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Molecular markers for rust and pyricularia leaf spot disease resistance in pearl millet

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Abstract Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is a warm-season grass used for food, feed, fodder and forage, primarily in countries of Africa and India but grown around the world. The two most-destructive diseases to pearl millet in the United States are rust (caused by *Puccinia substriata* var. *indica*) and pyricularia leaf spot (caused by *Pyricularia grisea*). Genes for disease resistance to both pathogens have been transferred into agronomically acceptable forage and grain cultivars. A study was undertaken to identify molecular markers for three rust loci and one pyricularia resistance locus. Three segregating populations were screened for RAPDs using random decamer primers and for RFLPs using a core set of probes detecting single-copy markers on the pearl millet map. The rust resistance gene Rr_1 from the pearl millet subspecies *P. glaucum* ssp. *monodii* was linked 8.5 cM from the RAPD OP-G8$_{350}$. The linkage of two RFLP markers, *Xpsm108* (15.5 cM) and *Xpsm174* (17.7 cM), placed the Rr_1 gene on linkage-group 3 of the pearl millet map. Rust resistance genes from both Tift 89D$_2$ and ICMP 83506 were placed on linkage-group 4 by determining genetic linkage to the RFLP marker *Xpsm716* (4.9 and 0.0 cM, respectively). Resistance in ICMP 83506 was also linked to the RFLP marker *Xpsm306* (10.0 cM), while resistance in Tift 89D$_2$ was linked to RAPD markers OP-K19$_{350}$ (8.8 cM) and OP-O8$_{350}$ (19.6 cM). Fragments from OP-K19 and OP-O8 in the ICMP 83506 population, and *Xpsm306* in the Tift 89D$_2$ population, were monomorphic. Only one RAPD marker (OP-D11$_{700}$, 5.6 cM) was linked to pyricularia leaf spot resistance. Attempts to detect polymorphisms with rice RFLP probes linked to rice blast resistance (*Pyricularia oryzae*; syn = *P. grisea*) were unsuccessful.

Key words Disease resistance · Genetic mapping · Pearl millet · *Pennisetum glaucum*

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is an annual warm-season cereal grown on about 37-million hectares worldwide (Anonymous 1996) with the largest areas of production in India and the Sahel region of Africa (Singh and King 1991). This important grain crop is grown as a protein-rich food source for humans as well as a forage/fodder crop for livestock. In the United States, pearl millet is grown in the southeast primarily as a summer annual forage crop for livestock and wildlife, or as a feed grain for poultry and livestock. HGM-100, the first dwarf grain hybrid adapted to the southeastern U.S., was released as an alternative crop for producers by Hanna (1993).

Breeding for disease resistance in pearl millet was reviewed by Williams and Andrews (1983). Kumar and Andrews (1993) later expanded this review to include over 140 qualitative traits including disease resistance, mutations, plant pigments, and others. As the acreage planted in forage and grain pearl millet increases, more emphasis must be placed on breeding for disease resistance to counteract the increased pressure exerted on the crop by pathogens. The two most destructive diseases of pearl millet in the U.S. are rust (caused by *Puccinia substriata* Ellis & Barth. var *indica* Ramachar

& Cummins) and pyricularia leaf spot [caused by *Pyricularia grisea* (Cke.) Sacc].

