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Germinal reversion of an unstable mutation for anthocyanin pigmentation in soybean

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Summary. Plants of the "*w4*-mutable" line of soybean [*Glycine max* (L.) Merr.] are chimeral for anthocyanin pigmentation. Mutable plants produce both near-white and purple flowers, as well as flowers of mutable phenotype with purple sectors on near-white petals. It is established here that the mutable trait is conditioned by an unstable recessive allele of the *w4* locus that conditions anthocyanin biosynthesis. The gene symbol *w4-m* is assigned to the mutable allele. Allele *w4-m* was derived from a stable, wild-type *W4* progenitor allele and reverts at high frequency to a stable, wild-type *W4* allele. Reversion occurs both early and late during the development of the germ line. Several experiments give estimates of germinal reversion frequency, indicating that approximately 6% of mutable alleles revert to wild-type from one generation to the next. Allele *w4-m* exhibits many features typical of an allele controlled by a transposable element.

Key words. Soybean – *Glycine max* – Genetic instability – Transposable element – Anthocyanin

Introduction

Anthocyanin biosynthesis in flowers and other plant parts of soybean [*Glycine max* (L.) Merr.] is dependent on the interaction of several genetic loci (Bernard and Weiss 1973; Palmer and Kilen 1987). Absence or near absence of anthocyanin pigmentation is conditioned, respectively, by recessive alleles of the *w1* and *w4* loci. The hue and intensity of pigmentation is modified by the *w2*, *W3*, and *wm* loci. A recently discovered mutation conditions a mutable trait for anthocyanin pigmentation in soybean (Groose et al. 1988). Most plants of the "*w4*-mutable" line are chimeral for anthocyanin pigmentation. Mutable plants bear both entirely near-white and entirely purple flowers, as well as flowers of mutable phenotype with purple sectors on near-white petals.

Results of the present study show that the *w4*-mutable line carries an unstable recessive ("mutable") allele of the *w4* locus and that the mutable allele reverts to wild type at high frequency in the germ line. Possible interaction of a transposable element with the *w4* locus is discussed. In a previous paper (Groose et al. 1988), the somatic behavior of the mutable allele was described, periclinal chimerism was illustrated, and periclinal chimeras were used to demonstrate that the germ line in soybean is of subepidermal origin.

Materials and methods

Mutable plants analyzed in this study descended from the *w4*-mutable line of soybean. The origin of the *w4*-mutable line and the pedigree of its sub-lines are described by Groose et al. (1988) and Weigelt et al. (1990). Most genetic analyses presented in this paper were performed with highly mutable F_{10} plants of sub-line "W4M-3-1-12-1", where "W4M" designates "*w4*-mutable" and "3", "1", "12", and "1" identify the F_6, F_7, F_8, and F_9 parentage, respectively.

The pleiotropic effects of the *w4* locus on anthocyanin pigmentation of flowers and hypocotyls of plants used in this study are summarized in Table 1. The symbols for phenotype, presented in Table 1 (WT, MU, NW, and GR), are used in subsequent tables. Materials used in the genetic analyses described in this paper were true-breeding for wild-type alleles at other anthocyanin loci (genotype *W1W1*, *W2W2*, *w3w3*, *WmWm*), as indicated by the absence of white, carmine, dilute-purple, and magenta flower phenotypes.

Genetic analyses of the mutable trait were performed in hybridization experiments using only "mutable" flowers (illustrated in Groose et al. 1988) in crosses. Periclinal chimeral flow-

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Table 1. Anthocyanin pigmentation as conditioned by the *w4* locus

Phenotype	Pigmentation		Genotype[a]
	Flower	Hypocotyl	
WT = wild type	purple	purple	*W4*––
NW = near-white	near-white	GR = green	*w4w4*
MU = mutable	mutable[b]	mutable[c]	*w4-mw4-m* or *w4-mw4*

[a] Genotypes of mutable plants are as determined by genetic analysis in the present study

[b] Flowers produced by mutable plants include flowers with purple sectors on a near-white background, as well as several periclinal chimeral types (illustrated in Groose et al. 1988) that result from somatic reversion in different cell layers

[c] Hypocotyls of mutable seedlings exhibit purple flecks and stripes on a green background

Table 2. Genetic analysis of cross between *w4*-mutable line and wild-type Harosoy line

