either drought-escape or grain yield potential. In such situations, there was no need to resort to the use of drought response indices that separate drought resistance specifically from drought-escape and yield-potential effects (Bidinger et al. 1987b). The drought-tolerance index of each trait estimated in this study was therefore simply the value obtained in the stress environment relative to that in the control. Drought tolerance so defined reflects the ability of a genotype to maintain normal (irrigated) trait expression under stress. Grain yield component traits (e.g. PN, PGN, SY) were however significantly correlated to flowering time (Table 3).

Genetic parameters of the testcross population

The effect of the moisture regime was highly significant ($P<0.01$) for virtually all traits observed in all three ex-

Table 4 Mean squares from experiment-wise combined analyses of variance for grain and stover yield-determining traits of pearl millet mapping-population testcrosses in terminal drought-stress environments and their counterpart fully irrigated controls, for three experiments at Patancheru, India, during the hot, dry summer seasons of 1997 and 1998

Trait	Stress environments 1 and 2 Experiment 1			Stress environment 3 Experiment 2			Stress environment 4 Experiment 3		
	Genotype	Moisture regime	Genotype ×moisture regime	Genotype	Moisture regime	Genotype ×moisture regime	Genotype	Moisture regime	Genotype ×moisture regime
Flowering time (FT), days	51.37***	9.32**	2.45*	18.55***	16.33**	2.04 ns	14.61***	158.34***	1.945 ns
Grain yield (GY), g m^{-2}	2,095***	825,296***	1,830***	2,289 ns	1,411,972***	3,208*	3,898***	8,639,154***	4,590***
Stover yield (SY), g m^{-2}	8,603***	228,274***	2,074***	14,337***	940,505***	4,966 ns	12,224***	8,179,862***	3,571 ns
Biomass yield (BMY), g m^{-2}	15,318***	2,137,115***	7,523***	26,616***	4,605,196***	15,025 ns	32,457***	37,563,580***	15,751***
Harvest index (HI), %	32.02***	3,220.09***	17.30***	41.58***	2,041.19***	14.72 ns	34.14***	7,763.13***	27.37**
100-grain mass (HGM), g	0.0457***	4.0100***	0.0055*	0.0393***	6.2833***	0.0036 ns	0.0292***	19.069***	0.0067 ns
Panicle number (PN), m^{-2}	80.25***	1,149.25***	14.31 ns	84.34***	11.06 ns	15.21 ns	82.42***	5475.42***	23.34***
Panicle grain number (PGN)	248,743***	153,349**	34,767*	375,832***	2,355,690***	55,282 ns	267,333***	14,837,438***	50,594*

*** $P<0.001$, ** $P<0.01$, * $P<0.05$, ns=non-significant

Table 5 Characteristics of pearl millet mapping population parental and progeny testcross hybrids for grain and stover yield-determining traits in four terminal drought stress environments from three experiments conducted at Patancheru, India during the hot, dry summer seasons of 1997 and 1998

Trait	Stress environment 1				Stress environment 2				Stress environment 3				Stress environment 4			
	Parental means		Mapping progenies		Parental means		Mapping progenies		Parental means		Mapping progenies		Parental means		Mapping progenies	
	H 77[a]	PRLT[b]	Range	Entry-mean h^{2c}	H 77	PRLT	Range	Entry-mean h^2	H 77	PRLT	Range	Entry-mean h^2	H 77	PRLT	Range	Entry-mean h^2
Flowering time (FT), days	39.0	37.5	32.0–46.0	90.6	39.8	37.7	32.0–46.0	89.9	35.5	36.5	30.0–42.0	79.6	35.5	35.3	30.0–42.0	75.7
Grain yield (GY), g m^{-2}	210	240	102–330	40.7	230	180	110–310	2.5	210	250	90–380	8.3	140	180	42–360	27.8
Stover yield (SY), g m^{-2}	300	270	100–400	41.2	260	210	130–310	66.8	200	230	100–590	65.3	200	220	100–380	71.4
Biomass yield (BMY), g m^{-2}	650	600	270–810	26.8	580	460	300–720	35.7	550	538	340–1,050	46.6	440	490	230–840	51.1
Harvest index (HI), %	32.2	38.8	22.4–48.6	46.4	40.2	38.8	28.9–49.5	51.0	48.7	43.7	23.3–52.1	56.1	31.9	35.3	14.5–49.7	42.3
100-grain mass (HGM), g	0.64	0.98	0.59–1.14	76.9	0.72	0.89	0.65–1.13	70.0	0.73	0.74	0.50–1.10	68.9	0.49	0.57	0.35–0.97	57.5
Panicle number (PN), m^{-2}	31.1	15.7	10.0–38.6	72.1	28.9	14.8	11.4–35.3	59.9	16.4	15.2	11.7–39.4	75.8	30.5	18.0	11.7–37.8	65.2
Panicle grain number (PGN)	1,060	1,600	710–2,040	76.1	1,130	1,390	730–1,910	78.1	2,220	2,190	450–3,190	75.9	940	1,720	460–2,430	64.4

[a] H 77, H 77/833–2
[b] PRLT, PRLT 2/89–33
[c] h^2; heritability

periments (Table 4), as expected from the major reductions in treatment-means in the stress environments (Table 2). The exception was PN in environment 3, which was largely determined before the onset of this relatively mild terminal drought stress (Table 2). Differences among testcrosses were also significant for all of the traits measured in all experiments, with the exception of GY in environment 3, which had a high level of experimental error (Table 3) and thus a low entry mean heritability (Table 5). Within experiments, differences among testcrosses were significant ($P<0.01$) for flowering, yield, and yield components in all three control environments, and for stress environments 1 and 4 (data not presented).

Genotype×moisture regime interactions were significant for GY in all experiments, indicating that testcrosses did react differently to stress even where their mean yields across treatments did not differ. The situation with the various yield components varied with experiment. There were significant genotype×environment interactions for most variables in environments 1, 2 and 4 (Table 4). However, in environment 3 there was a significant interaction only for GY. Apparently, in this experiment, small, non-statistically significant interactions for individual yield components resulted cumulatively in a significant interaction for yield itself.

