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Inheritance and Allelic Study of Brown Midrib Trait in Pearl Millet

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In pearl millet [*Pennisetum glaucum* (L.) R. Br.], two brown midrib lines are reported. One line (Pbmr) was developed at Purdue University through chemical mutagenesis, and the other line SDML 89107 developed by SADC/ICRISAT through selection from a germplasm accession from Zimbabwe. Allelism crosses among these two lines revealed that their brown midrib genes are allelic to each other. In another study, F_1, F_2, and test crosses derived from 10 crosses between six normal midrib parents and two brown midrib lines were studied. The results revealed that the brown midrib trait in most of the crosses was controlled by one major recessive gene in the nuclear genome of pearl millet. It appears that there are modifying and/or epistatic gene(s) that affect the expression of the brown midrib trait. The gene symbol *bm*$_1$*bm*$_1$ is proposed for the major gene present in SDML 89107 and Pbmr. The brown midrib trait in pearl millet is relatively simply inherited, and 20%–30% plants with brown midribs can be expected in F_2 populations segregating for this character.

The lignin content of a plant limits its use as a forage and its forage digestibility by ruminants. Genetic control of the lignification process offers the most direct method of reducing lignin content and increasing dry matter digestibility. Brown midrib mutations are seen as one way to favorably modify lignin quality and quantity (Cherney 1990; Cherney et al. 1991).

Brown midrib mutants in maize (*Zea mays* L.) were identified as early as 1924 (Jorgensen 1931). He described these lines as having brown pigmentation in the leaf midrib, stem tassel, cob, and roots. Four nonallelic brown midrib genes have been identified in maize (Kuć and Nelson 1964) and are designated *bm*$_1$ to *bm*$_4$, *bm*$_1$ being the first gene described by Jorgensen (1931). Inbreds and F_2 populations carrying these genes were evaluated for fiber composition by Muller et al. (1971) and Lechtenberg et al. (1972). They found the *bm*$_3$ gene to be the most effective in reducing lignin percentage. Barnes et al. (1971) and Lechtenberg et al. (1972) showed that the *bm*$_3$ mutant had the highest in vitro dry matter digestibility.

Porter et al. (1978) treated seeds from two grain sorghum [*Sorghum bicolor* (L.) Moench.] lines, 954114 (short) and 954104 (tall), with diethyl sulfate to induce mutations. Nineteen independently occurring brown midrib mutants were identified in segregating M_3 head rows and designated bmr-1 through bmr-19. Allelism tests among 12 of these brown midrib mutants of sorghum gave inconsistent results, but indicated that more than one gene locus is involved in the brown midrib trait (Bittinger et al. 1981).

Three low-lignin, brown midrib sorghum mutants, designated bmr-6, bmr-12, and bmr-18, were each backcrossed to normal grain and grassy type sorghums (Fritz et al. 1981). In both genetic backgrounds, the brown midrib mutants showed lower lignin percentages and higher digestibility than normal genotypes.

In 1989, four sorghum accessions with brown midrib plants were identified in Malawi germplasm growing at Makoka Research Station, Malawi (Gupta 1990). The brown midrib gene(s) in four naturally occurring brown midrib lines—IS 23787, IS 23789, IS 21549, and IS 23765—were allelic to each other and to bmr-6 (Gupta 1995). The brown midrib gene(s) in all these five lines were nonallelic to bmr-12 and bmr-18, whereas bmr-12 and bmr-18 were allelic (Gupta 1995), confirming the report by Bittinger et al. (1981).

In pearl millet, two brown midrib lines have been reported. One line, Pbmr, was developed at Purdue University by treating two inbreds derived from Tift $23D_2B_1$/2 × PI 185642 through chemical mutagenesis (Cherney et al. 1988). The other line, SDML 89107, was developed by SADC/ICRISAT through selection from a germplasm accession from Zimbabwe (Gupta et al. 1993).

Cherney et al. (1988) reported that brown midrib pearl millet was phenotypically similar to brown midrib mutants in sorghum. The lignin concentration in brown midrib pearl millet was 40 g/kg, compared to 50 g/kg, its normal counterpart. The brown midrib genotype's in vitro dry matter digestibility (IVDMD) was 726 g/kg, compared to the normal genotype 659 g/kg.

