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Mapping quantitative trait loci for downy mildew resistance in pearl millet

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Abstract Quantitative trait loci (QTLs) for resistance to pathogen populations of *Scelerospora graminicola* from India, Nigeria, Niger and Senegal were mapped using a resistant × susceptible pearl millet cross. An RFLP map constructed using F_2 plants was used to map QTLs for traits scored on F_4 families. QTL analysis was carried out using the interval mapping programme Mapmaker/QTL. Independent inheritance of resistance to pathogen populations from India, Senegal, and populations from Niger and Nigeria was shown. These results demonstrate the existence of differing virulences in the pathogen populations from within Africa and between Africa and India. QTLs of large effect, contributing towards a large porportion of the variation in resistance, were consistently detected in repeated screens. QTLs of smaller and more variable effect were also detected. There was no QTLs that were effective against all four pathogen populations, demonstrating that pathotype-specific resistance is a major mechanism of downy mildew resistance in this cross. For all but one of the QTLs, resistance was inherited from the resistant parent and the inheritance of resistance tended to be the result of dominance or over-dominance. The implications of this research for pearl millet breeding are discussed.

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a staple food crop of the semi-arid tropical regions of India and West Africa and is better adapted than any other cereal to regions of low rainfall and hot sandy soils. *Sclerospora graminicola* (Sacc.) Shröt. is an obligate biotrophic fungus that causes downy mildew on pearl millet, often resulting in devastating yield losses. With the introduction into India, in the late 1960s, of high-yielding hybrids produced on a single susceptible male-sterile line, downy mildew epidemics escalated. Subsequently released hybrids also proved to be non-durable. However, because the host-pathogen system was poorly understood, breeders could only select for resistance, rather than being able to incorporate known resistances, or to deliberately avoid resistances that were no longer effective.

The study of resistance has been hindered by the fact that resistance in the host shows continuous variation (Singh et al. 1980; Basavaraju et al. 1981a; Dass et al. 1984; Shinde et al. 1984). Conclusions drawn from previous studies on the inheritance of resistance have been conflicting, probably because such studies often dealt with only partially inbred parents, with pathogen populations that were likely to have been highly genetically variable, and involved experiments with low and uneven disease pressures. Such experimental conditions have probably contributed to the continuous distribution of the resistance phenotype. However, even when inbred parents and adequate and even disease pressure have been used, resistance has still shown continuous variation. No attemps have been made to study the inheritance of resistance to homogeneous isolates derived, for example, from single zoospores, mainly because of the difficulties involved in generating and maintaining isolates of this obligate biotroph. *S. graminicola* is heterothallic (Michelmore et al. 1982) so that homogeneous

isolates have to be continually maintained via asexual propagation, and no reliable cryopreservation protocol has as yet been developed that would enable isolates to be easily stored or transported.

A further hindrance to breeding is that resistance is regionally variable (Bhat 1973; Girard 1975; Shetty and Ahmad 1981; Singh and Singh 1987; Thakur 1987; ICRISAT 1989) so that breeding material has to be tested in expensive, time-consuming and often unreliable multilocational trials. This regional variability has been found to be principally due to genetic variability between pathogen populations rather than environmental differences between locations (Ball and Pike 1984). Selection for multilocational resistance could therefore theoretically be carried out by simultaneously screening breeding material in one location against a range of pathogen populations. However, this is not a feasible option for plant breeders within Africa and India because of strict quarantine regulations.

Molecular markers linked to resistance genes would allow resistances to different pathogen populations to be selected for at a single location in the absence of the pathogen. Linkage drag and the confounding effects of environmental variation associated with conventional breeding methods would also be eliminated. The ability to map genes contributing towards variation in complex traits with enough accuracy to be of use for plant breeding applications has only recently been made possible through the development of comprehensive molecular-marker maps. Loci contributing towards quantitative variation in disease resistance have now been mapped in tomato for resistance against insects (Nienhuis et al. 1987); in potato for resistance against cyst-nematodes (Kreike et al. 1993); in pea for resistance against Ascochyta blight (Dirlewanger et al. 1994); in potato for resistance against late blight (Leonards-Schippers et al. 1994); and in maize for resistance against gray leaf spot (Bubeck et al. 1993), northern corn leaf blight (Freymark et al. 1993) and stalk and ear rot (Pè et al. 1993).

The first molecular-marker map for pearl millet has recently been constructed (Liu et al. 1994) so that QTL analysis is now possible even in this little-studied crop. The present study aimed to map genes for resistance to downy mildew in pearl millet using molecular markers and QTL analysis so that linked molecular markers can be used in breeding programmes. An added advantage of mapping resistance genes using these methods is that homogeneous lines of the pathogen do not first have to be isolated, because QTLs effective against any individual pathogenic component that may exist within the pathogen population can be detected through inoculation with the whole pathogen population.

