Virus as a Mutagenic Agent in Maize

Abstract. *Evidence indicating that barley-stripe-mosaic virus has a mutagenic effect on maize is presented. The frequency of deficiencies of marker genes in F_1 seeds was significantly greater from pollinations involving virus-infected males than from healthy controls. A small percentage of F_2 ears, involving virus-infected P_1 males, exhibited marked ratio distortions for marker genes. Such distortions persisted in subsequent back-cross generations.*

Some cereal viruses induce host symptoms which are phenocopies of some of the known chlorophyll mutants. Although progeny tests of such virus-infected plants have failed to indicate that such induced symptoms are inherited, since virus infection involves the introduction of foreign genetic material it was considered possible that such alien nucleic acid might induce some recognizable genetic effects.

The idea that plant viruses may induce genetic changes in their hosts has been suggested in studies dealing with the pathology of virus-infected tobacco and related species (*1*) and virus-infected broadbean (*2*). Progenies from the last received limited study (*2*), but the evidence for the inheritance of virus-induced genetic changes can be regarded as only suggestive. More recently Nyland (*3*) suggested the possibility of virus-induced genetic abnormalities in tree fruits.

Our studies were begun in 1960. A strain of barley-stripe-mosaic virus (Argentina mild strain, seed-borne in barley) was used as a possible mutagen. It has not been determined whether this is an RNA virus. It is presumed to be, however, since no plant virus studied thus far has been found to be of the DNA type. Healthy and virus-infected maize plants are illustrated in Fig. 1.

Maize was used as the test organism. Two procedures were followed in the search for virus-induced genetic changes. The first involved comparison of frequencies of deficiencies or sectors for marker loci and the second, the frequency of mutations in F_2 and their subsequent breeding behavior. Plants of a dominant marker stock (A_1 *Su Pr C* R^r) were inoculated in the seedling stage. Pollen from infected plants was applied to multiply marked recessive stocks (a_1 *su pr C* R^s). The F_1 seeds were then scored for entire and sectorial or fractional losses of the dominant marker genes. Data from the treated and control series are presented in Table 1.

Table 1. Frequencies of entire and fractional deficiencies in F_1 progenies involving healthy and virus-infected male parental stocks of maize. A_1 is one of a series of genes affecting aleurone color, A_1 being colored and a_1 colorless; *Su*, starchy and *su*, sugary endosperm; *Pr*, purple and *pr*, red aleurone color.

Treatment	Total No. of seeds	Endosperm deficiencies per 1000 seeds					
		a_1		*Su*		*Pr*	
		Entire	Fractional	Entire	Fractional	Entire	Fractional
Virus-infected	1,300	3.1	3.8	2.3	0.8	0.0	3.1
Control	12,519	0.2	1.4	0.3	0.4	0.4	0.1

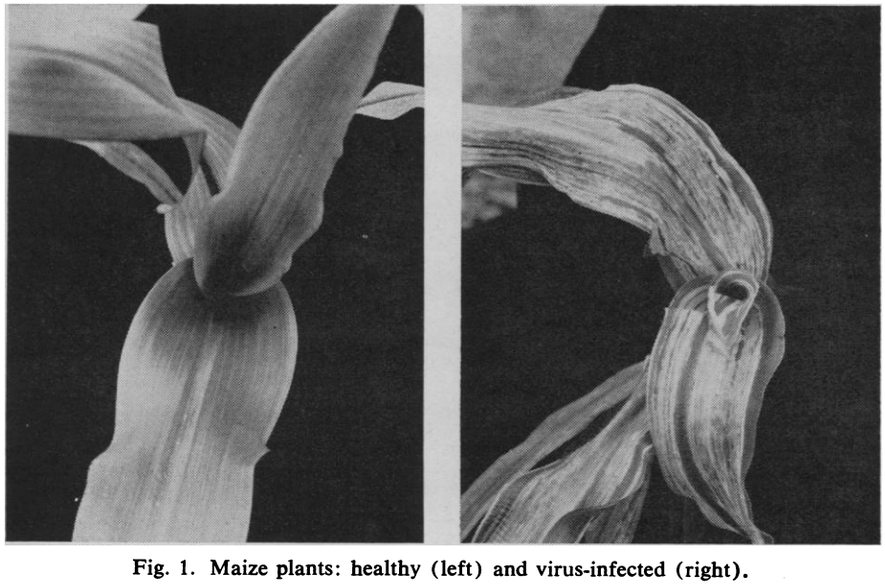

Fig. 1. Maize plants: healthy (left) and virus-infected (right).

Fractionals were scored according to the proportion of the kernel surface affected as ½, ¼, and so forth. The majority of the fractionals fell in the ½ class, suggesting deficiencies occurring in the cell progeny of one of the two cells produced by the first division of the triploid endosperm nucleus. On this assumption (entire = 1, fractional = ½) the frequencies presented in Table 1 can be summed over all loci, providing a simple and direct measure of the treatment effect. With this procedure the frequency of effects is 1 : 108 in the treated and 1 : 556 in the control series. The observed difference between treatment and control is significant at the 1-percent level. Corresponding ratios for endosperm deficiencies involving ultraviolet treatment and controls (*4*) were 1 : 42 and 1 : 740, respectively. Thus the estimated rate from the virus-infected males is slightly less than half of that observed for unfiltered ultraviolet radiation.

F_2 progenies were produced from both treatment and control pollinations. The ears were examined individually and, where marked deviations from expectancy for the marker genes were observed, the ear was shelled and classified. The ears were also examined for any possible mutations affecting the endosperm. In addition, a 25- to 50-kernel sample was planted in a sand bench and the seedlings were examined for mutations.

