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REVIEW

Genetic Studies on Glossy Leaves in Sorghum (*Sorghum bicolor* L. Moench)

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Abstract

The glossy leaf is a genetic trait that showed pleiotropic effects on disease and pest resistances as well as being a varietal marker in *Brassica oleracea* and *Zea mays*. The author aimed to clarify the genetic characteristics of glossiness that were newly found in sorghum leaves. The F_1, F_2 and F_3 populations derived from two crosses of (g×G) were evaluated for leaf glossiness by spraying water. Since the expected ratios of (g : G = 1 : 3) were well fitted in the segregations of F_2 and F_3 populations, a single recessive factor for the control of glossy (g) was confirmed, and the symbols *gl* and *Gl* were tentatively assigned for g and G. The F_4 lines with *gl* and *Gl* derived from two crosses of (g×G) were examined for pleiotropic effects on leaf digestion and leaf blight resistance. The 23 F_1 and F_2 between 7 varieties having non-glossy (G), glossy (g) and true-glossy (tg) phenotypes were evaluated for leaf glossiness by spraying water. The phenotypes of F_1 were tg in (tg×tg), g in (tg×g) and (g×g), and G in (tg×G) and (g×G). The expected ratios of (tg : g = 1 : 3), (tg : G = 1 : 3) and (g : G = 1 : 3) in F_2 segregations were fitted well. The results suggested that tg, g and G were multiple alleles located in a glossy locus. Consequently, the symbols gl^1, gl^2 and gl^+ were proposed to denote the multiple alleles for g, tg and G in the glossy locus, respectively.

Discipline: Plant breeding/Genetic resources
Additional key words: *Brassica oleracea*, inheritance, leaf digestion, multiple allele, phyletic evolution, pleiotropic effect, *Zea mays*

Introduction

The glossiness expressed by the lack of epicuticular waxes on leaf blades was reported in recessive mutants of *Brassica oleracea*[2] and *Zea mays*[4,6]. The series of glossy mutants with gly_1 to gly_9 in *Brassica oleracea*[1] and glossy mutants with gl_1 to gl_{10} in *Zea mays*[10] were listed. The glossiness has been generally used as a morphological marker in breeding and genetic studies of the above two species, and glossiness was reported to be related with insect resistance in *Brassica oleracea*[7,9,16] and with bacterial diseases in *Zea mays*[15].

In sorghum, Ayyangar and Ponnaiya[3] reported the inheritance of a bloomless trait that was absent of bloom (wax) on the surfaces of leaf sheaths and stems, and Hanna et al.[11] revealed the relationship between bloomless plants and *in vitro* digestion by rumen fluid. Tarumoto[17,20] and Tarumoto et al.[26] reported the visual and ultrastructural phenotypes of glossiness expressed on leaf blades in sorghum. However, no one reported the inheritance and pleiotropic effects of glossiness. Therefore, the author conducted genetic studies of non-glossy, glossy and true-glossy traits in sorghum pedigrees.

Genetic studies in non-glossy and glossy plants

In a rain or just after a shower, the author found the following non-glossy (G) and glossy (g) plants in sorghum (*Sorghum bicolor* L. Moench): rare and small droplets with water tension adhered on the leaf surfaces of non-glossy plants, whereas water adhered either on the whole surfaces or in large and irregular shaped droplets on the leaf surfaces of glossy plants[17]. The glossy plants in sorghum were similar with the normal plants in corn that expressed the non-glossy phenotype in seedling stage

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until the 6th leaf stage[4,12].

The series of glossy mutants with *gly*$_1$ to *gly*$_9$ in *Brassica oleracea*[1] and glossy mutants with *gl*$_1$ to *gl*$_{10}$ in *Zea mays*[12] were listed. The glossy traits were reported to relate with insect resistance in *Brassica oleracea*[11,16] and with bacterial diseases in *Zea mays*[15]. Thus, the author conducted genetic studies of non-glossy and glossy traits in sorghum pedigrees.