Materials and methods

Plant material

LGD-1-B-10 (Hash 1986), a downy mildew-susceptible, extra-early, inbred segregant of (B70 × Tift 756)-1-4-5 based on a germplasm from Togo and India, was crossed with ICMP 85410 (ICRISAT, unpublished), a downy mildew-resistant, dwarf, restorer, inbred segregant of (ICP 165 × ICP 220)-64 based on germplasm from Uganda, Mali and Nigeria. The parents had been selfed over sufficient generations for them to be considered inbred and therefore homozygous at most loci. F_2 seed was harvested from a single selfed F_1 plant and grown to provide leaf and stem material for DNA extraction and RFLP analysis. The F_2 and F_3 plants were selfed, and aliquots of seed from the plants in each F_3 family bulked so that single lineages were equally represented in the resulting F_4 families. The F_4 families were used to assess resistance to downy mildew. For screens carried out in Bangor, UK, the inoculum was grown on the universally susceptible genotype 7042. For the screen carried out at the ICRISAT Asia Centre in India, the inoculum was grown on 7042 and on the F_1 hybrid HB-3 (Dave 1987).

Pathogen material

Screens in Bangor

Pearl millet leaves containing abundant oospores of *S. graminicola* were collected from: 7042 and HB-3 growing in the downy mildew field screening nursery at the ICRISAT Asia Centre, India (oospores collected in 1989); a local landrace from Maiduguri in Nigeria (collected 1991); a local landrace from Bengou in Niger (1992); and local landraces from Doffane and Dimbetaba in Senegal (1992). For each pathogen population the leaves were ground into a fine powder and used to infect the universally susceptible genotype 7042 by coating the seed with the powder before sowing. Symptoms were apparent 3 weeks after inoculation. Each pathogen population was further asexually multiplied by spray-inoculating seedlings of 7042 at the 1–2 leaf stage with suspensions of sporangia collected from leaves of the oospore-infected plants, so providing sufficient inoculum for the disease screens.

Screens at the ICRISAT Asia Centre

Sporangia were collected from the leaves of infected genotypes 7042 and HB-3 growing in the downy mildew field nursery. These plants had been infected with a mixture of airborne sporangia and soilborne oospores.

Downy mildew screens

Screens in Bangor

Between 30 and 40 seeds of each pearl millet genotype (i.e. F_4 family, parental and control genotypes) were sown per pot (11.5 cm diameter) in low-nutrient peat and sharp sand compost (Chempak Seed Base, Chempak Products, UK: NPK 25-39-30 mg l^{-1}). Each pot represented a replicate of the pearl millet genotype and each screen had a varying number of replicates (Table 1) in a randomised design. A split-plot design was employed where genotypes were simultaneously screened against more than one pathogen population. Pots were placed on flood benching in a controlled environment glasshouse providing a 16-h day length (0600–2200 h) with a light intensity of between 500 and 1200 $\mu E m^{-2} s^{-1}$, and a temperature of 25–30 °C from 0600–1800 h and 20 °C from 1800–0600 h. The benches were flooded daily to an approximate depth of 1 cm for 30 min and then drained. When the seedlings were at the coleoptile to one-leaf stage the inoculum was prepared. Leaves from 2–3 month old infected plants were wiped clean of old sporangia using moist laboratory roll (Kimwipes Roll, Kimberly Clark, Kent, UK) and incubated in sealed plastic boxes lined with moist laboratory roll for 8 h at 20 °C in the dark. The resulting sporangia were collected into chilled (below 2 °C) distilled water and the concentration assessed using a haemocytometer and then adjusted to an appropriate concentration (Table 1). Each pot of seedlings was sprayed with approximately 4 ml of inoculum using a compressed-air cylinder-fed sprayer (Kestrel Eqpt. Ltd., London). The inoculum was maintained on ice throughout

inoculation to prevent zoospore release and so ensure a uniform sporangial concentration over time. The pots were covered with a polythene sheet to maintain a high level of humidity and incubated in the glasshouse at 20 °C for 15 h. Downy mildew disease was assessed 14 days later based on the percentage of infected plants within each pot. The disease score for each genotype was the mean of percent infection for individual pot replicates. This meant that the disease score for each entry could range from 0–100% and was a continuous variable.

Screen at the ICRISAT Asia Centre

Between 30–40 seeds of each F_4 family were sown in 10-cm diameter pots in a dry compost consisting of equal proportions of alfisol, farmyard manure and fine sand. Pots were placed in a lattice design in a glasshouse where cool air blowers were initiated when the day temperature exceeded 25 °C. Irrigation was carried out daily with an overhead hose. When the seedlings were at the coleoptile to one-leaf stage the inoculum was prepared. Leaves from 2–3 month-old infected plants of 7042 and HB-3 growing in the downy mildew field nursery were wiped free of old sporangia and incubated in covered boxes lined with moist blotting paper for 8 h at 20 °C. The resulting sporangia were collected into tap water at room temperature and the concentration assessed and adjusted to approximately 1.5×10^5 sporangia ml^{-1}. Each pot of seedlings was sprayed with approximately 5 ml of inoculum using a hand-pumped sprayer. The pots were covered with polythene sheeting and incubated at 20°C for 17 h. The percent downy mildew was assessed 14 days later. Only one screen was carried out in the ICRISAT Asia Centre, and this was against the local ICRISAT pathogen population.