For the two variables identified above as possible indicators of differential drought tolerance, HGM and HI, the results of the analysis of variance differed. HGM differed significantly ($P<0.001$) among testcrosses in all three experiments (as well as in all of the individual environments, data not presented), but testcross×moisture environment (g×e) interactions were significant ($P<0.05$) only in the 1997 experiment (Table 4). This suggests that the differences in HGM among testcrosses under stress, which were related to GY in the stress environments, may have been constitutive ones rather than drought specific (which would have been the interpretation if the g×e interaction for this trait had been significant). However, correlations of HGM in irrigated control environments with GY in stress environments were all non-significant indicating that the HGM differences observed under stress were not entirely constitutive (data not shown). There were significant g×e interactions for HI in two of the three experiments, indicating that the correlations of GY under stress with this variable may have more directly represented a differential response to stress (Table 4).

The contrasting phenotypes of the two parents of the mapping population (H 77 and PRLT) were exhibited in their testcrosses under stress (Table 5). In all stress environments, the testcross of the H 77 parent had a higher PN than that of the PRLT parent, but a lower HGM and PGN. The H 77 testcross had a higher BMY than the PRLT testcross in environments 1, 2 and 3 but this situation was reversed in the most-stress environment 4. The PRLT testcross had a higher GY in three of the four stress environments, including the most-severely stressed environment 4, where it out-yielded the H 77 testcross by nearly 30%. Only in interrupted early onset drought-stress environment 2, where later-flowering tillers could have effectively contributed to GY, did the high-tillering H 77 testcross have a GY superior to that of the low-tillering PRLT testcross. For most of the traits evaluated, the range observed in mapping-population testcrosses exceeded that of the parental testcrosses (Table 5). This provides a good range in the expression of yield components so that their contribution to yield under stress can be assessed (Table 3). The frequency histograms of testcross means were mono-modal and normally distributed (data not shown) in both stress and irrigated control environments for nearly all traits, suggesting polygenic inheritance.

QTLs associated with grain yield and yield component trait expression in stress environments

Significant QTLs were detected for all components of GY and BMY in all environments (Table 6, Fig. 1). These mapped to six of the seven pearl millet linkage groups; no QTLs were detected on linkage group 5. Alleles from both parents contributed to the increased expression of all observed traits except for PN and PGN.

Flowering time

Five different genomic regions were associated with FT and together these explained 60–70% of the phenotypic variation observed among the testcrosses in individual stress environments. Major QTLs for FT co-mapped on linkage groups 4 and 6 in all four environments. For the QTL on linkage group 4 the allele from H 77 increased flowering time, whereas for the QTL on linkage group 6 it was the allele from PRLT that increased flowering time (Fig. 1). An additional QTL on linkage group 4 was mapped in environment 3, again with the allele from H 77 increasing flowering time. QTLs of smaller significance were mapped on linkage groups 2 and 3 in one or more environments (Table 6).

Fig. 1 Map locations of QTLs detected in this study on linkage groups 1, 2, 4 and 6. For each linkage group a scale of genetic distance in Haldane cM is provided. One-LOD support intervals are indicated by *vertical bars* with the position of the maximum LOD peak indicated by ●. QTLs in which the PRLT parental allele conferred an increased trait value are indicated on the right-hand side of the linkage map and those in which the H 77 parental allele increase the trait value is on the left-hand side. *FT* Flowering time, *GY* Grain yield, *HGM* Hundred-grain mass, *PGN* Panicle grain number, *PN* Panicle number m^{-2}, *SY* Stover yield, *BMY* Biomass yield, *HI* Harvest index, *E1* 1997 late stress environment, *2* 1997 late stress environment, *3* 1998 field stress environment; *4* 1998 rainout shelter stress environment, *DT* refers to a QTL obtained using the drought tolerance index of that trait ▶

