Mapping New QTLS for Improvement of Downy Mildew Resistance in Pearl Millet

S. K. Gulia[1,2,3], C. T. Hash[2], R. P. Thakur[2], Wendy A. Breese[4], R. S. Sangwan[3]

[1]Fort Valley State University, Fort Valley, Georgia, USA. [2]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru-502 324, Andhra Pradesh, India; [3]Department of Plant Breeding, CCS Haryana Agricultural University, Hisar-125 004, India; [4]Centre for Arid Zone Studies, University of Wales, Bangor, United Kingdom

Downy mildew (DM) epidemics have repeatedly caused huge grain and straw production losses on pearl millet single-cross hybrids in India. The present study was undertaken to construct a skeleton genetic linkage map for a new pearl millet mapping population of 172 F_2-derived F_4 progenies derived from a single F_1 plant from plant × plant cross ICMB 89111-P6 (susceptible) × ICMB 90111-P6 (resistant), to study the inheritance of DM resistance (DMR) in this population, and to identify and map genomic regions controlling this DMR. More than 45% of polymorphic marker loci showed segregation distortion. A skeleton genetic linkage map of 748 cM (Haldane) was constructed using Mapmaker/Exp ver. 3.0. Of nine major putative DMR QTLs detected, one mapping on linkage group 4 (LG4) was common for all pathogen populations tested except that from Bamako, Mali. Another genomic region was identified on LG2 having major QTLs effective against most of the pathogen populations tested. A majority of the DMR QTLs detected exhibited over-dominant inheritance, and the more resistant alleles were largely from resistant parent ICMB 90111-P6. At least one DMR QTL was detected and mapped for each of the eight pathogen populations used. Marker-assisted selection (MAS) and backcrossing can now be used for improving DMR of elite pearl millet hybrid parental line ICMB 89111, as putative QTLs controlling resistance against a diverse range of DM pathogen populations have been identified.

INTRODUCTION

Pearl millet is grown on about 27 million ha annually, and its grain serves as the staple food for about 90 million people, mainly in dry semi-arid tropical environments. It is better adapted than other cereals to marginal lands of low fertility and low rainfall that

receive little or no inputs. Downy mildew (DM) is the most wide-spread and destructive disease of pearl millet. It is caused by *Sclerospora graminicola*, first reported on pearl millet in India by Butler (1907). Following the release and widespread adoption of genetically uniform pearl millet single-cross hybrids in India in the late 1960s (Dave, 1987), DM became an economically important disease and the first major epiphytotic in India occurred in the early 1970s (Singh and Singh, 1987; Hash, 1997).

In most previous studies, pearl millet DM resistance is reported to be a quantitative trait, controlled by a number of genes. The allogamous and highly variable nature of both the host and the pathogen (Thakur *et al.*, 1992) is a hindrance to breeding for host plant resistance to this disease. Resistance is generally dominant and variation in segregating populations is typically continuous (Singh *et al.*, 1980). Molecular marker technology can expedite conventional plant breeding for host plant resistance, and facilitate effective selection of pyramided resistance. Linkage drag and confounding effects of environmental variation associated with conventional selection can also be reduced.

The first detailed RFLP marker-based genetic linkage map of pearl millet was published by Liu *et al.* (1994) and extended by Devos *et al.* (2000) and Qi *et al.* (2004). Genetic linkage maps in pearl millet have also been constructed and quantitative trait loci (QTLs) have been identified and mapped for DM resistance (Jones *et al.*, 1995, 2002; Breese *et al.*, 2002; Gulia, 2004), rust and blast resistance (Morgan *et al.*, 1998), drought tolerance and grain yield (Yadav *et al.*, 2002, 2003, 2004), and for characters involved in domestication (Poncet *et al.*, 2000; 2002). The present study was conducted with the objectives of constructing a skeleton linkage map for a pearl millet mapping population and then using this to identify and map genomic regions controlling DM resistance effective against diverse pathogen populations of Indian and African origin.

MATERIALS AND METHODS

Parental lines and mapping population advancement: Two pearl millet inbred parental lines, ICMB 89111 (DM susceptible and elite d_2 dwarf line) and ICMB 90111 (DM resistant and genetically tall), were crossed, plant × plant, to produce F_1 hybrids. A single F_1 plant based on parental pair 6 was selfed to produce F_2 seeds for development of a segregating mapping population. About 35 seeds from each of 172 selfed individuals from the F_2 mapping population were sown in pots. Bulk tissue samples were harvested from each pot for isolation of DNA required for marker data generation. The seedlings from each pot were then transplanted to the field as $F_{2:3}$ (F_2-derived F_3) progenies, selfing of which provided $F_{2:4}$ families (=self-bulks), each representative of a single F_2 plant of the mapping population, for use in the DM screens against Indian and African pathogen populations (Fig. 1).

Genomic DNA isolation: Green leaf tissues were collected from 10-14 days old seedling bulks of each $F_{2:3}$ progeny. Genomic DNA was isolated from each tissue sample bulk as described by Sharp *et al.* (1988) modified as a pearl millet maxi-prep CTAB buffer protocol (Mace *et al.*, 2003; Gulia, 2004).

Genotyping of the mapping population: Marker data for 172 $F_{2:3}$ mapping population progenies was generated using 46 polymorphic marker loci including 26 SSRs and 20 RFLPs. Choice of polymorphic markers was initially based on maintaining a

minimum inter-marker linkage distance of 15-20 cM (ideal for QTL detection) based on the consensus map of Qi *et al.* (2004). However, final selection of markers used for genotyping the mapping population was based on clear and scorable polymorphism between the two parents and their F_1.