Statistics for screens

Individual replicates of genotypes with seedling counts < 10 were removed. Data sets were analysed by generalised linear modelling (GLM) using the SAS programme (SAS Institute Inc.). Parental and control genotypes were removed from the data sets so that heritabilities, and main and crossed effects were analysed using only F_4 family data. Before analysis, the data were transformed (arcsin, log or square-root transformations) with a function that gave maximum heterogeneic distribution of residual-fitted data. Appropriate error terms for the analyses were applied where split-plots designs had been used. The heritability was broad sense and was calculated from the GLM output by subtracting the error mean square (EMS) from the model mean square (MMS) and dividing by MMS.

RFLP and QTL analysis

The RFLP analysis on the F_2 population of LGD-1-B-10 × ICMP 85410 was as described by Liu et al. (1994).

For the downy mildew-trait data, each data set was transformed with a function that most closely normalised the distribution (arcsin, log or square-root transformations). The LOD score of association between the genotype and trait data was then calculated using the interval mapping programme Mapmaker/QTL (Lander and Botstein 1989) with the free model of QTL effect. Markers were selected at approximate 20-cM intervals to map between, so that occurrences of spuriosly high LOD scores were closer to independence. This resulted in 22 RFLP markers being utilised for QTL mapping that gave full map coverage and an average interval of 19.2 cM. This interval size allowed a LOD score of 2.0 to be chosen as the threshold level for the significance of a QTL. For each data set, there were 93–119 progeny with both trait and genotype information.

Results

General statistics for screens

For each screen the variation between F_4 families was highly significant. Heritabilities were high and ranged from 0.78 to 0.88 (Table 1). For all the screens, the resistance scores in the F_4 families followed a continuous distribution. The distributions in the disease score were generally skewed towards the resistant parent or around the parental mid-point, apart from one of the Indian pathogen population screens (IN3), where the disease pressure was very high and disease was skewed

Table 1 Inoculum concentrations, replication, disease scores and statistics for downy mildew screens

Screen[b]	Conc[c]	Rep[d]	Mean disease score (%) ± SD					Significance[a]		
			LGD[e]	ICMP[f]	7042[g]	F_4[h]	H[i]	F_4[j]	Path[k]	$F_4 \times p$[l]
IN1	7×10^4	4	69.9 ± 4.5	8.3 ± 5.5	75.6 ± 9.5	32.8 ± 16.1	0.78	***	**	***
NG3	7×10^4		54.4 ± 4.0	8.3 ± 4.6	50.2 ± 3.2	16.3 ± 12.9	0.80	***		
IN2	7×10^4	3	66.0 ± 13.1	4.1 ± 3.8	62.3 ± 16.7	26.1 ± 19.3	0.75	***		
NR2	5×10^4		40.3 ± 13.1	6.5 ± 4.1	39.0 ± 3.0	7.4 ± 7.6	0.67	***	**	***
NG2	6×10^4		71.0 ± 9.0	6.5 ± 5.3	70.5 ± 1.5	14.4 ± 16.0	0.71	***		
NR1	6×10^4	2	57.0 ± 4.2	5.2 ± 6.2	98.3 ± 1.4	19.6 ± 19.2	0.78	***	**	***
SN1	6×10^4		64.5 ± 3.0	0.0 ± 0.0	55.0 ± 3.0	10.3 ± 11.3	0.72	***		
NG1	5×10^4	3	58.0 ± 0.2	4.2 ± 1.1	69.0 ± 2.6	10.9 ± 11.8	0.82	***		
IN3	1.5×10^5	4	93.9 ± 9.7	49.1 ± 12.7	95.7 ± 4.5	84.5 ± 14.1	0.88	***		

[a] $^{**}P \leqslant 0.01$, $^{***}P \geqslant 0.001$

[b] Grouped screens were those that were carried out at the same time using a split-plot randomised design. The pathogen populations were from IN = India, NG = Nigeria, NR = Niger and SN = Senegal. Screens have been numbered to allow cross reference with corresponding QTL likelihood maps (Fig. 1 and Table 2)

[c] Concentration of inoculum (sporangia ml^{-1})

[d] Number of replicates within each screen

[e] LGD-1-B-10 (susceptible-parent genotype)

[f] ICMP 85410 (resistant-parent genotype)

