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Allelic Relationships and Inheritance of Brown Midrib Trait in Sorghum

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Four naturally occurring brown midrib mutants in sorghum [*Sorghum bicolor* (L.) Moench.] identified in 1989 in germplasm from Malawi, and three brown midrib lines previously developed at Purdue University through induced mutations (bmr-6, bmr-12, and bmr-18) were considered in this study. Allelism tests in these seven lines revealed that the four naturally occurring brown midrib lines are allelic to each other and to bmr-6. These five lines were non-allelic to bmr-12 and bmr-18, whereas bmr-12 and bmr-18 were allelic to at least those genes necessary to express brown midrib trait. Inheritance of the trait in F_1 and F_2 populations derived from 15 crosses between nine normal midrib parents and three brown midrib lines was then studied. Results revealed that the brown midrib trait is controlled, at least in part, by genes at two or more independent loci in the nuclear genome of sorghum. There is a strong possibility that some modifying genes may affect the expression of this trait. In general, 15% to 45% of plants with brown midrib can be expected in F_2 populations segregating for this character.

Forage utilization and digestibility by ruminants are limited by the lignin content in the plant. Genetic control of the lignification process offers a 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).

In maize (*Zea mays* L.), brown midrib mutants were identified as early as 1924 (Jorgensen 1931). Jorgensen 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 (Kuc' and Nelson 1964) and are designated bm_1 to bm_4, bm_1 being the first gene described by Jorgensen (1931). Inbred 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 the bm_3 mutant to have the highest in vitro dry-matter digestibility.

Porter et al. (1978) treated seeds from two 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. Numbers associated with the mutants (bmr) do not necessarily represent different loci. Corresponding normal segregants were designated N-1 through N-19.

Allelism tests among 12 of these brown midrib mutants of sorghum gave inconsistent results: when two mutants appeared to be allelic, they often did not show the same relationship with other mutants as expected. For example, when bmr-12 and bmr-18 were crossed, they produced a brown midrib F_1, indicating that they are allelic. However, when bmr-2 and bmr-12 were crossed, the F_1 was normal, whereas bmr-2 and bmr-18 produced a brown midrib F_1 (Bittinger et al. 1981). These tests further indicated that more than one locus is involved in the expression of brown midrib trait, and there is a strong possibility that some modifying genes may affect the expression of this trait.

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 were lower in lignin percentage and higher in digestibility than their normal counterparts.

In 1989, four sorghum accessions with brown midrib plants were identified in Malawi germplasm grown at Makoka Research Station, Malawi (Gupta 1990). The number of loci and the allelic relationships of genes controlling the brown midrib trait in these accessions are of interest to plant breeders. The first purpose of the study reported here was to evaluate the allelic relationships of these newly identified naturally occurring brown midrib sorghum lines and compare them with previously described, chemically induced brown midrib mutants. The second purpose was to study the inheritance of brown midrib trait in sorghum.

Materials and Methods

Allelic Study

For allelism testing, I used seven brown midrib sorghum lines, including three (bmr-6, bmr-12, and bmr-18) developed at Purdue University (Porter et al. 1978) through chemically induced mutation, and four (IS 23787, IS 23789, IS 21549, and IS 23765) that I identified in Malawi germplasm grown at Makoka Research Station in 1989. These seven lines were sown at Muzarabani, Zimbabwe, on May 26, 1991. They were crossed in all possible combinations (excluding reciprocals) using hand emasculation. The F_1 seed was sown at Henderson, Zimbabwe, on December 14, 1991. All F_1 plants were self-pollinated to produce pure F_2 seed. Four F_2 populations derived from crosses of bmr-6 with IS 23787, IS 23789, IS 21549, and IS 23765 were sown at Bulawayo, Zimbabwe, on October 1, 1992.

Inheritance Study

For inheritance testing, I used nine sorghum lines with normal midrib and three sorghum lines (IS 23787, bmr-12, and bmr-18) with brown midrib. All seed was sown at Muzarabani, Zimbabwe, on April 13, 1990. Then, the normal midrib lines were crossed onto brown midrib lines using hand emasculation. Fifteen crosses were produced. The F_1 seed from these 15 crosses was sown at Aisleby, Zimbabwe, on October 18, 1990 with a plant population of 90–100 plants per plot. All F_1 plants were self-pollinated to produce pure F_2 seed. The F_2 populations were sown at Aisleby, Zimbabwe, on October 31, 1991.

