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New Cases of Somatic Conversion (Paramutation) in Tomato (*Lycopersicon esculentum* Mill.)[1]

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Summary. The genetic behaviour of three chlorophyll variegated F_1 tomato plants, derived from irradiated gametophytes, was analyzed over several generations of selfing and backcrossing. The results suggest that irradiation has put genes, different in all three mutants, into a labile state, n^*, remaining so after fertilization. This state had the power of converting the associated wild allele N into a deficient form.

'Somatic conversion' was soon followed, in Plant C_{11} always and nearly always in Plant C_{12}, by stabilization of both alleles in a conversion-inactive recessive state, genetically similar, and stable except in special conditions.

In the other type, found seldom in C_{12}, always in C_6, the n^* state was permanent and transmissible. Conversion occurred with a certain frequency determined by developmental and genotypic influences, and the converted allele also acquired conversion power, so that gametes from an N n^* plant were of three kinds: N, n^* and n'^*. This process corresponds to 'paramutation' (Brink 1958).

Results were compared and contrasted with other published data.

Introduction

The heritable alteration of an allele by heterozygous association with another allele has been termed 'paramutation' by Brink (1958), and sometimes called somatic conversion (Hagemann 1958, Renner 1959) by reference to Winkler's terminology (1930).

This phenomenon, which transgresses Mendelian laws, has been most extensively studied in maize at the *R* locus (review in Brink et al. 1968) and at the *B* locus (summary in Coe 1968), as well as in tomato at the *sulf* locus (summary in Hagemann 1969). Early cases of unusual hereditary behaviour described by some authors in other plant materials could be ascribed to the same phenomenon (references in Brink 1964 and Hagemann 1969).

Although different models have been proposed to account for this category of gene instability (Sastry et al. 1965, Coe 1966), no direct proof has yet been given of its mechanism at cell level. The idea that some heterochromatization of the chromosome, at or adjacent to the locus, causes a repression effect on the gene (Brink 1964) seems now to be generally accepted. Recent data have shown that the repression can be alleviated by chemical mutagens (Axtell and Brink 1967) or by ionizing radiation (Shih 1969).

Contradictory results have been published about the inherent gene instability at those loci where paramutation takes place. Whereas the *R* allele of maize proved to undergo, at least partially, directed changes in the absence of any paramutagenic partner (Brink et al. 1968), the gene remains remarkably stable under the same conditions in the other systems (Hagemann 1965, Coe 1968). This difference is a source of controversy about the definition of paramutation (Hagemann 1969, Styles 1970) and emphasizes the difficulty of determining what is general and what is special in the various systems considered.

Further studies are therefore needed to discern the nature and role of the allelic interaction in the 'directed changes of alleles'. In particular, the discovery of new systems should show how widespread this form of gene instability is. Recently two new examples were discovered in maize, for the *C* locus (Mazoti 1968) and the *Ifd* locus (Greenblatt 1968). It is to be hoped that these studies, published in abstract form, will be developed.

This paper describes the behaviour of three unstable genes controlling chlorophyll expression recently found in irradiated tomatoes. They are similar to the gene described by Hagemann, but have a wider range of behaviour.

Material and Methods

The initial material was found in the F_1 hybrid of a *L. esculentum* cross, var. Moneymaker × line 121, where the Moneymaker parent, a commercial cultivar, had been exposed in haplophase to various irradiation treatments.

The line 121, kindly provided by R. D. Brock (CSIRO, Canberra), is characterized by three recessive seedling markers, and was initially introduced at our laboratory to provide a parameter of genetic radiosensitivity (Ecochard and de Nettancourt 1969, Ecochard 1970).

In addition to the specific mutants, a number of chlorophyll variegated individuals were detected in F_1 and given the numbers C_1, $C_2 \ldots C_{12}$. Ten of them derive from thermal neutron irradiation of pollen of P generation with about 2000 rad for 48 h. Plants C_{11} and C_{12} derive from X-ray treatment of 1000 rad in 1 h of female flowers of P generation 24 h before pollination, the egg-cells being in G_2 (material kindly provided by D. de Nettancourt, ITAL Wageningen).

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The extent of the mutated tissue differed greatly among the 12 plants. In one, intensely chlorophyll-deficient tissues soon prevailed and the plant died before flowering; in eight others, the size and number of virescent sectors decreased with plant growth, and only a few offspring exhibited mericlinal variegation (see Dommergues 1962).