1. Inheritance

The bl CK-60 (g), bmr-18 (g), Sweet Sudan (G), two F_1 of g×G, four F_2 populations derived from two F_1, and 58 F_3 progenies selected from four F_2 populations in Table 1 and 2 were seeded in the field in 1979, and were tested for their glossiness at 50 days after seeding, approximately the 11th leaf stage, by the alternative judgment of G (non-glossy) and g (glossy) in spraying water from a small-holed sprayer[2,4]. The F_1 of bl CK-60×Sweet Sudan and bmr-18×Sweet Sudan were classified into G, suggesting that G was dominant to g. The segregations in F_2 populations were fitted well with the expected ratio of (g : G = 1 : 3) in Table 2[17]. The F_3 progenies from glossy F_2 plants were homogeneous ones with glossy leaves, while F_3 progenies from non-glossy F_2 plants consisted of homogeneous progenies with non-glossy leaves and heterogenous progenies in which the segregation of g and G plants fitted with the expected ratio of (g : G = 1 : 3). From the above results, a single recessive factor for the control of glossy (g) was confirmed, and the symbols *gl* and *Gl* were tentatively assigned for g and G. The glossy mutants in broccoli (*Brassica oleracea* L. var. *italica*)[2] and corn (*Zea mays* L.)[6,10] were recessive to normal plants. Therefore, it is considered that the non-glossy plant with *Gl* would be a wild plant and the glossy plant with *gl* would be a mutant in sorghum.

2. Pleiotropic effects of glossiness

(1) Leaf structure: The 11th leaves of SC112 (*gl*), Rancher (*gl*) and Zairai-Tokin (*Gl*) taken at the 12th leaf stage after they had expressed the final phenotypes of glossiness[17,26] were used. The leaf blades were sectioned with a sharp razor, were dehydrated in a graded ethanol series, placed into 100% isoamyl acetate, and were finally dried in a critical-point dryer. The dried specimens were mounted on metal discs with silver conductive cement and were then coated with carbon and gold in a vacuum evaporator. Coated samples were examined with a scanning electron microscope (HHS-2R Hitachi) at 15 kv accelerating voltage.

In the ultrastructure of leaf cross-sections (Fig. 1)[19], SC112 and Rancher with *gl* had thicker and harder cell walls on the outer side than Zairai-Tokin with *Gl*. The glossy plants were characterized in the appearance of their shiny dark green leaves and by the lack of a whitish bloom (abundant star-shaped epicuticular waxes), which was attached on the non-glossy plants[2,4,12]. In general, the epicuticular waxes on the leaf surface protect the leaves from damages. This would be the case of non-glossy leaves in sorghum. Also, the thick and hard cuticular cell wall on the glossy leaf surface would serve as a protector.

The relationship between trichome development and resistance to sorghum shoot fly at the seedling stage was reported by Blum[5] and Maiti and Bidinger[13]. The adult of the shoot fly is approximately 5 mm long, while the tri-