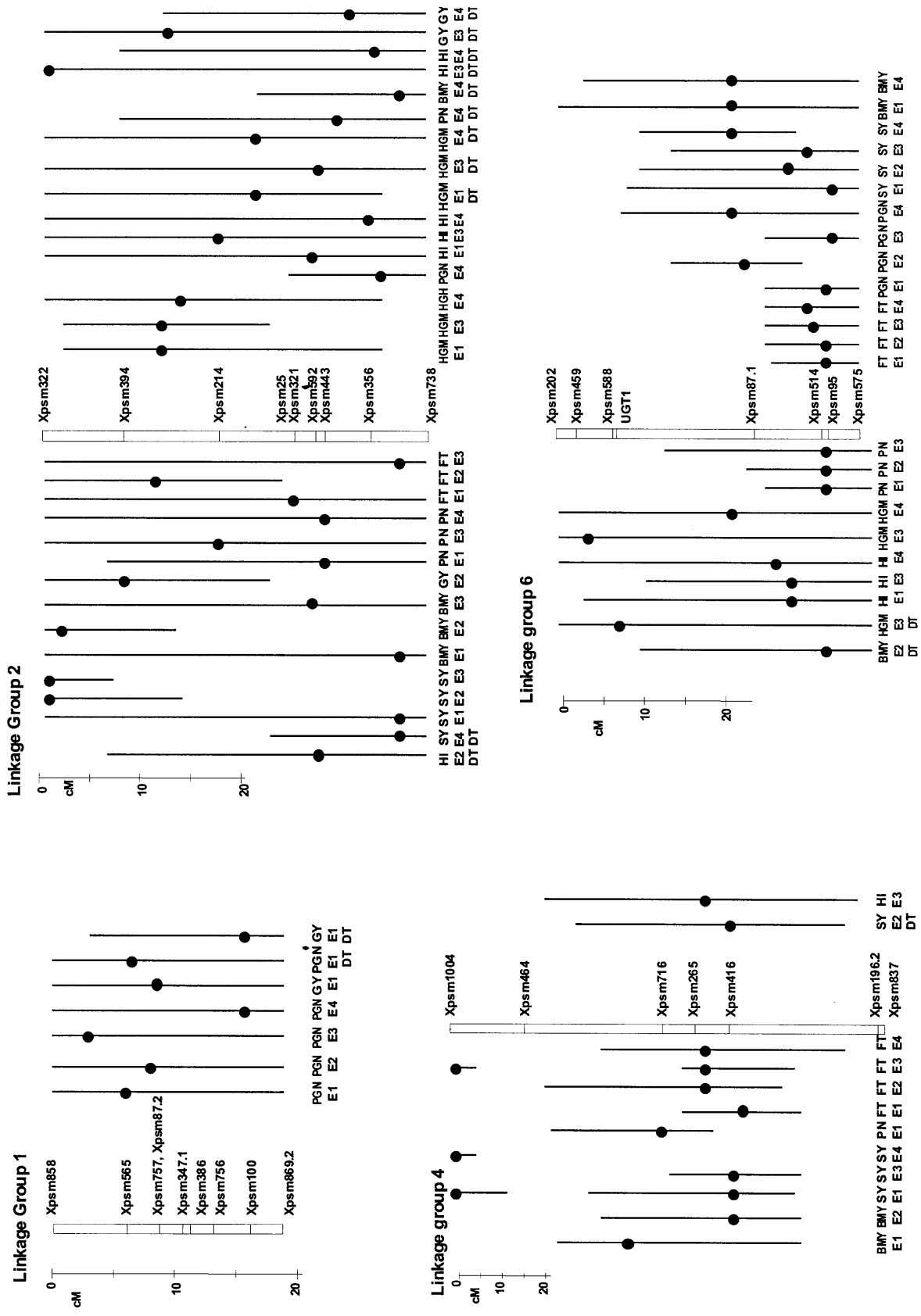
Linkage Group 2
Xpsm322
Xpsm394
Xpsm214
Xpsm25
Xpsm321
Xpsm392
Xpsm443
Xpsm356
Xpsm738
cM
0
10
20
Linkage Group 1
Xpsm858
Xpsm565
Xpsm757, Xpsm87.2
Xpsm347.1
Xpsm386
Xpsm756
Xpsm100
Xpsm869.2
Linkage group 4
Xpsm1004
Xpsm464
Xpsm716
Xpsm265
Xpsm416
Xpsm196.2
Xpsm837
Linkage group 6
Xpsm202
Xpsm459
Xpsm588
UGT1
Xpsm87.1
Xpsm514
Xpsm95
Xpsm575

Table 6 QTLs associated with grain and stover yield determining traits of pearl millet mapping progeny testcross hybrids in four terminal drought-stress environments from three experiments conducted at Patancheru, India, during the hot, dry summer seasons of 1997 and 1998. Estimates of the total LOD values and R^2 values shown in bold letters are those obtained using the combined model. QTLs indicated in italics are those that were not detected at a LOD threshold of 2.0 but significantly contributed to the optimal combined model

Trait	Stress Environment 1						Stress Environment 2						Stress Environment 3						Stress Environment 4					
	Marker intervals	LG	Position[a]	LOD	R^2	Additive effect[b]	Marker intervals	LG	Position	LOD	R^2	Additive effect	Marker intervals	LG	Position	LOD	R^2	Additive effect	Marker intervals	LG	Position	LOD	R^2	Additive effect
Flowering time (FT), days	*Xpsm25–Xpsm321*	*2*	*0*	*1.79*	*20.3*	*–0.84*	*Xpsm394–Xpsm214*	2	3	2.23	12.4	–1.27	*Xpsm356–Xpsm738*	2	4	2.64	13.7	–0.86	*Xpsm686–Xpsm525*	3	0	3.13	12.9	0.83
	Xpsm416–Xpsm196.2	4	6	5.50	35.1	–1.76	*Xpsm265–Xpsm416*	4	4	5.59	26.2	–1.98	*Xpsm1004–Xpsm464*	4	0	4.38	22.6	0.15	*Xpsm265–Xpsm416*	4	4	3.56	18.6	–1.13
	Xpsm95–Xpsm575	6	0	9.57	38.1	2.02	*Xpsm95–Xpsm575*	6	0	10.78	41.7	2.56	*Xpsm265–Xpsm416*	4	4	4.75	26.0	–1.19	*Xpsm87.1–Xpsm514*	6	6	8.23	38.7	1.64
													Xpsm87.1–Xpsm514	6	8	8.19	34.9	1.49						
	Total			**20.29**	**72.7**					**23.33**	**70.8**					**21.08**	**71.0**					**17.23**	**62.9**	
Panicle number (PN)	*Xpsm443–Xpsm356*	2	0	4.26	19.3	–2.15	*Xpsm95–Xpsm575*	6	0	8.04	33.1	–2.64	*Xpsm214–Xpsm25*	2	0	2.40	11.5	–1.11	*Xpsm443–Xpsm356*	2	0	2.58	10.7	–0.93
	Xpsm716–Xpsm265	4	0	2.18	16.4	–1.23	*Xpsm618–Xpsm717*	7	0	2.56	13.0	–1.15	*Xpsm95-Xpsm575*	6	0	4.22	19.0	–1.42	*Xpsm686–Xpsm525*	4	0	2.08	10.0	–0.79
	Xpsm95–Xpsm575	6	0	7.57	31.6	–2.92							*Xpsm618–Xpsm717*	7	0	3.67	16.8	–1.18						
	Total			**16.13**	**58.9**					**10.14**	**41.0**					**10.12**	**39.9**					**5.37**	**23.8**	
100-grain mass (HGM), g	*Xpsm394–Xpsm214*	2	4	4.46	21.8	0.05							*Xpsm394–Xpsm214*	2	4	4.68	23.1	0.06	*Xpsm394–Xpsm214*	2	6	4.08	20.3	0.05
	Xpsm718–Xpsm266	7	16	2.96	17.2	–0.05							*Xpsm459–Xpsm588*	6	2	2.76	13.8	–0.04	*Xugt1–Xpsm87.1*	*6*	*10*	*1.90*	*11.3*	*–0.04*
													Xpsm718–Xpsm266	7	16	2.26	12.6	–0.04						
	Total			**7.70**	**35.7**											**9.49**	**40.8**					**6.08**	**29.6**	
Panicle grain number (PGN)	*Xpsm565–Xpsm757*	1	0	4.44	19.9	130	*Xpsm757–Xpsm87.2*	1	0	2.28	10.8	84	*Xpsm858–Xpsm565*	1	2	3.04	14.7	124	*Xpsm100–Xpsm869*	*1*	*0*	*1.88*	*9.0*	*73*
	Xpsm95–Xpsm575	6	0	9.30	37.2	168	*Xugt1–Xpsm87.1*	6	12	11.72	49.6	193	*Xpsm95–Xpsm575*	6	0	6.77	28.7	173	*Xpsm356–Xpsm738*	*2*	*2*	*1.85*	*9.9*	*86*
	Xpsm618–Xpsm717	7	0	2.07	9.9	82							*Xpsm875–Xpsm618*	7	6	3.08	15.7	126	*Xugt1–Xpsm87.1*	6	10	4.93	27.2	155
																			Xpsm875–Xpsm618	7	4	3.06	14.6	104
	Total			**15.33**	**53.7**					**13.31**	**52.9**					**12.61**	**48.5**					**11.15**	**48.5**	

