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Genetica. — *Induced instability at a paramutagenic* R *locus in* Zea Mays(*). Nota di CARLO COLELLA(**) e GIUSEPPE GAVAZZI, presentata(***) dal Corrisp. C. BARIGOZZI.

RIASSUNTO. — Il locus *R*, nel mais, controlla la produzione di pigmenti antocianici nell'aleurone e nei tessuti sporofitici. Gli alleli della serie *R* sono caratterizzati dall'attività paramutagenica e paramutabile, definibili come la capacità di indurre e subire rispettivamente la paramutazione. In questo lavoro si è fatto uso di un allele paramutagenico di *R* (R_2^{mc}) per studiare l'azione di un agente alchilante, il metansolfonato d'etile, e dei raggi X sull'espressione di R_2^{mc}. Ambedue questi agenti inducono alterazioni che portano a un aumento nel contenuto di pigmentazione aleuronica. Vi sono prove sperimentali che queste alterazioni sono associate al locus *R* e sono trasmissibili per via germinale. Nelle generazioni successive si osserva una reversione parziale al livello di pigmentazione iniziale. I dati raccolti suggeriscono che l'espressione di R_2^{mc} sia regolata da un sistema genetico a due elementi, uno paramutagenico e uno paramutabile, localizzati sullo stesso cromosoma.

INTRODUCTION

The term paramutation was introduced by Brink (1958) to describe the heritable decrease in gene action observed in a sensitive *R* allele in maize after heterozygous association with an inducing *R* allele. Alleles inciting such a change are referred to as paramutagenic, and factors sensitive to the inducer are termed paramutable. The phenomenon is now envisaged as a process of magnification of a built-in capacity of paramutable *R* alleles to self-regulate their expression (Brink *et al.*, 1968).

Following the observation, first reported by Linden (1963), that the paramutation process is radiosensitive, efforts have been directed toward the study of the action of chemical and physical agents on paramutation. Particular emphasis has been given to the study of their effect upon the expression of paramutant *R* alleles (*R'*), i.e. alleles whose pigmentation potential has been previously reduced by paramutation (Axtell and Brink, 1967; Shih and Brink, 1970; Shih, 1970). These studies indicated that there are mutagens capable of inducing frequent changes, though of low degree, toward a higher level of gene expression. The induced change is heritable and it is *R* locus dependent. Such results are in agreement with the hypothesis (Brink 1964) that a paramutable *R* factor is composed of a structural gene or gene complex, controlling anthocyanin production, and of an adjacent chromosomal region consisting of a variable number of repeats endowed with repressive activity upon *R* expression. The degree of repression may be a function of the number of repeats.

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The response of a paramutable *R* factor to an inducing *R* allele or to physical and chemical agents is interpretable, according to this view, in terms of changes in either the number of the repressive repeats or in their functional state.

Evidence from different studies on recombination and mutation of the R^{st} (stippled) paramutagenic factor seems to point to a complex organization of its genetic material. There is a suggestion (Ashman, 1970; Kermicle, 1970) that R^{st} is divisible into a gene or gene complex for anthocyanin biosynthesis, a component involved with paramutagenic activity and a genetic element with color inhibiting functions. The latter would control the pigment genes; its loss in the germ cells would lead to self-colored stable revertants (R^{sc}). The study of the heritable changes of a paramutagenic *R* allele induced by either physical or chemical mutagens could be of value in interpreting its structural organization and the paramutation process.

MATERIAL AND METHODS

Material.

The paramutagenic *R* allele chosen for the present study is a derivative of stippled, symbolized R^{nc} (nearly colorless), originally isolated from a homozygous R^{st} stock (Gavazzi, 1967). It displays a generally colorless aleurone with a few limited spots of faint pigmentation and colorless sporophytic tissues, and it exhibits the paramutagenic action of R^{st}. A characteristic feature of nearly colorless (Gavazzi and Colella, 1973) is its high reversion rate to R^{sc}. This event is limited mostly to meiosis and its frequency is not affected by X rays or ethylmethanesulfonate (EMS). R^{nc} was chosen for the present study because any change induced in its gene action toward a higher level of expression can be easily detected by visual inspection of the aleurone pigmentation.

In the test, the symbol R^{nc} will be used to refer to the standard nearly colorless allele while the symbol R' will apply to its changed form, in which pigmentation ranges between the initial nearly colorless and the heavily mottled phenotype.