Cross no.	Parents		F_1	F_2 Segregation		
	Female	Male		No. seedlings		
				WT	MU	$\chi^2_{1df}(3:1)$
1	Harosoy	W4M-3-1-12-1	WT	72	24	0.000
2	Harosoy	W4M-3-1-12-1	WT	77	15	3.711
3	Harosoy	W4M-3-1-12-1	WT	85	16	4.519*
4	Harosoy	W4M-3-1-12-1	WT	88	37[a]	1.411
5	Harosoy	W4M-3-1-12-1	WT	72	24	0.000
6	Harosoy	W4M-3-1-12-1	WT	95	7[b]	17.896***
7	W4M-3-1-12-1	Harosoy	WT	74	22	0.223
8	W4M-3-1-12-1	Harosoy	WT	110	0	36.667***
9	W4M-3-1-12-1	Harosoy	WT	78	22[c]	0.480
10	W4M-3-1-12-1	Harosoy	WT	80	30[c]	0.303
						χ^2_{9df} homogeneity = 48.533***

* $p < 0.05$

*** $p < 0.005$

[a] Includes eight seedlings of GR phenotype

[b] Includes one seedling of GR phenotype

[c] Includes four seedlings of GR phenotype

ers (also illustrated in Groose et al. 1988), some of which are revertant in the germ line, were avoided in making crosses. Mutable plants were crossed with plants of the wild-type purple-flowered Harosoy line to determine inheritance of the mutable trait. Allelism to the *w4* locus was tested by crossing mutable plants to the near-white-flowered Harosoy isoline (designated "Harosoy-*w4*"), obtained from the Soybean Genetics Collection (Dr. R. L. Bernard, Curator, Department of Agronomy, University of Illinois, Urbana/IL).

The distribution of germinally revertant progeny among pods was analyzed with seven mutable F_8 plants, each descended from a different F_7 plant of *w4*-mutable. All pods were threshed individually, and the number of germinally revertant (wild-type) and nonrevertant (mutable or near-white) progeny borne by each pod was determined by observation of the F_9 plants at flowering.

Results and discussion

The mutable trait is conditioned by an unstable recessive allele of a single locus

When mutable plants of *w4*-mutable were crossed to the wild-type purple-flowered Harosoy line, all F_1 progeny were wild-type (Table 2). Seven F_2 progenies segregated approximately three wild-type: one mutable, and three F_2 families produced an excess of wild-type segregates. These results indicate that mutability is conditioned by an unstable recessive ("mutable") allele of a single genetic locus and that the mutable allele is capable of reversion to wild-type. The deviation from the expected 3:1 ratio in some F_2 families and the lack of homogeneity among families may be attributed to germ line reversion early in the development of F_1 plants or in the mutable parent of the F_1.

F_2 families such as no. 3 and no. 6 (Table 2) that segregate for an excess of wild-type progeny are probably descended from F_1 plants in which the mutable allele reverted to wild-type in the germ line early in development. Early reversion in an F_1 plant would result in a large homozygous wild-type sector that would bear only wild-type progeny, leading to the production of an overall excess of wild-type progeny by the F_1 plant. Somatic analysis of mutable plants of the *w4*-mutable line indicated that the mutable allele can revert early in development to produce large revertant sectors (Groose et al. 1988). An F_2 family such as no. 8 that breeds true for wild-type is probably descended from an F_1 individual that received a germinally revertant (wild-type) allele from the mutable parent. We observed several segregates of green phenotype (no purple sectors) in four F_2 families.

Additional evidence that mutability in *w4*-mutable is conditioned by a single unstable recessive allele (including F_3 generation data from the cross of *w4*-mutable with Harosoy, as well as F_1 and F_2 generation data from crosses of *w4*-mutable to several other wild-type lines, including wild relatives of soybean) are reported elsewhere (Groose and Palmer 1990).

