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Characteristics, Inheritance, and Allelic Relationships of Midribless Mutants in Pearl Millet

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Three spontaneous midribless mutants in pearl millet [*Pennisetum americanum* (L.) Leeke] were identified after screening the world collection of over 17,000 germ plasm accessions. The midribless mutants are characterized by leaf blades that tend to droop because of the absence of a keel in the midrib portion of the leaf lamina. Seed set was drastically reduced in J 561 (India) and IP 6534 (Mali) midribless mutants as the gynoecium and androecium were affected. In another midribless mutant, IP 10154 (Mali), the gynoecium was absent or rudimentary, but the androecium was more prominent with prolific pollen shedding. Studies of F_2 segregation in reciprocal crosses between normal and their respective midribless mutants indicated that a single recessive gene controlled the midribless trait in each of the three mutants. Tests for complementation among the midribless mutants indicated that J 561 and IP 6534 have the same gene for the trait designated *mrl₁*, and IP 10154 has a different gene designated *mrl₂*. The midribless trait in J 561 (*mrl₁*) showed independent assortment with three qualitative seedling traits, viz., bright yellow (*by by*), glossy (*gl gl*), and trichomeless (*tr tr*).

The occurrence of plants with weak midribs in pearl millet [*Pennisetum americanum* (L.) Leeke] has been reported by Kumar and Joshi and Krishnaswamy and Rangaswamy Ayyangar.[8,9] Although the inheritance of several mutants in pearl millet has been reported,[5,7] this is the first report on the inheritance of the midribless trait. During the course of screening pearl millet germ plasm for seedling markers, we identified three midribless mutants in the world collection maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. This paper describes the morphological differences between normal plants and the midribless mutants, the mode of inheritance, allelic relationships, and linkage of the midribless trait with bright yellow, glossy, and trichomeless seedling traits that are qualitatively inherited.[2,3,10]

Materials and Methods

Over 17,000 accessions of pearl millet germ plasm were grown in boxes filled with sand in batches of 2,000 to screen them for seedling markers, and 15-day-old seedlings were screened for midribless mutants. Morphological characters were recorded using the standard pearl millet descriptors.[6] Ovule fertility and seed set of the mutants were studied by pollinating with pollen from their respective normal plants. The pollen fertility of the mutants was studied by pollinating the male sterile line (5141 A) with pollen from the midribless mutants.

To study the mode of inheritance, the three midribless mutants were crossed to their counterparts with normal midribs as described by Burton.[4] The resultant segregating populations were scored for normal and midribless plants. To study allelic relationships, crosses were made among the midribless mutants. If the F_1 between two midribless lines was midribless and the F_2 did not segregate, it was assumed that both of them carry the same midribless gene. To determine linkage, J 561 was used as a tester line for midribless, D 348 for bright yellow (*by by*), IP 7044 for glossy (*gl gl*), and Tift 23DB for trichomeless (*tr tr*), which are inherited as monogenic recessive traits.[1,3,10]

Results and Discussion

Description of the Mutants

Two germ plasm lines from Mali (IP 6534 and IP 10154) and one from India (J 561) segregated for midribless and normal plants. Two of the midribless mutants (IP 6534 and J 561) bred true in subsequent

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Figure 1. Pearl millet plants with normal midrib (left) and midribless mutant (right).

Table 1. Morphological differences between plants with normal midribs (NM) and midribless (ML) mutants

Character	J 561 Mean ± SE	IP 6534 Mean ± SE	IP 10154 Mean ± SE
Days to 50% flowering			
NM	67.2 ± 1.6	137.4 ± 2.4	102.4 ± 2.5
ML	69.4 ± 4.2	131.8 ± 4.8	109.6 ± 3.6
Plant height (cm)			
NM	245.6 ± 3.5	300.2 ± 2.6	310.0 ± 3.2
ML	250.8 ± 2.4	270.4 ± 2.4	225.5 ± 1.8
Tillers (No.)			
NM	3.0 ± 2.6	3.0 ± 4.2	2.7 ± 2.8
ML	2.1 ± 1.2	5.7 ± 2.3	2.0 ± 2.2
Stem thickness (mm)			
NM	10.2 ± 1.6	9.8 ± 2.1	8.2 ± 2.8
ML	11.4 ± 1.8	7.2 ± 1.4	7.4 ± 2.3
No. of leaves			
NM	14.2 ± 1.4	20.3 ± 1.8	22.0 ± 2.3
ML	15.7 ± 1.5	21.7 ± 1.2	19.2 ± 2.0
Leaf blade length (cm)			
NM	68.2 ± 3.1	70.4 ± 3.2	59.7 ± 2.7
ML	80.7 ± 3.6	49.8 ± 2.8	58.2 ± 2.8
Leaf blade width (mm)			
NM	36.2 ± 2.2	36.3 ± 1.4	43.2 ± 1.8
ML	50.4 ± 1.8	32.9 ± 1.6	47.6 ± 1.3
Spike length (cm)			
NM	22.2 ± 3.8	24.8 ± 4.2	31.8 ± 5.4
ML	27.4 ± 2.4	19.4 ± 2.6	30.4 ± 3.8
No. of stigmas/spike			
NM	1,735.2 ± 4.2	2,256.6 ± 3.3	2,757.4 ± 5.1
ML	192.4 ± 15.5	268.3 ± 14.2	—
No. of grains/spike			
NM	1,643.4 ± 4.6	2,149.8 ± 3.2	2,612.5 ± 6.4
ML	178.2 ± 12.2	242.4 ± 13.5	—
1,000-grain mass (g)			
NM	9.35 ± 2.2	9.40 ± 2.7	18.64 ± 1.9
ML	7.07 ± 4.8	11.92 ± 3.7	—