Rust was first reported on pearl millet in 1904 (Zimmerman 1904 in Singh and King 1991). It was first reported in the U.S. in 1954 as *P. penniseti* Zimm. (Luttrell 1954) and as *P. substriata* var *indica* in 1973 (Wells et al. 1973). Rust can infect pearl millet at any stage during the growing season. Early infection of seedlings can completely destroy a crop. Monson et al. (1986) found a 51% decrease in digestible dry matter-yield over a 2-year period of natural rust infection. A significant loss in digestible dry matter-yield occurs at low rust severities which favors the use of highly effective resistance over slow-rusting, or partial rust resistance, in forage pearl millet (Wilson et al. 1991). At least 11 races of *P. substriata* var. *indica* have been identified in south Georgia (Tapsoba and Wilson 1996). Resistance genes are the primary control method for rust on pearl millet. Many germplasm lines, mainly from Africa, have been identified as rust-resistant by ICRISAT (Singh and King 1991). Currently, two sources of major gene resistance are employed in breeding-lines used to produce adapted cultivars for the southeastern U.S. The original source of rust resistance came from a wild subspecies [*P. glaucum* subspecies *monodii* (Maire) Brunken] of pearl millet from Senegal (Hanna et al. 1985). This resistance gene was labeled Rr_1 and was used in the parental lines 'Tift 85DB' (Hanna et al. 1987) and 'Tift 65' (Burton and Wilson 1995), a grain hybrid (Hanna 1993), and a forage cultivar (Hanna et al. 1988). Beginning in 1992, the complete resistance conferred by Rr_1 was overcome by a new race or races of the rust pathogen (Wilson 1993), and today rust can be found on all lines with this gene. However, the Rr_1 gene still confers resistance to many rust isolates purified from natural rust populations and therefore should be used in any new resistant cultivar. The second source of rust resistance comes from the Senegalese pearl millet landrace 'Sa Fe'. This resistance is believed to be controlled by more than one gene (Hanna and Wells 1993). Screening with different isolates of *P. substriata* var. *indica* showed that the gene(s) for this second type of resistance are different from the Rr_1 gene (Tapsoba and Wilson 1996). This resistance has been incorporated into the breeding line 'Tift $89D_2$' (Hanna and Wells 1993) and is effective against most isolates virulent to the Rr_1 gene. While plants with the Tift $89D_2$ resistance appear relatively disease-free in the field, purified rust isolates from bulked, field-collected rust spores have been found that can infect Tift $89D_2$ plants (Tapsoba and Wilson 1996). Combining the resistance genes from Tift $89D_2$ along with Rr_1 makes a plant resistant to 9 of 11 isolates of rust characterized in Georgia.

Pyricularia leaf spot is the second major foliar disease of pearl millet in the U.S. *P. grisea* causes grayish, water-soaked foliar lesions that enlarge and become necrotic, resulting in extensive chlorosis (Wilson et al. 1989). This disease becomes most severe during humid weather conditions especially with dense plant stands (Hanna and Wells 1989). Leaf blight on pearl millet caused by *P. grisea* was negatively correlated with green-plot yield, dry matter yield, and digestible dry matter. Loss of digestible dry matter yield to this disease in the forage hybrid 'Tifleaf 1' (Burton 1980) averaged 18% annually in the first harvest (Wilson and Gates 1993). The related pathogen *P. oryzae* was shown to reduce overall growth rate, leaf-area formation, dry matter accumulation, spikelet number, 1000 kernel weight, and grain yield in rice (*Oryza sativa* L.) (Bastiaans 1993). Resistance to pyricularia leaf spot in pearl millet was derived from the same *P. glaucum* ssp. *monodii* accession in which the Rr_1 rust gene was found (Hanna et al. 1987). Pyricularia resistance in *monodii* was characterized as three independent, dominant genes (Hanna and Wells 1989), although Tift 85DB, with resistance derived from *monodii*, was shown to have a single resistance gene (Wilson et al. 1989). This resistance gene is effective against all isolates of *P. grisea* tested to-date. Several other sources of pyricularia leaf spot resistance have been identified from Burkina Faso landraces. Each has been characterized as having dominant, single-gene resistance that is independent of the *monodii* resistance gene. No host-pathogen race interactions are currently known for *P. grisea* and pearl millet. Since race pathogenicity is seen in related *Pyricularia* pathogens (i.e., *P. oryzae*), it would be prudent to collect sources of resistance genes and deploy them in pearl millet in anticipation of race interactions to prevent disease outbreaks in the future.

A study was undertaken to identify molecular markers for three rust loci and one pyricularia resistance locus. One of the most efficient ways to screen breeding lines for multiple resistance genes is by using molecular markers linked to these genes in marker-assisted selection (MAS) (Harms 1992; Michelmore 1995 a). MAS is being used in many agricultural species, both plants and animals. Examples of successful MAS in crop plants include selection for blast resistance in rice (Hittalmani et al. 1995) and bean common mosaic-virus resistance in *Phaseolus vulgaris* L. (Kelly et al. 1995). MAS relies on the isolation of molecular markers closely linked to the resistance genes. Given the necessity of controlling multiple races of the rust pathogen and the opportunity to deploy different resistance genes against pyricularia leaf spot, stacking or pyramiding resistance genes into cultivars of pearl millet using MAS could be beneficial for the southeastern U.S.