At 50% flowering, the whole-plant IVDMD of SDML 89107 was 10.7% higher (690 g/kg versus 623 g/kg) than that of its normal counterpart, whereas its stem IVDMD was 16.2% higher (Gupta et al. 1993).

The number of loci and the allelic relationships of genes controlling the brown midrib trait in these two pearl millet brown midrib mutants are of interest to plant breeders. The purpose of this study was to (a) study the inheritance of the brown midrib trait in pearl millet and (b) evaluate the allelic relationship of the newly identified, naturally occurring, brown midrib line with the previously described chemically induced brown midrib mutant.

Materials and Methods

Inheritance

The material consisted of two brown midrib pearl millet lines: Pbmr, developed at Purdue University through induced mutation (Cherney et al. 1988), and SDML 89107, developed by SADC/ICRISAT through selection (Gupta et al. 1993). Four advanced grain varieties—SDMV 89005, SDMV 89007, ICMV 82132, and ICMV 87901—were used as normal midrib parents.

These six entries were sown at Muzarabani, Zimbabwe on 14 April 1990 to produce F_1 crosses (including reciprocals) between brown midrib and normal midrib parents. The F_1 seeds and the brown midrib parents were sown at Aisleby, Zimbabwe on 18 October 1990 to classify their midrib color. The brown midrib parents and the F_1 seeds for eight crosses where brown midrib lines were used as females were sown in a greenhouse at Matopos, Zimbabwe on 22 June 1992. F_2's were produced, and test crosses were made using brown midrib lines as female parents. In addition to the above crosses, the F_1 seeds for two crosses—Pbmr × Purdue normal (Pnormal), and SDML 89107 × SDMV 89107 (normal counterpart of SDML 89107)—were produced at Muzarabani in the material sown on 26 May 1991. The brown midrib parents and F_1 seeds of these two crosses were sown in a greenhouse at Matopos on 2 April 1992 to produce F_2's and test crosses (using brown midrib lines as female parents). The resultant 10 F_2 populations and 10 test crosses (Table 1) were sown at Bulawayo, Zimba-

Table 1. Segregation in F_2 populations, derived from crosses between brown midrib and normal midrib lines, for midrib color in pearl millet

F_2 population (brown × normal)	Number of plants		χ^2 value at	
	Normal	Brown	3:1	13:3
Pbmr × SDMV 89005	600	158	6.98**	2.18 ns
Pbmr × SDMV 89007	791	231	3.13 ns	9.96**
Pbmr × ICMV 82132	283	87	0.44 ns	5.51*
Pbmr × ICMV 87901	395	153	2.49 ns	30.24**
Pbmr × PNormal	388	109	2.50 ns	3.30 ns
SDML 89107 × SDMV 89005	452	118	5.62*	1.42 ns
SDML 89107 × SDMV 89007	509	112	16.06**	0.21 ns
SDML 89107 × ICMV 82132	260	52	11.56**	0.89 ns
SDML 89107 × ICMV 87901	563	213	2.48 ns	38.54**
SDML 89107 × SDMV 89107	240	71	0.78 ns	3.40 ns

* Significance at $P = .05$.
** Significance at $P = .01$.
ns = nonsignificant.

bwe on 16 January 1993 to classify the plants for midrib color.

Allelism Study

Two brown midrib lines (Pbmr and SDML 89107) were sown at Muzarabani on 26 May 1991 to produce reciprocal crosses. The F_1 seeds of these two crosses were sown in the greenhouse at Matopos on 2 April 1992 to classify their midrib color and to produce F_2 seeds. The resultant F_2 population was sown at Bulawayo on 1 October 1992.

Classification of Plants for Midrib Color

The classification for normal midrib and brown midrib plants was done at the seventh leaf stage (the brown midrib color appears at sixth leaf stage and becomes clear at the seventh leaf stage). All the brown midrib plants were identified and tagged.

Statistical Analysis

The χ^2 test was used to test the data for goodness of fit (Steel and Torrie 1980).

Results and Discussion

Inheritance

In 10 crosses involving two brown midrib lines and six elite normal midrib lines, all the F_1 plants had normal midribs. This indicates that the brown midrib trait in pearl millet is controlled by recessive allele(s).