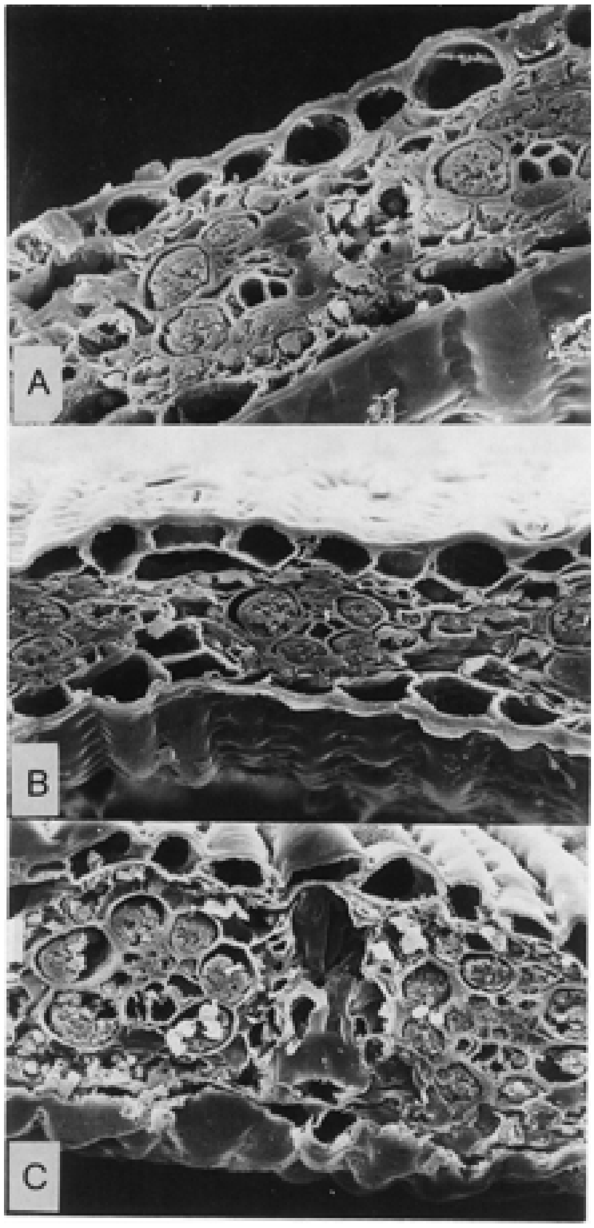

Fig. 1. Scanning electron micrographs of cross sections of 11th leaves (×1,000)
A: SC112, B: Rancher, C: Zairai-Tokin.

Table 1. Materials and their character expression of glossiness

	Glossiness at the following stage*			Phenotype
	4th leaf stage	8th leaf stage	Flag leaf	
bl CK-60	G	g	g	Glossy (g)
bmr-18	G	g	g	Glossy (g)
Sweet Sudan	G	G	G	Non-glossy (G)

*: Tarumoto (1980)[17].

Table 2. Segregation of glossy (g) and non-glossy (G) plants in F_2 and F_3 progenies of bl CK-60 × Sweet Sudan and bmr-18 × Sweet Sudan

	Plant phenotype				P
	Total no. of plants	Non-glossy (G)	Glossy (g)	χ^2*	
		bl CK-60 × Sweet Sudan			
F_1 : F_2-1	144	105	39	0.333	0.75−0.50
F_2-3	114	87	27	0.105	0.75−0.50
F_3 :					
9 progenies from glossy F_2 plants		None	All		
9 progenies from non-glossy F_2 plants		All	None		
6 progenies from glossy F_2 plants:					
1	99	72	27	0.295	0.75−0.50
2	83	65	18	0.486	0.50−0.25
3	71	52	19	0.400	0.75−0.50
4	58	45	13	0.207	0.75−0.50
5	30	21	9	0.400	0.75−0.50
6	102	76	26	0.013	>0.90
		bmr-18 × Sweet Sudan			
F_2 : F_2-4	119	87	32	0.227	0.75−0.50
F_2-7	65	48	17	0.046	0.90−0.75
F_3 :					
19 progenies from glossy F_2 plants		None	All		
5 progenies from non-glossy F_2 plants		All	None		
10 progenies from non-glossy F_2 plants:					
1	83	63	20	0.036	0.90−0.75
2	126	96	30	0.095	0.90−0.75
3	73	56	17	0.114	0.75−0.50
4	121	89	32	0.135	0.75−0.50
5	87	64	23	0.096	0.90−0.75
6	99	71	28	0.569	0.50−0.25
7	66	49	17	0.020	0.90−0.75
8	96	76	20	0.889	0.50−0.25
9	165	126	39	0.164	0.75−0.50
10	104	79	25	0.051	0.90−0.75

*: Calculated on the basis of 3:1 segregation.

chome on the first five leaves was about 0.02 mm in length as estimated from the ultrastructures[19,26]. The fly is about 250 times larger than the sorghum trichome. Possibly, even though the trichome is a mechanical obstacle against the fly, the thick and hard cuticular cell wall on the glossy leaves also takes part in the shoot fly resistance.

(2) Leaf digestion: The semi-isogenic F_4 lines with (*bm, gl*) and (*bm, Gl*) derived from the cross of bl CK-60 (*bm, gl*)×Sweet Sudan (*Bm, Gl*) and the semi-isogenic F_4 lines with (*bmr, gl*) and (*bmr, Gl*) from the cross of bmr-18 (*bmr, gl*)×Sweet Sudan (*Bmr, Gl*) in Table 3 were studied. The uppermost fully expanded leaves from 10 plants showing uniform morphological characteristics but segregating for glossiness were sampled in each line on July 7 (10th leaf stage) and July 31 (flag leaf stage), 1980. Each set of the dried leaf sections was hydrolyzed in 1% cellulase with 0.1 M acetate buffer (pH 4.0) at 40ºC for 48 h without shaking. After digestion, the specimens were washed, dried and weighed, and *in vitro* dry matter digestibility (IVDMD) was calculated as reported by Tarumoto and Masaoka (1980, 1981)[24,25].