Variegation was permanent in the last three individuals, namely C_6, C_{11} and C_{12}, and the chlorophyll mutation was transmitted in various ways. These three plants were vegetatively propagated and phenotype and genotype were analyzed in detail through successive generations of selfings and back crosses. Allelism was also tested by crossing C_6, C_{11} and C_{12} with one another.

A number of flower buds in meiosis were screened by the Ramanna-Prakken technique (1967) to find chromosome abnormalities, if any. In addition, sections of embedded ripe pollen grains of 'Moneymaker' were examined by ultramicroscope to see whether the sperm cell contained visible proplastids, which might be responsible for plastid inheritance (work at TFDL by S. Henstra).

Results

The diallel cross between C_6, C_{11} and C_{12} yielded many seeds. As most of the progeny of all three combinations were normal green, in contrast to the selfed progeny, the mutations were at different loci.

I. Plant C_{11}

Phenotypic behaviour. In the early stages, Plant C_{11} was variegated with yellowish, pale green and normal sectors, irregular but clearly outlined. Above the tenth leaf the structure became stable, yellowish parts being limited to the blade margins (Fig. 1), and remained so throughout the clone derived from C_{11}, under normal conditions. With age the mutant area decreased in size, but young leaves again manifested the typical phenotype.

Low magnification of leaf sections revealed a periclinal structure: the palisade tissue, or its upper layer only in thicker parts, contained smaller and paler chloroplasts; the same was true for the spongy tissue; the internal parenchyma had a normal chlorophyll system. The superficial pattern is explained by the absence of this mass from the leaf margins.

The green part of the vegetative tissue could be forced to emerge into shoot by callus formation on decapitated individuals, and a clone was derived from it.

Fig. 1. Phenotype of Plant C_{11} when become stable

Fig. 2. Self progeny of Plant C_{11}

Genotypic behaviour. The first inflorescences were formed above the tenth leaf and no lateral shoot was isolated below: the genetic observations thus correspond to the typical periclinal pattern described above. Flowering was normal, and meiosis displayed no abnormality in chromosome number, structure or pairing, although minor changes in heterochromatin distribution may have been overlooked. Pollen and seed fertility were good.

The observations made on selfed and backcrosses over two generations are summarized in Table 1. The results show an unexpected phenomenon: although Plant C_{11} was actually hybrid, as substantiated by expression of the recombination of the marker genes, its gametes were all mutant, and the mutant gene behaved as a monofactorial recessive (Fig. 2). Its phenotype was virescent yellow without variegation. Plants developed rather slowly but were strong and fertile. Backcrosses were entirely green and segregated 3:1 after selfing.

In contrast, the selfings of those green plants obtained by decapitation of the original C_{11} segregated 3:1.

Stability of the mutated gene. Dry seeds derived from selfing Plant C_{11} were soaked in ethylmethylsulphonate (EMS) at 0,8% for 24 h. Whereas the untreated control always yielded homogeneous virescent yellow offspring, about 25% of the plantlets in the EMS treated batch exhibited noticeable green sectors on the first leaves (Fig. 3). Natural diplontic

Table 1. *Genetic behaviour of Plant C_{11}, after selfing, backcrossing and both*

	Generation	Number of plants	Segregation normal %	mutants %
Selfing				
C_{11}.S	F_2	973	0	100
C_{11}.S.S	F_3	2560	0	100
Backcrossing				
$C_{11} \times M$	F_2	548	100	0
$C_{11} \times L$	F_2	113	100	0
$M \times C_{11}$	F_2	486	100	0
$L \times C_{11}$	F_2	438	100	0
Selfing + backcrossing				
C_{11}.S $\times$ M	F_3	111	100	0
C_{11}.S $\times$ L	F_3	103	100	0
$M \times C_{11}$.S	F_3	89	100	0
$L \times C_{11}$.S	F_3	71	100	0
Backcrossing + selfing				
($C_{11} \times M$).S	F_3	346	80.4	19.7
($C_{11} \times L$).S	F_3	232	84.5	15.5
($M \times C_{11}$).S	F_3	565	71.7	28.3
($L \times C_{11}$).S	F_3	—	—	—
	Total	1143	76.7	23.3

S = selfing
M = *L. esculentum* 'Moneymaker'
L = *L. esculentum* line 121.