The segregation data for midrib color in F_2 populations and test crosses are presented in Tables 1 and 2. In three F_2 populations, involving Pbmr and three normal midrib lines (PNormal, ICMV 82132, and ICMV 87901), midrib color segregation fit a 3:1 (normal/brown) ratio. The segregation in test cross data for midrib color in these crosses fit a 1:1 ratio. These data suggest that the brown midrib trait is controlled by a single recessive gene in these crosses.

In one cross between Pbmr and SDMV 89007, the segregation data in the F_2 population for midrib color fit a 3:1 (normal/brown) ratio. However, this is not supported by test cross data for this cross as the χ^2 value at the expected ratio, i.e., 1:1, was significant. In another F_2 population, Pbmr × SDMV 89005, the χ^2 value at the expected ratio of 13:3 was not significant, indicating a good fit of the observed segregation to this ratio. This was supported by segregation in test cross data where the midrib color segregation fit a 1:1 (normal/brown) ratio.

In F_2 populations, involving SDML 89107 and the normal midrib line SDMV 89107, midrib color segregation fit a 3:1 (normal/brown) ratio. This indicates that the brown midrib trait is controlled by a single recessive gene in this cross. This is supported by segregation in test cross data involving this cross in a 1:1 ratio.

In another F_2 population, SDML 89107 × ICMV 87901, the χ^2 value at 3:1 (normal/brown) ratio is not significant, indicating that this trait is controlled by a single gene. However, this is not supported by test cross data for this cross as the χ^2 value at the expected ratio, i.e., 1:1, was significant.

In the remaining three crosses, involving brown midrib line SDML 89107, a two-gene epistasis model fit better than any other ratio. The F_2 populations involving brown midrib line SDML 89107 and three normal midrib lines, the χ^2 values at the expected ratio 13:3 are not significant, indicating a good fit of the observed segregations to this ratio. The segregation in test cross data of two crosses fit a 1:1 ratio, and for the third cross (SDML 89107 × SDMV 89005), the χ^2 values at both 1:1 and 3:1 (normal/brown) ratios are significant, indicating a poor fit of the observed segregations to either of these ratios.

It appears that the brown midrib trait in pearl millet, in most of the crosses involving brown midrib lines, is controlled by one major gene. In a few crosses, this trait is controlled by two genes. Alternatively, modifying genes are present, which affect the expression of the trait.

Allelic Relationships

The F_1 plants (194) in a cross between Pbmr and SDML 89107, including a reciprocal cross, had uniformly brown midribs. All the 312 F_2 plants derived from this cross also had brown midribs. This indicates that the gene(s) controlling this trait in these two brown midrib lines are allelic. The gene symbol bm_1bm_1 is proposed for this gene in both brown midrib lines SDML 89107 and Pbmr.

Table 2. Segregation in test crosses, derived by crossing brown midrib line with F_1 crosses between brown midrib and normal midrib lines, for midrib color in pearl millet

Test cross (brown × normal)	Number of plants		χ^2 value at	
	Normal	Brown	1:1	3:1
Pbmr × (Pbmr × SDMV 89005)	43	32	1.61 ns	12.48**
Pbmr × (Pbmr × SDMV 89007)	243	129	34.94**	18.58**
Pbmr × (Pbmr × ICMV 82132)	58	57	0.01 ns	37.01**
Pbmr × (Pbmr × ICMV 87901)	138	135	0.03 ns	87.04**
Pbmr × (Pbmr × PNormal)	59	50	0.74 ns	25.32**
SDML 89107 × (SDML 89107 × SDMV 89005)	223	117	33.05**	16.06**
SDML 89107 × (SDML 89107 × SDMV 89007)	111	89	2.42 ns	40.56**
SDML 89107 × (SDML 89107 × ICMV 82132)	67	69	0.03 ns	48.04**
SDML 89107 × (SDML 89107 × ICMV 87901)	244	96	64.42**	1.90 ns
SDML 89107 × (SDML 89107 × SDMV 89107)	45	61	2.42 ns	59.89**

* Significance at $P = .05$.
** Significance at $P = .01$.
ns = nonsignificant.