[g] Universal susceptible check

[h] Mean disease score over all F_4 families

[i] F_4 family mean heritability

[j] Significance of variation between F_4 families

[k] Significant effect of pathogen population

[l] F_4 family × pathogen population interaction

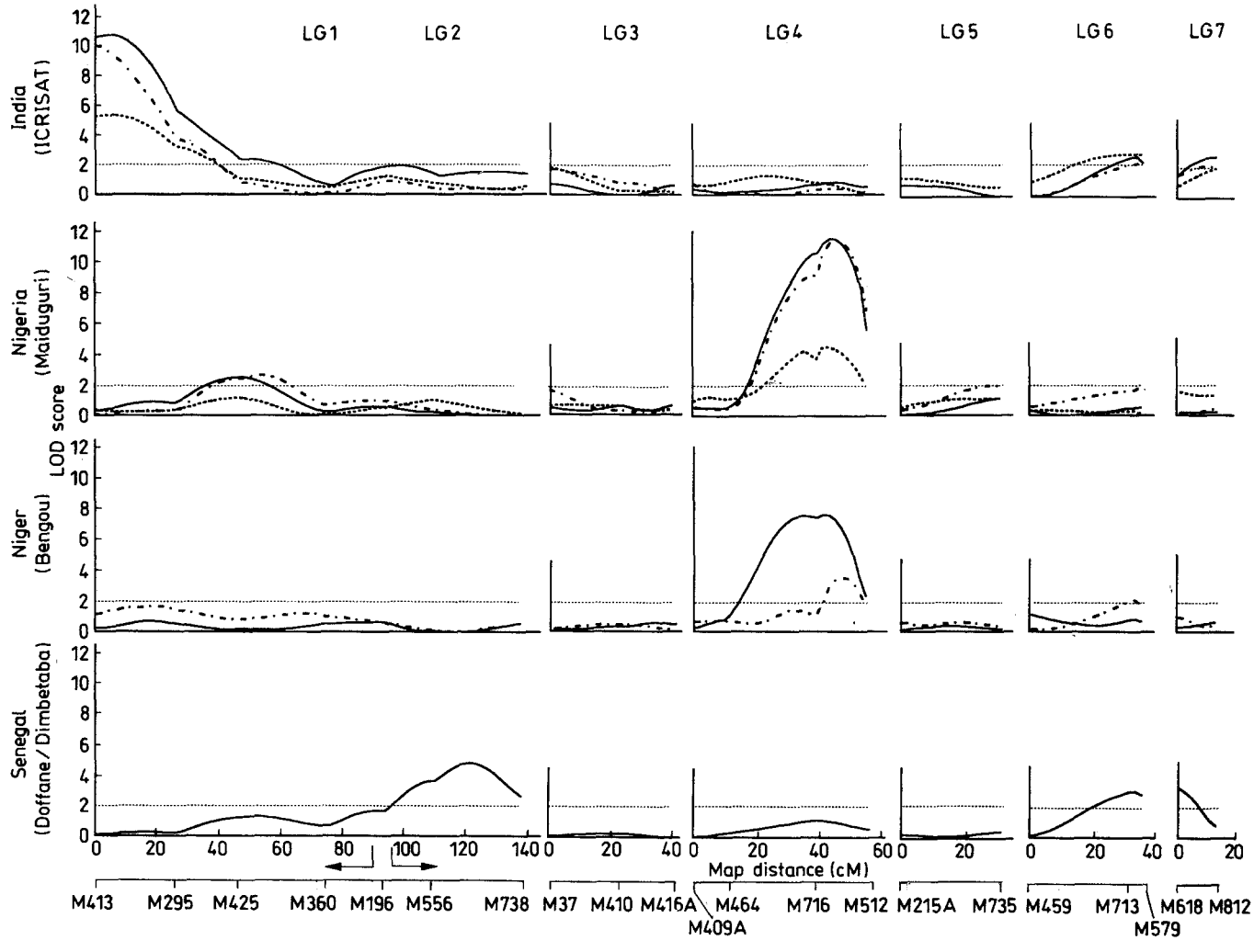
towards the susceptible parent (graphs of the distributions not shown, but direction of skewness is indicated by mean data for F_4 families in Table 1).

When screens were against more than one pathogen population, highly significant effects of pathogen population and F_4 family × pathogen-population were found (Table 1). When host genotypes were inoculated simultaneously with the Indian and an African pathogen population (screens IN1 and NG3, and IN2 and NR2), the Indian pathogen population was significantly more pathogenic on the F_4 families, the susceptible parent LGD, and the universally susceptible control 7042. There was no significant difference in the pathogenicity of Indian and African populations on the resistant parent, ICMP 85410. For the screen against three African pathogen populations (NG2, NR1 and SN1) the population from Nigeria was more pathogenic on the F_4 families and the susceptible control 7042 than was the population from Niger, and that from Niger was more pathogenic on these genotypes than the population from Senegal. There was no significant difference in the pathogenicity of these populations on the parental controls.