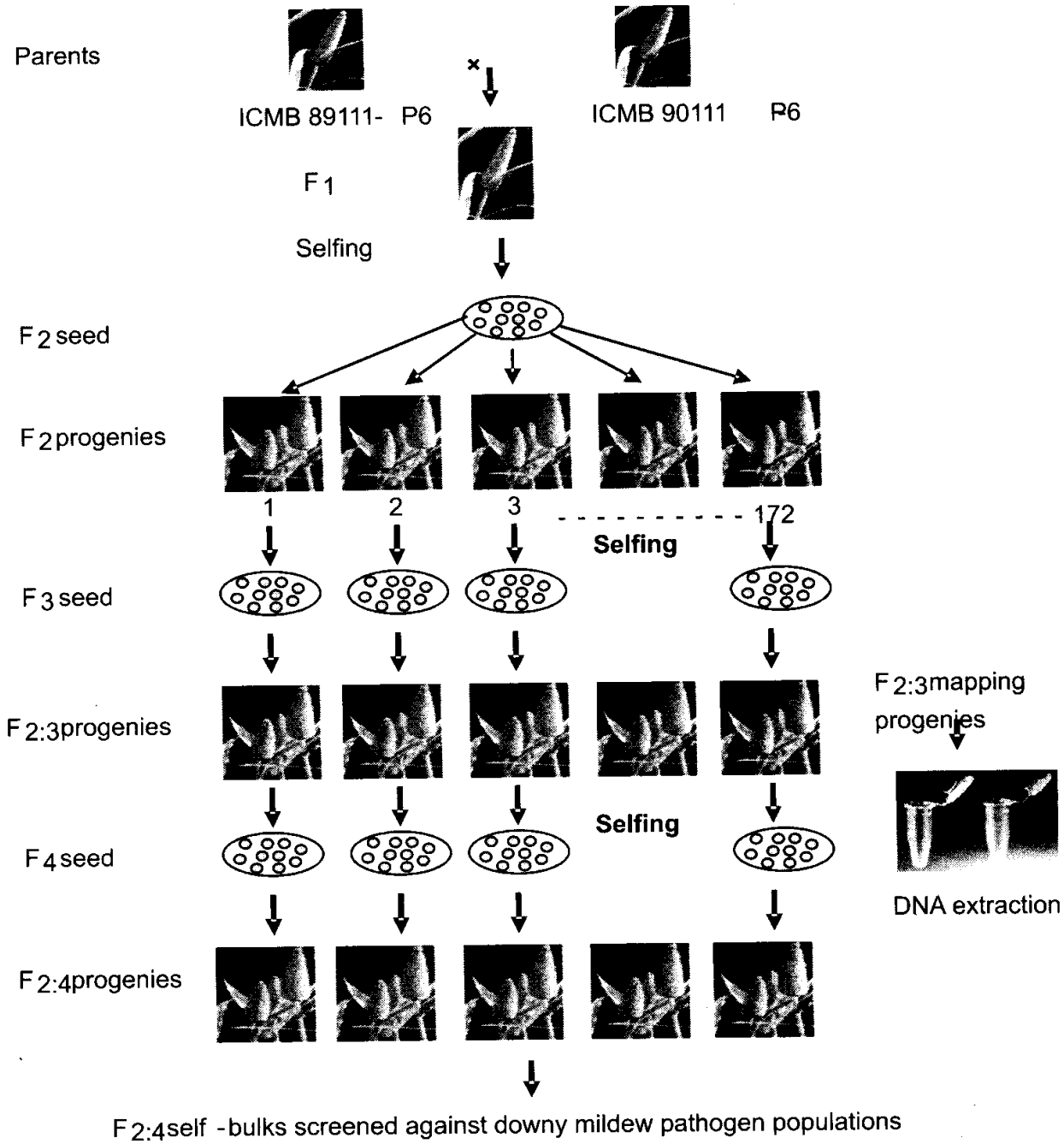

FIG. 1: Advancement of mapping population based on pearl millet cross ICMB 89111-P6 x ICMB 90111-P6, used for genotyping and phenotyping.

Pathogen populations used for DM screening: The mapping population was screened at ICRISAT-Patancheru against six Indian pathogen populations [from ICRISAT, Patancheru (*Sg*153), Maharashtra Hybrid Seeds Company, Jalna (*Sg*150), Gujarat Agricultural University Millet Research Station, Jamnagar (*Sg*200), Central Arid Zone Research Institute, Jodhpur (*Sg*139), Rajasthan Agricultural University Agricultural Research Station Durgapura, Jaipur (*Sg*151) and Indian Agricultural Research Institute, New Delhi

(*Sg*298)] in three time replications, and at Bangor, UK, against two African pathogen populations from Maiduguri, Nigeria and Bamako, Mali, in three replicates. The pathogen populations were maintained on plants of highly downy mildew susceptible host genotypes 7042(S) and F_1 hybrid NHB 3, both of which show >80% infection under heavy inoculum pressure (Thakur *et al.*, 2001).

DM screening procedure for phenotyping: For each $F_{2:4}$ mapping population progeny, 40-45 seeds were sown in a pot (15 cm diameter) filled with autoclaved potting mix of soil, sand and farmyard manure (2:1:1, v/v/v). Seedlings at the coleoptile-to-two-leaf growth stage were counted prior to spray-inoculation with a chilled aqueous suspension of sporangia (about 1×10^6 sporangia mL^{-1}) using a hand sprayer. After 16 hours of incubation under a polyethylene sheet in the dark at 20°C and >95% relative humidity (Jones *et al.*, 2001), pots were shifted from the incubation room to greenhouse benches (25 ± 2°C) and arranged in a completely randomized block design. Downy mildew infection was recorded two weeks later by counting the number of diseased seedlings in each pot and downy mildew incidence (DMI) percentage was calculated. GenStat was used to analyze the mapping progeny DM screening data set obtained with each of the eight pathogen populations from India and Africa, providing entry means, heritability estimates, and tests of significance for each screen and across screens.

