Mapping the *d₁* and *d₂* Dwarfing Genes and the Purple Foliage Color Locus *P* in Pearl Millet

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Abstract

The d_1 and d_2 dwarfing genes and the P purple foliage color gene were placed on the restriction fragment length polymorphism (RFLP)-based molecular marker linkage map of pearl millet [*Pennisetum glaucum* (L.) R. Br.] using a mapping population based on a cross of inbred lines IP 18293 (D_1/D_1, d_2/d_2, P/P) and Tift 238D1 (d_1/d_1 D_2/D_2 p/p). A skeleton genetic linkage map of 562 cM (Haldane function) was constructed using 33 RFLP markers and these three morphological markers. The D_1/d_1 plant height locus mapped to pearl millet linkage group 1, while the D_2/d_2 plant height locus and the P/p foliage color locus mapped to pearl millet linkage group 4. Loose genetic linkage was observed between the D_2/d_2 and P/p loci, with 42% repulsion-phase recombination corresponding to 92 cM (Haldane). This loose linkage of morphological marker loci detected on pearl millet LG4 can likely find use in applied pearl millet breeding programs, as host plant resistances to both downy mildew and rust have previously been identified in this genomic region. Such exploitation of these morphological markers in an applied disease resistance breeding program would require development of appropriate genetic stocks, but the relatively loose genetic linkage between d_2 and P suggests that this should not be difficult.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is widely grown as a food crop of subsistence agriculture in sub-Saharan Africa and the Indian subcontinent. It is cultivated on about 27 million ha (FAO and ICRISAT 1996; Rachie and Majmudar 1980), and has an average grain yield of 500–600 kg/ha. Pearl millet has a number of advantages that have made it the traditional staple cereal crop in subsistence or low-resource agriculture in these hot semiarid regions. Morphological variants with distinct phenotypic expression can be used to establish linkage studies. In pearl millet, such variations have been observed in plant height and foliage color. Genetic variation for purple pigmentation of nodes, internodes, leaf blades, anthers, and glumes is observed in pearl millet, and at least five sources of genetic dwarfism are known in this crop (Anand Kumar and Andrews 1993; Appa Rao et al. 1986; Burton and Fortson 1966; Koduru and Krishna Rao 1983).

Dwarfing genes have been successfully utilized in short-statured cultivars of wheat, rice, barley, sorghum, and pearl millet, with improved lodging resistance. Semidwarf grain cultivars are better able than their tall counterparts to respond positively to high levels of nitrogen application because of their reduced lodging vulnerability. Therefore, under well-managed conditions, dwarf cereal genotypes frequently yield more harvestable grain than their tall counterparts. Semidwarf cereals also tend to have a higher grain harvest index, a higher leaf-to-stem ratio, and easier manual and mechanical harvesting characteristics than their tall counterparts. It is also easier to manage damage by grain-feeding birds on semidwarf cultivars of sorghum and pearl millet. Dwarf plants in pearl millet were discovered almost simultaneously in the United States and India. Burton and Fortson (1966) reported the inheritance of reduced plant height from five different sources, named D_1 to D_5, in pearl millet. Dwarfness in source lines D_1 and D_2 was controlled largely by one or two recessive genes. When transferred to near-isogenic backgrounds, dwarfness from source lines D_1 and D_2 is controlled by single independently segregating recessive genes, designated as d_1 and d_2. The d_2 dwarfing gene in pearl millet has several pleiotropic effects on plant phenotype. Principally, it reduces plant height by 50%

through a reduction in the length of all stem internodes except the peduncle (Burton and Fortson 1966), leading to a higher proportion of leaves (Rai and Hanna 1990). Based on the composition and digestibility of dwarf pearl millet forage, Johnson et al. (1968) concluded that the d_2 gene could be used to improve the nutritive value of pearl millet forage. Green forage from d_2 dwarf plants had significantly higher in vitro dry matter digestibility (IVDMD) than that from tall plants (Hanna et al. 1979). A similar response reported previously (Burton et al. 1969) for the d_2 gene was attributed to increased leaf percentage and improved nutritional quality of the stem fraction of pearl millet forage.