generations and are being maintained by selfing. The other mutant (IP 10154) is female sterile: Its gynoecium is rudimentary or completely absent. Hence, it is maintained by crossing heterozygous normal plants with pollen from midribless plants. All the leaves of the midribless mutants characteristically droop in appearance (Figure 1) and are distinguishable from emergence to maturity. The leaf lamina and leaf sheath of normal plants have a prominent keel, which is absent in midribless mutants. Normal leaves have a deep furrow on the abaxial side and are curved inward and on the adaxial side there is a prominent midrib (Figure 2). However, in mutants, both abaxial and adaxial sides are alike and the leaf resembles a ribbon (Figure 2). Before internode elongation, the stem of normal plants is elliptical, whereas in mutants it is round. Days to 50% flowering, plant height, tiller number, stem thickness, and leaf number did not differ significantly between normal plants and midribless mutants (Table 1). However, they differed considerably for leaf blade length, leaf blade width, spike length, and grain size (Table 1). Because of the drooping leaf blades, mutant canopy height before floral initiation was less than that of normal plants.

Two mutants, IP 6534 and J 561 have similar floral morphology. Compared to normal plants, only about 9% of stigmas and anthers emerged in the mutants, resulting in reduced seed number per spike. Some florets have a rudimentary gynoecium and androecium. Seed number per spike in mutants was drastically reduced to about 9% of normal on selfing, open-pollination, or crossing, suggesting reduced female fertility. The mutants have normal pollen fertility as they produced seeds when crossed to a male sterile line (5141 A). The midribless mutants reported here have different levels of fertility, whereas the weak-midrib plants previously reported were completely sterile.[8,9]

Before anthesis, numerous white plumose stigmas are seen in normal plants of IP 10154, but they are completely absent in the mutant. However, the androecium is normal with plump anthers, producing abundant viable pollen.

Inheritance

Crosses between normal and midribless plants produced plants with midribs, thus suggesting that the midribless trait is recessive. Segregation in the F_2 generation showed a good fit to 3 normal:1 mutant ratio (Table 2), thus suggesting a single-gene difference. All the midribless plants in F_2 that set seed by selfing bred true in the F_3 generation. Of the 10 normal F_2 plants, 3 plants bred true, whereas the rest segregated into normal and mutant in a 3:1 ratio in the F_3 generation, confirming single-gene difference. Both the midribless character and the absence of female parts were found to be inherited together, and it was not possible to separate these characters in subsequent generations, indicating either (1) very tight linkage (no recombinants among 572 F_2 plants), (2) pleiotropic effect of the same gene, or (3) cryptic changes in the chromosome. As pearl millet is normally allogamous, these mutants are concealed in a heterozygous condition. Because of their reduced fertility, their transmission is very low, and they are masked by more vigorous normal plants and eliminated in subsequent generations.

Allelic Relationships

To determine allelic relationships, diallel crosses were made among the three midribless mutants. The F_1 hybrids produced normal or midribless plants depending upon the parents involved in the cross (Table 3). In crosses involving J 561 with IP 6534, the F_1 was midribless and the F_2 did not show segregation, thus indicating that the midribless gene in both parents

Table 2. F_2 segregation of the reciprocal crosses between normal plants (NM) and midribless (ML) mutants of pearl millet

Parents	Cross	No. progenies	F_1	F_2 plants		χ^2	P
				NM	ML	(3:1)	
J 561	NM × ML	6	NM	219	68	0.26	0.7–0.5
	ML × NM	4	NM	102	28	0.83	0.5–0.3
IP 6534	NM × ML	5	NM	175	54	0.25	0.7–0.5
	ML × NM	7	NM	79	21	0.85	0.5–0.3
IP 10154[a]	NM × ML	5	NM	264	79	0.71	0.5–0.3

[a] Reciprocal cross was not possible as the ML mutant was female sterile.