Materials and methods

Plant culture

All plants for disease-resistance screening were greenhouse-grown in pots (10-cm square or 17-cm round) in a potting mix of peat

moss : sand : Perlite (3 : 2 : 2) supplemented with lime and time-release fertilizer (15-15-15). Plants were fertilized 2 weeks after planting with complete Hoagland's solution and once per week thereafter.

Three populations were developed for mapping disease resistance, two for rust and one for pyricularia leaf spot (Table 1). The first rust-resistant population (referred to as R1) segregated for the Rr_1 gene as well as rust resistance from the line 'ICMP 83506' developed at the International Crops Research Institute for the Semiarid Tropics (ICRISAT, India). ICMP 83506 was crossed to Tift 85DB and a single F_1 individual was selfed to produce an F_2 population of 54 individuals segregating for two sources of rust resistance. The R1 population was screened with two different races, 93-3 (avirulent to Rr_1) and 92-1 (avirulent to ICMP 83506) (see Tapsoba and Wilson 1996 for rust race information).

The second population segregating for rust resistance (R2) was made by pollinating the susceptible parent Tift 23DB with the resistant parent Tift $89D_2$. A single F_1 individual was selfed to produce an F_2 population of 62 individuals which was screened with the rust race 92-1 (avirulent to Tift $89D_2$).

The third population segregating for pyricularia resistance (204-3) was made by crossing the resistant parent *P. glaucum* ssp. *monodii* 'Ps34' (rp_1rp_1 *TrTr*, pyricularia-resistant) onto the pyricularia-susceptible genetic stock, red trichomeless (Rp_1Rp_1 *trtr*) in a Tift 23 background (Hanna and Burton 1992). A red, trichomed F_1 plant was selfed to produce an F_2 population of 62 individuals segregating for pyricularia disease resistance. This population was planted 5–10 seeds per pot and inoculated with *P. grisea*. Plants were scored as susceptible or resistant and selfed to produce F_3 seed that also were planted and inoculated with *P. grisea*. These plants were scored for resistance and tillers were harvested in bulk from each F_3 family for DNA isolation.

Disease inoculation

Rust isolate selection was based on preliminary data gathered with the isolates and genotypes reported in Tapsoba and Wilson (1996). Urediniospores from *P. substriata* var. *indica* isolates 93-3 and 92-1 were increased on the susceptible cultivar Tifleaf 1 and stored in vials at $-80°C$ (approximately 0.5 cm^3, one inoculation quantity). Plants at the three-leaf stage (2 weeks after planting) were misted for 5 min to moisten the leaves and saturate the air with humidity. Urediniospores were removed from the freezer and heated in a water bath to 37°C for 45–60 s to break dormancy. Spores for inoculation were placed in approximately 500 ml of water with one drop of Triton X-100 for a final inoculum concentration of 1×10^5 conidia ml^{-1}. Inoculum was sprayed onto plants with an air sprayer. Inoculation chambers were closed and relative humidity was maintained at 100% by intermittent misting. After 18 h, plants were allowed to air dry and moved to the greenhouse. Pustule development was scored 8–10 days later.

P. grisea spores were collected in the field from pearl millet leaves with lesions and stored at 7°C. For spore isolation, lesions were cut into 2–5-mm pieces, surface-sterilized with 0.5% sodium hypochlorite for 1 min, and plated on 20% V-8 agar. Plates were incubated 2–4 days at 25°C under continuous fluorescent light and *P. grisea* was transferred to fresh V-8 plates. Microscopic observation of spores was used to confirm the growth of *P. grisea*. Conidia from 5–7-day-old cultures were suspended in water for a final inoculum concentration of 3.5×10^4 conidia ml^{-1}. Plants at the three-leaf stage were misted with inoculum and incubated for 24 h in the dark at 100% humidity. Plants were allowed to dry and then evaluated for resistance after 6 days. All populations inoculated for rust or pyricularia included the susceptible cultivar Tifleaf 1 as a control.