Pigmented plant parts constitute easily recognizable morphological genetic markers for use in several aspects of studies on higher plants. Because of this distinct advantage, the genetics of pigmentation have been worked out in several model plant systems such as maize, rice, and *Petunia*. The purple coloration is due to three anthocyanidins—cyanidin, delphinidin, and pelargonidin (Raju et al. 1985). Inheritance of purple pigmentation of the coleoptilar leaf in pearl millet was reported to be controlled by a single dominant gene (Yadav 1976). Koduru and Krishna Rao (1979) reported that purple seedling base, which is recognizable from the one-leaf or two-leaf stage, is controlled by the complementary interaction of two dominant genes, Pb_1 and Pb_2. Gill (1969) reported that purple foliage pigmentation is controlled by a single dominant gene Rp, whereas Minocha et al. (1980) and Minocha and Sidhu (1981) reported this trait is controlled by two complementary genes. Appa Rao et al. (1988) concluded that purple pigmentation on all plant parts is controlled by a single dominant gene. They observed that purple coloration of the leaf sheath, leaf blades, internodes, bristles, and glumes is inherited as a single unit, indicating pleiotropic effects of a single gene. They proposed the symbol P for this trait.

The modes of inheritance and linkage relationships of genes responsible for purple coloration of seven plant parts in pearl millet were discussed by Manga et al. (1988). They reported linkage between genes for purple node, auricle and purple leaf midrib, margin and leaf sheath, purple internode, and purple apicule. Singh et al. (1967) reported that the purple color of lemma, palea, and bristles (scored together) is a monogenic dominant trait. Gill (1969) reported that the purple bristling (Bep_1 and Bep_2) and purple glume (Pg_1 and Pg_2) traits are controlled sets of dominant complementary genes. Hanna and Burton (1992) reported that red and purple foliage color are controlled by alleles Rp_1 and Rp_2, respectively, at a single locus that is loosely linked (27%–28% recombination) with the d_2 dwarfing gene.

A potential revolution in the understanding of and ability to manipulate oligogenic and quantitative traits is offered to plant breeders by recent advances in genetic marker technology (Young 1999). Once linkage between a marker locus and the gene for an agronomic trait of interest has been established, DNA diagnostic tests can be used to guide plant breeding. Precise mapping of these three morphological marker loci—d_1, d_2, and P—would help pearl millet breeders and geneticists to utilize them more efficiently in their programs, and facilitate comparative genome mapping of pearl millet with other grasses (Devos and Gale 2000; Devos et al. 2000).

In this study, an attempt was made to locate pearl millet dwarfing genes d_1 and d_2, along with the purple foliage color gene P, on a restriction fragment length polymorphism (RFLP)-based skeleton linkage map for this crop.

Materials and Methods

Two pearl millet inbred lines, IP 18293 and Tift 238D1, were used as parents. Appa Rao et al. (1996) report IP 18293 was isolated at ICRISAT-Patancheru from a segregating population from the cross IP 10399 (India) × IP 10729 (Sudan). The subselection of IP 18293 used in the present study is a P purple-foliaged (Appa Rao et al. 1990), d_2 dwarf genetic stock that is highly resistant to downy mildew [*Sclerospora graminicola* (Sacc.) J. Schröt.] (Appa Rao et al. 1996). Tift 238D1 is a downy mildew-susceptible, green-foliaged, d_1 dwarf line reported by Burton (1966) to carry a single dominant gene for fertility restoration in the A_1 cytoplasmic-genetic male sterility system. Tift 238D1 was developed at the Coastal Plain Experiment Station (Tifton, GA).

A mapping population was produced at ICRISAT-Patancheru from the cross IP 18293 × Tift 238D1 by selfing 10 F_1 plants derived from a single plant × plant cross. The single F_1 plant that produced the largest number of seeds when self-pollinated was selected to provide F_2 seeds from which to generate the mapping population. F_2 plants were raised with their parental lines and the green-foliaged, d_2 dwarf, A_1 cytoplasmic male-sterile line 81A (Anand Kumar et al. 1984) at the Agricultural College and Research Institute, Tamil Nadu Agricultural University (Madurai, India) during the summer (hot postrainy) season of 1999 (February sowing). Each of the individual F_2 plants was selfed to produce F_3 seed. Single-plant testcrosses were also made to 81A using the individual F_2 plants as male parents. Among the segregating F_2 plants, purple-foliaged individuals were selected, and pollen collected from these was also dusted on the stigmas of d_1 dwarf, green-foliaged parental line Tift 238D1. Observations were recorded on the segregating F_2 plants for traits such as plant height (both quantitative and qualitative), foliage color, and panicle length.

Approximately 30 selfed seeds harvested from each of 142 individual F_2 plants were sown in pots (one pot per F_2 plant) to produce unselected seedlings for DNA sampling and transplanting. The resulting plants were raised under field conditions as F_2:F_3 (F_2-derived F_3) progenies in plot RL 18, at ICRISAT-Patancheru, during the late *kharif* (rainy) season of 1999 (August sowing). Immediately before transplanting these F_3 plants to the field, bulk leaf samples were collected from all F_3 seedlings in the progeny of an individual F_2 plant for use in DNA isolation. In the F_3 progeny rows, observations were recorded for plant height (both quantitative and qualitative) and foliage color. Testcrosses were made on d_2 dwarf, green-foliaged male-sterile line 81A using bulk pollen collected from all of the plants in a given F_2:F_3 progeny row.