In each of the segregating F_4 lines, non-glossy (*Gl*) leaves were more digestible than glossy (*gl*) leaves as shown in Table 3[19]. Although the difference in digestibility was small in F_4-6-1 sampled on July 7, it became larger on July 31, and the non-glossy leaves invariably had higher IVDMD values than glossy leaves in all lines. As Hanna et al.[11] have postulated, the powdery-appearing epicuticular wax would serve as a barrier against intrusion of microorganisms or enzymes in rumen fluid. This would hold true in the digestion of leaf section by cellulase. The result in Table 3 suggests that the thick and hard cuticular cell wall of glossy leaves (Fig. 1) would be a barrier to digestion by cellulolytic enzymes, which forms the major factor for their low digestibility compared to non-glossy leaves with dense epicuticular wax.

(3) Leaf blight resistance: Sixty four sudangrass-type F_4 lines selected for either (*bm, Gl*) or (*bmr, Gl*) phenotype, having the same genetic background as those used in the test of leaf digestion (segregating for *Gl : gl*), were grown in a disease nursery for sorghum leaf blight, *Helminthosporium turcicum*. From among those lines, four with *bmbm* and another four with *bmrbmr*, uniform morphologically, were selected and evaluated for infection grades of the leaf blight used in Tarumoto and Isawa[22].

In comparison with leaf blight resistance for non-glossy and glossy F_4 lines, the result showed no relationship between glossiness and the disease resistance (Fig. 2)[19]. The present author and coworkers (Tarumoto and Isawa[22]; Tarumoto et al.[23]) reported that the resistance to leaf blight was controlled by a single dominant gene in sorghum-sudangrass hybrids. One of the hybrids used previously was the cross between CK-605 (glossy, resistant) and Sweet Sudan (non-glossy, susceptible). The F_2

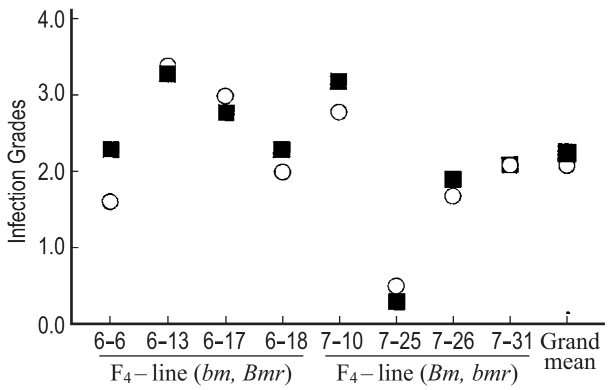

Fig. 2. Infection grades of sorghum leaf blight, *Helminthosporium turcicum*, compared between glossy (■) and non-glossy (○) plants in eight F_4 lines

Table 3. IVDMD (*in vitro* dry matter digestibility) values for non-glossy and glossy leaves obtained in F_4 plants segregating within each line

Sampling time	Glossiness	F_4-line (*bm, Bmr*)				F_4-line (*Bm, bmr*)				Grand mean
		6-1	6-6	6-23	Mean	7-3	7-16	7-26	Mean	
July 7	Non-glossy	44.8	48.2	45.3	46.1	36.6	31.2	36.8	34.9	40.5
	Glossy	43.9	39.3	40.2	41.1	32.2	27.9	28.2	29.4	35.3
	Difference	0.9	8.9	5.1	5.0	4.4	3.3	8.6	5.5	5.2
July 31	Non-glossy	32.5	28.3	–	30.4	32.1	–	23.8	28.0	29.2
	Glossy	29.6	25.4	–	27.5	25.3	–	21.8	23.6	25.5
	Difference	2.9	2.9	–	2.9	6.8	–	2.0	4.4	3.7

Mean IVDMD values for two digestion tests are given in table. The uppermost fully expanded leaves of about 10 plants were sampled and used in each test.

population would have segregated into 9 resistant, non-glossy: 3 resistant, glossy: 3 susceptible, non-glossy: 1 susceptible, glossy, if there is no genetic correlation between these two traits. The result in Fig. 2[19] suggests that the resistance and *gl* gene are independent.