DNA isolation

Culms were harvested just above the terminal node. Outer leaf-sheaths were removed, as were the leaves at the ligule, leaving the soft inner-leaf whorls for DNA isolation. Two DNA isolation protocols were followed, modifications of either Tai and Tanksley (1990) or Williams and Ronald (1994). Ground tissue was incubated in pre-warmed (65°C) Tai and Tanksley isolation buffer or modified PEX isolation buffer [10 ml buffer per g of tissue; 100 mM Tris-HCl pH 7.5, 12.5 mM potassium ethyl xanthogenate (Fluka Chem., Ronkonkoma, N.Y., USA), 10 mM EDTA pH 8.0, 700 mM NaCl, and 1.25% SDS; stored at 4°C in a brown bottle] for 30 min to 1 h, with 1 hour giving the better yield. One milliliter of 5 M sodium acetate for each 10 ml of buffer was added, mixed well, and placed on ice for 20–30 min, then centrifuged at 2000*g* for 20 min at 4°C, after which the supernatant was filtered through Miracloth into a sterile bottle. Isopropanol was added (7.5 ml per 10 ml of buffer) and the DNA was allowed to precipitate at $-20°C$ for 1–3 h. Pelleted or spooled DNA was washed in ethanol (80% ethanol with 15 mM of sodium acetate) for 20 min. The DNA was dissolved in TE containing 100 µg/ml of RNase. DNA quantity was measured using a Hoefer TKO-100 fluorometer and Hoechst 33258 dye, with calf thymus DNA as a standard.

RAPD analysis

DNAs from parental plants were screened for polymorphisms using the polymerase chain reaction (PCR) with 480 Operon (Alameda,

Table 1 Genetic materials used for marker isolation and mapping

Genotype	Resistance loci	Source of resistance loci	Recurrent parent of resistant inbreds
Inbred/resistant lines			
Tift383	None	–	–
Tift23DB	None	–	–
PS34	Rust race 93-3^r (= Rr_1); pyr^r	Subspecies	–
ICMP83506	Rust race 92-1^r	Unknown	–
Tift85DB	Rr_1; pyr^r	PS34	Tift23DB
Tift65	Rr_1; pyr^r	PS34	Tift383
Tift$89D_2$	Rust race 92-1^r	Sa Fe	Tift23DB
Populations			
R1: Tift85DB × ICMP83506	Rr_1; 92-1^r; pyr^r	PS34/unknown	–
R2: Tift23DB × $89D_2$	92-1^r	Sa Fe	–
R3: Tift23DB × PS34	pyr^r	PS34	–

Calif., USA) random decamer primers (from kits A to Y) in a Biotherm forced-air thermocycler programmed for five cycles of 15 s at 93°C, 30 s at 36°C, 60 s at 72°C, followed by 36 cycles of 15 s at 93°C, 30 s at 40°C, and 60 s at 72°C. Amplified products from all PCR reactions were separated by electrophoresis in 1.5% agarose gels and stained with ethidium bromide. RAPD bands identified as linked to resistance genes were gel-isolated and cloned into the pZErO-1 vector (InVitrogen, Carlsbad, Calif.) by first blunt-ending the amplified fragment with T4 polymerase, then ligating into an *Eco*RV-digested plasmid. Ligations (2 µl) were transformed into electrocompetent *Escherichia coli* cells (30 µl Top 10F′) using an Electro Cell Manipulator 600 (BTX, Inc., San Diego, Calif.; high voltage mode, resistance 129 ohms, charging voltage 1.2 kV, pulse length 4.6–4.7 ms, using a 1-mm chamber gap). The sequence-characterized amplified region (SCAR) marker G8 was produced by end-sequencing a cloned 350-bp band that was amplified using the random primer OP-G8 (SCAR G8 primer sequences: 5′TCACGTCCACCAGGGGCTCGA3′, 5′CCACAGCGGATTGAGATGCCC3′). Amplification of the SCAR was carried out for 35 cycles of 30 s at 93°C, 60 s at 45°C, and 60 s at 72°C. Data from RAPDs were compared to the observed resistance reactions for individuals using the Mapmaker program (Lander et al. 1987), analyzing 2-point or multi-point comparisons (LOD score 3.0) with the Haldane mapping function.

RFLP analysis

A core set of 50 RFLP probes that detect single-copy markers on the pearl millet linkage map was obtained from M. D. Gale and K. M. Devos (John Innes Institute, Norwich, UK). The database Rice-BS125/2/BS125/WLO2 (S. R. McCouch, Cornell University, N.Y.) was searched for markers linked to pyricularia resistance and selected probes from a rice RFLP set (courtesy of S. R. McCouch) were used on pyricularia-resistant and -susceptible pearl millet parents.