Linkage map construction and QTL analysis: Linkage analysis was accomplished using MapMaker/Exp version 3.0b (Lincoln *et al.*, 1992a). centiMorgan (cM) distances were calculated using the Haldane function. The pearl millet base map (Liu *et al.*, 1994) and integrated consensus map (Qi *et al.*, 2004) were used for comparison. Mapchart was then used to draw all linkage groups of the genetic linkage map. QTL mapping was performed using simple interval mapping (SIM) as implemented in MapMaker/QTL ver. 1.1b (Lincoln *et al.*, 1992b) and composite interval mapping (CIM) as implemented in PlabQTL ver. 1.1 (Utz and Melchinger, 2000) using 2.5 as LOD threshold to declare the presence of a QTL.

RESULTS

Downy mildew incidence (DMI): Resistant parent ICMB 90111-P6 was resistant to highly resistant against all pathogen populations except Jalna (24% DMI), while susceptible parent ICMB 89111-P6 was highly to very highly susceptible (69-90% DMI) to all pathogen populations, except those from Jalna and Maiduguri, to which it was moderately susceptible (14-35% DMI). Among all screens, the pathogen population from Jodhpur (36.9% mean DMI) was most virulent, while that from Jalna was the least virulent (12.7% mean DMI) on the mapping population progenies (Table 1). For each screen, the variation in DMI among $F_{2:4}$ self-bulks was highly significant. The pooled ANOVA from replicated data of $F_{2:4}$ self-bulks across all eight screens from India and Africa, revealed no significant variability in entry mean DMI. However, significant variability in entry mean was detected across the six pathogen populations from Asia and across the two pathogen populations from Africa. All screens exhibited high operational heritabilities (Table 1), ranging from 0.66 to 0.95 on mean basis and 0.43 to 0.81 on plot basis.

TABLE 1: Mean downy mildew incidence (%) of parental lines, susceptible control and $F_{2:4}$ mapping population progenies, and their analyses of variances

Pathogen	Jodhpur	Patancheru	New Delhi	Jamnagar	Jaipur	Jalna	Asian Isolates	Maiduguri	Bamako	African Isolates	All 8 Isolates
ICMB 89111-P6	72	91	69	94	95	14		35	82		
ICMB 90111-P6	9	3	6	4	10	24		0	1		
7042(S)	100	99	96	99	99	95		75	85		
$F_{2:4}$ progenies mean	37.9±9.7	23.7±7.3	20±7.0	28±6.6	31±7.7	13±5.9	25±3.0	13±4.7	34±6.9	23±4.5	25±3.0
Significance of ANOVA (Entry)	**	**	**	**	**	**	**	**	**	**	ns
Significance of ANOVA (Entry x Pathogen)							**			**	**
h^2 (plot basis)	0.61	0.75	0.58	0.79	0.69	0.42	0.57	0.81	0.78	0.43	0.56
aSEM											

**P ≤ 0.01, ns – non-significant at $P = 0.05$

Genetic linkage map: A genetic linkage map of seven linkage groups (LGs) with a total length of 747.9 cM was constructed (Fig. 2). Most LGs accommodated both SSR and RFLP markers, except LG5, which consisted of eight SSRs markers only. LG3 was the shortest (30.2 cM) while LG7 was the longest (195.2 cM). Inter-marker distances among all marker loci across LGs ranged from a minimum of 1.3 cM between *Xpsmp*2276 and *Xpsmp*2277 on LG5 to the maximum of 65.9 cM between *Xpsm*409.1 and *Xpsm*648 on LG4. The remaining marker loci in LG4 were located close to each other with inter-marker intervals ranging from 2.4 cM to 9.6 cM. The inter-marker distances between centrally placed SSRs were much lower than the distally located RFLPs on LG7. SSR marker loci *Xpsmp*2261, *Xpsmp*2276 and *Xpsmp*2277, which had been mapped in earlier studies, were added at the bottom of LG5.

QTLs for downy mildew resistance (DMR): The effects, number and positions of QTLs identified by the two software packages on different LGs differed slightly. For example, the number of major DMR QTLs identified by Map Maker/QTL ranged from a minimum of only one (Maiduguri) to a maximum of five (Jodhpur) (Table 2), while for PlabQTL (not shown) this ranged from one (Maiduguri) to four (Jodhpur, Patancheru and Jaipur). The best single-QTL models identified by Map Maker/QTL recorded LOD scores ranging from 10.1 in case of Jaipur (explaining 68.5% of observed phenotypic variation among progenies) to 16.2 in case of Maiduguri (explaining 69.5% of observed phenotypic variation in disease reaction). Multiple QTL-models of Map Maker/QTL (Table 2) generally confirmed the numbers and positions of DMR QTLs identified for all pathogen populations except for Maiduguri where the qualifying criterion for accepting a multiple QTL-model could not be satisfied. The most complex qualified multiple-QTL model detected was a four-QTL-model for DMR effective against the Patancheru pathogen

population, which included two QTLs on LG2 and one each on LG3 and LG4, had a high LOD value of 25.1 (>21.9+2.0 = 23.9), and explained 90.4% of observed phenotypic variation.