Table 3. Allelic relationships of different midribless (ML) mutants of pearl millet

Cross	F_1	F_2 plants (No.)		Ratio	χ^2	P	Allelic relationship
		NM[a]	ML				
J 561 × IP 6534	ML	0	178	—	—	—	Allelic
IP 6534 × J 561	ML	0	214	—	—	—	Allelic
IP 6534 × IP 10154[b]	NM	127	88	9:7	0.695	0.5–0.3	Nonallelic

[a] NM = normal midrib.

[b] Sterile female.

Table 4. F_2 segregation for midribless trait with bright-yellow, glossy, and trichomeless traits in pearl millet

Cross[a]	F_2 plants (No.)[b]				χ^2	P
	a	b	c	d	(9:3:3:1)	
byby MrlMrl × *ByBy mrlmrl*	302	115	105	33	1.57	0.7–0.5
glgl MrlMrl × *GlGl mrlmrl*	387	129	159	42	5.54	0.2–0.1
trtr MrlMrl × *TrTr mrlmrl*	132	51	57	18	3.09	0.5–0.3

[a] *By*, green plant; *byby*, yellow plant; *Gl*, nonglossy; *glgl*, glossy; *Tr*, trichome; *trtr*, trichomeless; *Mrl*, midrib; and *mrlmrl*, midribless.

[b] a = number of individuals carrying dominant alleles at both loci (*A-B-*); b = number of individuals carrying a dominant allele only for the *A* locus (*A-bb*); c = number of individuals carrying a dominant allele only for the *B* locus (*aaB-*); and d = number of individuals homozygous recessive at both loci (*aabb*).

is the same. The gene symbol mrl_1 is proposed for this gene in J 561 and IP 6534.

When the midribless mutant IP 6534 was crossed with IP 10154, which is another midribless mutant, the F_1 plants produced leaves with normal midribs showing complementation. In the F_2 generation, there was segregation for normal and midribless in a 9:7 ratio confirming that these two midribless mutants are complementing each other for the presence of midrib. The gene symbol mrl_2 is proposed for the midribless gene in IP 10154.

Linkage Relationships

To find out the linkage relationships of the midribless trait, J 561 (mrl_1) was used as a tester line. Crosses between midribless and bright yellow produced green plants with normal midribs. The F_2 populations were classified into four phenotypic classes, and their segregation ratio of 9:3:3:1 (Table 4) indicated an independent assortment of the midribless gene with the bright-yellow gene (*by by*), which was reported to be monogenic recessive to green.[2]

Crosses between midribless and glossy (IP 7044) produced normal plants. The F_2 segregated into four phenotypic classes in a 9:3:3:1 ratio (Table 4). Glossy trait was reported to be due to a single recessive gene *gl*.[3] The F_2 digenic segregation ratio of 9:3:3:1 clearly indicates independent assortment of the midribless gene (mrl_1) with the glossy gene (gl_1).

Crosses between midribless and trichomeless produced plants with trichomes and normal midribs. The F_2 populations segregated into four phenotypic classes in a 9:3:3:1 ratio (Table 4). The digenic segregation ratio indicates independent assortment of the midribless gene with the trichomeless gene (*tr tr*), which has been reported to be monogenic recessive.[10]

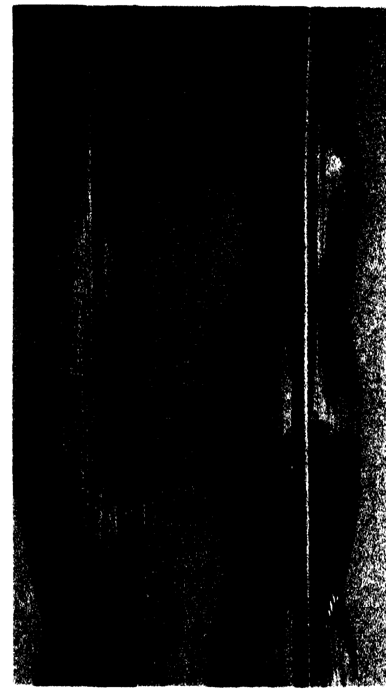

Figure 2. Normal pearl millet leaf blade with prominent midrib (left), and leaf blade of midribless mutant (right).

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