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GENETIC EFFECTS OF ULTRA-VIOLET RADIATION IN MAIZE. I. UNFILTERED RADIATION

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The genetic effects of ultra-violet light are of interest chiefly because of the possibility of differences in the effects of different wave-lengths. Comparison of x-rays of different wave-length range, reported by various investigators, has shown no qualitative differences in genetic effect. If the diverse genetic effects induced by radiation are dependent on different initial alterations in the irradiated material, it is more probable that distinctive wave-length relations of specific genetic alterations might be found in the ultra-violet than in the x-ray spectrum. Since more is known of the chemical effects of ultra-violet radiation than of x-ray, and since monochromatic radiations may be compared, it is possible that the investigation of variations in genetic effect between wave-lengths of the ultra-violet spectrum may ultimately provide a sounder basis for speculation as to the physical nature of induced mutation.

It is known from the studies of Altenburg,[1] Noethling and Stubbe[2] and others, that ultra-violet radiation induces mutation. The bactericidal effects of monochromatic ultra-violet radiations, as determined by numerous investigators (see Duggar[3]), show characteristic variations in the effectiveness of different wave-lengths, which, according to Gates,[4] parallel the absorption curves for certain derivatives of the nucleo-proteins. These substances, as well as various other proteins which have been studied,

show little absorption above 3100 Å. If genetic alterations induced by ultra-violet radiation are dependent upon absorption by proteins, therefore, it is probable that a limiting wave-length for genetic effectiveness may be found in the ultra-violet.

The chief obstacle to a survey of genetic effectiveness in this region of the spectrum is the difficulty of reaching the germ plasm with ultra-violet light. Altenburg's experiments with irradiation of mature flies showed that little, if any, of the radiation reaches the germ cells. The irradiation of the polar cap cells of the Drosophila egg, as developed by Geigy[5] and by Altenburg, makes possible the effective treatment of chromosomes of the germ line, but the rather involved genetic technique required probably precludes the use of this method in extensive comparisons of effects of specific wave-lengths. Penetration difficulties are less serious in the treatment of pollen, but there are few plant species in which genetic conditions are sufficiently favorable to make possible the extensive production and efficient classification of genetic alterations on the scale required.

Maize has unusual advantages for this sort of investigation, particularly in the numerous endosperm characters which may be used for the immediate indentification of deficiencies in the seeds produced by the use of treated pollen. Comparisons of the effects of radiations of different wave-length on the occurrence of endosperm deficiencies may be made quickly and almost unlimited numbers of individuals may be examined for the determination of critical regions of the spectrum. Detailed analysis of effects of radiation on the frequency of other genetic alterations may then be confined to the critical regions.

The genetic technique for the identification of germinal alterations in maize other than deficiencies is also fairly simple. The technique used is similar to that followed in experiments with x-ray treatment of pollen.[6] Mature pollen of a multiple dominant stock is treated and applied to silks of a multiple recessive. The occurrence of induced deficiencies at the marked loci is indicated in the F_1 seeds and plants by the appearance of the recessive characters. The occurrence of additional deficiencies and of translocations is shown by the segregation of defective pollen in the F_1 plants. Deficiencies and translocations are distinguished and genetically located by simple additional tests. Induced mutations are identified in the F_2 seeds and seedlings from the self-fertilized F_1 plants.

The experiments here reported were designed for the preliminary determination of the types of genetic effect produced in maize by ultra-violet treatment of pollen, and the identification of regions of varying effectiveness in the ultra-violet spectrum. It is planned to continue the study with monochromatic radiations. The results of the preliminary experiments indicating the genetic effects of different wave-lengths are summarized in this and the two accompanying papers. Genetic evidence on the induced

alterations will be reported in detail in a research bulletin of the Missouri Agricultural Experiment Station.