Two common blocks of DMR QTLs effective against most of the pathogen populations were identified – one each in the vicinity of marker loci *Xpsm*409.1 on LG4 and *Xpsm*708.1 on LG2. In addition, a number of pathogen-population-specific DMR QTLs were detected (Table 2).

Inheritance of DMR QTLs: The predominant mode of inheritance for DMR QTLs identified by single- as well as multiple-QTL models was dominance to over-dominance, with the more resistant alleles being contributed by resistant parent ICMB 90111-P6 for nearly all QTLs. Some exceptional DMR QTLs with recessive, additive, or partially dominant resistance alleles were observed, with by resistance at some of these coming from susceptible parental line ICMB 89111-P6 (Table 2).

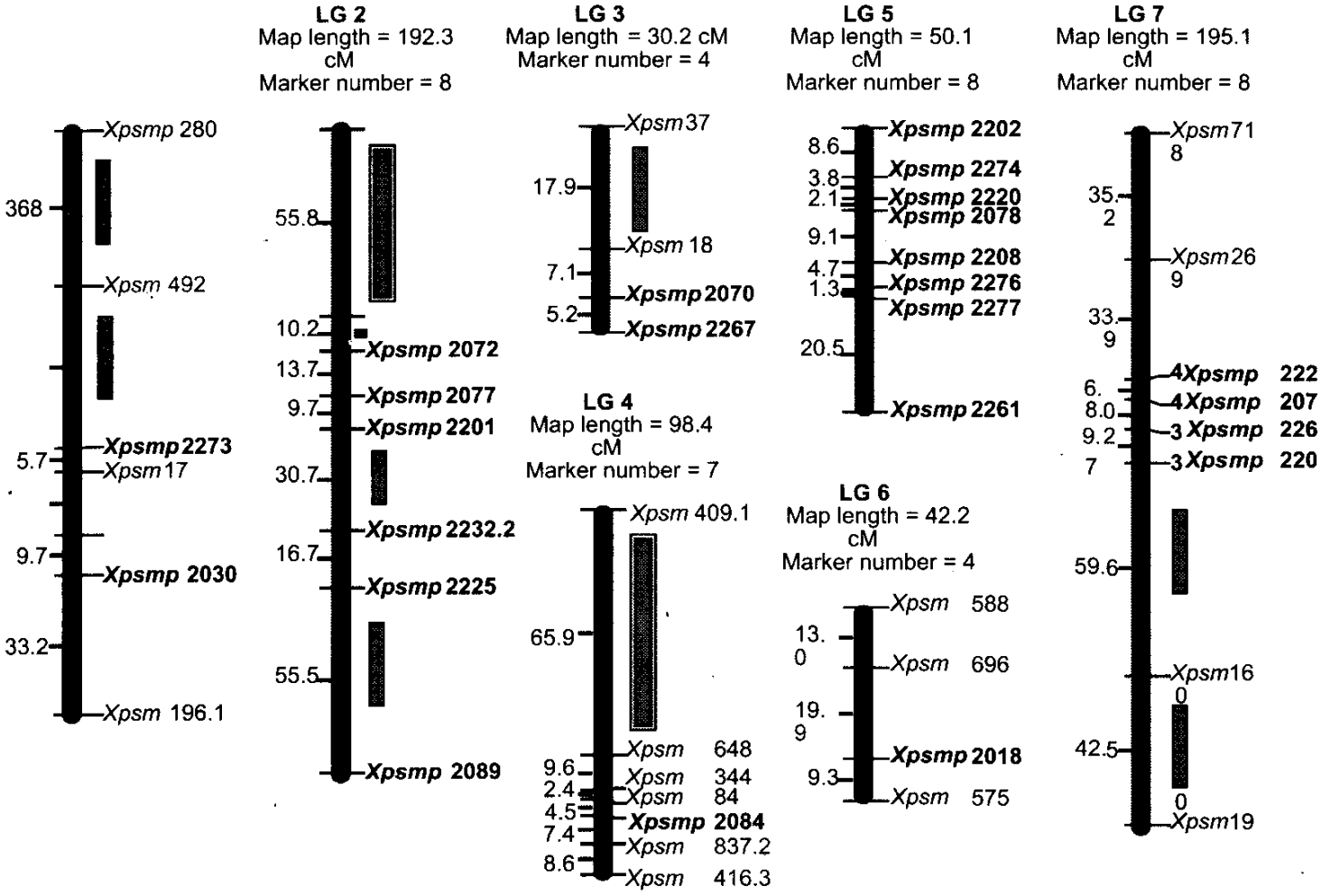

FIG. 2: Skeleton linkage map of pearl millet from cross ICMB 89111-P6 x ICMB 90111-P6. Markers in bold black are SSR (microsatellite) loci and those in black are RFLP loci. Inter-marker distances (Haldane cM) are given to the left each linkage group (LG). Light black vertical bars indicate positions of putative DMR QTLs effective against various pathogen populations. Double lined bars on LG2 and LG4 are the common blocks of QTLs effective across pathogen populations.