The pigment distribution in the aleurone of the changed R^{nc} form is phenotypically like that of paramutable *R*. R^{nc} and the other *r* alleles used in this study (r^r, red seedling and colorless aleurone; r^g green seedling and colorless aleurone) have been previously introduced into the genetic background of the inbred strain known as W22.

Mutagen treatments.

Dormant seeds were irradiated with 6 KR (210 KV, 15 mA, 0.5 cm CU, 1 mm Al filter, 55 R/min.)

For the chemical mutagenic treatment, the alkylating agent ethyl methanesulphonate (EMS) was chosen because of its well known mutagenic efficiency in maize (Neuffer and Ficsor, 1963; Amano and Smith, 1965) as well as in other higher plants (Loveless, 1966; Ehrenberg, 1971). The mutagen was administered at different stages of growth with the aim of detecting whether *R* gene action is differentially affected by exposure to the mutagen at different phases of development. The following treatments were performed:

1) Seeds: soaked for 24 hours at room temperature in the mutagen solution (100 seed/200 ml).

2) Seedlings: at the two-leaf stage through cut roots for 24 hours.

3) Plants: at the time of microsporogenesis by injection (10 cc) into the stem.

For each treatment a freshly prepared 0.2% EMS solution in pH7 phosphate buffer (5×10^{-2} M) was used.

Scoring aleurone pigmentation.

To evaluate changes in pigmentation of R^{nc} induced by the mutagens, all the kernels of ears produced by testcrosses of R^{nc} R^{nc} females with an *rr* line were matched with a standard set of four kernels defining five color classes ranging from colorless (class 0) to heavily mottled (class 40) aleurone and these values were then used to estimate the mean aleurone pigment level of the ears.

When the testcrossed ears were segregating for R^{nc} and *r*, the mean score was doubled to take account of the fact that one half of the segregating kernels were expected to be genotypically *rr* and would not contribute to the measurement of R^{nc} gene action.

Mating scheme.

The following progenies were analyzed:

a) In 1970 control and EMS treated R^{nc} R^{nc} females were reciprocally crossed with an $r^g r^g$ tester. The resulting ears, referred to as M_1 ears in the text, were scored for production of kernels with a darker aleurone phenotype. These were pooled and planted in 1971 to produce the following mating:

b) $R' r^g \times r^r r^r$. Kernels on M_2 ears exhibiting a high level of aleurone pigmentation were divided into 5 classes by matching them with the standard set of four kernels and planted to test the heritability of level of aleurone pigmentation. They yielded the plants used as females on the cross:

c) $R' r^r \times r^g r^g$. The mean score of each M_3 ear was evaluated and the values obtained were compared with the parental one in order to estimate the transmission of the dark aleurone color character.

Results

Increase in level of the aleurone pigmentation induced by EMS *and* X *rays.*

In 1970 homozygous R^{nc} individuals were treated with EMS at various developmental stages (see Materials and Methods) and reciprocally crossed with an $r^g r^g$ tester. On the testcrossed ears two nonparental phenotypes were observed:

a) selfcolored kernels (R^{sc}). They occur mostly at meiosis with a rate of 0.75 % in the female and 1.53 % in the male germ line. EMS treatment does not change their spontaneous frequency. A detailed study of their origin is presented elsewhere (Gavazzi and Colella, 1973).

b) Kernels with a distinctly dark aleurone pigmentation. They form a heterogeneous class of phenotypes ranging from slightly pigmented to heavily mottled aleurone. As the data (Table I) clearly show, they are more frequent in the treated population.

Using R^{nc}/R^{nc} as pistillate parent significantly increased the frequency of kernels with dark aleurone on M_1 ears of individuals treated at the seed or seedling stage, while premiotic treatment was without effect. Similar effects on aleurone pigmentation were not observed when R^{nc} were used as a male parent though more than 16000 kernels were analyzed. Dark pigmentation of the aleurone was observed also in the control series.

TABLE I.