Results of an earlier study are also consistent with the hypothesis that mutability is conditioned by an unstable allele of a single locus (Groose et al. 1988). Reversion of the unstable allele in independent cell-layers in the meristem resulted in the production of periclinal chimeral sectors on mutable plants. It was possible to identify periclinal chimeral sectors because expression of anthocyanin genes in flowers is cell-layer specific. Mosaic mapping with periclinal sectors established that the germ line in soybean is derived from the subdermal cell-layer (cell-layer LII). Reversion in cell-layer LII resulted in flowers of a distinctive phenotype ("LII revertant") that produced self-progeny that segregated approximately three wild-type: one mutable, indicating reversion of a single

Table 3. Genetic analysis of cross between *w4*-mutable line and near-white-flowered Harosoy-*w4* isoline

Cross no.	Parents		F_1	F_2 Segregation				
	Female	Male		No. seedlings				
				WT	MU	WT+MU	GR	χ^2_{1df} (3:1)
1	Harosoy-*w4*	W4M-3-1-12-1	MU	8	77	85	25	0.303
2	Harosoy-*w4*	W4M-3-1-12-1	MU	11	22	33	5	2.843
3	Harosoy-*w4*	W4M-3-1-12-1	MU	10	55	65	23	0.060
4	Harosoy-*w4*	W4M-3-1-12-1	MU	2	82	84	12	8.000***
5	W4M-3-1-12-1	Harosoy-*w4*	MU	0	70	70	28	0.667
6	W4M-3-1-12-1	Harosoy-*w4*	MU	3	71	74	21	0.424
7	W4M-3-1-12-1	Harosoy-*w4*	MU	3	56	59	30	3.599
8	W4M-3-1-12-1	Harosoy-*w4*	MU	0	74	74	25	0.004
9	W4M-3-1-12-1	Harosoy-*w4*	MU	3	66	69	35	4.153*
10	W4M-3-1-12-1	Harosoy-*w4*	MU	8	56	64	23	0.096
11	W4M-3-1-12-1	Harosoy-*w4*	WT	82	0	82	23	0.536
12	W4M-3-1-12-1	Harosoy-*w4*	WT	75	0	75	27	0.117
13	W4M-3-1-12-1	Harosoy-*w4*	MU	3	78	81	30	0.243
								χ^2_{12df} homogeneity = 21.036*

* $p<0.05$
*** $p<0.005$

unstable allele. In one instance, an LII-revertant shoot produced only wild-type progeny, indicating coincidental reversion of both mutable alleles in the germ line.

w4-m, an unstable recessive allele of the w4 locus, conditions the mutable trait

In an allelism test, mutable plants were crossed with the Harosoy-*w4* isoline. F_1 and F_2 generation data for crosses to Harosoy-*w4* indicate allelism of the mutable trait to the *w4* locus (Table 3). Of 13 F_2 plants, 11 were mutable, and 10 of these produced progeny that segregated three mutable (or wild-type) : one green in the F_2 generation. The aberrant segregations of two of the F_2 progenies (nos. 4 and 9) might be attributed to chance. The appearance of wild-type segregates among F_2 progeny of mutable F_1 plants is consistent with the hypothesis that mutability is conditioned by an unstable recessive allele that reverts to wild-type. Wild-type segregates among F_2 progeny of a mutable F_1 plant may be attributed to reversion in the germ line of the F_1 plant. Two F_1 plants were wild-type (nos. 11 and 12, Table 3) and produced F_2 families that segregated three wild-type : one green. These F_1s may be attributed to germinal reversion in the mutable parent.

Additional evidence that mutability is conditioned by an unstable recessive allele of the *w4* locus (including F_3 generation data from the cross of *w4*-mutable with Harosoy-*w4*, as well as F_1 and F_2 generation data from a cross between *w4*-mutable and a white-flowered line carrying recessive alleles of the *w1* locus) are reported elsewhere (Groose and Palmer 1990).

Finally, the phenotype of flowers of the *w4*-mutable line confirms allelism to the *w4* locus (illustrated in Groose et al. 1988). Most flowers on mutable plants exhibit the faint blush of anthocyanin in the throat of the standard petal that is typical of plants homozygous for recessive alleles at *w4* and for dominant alleles at *w1*.

In summary, an unstable recessive allele of the *w4* locus conditions mutability. As a trait, mutability may be considered recessive to wild-type but dominant to the near-white phenotype conditioned by *w4* alleles. The gene symbol *w4-m* is assigned to the mutable allele. Several lines of evidence indicate that *w4-m* arose from a stable wild-type (*W4*) progenitor allele (Weigelt et al. 1990).

w4-m reverts to a stable wild-type W4 allele

Most germinal revertants exhibit a phenotype that is indistinguishable from wild-type (illustrated in Groose et al. 1988). Although more than 99% of revertant alleles are identical to wild-type (*W4*), reversion to an intermediate form may occasionally occur (S. M. Schulte and R. W. Groose, unpublished results). Thus far, we have identified four instances of reversion to intermediate phenotypes that appear to be conditioned by partially revertant or "pale" alleles. The putative pale alleles are the subject of current research.