Table 6 (continued)

Trait	Stress Environment 1						Stress Environment 2						Stress Environment 3						Stress Environment 4					
	Marker intervals	LG	Position[a]	LOD	R^2	Additive effect[b]	Marker intervals	LG	Position	LOD	R^2	Additive effect	Marker intervals	LG	Position	LOD	R^2	Additive effect	Marker intervals	LG	Position	LOD	R^2	Additive effect
Grain yield (GY) g m^{-2}	*Xpsm87.2–Xpsm347.1*	1	0	1.95	9.3	9.29	*Xpsm394–Xpsm214*	2	0	2.25	10.8	–10.70												
Stover yield (SY), g m^{-2}	*Xpsm356–Xpsm738*	2	4	3.61	17.5	–22.90	*Xpsm322–Xpsm394*	2	2	2.70	13.6	–12.50	*Xpsm322–Xpsm394*	2	0	2.09	11.4	–13.00	*Xpsm686–Xpsm525*	3	0	2.47	11.7	9.17
	Xpsm1004–Xpsm464	4	0	2.49	14.8	–1.40	*Xpsm87.1–Xpsm514*	6	4	4.97	24.3	17.60	*Xpsm416–Xpsm196.2*	4	0	3.38	15.6	–17.00	*Xpsm1004–Xpsm464*	4	0	2.74	17.3	–1.10
	Xpsm416–Xpsm196.2	4	0	3.01	14.0	–19.80							*Xpsm87.1–Xpsm514*	6	8	3.86	18.2	18.40	*Xugt1–Xpsm87.1*	6	10	7.91	39.8	19.50
	Xpsm95–Xpsm575	6	0	6.52	27.8	30.10																		
	Total			**15.73**	**56.0**					**7.39**	**34.3**					**10.24**	**41.4**					**12.09**	**52.7**	
Biomass yield (BMY), g m^{-2}	*Xpsm356–Xpsm738*	2	6	4.25	20.0	–34.20	*Xpsm322–Xpsm394*	2	2	2.49	12.3	–19.90	*Xpsm592–Xpsm443*	*2*	*0*	*1.75*	*8.4*	*–13.90*	*Xugt1–Xpsm87.1*	6	10	4.21	23.1	27.0
	Xpsm464–Xpsm716	4	4	2.89	15.1	–27.57							*Xpsm416–Xpsm196.2*	4	0	2.64	14.8	–21.40						
	Xugt1–Xpsm87.1	6	8	2.14	12.2	29.17																		
	Total			**8.97**	**43.1**											**4.60**	**22.9**							
Harvest index (HI), %	*Xpsm595–Xpsm443*	2	0	2.32	11.4	1.51	*Xpsm717–Xpsm812*	7	2	2.21	11.3	–0.44	*Xpsm214–Xpsm25*	2	6	2.73	13.3	1.66	*Xpsm356–Xpsm738*	2	0	4.00	18.2	2.62
	Xpsm87.1–Xpsm514	6	6	3.04	16.1	–1.83							*Xpsm265–Xpsm416*	4	4	2.45	12.9	1.59	*Xpsm87.1–Xpsm514*	6	2	2.11	11.4	–2.02
													Xpsm87.1–Xpsm514	6	4	5.61	26.7	–2.36						
	Total			**5.95**	**27.6**											**12.25**	**49.4**					**7.29**	**32.7**	

[a] Distance (Haldane cM) from the marker on the left side of the interval
[b] Average additive effect of substituting the H 77 allele with the PRLT allele

Panicle number, panicle grain number, hundred-grain mass

Up to four QTLs associated with PN, PGN and HGM were obtained in each individual stress environment (Table 6). Many of these QTLs overlapped with those associated with FT in the same environment (Fig. 1). In all four stress environments, H 77 contributed all alleles for increased PN, while PRLT contributed all alleles for increased PGN. In environments 1, 2 and 3 the QTL for PN with the largest effect mapped on linkage group 6 and explained up to 33% of the phenotypic variation observed. The major QTL for PGN also mapped to a similar region of linkage group 6 in all four environments (Fig. 1) and explained up to 50% of the phenotypic variation observed. A QTL for PGN also mapped on linkage group 1 in all four environments. This QTL was not associated with a QTL for FT or for any other grain yield component traits (Fig. 1). The major QTL for HGM was mapped on linkage group 2 with the positive allele coming from PRLT and explaining up to 23% of the phenotypic variation.