Frequency (%) of kernels with dark or selfcolored aleurone pigmentation observed on M_1 *ears produced by pollinating* $R^{nc}\ R^{nc}$ *individuals with an* $r^g\ r^g$ *tester.*

Stage of treatment	$R^{nc}\ R^{nc}$ used as	No. of kernels tested (1)	% R^{sc}	% dark pigmented
None	female	4905	0.71	0.18
Seed	female	746	0.80	1.54
Seedling	female	2576	0.62	2.15
Premeiotic	female	1191	0.92	0.17
None	male	2539	1.61	0.00
Seed	male	4001	1.35	0.00
Seedling	male	3035	1.58	0.00
Premeiotic	male	1504	1.66	0.00

(1) Adjusted for proportion of selfcolored revertants verified.

TABLE II.

Frequency (%) of ears showing kernels with pigmented phenotype obtained by reciprocal crosses of $R^{nc}\ R^{nc}$ *individuals with an rr tester.*

Entering the cross as	Treatment of seeds	# of ears	Dark (1) ears	Freq. %
Female	nil	36	0	0
Female	EMS	18	8	44.4
Female	X rays	61	3	4.9
Male	nil	20	0	0
Male	EMS	60	0	0
Male	X rays	47	0	0

(1) Ears with distinctly dark kernels (aleurone of class 20 and higher).

Progeny tests of the dark kernels showed germinal transmission of 68.8% (31/45) in the treated population while no transmission was observed in the control population (0/5). Previous tests had already proved that the occasional kernels with a dark color that appear on untreated homozygous $R^{nc}\ R^{nc}$

ears do not show germinal transmission of the dark color effect. The same mating scheme was repeated in 1972, after treating seeds with both EMS and X rays. These results, (Table II) confirmed previous observations, indicating that an X ray dose of 6 KR is less effective than the particular EMS treatment applied (0.2 % solution for 24 hr) in inducing the high pigmentation of the aleurone.

Germinal transmission of the change in aleurone pigmentation.

To see whether the induced change in the level of aleurone pigmentation is germinally trasmissible, a sample of $R^r r^g$ kernels with a distinctly dark aleurone was selected from M_1 ears and tested. Plants were testcrossed as pistillate parents with a homozygous $r^r r^r$ tester and the resulting M_2 ears were classified according to the level of aleurone pigmentation. The results (Table III), refer to the plants that showed germinal transmission of the increase in aleurone pigmentation. The mean scores of these ears ranged from 0.06 to 8.61, while the control series had a value of 0.04.

It is thus apparent that a portion of M_1 plants grown from EMS treated individuals showed an inherited effect of the treatment, though there was a high degree of variation in the transmission. In this experiment the aleurone pigmentation of the parent was not scored, thus the observed variation in the progeny ears could indicate either a different degree of transmission or differences in the parental values in aleurone pigmentation.

Inheritability of the induced changes in the level of aleurone pigmentation.

To estimate more precisely the level of germinal transmission of the change in aleurone pigmentation, samples of dark kernels, assumed to be R^r/r^r, from 11 M_2 ears were planted separately according to their color class and parentage. Sib kernels of 0 class were used as control. The resulting plants were crossed as pistillate parent with an $r^g r^g$ tester.

Among 70 plants grown from class 0 kernels, all but two bred true. The two exceptions gave ears with a mean color score of 0.177 and 0.906. The two kernels were either misclassified or they had discordant embryo and endosperm phenotype. Data referring to the other testcross ears are shown in Table IV where the mean color scores (M.S.) of the M_3 ears (the overall M.S. of M_3 ears is the average of the M.S. of M_3 ears derived from sib plants of each M_2 family tested) are listed according to the parentage and the phenotype (expressed as mean aleurone color scores) of the parental M_2 ears.

From the comparison between the mean color scores (M.S.) of M_2 and M_3 ears (column 2 and 8 respectively of Table IV) it is apparent that the amount of pigmentation is markedly reduced while passing from the M_2 to the M_3 generation, although the induced increase in level of aleurone pigmentation tends to be transmitted.

TABLE III.

Frequency distribution and mean aleurone color scores for M_2 *ears obtained by crossing a sample of* $R^{nc}\ r^g$ *individuals, isolated from* M_1 *ears as seeds with elevated aleurone pigmentation, with an* $r^r\ r^r$ *tester line.*

