2. Induction of heritable change at the R locus by abnormal chromosome K10.

Abnormal chromosome K10 has a novel effect on the R paramutation system. Following introduction into a K10 chromosome a paramutable R factor becomes relatively insensitive to stippled action in R K10/Rst heterozygotes. It has now been observed that the insensitivity regularly persists, in one or another degree, after return of R by crossing over to a structurally normal chromosome 10. Recent data also show that the change in sensitivity does not occur in plants in which paramutable R and the K10 knob are carried by homologous chromosomes, that is, in repulsion. The large, terminal knob characterizing abnormal chromosome 10 is distal in 10L to striate-2 which, in turn, is distal to R. Since striate-2 and R show about 35 per cent recombination, the R locus and the K10 knob are far apart. The evidence suggests that the observed change in R sensitivity is the result of a stimulus originating in the distinctive, terminal K10 segment that is propagated along the same chromosome to the R locus, at which the heritable alteration in sensitivity to Rst action is then effected.

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Addendum:

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1. Investigations on WF9 mutants.

In 1960-61 mutants were induced in the WF9 line by X-rays of 7000 R and 15000 R. On the basis of morphological and biochemical investigations, 60 mutants were identified.

In 1965, the protein and oil content was determined in seeds of 37 mutants. The results of these investigations are shown on Table 1.

In 1967 a study of the resistance of 60 mutants was made at our request by the Agricultural Research Institute of the Hungarian Academy of Sciences, where Dr. I. Manninger had the goodness to include our mutants in an experiment together with his own breeding material.

During one series utilizing comparative experiments of standard methodology, in two experiment places (per 10 plants) he studied resistance to the head and the common smut with the provocation method, and resistance to Fusarium in a natural infection.

In the starting material the frequency of infection was 5.26 per cent for head smut, 2.17 per cent for common smut, and 7.3 per cent for Fusarium. Among the 7 kinds of WF9 breeding material (including 3 kinds of male sterile) selected by the Institute or received from abroad, two kinds appeared to be fully resistant to all 3 pathogens.

Table 1
The results of investigations of protein and oil content in 37 WF9 mutants (M_5 generation, 1965).

Stock	Protein %	Oil %
Control	9.8 ± 1.6	4.47 ± 1.0
561 1	13.01	6.89
517 1	11.8	3.83
518 1	12.1	3.01
520 1	11.8	4.52
522 1	13.3	3.59
523 1	12.7	6.52
524 2	11.5	5.91
527 1	13.8	4.78
528 3	12.7	4.96
530 1	11.5	4.71
530 2	11.5	3.62
530 3	11.8	3.92
531 1	12.7	4.26
532 2	12.9	4.81
533 2	12.1	4.32
534 1	10.7	4.68
537 1	11.8	5.06
538 1	9.4	5.09
538 3	11.6	4.49
539 1	13.3	4.51
542 1	9.8	5.41
542 2	9.0	5.19
547 1	10.7	4.27
547 2	13.3	4.51
548 1	14.4	4.26
549 1	11.6	3.68
549 3	10.0	4.41
553 1	8.9	2.48
553 2	12.8	3.74
555 1	14.2	4.03
556 3	11.6	4.45
558 1	10.0	4.43
559 1	9.0	4.20
559 2	8.0	4.82
135 2	9.4	4.26
136 1	10.6	4.00
137 1	9.8	4.47

Among the mutants, 43 out of 60 showed total resistance to head smut. Fourteen of the mutants, however, had a frequency of infection surpassing the standard value significantly, from 10 to 44 per cent.

Thirty-six lines were fully resistant to common smut in the provocation experiment. Of these, 28 lines were totally resistant in the provocation experiment for head smut; therefore, in 28 mutants the two kinds of resistance were to be found together. Some mutants, however, in the control experiment showed a much higher susceptibility for common smut as well (10-33%). As for Fusarium, in two experiment places 14 mutants were fully resistant, and 7 mutants were fully resistant to the two smut and the Fusarium damages together.

The investigations will be repeated in 1968. The genetical analysis of resistance in the stably resistant form will be begun after this control only.

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1. Lysine microbiological assay.

A procedure has been developed using a microbiological assay to rapidly and accurately determine the lysine content of corn grain samples. Samples are prepared for analysis by hydrolyzing a measured scoopful (approximately 200 mg) of ground corn in 10 ml of 6 N HCl at 110°C for 24 hours. Hydrolysis is carried out in a 10 ml screw cap vial. The caps are fitted with Teflon liners and care is taken to insure that the caps fit tightly. After hydrolysis the sample is filtered into a 50 ml beaker and evaporated on a hot plate to dryness. The samples are stored dry in a freezer until ready for assay. Just prior to assay the samples are resuspended in 25 ml of 0.05 M phosphate buffer pH 6.8.

The assay itself is a modified version of the one described by Difco Laboratories. The organism used is Leuconostoc mesenteroides and all procedures are identical except that a 3 ml assay volume is used instead of 10 ml. This results in a considerable saving of media and the assay is read directly in the culture tubes. Growth is determined by increase in turbidity at 660 mu.

Nitrogen is determined on an aliquot of the hydrolyzed sample by Nessler's procedure. Nitrogen determination on an aliquot of the same sample as is used for the lysine assay permits direct expression of lysine in terms of the nitrogen content of the sample and also does not require that gravimetric procedures be used in sample preparation, i.e., the sample does not have to be weighed and the filter paper need not be washed quite so thoroughly .