Stover and biomass yield, harvest index

At least two QTLs associated with SY were detected in each environment; in all four environments the major QTL was found on linkage group 6. This QTL explained up to 40% of the phenotypic variation and the allele from PRLT increased SY. QTLs of smaller effect were obtained on linkage groups 2 and 4 in three of the four environments, and in each case it was the allele from H 77 that enhanced SY. In most cases, the parental alleles that were associated with increased FT were also associated with increased SY and BMY (Fig. 1). Up to three QTLs associated with HI were mapped in each environment. In environments 1, 3 and 4 a QTL on linkage group 6 was mapped with the H 77 parental allele enhancing HI. A QTL for HI on linkage group 2 was also obtained in these environments, and in this case the PRLT parental allele provided an increased HI. This QTL was of particular significance in the most-severe drought environment (stress environment 4) whereas in the relatively mild stress environments 1 and 3 it was the QTL on linkage group 6 that was of greater importance.

Grain yield

Two QTLs associated with GY in drought were mapped: one on linkage group 1 in stress environment 1 and the other on linkage 2 in stress environment 2 (Table 6). For the QTL on linkage group 1, the PRLT parental allele provided enhanced grain yield in the late stress of environment 1. In the rain interrupted environment 2, it was the H 77 parental allele for the QTL on linkage group 2 that enhanced grain yield. No QTLs for GY were obtained in stress environments 3 and 4.

For the QTL with the largest additive effect on FT (on linkage group 6), the parental alleles that increased FT were generally associated with an increase in PGN and decreases in HI and PN in mild drought-stress environments (1, 2 and 3) or with decreases in HI and HGM in the severe drought-stress environment 4 (Table 6). The commonality among QTLs for FT, SY, BMY and HI was not unexpected in non-stress or mild-stress environments, since later flowering in pearl millet is normally associated with increased vegetative growth that results in increased BMY – usually with a smaller fraction of this total dry matter being harvested as grain (i.e. lower HI). This inverse relationship of HI and FT is expected to be accentuated in terminal drought-stress environments, as later-flowering genotypes are subjected to more severe stress during their grain filling than are earlier-flowering genotypes, and there is consequently a greater reduction in their GY and HI. Since HGM is the GY component most-adversely affected by increases in drought-stress severity (Table 2), the negative association of some QTLs for FT and HGM in the most severe of these four terminal stress environments was not unexpected. This result, however, suggests that despite the overall independence of FT and GY in these experiments, drought escape did contribute to the observed variation among mapping-progeny testcrosses in at least one GY component, HGM, in at least the most-severe stress.

Whether these associations of QTLs for FT and various GY component traits are due to pleiotropic effects of the individual QTLs for FT, or whether there were specific QTLs for each trait tightly linked to individual QTLs for FT, was difficult to establish in this study due to the relatively small number of mapping progenies that were phenotyped. However, close examination of phenotypic data for testcrosses derived from skeleton-mapped F_2 plants with marker genotype recombination in these regions of closely associated QTLs revealed some with recombinant phenotypes. This suggests that linkage was responsible for at least part of these QTL associations, in addition to the overall (pleiotropic) consequences of a longer vegetative period on yield-component patterns. Fine mapping of these genomic regions should help further resolve this issue.

Several QTLs for PN, PGN and HGM under stress mapped to identical or immediately adjacent intervals (Table 6, Fig. 1). In each case, alleles for higher PN were associated with those for reduced PGN, and/or HGM, so that there was no net effect on GY itself. This co-mapping was consistent with the correlations obtained previously between these GY component traits and in keeping with the mathematical relationship between them and GY (Bidinger et al. 1987a). For example, the product of these three components is constant at a given level of GY, so if one increases then at least one of the remaining two must decrease commensurately. This was particularly evident in several stress environments where H 77 alleles on linkage group 2 increased PN, SY and BMY but decreased HGM and HI (Table 6). A similar situation

was evident for linkage group 6, where H 77 alleles that increased PN (as well as reducing FT) were associated with reduced PGN in all stress environments.

Despite these (apparent) antagonistic associations between QTLs for GY component traits, two genomic regions were found to be associated with GY in drought. In the rain-interrupted, early onset drought-stress environment 2, linkage group 2 alleles for later flowering from H 77 were associated with increased GY, SY and BMY. Apparently, the later-flowering testcrosses with these alleles were able to take advantage of the unexpected late rainfall to prolong normal growth periods, but without either increasing effective tiller number (PN) or reducing HI (at least not by amounts necessary to generate statistically significant QTLs for these traits in this genomic region). In the late-onset drought stress of environment 1, linkage group 1 alleles from PRLT improved grain yield by their significant effect on the PGN component of GY.

QTLs associated with the drought-tolerance index of grain yield and its component traits

In the mild, interrupted, late-onset drought stress environment 1, one QTL on linkage group 1 was associated with the GY drought-tolerance index (percentage of the irrigated control GY maintained in the stress environment). The drought-tolerance index of PGN also mapped to linkage group 1 in this environment (Table 7, Fig. 1). PRLT alleles at both these QTLs were associated with better drought tolerance, suggesting that PRLT alleles in this genomic region contributed to better GY drought tolerance in mild stress through better maintenance of PGN. PRLT alleles on linkage group1 contributed to both increased PGN and GY under stress (Table 6), as well as to the superior drought tolerance of PGN and GY. There was also a QTL for better maintenance of HGM on linkage group 2 in stress environment 1, again associated with PRLT alleles, but it had no significant effect on GY drought tolerance in this environment (Table 7). This was because the alleles for superior maintenance of HGM under stress in this mild-stress environment were associated with increased HGM and HI per se but had reducing effects on PN, SY and BMY (Table 6). These effects of PRLT alleles in this region of linkage group 2 effectively nullified each other in this environment, with the result that this QTL for the maintenance of HGM under drought did not contribute significantly to either GY or to the GY drought-tolerance index.