TABLE 2: Summary of significant QTLs for downy mildew resistance effective against Indian and African pathogen populations of *S. graminicola* from results of simple interval mapping as implemented in Map Maker/QTL. A negative sign for additive effects indicates that resistance is inherited from the more resistant parent (ICMB 90111-P6), while a positive sign indicates resistance is inherited from the more susceptible parent (ICMB 89111-P6)

Pathogen populations	Best single-QTL models								Highest qualified multiple-QTL models						
	LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]		LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]
Indian															
Jodhpur	1	*Xpsm*280+36.2	3.3	9.7	-10.6	3.4	Rec								
	2	*Xpsmp*2201+17.7	3.9	16.7	-7.3	16.0	Rec								
	3	*Xpsm*37+16.3	3.0	9.1	10.1	-0.6	Add		2	*Xpsm*708.1+36.1			-14.1	-7.6	OD
	4	*Xpsm*409.1+31.0	**11.0**	65.3	-27.2	-10.0	OD		3	*Xpsm*18+1.7			10.0	-0.5	Add
	7	*Xpsm*160+40.2	4.2	12.2	-10.9	5.5	Rec		4	*Xpsm*409.1+29.4	**19.2**	83.2	-24.7	-9.4	OD
Jalna	2	*Xpsmp*2225+30.8	4.5	55.4	-12.2	-12.0	OD		4	*Xpsm*409.1+40.7			-13.2	-10.1	OD
	4	*Xpsm*409.1+40.9	**10.2**	62.9	-13.2	-10.1	OD		7	*Xpsmp*2224+5.0	**13.9**	66.8	3.0	-2.9	Rec
Jaipur	2	*Xpsm*708.1+27.3	7.0	58.2	-21.9	-17.7	OD		2	*Xpsm*708.1+26.1			-21.8	-17.0	OD
	4	*Xpsm*409.1+39.1	**10.1**	68.5	-28.3	-14.5	OD		2	*Xpsmp*2225+51.2			-3.3	-4.5	OD
	7	*Xpsm*160+34.5	3.0	14.3	-10.8	-5.4	OD		4	*Xpsm*409.1+37.2	**15.0**	82.3	-14.8	-4.2	Dom
Patancheru	2	*Xpsm*708.1+28.1	10.4	66.5	-28.4	-22.5	OD		2	*Xpsm*708.1+26.2			-12.5	-6.6	OD
	4	*Xpsm*409.1+37.3	**12.9**	70.5	-30.6	-22.8	OD		2	*Xpsmp*2225+39.2			-6.2	-9.2	OD
	7	*Xpsmp*2203+27.3	8.8	67.1	-27.5	-30.5	OD		3	*Xpsm*37+5.4			5.1	1.3	Dom
									4	*Xpsm*409.1+36.7	**25.1**	90.4	-25.3	-20.3	OD
Jamnagar	2	*Xpsm*708.1+26.3	**12.7**	67.2	-27.5	-21.0	OD								
	4	*Xpsm*409.1+37.3	11.2	68.3	-29.2	-19.4	OD		2	*Xpsm*708.1+25.1			-27.6	-19.5	OD
	7	*Xpsmp*2203+29.9	7.7	66.8	-23.5	-25.6	OD		4	*Xpsm*409.1+40.1	**19.8**	88.8	-14.4	-7.4	OD
New Delhi	2	*Xpsm*708.1+29.9	5.6	56.2	-16.2		-14.4	OD							
	4	*Xpsm*409.1+39.0	**10.5**	63.6	-20.1		-12.7	OD	2	*Xpsm*708.1+26.2			-9.4	-8.8	OD

Pathogen populations	Best single-QTL models								Highest qualified multiple-QTL models						
	LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]		LG[a]	Position[b]	LOD[c]	Var[d]	Add[e]	Dom[f]	Inher[g]
	7	*Xpsmp*2203+29.9	3.5	54.6	-16.1		-17.2	OD	4	*Xpsm*409.1+39.5	**17.0**	82.1	-19.5	-13.2	OD
African															
Maiduguri	2	*Xpsm*708.1+29.2	**16.2**	69.5	-24.6		-21.1	OD							
Bamako	1	*Xpsm*492+12.3	5.9	31.7	-20.3		-4.5	Dom							
	2	*Xpsmp*2201+14.2	3.2	15.8	-10.0		13.0	Rec	2	*Xpsmp*2201+20.5	**19.6**	80.3	-6.0	6.5	Rec
	4	*Xpsm*409.1+20.8	**15.5**	73.9	-28.9		-4.4	PD	4	*Xpsm*409.1+19.8			-27.3	-5.2	PD
	7	*Xpsm*160+42.1	5.0	15.0	-13.3		-0.6	Add	7	*Xpsm*160+35.7			-5.6	-3.6	OD

[a] Linkage groups; [b] Position of the QTL with the marker loci; [c] Log likelihood score; [d] Percentage of variation explained for downy mildew resistance; [e] Additive effect; [f] Dominance effect; [g] Estimated mode of inheritance for resistance: OD = Over-dominance, Dom = Dominance, PD = Partial dominance, Add = Additive, Rec = Recessive

DISCUSSION

DM screens: The highly significant variability observed among $F_{2:4}$ self-bulks and high heritabilities (Table 1) demonstrated that a large portion of the variation in DMI among mapping population progenies in this study was attributable to genetic variation, as also reported by Jones *et al.* (1995, 2002). The distribution of host plant resistance was continuous, as reported in most previous studies (Singh *et al.*, 1980; Basavaraju *et al.*, 1981o; Dass *et al.*, 1984; Shinde *et al.*, 1984; Jones *et al.*, 1995). Mapping population progeny × pathogen interactions were highly significant. Intercontinental variation among pathogen populations of *S. graminicola* has previously been reported by Ball (1983) and Ball and Pike (1984), based on a more genetically diverse set of pearl millet hosts. Significant effects of entries and entries × pathogen population interactions were also observed for downy mildew incidence by Jones *et al.* (1995) and Thakur *et al.* (2001) in their studies of the genetics of resistance to downy mildew in pearl millet.