Pattern of inheritance

There were clear differences in the locations of the QTLs that were found for resistance against the pathogen populations tested (Fig. 1). A major QTL for resistance against the pathogen population from India was detected on linkage group 1 (LG1), against the pathogen populations from Nigeria and Niger on LG4, and against the pathogen population from Senegal on LG2. The variation explained by the major QTLs noted

Fig. 1 QTL likelihood maps indicating LOD scores for resistance to downy milldew. The maps are the result of F_4 families being screened against pathogen populations (labelled on the Y-axis) from India (ICRISAT), Nigeria (Maiduguri), Niger (Bengou) and Senegal (Doffane/Dimbetaba). For cross reference to tabulated data: IN1____, IN2_·_·, IN3....., NG1____, NG2_·_·, NG3....., NR1____, NR2_·_·, SN1____. The screen IN3 was carried out at ICRISAT Asia Centre, India, whilst all other screens were carried out in Bangor, UK. Each graph represents a linkage group (LG), and the RFLP markers chosen to map between are shown on the X-axis. There were no QTLs detected on the A and B linkage groups found by Liu et al. (1994) and so these are not shown. ..... level of significance for the presence of a QTL (LOD 2). ← → region of translocation event (see Liu et al. 1994)



above were high, and ranged from 19.4 to 48.9% (Table 2).

QTLs with a lower LOD score, and contributing towards a smaller proportion of the variation in the trait (7.8–15.3%), were also detected. There was a QTL of small effect for resistance against the Nigerian pathogen population on LG1 (this QTL was significant in only two of the screens, NG1 and NG2, but its effect was present at a non-significant level for NG3), against the Indian pathogen population on LG2 (significant for IN1 and present at a non-significant level for IN2 and IN3), and against the pathogen populations from India, Niger (significant only for NR2) and Senegal on LG6. On LG7 it was possible that there were two QTL locations; one for resistance against the Indian pathogen population and one against the Senegalese pathogen population.

The total variation explained by the QTLs detected within a screen (not including putative QTLs) varied from 22 to 64.5% (Table 2).

Repeatability

In repeated screens similar QTLs were detected, although the locations of the QTLs were sometimes shifted a few cM (Fig. 1 and Table 2). However, this shift did not exceed the 100:1 support interval for the locations of the QTLs (a LOD score drop of 2.0 either side of the maximum likelihood) so that it is likely that in repeated screens the same QTLs for resistance were being detected. The relative variation explained by a QTL often varied between repeated screens, for example, for the screens IN1 and IN3; equivalent variation was

Table 2 Significant and putative[a] QTLs for downy mildew resistance

QTL map[b]	LG[c]	Position[d]	LOD[e]	%Var[f]	T. Var[g]	W[h]	D[i]	4D[j]
IN1	1	M413+4cM	10.8	40.0		−9.2	−2.2	−8.8
	1	M196+4cM	2.0	9.3		−4.2	−3.6	−14.4
	6	M713	2.7	10.5		−5.0	0.9	3.6
	7	M812	3.2	12.9		−6.1	0.2	0.8
					64.5			
IN2	1	M413	10.1	35.0		−11.0	−4.8	−19.2
	1	*M196*	*0.9*	*3.6*		*−2.9*	*−3.7*	*−14.8*
	6	M713	2.2	8.8		−5.9	−0.3	−1.2
	7	*M618+4cM*	*1.9*	*10.1*		*−7.5*	*−0.2*	*−0.8*
					40.7 (45.4)			
IN3	1	M413+4cM	5.4	19.4		−7.5	−2.5	−10.0
	1	*M196*	*1.2*	*4.1*		*−2.7*	*−3.4*	*−13.6*
	6	M713	2.8	9.1		−5.3	0.5	2.0
	7	M812	2.1	8.0		−5.6	−0.5	−2.0
					42..8 (46.6)			
NG1	1	M425	2.5	9.9		3.9	−3.2	−12.8
	4	M716+4cM	11.5	46.8		−11.6	−5.2	−20.8
					49.0			
NG2	1	M425+6cM	2.7	15.3		0.9	−1.0	−4.0
	4	M716+6cM	11.5	48.9		−2.3	−1.1	−4.4
					56.5			
NG3	*1*	*M425*	*1.1*	*4.4*		*0.2*	*−0.7*	*−2.8*
	4	M716+4cM	4.5	22.0		−0.7	−0.5	−2.0
					22.0 (39.4)			
NR1	4	M716+2cM	7.7	37.1		−2.5	−0.5	−2.0
					37.1			
NR2	4	M716+8cM	3.5	29.0		−6.6	−7.6	−30.4
	6	M713	2.0	7.8		−3.2	2.0	4.0
					31.8			
SN1	2	M556+12cM	4.9	33.2		−1.4	−1.0	−4.0
	6	M713	3.1	12.1		−0.8	−0.2	−0.8
	7	M618	3.3	13.0		−1.0	−0.1	−0.4
					34.0			

[a] Where the LOD score did not rise above the significance level but the shape of the map suggested a QTL could be present and the QTL was significant in a repeat screen, data were included in the Table (italicised)