Parental blots contained 7.5 µg of DNA of each genotype digested with three restriction enzymes (*Eco*RI, *Dra*I, and *Hin*dIII). The DNA was transferred to Genescreen Plus (Dupont/NEN) nylon filters and baked at 80°C for 2 h. Probe DNA was amplified from plasmids using M13 primers and 1–5 µl of the PCR product was radiolabeled with ^{32}P dCTP by the random primer method (DECAprime II labeling kit, Ambion, Austin, Tex.). Un-incorporated, radiolabeled nucleotides were separated from the labeled probe on Sephadex-G50 spin columns. Filters were hybridized with the probe overnight at 65°C then washed three times with 2 × SSPE + 1% SDS, 5 min at room temperature, 0.5 × SSPE + 1% SDS, 30 min at 65°C, and 0.1 × SSPE + 1% SDS, 30 min at 65°C, before exposing to X-ray film (Fuji medical X-ray film, Fujifilm) for 2–5 days at − 80°C. Filters that hybridized weakly with probes were washed with 2 × SSPE + 1% SDS, 5 min at room temperature, and twice with 1 × SSPE + 1% SDS for 30 min at 65°C.

Results

Polymorphism of RAPD loci

Out of a total of 480 primers screened, 448 amplified an average of nine scorable fragments per RAPD primer. More polymorphism was observed between Tift89D2 and its recurrent parent, Tift23DB (107 polymorphic fragments from 88 primers; 2.6% of the total number of fragments), than between Tift85DB and the same recurrent parent (31 polymorphic fragments from 30 primers; 0.8% of the total number of fragments), even though all three genotypes were screened with 480 primers. Tift65 and Tift85DB, both of which have rust resistance from the same source, shared 17 RAPD fragments that were derived from the accession PS34, and not from the recurrent parent.

Rr_1 rust resistance locus

The R1 F_2 population of 54 individuals segregated 3 : 1 for each of two rust resistance genes, Rr_1 from 85DB ($\chi^2 = 0.025$, $P = 0.87$) and a second gene from ICMP 83506 ($\chi^2 = 0.617$, $P = 0.43$), which could be differentiated by pathotype reaction. Five RAPD primers consistently produced polymorphic bands between multiple individuals of Tift 65 or Tift 85DB and Tift 23DB. These polymorphic fragments were putatively linked to rust (Rr_1) or pyricularia resistance and were screened on the R1 population as well as the R3 population (see below). A 350-bp RAPD fragment that amplified using OP-G8 mapped 8.5 cM from the Rr_1 locus and was the closest marker found to the Rr_1 gene. This 350-bp fragment was cloned and end-sequenced to produce 24-nucleotide SCAR primers. Amplification of the G8 SCAR produced a single fragment (Fig. 1 a) that corresponded in size and segregation pattern with the 350-bp fragment obtained using the RAPD primer OP-G8.

Eight pearl millet probes detected polymorphisms in bulked DNA of resistant individuals from the R1 population (two bulks, one with the Rr_1 gene only and the other with resistance to race 92-1). When the entire R1 population was probed, two of the markers were linked with Rr_1, *Xpsm108* (15.5 cM) and *Xpsm174* (17.7 cM), both distal to SCAR $G8_{350}$. Comparison of the distances from the two RFLP markers to the resistance locus places the Rr_1 gene near the center of linkage-group 3 on the pearl millet molecular map (Fig. 2). Analysis of other RFLP loci that would appear to be closer to the gene according to the molecular map (*Xpsm248* and *Xpsm473*) produced no polymorphisms in this population when surveyed with three enzymes.

Resistance to rust isolate 92-1

Three RFLP markers from ICMP 83506 were identified that were linked to the rust resistance gene in the R1 population. The marker *Xpsm716* did not show recombination with the rust resistance locus in 54 individuals and mapped to linkage-group 4 (Fig. 2). The marker *Xpsm306* mapped 10 cM proximal to the resistance gene, while *Xpsm464*, the nearest marker distal to the resistance gene, mapped 45.5 cM away in this population (Fig. 2). On the pearl millet map (Liu et al. 1994), distances of 6.4 cM between *Xpsm306* and *Xpsm716* and 28.0 cM between *Xpsm716* and *Xpsm464* were reported.