The germinal stability of revertant *W4* alleles was demonstrated with progenies of two F_9 sub-lines that were true-breeding (*W4W4*) for revertant alleles. Both of these lines, W4M-2-9-6 and W4M-3-6-4, were descended from mutable F_7 plants (*w4-mw4-m*). F_{10} sub-lines were derived by threshing 63 and 84 plants, respectively, of W4M-2-9-6 and W4M-3-6-4. Every F_{10} progeny (7,114 seedlings for W4M-2-9-6 and 16,146 seedlings for W4M-3-6-4) exhibited wild-type pigmentation. If a revertant allele had lost function in a germ-line sector of any F_9 plant, one-fourth of the progeny produced by that sector would be recessive (or perhaps mutable). The production of only wild-type progeny in this experiment indicates that revertant alleles are highly stable.

Revertant alleles also exhibit a high level of somatic stability. We have examined thousands of revertant sectors on mutable plants but have never observed a nonpigmented sector within a revertant sector (as evidence of loss of function of a revertant allele). Similarly, we have never observed a mutable or nonpigmented sector on a wild-type plant that was heterozygous for revertant *W4* alleles and mutable *w4-m* or recessive *w4* alleles (i.e., genotypes *W4w4-m* and *W4w4,* respectively).

w4-m can revert both early and late during the development of the germ-line

Somatic analysis of mutable plants revealed that reversion of *w4-m* can occur at various times during development and that the size of revertant sectors on mutable plants is dependent on the developmental timing of reversion (Groose et al. 1988). Early and late reversions produce, respectively, large and small revertant sectors. Both early and late reversion of *w4-m* in the development of the germ line were demonstrated by somatic and genetic analyses of clonal sectors of different phenotypes on mutable plants.

Reversion of *w4-m* early in the development of the germ-line is also indicated by the genetic analyses reported in the present study. Early germinal reversion in F_1 plants is a probable source of the deviation from expected segregation ratios in some F_2 families and the lack of homogeneity among F_2 families (Table 2).

Germinal reversion of *w4-m* late in development was confirmed in an experiment in which a large number of F_1 progeny were produced in reciprocal crosses between *w4*-mutable (using only "mutable" flowers) and Harosoy-*w4* (Table 4). Reciprocal crosses produced some wild-type F_1 progeny, indicating that late germinal reversion can occur in either the female or male reproductive organs. The data in Table 4 provide additional evidence of allelism of the mutable trait to the *w4* locus.

Table 4. Anthocyanin pigmentation in F_1 progeny of reciprocal crosses between *w4*-mutable line (using only "mutable" flowers) and near-white-flowered Harosoy-*w4* isoline

Parents		No. F_1 seedlings		
Female	Male	WT	MU	GR
W4M-3-1-12-1	Harosoy-*w4*	11	129	12
Harosoy-*w4*	W4M-3-1-12-1	6	110	7
Total		17	239	19

Reversion of *w4-m* late in the development of the germ line and after differentiation of the female and male reproductive tissues was demonstrated by an analysis of the distribution of germinally revertant progeny among pods borne by mutable F_8 plants. If all germinal revertants are produced by flowers in which the male and female germ lines are genetically concordant and heterozygous (i.e., *W4w4-m,* with the assumption that coincidental reversion of both unstable alleles is rare), then all progeny of germinally revertant flowers should segregate approximately three revertant : one nonrevertant. On the basis of this assumption, the expected relative frequencies of two- and three-seeded pods that would bear one, two, and three germinally revertant progeny are calculated in Table 5. An excess of pods that bore a single germinal revertant was observed among two- and three-seeded pods produced by seven mutable plants (Table 5). The observed distributions of the different types of revertant pods differed significantly (for two-seeded revertant pods $\chi^2_{1df}=10.417$, $P<0.005$; and for three-seeded revertant pods $\chi^2_{2df}=46.181$, $P<0.005$) from the distributions that are expected if all germinal revertants are produced by heterozygous and genetically concordant female and male germ lines. (Note: the lack of fit between expected and observed distributions would be even greater if the possibility of coincidental reversion of both alleles in the germ line had been taken into account. Any flowers that are homozygous and genetically concordant in the female and male germ lines would produce pods bearing only revertant progeny.)

