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FIGURE 1 On glossy leaves (right) water accumulates as droplets, while on nonglossy leaves (left) such droplets are not formed

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Glossy genes in pearl millet

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ABSTRACT A new seedling marker named glossy was found in pearl millet [*Pennisetum americanum* (L.) Leeke]. Subsequently, seven more glossy lines were identified after screening a world collection of 16 480 germplasm accessions. The glossy characteristic was distinguishable at seedling emergence and persisted for 28 days. Similar to sorghum and maize, mist accumulated as droplets on glossy leaves of pearl millet. Intercrosses among the eight glossy lines indicated that three different genes control glossiness. The gene symbols assigned were gl_1, gl_2, and gl_3. Studies of F_2 segregation in reciprocal crosses between the glossy and nonglossy plants showed a segregation ratio of 3:1 and no reciprocal differences. The F_2 segregation ratios also showed that genes controlling purple plant color (*P*), long bristles (*Bl*) and trichomeless (*tr*) were independent of gl_1 and gl_2.

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EASILY IDENTIFIABLE seedling markers are of immense value in mapping chromosomes. Seedling markers facilitate screening a large number of genetic resources collections or segregating populations if they happen to be associated with economically useful traits that may otherwise be difficult to identify. In sorghum (*Sorghum bicolor* (L.) Moench) an easily identifiable seedling marker glossy was found to be associated with shootfly resistance and seedling drought tolerance[1,2]. As a result the glossy trait is now being used as a seedling marker in selecting for shootfly resistance from sorghum germplasm at ICRISAT.

Glossy genes were reported in several crop species and were extensively studied in maize[3], where the maximum expression of glossiness was on the first 5 or 6 leaves. In sorghum glossiness was reported from the sixth leaf onwards with maximum expression at the 12–15 leaf stage (approximately 50 days after emergence)[4]. Maiti et al.[5] observed glossiness from emergence with maximum expression at 10–15 days after emergence, and disappearing at later stages.

In the trichomeless mutant of pearl millet[6] virtually all shoot trichomes are suppressed; it produces light green shiny leaves from germination that persist until maturity. Although the inheritance of several characters in pearl millet is known[7,8,9,10], the glossy trait has not been reported. We identified eight lines from five countries in the world collection of pearl millet maintained at ICRISAT Center, Patancheru, which segregated for glossy plants. In this paper we report on the allelic relationships of eight glossy lines, their mode of inheritance, and linkage with purple plant color, long bristles, and trichomeless.

Materials and Methods

The world collection of pearl millet [*Pennisetum americanum* (L.) Leeke] maintained at

ICRISAT Center, Patancheru, was screened to identify the glossy trait. The entire collection of 16 480 accessions from 31 countries was screened in batches of 1000. Over 200 seed of each accession were sown in nursery beds. Initially, glossy and nonglossy lines of sorghum were planted for comparison. Pearl millet seedlings were carefully examined for glossy character from emergence onwards, using the glossy sorghum line for comparison. Once the pearl millet glossy line IP 8275 was identified, it was used for comparison in subsequent screenings. Morphological characters were scored in accordance with the standard pearl millet descriptors[5].

Allelic relationships were established by intercrossing the glossy lines. If the F_1 between two glossy lines was glossy and the F_2 did not segregate, it was assumed that both of them carry the same glossy gene. To study the inheritance of the glossy character, segregation of glossy and nonglossy plants were scored in reciprocal crosses between true breeding glossy and nonglossy lines. To test for linkage of the glossy genes with purple plant color, long bristles, and trichomeless, true breeding glossy lines- IP 8275 (gl_1), IP 8276 (gl_2), and IP 8279 (gl_3) were each crossed with a true breeding nonglossy line with purple leaves and long bristles derived from IP 8140 and Tift 23B *tr* for trichomeless. To establish linkage, F_2 progenies were grown and 14- to 20-day-old seedlings were classified as glossy or nonglossy and were transplanted separately into the field. The plants were further classified for purple foliage, bristle length, and trichomeless. Linkage was calculated by the product method as described by Immer and Henderson[6].