QTLs associated with the drought tolerance of HI and SY were detected in rain-interrupted early onset stress environment 2 (Table 7). That associated with HI drought tolerance mapped to the same linkage group (linkage group 2) as the QTLs for FT, GY, SY and BMY per se in this environment (Table 6, Fig. 1). The H 77 allele at this QTL was associated with better HI drought tolerance, as well as later FT, and increased GY, SY and BMY per se in this particular stress environment. It is interesting to note that H 77 alleles on linkage group 2 were not associated with increased HI in any of the other stress environments, indicating that this may have been a response to the specific timing of the stress (or the interruption of stress) in environment 2. The most-plausible explanation is that the rain received after the initial onset of drought stress preferentially benefited continued vegetative and/or reproductive growth of later-flowering basal tillers of later-flowering entries with H 77 alleles in this genomic region. Earlier-flowering entries with the PRLT alleles in this region were either too far advanced in their development to be able to respond to the late rain, or had effectively terminated the development of later-flowering basal tillers in response to the initial drought stress, and were unable to maintain an additional sink capacity (PGN or seed size) in order to exploit the more-favourable growth conditions provided by the shower. Thus, it is likely that either a QTL for flowering time and/or a QTL controlling the allocation of photosynthate to later-flowering basal tillers (following relief of intermittent drought stress) has been detected as a HI drought-tolerance QTL in this particular stress environment. The capacity to maintain an effective photosynthate sink (and therefore a high potential HI) under intermittent stress has been reported to be associated with increased GY drought tolerance in both maize (Edmeades et al. 1999) and pearl millet (Bidinger et al. 1987a, b). Such a capacity will thus provide GY advantages in stress environments by maintaining the capacity for a high level of the partitioning of dry matter to grain production.

In the uninterrupted drought-stress environments 3 and 4, a QTL was detected for the GY drought-tolerance index on linkage group 2, explaining up to 23% of the phenotypic variation (Table 7). In both environments, a QTL for the drought-tolerance index of HGM and HI also mapped to this linkage group (Fig. 1). PRLT alleles were associated with the increased drought tolerance of GY as well as its component traits. In the most-severe stress regime studied (stress environment 4), these were accompanied by QTLs for the drought tolerance of PN, SY and BMY, and again PRLT alleles were associated with increased drought tolerance (Table 7). PRLT alleles in this region thus conferred a superior capacity to maintain grain filling (HGM), and thereby maintain both HI and GY, under unrelieved terminal drought. PRLT alleles in this linkage group were also associated with better PN and BMY drought tolerance, but this was at the cost of SY drought tolerance (for which H 77 alleles were superior). The superior drought tolerance of HGM, PN, BMY and HI conferred in this severe drought-stress environment by PRLT alleles on linkage group 2 was associated (by linkage or pleiotropy) with reduced PN and/or reduced SY in each of the four stress environments (Table 6, Fig. 1).

The effectiveness of these linkage group 2 alleles for drought tolerance was confirmed by their association with QTLs for most of these GY component traits per se in stress environment 4 (Table 6, Fig. 1). As with the ex-

Table 7 QTLs associated with the drought-tolerance index (calculated by dividing each testcross entry trait mean in a particular stress environment by its corresponding mean in the paired irrigated control environment) of grain and stover yield determining traits of pearl millet mapping-progeny testcross hybrids in four terminal drought-stress environments from three experiments conducted at Patancheru, India, during the hot, dry summer seasons of 1997 and 1998. Estimates of the total LOD values and R^2 values shown in bold letters are those obtained using the combined model

Trait	Stress environment 1						Stress environment 2						Stress environment 3						Stress environment 4					
	Marker intervals	LG	Position[a]	LOD	R^2	Additive effect[b]	Marker intervals	LG	Position	LOD	R^2	Additive effect	Marker intervals	LG	Position	LOD	R^2	Additive effect	Marker intervals	LG	Position	LOD	R^2	Additive effect
100-grain mass (HGM)	*Xpsm214–Xpsm25*	2	4	2.22	11.5	0.025							*Xpsm592–Xpsm443*	2	0	2.49	11.8	0.023	*Xpsm214–Xpsm25*	2	4	3.19	16.1	0.034
													Xugt1–Xpsm87.1	6	0	2.04	11.6	–0.022						
	Total															**4.51**	**21.5**							
Panicle number (PN)																			*Xpsm443–Xpsm356*	2	2	2.97	15.3	0.048
Panicle grain number (PGN)	*Xpsm565–Xpsm757*	1	2	2.17	10.3	0.065																		
Grain yield (GY)	*Xpsm100–Xpsm869*	1	0	2.85	13.3	0.053							*Xpsm394–Xpsm214*	2	4	2.30	13.5	0.057	*Xpsm443–Xpsm356*	2	2	4.68	23.0	0.060
Stover yield (SY)							*Xpsm416–Xpsm196.2*	4	0	2.23	10.6	0.063							*Xpsm356–Xpsm738*	2	4	2.50	13.2	–0.022
																			Xugt1–Xpsm87.1	6	4	2.98	18.1	0.038
	Total																					**5.37**	**28.1**	
Biomass yield (BMY)							*Xpsm95–Xpsm575*	6	0	2.27	10.7	–0.048							*Xpsm356–Xpsm738*	2	4	3.27	16.2	0.033
Harvest index (HI)							*Xpsm592–Xpsm443*	2	0	2.97	13.8	–0.037	*Xpsm322–Xpsm394*	2	0	1.92	10.1	0.032	*Xpsm443–Xpsm356*	2	4	3.77	18.0	0.064

[a] Distance (Haldane cM) from the marker on the left side of the interval
[b] Average additive effect of substituting the H 77 allele with the PRLT allele

pression of drought tolerance, the more-favourable allele was provided by PRLT for all traits except PN (i.e. HI, HGM, PN and PGN). Therefore, it appears that the PRLT alleles on linkage group 2, although associated with a reduced PN, increased PGN and HGM under unrelieved terminal drought stress resulting in improved HI. Under the severe terminal stress in environment 4, this resulted in better stability (tolerance index) of both grain and biomass production. Under the less-severe terminal drought of stress environment 3, the reduction in PN associated with the PRLT alleles on linkage group 2 resulted in significant reductions of SY and BMY, indicating a trade-off between GY drought-tolerance and SY drought performance and drought tolerance. Careful consideration will need to be made of the frequency and severity of terminal drought-stress occurrence in the target environment before deploying this GY drought-tolerance QTL in dual-purpose pearl millet cultivars intended for both grain and stover production. No significant QTL was found on linkage group 2 for GY per se, in either of these two stress environments (Table 6). In stress environment 3, QTLs for FT, HGM, HI, PN, SY and BMY per se, also mapped to this region on linkage group 2 (Table 6, Fig. 1). Although PRLT alleles in this region were associated with earlier flowering and increased HGM and HI per se, their effects on PN, SY and BMY per se were negative in this stress environment. Similarly for stress environment 4, PRLT alleles in this region were associated with increased HGM, HI and PGN per se, but with reduced PN per se (Table 6). Apparently these antagonistic effects on various GY-determining component traits, prevented the detection of significant direct effects of this genomic region on GY itself in either of these two stress environments, although its effect on GY drought-tolerance was significant in both. The low heritability of GY (Table 5) potentially also contributed to this inability to detect direct effects of this, or any other, genomic region on GY per se in these two stress environments.