These results indicate that germinal reversion can occur very late in development, after sexual differentiation in the germ line. Perhaps *w4-m* reversion occurs in the gametophytes or in the progeny zygote. Further analysis (not presented) revealed a heterogeneous distribution of revertant progeny among branch shoots of these mutable plants and indicated that reversion had also occurred early in the development of the germ line of some of these plants.

In summary, germinal reversion of *w4-m* can occur both early and late in development of the germ line.

w4-m reverts to wild type at very high frequency in the germ line

The germinal frequency of reversion of *w4-m* was estimated in several different genetic backgrounds on the basis of the frequency of revertant alleles contributed to progeny by mutable parents (Table 6). (It should be emphasized that this measure of reversion frequency may not be perfectly correlated to the frequency of individual

Table 5. Frequencies of multiple-seeded pods produced by revertant sectors of mutable plants of the *w4*-mutable line

Type of pod	No. progeny		Expected frequency[a]	Expected frequency of revertant pods[b]	Observed revertant pods[c]	
	Wild-type	Mutable			No.	Frequency
2-seeded	2	0	0.563	0.600	14	0.350
	1	1	0.375	0.400	26	0.650
	0	2	0.067			
Total			1.000	1.000	40	1.000
3-seeded	3	0	0.422	0.429	6	0.188
	2	1	0.422	0.429	8	0.250
	1	2	0.141	0.143	18	0.563
	0	3	0.016			
Total			1.000	1.000	32	1.000

[a] With the assumption that all germinally revertant progeny are produced by flowers in which the female and male germ lines are genetically concordant and heterozygous, the expected frequencies of the different types of two- and three-seeded pods were calculated by expanding the binomials $(0.75\ \text{wild-type}+0.25\ \text{mutable})^2$ and $(0.75\ \text{wild-type}+0.25\ \text{mutable})^3$, respectively

[b] Expected frequencies of the different types of two- and three-seeded pods that carry at least one germinally revertant progeny, with the assumption that all germinal revertants are produced by heterozygous and genetically concordant female and male germ lines

[c] Overall, seven plants produced 117 wild-type progeny (12.0%) among a total of 972 progeny. Eleven revertant progeny were borne by one seeded pods. (Note: a small proportion of plants of no mutability or very low mutability was noted among the nonrevertant progeny)

reversion events, because early germinal reversion can produce a large number of revertant gametes that are clonally derived from the same event.) In two experiments, it was possible to determine the exact number and percentage of germinally revertant alleles that were contributed by mutable parents to their progeny. For the other four experiments, a range of possible values is presented. The exact values are not known because the genotypes of individual revertant progeny were not determined. It is likely, however, that the true values are near the minimum values. The heterozygous condition of the germ line in most large revertant sectors, as well as the frequent occurrence of germinal reversion late in development, are expected to result in the production of a preponderance of heterozygous germinal revertants by mutable plants (see previous section).

Overall, the results presented in Table 6 indicate that approximately 6% of mutable alleles revert to wild type from one generation to the next. These results are consistent with the results of somatic analysis of mutable plants (Groose et al. 1988), where the proportions of different flower types and the frequency of revertant progeny produced by each flower type were determined. On the basis of results of the somatic analysis, approximately 12.6% of germinally revertant progeny would be expected among the progeny of mutable plants.

Mutable alleles carried by different sub-lines of the *w4*-mutable line may differ in reversion frequency (R. W. Groose, unpublished results). We are currently investigating variation in somatic reversion frequency among sub-lines of the *w4*-mutable line. A few sublines exhibit a stable recessive phenotype, indicating that the mutable allele may occasionally lose its ability to revert to wild type. In the genetic analyses of the present study, we have noted the appearance of a small proportion of green or near-white segregates in recessive classes where only mutable segregates might have been expected (Tables 2, 4, and 5).