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Localization of nod-3, a Gene Conditioning Hypernodulation, and Identification of a Novel Translocation in *Pisum sativum* L. cv. Rondo

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The gene *nod-3*, which conditions hypernodulation in pea (*Pisum sativum* L.), was localized to linkage group I by linkage to markers *d* (anthocyanin ring at the base of stipules) and *Idh* (isocitrate dehydrogenase). This region of the pea genome has an unusual concentration of genes involved in the legume-*Rhizobium* symbiosis. Line Nod 3 and its parent cv. Rondo have a novel translocation which was characterized cytogenetically. The translocation does not effect segregation of the genes *nod-3, d* and *Idh*. One of its breakpoints is situated near gene *b* (pink flowers) of linkage group III, which corresponds to chromosome 5. The second partner of the translocation remains to be determined.

Legume mutants with an altered symbiosis provide useful material for studying the role of the plant in establishing effective nitrogen fixation. In pea, about 30 nonallelic *nod* or *sym* genes have been identified. Some genes, conferring strain specificity, were found as naturally occurring variants among primitive cultivars from the Middle East (Kneen and LaRue 1984; Lie 1984). However, most of the genes have been identified in symbiotic mutants induced in commercial cultivars of *Pisum sativum* (Duc and Messager 1989; Jacobsen 1984; Kneen and LaRue 1988). Compilations of these genes are presented in the reviews by LaRue and Weeden (1992) and Brewin et al. (1993).

Although most induced mutants have few or no nodules, some have excessive nodulation. Nine such mutants in soybean are alleles of a single gene *nts* (Delves et al. 1988). Among six hypernodulating mutants in pea—nod-3 (Jacobsen and Feenstra 1984), 190F, 191F, P77, P79 (Duc and Messager 1989), and k301 (Sidorova and Uzhintseva 1992)—two (190F and 191F) have been found to be allelic (Duc and Messager 1989), but other allelism tests have not been made, and the number of complementation groups remains uncertain. The two mutant lines Nod 3 and 190F exhibit considerably different phenotypes, and corresponding genes are tentatively treated as different loci (LaRue and Weeden 1992). In the absence of a thorough complementation analysis, the mapping of the mutants can partially answer the question of how many genes are affected by the hypernodulating mutations and where they map in relation to other genes involved in symbiotic nitrogen fixation.

A not uncommon problem with mapping studies in pea is appearance of unexpected chromosomal rearrangements. Although most cultivated forms of peas from the northern temperate zone are believed to possess the standard karyotype as defined in Blixt (1958), some spontaneous translations have been identified in European cultivars (see references in Yarnell 1962). Lamprecht (1939) had observed the spontaneous appearance of new chromosomal rearrangements in his experimental material with an average frequency of ~1/100,000, but in some lines reciprocal translocations occurred with the frequency as high as 1/1,000 (Lamprecht 1949).

In this communication we report the location of the gene *nod-3* as well as the presence of a novel translocation in the mutant line Nod 3 and its parent, the cultivar Rondo.

Materials and Methods

Seeds of the mutant line Nod 3 and its parent cv. Rondo were kindly provided by Dr. Jacobsen (University of Groningen, The Netherlands). Multiple marker lines JI 73 (NGB 1238; obtained from the John Innes Institute, Norwich, U.K.) and Slow (developed at Cornell University, Ithaca, New York) were used as alternative parents for crossing with the Nod 3 line. Both marker lines have normal karyotype and are recommended for mapping studies in pea (Weeden et al. 1993). Nod 3 × JI 73 will be referred as cross A, and Nod 3 × Slow as cross B.

Plants were grown in small conical pots (50 cm^3) containing vermiculite and watered with a low N-nutrient solution. Seedlings were inoculated with *R. leguminosarum*, strain 128C53, grown in a 20°/15°C 16 h/18 h day/night regime in a light room, and scored for nodulation at 3–4 weeks after planting as described earlier (Kneen and LaRue 1988). After nodulation tests, plants were transplanted into potting soil, and scored later for morphological markers, isozyme markers, and pollen fertility.

Isocitrate dehydrogenase phenotypes were determined as described (Weeden