Results and Discussion

Among the 16 480 accessions screened, none was found with exclusively glossy plants. However, eight accessions (IP 8275 to IP 8282) segregated for glossy and nonglossy plants in varying proportions. The occurrence of glossy plants as segregants in landrace populations suggest they are predominantly maintained in heterozygous condition. Selfing of heterozygous populations led to homozygosity of recessive alleles and exposed a large number of recessive genes concealed in heterozygous condition[5]. In self-pollinating crops like sorghum, 2.8 percent of the 22 898 accessions of the world collection were exclusively glossy[12].

Characteristics of glossy plants: The glossy mutants were clearly distinguishable from emergence up to 28 days by their shiny light-green foliage. Both the lower and upper surfaces of leaf blades and sheaths of glossy plants were shiny in bright sunlight compared to the dark green, dull leaves of nonglossy plants. Hairs on leaf blades, sheaths, barbs along leaf margins, short hairs and claws on veins of upper and lower leaf surfaces were present on all glossy lines. Glossiness is not due to the absence of trichomes. Glossy leaves also are detectable by spraying water. On glossy leaves water accumulated as droplets, while on nonglossy leaves such droplets were not seen (Figure 1). Such differences between glossy and nonglossy leaves also were found in sorghum[7, 8]. Water repulsion by nonglossy leaves was attributed to the presence of epicuticular waxes as reported in maize[3] and sorghum[9].

Glossy plants are distinguishable from emergence and persist for varying periods in different genotypes. However, glossiness gradually increases, reaching maximum intensity between 12–18 days after emergence in different genotypes and then decreases gradually. Glossiness practically disappears before flowering. The young leaves are more glossy than old leaves. The entire leaf blade and leaf sheath are either glossy or nonglossy unlike some chlorophyll-deficient mutants that change their color from leaf tip towards the base[7]. In general, the glossy lines do not differ considerably from their respective nonglossy lines for various morphological characters except IP 8277 from Lebanon. This glossy line flowered 37 days earlier with 73 cm reduction in plant height and produced nearly double the grain size of its normal counterpart.

Allelic relationships of glossy lines: When IP 8275 (assigned gene symbol gl_1) was crossed with the remaining seven glossy lines, the F_1s were glossy in five crosses that bred true for glossiness in subsequent generations (Table I), indicating that the glossy gene is the same in six lines. The glossy trait in IP 8275 (India), IP 8277 (Lebanon), IP 8278 (India), IP 8279 (Mali), IP 8281 (Sudan), and IP 8282 (Sudan) is controlled by the same glossy gene and is designated as gl_1. When IP 8275 was crossed with IP 8276 and IP 8280, the F_1 in both the crosses was nonglossy and the F_2 segregated for nonglossy and glossy plants in a 9 : 7 ratio (Table I), indicating that the glossy gene is nonallelic in these two lines. When IP 8276 was crossed with IP 8280, the F_1 was again nonglossy and the F_2 segregated into nonglossy and glossy in a 9 : 7 ratio (Table I), indicating that the glossy gene in these two lines also is different. The gene symbols gl_2 and gl_3 were proposed for IP 8276 (Senegal) and IP 8280 (Mali), respectively.

Inheritance of glossiness: Reciprocal crosses between the glossy and their respective nonglossy plants produced nonglossy F_1 plants indicating that nonglossy is dominant to glossy. In the F_2 generation, nonglossy and glossy seedlings segregated in a 3:1 ratio (Table II), suggesting monogenic recessive inheritance of the glossy character. The heterogeneity chi-square indicated that all the six allelic gl_1 glossy lines showed a 3:1 segregation ratio (the pooled data are given in Table II). When F_2 plants were advanced to the F_3 generation, all the glossy plants bred true. Of the 80 nonglossy F_2 plants tested, 31–36 segregated in F_3, while the rest bred true, thus confirming monogenic inheritance of the character. In maize and sorghum, the mode of inheritance is similar[4, 10]. All the glossy mutants reported so far are monogenic recessive to nonglossy lines[4, 10].