A more-detailed analysis of the role of the QTLs for drought tolerance identified on linkage group 2 in stress environments 3 and 4 was carried out by Yadav et al. (1999) to determine whether the superior drought tolerance of GY conferred by this region on linkage group 2 was causally related to a reduced GY in these stress environments, or whether the inherent differences in PN in the mapping-population testcrosses confounded this relationship. These authors calculated a predicted GY in stress environments 3 and 4 for each testcross normalising on the actual PN produced in its paired irrigated control environment. The QTLs associated with these predicted values for GY per se in stress environments 3 and 4 mapped to linkage group 2, along with the other QTLs for drought tolerance of the yield components described above. Moreover, the parental alleles derived from PRLT that contributed to the superior drought tolerance of HI and other GY component traits in these stress environments also contributed to greater predicted GY. Interestingly, the probability threshold of this QTL for predicted GY increased from LOD 2.48 in moderate stress environment 3 to LOD 5.56 in severe stress environment 4. Similar results were obtained when QTLs were analyzed based on a subset of mapping progeny testcrosses having a similar PN in these stress environments (data not shown). This suggests that it was constitutive differences in PN (rather than an effect of stress on PN) among the mapping population testcrosses that resulted in the QTL for GY drought tolerance on linkage group 2 failing to be translated into a QTL for GY per se in these terminal drought-stress environments. From within the test entries it was, however, possible to select entries that had high PN and both a high drought-tolerance index and a high GY per se in these stress environments. Subsets of entries have been selected based on trait performance alone and on genotype at the QTL of interest. These are currently under evaluation to validate further the effect of QTLs on trait performance and on the efficiency of marker-assisted selection relative to phenotypic selection. They are also being evaluated in the background of a number of other testers to investigate the interaction of genetic background on the expression of the QTL identified here.

Conclusions

Drought stress is a widespread but unpredictable phenomenon in many areas in which pearl millet is grown. It can drastically reduce grain and stover yields and yield stability. In this study, a number of QTLs associated with the drought tolerance of pearl millet GY and its component traits have been identified. These provide a new opportunity for plant breeding to increase grain and stover yields of this crop in water-limited environments, if their incorporation into otherwise adapted genotypes can be shown to improve yield under stress. Two of the genomic regions identified (on linkage groups 1 and 2) were associated with a superior GY or better maintenance of GY in terminal drought stress. In addition, a number of genomic regions associated with traits that determine GY under stress have also been identified, but they were often not associated with better maintenance of GY under stress or with actual GY under stress. This lack of an overall effect on GY appears partly due to the negative correlations between the various yield-component traits, which may have been amplified under terminal drought stress. However, the relatively low heritabilities of GY under drought stress were also a contributing factor (Table 5). Grain yield is a complex trait and it is the various components that make up yield that are inherited rather than grain yield itself. Therefore the heritability of grain yield is in general not high and it becomes especially low under stress (Blum 1988). It is possible that some of the QTLs for the components of GY, did contribute directly to GY but that these effects were undetected due to the numbers of mapping population progenies and replications used in the field experiments upon which this QTL analysis was based. A more-de-

tailed spatial analysis of GY may help overcome these limitations, but it is likely that even larger numbers of replications for the drought-stress environments, or larger numbers of mapping progeny crosses to a common tester, will be required before detection of such contributions to GY under stress will be possible.

Nevertheless, some genomic regions have been identified in this study that are associated with GY drought tolerance as well as GY in one or more water-limited environments. PRLT alleles from the genomic region detected on linkage group 1 should be useful in improving grain yield in mild late-onset drought-stress environments such as stress environment 1. This QTL is also of interest as it is not associated with one for PN. Introgression of this genomic region into the high-tillering H 77 parent could be of importance not only in a mild late stress but also in unpredictable drought stress where high-tillering types are better adapted due to their greater developmental plasticity if rain returns. In interrupted early onset drought-stress environments such as stress environment 2, H 77 alleles on linkage group 2 (which appear to be associated with superior tillering capacity) and PRLT alleles on linkage group 6 (that are associated with later flowering) could help to improve grain yield. In the more-readily predictable, uninterrupted, severe drought-stress environments, which reduce grain yields much more, PRLT alleles on linkage group 2 (which appear to be associated with limited tillering capacity but larger individual panicle sink size) could help improve grain yield stability, but at some cost to stover yield in less-severe drought stress.

In each of these drought-stress environments, the increases in both grain yield and in drought tolerance of grain yield were due to the contributions of these genomic regions to increased harvest index, often in association with relatively minor (but apparently important) changes in flowering time. By using testcrosses on 843A to identify these QTLs, we have demonstrated that they will be expressed in hybrid combinations. Some of the QTLs identified in this study could have an almost immediate application in new versions of currently popular hybrids such as HHB 67 (843A × H 77/833–2). However, the effects of other QTLs on the expression of GY under both stress and non-stress need further study before their deployment should be considered. It is important to consider yield potential when improving drought tolerance. Results will be presented later on QTL analysis of the control data sets produced in the experiments reported here. Knowledge of such QTLs will minimise potential deleterious effects in transferring QTLs for drought tolerance. We are currently producing near-isogenic lines for each QTL in the background of the more-agronomically elite parent of the mapping population (i.e. H 77/833–2) to further confirm their effects in a uniform and economically important genetic background. The near-isogenic lines so developed will not only help evaluate the effects of these QTLs in different backgrounds (by evaluating their crosses with a wide range of genetically diverse male-sterile lines) but will also provide genetic tools to further our understanding of the physiological and biochemical pathways that might be involved in the improved drought tolerance conditioned by these QTLs.