[b] See Table 1 for explanation of the abbreviations

[c] Linkage group

[d] Position of the QTL relative to the nearest probe to the left

[e] Maximum-likelihood LOD score for the QTL

[f] Phenotypic variation explained by QTLs

[g] Total phenotypic variation explained by the simultaneous examination of significant QTLs, and significant plus putative QTLs (in brackets), for each QTL map

[h] Weight calculated by Mapmamker/QTL. A negative weight meant that resistance was inherited from the resistant parent, and a positive weight that resistance was inherited from the susceptible parent

[i] Dominance factor calculated by Mapmaker/QTL

[j] 4 × dominance factor; considers segregation of heterozygotes over 2 generations to allow comparison to the weight factor so that inheritance can be estimated

explained by the QTLs on LGs 6 and 7, yet the variation explained by the QTL on LG1 for IN3 was half of that explained in screen IN1 (Fig. 1 and Table 2).

Mode of inheritance

Weight and dominance factors calculated by Mapmaker/QTL demonstrated how the QTL was inherited. Where there was a negative weight component, the effect of the resistant-parent allele was to decrease susceptibility, i.e. increase resistance. This was the case for all of the QTLs except that on LG1 against the Nigerian pathogen population (NG1, NG2 and NG3), where the weight was consistently positive, demonstrating that the susceptible-parent allele at that QTL was causing the increase in the resistance (Table 2).

As genotype data were assessed on an F_2 population and the trait data were assessed on F_4 families, segregation of heterozygotes over two generations meant that the effective value of the dominance factor was reduced to 1/4. When the dominance factor calculated by Mapmaker/QTL was multiplied by 4 to accomodate for this segretation, and so allow a comparison with the weight factor (Table 2), dominance or over-dominance was estimated to be the mode of inheritance for resistance at all the QTLs of major effect. For the QTLs of smaller effect, the inheritance of resistance tended to be more variable. For example, resistance at the QTL on LG6 appeared to be recessive in screens IN1, IN3 and NR2, but additive in screen IN2, and dominant in screen SN1. However, for the QTL on LG7, 4D was consistently much lower than W suggesting that the inheritance of resistance was additive.

There was some evidence for interactions occurring between QTLs. Where QTLs were examined together, the variation explained was generally lower than when they were examined separately (Table 2), suggesting that non-allelic interactions were occurring. Where two QTLs were examined simultaneously, the LOD score was frequently lower than the sum of the QTL's independent likelihoods, for example, between the QTLs on LGs 1 and 4 for resistance against the Nigerian pathogen population, and between the QTLs on LGs 1 and 7 against the Senegalese pathogen population (data not shown). However, a lower LOD score and the variation explained does not necessarily indicate epistasis, but could be due to data errors or non-normal trait data (Mapmaker/QTL manual).

Discussion

General statistics for screens

Highly significant differences between F_4 families and high heritabilities demonstrated that resistance was segregating in the population and much of the variation in the phenotype was attributable to genetic variation. Host resistance was continuously distributed in the progeny, as has been found in most previous studies on the genetics of resistance to downy mildew in pearl millet (Singh et al. 1980; Basavaraju et al. 1981a; Dass et al. 1984; Shinde et al. 1984). However, this does not necessarily imply that the inheritance is complex and that many genes are segregating, as has been suggested by Basavaraju et al. (1981a). Even with the fairly high heritabilities found here, segregation of just two genes could result in a continuous distribution, particularly as the progeny screened were F_4 families where the segregation of heterozygotes would have resulted in less distinct phenotypic classes than if the F_2 had been screened.

Where F_4 families were screened against similar inoculum concentrations of two or more pathogen populations in one screen, the Indian pathogen population was found to be more pathogenic than the African pathogen populations. Among the African pathogen populations, the pathogen population from Niger was the most pathogenic on F_4 families, followed by the populations from Nigeria and then Senegal (Table 1). Ball et al. (1986) also showed that the populations from Niger and Nigeria were more pathogenic than those from Senegal. However, they found no differences between those from Nigeria and Niger and noted that the Indian pathogen population had as low a pathogenicity as that from Senegal. The differences between this study and that of Ball et al. (1986) could be due to dissimilarities in the hosts and pathogen populations studied or to the screening protocols used. In particular, unlike most of those studied by Ball et al. (1986), the Indian pathogen population used in our study was not typical of a population that would be found in a farmer's field, as it was collected from the downy mildew nursery at the ICRISAT Asia Centre. Here, cultivars of diverse origins are screened, facilitating the selection for a broader spectrum of virulence and increased pathogenicity.

The same ordering of pathogenicity on F_4 families was found on 7042, which is a genotype considered to contain no resistance genes. Therefore, high pathogenicity on the F_4 families can be attributed, at least in part, to the increased aggressiveness of pathogen populations.