Glossy trait in wild Pennisetums: Of the 52 accessions of 23 *Pennisetum* species screened, all five accessions of *Pennisetum schweinfurthii* were glossy. In this species, we did not find a single nonglossy plant. As we were not success-

Table I. Allelic relationships based on F_1 behavior and F_2 segregation of different glossy lines of pearl millet

Cross	F_1 phenotype	No. F_2 plants: non glossy	No. F_2 plants: glossy	Ratio	χ^2	P	Allelic relation*
IP8275 × IP8276	nonglossy	412	283	9:7	2.89	0.2–0.1	NA
IP8275 × IP8277	glossy	0	475				AI
IP8275 × IP8278	glossy	0	537				AI
IP8275 × IP8279	glossy	0	406				AI
IP8275 × IP8280	nonglossy	504	373	9:7	0.83	0.5–0.3	NA
IP8275 × IP8281	glossy	0	483				AI
IP8275 × IP8282	glossy	0	526				AI
IP8276 × IP8280	nonglossy	436	308	9:7	1.67	0.2–0.1	NA

* NA = nonallelic; AI = allelic

Table II. Inheritance of different glossy genes in pearl millet

Parents	Progenies	No. F_2 plants*: NGl	No. F_2 plants*: Gl	χ^2 (3:1)	P
gl_1 × NGl†	26	2634	831	1.91	0.2–0.1
NGl × gl_1‡	20	1873	598	0.84	0.5–0.3
gl_2 × NGl	2	496	178	0.71	0.5–0.3
NGl × gl_2	3	427	156	0.96	0.5–0.3
gl_3 × NGl	3	1134	369	0.16	0.7–0.5
NGl × gl_4	1	908	298	0.05	0.9–0.8

* NGl = non-glossy; Gl = glossy
† Pooled data of 6 glossy lines that have the same gl_1 gene. Heterogeneity $\chi^2 = 2.45$
‡ Heterogeneity $\chi^2 = 3.11$

ful in crossing this species with any other nonglossy species, the inheritance of glossiness in *Pennisetum schweinfurthii* could not be studied.

Linkage relationships: F_2 segregation data for glossy with purple plant color, trichomeless, and long bristles is given in Table III. Purple color on leaves, internodes, glumes, bristles, and seed was found to be controlled by a single dominant gene P[10]. Joint F_2 segregation data indicated independent assortment of the purple gene P with gl_1 and gl_2 (Table III). Long bristles were found to be due to a single dominant gene Bl[10]. The gene for long bristles Bl also showed independent assortment with gl_1 and gl_2. The trichomeless mutant, which is due to a single recessive gene[14], was crossed with gl_1 and gl_2. The F_1 plants had trichomes and were nonglossy and independent assortment for glossy and trichomeless was observed in the F_2 generation (Table III). Trichomeless glossy plants that were obtained as recombinants can be distinguished easily from the trichomeless plants until 20 days but are indistinguishable after one month.

Table III. F_2 segregation for glossiness with purple plant color, bristle length, and trichomeless traits

Cross	No. F_2 plants*				$\chi^{2\dagger}$	P
	P- Gl	*P- glgl*	*ppGl-*	*ppglgl*		
Purple × gl_1	558	208	187	71	2.79	0.5–0.3
gl_1 × Purple	794	273	235	98	4.62	0.3–0.2
gl_2 × Purple	612	196	214	72	1.00	0.9–0.8
	Bl-Gl-	*Bl- glgl*	*blblGl-*	*blblglgl*		
Bristles × gl_1	566	203	185	70	1.62	0.7–0.5
gl_1 × Bristles	789	261	248	102	3.22	0.5–0.3
gl_2 × Bristles	614	194	207	76	1.47	0.7–0.5
	Tr-Gl	*Tr- glgl*	*trtrGl-*	*trtrglgl*		
Trichomeless × gl_1	307	87	92	33	2.17	0.7–0.5
Trichomeless × gl_1	576	195	187	63	0.19	0.95–0.90
Trichomeless × gl_2	264	80	68	24	4.17	0.3–0.2

* *P-* = purple plant; *p* = green plant; *Bl-* = bristled spike and *bl* = nonbristled spike; *Tr-* = trichome plant and *tr* = trichomeless plant; *Gl-* = nonglossy plant and *gl* = glossy plant

† 9:3:3:1

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