Classification of Plants for Midrib Color

I classified normal midrib and brown midrib plants at the 7-leaf stage in both the F_1 and the F_2 populations, as the brown midrib color appears at the 6-leaf stage and becomes clear at the 7-leaf stage. All brown midrib plants were identified and tagged.

Table 1. Midrib color (N for normal and bmr for brown) of F_1 hybrids among seven brown midrib lines in sorghum[a]

Line	bmr-12	bmr-18	IS 23787	IS 23789	IS 21549	IS 23765
bmr-6	N (26)	N (25)	bmr (39)	bmr (27)	bmr (21)	bmr (40)
bmr-12	—	bmr (100)	N (82)	N (63)	N (118)	N (82)
bmr-18		—	N (27)	N (27)	N (64)	N (38)
IS 23787			—	bmr (87)	bmr (69)	bmr (88)
IS 23789				—	bmr (73)	bmr (68)
IS 21549					—	bmr (91)

[a] The number of F_1 plants is given in parentheses.

Statistical Analysis

I used the chi-square test to test the data for goodness of fit.

Results and Discussion

Allelic Relationships

The F_1 plants from crosses of bmr-6 with the Malawi brown midrib lines, IS 23787, IS 23789, IS 21549, and IS 23765, were uniformly brown midrib (Table 1), and all the F_2 plants derived from these crosses were also brown midrib (Table 2). Furthermore, the F_1 plants from crosses among the four Malawi brown midrib lines were uniformly brown midrib (Table 1). This indicates that the gene(s) controlling this trait in these four brown midrib lines are allelic to those of bmr-6.

The F_1 crosses of the above five lines with bmr-12 and bmr-18 were normal. The cross bmr-12 × bmr-18 produced a brown midrib F_1 (Table 1). These crosses confirm that genes conferring brown midrib trait in bmr-12 and bmr-18 are nonallelic to those in bmr-6, and that those of bmr-12 and bmr-18 are allelic to at least those genes which are necessary to express brown midrib trait. These results with Purdue bmr lines are in agreement with the findings of Bittinger et al. (1981) and suggest that allele(s) conferring the brown midrib trait in sorghum are located on at least two independent loci.

Inheritance

In the second study, of 15 crosses involving three brown midrib lines IS 23787, bmr-12, and bmr-18 and a range of elite lines having normal midrib (Table 3), all the F_1 plants had normal midribs. This indicates that the brown midrib trait is controlled by recessive allele(s).

The segregation data for midrib color in these 15 F_2 populations are presented in Table 3. The chi-square values at expected F_2 ratios 3:1 and 15:1 are significant at the 5% significance level for most of the crosses, indicating poor fit of the observed segregations to these ratios. Only in four F_2 populations (involving brown midrib lines IS 23787 and bmr-12), did the midrib color segregation fit a 3:1 (normal : brown) ratio, but other ratios fit better. The 15:1 ratio does not fit in any of these populations.

In all the crosses involving brown midrib line IS 23787, epistasis models fit better than any other ratio. In four F_2 populations, the midrib color segregation fit a 171:85 (normal : brown) ratio. In one case, IS 23787 × WSV 387, the F_2 ratio fit a 13:3 ratio.

However, in the F_2 populations involving brown midrib line bmr-12, the chi-square values at expected ratio 171:85 are significant at the 1% significance level for all the crosses, indicating poor fit of the observed segregations to this ratio. In five F_2 populations involving brown midrib line bmr-12, the chi-square values at the expected ratio 13:3 (normal : brown) are not significant, indicating good fit of the observed segregations to this ratio. In one case, bmr-12 × Tegemeo, the F_2 ratio fit a 9:7 ratio.

In the three F_2 populations involving brown midrib line bmr-18, the chi-square values at expected ratios 13:3 (normal : brown) and 55:9 (normal : brown) are not significant, indicating good fit of the observed segregations to either of these ratios. The homogeneity chi-square for brown midrib trait segregation over these three F_2 populations was not significant. Based on the pooled analysis, a 55:9 ratio gave the best fit. In one case, bmr-18 × SV 1, the F_2 ratio fit a 171:85 ratio.