Fig. 3. De-repression effect of EMS upon C_{11} self progeny

Fig. 4. De-repression effect of gamma-rays in one offspring of an irradiated C_{11} self progeny

selection progressively eliminated these sectors during plant development in all but one individual. In that one, a completely green shoot was formed, where the normal chlorophyll pigmentation was accompanied by growth retardation; no fertile inflorescence has developed on that part of the plant, so far.

In another experiment, 72 plants from selfed C_{11} were simultaneously exposed to chronic gamma-rays from a ^{137}Cs source. Dose-rate ranged from 4.0 to 0.4 rad/h. No significant difference was observed between the plants in chlorophyll expression during growth. However, progeny from one selfed plant, which received about 1 rad/h, produced 3 seedlings out of 24, with mottled cotyledons followed by green spots on the leaves (Fig. 4). These plants are still mixochimeric.

Discussion

The histological examination of Plant C_{11} confirmed that the subepidermal L_{II} layer had mutated, whereas the cortical layer L_{III} had a normal phenotype (*Poinsettia* type, in Dermen 1960; see also Dulieu 1967). This L_{III} was uncovered by decapitation and was uniformly green; it produced both types of gametes. Conversely, the L_{II}, which alone makes up the sporogenic tissue in normal conditions, was homozygous for the induced mutation, and this point must now be explained.

The meiotic behaviour eliminates any interpretation based on chromosomal abnormality. To account for the facts at gene level, one may first consider a somatic recombination very early in the proembryo, at the mitosis responsible for the differentiation between the L_{II} and L_{III} initiators (Vallade 1970). Reciprocal recombination, or somatic crossing over, would most likely have produced a homozygous dominant L_{III}; this did not appear, whereas gene conversion could explain why it remains heterozygous.

Gene conversion, a well known phenomenon in yeast and fungi (Roman 1963), is usually interpreted as a miscopying of replicating DNA in a heterozygous cell, leading to non-reciprocal recombination, $AAaa \rightarrow Aa + aa$. This process has not been demonstrated in higher plants until very recently (Cornu and Dommergues 1971, Dulieu et al. 1971).

One may then postulate an influence of the mutant allele *a* upon its untreated partner, not necessarily bound to chromatid exchange, and without anticipating its exact nature ('somatic conversion', Winkler 1930). The point here is that the phenomenon no longer operated after a few mitotic divisions, since the L_{III} tissues, as well as the heterozygous backcrosses, remain green.

It is known (Evans 1967) that a primary lesion induced by irradiation of sperm nuclei in G_2 remains in a labile state until the gametes have fused. Moreover, Cornu (1970) demonstrated that in plant proembryos the initial effect of irradiation may not lead to a true mutation for a few mitotic divisions. If, then, conversion power is associated with such a labile state of the locus, say a^0, it follows that Aa^0 and aa^0 cell lineages may differentiate and make up the L_{III} and L_{II} tissues, respectively; soon afterwards the somatic tissues will reach the stable states *Aa* and *aa*.

This hypothesis cannot be directly tested in Plant C_{11}, since the process is complete. However, comparison with C_6, where conversion is permanent, and C_{12}, which presents both types of process, will suggest a comprehensive interpretation in terms of 'somatic conversion'.

The results of treatments with EMS and gamma-rays show that expression of the altered gene can be partially restored in the progeny of Plant C_{11}. The frequency of such events is too high to be ascribed to back-mutation. Although this effect has not yet proved to be transmissible, it seems to concern gene function and not gene structure. The hypothesis of de-repression put forward for the paramutated *R* allele of maize (Axtell and Brink 1967, Shih 1969) may also hold true for the present material.

II. Plant C_6

Phenotypic behaviour. In the early stages the variegation of Plant C_6 was similar to that of C_{11}, but, unlike C_{11}, it remained unchanged throughout clonal development. Contrasting and often small sectors gave the foliage a mosaic pattern with various pigmentation colours (Fig. 5).

Microscopic observations revealed complete mixochimerism in every leaf tissue. Single cells might differ from their neighbours (Fig. 6) but a given cell always contained only one kind of chloroplast.

Genetic behaviour. Like C_{11}, the Plant C_6 flowered normally, and was fairly fertile on selfing as well as on backcrossing.