The R2 population of 62 individuals, screened using the rust isolate 92-1, segregated in a 3 : 1 ratio ($\chi^2 = 0.022$, $P = 0.88$) characteristic of single, dominant gene

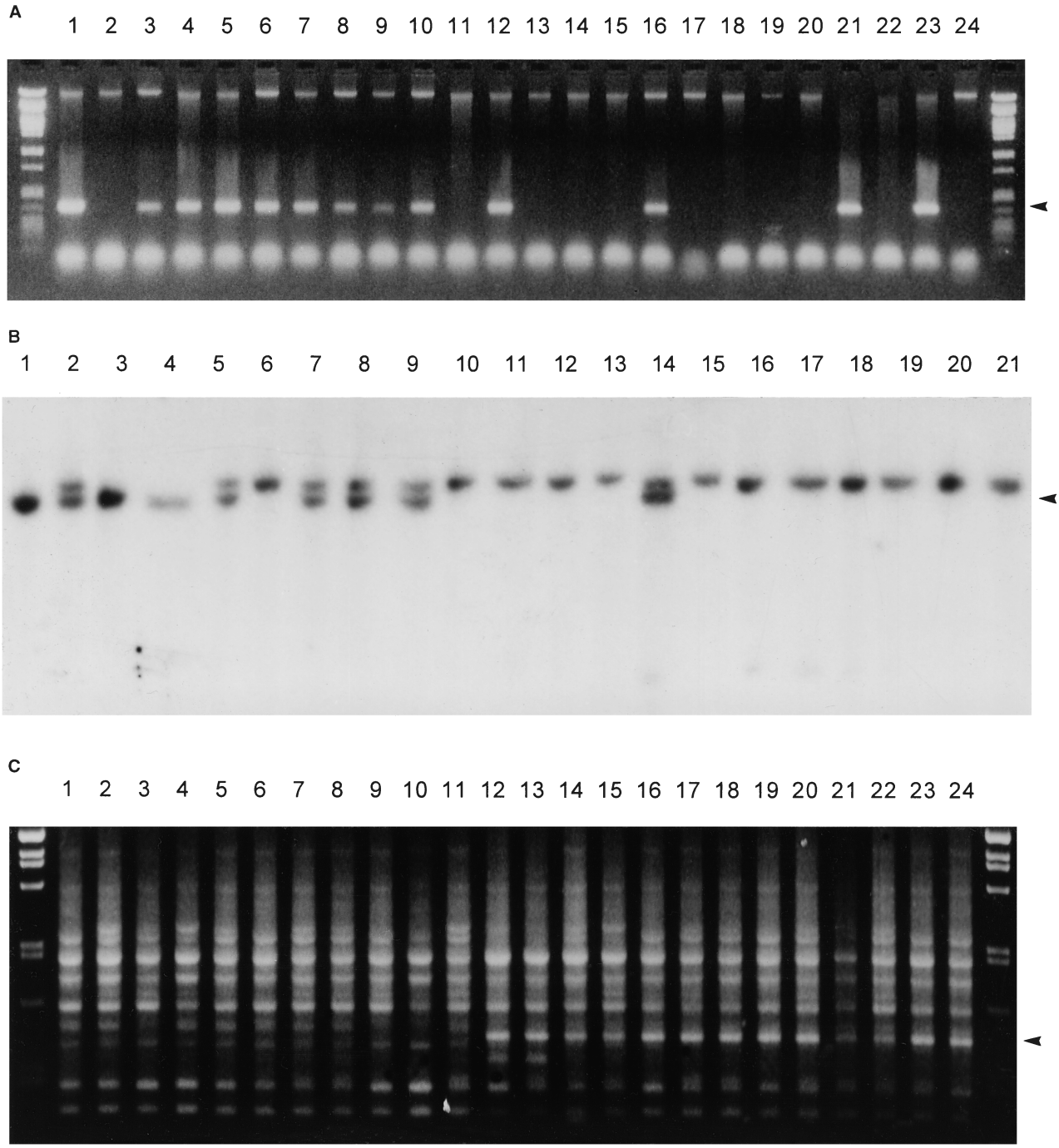

Fig. 1A–C A PCR amplification of SCAR $G8_{350}$ (*arrow*) from individuals of the R1 pearl millet population segregating for the Rr_1 rust resistance gene. *Lanes 1–10* resistant, *11–19* susceptible, and *20–24* parental cultivars Tift383, Tift65, Tift23DB, Tift85DB, and $Tift89D_2$, repectively. Recombinant individuals were identified in *lanes 2, 12, and 16. Unlabeled lanes* contain lambda DNA digested with *Pst*I. **B** Southern-blot hybridization of the RFLP probe *Xpsm716* to *Eco*RI-digested genomic DNA from the R2 pearl millet population segregating for the rust resistance gene from $Tift89D_2$. *Lanes 1–9* resistant and *10–21* susceptible. Recombinant individuals were identified in *lanes 6 and 14. Arrow* indicates band from resistant parent. **C** PCR amplification of DNA from the R3 pearl millet population segregating for the pyricularia leaf spot resistance gene from *P. glaucum* ssp. *monodii* using the primer OP-D11. A band at 700 bp (*arrow*) was scored. *Lanes 1–11* susceptible and *12–24* resistant. No recombinant individuals were observed in this group. Unlabeled lanes contain lambda DNA digested with *Pst*I

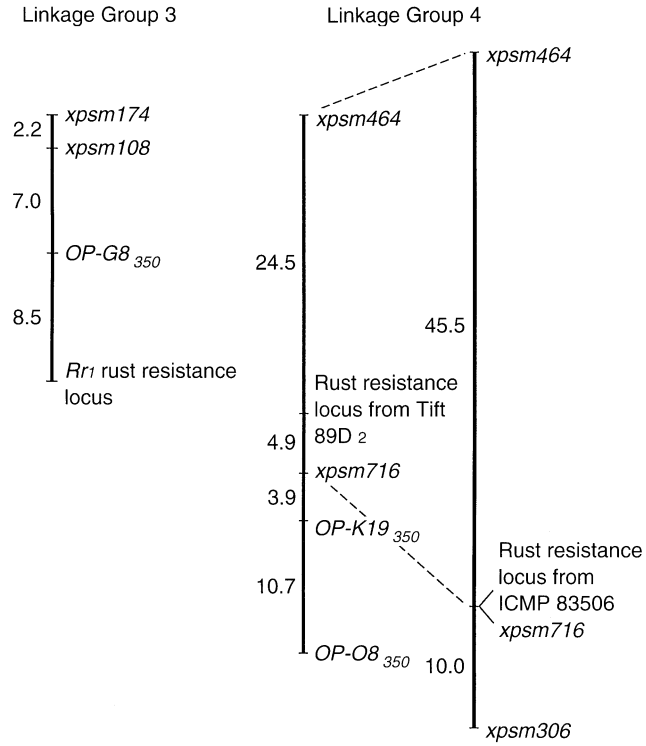

Fig. 2 Maps of pearl millet linkage groups containing rust resistance loci. The map of linkage-group 3 was constructed from data collected from the R1 population screened with rust isolate 93-3. The map of linkage-group 4 (left) was constructed from data collected from the R1 population screened with rust isolate 92-1 and (right) was constructed from data collected from the R2 population screened with rust isolate 92-1. The map distance is shown in cM