Genetic linkage map: A linkage map of 747.9 cM (Haldane) was constructed using 46 marker loci with an average inter-marker map distance of <20 cM, which is ideal to locate QTLs of interest. The total genome length of previously constructed pearl millet marker-based genetic linkage maps ranged from 287.7 cM (base map, LGD 1-B-10 × ICMP 85410-P7) to 695.7 cM (81B-P6 × ICMP 451-P8) with an average map length of 587.2 cM, which is moderately less than the newly constructed map (Table 3). Despite substantial increases in lengths over the original pearl millet base map of Liu *et al.* (1994), more recent studies continue to confirm that pearl millet has a short linkage map compared to all other major cereals.

There are clear indications (Table 3) of increases in the map lengths of LG1, LG2, LG4, LG6 and LG7 since the pearl millet base map (Liu *et al.*, 1994) was first published. The identification of additional polymorphic RFLP and SSR markers located on both upper and lower distal ends of several linkage groups in pearl millet, quite far from putative centromeric regions, has resulted in this increase in total linkage map length, as previously reported by Devos *et al.* (2000). Other possible reasons for this apparent increase in map length could be sizes of the various mapping populations, genetic constitution of their parental lines, number and polymorphism of marker loci obtained for these parental lines, segregation distortion and putative double crossovers in later studies. Marker order on all seven linkage groups obtained in this study (Fig. 2) is essentially in complete agreement with the published pearl millet base map of Liu *et al.* (1994) and consensus map of Qi *et al.* (2004). In the original base map (Liu *et al.*, 1994) pseudo-linkage of LG1 and LG2 was observed, but the present study witnessed a clear separation of LG1 and LG2, also supported by (Qi *et al.*, 2004). Map lengths reported for LG2 range from the minimum of 36.2 cM (Liu *et al.*, 1994) to the high of 192.3 cM in the present study. LG3 was the shortest among all seven linkage pearl millet groups in this study, as in most previous ones (Devos *et al.*, 2000; Qi *et al.*, 2004; and Yadav *et al.*, 2004). The mapped portion of LG4 (98.3 cM) in this study was relatively larger than in the base map (46.0 cM) but shorter than in a few recently mapped pearl millet populations. The addition of three new SSR markers (*Xpsmp*2261, *Xpsmp*2276 and *Xpsmp*2277) to the lower distal end of LG5 in the current study contributed to the 20 cM increase in its length compared to the base map. LG6 was slightly longer (42.2 cM) than the published base map of 32.5 cM (Liu *et al.*, 1994). However, the only four polymorphic markers on LG6 in this study were mapped to the centromeric region as there was a lack of polymorphic markers for both its

upper and lower distal regions. Linking of two RFLP markers (*Xpsm*160 and *Xpsm*190) to the lower distal end of LG7 in the current study provided a further increase of 102.1 cM in the total map length of LG7. In the earlier study by Liu *et al.* (1994), these two linked RFLP markers formed a sub-linkage group with 5.9 cM intermarker distance that segregated independently from the seven expected major pearl millet linkage groups. In an earlier study of cross 841B × 863B, RFLP marker loci *Xpsm*269 and *Xpsm*718 together with SSR locus *Xpsmp*2203 added 100 cM to the other end of LG7 (Yadav *et al.*, 2004).

TABLE 3: Total mapped genome and linkage group lengths for four pearl millet mapping populations. The map distances are shown in Haldane centimorgans (cM) except for that reported by Liu *et al.* (1994), for which the Kosambi mapping function was used

Mapping population pedigree	LGD 1-B-10 x ICMP 85410-P7	81B-P6 x ICMP 451-P8	ICMB 841-P3 x ICMB 863-P2	ICMB 89111-P6 x ICMB 90111-P6
LG				
1	73.4	77.3	104.9	139.6
2	36.2	175.8	179.0	192.3
3	38.2	52.2	15.4	30.2
4	63.2	132.4	64.3	98.3
5	30.9	102.8	26.9	50.1
6	32.5	58.3	113.1	42.2
7	13.3	96.9	113.8	195.2
Total mapped genome length	287.7	695.7	617.4	747.9
Mapped (F_2) population size	133	184	147	172
Reference	Liu *et al.*, 1994	Devos *et al.*, 2000	Yadav *et al.*, 2004	This study (Gulia, 2004)

QTL mapping for downy mildew resistance (DMR): A minimum of nine host-plant DMR QTLs were identified in this study. These were distributed across all pearl millet linkage groups except LG5 and LG6. At least one major DMR QTL was identified for each pathogen population from India and Africa against which the mapping population progenies were screened. DMR QTLs explaining more than 50% of observed phenotypic variation in a particular screen and having comparatively high LOD score values were considered major resistance QTLs as suggested by Mackill and Junjian (2001). A summary of these QTLs is presented in Table 2. The largest number of single QTLs for host-plant resistance were detected in screens against the pathogen population from Jodhpur (5 QTLs distributed across LG1, LG2, LG3, LG4 and LG7). QTL analyses from DM screens against pathogen populations from Jaipur, Patancheru, Jamnagar and New Delhi each detected three QTLs mapped on LG2, LG4 and LG7. These QTL mapping results suggested only limited differences in the genetic constitution of virulence in these four pathogen populations of Indian origin (at least with regards to host plant resistances segregating in this mapping population). However, detection of major pathogen-population-specific QTLs with other DM screening data sets suggested significant differences in the genetic structure of pathogenicity and virulence across the wider set of eight pathogen populations used in this study. This is supported by previous studies by Ball and Pike (1984), where virulence differences between pathogen populations from India and Africa were found. Results from the screen against the Maiduguri pathogen population detected

only a major DM resistance QTL on LG2, perhaps because of epistatic masking other QTLs of small effects, insufficient numbers or genomic coverage of marker loci to locate other resistance QTLs, or insufficient power and precision of the analysis to detect QTLs of small effect with the relatively small mapping population used.