Pattern of inheritance

For the first time, the independent inheritance of resistances to differing pathogen populations of *S. graminicola* has been demonstrated in pearl millet. The QTLs that were detected highlighted clear differences in the pathogen populations examined. This is in accordance with the many previous reports of across-locational differences in the resistance of pearl millet lines to *S. graminicola* (Bhat 1973; Girard 1975; Shetty and Ahmad 1981; Singh and Singh 1987; Thakur 1987; ICRISAT 1989) and the determination that much of this variation could be attributed to genetic differences in the host and in the

pathogen (Ball 1983; Ball and Pike 1984; Ball et al. 1986). Ball and Pike (1984) found that pearl millet genotypes of Indian origin were more susceptible to West African than India pathogen populations, and West African host genotypes were vulnerable to the Indian pathogen populations. This was attributed to the separate centres of diversity that had been established as a result of the host and the pathogen co-evolving on separate continents following the introduction of pearl millet from Africa into India around 3000 years ago (Purseglove 1976). We found that, not only were there different QTLs for resistance against the Indian and West African pathogen populations, but there were different QTLs for resistance against the pathogen populations from Senegal and those from Nigeria and Niger. Although the resistance and virulence factors examined in the present paper may not be representative of the species as a whole, these results suggest that there are distinct geographical differences in the virulence of pathogen populations from within Africa, as well as between Africa and India.

There were no QTLs that were effective against all the pathogen populations tested demonstrating that pathotype-specific resistance is a major mechanism of downy mildew resistance in this cross.

Relative effects of the QTLs

For each screen, QTLs of large effect, contributing towards a large proportion of the variation in resistance, were detected. Other QTLs of smaller effect were also usually detected. The variance explained by each QTL could be proportional to the importance of the resistance gene, or genes, in that region in preventing or slowing down infection of the pathogen population. For example, the QTLs of major effect could be involved in specific recognition of the pathogen, whilst the QTLs of smaller effect could be the result of modifier genes that effect fungal growth and symptom expression. However, the sexual phase of *S. graminicola* is a significant part of its life cycle, with oospores usually being the only means of perenneation. It is likely, therefore, that, in addition to the variability that has been found between pathogen populations, there is considerable variation within pathogen populations. This is supported by the work of Thakur et al. (1992) who found that distinct populations could be rapidly selected for from single pathogen populations on different host genotypes. In this case, the variation explained by a QTL may be proportional to the frequency of a corresponding virulence factor (or avirulence gene, if this host-obligate biotropic pathogen system is comparable to other such systems that have already been characterised), or to the aggresiveness of individual isolates, within the pathogen population. Whilst such a situation may have meant that the inheritance of these resistances could have been studied following isolation of homogeneous isolates from pathogen populations, QTL analysis had enabled these resistances to be characterised, and also mapped, without this arduous task having to be first carried out.

Undetected QTLs

For screen NRI, only a single QTL was detected, despite segregation for resistance being continuous and the heritability being 0.78. It is therefore likely that other QTLs were segregating but were not detected. This could have been because these QTLs explained a low proportion of the variation in resistance (for example, as a result of the corresponding virulence being at a low frequency within the pathogen population), or that the effects of the QTLs were masked by inter-allelic interactions, or else that the power of the analysis was insufficient to separate the effects of linked resistance genes in the region of the QTL that was detected on LG4.

If QTLs remained undetected in this screen, then it is likely that there were also QTLs contributing towards resistance that remained undetected in other screens. This is also suggested by the fact that the total variation explained for each screen did not exceed 65%.

Repeatability

Where there were repeated screens using the same pathogen population, QTLs at similar locations were detected, demonstrating the stability of the expression of the resistance and virulence factors over environments. Even where a variable disease pressure between repeated screens resulted in very different disease distributions in the progeny (IN3, as compared with IN1 and IN2), the same QTLs were effective.

Minor discrepancies in repeated screens generally arose where QTLs had low LOD scores which were significant in some screens and non-significant in others, although the shape of the QTL map suggested that the QTL was still having some effect. Changes in the LOD score and variation explained by a QTL between repeated screens would have been due to environmental variation, such as alterations in the inoculum concentration, and differences in the oospore population that successfully infected the 7042 plants used to grow the inoculum.

Mode of inheritance of QTLs

For all but one of the QTLs, an increase in resistance was inherited from the resistant parent (Table 2). The exception was for the QTL on LG1 effective against the Nigerian pathogen population, where the resistance was inherited from the susceptible parent LGD-1-B-1-10. This is not completely unexpected, as LGD-B-1-10 does not show complete susceptibility in any of the screens, as, indeed, ICMP 85410 does not show complete resistance. This is not because escapes are occurring for the susceptible genotype, or because disease pressures are

too high for the resistant genotype, as genotypes are known that are more susceptible than LGD-1-B-10 and more resistant than ICMP 85410.