resistance. Twelve RAPD primers and three pearl millet probes that consistently produced a polymorphic band between Tift $89D_2$ and Tift 23DB were used to screen the R2 population. Two RAPD fragments and two RFLP markers were identified as linked to this resistance locus. The RAPDs OP-$K19_{350}$ and OP-$O8_{350}$ mapped 8.8 cM and 19.5 cM, respectively, from the resistance locus in the R2 population (Fig. 2). The RFLP locus *Xpsm716* (Fig. 1 b) was identified 4.9 cM proximal to the resistance locus on the linkage-group 4 map (Fig. 2). A second RFLP marker, *Xpsm464*, mapped 24.5 cM distal to the resistance locus. These two RFLP markers were the same markers identified as linked to the 92-1 resistance locus in the R1 population.

P. glaucum ssp. *monodii* pyricularia resistance

The RAPD OP-$D11_{700}$ (Fig. 1 c) that was identified in Tift85DB and Tift65 was shown to be located 5.6 cM from the pyricularia resistance locus in population R3 (62 individuals, 3:1 segregation for resistant:susceptible phenotypes, $\chi^2 = 1.053$, $P = 0.30$). Although three pearl millet probes detected polymorphisms between Tift 85DB and Tift 23DB; none of the loci detected with these probes were linked to the resistance locus in the R3 population. Probes that detected RFLPs linked to resistance to the related pathogen *P. oryzae* (syn = *P. grisea*) in rice were used to determine whether the locus conferring resistance in pearl millet was conserved in rice. Twelve RFLP probes that detect loci linked to nine rice resistance loci were used on the R3 population. Hybridization was weak for most probes, but all loci scored were monomorphic.