Although previous studies have reported independent inheritance of DMR to different populations of *S. graminicola* across India and Africa, in the current study two common DMR QTLs were obtained against most of pathogen populations. These common blocks of DMR QTLs were located on LG4 (interval *Xpsm*409.1–*Xpsm*648) and LG2 (interval *Xpsm*708.1–*Xpsmp*2237), with their exact positions within these intervals varying across pathogen populations. The map positions of these QTLs could not be more precisely located because of a lack of polymorphic marker loci between the two markers flanking them on LG4 and LG2. Such common blocks of DMR QTLs are of great interest to pearl millet breeders, as they could confer broad-spectrum and durable resistance. Failure to detect these QTLs in a few screens could be either due to the lack of an appropriate virulence gene to overcome alleles of susceptible parent ICMB 89111-P6 or due to presence of a virulence gene in the pathogen population capable of overcoming the resistant allele from resistant parent ICMB 90111-P6. In addition to the above results, there were several major QTLs conferring DMR to specific pathogen populations, which mapped either to common positions on a particular linkage group or on different positions on different linkage groups, as reported by Jones *et al.* (1995). These QTLs were identified as major host plant resistance QTLs with varying LOD scores (5.6 to 12.7) and typically explained substantial portions of the observed phenotypic variation in a particular screen (from 56.2% to 67.2%).

Mode of inheritance of DMR QTLs: For a majority of DMR QTLs identified in this study, an increase in resistance was inherited from the resistant parent, as previously reported by Jones *et al.* (1995). Similarly, the inheritance of resistance was most often dominant to over-dominant as also reported by Jones *et al.* (1995), except a few QTLs where it was recessive, additive, or partially dominant. Singh *et al.* (1978) reported a recessive resistance gene governing downy mildew inheritance in pearl millet. Recessive resistance has also been observed in other plant-pathogen systems (Day, 1974; de Wit, 1992). It was noticed that for some QTLs, the mode of inheritance appeared to vary across different pathogen populations. Possible explanations for such variation in mode of inheritance include error in assessing QTLs of small effect, closely linked genes, or different alleles at the same locus or inter-allelic dominance interactions affecting the apparent inheritance of resistance (Jones *et al.*, 1995), and differences in the virulence of pathogen populations used to detect these QTLs.

CONCLUSIONS AND FUTURE IMPLICATIONS

In this study, a pearl millet genetic linkage map of 747.9 cM (Haldane) was constructed with 46 co-dominant marker loci, which provided fairly good genomic coverage. Development and mapping of additional polymorphic markers to better saturate gaps in its linkage map would make this pearl millet mapping population ideal for QTL detection. A total of nine different DMR QTLs linked to flanking markers were identified using disease screening data from eight downy mildew pathogen populations. Among these, blocks of DMR QTLs identified on LG2 and LG4 were effective against most of the

pathogen populations used in this study. Such blocks of disease resistance genes, appropriately backstopped by pyramiding with additional resistances, are expected to provide resistance that is durable for longer periods. Several pathogen population-specific DMR QTLs were also identified on LG1, LG2, LG4 and LG7. Although such QTLs are not considered likely to provide durable resistance, their use in pathogen population- and/or region-specific breeding programs can be recommended. In all such cases, MAS provides an opportunity for plant breeders to select for DMR in the absence of the pathogen and allows efficient development of genotypes with pyramided resistances. The best option would be introducing and pyramiding DMR genes from different sources of resistance to a variety of pathogen populations into the genetic background of a few genetically diverse elite seed parent maintainer lines. Then, these genotypes with pyramided resistances could be used to breed high yielding hybrids expected to have more durable DM resistance.

REFERENCES

Ball, S.L., 1983. Pathogenic variability of downy mildew (*Sclerospora graminicola*) on pearl millet. I. Host cultivar reactions to infection by different pathogen isolates. *Ann. Appl. Biol., 102*: 257-264.

Ball, S.L. and Pike, D.J., 1984. Intercontinental variation of *Sclerospora graminicola*. *Ann. Appl. Biol., 104*: 41-51.

Basavaraju, R., Safeeulla, K.M. and Murty, B.R., 1981o. Inheritance of resistance to downy mildew in pearl millet. *Indian J. Genet., 41(1)*: 144-149.

Breese, W.A., Hash, C.T., Devos, K.M. and Howarth, C.J., 2002. Pearl millet genomics: An overview with respect to breeding for resistance to downy mildew. *In*: Sorghum and Millets Diseases. (Leslie, J.F., Ed.). Iowa State Press. Ames, Iowa, USA: Pp. 243-246.

Butler, E.J., 1907. Some diseases of cereals caused by *Sclerospora graminicola*. Memoirs of the Department of Agriculture in India. Botanical Series, 2: 1-24.

Dave, H.R., 1987. Pearl millet hybrids. *In*: Proceedings of the International Pearl Millet Workshop. (Witcombe, J.R. and Beckerman, S.R., Eds.). Patancheru, India: ICRISAT, pp. 121-126.

Day, P.R., 1974. Genetics of host parasite interactions. W.H. Freeman and Company, San Francisco, USA.

de Wit, P.J.G.M., 1992. Molecular characterization of gene-for-gene system in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. *Ann. Rev. Phytopathol., 30*: 391-418.

Devos, K.M., Pittaway, T.S., Reynolds, A. and Gale, M.D., 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.

Hash, C.T. and Witcombe, J.R., 2002. Gene management and breeding for downy mildew resistance. *In*: Sorghum and Millets Diseases. (Leslie, J.F., Ed.). Iowa State Press. Ames, Iowa, USA. pp. 27-36.