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References

Andrews DJ, Anand Kumar K (1996) Use of the West African pearl millet landrace Iniadi in cultivar development. Plant Genet Resour Newslett 105:15–22

Beavis WD, Smith OS, Grant D, Fincher R (1994) Identification of quantitative trait loci using a small sample of topcrossed and F_4 progeny from maize. Crop Sci 34:882–896

Bidinger FR, Mahalakshmi V, Rao GDP (1987a) Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. 1 Factors affecting yield under stress. Aust J Agric Res 38:37–48

Bidinger FR, Mahalakshmi V, Rao GDP (1987b) Assessment of drought resistance in pearl millet. II. Estimation of genotype response to stress. Aust J Agric Res 38:49–59

Bidinger FR, Witcombe JR (1989) Evaluation of specific drought avoidance traits as selection criteria for improvement of drought resistance. In: Baker FWG (ed) Drought resistance in cereals. CAB International, Wallingford, UK, pp 151–164

Blum A (1988) Plant breeding for stress environments. CRC Press, Florida, USA

Ceccarelli S, Grando S (1996) Drought as a challenge for the plant breeder. Plant Growth Reg 20:149–155

Cowen NM (1988) The use of replicated progenies in marker-based mapping of QTLs. Theor Appl Genet 75:857–862

Devos KM, Pittaway TS, Reynolds A, Gale MD (2000) Comparative mapping reveals a complex relationship between the pearl millet genome and those of foxtail millet and rice. Theor Appl Genet 100:190–198

Edmeades GO, Bolaños J, Chapman SC, Lafitte HR, Bänziger M (1999) Selection improves drought tolerance in tropical maize populations. I. Gains in biomass, grain yield, and harvest index. Crop Sci 39:1306–1315

FAO and ICRISAT (1996) The world sorghum and millet economies: facts, trends and outlook. Food and Agricultural Organization of the United Nations (FAO), Rome, Italy, and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, AP, India

Fischer RA, Maurer R (1978) Drought resistance in spring wheat cultivars. 1. Grain yield response. Aust J Agric Res 29:897–912

Frova C, Krajewski P, di Fonzo N, Villa M, Sari-Gorla M (1999) Genetic analysis of drought tolerance in maize by molecular markers. 1. Yield components. Theor Appl Genet 99:280–288

Fussell LK, Bidinger FR, Bieler P (1991) Crop physiology and breeding for drought tolerance: research and development. Field Crops Res 27:183–199

GENSTAT 5 Committee (1993) GENSTAT 5 reference manual. Clarendon Press, Oxford, UK

Jones ES, Liu CJ, Gale MD, Hash CT, Witcombe JR (1995) Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theor Appl Genet 91:448–456

Kapoor RL, Kakkar PS, Khairwal, IS, Bainiwal CR, Nijhawan DC, Yadav HP (1989) Bajra hybrid HHB 67 – a major breakthrough. Haryana Farming XVIII:17, 21

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Liu CJ, Witcombe JR, Pittaway TS, Nash M, Hash CT, Busso CS, Gale MD (1994) An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). Theor Appl Genet 89:481–487

Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron 43:107–153

Mahalakshmi V, Bidinger FR, Raju DS (1987) Effect of timing of water deficit on pearl millet (*Pennisetum americanum*). Field Crops Res 15:327–339

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M (1997) Genome mapping, molecular markers and marker-assisted selection in crop improvement. Mol Breed 3:87–103

Oosterom EJ van, Bidinger FR, Mahalakshmi V, Rao KP (1996) Effect of water availability patterns on yield of pearl millet in semi-arid tropical environments. Euphytica 89:165–173

Prioul J-L, Quarrie S, Causse M, de Vienne D (1997) Dissecting complex physiological functions through the use of molecular quantitative genetics. J Exp Bot 48:1151–1163

Quarrie SA, Stojanovic J, Pekic S (1999) Improving drought resistance in small-grained cereals: a case study, progress and prospects. Plant Growth Reg 29:1–21

Ribaut JM, Jiang C, Gonzalez de Leon D, Edmeades GO, Hoisington D (1997) Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor Appl Genet 94:887–896

Richards RA (1996) Defining selection criteria to improve yield under drought. Plant Growth Reg 20:157–166

Schön CC, Melchinger AE, Boppenmaier J, Brunklaus-jung E, Herrmann RG, Seitzer JF (1994) RFLP mapping in maize: quantitative trait loci affecting testcross performance of elite European flint lines. Crop Sci 34:378–389

Soller M, Beckmann JS (1990) Marker-based mapping of quantitative trait loci using replicated progenies. Theor Appl Genet 80:205–208

Stegmeier WD, Andrews DJ, Rai KN, Hash CT (1998) Pearl millet parental lines 843A and 843B. Int Sorghum and Millets Newslett 39:129–130

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27: 205–234

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G (1997) Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol Breed 3:439–448

Turner NC (1997) Further progress in crop water relations. Adv Agron 50:293–338

Witcombe JR, Soman P (1992) Selection for seedling emergence using a glasshouse test in pearl millet. Plant Breed 108:75–80

Yadav R, Courtois B, Huang N, McLaren G (1997) Mapping genes controlling root morphology and root distribution in a double-haploid population of rice. Theor Appl Genet 94:619–632

Yadav RS, Hash CT, Bidinger FR, Howarth CJ (1999) QTL analysis and marker-assisted breeding of traits associated with drought tolerance in pearl millet. In: Ito O, O'Toole J, Hardy B (eds) Genetic improvement of rice for water-limited environments. International Rice Research Institute, Manila, The Philippines, pp 221–233