Genomic DNA was extracted from leaf tissue samples as described by Sharp et al. (1988), using F_3 bulks representing each of the 142 individual entries from the segregating F_2 population. Each DNA sample was then digested with several restriction endonucleases. DNA fragments separated electrophoretically after digestion were transferred from agarose gels on to nucleic acid nylon transfer membranes (Hybond-N[+]; Amersham Pharmacia Biotech, Ltd.) following the procedure of Southern (1975). We received homologous clones for probing from a *Pst*I genomic library (Liu et al. 1994) from M. D. Gale and Katrien M. Devos of the John Innes Center, Cambridge Laboratory, Norwich, United Kingdom. RFLP genotyping was performed following Liu et al. (1994).

Testcross trials were conducted at ICRISAT-Patancheru (field numbers RP 11C and RP 8A). Testcrosses were sown along with the parental lines and tester 81A in single-replication augmented design trials. Testcross trial I, including the crosses of 81A × all segregating F_2 plants and Tift 238D1 × purple-foliaged F_2 plants, was evaluated during the summer season of 1999–2000 (January sowing) in field RP 11C. Testcross trial II, of crosses produced on green-foliaged d_2 dwarf male-sterile line 81A using bulk pollen from the F_2:F_3 progeny rows, were evaluated in the *kharif* season of 2000 (June sowing) in field RP 8A. In each trial, each entry was sown as a single 4 m row with 75 cm distance between rows and thinning was done 2–3 weeks after sowing to maintain a plant-to-plant distance of approximately 15 cm and a total population of about 29 plants to be observed for each testcross progeny in each trial. A basal fertilizer dose of diammonium phosphate (100 kg/ha) was applied and a topdressing of urea (100 kg/ha) was provided 30 days after sowing. Observations were recorded in both trials on traits such as plant height (qualitative and quantitative), foliage color, and uniformity. Data from these morphological traits were used to score individual F_2 plants as heterozygous or homozygous for alleles of one of the two parental lines and these scores were then used, along with the RFLP marker genotype data for individual F_2 plants, for linkage map construction.

Multipoint linkage analysis was performed using the program MapMaker/Exp version 3.0b (supplied by E. S. Lander, Whitehead Institute for Biomedical Research, Cambridge, MA) (Lincoln et al. 1992a), with a threshold LOD value of 3.0 and recombination fraction of 0.5, and with the error detection algorithm implemented assuming an a priori error rate of 1%. CentiMorgan (cM) distances were calculated using the Haldane function. Linkage group numbers were assigned as per Liu et al. (1994), using RFLP loci identified by common single-copy probes. Quantitative data collected on plant height from the F_2 segregating population was used to detect quantitative trait loci (QTL) for this trait controlled by the d_1 and d_2 dwarfing genes. QTL mapping was initially conducted using the interval mapping method implemented in MapMaker/QTL version 1.1b (Lander and Botstein 1989; Lincoln et al. 1992b) and results from this were confirmed using the composite interval mapping method implemented in QTL Cartographer (Basten et al. 1997).
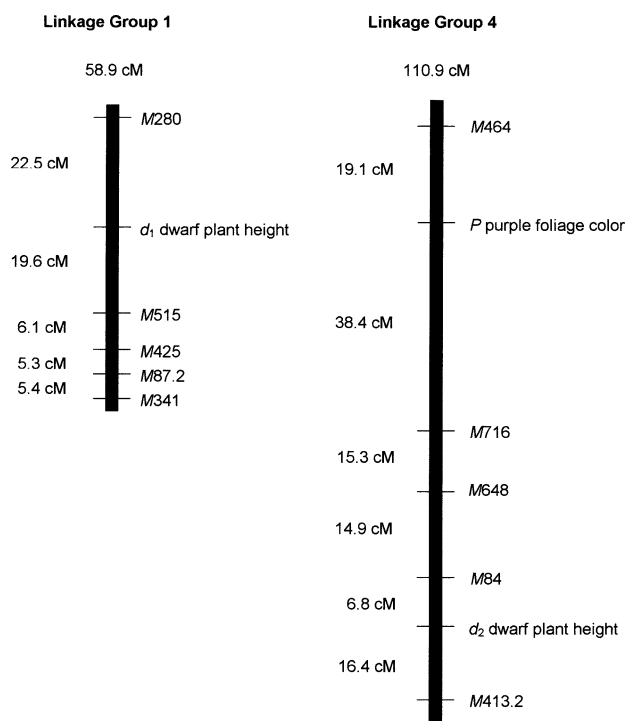

Figure 1. Positions of morphological markers d_1, d_2, and P on pearl millet linkage groups 1 and 4. To the left of each linkage group are map distances (Haldane function) for each interval, to the right of each linkage group are marker locus names with the *Xpsm* prefix for RFLP loci abbreviated as *M*.