Instability of w4-m suggests the action of a transposable element

The high frequency of reversion of *w4-m* is analogous to the behavior of many mutable alleles in plants that are controlled by transposable elements (for reviews, see Döring and Starlinger 1986; Fedoroff 1983; Nevers et al. 1986; Peterson 1987). Other aspects of the behavior of *w4-m* that are indicative of transposable element action include: (i) variation in reversion frequency that is suggestive of "changes in state" of *w4-m;* (ii) loss of mutability, which could result from imprecise excision of an element from the *w4* locus; and (iii) partial reversion to "pale" alleles, which might also result from imprecise excision. In the materials used in this study, *w4-m* is behaving as if it were controlled by an autonomous transposable element inserted at the *w4* locus.

Table 6. Estimates of the germinal reversion frequency of *w4-m*

Mutable parents[a]			Progeny				Alleles contributed to progeny by mutable parents			Source of data
Genetic background	Generation	No. plants	Type	Total	Wild-type		Total	Revertant alleles[b]		
					No.	%		No.	%	
w4-mutable	F_7	51	F_8 self	288	26	9.0	576	31[c]	5.4	[d]
w4-mutable	F_8	7	F_9 self	972	117	12.0	1 944	117–234	6.0–12.0	Table 5
w4-mutable	F_{10}	>50[e]	F_1 hybrid[f]	275	17	6.2	275	17	6.2	Table 4
w4-mutable × Harosoy	F_2	12	F_3 self	512	58	11.3	1 024	58–116	5.7–11.3	[g]
w4-mutable × Harosoy-*w4*	F_1	11	F_2 self	809	51	6.3	1 079[h]	51–102	4.7– 9.5	Table 3
w4-mutable × Minsoy	F_2	12	F_3 self	479	38	7.7	958	76–152	7.9–15.9	[i]

[a] All mutable parents were homozygous for mutable alleles (*w4–mw4–m*) except for the mutable F_1 plants from the cross between *w4*-mutable and the Harosoy-*w4* isoline. These mutable parents were heterozygous for mutable and stable recessive alleles (*w4-mw4*)

[b] In four cases, the exact number and percentage of revertant alleles is not known because the genotypes of individual progeny were not determined. In these cases, the number and percentage are presented as the range that is possible from the minimum (if all germinal revertants are heterozygous) to the maximum (if all germinal revertants are homozygous)

[c] Exact number is known because F_8 genotypes were determined on the basis of segregation of F_9 progenies. Of 26 F_8 germinal revertants, 5 were homozygous (*W4W4)* and 21 were heterozygous (*W4w4–m*)

[d] The 288 mutable F_8 progeny descended from 51 mutable F_7 plants (from W4M-1, W4M-2, and W4M-3) that were homozygous for mutable alleles

[e] Special case: more than 50 mutable plants of subline W4M-3-1-12-1 were used as parents in reciprocal testcrosses

[f] Progeny of testcross between W4M-3-1-12-1 and Harosoy-*w4* isoline

[g] These F_3 progenies were produced by mutable F_2 plants descended from the cross between Asgrow Mutable line and wild-type Harosoy line (Groose and Palmer 1990)

[h] Estimated, assuming that a single *w4* allele from the Harosoy *w4* isoline is carried by two-thirds of the 809 mutable and wild-type progeny of the 11 mutable (*w4-mw4*) F_1 plants of Table 3

[i] These F_3 progenies were produced by mutable plants grown from remnant seed of four F_2 families from a *w4*-mutable × Minsoy cross of Groose and Palmer (1990)

New mutations for chlorophyll deficiency, partial sterility, and necrotic roots have been identified in the *w4*-mutable (Groose and Palmer 1987). Mutations for chorophyll deficiency have occurred more than once and appear to be allelic (Palmer et al. 1989). Some of the new mutations are unstable and provide circumstantial evidence of transposition of a mobile genetic element.

The unstable *y18-m* allele of the *y18* locus for chlorophyll pigmentation and the *r-m* allele of the *R* locus for seed coat pigmentation in soybean may also be controlled by transposable elements (Peterson and Weber 1969; Chandlee and Vodkin 1989). Like many mutable alleles, the mutability of *y18-m* is affected by temperature (Sheridan and Palmer 1977). In contrast, temperature does not affect the mutability of *w4-m* (Groose and Palmer 1988).

Sequence analysis of an inserted element (*Tgm1*) in recessive alleles of the *le* locus for seed lectin in soybean revealed characteristics typical of one class of transposable elements in plants (Rhodes and Vodkin 1985, 1988). Perhaps the behavior of *w4-m* is the result of the action of an active form of *Tgm1* at the *w4* locus.

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