Offspring were studied to F_4.

Table 2 shows that the segregations were more complex than in C_{11}, and that they continued to give

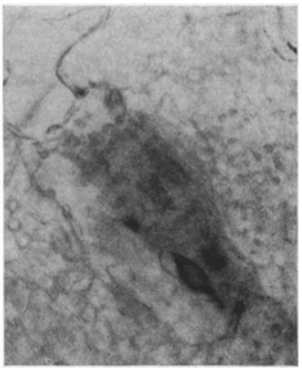

Fig. 5 (left). Phenotype of Plant C_6

Fig. 6 (right). Transversal section in the leaf blade of Plant C_6, showing extreme expression of mixochimerism

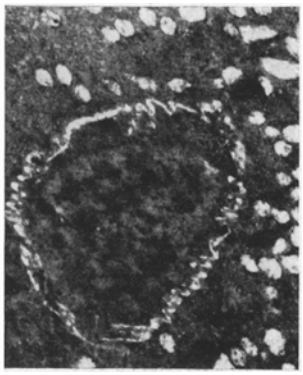

Fig. 7 (left). Self progeny of Plant C_6

Fig. 8 (right). Ultramicrograph of a pollen grain of *L. esculentum* 'Moneymaker', showing no visible proplastid in the germ cell (magnification 15,000, photograph S. Henstra, TFDL)

rise to variegated offspring (Fig. 7). The phenotypes were scored in four classes:

+: uniformly pigmented with normal chlorophyll.

V_1: normal green cotyledons; leaves variegated to different extents; plants later either gradually revert or the mutation becomes accentuated.

V_2: pale mottled cotyledons; leaves variegated, often mainly chlorotic; mutation accentuated over the course of time; plants scarcely reach flowering.

l: lethal; uniformly yellow cotyledons; the plants die before leaf emergence.

Backcrossing of V_1 plants yielded only + and V_1 offspring, in various proportions. When selfed, most V_1 plants and also some + plants displayed the four types of offspring. A few selfings of the first backcross segregated only as + and l seedlings in the proportion 3:1.

As in Hagemann's material (1965), the ratio in any generation departed from Mendelian expectations according to the severity and extent of chlorosis in the parent plant or part of plant.

Discussion

Electron-microscopy of tomato pollen revealed the absence of visible proplastids in the generative cell (Fig. 8). This species would then belong to the *Mirabilis jalapa* group and not to the *Pelargonium zonale* group (Lombardo and Gerola 1968), which is known to transmit plastid mutations through the male gametes also. In backcrosses, the mutation is recovered at least as frequently from the male side. It persists with a high frequency in successive backcrosses where variegated plants are used as pollen donor. It is thus probable that the genetic mechanism involved is nuclear. The normality of meiosis further suggests that it operates at gene level.

The segregation observed after selfing C_6 markedly differs from a Mendelian ratio and includes only 1.43% green plants. The untreated allele is transmitted unchanged only in a reduced proportion. Results now strongly suggest that it is converted at a definite frequency to a deficient form, through heterozygous association with the induced mutation, at any stage of somatic development of the plant C_6 and in its progeny. This meets the definition of paramutation (Brink 1958). The similarity with the *sulf* system in tomato is apparent.

One can tentatively search for the most probable parental genotype producing each segregation type displayed in Table 2.

Half the backcrosses are initially heterozygous: the V_1 plants represent paramutants. In the selfed progeny of C_6, the V_1 plants must have similar origin:

BB: phenotype +

Bb: phenotype + or V_1

Now C_6 produces three kinds of gametes:

b with the mutant allele: 50%

b′ paramutant
B with the allele that escaped paramutation } together 50%

The V_2 and l segregants, totalling 84.7%, probably represent the combinations where B is absent, hence $b' = 42\%$ and $B = 8\%$. The V_2 plants, at 26.3%, might correspond to the bb genotype, and b′, dominant over b, is lethal.

The Bb′ plants, theoretically 6.72%, cannot all be +. That a proportion of them are V_1 means that a paramutated allele can in turn be paramutagenic (Brown and Brink 1960) and that the phenomenon is recurrent.

If the conversion power differs between the two alleles involved in the process in a given generation and is perhaps influenced by the genetic background, one might expect to find exceptions to the general parent-progeny correlation and an increasing conversion power can probably be selected for; the conversion power (Hagemann's conversion activity, 1965) is the proportion of variegated plants in a population heterozygous for a given deficient allele.