Discussion

Molecular markers linked to disease resistance loci have been identified in several crop species, e.g., wheat (Autrique et al. 1995), rice (Hittalmani et al. 1995), barley (Horvath et al. 1995), bean (Melotto et al. 1996), lettuce (Anderson et al. 1996), tomato (Chague et al. 1996; Ganal and Tanksley 1996), and in some cases have provided landmarks for map-based cloning of resistance genes (Michelmore 1995 b). Molecular markers also have become a powerful tool for marker-assisted selection of resistant genotypes (Horvath et al. 1995). In pearl millet, our results will provide tools for marker-aided selection of multiple disease resistance loci during cultivar development. Specifically, resistance genes to two rust isolates, 92-1 and 93-3, have been located to two different linkage groups, pearl millet 3 (93-3 resistance) and 4 (92-1 resistance). Resistance to rust isolate 92-1 was observed in two populations with presumably divergent sources of resistance genes, ICMP 83506 and Tift $89D_2$. The two sources of resistance displayed different pathotype specificities when challenged with 11 races of rust identified in the southeastern USA (Tapsoba and Wilson 1996), although both mapped at or near the marker *Xpsm716*, an RFLP marker at the proximal edge of a 28–45-cM segment in which no other markers have been identified (Liu et al. 1994; Busso et al. 1995). This linkage-group location near *Xpsm716* may be an important resistance locus in pearl millet. Interestingly, a major QTL for resistance to downy mildew pathogen populations from Nigeria/Niger also resides on pearl millet linkage-group 4 near *Xpsm716* (Jones et al. 1995). Genetic tests for allelism of the two rust pathotype specificities mapping to linkage-group 4 have not been carried out, thus it is possible that the 92-1 resistance from the two populations represents either two alleles at a single locus or a cluster of genes at a complex locus (Crute and Pink 1996). Rust resistance genes at homoeologous loci in flax have followed different evolutionary pathways, resulting in different pathotype specificities as a function of either multiple alleles at a single locus or multiple closely linked genes at a complex locus (Ellis et al. 1995). Thirteen different rust resistance specificities mapped to the L locus of flax, but efforts to link any of these specificities in coupling have been unsuccessful. At the complex M locus, however, recombination

between different genes conferring different specificities has been observed. If two genes at a complex locus are responsible for 92-1 resistance in pearl millet, pyrimiding of the two, linked in coupling, into a parental line would be useful for hybrid production and might offer a broader spectrum of resistance.

The use of these markers linked to rust- and pyricularia-resistance loci is currently limited to marker-assisted selection. Mapped-based cloning of these resistance genes is not feasible at this time due to the absence of a saturated genetic map for pearl millet and the presence of large gaps between markers. The most important genetic "gap" relative to cloning resistance genes is the 28–45-cM distance between *Xpsm716* and *Xpsm464* on linkage-group 4 containing the rust resistance loci from Tift $89D_2$ and ICMP 83506. It is not known whether this large genetic distance reflects a large physical distance or a "hot-spot" for recombination.

A plant carrying the Rr_1 resistance gene, the resistance gene from Tift $89D_2$, and pyricularia resistance from *P. glaucum* ssp. *monodii*, could be identified with a good probability using three molecular markers (SCAR-G8, OP-K19, and OP-D11) in the PCR reaction. Further work to obtain informative SCAR primers for OP-D11 and sequence-tagged site primers for *Xpsm716* would increase the speed of selecting a plant having all three loci. Tapsoba and Wilson (1996) gave evidence for at least 11 races of rust isolated in southern Georgia. Sources of resistance have been identified for most of these races and rust-resistant, segregating populations have been produced from many of these sources. Isolating molecular markers from these rust resistance sources would be easier than the original search for several reasons. Established and proven protocols for RAPD and RFLP analysis of pearl millet are available, the DNA isolation protocol has been improved to speed up sample preparation, and with the mapping of two loci for rust resistance, other resistant genotypes could be screened quickly for these loci. For pyricularia resistance in pearl millet, only one race is known but the related pathogen that infects rice has evolved into many known races. Continued research is needed to map the currently deployed pyricularia resistance gene to a linkage group and to develop more efficient markers for this locus, as well as to search for other pyricularia resistance loci.

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