Female (1) Parent	# of $R^{nc}\ r^r$ kernels	Aleurone color classes					Mean aleurone color score
		0	10	20	30	40	
Control (2)	4945.0	4925.0	20				0.04
21.2.	138.5	130.5	7	1			0.65
21.3.	104.0	82.0	20	2			2.31
21.4.	129.5	126.5	3				0.23
21.6	194.0	185.0	8	1			0.52
21B.1	154.5	149.5	5				0.32
21B.2	129.5	123.5	3	3			0.69
21B.3	53.0	51.0	2				0.38
21B.4	122.5	112.5	10				0.82
21B.5	18.5	16.5		2			2.16
21B.10	81.5	77.5	4				0.49
21A.1	153.5	105.5	25	13	8	2	5.41
21A.2	121.5	93.5	19	8	1		3.13
21E.3	169.5	168.5	1				0.06
21E.4	156.5	146.5	10				0.64
21F.1	73.0	64.0	6	3			1.64
21F.2	131.5	124.5	4	3			0.76
21F.3	58.5	55.5	3				0.51
21H.1	153.5	144.5	4	3	1	1	1.11
21H.2	114.5	73.5	23	12	5	1	5.76
21H.3	124.0	109.0	15				1.21
21H.4	116.0	103.0	9	3	1		1.55
21L.1	69.5	67.5	2				0.29
21L.2	121.5	120.5	1				0.08
21L.3	107.0	96.0	9	2			1.21
21L.4	191.0	170.0	16	5			1.36
21L.9	118.0	115.0	3				0.25
21L.12	81.0	71.0	9	1			1.36
21M.1	160.0	154.0	6				0.38
21.1	30.5	20.5	2	8			5.90
23.2	90.5	45.5	20	17	8		8.62

(1) Numbers with a common letter refer to plants produced by sib kernels on the M1 ears.

(2) Pool of 27 ears produced in the same year and locality os the M2 ears, by crossing $R^{nc}\ R^{nc}$ plants with an *rr* tester line.

TABLE IV.

Heritability of the induced increase in aleurone pigmentation. M_3 ears were obtained by crossing $R' r^r$ individuals of the four aleurone classes indicated in the table, with pollen of an r^g r^g tester.

Family N. of M_2 ears	Mean aleurone color score (M. S.)	Weighed M.S. (a)	Parental (M_2) aleurone color scores				Overall M. S. (c) of M_3 ears	Frq. ($\times 10^{-3}$) of dark seeds on M_3 ears
			10	20	30	40		
			Progeny (M_3) scores					
			(b)					
23–2	8.62	20.83	0(2)	1.72	0.95		0.65	0.34
			0.24	1.81	0.00			
				0.00	1.46			
				0.26	0.90			
				0.44				
21 A–1	5.41	19.16	0 (4)	0.00	0.00	0.00	0.50	0.32
			0.21	3.28	1.19			
			0.43		0.86			
23–1	5.90	16.66	0.31	0 (2)			0.10	0.10
21 A–2	3.13	15.00	0 (5)	0 (2)	0.00		0.00	0.00
21 H–2	5.76	14.42	0.49	0.00	1.60	0.89	0.54	0.31
			0 (4)	0.49				
			0.16	1.75				
			0.26	0.94				
			0.97					
21 H–4	1.55	14.28	0 (2)	0.00	0.00		0.23	0.13
			0.70					
			0.36					
			0.58					
21 F–1	1.64	13.33	0 (2)	0.00			0.00	0.00
21 H–3	1.21	10.00	0 (3)				0.05	0.03
			0.06					
			0.12					
			0.10					
21–6	0.52	10.00	0 (2)				0.00	0.00
21 L–12	1.36	10.00	0 (5)				0.00	0.00
21 B–4	0.82	10.00	0 (5)				0.00	0.00

(a) Mean color score (M.S.) of the sample of dark $R' r^r$ kernels that was successfully grown and progeny tested.

(b) Number in brackets refer to the M_3 ears with a mean score of 0 value.

(c) Each value entering in the last two columns is the average of the M.S. of M_3 ears derived from sib plants of each M_2 family tested.

A regression analysis was made on the parent-progeny data by taking the weighed M.S. of M_2 ears as X values and the overall M.S. of M_3 ears as Y values. The weighed M.S. (Table IV, third column) expresses the average degree of pigmentation of the sample of kernels that, for a given M_2 ear, was successfully progeny tested (e.g. for ear 23–2 we obtained progeny from 3 kernels of color class 10,5 of 20 and 4 of 30. The weighted M.S. is thus equal to $(3 \times 10) + (5 \times 20) + (4 \times 30)/12 = 20.83$).

The regression analysis of variance, performed after appropriate transformation of the data to approximate a normal distribution, showed high significance (F = 12.87).