In conclusion, 15% to 45% of plants with brown midrib can be expected in F_2 populations segregating for this character. The results revealed that the brown mid-

Table 2. Segregation for midrib color in F_2 populations, derived from crosses between brown midrib lines, in sorghum

F_2 population	No. of plants	
	Normal	Brown
bmr-6 × IS 23787	0	640
bmr-6 × IS 23789	0	658
bmr-6 × IS 21549	0	719
bmr-6 × IS 23765	0	612

Table 3. Segregation for midrib color in F_2 populations, derived from crosses between brown midrib and normal midrib lines, in sorghum

F_2 population (brown × normal)	Observed no. of plants			Chi-square value at expected ratio				
	Normal	Brown	Total	3:1	13:3	9:7	55:9	171:85
IS 23787 × SV 1	140	69	209	7.16**	27.91**	9.79**	62.12**	0.00
IS 23787 × Town	186	79	265	3.27	21.28**	20.92**	54.39**	1.37
IS 23787 × SDS 1513	147	73	220	7.85**	30.08**	9.98**	66.55**	0.00
IS 23787 × WSV 387	170	45	215	1.90	0.67	45.49**	8.39**	14.60**
IS 23787 × SDS 1594-1	181	82	263	5.35*	26.67**	16.89**	63.76**	0.49
Homogeneity chi-square (4 df) 10.575*								
Homogeneity chi-square excluding cross IS 23787 × WSV 387 (3 df) 0.859								
Total[a] (1 df)	303	654	957	22.64**	104.7**	56.82**	245.2**	1.02
bmr-12 × SV 1	177	38	215	6.15*	0.16	59.40**	2.32	23.38**
bmr-12 × SDS 1513	147	40	187	1.30	0.86	37.99**	8.31**	11.77**
bmr-12 × Tegemeo	112	90	202	41.19**	88.29**	0.05	155.41**	11.74**
bmr-12 × SDSL 88154	147	34	181	3.73	0.00	45.84**	3.34	16.97**
bmr-12 × WSV 387	167	33	200	7.71**	0.66	60.35**	0.98	25.16**
bmr-12 × SDS 1594-1	201	34	235	13.90**	2.83	81.88**	0.03	37.19**
Homogeneity chi-square (5 df) 74.531**								
Homogeneity chi-square excluding cross bmr-12 × Tegemeo (4 df) 3.787								
Total[a] (1 df)	179	839	1018	29.86**	0.91	283.2**	10.44**	111.9**
bmr-18 × SV 1	127	59	186	4.48*	20.54**	10.94**	47.99**	0.18
bmr-18 × Segaolane	194	31	225	15.11**	3.65	82.13**	0.01	38.28**
bmr-18 × SDS 1594-1	146	33	179	4.11*	0.01	46.61**	2.83	17.60**
bmr-18 × SDSL 87019	185	31	216	13.06**	2.74	75.86**	0.02	34.61**
Homogeneity chi-square (3 df) 26.493**								
Homogeneity chi-square excluding cross bmr-18 × SV 1 (2 df) 1.908								
Total[a] (1 df)	95	525	620	30.96**	4.78*	203.5**	0.81	89.37**

* indicates significance at $P = .05$.

** indicates significance at $P = .01$.

[a] Only for those crosses for which homogeneity chi-square is not significant.

rib trait is controlled, at least in part, by genes at two or more independent loci in the nuclear genome of sorghum. There is a strong possibility that some modifying genes may affect the expression of this trait. The allele(s) for brown midrib trait located on different loci can be combined into a single genotype. It will be useful to compare the dry-matter digestibility of the resulting genotypes to that of their parental brown midrib sources that are currently available.