These assumptions are completely confirmed by the segregation patterns in the offspring of backcrosses (Table 2): Types 2 to 8 represent various combinations of high, low or nil conversion activity

Table 2. *Genetic behaviour of Plant C_6, after selfing, backcrossing, and both. S, M and L as in Table 1*

	Generation	Parent genotype	Segreg. type*	Number of plants	Segregation: +%	V_1%	V_2%	1%
Selfing								
C_6.S	F_2	V_1		558	1.43	13.8	26.3	58.4
C_6.S.S	F_3	+	t_1	71	100	0	0	0
			t_2	59	75.3	0	0	24.7
		V_1	t_3	116	19.0	36.2	0	44.8
			t_4	291	0	30.6	18.9	50.5
Simple backcrossing								
$C_6 \times M$	F_2	V_1		509	91.7	8.3	0	0
$C_6 \times L$	F_2	V_1		232	91.8	8.2	0	0
$M \times C_6$	F_2	V_1		547	87.8	12.2	0	0
$L \times C_6$	F_2	V_1		177	87.0	13.0	0	0
Double backcrossing								
$M \times (M \times C_6)$	F_3	V_1**		363	68.5	31.5	0	0
Triple backcrossing								
$M \times [M \times (M \times C_6)]$	F_4	V_1**		449	35.4	64.6	0	0
Backcrossing + selfing								
$(C_6 \times M).S$, $(C_6 \times L).S$, $M \times C_6.S$, $L \times C_6.S$	F_3	+	t_1	13	100	0	0	0
			t_2	212	78.8	0	0	21.2
			t_3	641	74.7	3.6	2.2	19.5
			t_4	252	66.7	33.3	0	0
		V_1	t_5	1241	66.6	10.8	2.4	20.1
			t_6	195	0	35.4	49.2	15.4
			t_7	45	0	0	4.4	95.6
			t_8	203	72.4	0	0	27.6
$[M \times (M \times C_6)].S$	F_4			1304	same types except t_2 and t_8			

* Segregating populations scored per fruit.
** Selecting the more intensely variegated individuals.
The phenotypes +, V_1 and V_2 are explained in the text

in a heterozygous genotype and in its offspring, whereas Type 1 corresponds to a homozygous dominant. In repeated backcrosses with 'Moneymaker', however, the conversion activity was regularly increased by selecting the more variegated individuals as parent at every generation. The fact that the proportion of V_1 individuals soon exceeded 50% is new evidence of secondary paramutation.

III. Plant C_{12}

The above results, considered alone, might imply that Plant C_{11} and Plant C_6 represent two different and unrelated phenomena. The data on C_{12} represent a third, intermediate position between the other two, and are important for interpretation of the genetic mechanism.

Phenotypically C_{12} resembled C_{11}, though with a paler foliage especially in the mutated part. The leaf tissues, variegated in the early stages, were also soon redistributed in such a way that L_{II} was pale yellow and L_{III} green.

After selfing, one could observe the following segregation among 537 offspring:

+: 0.2% (1 plant); V_1: 0; V_2: 1.7%; 1: 98.1%.

The + plant unfortunately did not produce seeds. One of the V_2 plants (which was probably a misinterpreted V_1) gave, upon selfing, the following segregation:

+: 41.3%; V_1: 30.4%; V_2: 5.4%; 1: 22.8%.

After backcrossing the original C_{12} plant, 1008 progeny plants, representing the four possible combinations, were all normal green and, upon selfing, segregated 3:1.

The interpretation that emerges from these observations is as follows. A phenomenon similar to that occurring in C_{11} resulted in the formation of a homozygous mutant L_{II} layer. This mutation was recessive and did not keep its conversion power after a few mitotic divisions. Unlike C_{11} it was lethal in homozygous offspring, although L_{II} tissues themselves were viable, thanks to contact with adjacent functional tissues. A very small proportion of the L_{II} cells, however, remained heterozygous, and also retained the conversion power attached to this. The gametes derived from this cell lineage gave rise to the normal green individual observed after selfing, and also to a few variegated heterozygous offspring, one of which displayed in the next generation a segregation typical of paramutation.