This result is taken as evidence that the overall mean color score of M_3 ears is related to the weighed M.S. of the parental ears. The observed variation within the progeny seems thus related to the differences among parental values of aleurone pigmentation. In the last column of Table IV the dark effect on M_3 ears is expressed as percentage of dark kernels on the total number of seeds. These values suggest the existence of a positive relationship between the degree of aleurone pigmentation of the parents and the frequency (%) of darker kernels in the progeny ears. Stated in a different way this means that the probability of appearance of dark kernels in the progeny of plants grown from kernels of a dark aleurone class (e.g. 40) is higher than in the progeny of a plant of low aleurone value (e.g. 10).

Association of the higher aleurone pigmentation with R^{nc}.

A test was devised to establish whether the heritable dark aleurone effect is due to a change of R^{nc} or of another genetic factor, either chromosomal or extrachromosomal, affecting anthocyanin production in the aleurone tissues.

Samples of $R'\ r^r$ sib kernels of aleurone class 20 and 30 were selected on M_2 ears and planted to be used as female parents in the following matings:

(1) $R'\ r^r \times r^g\ r^g$

(2) $R'\ r^r \times R^{nc}\ R^{nc}$.

The first mating (average color scores = 0.795; $n = 15$ ears) yields kernels of two genotypes $r^r\ r^g$ and $R'\ r^g$, the former with colorless aleurone, the latter with aleurone pigmentation ranging from colorless to heavily mottled. Because of the partial overlapping in the aleurone phenotype conditioned by the two genotypes, assigning the kernels to their proper genotype requires germination: $r^r\ r^g$ seedling in fact are red, while $R'\ r^g$ are green in their sporophytic tissues.

Accordingly, a first test of association of the dark aleurone pigmentation with R^{nc} was accomplished by taking two samples, from the M_2 ears, 120 kernels each, one of class 0 and the other of class 20, 30 or 40 phenotypes, respectively. The seeds were germinated and their seedlings classified as " red " and " non red " according to their pigmentation (Table V, first two rows). The majority of the kernels with dark aleurone did not synthesize pigment in their sporophytic tissues (the two exceptions were probably contaminats or recombinants with both the red seedling marker of r^r and the dark aleurone property carried on the same chromosome) while more than half of those with colorless aleurone produced pigmented seedlings. These results do not allow one to discriminate between the two hypotheses previously formulated. They simply indicate that the presence of R^{nc} is necessary for the

expression of the dark aleurone phenotype. If the dark aleurone results from an interaction of R^{nc} with an unlinked color modifier (M^c), then one would assume that in a heterozygous $R'\,r^r$ individual testcrossed with an $r^g\,r^g$ tester, M assorts, at meiosis, with R' as well as with r^r gametes so that both $R'\,r^g$ and $r^r\,r^g$ progeny kernels should carry it in about the same frequency. However, its presence in the latter genotype would pass undetected since no genetic information for aleurone pigmentation is available, while the former genotype would disclose its presence by producing a dark aleurone as a result of the interaction of the color modifier with R^{nc} gene expression.

TABLE V.

Results of crosses performed to test the association of the induced increase in aleurone pigmentation with R^{nc} *(see text for explanations).*

Cross (1)	Aleurone pigmentation	Seedling	
		red	nonred
$R'\,r^r \times r^g\,r^g$	dark	2	116
$R'\,r^r \times r^g\,r^g$	colorless	66	50
$R'\,r^r \times R^{nc}\,R^{nc}$	dark	1	118
$R'\,r^r \times R^{nc}\,R^{nc}$	colorless	68	49

(1) The first genotype of each cross refers to the pistillate parent.

One way of detecting M^c would be to cross $R'\,r^r$ plants with an $R^{nc}\,R^{nc}$ tester. The presence of one dose of R^{nc}, contributed by the male parent, in the $r^r\,R^{nc}$ aleurone of the progeny kernels should in fact disclose its activity. Accordingly pollen of $R^{nc}\,R^{nc}$ plants was applied on silks of $R'\,r^r$ plants grown from kernels of class 20–30 aleurone (see mating 2). The same male parent was used with $R^{nc}\,r^r$ females as a control.

