

We have tested this possibility by using pollen from two different forms of inflorescences borne on the same plant. The planting was widely spaced in the field (six sq. ft./plant) and extra fertilizer and water were applied. The plant chosen had a grade two lateral inflorescence that terminated a branch which originated above ground level and could not have been derived from a different embryo than that which produced the main stalk. The terminal inflorescence to the main stalk of this plant was normal (grade 5). The data were gathered from our winter crop planting in Goulds, Florida.

The results (Table 1) of a heritability test of variation through the male side confirm the previous one made on the female side. The hybrid progeny produced from crossing A158 with pollen from a 'vegetative' type tassel (grade 2) borne on a lateral branch was significantly more vegetative than that produced with pollen from the normal type tassel (grade 5) terminating the main stalk ( $P = < .01$ ).

Table 1  
Frequency distributions for hybrid progeny from A158 crossed by two grades of corn grass tassels borne on a single plant

Parental Tassel	Progeny Tassel Grades				Totals
	2	3	4	5	
Grade 2 (veg.)	9	18	35	16	78
Grade 5 (normal)	1	9	13	54	77

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### 1. Genetic instability of $R^{st}$ and derivatives in somatic and germinal tissues.

In a previous report we showed that after introduction of  $Mp$  in a homozygous  $R^{st} R^{st}$  stock, some kernels are produced exhibiting a new spotting pattern among the standard stippled kernels. These stippled derivatives, that breed true in successive generations, are here symbolized as  $R_{sk}$ ,  $R_{l.sk.}$ ,  $R_{l.st.}$ , and  $R_{n.c.}$  to indicate respectively smoky, light smoky, light stippled and nearly colorless. Like  $R^{st}$  these new forms do not synthesize anthocyanin in the sporophytic tissues and they are strong inducers of paramutation. On the other hand, they differ from stippled in the number and size of dots that they form in the aleurone.

The mosaic phenotype of the aleurone is presumably caused by the frequent somatic reversion of  $R$  from an inactive to an active form. Each of the spots therefore registers one such event which occurred sometime

during endosperm development. Accordingly, the various stippled derivatives differ in the time and frequency at which the somatic reversion of R occurs. When the reversion involves germinal tissues it gives rise to self-colored revertants, designated R<sup>sc</sup>, that are germinally transmissible.

In the present report we analyze the capacity of the various stippled derivatives to undergo reversion toward R<sup>sc</sup> in the germ cells and in the somatic tissues of the aleurone.

#### A. Reversion rate toward R<sup>sc</sup> in the germ cells.

In order to measure the reversion rate in the germ line, plants carrying the stippled allele under test (heterozygous for r and outside markers) were pollinated with a stock homozygous for r and outside markers. The genetic markers here used are the gene golden (g, 14 units proximal) and M<sup>st</sup> (six units distal to R). The matings made and the non-parental phenotypes selected were as follows:

<u>Cross</u>	<u>Non-parental phenotypes selected</u>
<u>+</u> <u>R<sup>st</sup></u> <u>+/g</u> <u>r<sup>g</sup></u> <u>m<sup>st</sup></u>	1. colored endosperm and scutellum
<u>g</u> <u>r<sup>g</sup></u> <u>m<sup>st</sup></u> / <u>g</u> <u>r<sup>g</sup></u> <u>m<sup>st</sup></u>	2. colored endosperm only
	3. colored scutellum only

The same scheme has been adopted for the stippled derivatives. Class 1 kernels represent instances in which the megaspore is revertant. If reversion of stippled to self-colored occurs during development of the megagametophyte, class 2 and 3 kernels are the reciprocal types expected. Kernels carrying the non-parental phenotype have been progeny-tested by selfing them. The reversion rate has then been estimated by subtracting the contaminants and germinally non-transmissible revertants from the total number of presumed revertants initially isolated. The figures to subtract were determined by extrapolating from those computed in the tested sample (since not all the revertants isolated germinated). Class 3 revertants have not yet been progeny-tested. The reversion rates of the three classes of revertants are reported in Table 1.