It is remarkable that the genes involved in all three plants, C_6, C_{11} and C_{12}, were different. This might indicate that somatic conversion is a general property of these conditions of irradiation and that the present material allows its detection.

General Discussion and Conclusion

The experimental results suggest that the three plants, C_6, C_{11} and C_{12} (perhaps also the other variegated plants found), represent one genetic phenomenon, differing slightly in its manner of expression.

Interference by an extranuclear factor cannot be absolutely ruled out, for the absence of visible proplastids in the pollen generative cell does not necessarily mean absence of a cytoplasmic factor. Still, in the light of the genetic behaviour of this material, a mechanism at gene level seems reasonable. An interpretation compatible with all the observed facts is that haplophasic irradiation placed three different genes necessary for chlorophyll formation in a labile state, n^*, maintained through successive mitotic cycles after fertilization. In this state, n^* had the power to convert the wild allele N into a deficient form. Conversion was accompanied or soon followed, always in C_{11} and most of the time in C_{12}, by stabilization of both alleles in a recessive inert state, n and n', genetically similar. In other plants, always in C_6 but seldom in C_{12}, the n^* state was permanent and transmissible (paramutation). Conversion occurs with a certain frequency, determined by ontogenic as well as genetic factors, producing variegation of the leaves in successive generations. Secondarily, such a converted allele may also acquire conversion power so that the gametes issuing from a Nn^* individual are of three kinds: N, n^* and n'^*. This is closely related to the behaviour of the *sulf* gene (Hagemann 1958, 1969).

There were a few variegated offspring in the first backcross, segregating 3:1 upon selfing, thus indicating a loss of the conversion power. Whether this loss is irreversible, and whether the inert allele can be selected for in C_6 offspring, have not been determined. In contrast, it was possible to obtain by selection in repeated backcrosses more than 60% variegated offspring. Backcrosses are now being repeated to reach the maximum conversion power possible, and to restore the 'Moneymaker' genotype.

Attempts to keep lethal or sublethal recessive homozygous genotypes alive after grafting, as in Hagemann 1958, failed except on rare occasions.

Whereas the behaviour of our C_6 material closely resembles that of Hagemann's *sulf* locus, the behaviour of C_{11} and C_{12} reveals other aspects of the somatic conversion mechanism. In C_{11}, the mutation, though detrimental for chlorophyll expression, is perfectly viable, and thus expresses a less drastic alteration of the gene concerned. More important is the fact that conversion now concerns the entire L_{II} layer and only this layer, and that here, as well as in most C_{12} cells, both mutant and converted alleles become stable very early, and subsequently behave as normal recessives. It would be of interest to know whether, after one or more generations, a loss of conversion power in the C_6 progeny can also sometimes be irreversible. Hagemann (1969) found *sulf* alleles with a conversion power ranging from 0 to 100% for particular years, but none was permanently inactive.

Anyhow, the situation in C_{11} and C_{12} does manifest new features of somatic conversion. These features will have to be taken into account in future attempts to explain the genetic mechanism.

Not much can be said yet about the mechanism at cell level. It is already clear, however, that somatic recombination, either reciprocal or not, is not involved in the formation of the new alleles. Another point that has to be stressed is that the wild allele, despite such changes in the present material, is stable in normal conditions, as is *sulf*$^+$ (Hagemann 1965). This contrasts with the inherent metastability of the *R* allele of maize (Brink et al. 1968). Undoubtedly an interallelic relationship, whatever its features, is the origin of the observed changes in genetic expression.

As to whether this effect concerns the structure of the genes or control over their action, the experiments with EMS and gamma-rays suggest the latter. The first results are similar to those observed by Axtell and Brink (1967) and Shih (1969) at the *R* locus of maize and which they interpreted as a de-repression. It remains to be seen whether this will hold true also for the C_6 mutation. We must also compare the efficiency of other treatments in order to determine the correlation, if any, of this effect with conventional mutagenic efficiency.

Fincham (1970), discussing the behaviour of some highly mutable systems in plants, thinks that 'the frequent mutation does not represent the creation of new genetic information but rather the unmasking of structural genes which were already present'. He also notes in the same paper that the phenomenon of paramutation is possibly related to the other examples of gene instability he deals with.

It is to be hoped that by accumulating data on various systems the relationship between paramutation and other forms of gene instability will soon be clarified.

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