The first cross gave a mean score of 2.81 for the aleurone (9 ears). This is an extremely high value that cannot be accounted for simply by the presence of three doses of R^{nc} as the low value (0.16 for 10 ears) of the second cross clearly shows. No explanation is available yet for the observed high value. However, the significantly higher pigmenting level in the progeny of mating (2) compared to that of mating (1) is indicative of an interaction between the EMS induced change in the maternal R' and the R^{nc} paternal allele. The second test of association to be described is based on this unexpected interaction. $R'\,R^{nc}$ and $r^r\,R^{nc}$ kernels obtained from cross (2) were divided into two classes according to their aleurone phenotype and scored, upon germination, for seedling pigmentation. The hypothesis of an unlinked color modifier (M^c) would assume that approximately a half of $R'\,R^{nc}$ and $r^r\,R^{nc}$ kernels produced by cross (2) would carry M^c and would be dark in their aleurone.

Accordingly about one half of the dark kernels should yield, upon germination, red seedlings.

Contrary to the expectations the results, reported in the last two rows of Table V, show that the seeds with dark aleurone are green in their sporophytic tissues, suggesting that the dark phenotype is associated with the R^{nc} allele. Thus an R unlinked or nonchromosomal factor could hardly be involved unless the further assumption is made that in an R' R^{nc} endosperm this factor interacts with the paternal R^{nc} only if the maternal R' allele is present too, a possibility that appears somewhat remote.

DISCUSSION

Before evaluating the results presented above it seems appropriate to consider some features of paramutation pertinent to the data.

Paramutation is describable as a quantitative change in the phenotypic expression of an R allele. The change may be to either a higher or lower pigmentation level and is, at least in part, genetically transmissible.

Factors eliciting this change are referred to as paramutagenic, while those sensitive to the change are termed paramutable.

Both chemical and physical mutagens are known to affect the paramutation system.

Specifically it has been shown with both X rays and alkylating agents that gene action of a paramutable R is consistently increased to a higher level in response to treatment. The induced change in R gene action occurs with too high a frequency to be accounted for by gene mutation. Similar changes in R gene action occur autonomously, even though less dramatically (Styles and Brink, 1966) thus proving that the change in gene action is not necessarily mediated by a partner allele or by the activity of the mutagen exclusively.

To account for these observations Brink proposed a model of R gene repression that depicts a paramutable R as a chromosomal region consisting of a gene or gene complex controlling anthocyanin biosynthesis and of an adjacent region called Repressor, composed of a series of repeated subunits, the effect of which is to repress R action, the amount of repression being directly related to the number of active subunits present.

According to this hypothesis paramutation is a change in the number of subunits in the Repressor segment. In particular the increase in phenotypic expression of paramutable R' following physical or chemical treatments might well be due to physical or functional loss of subunits of the R Repressor.

The results presented in this paper show that the partial restoration of gene action induced by mutagenic agents to a paramutable R factor can also take place at a paramutagenic allele highly suppressed in its gene action. Evidence has been obtained that the induced increase in level of pigmentation is R locus associated, thus excluding the possible involvement of other chromosomal or extrachromosomal genetic units influencing R^{nc} expression. The partially repressed R^{nc} ($R^{nc'}$) is germinally transmissible in its new state even though it shows a partial reversion, in succeeding generations, to its original level of gene expression. It has also been shown that $R^{nc'}$ exhibits a trans-effect, consisting in an induced increase in the level of gene action of an R^{nc} allele that is made heterozygous with $R^{nc'}$.

These results can be interpreted by assuming that the nearly colorless phenotype is the result of a self-paramutational event. One way of envisaging such events is to postulate that the R^{nc} region carries both paramutagenic and paramutable functions along the same chromosome. According to this view the induced increase in aleurone pigmentation of R^{nc} would reflect changes occuring at the paramutable component conducive to a decrease in the suppression exerted by the latter upon R gene action or alternatively it could be due to changes in the paramutagenic component.

The observed partial reversion of changed R^{nc} towards its original form as observed while passing from M_2 to M_3 generation would suggest that a self regulatory mechanism is operative that tends to reestablish a fixed level of R suppression. However, no evidence for the mechanism leading to R suppression and to its partial release is yet available. Even though the data obtained do not allow one to make sound inferences on the functional relationship between these two components it is tempting to speculate that activity of the paramutable function rests upon the presence of an active paramutagenic component. The paramutational process would demand that the latter is active both in *cis* and *trans* while the former would be *cis* limited in its activity.

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