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Shikimate Dehydrogenase Allozymes: Inheritance and Close Linkage to Fruit Color in the Diploid Strawberry

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The inheritance of shikimate dehydrogenase (SDH) allozymes, and their linkage relationships with phosphoglucoisomerase (PGI-2) and a locus governing red versus yellow fruit color were studied in the diploid ($2n = 2x = 14$) strawberry. SDH behaved as a monomeric enzyme with alternate, codominant alleles at a single locus. In the F_2 generation of reciprocal crosses between *Fragaria vesca* Alpine cultivars Baron Solemacher (red fruit) and Yellow Wonder (yellow fruit), SDH segregated in a 1:2:1 ratio, and fruit color segregated 3 red / 1 yellow. The SDH and fruit color segregations were highly correlated, with a recombination frequency of 1.1%. Diploid USDA accession FRA 364 was heterozygous with respect to both SDH and PGI-2. Both isozymes segregated 1:1, and assorted independently of each other in the F_1 generation of crosses between FRA 364 (as male) and the two Alpine cultivars. The close (1.1 cM) linkage detected between the SDH and fruit color loci constitutes the first report of quantified genetic linkage in the strawberry.

The diploid ($2n = 2x = 14$) strawberry, *Fragaria vesca* L., and the octoploid ($2n = 8x = 56$), commercially cultivated strawberry (*Fragaria* × *ananassa* Duch.) are closely enough related to be intercrossable (Scott and Lawrence 1975) and for the *F. vesca* genome to participate in meiotic chromosome pairing with a corresponding octoploid-derived genome in pentaploid interspecific hybrids (Senanayake and Bringhurst 1967). A similar spectrum of character variation (e.g., seasonal versus perpetual flowering, runnering versus nonrunnering) is seen within diploid and octoploid strawberry species. As a diploid, *F. vesca* is a simpler subject than *F.* × *ananassa* for qualitative genetic research. Therefore, the diploid strawberry is an appropriate and useful "model system" for strawberry genetic research (Arulsekar and Bringhurst 1983; Brown and Wareing 1965).

Several examples of monogenically controlled morphological variants have been described in *F. vesca* (reviewed by Galletta and Maas 1990; Scott and Lawrence 1975). Among these, red fruit color is dominant to white or yellow (Brown and Wareing 1965; Richardson 1914, 1923). The allele symbols *C* (red) and *c* (white) were assigned by Brown and Wareing (1965) with reference to fruit color segregation in a cross between the white-fruited Alpine variety Bush White and a red-fruited wild type. Alpine *F. vesca* varieties are distinguished by their perpetual, as opposed to seasonal, flowering habit and their European origin.

Of the few isozyme systems studied in the strawberry, phosphoglucoisomerase (PGI) is the most well characterized. Variation in the dimeric, cytosolic form, PGI-2, is governed by a single locus in *F. vesca* and by four loci (one for each of the four homoeologous genomes) in the octoploid *Fragaria* species (Arulsekar and Bringhurst 1981, 1983; Arulsekar et al. 1981). In diploid and octoploid species, an invariant anodal isozyme, designated PGI-1, is considered to be a chloroplast isozyme.

Shikimate dehydrogenase (SDH) isozymes have not been studied previously in the strawberry. In the study presented here, we examined SDH allozyme inheritance and genetic linkage relationships to PGI-2 and yellow fruit.

Materials and Methods

Plant Material

Alpine *F. vesca* cultivars Baron Solemacher and Yellow Wonder were obtained as seed from Johnny's Selected Seeds, Albion, Maine, and W. Atlee Burpee and Company, Warminster, Pennsylvania, respectively. FRA 364 was obtained as a runner clone from the National Clonal Germplasm Repository at Corvallis, Oregon, and was described on the Germplasm Repository information sheet as an *F. vesca* clone originally collected from the wild in California. F_2 populations generated from reciprocal crosses between Baron Solemacher (red fruit) and Yellow Wonder (yellow fruit) provided data for analysis of SDH inheritance patterns and linkage to red versus yellow fruit color. Unidirectional crosses of both cultivars (as females) with FRA 364 (red fruit) provided data for joint segregation analysis of SDH and PGI-2.

Isozyme Analyses

Young leaf lamina tissues were picked from greenhouse-grown plants and ground in extraction buffer (pH 7.5) containing 2.4 g Tris-HCl, 4.2 g citric acid (monosodium salt), 1.0 g ascorbic acid, 100 mg L-cystine, and 100 g PVP-40 per liter (Bringhurst et