At many QTLs, the inheritance of resistance was estimated to be due to dominance or over-dominance. The majority of previous research into the genetics of downy mildew resistance in pearl millet have found dominance to be an important component of resistance (Appardurai et al. 1975; Gill et al. 1978; Pethani et al. 1980; Basavaraju et al. 1981b; Shinde et al. 1984; Mehta and Dang 1987), and over-dominance has also been detected (Singh et al. 1978; Basavaraju et al. 1981b; Dass et al. 1984). Over-dominance could be explained in terms of the buffering effects of heterozygosity, although apparent over-dominance could have arisen from the linkage of RFLP loci to more than one dominant, or partially-dominant, resistance locus in repulsion. However, a repulsion phase of resistances is unlikely as most of the resistance genes would have been inherited from the resistant parent. For this cross, it should be noted that, as well as the segregation of heterozygotes resulting in reduced dominance, a further complication was that segregation of many of the marker alleles were highly skewed (Liu et al. 1994) which can result in a distortion of dominance effects. For example, skewed segregation from the F_2 heterozygote that resulted in over-representation of the dominant resistance allele in the F_4 would have resulted in apparent over-dominance of resistance.

There was some evidence for the inheritance of resistance at the QTL on LG6, against the pathogen populations from India and Niger, being recessive. One previous study on the inheritance of downy mildew resistance in pearl millet found resistance to be recessive (Singh et al. 1978), and recessive resistance genes, although uncommon, have been found in other plant-pathogen systems (Day 1974; De Wit 1992). At what appeared to be the same locus, inheritance of resistance against the Senegalese pathogen population seemed to be dominant. These different modes of inheritance at the same QTL could be due to the effects of closely linked genes, different alleles at the same locus, or merely errors in assessing inheritance at QTLs of such small effect.

Several previous studies have found that epistatic effects played a significant role in the inheritance of resistance (Singh et al. 1980; Dass et al. 1984; Shinde et al. 1984). In the present study, limited interactive effects were detected but, as they all involved low LOD scoring QTLs, no conclusions could be drawn on the basis of these second order statistics.

Implications for breeding programmes

Now that resistance QTLs have been located, linked markers can be used to select for the genes in plant breeding programmes. Marker-assisted selection will enable the plant breeder to select for an identified resistance in the absence of the pathogen, and so allow resistances against any pathogen population to be selected for in any country.

Commercial seed is presently produced on open-pollinated cultivars and single-cross hybrids. Hybrids yield more than open-pollinated cultivars, but have never matched their disease durability and usually become susceptible to downy mildew within 5 years of their widespread cultivation. This is principally because the male-sterile lines on which the hybrids are produced are extremely difficult to breed. They are therefore utilised over long periods during which time they become susceptible. Thus, a priority for marker-assisted selection is to breed resistance into male-sterile lines to make both them, and the hybrids produced on them, more durably resistant.

The options that are available for the introduction of resistance into these male-sterile lines are to pyramid resistance, to sequentially deploy resistance genes over time, or to create a male-sterile line that is genetically heterogeneous for disease resistance genes. However, in the case of pyramiding, the pathogen could prove to be highly efficient at accumulating all the virulence genes needed to overcome the pyramided resistance. Selection for multiple virulence is more efficient where the pathogen is an obligate biotroph, can sexually reproduce, and disease is rapidly spread by airborne spores (Day 1974). *S. graminicola* has all three of these characteristics. Further, the rapid selection of host-specific virulences from heterogeneous field populations has already been demonstrated (Thakur et al. 1992). Pyramided resistance may therefore prove to be unstable for this host-pathogen system and could encourage the evolution of complex pathogen races against which new resistances would then have to be found. Moreover, farmers often retain grain for sowing in subsequent seasons so that recombination will expose individual resistances and thus reduce the durability of the resistance. This practise of retaining grain would also mean that the sequential deployment of resistance genes over time would not be possible, as a released resistance could not be withdrawn.

The best option would be to produce a male-sterile line which is genetically heterogeneous for resistance genes, thus mimicking the durable disease resistance of open-pollinated cultivars. This could be accomplished by producing a set of backcross lines, each differing for a single resistance gene, and allowing these lines to recombine during the multiplication of the male-sterile line in the hybrid seed production process. Durability may be increased by incorporating more than one resistance gene against pathogen populations into each line.

Marker-assisted selection will make it possible to produce a range of genotypes that will allow the durability of different resistent gene combination/deployment strategies to be compared in serial passage experiments. Similar studies have been proposed by Pederson and Leath (1988) to evaluate the effectiveness of pyramided resistance to control *Puccinia graminis* in wheat.

This research has greatly furthered our knowledge of the genetics of downy mildew resistance in pearl millet and this has been achieved without having to undertake the lengthy procedure of deriving homogeneous isolates and identifying pathotypes. In pearl millet, a crop that has previously had only limited genetic investigation, the potential now exists for a breeding programme to be able to incorporate across-locational resistance into single genotypes and to implement a multi-line strategy to improve resistance durability.

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