Results and Discussion

A skeleton genetic linkage map of 562 cM (Haldane function), comprising the seven expected pearl millet linkage groups, was constructed using 33 homologous RFLP markers and the three morphological markers d_1, d_2, and P. The RFLP marker locus order of the skeleton map of the (IP 19283 × Tift 238D1)-based mapping population used in this study was comparable to that found in previously developed RFLP-based genetic linkage maps of this species (Azhaguvel 2001; Busso et al. 1995; Devos et al. 2000; Hash et al., unpublished data; Kolesnikova 2001; Liu et al. 1994; Yadav et al. 2002), with only one pair of markers on linkage group 2 inverted relative to orders in two unpublished skeleton maps (not shown). Total linkage map length and the locations of larger gaps between linked RFLP loci were also comparable to these earlier studies (not shown). Flanking RFLP markers linked to each of the three target morphological markers were detected (Figure 1).

Pearl millet plant height is considered to be a relatively simply inherited trait with a few loci largely affecting its expression. The d_1 plant height locus was placed in pearl millet linkage group 1 (LG1). It was 22.5 cM from marker locus *Xpsm*280 on the top of this linkage group. The other flanking marker was *Xpsm*515, located 19.5 cM from the d_1 dwarf locus. The total mapped length of LG1 in this population was 58.9 cM (Figure 1).

The d_2 dwarf plant height locus was placed near the bottom of pearl millet linkage group 4 (LG4) with *Xpsm*84 and *Xpsm*413.2 as flanking RFLP marker loci. The purple foliage color marker locus *P* was placed between RFLP marker loci *Xpsm*464 and *Xpsm*716 near the top of LG4. The total length of LG4 in this population was 110.9 cM (Figure 1). The LOD for this LG4 marker order was 252.7, and the most likely candidate error (individual segregant having an unlikely three-point marker genotype based on this marker order) had an LOD of 1.35. The map distance observed between the *P* purple foliage color locus and that for d_2 dwarf plant height was 91.8 cM (Haldane), corresponding to 42% recombination (95% confidence interval [CI] of 33–50%), which was marginally greater than the 27%–28% recombination previously reported by Hanna and Burton (1992) for linkage between the d_2 dwarfing gene and the *Rp* locus with alleles conferring green, red, and purple foliage color. Observed linkages of morphological markers d_2 and *P* with the three RFLP loci that mapped between them provide additional evidence for this loose linkage, since linkage is transitive. More precise estimates of the genetic distance between these two morphological marker loci would require use of substantially larger segregating F_2 populations, preferably with recessive alleles in both coupling and repulsion phases and the use of polymerase chain reaction (PCR)-compatible DNA marker loci mapping to the region of LG4 between *P* and d_2. As the d_2 dwarfing genes used in these two studies were identical by descent, but the *Rp* and *P* loci controlling foliage color were not, allelism tests will be necessary to ascertain whether these differences in map distance are due to the small population size used in the current study or the involvement of more than one gene on pearl millet LG4 that can confer purple foliage color.

The results of interval mapping as implemented in MapMaker/QTL suggested that there were two QTLs associated with d_1 dwarf plant height, one near the top of LG1 and the other near the bottom of this linkage group. However, QTL Cartographer results indicated that there was only one plant height QTL associated with d_1 dwarf plant height on LG1, with dwarf height conferred by recessive alleles from Tift 238D1. A plant height QTL corresponding to the d_2 dwarfing gene from parent IP 18293 was located on LG4 by both MapMaker/QTL and QTL Cartographer. A statistically significant two-QTL model (LOD = 23.8, R^2 = 0.64) for plant height from MapMaker/QTL and a non-significant three-QTL model for this trait confirmed that the d_1 and d_2 loci, mapping to LG1 and LG4, respectively, were responsible for most of the genetic variation in plant height in the F_2 population used in this study. The loose linkage detected for the d_2 and *P* loci on pearl millet LG4 can likely find use in applied pearl millet breeding programs, as host plant resistances to both downy mildew (Jones et al. 1995) and rust (Morgan et al. 1998) have previously been identified in this genomic region. Such exploitation of these morphological markers in an applied disease resistance breeding program would require development of appropriate genetic stocks, but the relatively loose genetic linkage between d_2 and *P* suggests that this should not be difficult.

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