Hash, C.T., 1997. Research on downy mildew of pearl millet. *In*: Integrating research evaluation efforts: Proceedings of an International Workshop, 14-16 Dec 1994. (Bantilan, M.C.S. and Joshi, P.K., Eds.). ICRISAT. Patancheru, India. Pp. 121-128.

Jones, E.S., Breese, W.A. and Shaw, D.S., 2001. Infection of pearl millet by the downy mildew fungus *Sclerospora graminicola*: Chilling inoculum to prevent zoospore release and subsequent spray damage to zoospores. *Plant Pathol.*, *50*: 1-8.

Jones, E.S., Breese, W.A., Liu, C.J., Singh, S.D., Shaw, D.S. and Witcombe, J.R., 2002. Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Field and greenhouse screens detect the same QTL. *Crop Sci.*, *42*: 1316-1323.

Jones, E.S., Liu, C.J., Gale, M.D., Hash, C.T. and Witcombe, J.R., 1995. Mapping quantitative trait loci for downy mildew resistance in pearl millet. *Theor. Appl. Genet.*, *91*: 448-456.

Lincoln, S., Daly, M. and Lander, E., 1992a. Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report. 2nd edition.

Lincoln, S., Daly, M. and Lander, E., 1992b. Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report. 3rd edition.

Liu, C.J., Witcombe, J.R., Pittaway, T.S., Nash, M., Hash, C.T., Busso, C.S. and Gale, M.D., 1994. An RFLP-based genetic linkage map of pearl millet (*Pennisetum glaucum*). *Theor. Appl. Genet.*, *89*: 481-487.

Mace, E.S., Buhariwalla, H.K. and Crouch, J.H., 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant. Mol. Biol. Rep.*, *21*: 459-460.

Mackill, D.J. and Junjian, Ni, 2001. Molecular mapping and marker assisted selection for major gene traits in rice. *In*: Proceedings of the 4th International Rice Genetics Symposium (Khush, G.S., Brar, D.S. and Hardy, B., Eds.). International Rice Research Institute. Los Banos, Philippines.

Morgan, R.N., Wilson, J.P., Hanna, W.W. and Ozias-Akin, P., 1998. Molecular markers for rust and pyricularia leaf spot disease resistance in pearl millet. *Theor. Appl. Genet.*, *96*: 413-420.

Poncet, V., Lamy, F., Devos, K.M., Gale, M.D., Sarr, A. and Robert, T., 2000. Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L., Poaceae). *Theor. Appl. Genet.*, *100*: 147-159.

Poncet, V., Martel, E., Allious, S., Devos, K.M., Lamy, F., Sarr, A. and Robert, T., 2002. Comparative analysis of QTLs affecting domestication traits between two domesticated × wild pearl millet (*Pennisetum glaucum* L., *Poaceae*) crosses. *Theor. Appl. Genet.*, *104*: 965-975.

Qi, X., Pittaway, T.S., Lindup, S., Liu, H., Waterman, E., Padi, F.K., Hash, C.T., Zhu, J., Gale, M.D. and Devos, K.M., 2004. An integrated genetic map and a new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. *Theor. Appl. Genet.*, *109*: 1485-1493.

Sharp, P.J., Kries, M., Sherry, P.R. and Gale, M.D., 1988. Location of β-amylase sequences in wheat and its derivatives. *Theor. Appl. Genet.*, *75*: 286-290.

Singh, S.D. and Govind Singh, 1987. Resistance to downy mildew in pearl millet hybrid NHB 3. *Indian Phytopathol.*, *40*: 178-180.

Singh, F., Singh, R.K., Singh, R.M. and Singh, R.B., 1978. Genetic analysis of downy mildew (*Sclerospora graminicola*) resistance in pearl millet [*Pennisetum typhoides* (Burm.) S. and H.]. *Z. Pflanzenzücht.*, *81*: 54-59.

Singh, F., Singh, R.M., Singh, R.B. and Singh, R.K., 1980. Genetics studies of downy mildew resistance in pearl millet. *In:* Trends in Genetical Research on *Pennisetums*. (Gupta, V.P. and Minocha, J.L., Eds.). Punjab Agricultural University. Ludhiana, India.

Thakur, R.P., Rai, K.N., Rao, V.P. and Rao, A.S., 2001. Genetic resistance of pearl millet male-sterile lines to diverse Indian pathotypes of *Sclerospora graminicola*. *Plant Dis.*, *85(6)*: 621-626.

Thakur, R.P., Shetty, K.G. and King, S.B., 1992. Selection for host-specific virulence in asexual population of *Sclerospora graminicola*. *Plant Pathol., 41*: 626-632.

Utz, H.F. and Melchinger, A.E., 2000. PLABQTL: A computer program to map QTL, Version 1.1. University of Hohenheim, Germany.

Yadav, R.S., Bidinger, F.R., Hash, C.T., Yadav, Y.P., Yadav, O.P., Bhatnagar, S.K., and Howarth, C.J., 2003. Mapping and characterization of QTL × E interactions for traits determining grain and stover yield in pearl millet. *Theor. Appl. Genet., 106*: 512-520.

Yadav, R.S., Hash, C.T., Bidinger, F.R., Cavan, G.P. and Howarth, C.J., 2002. Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought stress conditions. *Theor. Appl. Genet., 104*: 67-83.

Yadav, R.S., Hash, C.T., Bidinger, F.R., Devos, K.M. and Howarth, C.J., 2004. Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and tester background. *Euphytica, 136(3)*: 265-277.