In the control series three presumed mutations were observed in the approximately 1000 F_2 ears classified. These involved two white and one virescent seedling types. No ratio distortions were observed for the marker genes.

The situation in the treated series was quite different for the approximately 1000 F_2 ears classified. Two main types of effects were noted. First, mutations were observed for several traits; vivipary, aleurone color, and white and virescent seedlings. F_2 frequencies suggested that each of these was monogenic, and limited F_3 progeny tests supported this interpretation.

The second effect noted in the treated series was a marked departure from expectation for individual marker genes in approximately 1 percent of the F_2 ears studied. These distorted ratios were of a type which might be expected if gametophyte factor mutation or a chromosomal deficiency were involved. Progeny tests (Aa × aa and reciprocal) should distinguish these two alternatives. If a gametophyte factor were involved the distortion should appear in crosses of the type aa ♀ × Aa ♂ and be absent in the reciprocal. This expectation was not realized. A chromosomal deficiency should be revealed in the Aa × aa cross by a characteristically reduced seed set. A reduced seed set was not observed for any of the progenies tested. The departures from expected equality were similar for both types of back-crosses. In one case, involving the starchy and sugary alleles, the percentages of sugary seeds ranged from 24 to 43 instead of the expected 50 percent. Each of these deviations from expectancy was significant. No satisfactory explanation has been devised for such results but there can be little doubt that some disturbing influence is operative.

Any genetic effects from viral infection are thought to be limited to effects produced prior to or during gamete formation in the treated plants. Recognizable virus symptoms have never been detected in F_1 progeny involving treated gametes. Also we have not succeeded in demonstrating seed transmission of this virus in maize. Inoculation-transfer tests have failed to indicate the presence of virus in normal or chlorophyll-deficient F_2 seedlings.

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Regulation of Enzyme Activity by Specific Reversal of Feedback Inhibition

Abstract. *Studies with a photosynthetic bacterium have revealed a novel type of regulatory control of enzymes participating in amino acid biosyntheses. Feedback inhibition of enzyme activity can be specifically reversed by ultimate end products of a branched pathway.*

Current understanding of the metabolic regulation of biosynthetic pathways is based on two general mechanisms: the control of enzyme synthesis by repression and induction, and the control of enzyme activity by end-product (feedback) inhibition (*1*). In the latter mechanism, the end product inhibits the activity of the enzyme, which catalyzes the first reaction of a sequence leading specifically to the product in question, thus avoiding oversynthesis.

Inhibition by the end product can in some instances be reversed or counteracted by substrate (*2*), by a heavy-metal cation (*3*), or by relatively high concentrations of certain amino acids which are not direct participants in the biosynthetic sequence in question (*4*). We have uncovered a novel type of reversal, in which the ultimate amino acid end products of a branched biosynthetic pathway reverse feedback inhibition caused by an intermediate or an amino acid derived from an intermediate.

As shown in Fig. 1, the amino acid homoserine is the branch point for the synthesis of threonine and methionine. Threonine has a dual role in that it is an "end product" utilized in protein synthesis and is also an intermediate in the synthesis of isoleucine.

After considering the relationships indicated, it seemed possible that homoserine dehydrogenase, which catalyzes the interconversion of L-aspartic β-semialdehyde and homoserine, might be subject to some sort of regulatory control by the several end products produced from homoserine. Cell-free extracts of the photosynthetic bacterium *Rhodospirillum rubrum*, grown in a synthetic medium with glutamate as nitrogen source (*5*), exhibit high homoserine dehydrogenase activity as measured by reduction of triphosphopyridine nucleotide (NADP) with homoserine as the substrate. As with the enzyme from certain other bacteria (*6, 7*), activity is strongly inhibited by addition of L-threonine; D-threonine does not inhibit. The data of Fig. 2 show that inhibition of the *Rhodospirillum* enzyme by L-threonine is strictly competitive with homoserine; in contrast, noncompetitive kinetics have been reported for the homoserine dehydrogenase of *Escherichia coli* (*7*).

From the standpoint of regulatory control, it is of particular significance that the inhibition of homoserine dehydrogenase activity by L-threonine can be completely overcome by either L-isoleucine or L-methionine, that is, by the ultimate end products of the branched pathway. These effects are illustrated by the data of Fig. 3.

With increasing concentrations of isoleucine or methionine, the inhibition due to threonine is gradually reversed. With isoleucine, a complete reversal is attained when the ratio of isoleucine to threonine is approximately 2; methionine appears to be slightly less effective. A homolog of methionine, L-ethionine, also reverses the threonine inhibition. Inhibition of homoserine dehydrogenase activity by L-threonine and reversal by L-isoleucine or L-methionine have also been observed with preparations purified a hundred fold. Furthermore, essentially similar results were obtained when enzyme activity was assayed by measuring reduction of L-aspartic β-semialdehyde with $NADPH_2$. With regard to specificity,

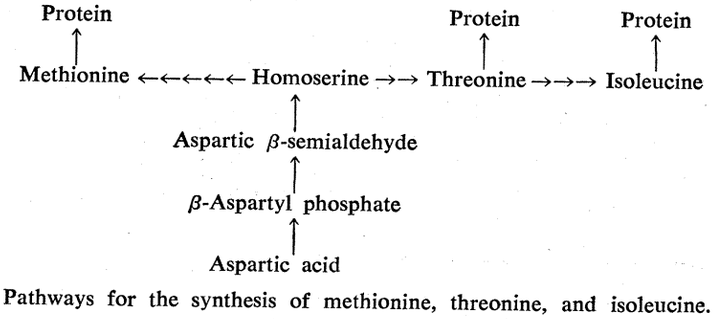

Fig. 1. Pathways for the synthesis of methionine, threonine, and isoleucine.