3. Relationship between phyletic evolution and glossiness

In order to find the important traits in specific and varietal classification in sorghum, a total 76 accessions belonging to 14 species in the genus *Sorghum* were grown in a field and surveyed for 46 traits concerned with plant, panicle, seed, and maturity. According to higher correlation coefficients, factor loading values and contribution percentages in a principal component analysis (PCA), 21 traits were selected. Seven morphological traits (glossiness, stalk diameter, basal tillering, leaf width, head shape, kernel shape and kernel covering) were high factor loading ones in the 1st principal component (X_1), two ecological and two morphological traits (days to heading, days to maturity, culm length, and leaf length) were high factor loading ones in the 2nd principal component (X_2), and four traits concerned with seed quality (kernel color, endosperm texture, endosperm color, and presence of testa) were high factor loading ones in the 3rd principal component (X_3). In the scatter diagrams of 76 sorghum accessions in the X_1–X_2 plane (Fig. 3)[18], six groups of I to VI were classed (Table 4)[18]. The numerical classification was fundamentally fitted with the botanical one by de Wet et al.[7,8], and the evolution progress from wild-relative to cultivated species was sited from the left to right side in Fig. 3. The wild-relative species which belonged to series Spontanea and subsection *halepensia* were almost all classified into the IV, V and VI groups. The wild-relative species classified into the IV, V and VI groups commonly had non-glossy leaves, thin stalks and leaves, loose panicles, and long grain fully covered by glumes that are the characteristics

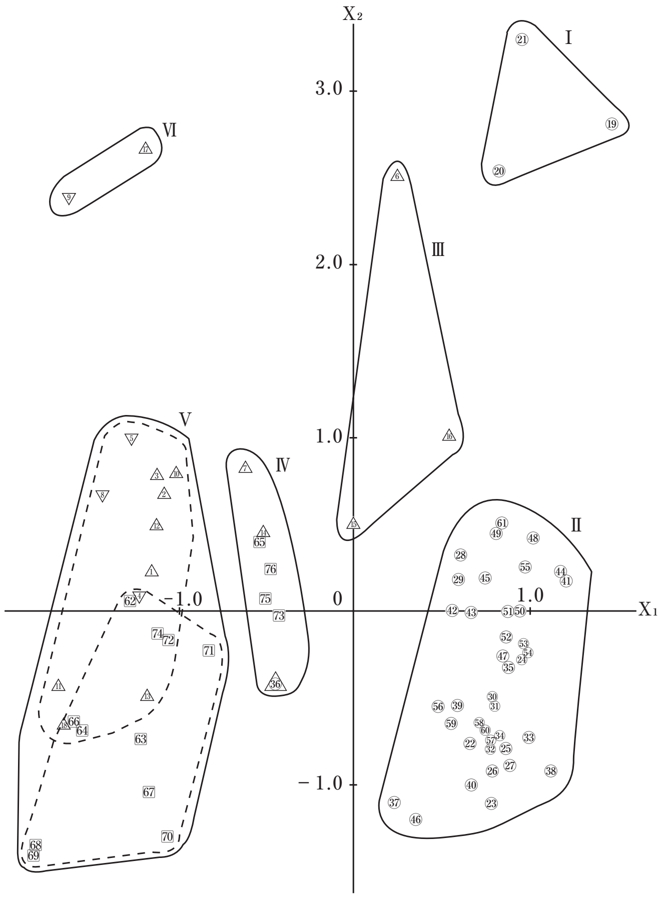

Fig. 3. Scatter diagram of 76 *Sorghum* lines in the plane of first (X_1) and second (X_2) principal components by principal component analysis based on 21 characters

○ : *S. bicolor*,
△ : Species belonging to series Spontanea,
□ : *S. sudanense*,
▽ : Species belonging to subsection *halepensia*.
Figures in the above symbols show the experimental number in Tarumoto[18].