Table 1  
 Reversion rate of  $R^{st}$  and its derivatives to  $R^{sc}$  in the germ cells. (Class  
 1 = colored endosperm and scutellum; Class 2 = colored endosperm; Class  
 3 = colored scutellum)

Class 1 and 2 Revertants					
Allele tested	# gametes tested	Class 1 revertants	Reversion rate $\times 10^{-4}$	Class 2 revertants	Reversion rate $\times 10^{-4}$
Nearly colorless	4536	21.5	47.4	0.0	0.0
Stippled	3030	9.4	31.1	8.2	27.0
Smoky	4080	3.4	8.4	19.2	46.5
Light stippled	3131	0.9	3.0	6.0	19.1
Light smoky	6790	0.0	0.0	0.0	0.0

  

Class 3 Revertants			
Allele tested	#gametes tested	Class 3 revertants	Reversion rate $\times 10^{-4}$
Nearly colorless	4370	0.0	0.0
Stippled	4888	2.0	4.1
Smoky	6211	3.0	4.8
Light stippled	5403	5.0	9.2
Light smoky	6442	0.0	0.0

Although the population of gametes tested for each stippled derivative is not very large, the results seem to suggest that:

1. Within each of the three classes of revertants, some of the stippled derivatives exhibit striking differences in their capacity to revert toward  $R^{sc}$ .
2. A particular allele can exhibit differential capacity to revert to  $R^{sc}$  at various times (see the reversion rate of  $R^{n.c.}$  as measured in Class 1 and 3).

#### B. Reversion rate toward $R^{sc}$ in somatic cells.

In order to compare the reversion rate of a given allele in its somatic and germinal cells, we measured the frequency of somatic reversion as the rate of reversion per cell per division. This is accomplished by scoring the stippled kernels for half seed and quarter seed sectors. These sectors represent reversions occurring when the endosperm was at a two and four cell stage respectively. The estimate of somatic reversion rate has been limited to stippled, smoky and light smoky kernels. In fact, their somatic sectors are frequent enough and their identification easier than

is true for seeds carrying other stippled derivatives. The results are reported in the following table.

Table 2  
Reversion rate of different stippled derivatives in germinal and somatic tissues

Allele tested	#seeds scored	Germinal revertants	$\times 10^{-3}$	Half-seed sectors	$\times 10^{-3}$	Quarter-seed sectors	$\times 10^{-3}$
Stippled	1397	6.6	4.7	11	3.9	26	4.6
Smoky	3672	3.1	0.8	45	6.1	72	4.9
Light smoky	4080	0.0	0.0	0	0.0	4	0.3

These data suggest that when the frequency of reversion is expressed as reversion rate per cell per generation, it is possible to group the alleles into 3 categories:

1. The stippled allele exhibiting the same chance of reversion for all the cells and at different times of the development.
2. The smoky allele exhibiting an increase of approximately 5 times in its somatic reversion rate when compared to that in the germ cells.
3. The light smoky allele with high stability both in the germ cells and at the first cell divisions during endosperm development.

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## 2. Paramutagenic action of some stippled derivatives.

The various stippled derivatives can be distinguished by means of different criteria such as their capacity to induce paramutation and their reversion rate toward  $\underline{R}^{sc}$  in somatic and germ cells (see previous note). The first criterion is particularly important in relation to the problem of the genetic basis of paramutation. In fact, the mosaic phenotype of the aleurone, conditioned by  $\underline{R}^{st}$ , could be controlled by a genetic component associated with the  $\underline{R}$  region that is also responsible for inducing paramutation. Accordingly, one should find that any genetic alteration leading to a change in the stippling pattern is coupled with an alteration in its paramutagenic potentialities. This possibility has been tested by comparing the paramutagenic capacity of  $\underline{R}^{st}$  and  $\underline{R}^{n.c.}$  that differ markedly in their phenotypic expression. This test has been accomplished by crossing standard stippled and nearly colorless sib plants with a homozygous  $\underline{R}^R/\underline{R}^R$  stock and then testcrossing them on W23  $\underline{r}^g/\underline{r}^g$ . Individual testcross kernels were scored for pigmentation according to the standard matching technique (see Brink et al., 1960).