Table 4. Sorghum species classified by the scatter diagram in Fig. 3

Group	Species and lines belonging to the group
I	*S. bicolor*
II	*S. bicolor*
III	*S. hewisonii* (6)[a)], *S. saccharatum* (15), *S. versicolor* (16)
IV	*S. hewisonii* (7), *S. saccharatum* (14), *S. sudanense* (65, 73, 75, 76), KS30 (36: *S. bicolor* × *S. virgatum*)
V	*S. aethiopicum*, *S. arundinaceum*, *S. niloticum*, *S. plumosum*, *S. pugioniflolium*, *S. controversum*, *S. milliaceum* (9)
VI	*S. milliaceum* (8), *S. verticilliflorum*

a): Figures in parentheses show the experimental number in Tarumoto[18].

of sudangrass, *Sorghum bicolor* var. *sudanense*, derived from the crosses between wild-relative and cultivated species[8]. Since the non-glossy leaf is the symbolic trait of wild-relative species in sorghum, the glossiness is considered to be a useful character for identifying the phyletic evolution in the genus *Sorghum*.

Inheritance in true-glossy, glossy and non glossy plants

Maiti and Bidinger[13] studied the relationship between trichomes development and resistance to sorghum shoot fly, and noted that the trichomed cultivars had the "glossy trait" which was characterized by having a glossy appearance during the first three weeks. While

Table 5. Materials used for crossing

Entry no.	Variety	Distributor	Glossiness	
			Phenotype [a)]	Genotype [b)]
1	IS8962	ICRISAT, India	True-glossy (tg)	Unknown
2	IS4634	do	True-glossy (tg)	Unknown
3	IS5604	do	True-glossy (tg)	Unknown
4	IS1316	do	Glossy (g)	*glgl*
5	SC112	Texas A & M Univ., USA	Glossy (g)	*glgl*
6	Rancher	do	Glossy (g)	*glgl*
7	Zairai-Tokin	Hiroshima Pref. A.E.S., Japan	Non-glossy (G)	*GlGl*

a): Tarumoto (1980, 1981, 1986b), b): Tarumoto (1981).

Table 6. F_1 phenotype, F_2 segregation and probability of fit to (3:1) ratio in the result of allelism test for glossiness

Type	Cross	F_1 phenotype	F_2 segregation				Probability of $\chi^2(3:1)$
			Total	tg	g	G	
tg×tg	1×2	tg	130	130			
	2×1	tg	53	53			
	3×2	tg	132	132			
g×g	4×5	g	269		269		
	5×4	g	280		280		
	5×6	g	170		170		
	6×5	g	136		136		
	6×4	g	175		175		
tg×g	1×4	g	361	94	267		.75−.50
	4×1	g	225	60	165		.75−.50
	1×5	g	284	78	206		.50−.25
	1×6	g	300	80	220		.75−.50
	2×4	g	328	85	243		.75−.50
	4×2	g	329	79	250		.75−.50
	2×5	g	209	50	159		.75−.50
	2×6	g	210	59	151		.50−.25
	6×2	g	310	85	225		.50−.25
	3×4	g	316	85	231		.50−.25
	3×5	g	467	122	345		.75−.50
tg×G	1×7	G	308	83		225	.50−.25
	2×7	G	440	111		329	over .90
g×G	5×7	G	386		106	280	.50−.25
	6×7	G	305		77	228	over .90

the glossy plants noted by Tarumoto[17] showed a "non-glossy" appearance until the 4–5th leaf stages (during the first three weeks in Japan). The discrepancy between the definitions of glossy trait by Tarumoto[17] and by Maiti and Bidinger[13], was resolved by Tarumoto (1980)[20] who found true-glossy (tg) plants in addition to glossy (g) and non-glossy (G) plants[17]. The inheritance of the true-glossy plants has been unknown. Thus, the author conducted inheritance studies in the three phenotypes of glossiness, including true-glossy (tg), glossy (g) and non-glossy (G).

The 7 varieties listed in Table 5 were used for crossing, and the 23 F_1 and F_2 populations derived from the crosses of (tg×tg), (g×g), (tg×g), (tg×G), and (g×G) were prepared. The above materials were grown in the greenhouse in winter of 1982–83, and were tested for their glossiness of leaves from the 1st to flag leaf stage, for the judgment of tg (true-glossy), g (glossy) and G (non-glossy) by spraying water from a small-holed sprayer[2,4]. The phenotypes of F_1 were tg in the combination of (tg×tg), g in (tg×g) and (g×g), and G in (tg×G) and (g×G), respectively, in Table 6[21]. In the F_2 of (tg×g), (tg×G) and (g×G) where segregations were observed in glossiness (Table 6), the expected ratio of (tg : g = 1 : 3), (tg : G = 1 : 3) and (g : G = 1 : 3) were well fitted[21]. The results suggest that tg, g and G would be multiple alleles located in a glossy locus. Although the symbols *gl* and *Gl* were proposed for g and G in the previous section[17], the symbols gl^1, gl^2 and gl^+ are newly proposed to denote the multiple alleles for g, tg and G in the glossy locus, respectively. The glossy plants are mutants and recessive to normal plants in broccoli (*Brassica oleracea* L. var. *italica*)[2] and corn (*Zea mays* L.)[6,10]. In sorghum (*Sorghum bicolor* L. Moench), the non-glossy plants (gl^+-) are dominant to glossy (gl^1gl^1, gl^1gl^2) and true-glossy (gl^2gl^2) plants and the wild relative species have non-glossy leaves. Therefore, it is concluded that the non-glossy plant (gl^+-) is a wild type, and the glossy (gl^1gl^1) and true-glossy (gl^2gl^2) plants are mutant types in sorghum. The relationship between genotypes, phenotypes and time of character expression in three types of glossiness in sorghum is illustrated in Fig. 4.

Conclusion

In the inheritance study on the three phenotypes of glossiness in sorghum, including true-glossy (tg), glossy (g) and non-glossy (G), it was clarified that the genes gl^2 (tg), gl^1 (g) and gl^+ (G) were multiple alleles located in a glossy locus; gl^+ was a simple dominant gene to gl^2 or gl^1; and gl^1 was a simple dominant gene to gl^2. The relationship between genotypes, phenotypes and time of character expression in three types of glossiness is summarized in Fig. 4. The gl^1 gene (g) was found to produce pleiotropic effects not only on the cellular structures but on digestibility and possibly the sorghum shoot fly resistance of the leaves also. The glossiness was revealed to be a useful character for identifying the phyletic evolution in the genus *Sorghum*, since the non-glossy leaf (gl^+-) was the symbolic trait of wild-relative species in sorghum.

All of the varieties having "glossy trait" in Maiti and

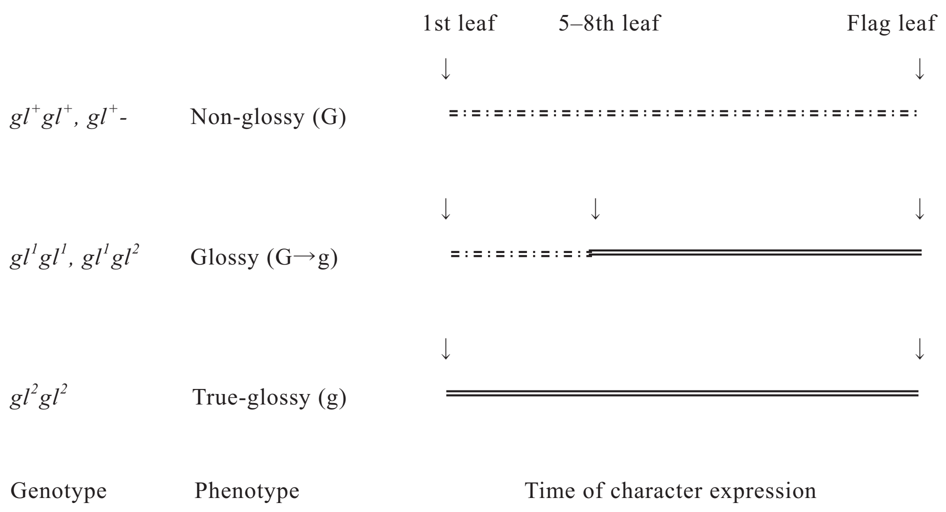

Fig. 4. Genotype, phenotype and time of character expression in three types of glossiness

═══ : Water attached. =:=:=:=: : Water shed.

Bidinger (1980)[13] were not ones having true-glossy leaves[20], but they were reported to have the shoot fly resistance and drought tolerance in the seeding stage[13,14]. Thus, it is necessary to reveal the pleiotropic effects of the gl^2 (tg) gene on such traits as pest resistance, drought tolerance and leaf structures in order to utilize glossiness more in breeding and genetic studies.

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