Results with Unfiltered Radiation.—The radiation here referred to as "unfiltered" ultra-violet is the radiation from a commercial quartz mercury-vapor arc (Cooper-Hewitt type *EAC*) operated at 4.8 amperes and 110 volts a. c. The treating distance was 19 cm. The pollen was irradiated in a single layer in a Petri dish, which was covered by a sheet of thin Cellophane. The filtering effect of the Cellophane is not sharply selective, all ultra-violet wave-lengths emitted by the lamp being transmitted, though with somewhat greater loss of intensity in the shorter than in the longer wave-lengths. The heating effect on the pollen was reduced (but not eliminated) by an electric fan ventilating the space between the arc and the Petri dish. Under these conditions treatments of 2 to 8 minutes could be made, but few seeds were produced by pollen irradiated for 16 minutes.

The effects of unfiltered radiation were determined from the results of a series of treatments made in 1934. The treated pollen was applied to ears of two stocks recessive for several genes affecting endosperm characters: (1) *a a wx wx su su pr pr y y* (here designated "*a*-recessive"), and (2) *c c wx wx su su pr pr y y* (designated "*c*-recessive"). Since the pollen stock used in this series of trials was *A A C C Wx Wx Su Su Pr pr Y y*, deficiencies affecting the entire endosperm could be detected only for the genes *A*, *C*, *Wx* and *Su*, but fractionals for *Pr* and *Y* could be detected in half of the seeds produced.

Endosperm Deficiencies.—The ears resulting from these pollinations showed a distinct increase in the frequency of deficiencies marked by the endosperm characters. In addition to deficiencies affecting the entire endosperm, many deficiencies affecting only part of the endosperm ("fractionals") were observed. Fractionals were classified, according to the approximate fraction of the surface showing the recessive character, as "$^{15}/_{16}$", "$^{7}/_{8}$", "$^{3}/_{4}$", "$^{1}/_{2}$", "$^{1}/_{4}$", "$^{1}/_{8}$", "$^{1}/_{16}$" and "small". Comparison with the control in this experiment, and with standard control values previously determined in large populations of the same stocks, showed no significant increase in the frequency of the smaller fractionals ("$^{1}/_{16}$" and "small"). There was a pronounced increase in the frequency of fractionals of the "$^{1}/_{2}$" class, and a smaller, though clearly significant, increase in the frequency of fractionals of other classes from "$^{7}/_{8}$" to "$^{1}/_{8}$", those larger than "$^{1}/_{2}$" being increased in frequency to about the same extent as those smaller. It seems probable, therefore, that the fractionals induced by ultra-violet treatment of pollen are in general the result of deficiency affecting the cell progeny of one of the two cells produced by the first division of the endosperm-fusion-nucleus.

The "*c*-recessive" stock included the markers *c* and *wx*, which are linked with about 26% crossing-over. Losses of *C* and *Wx* were usually associated,

indicating that losses were due in most cases, if not in all, to deficiency rather than to recessive mutation. With the other genes used it is assumed that the loss of the dominant marker was due similarly to deficiency, although there is no way of excluding the possibility that some of these losses were due to induced mutation.

The frequency of endosperm deficiencies involving *A*, *Su*, *Wx* and *Pr* is shown in table 1. Data on fractional deficiencies involving *Wx* and *Y* are omitted from the table, since, in seeds with colored aleurone, it is impossible to insure the detection of all fractionals for these characters.

Ultra-violet treatment of the pollen, like x-ray treatment, increases the frequency of endosperm deficiency. The number of deficiencies affecting the entire endosperm, with the ultra-violet doses used in this experiment, is considerably lower than the number resulting from heavy doses of x-rays. The effect of the ultra-violet treatment on the frequency of endosperm

TABLE 1

EFFECT OF UNFILTERED ULTRA-VIOLET RADIATION ON FREQUENCY OF ENDOSPERM DEFICIENCIES

	"*a*-RECESSIVE" STOCK									"*c*-RECESSIVE" STOCK								
	NO. SEEDS EXAM-	DEFICIENCIES OBSERVED								NO. SEEDS EXAM-	DEFICIENCIES OBSERVED							
		ENTIRE				FRACTIONAL					ENTIRE				FRACTIONAL			
TREAT-MEMT	INED	*A*	*Su*	*Wx*	TOTAL	*A*	*Su*	*Pr*	TOTAL	INED	*C*	*Su*	*Wx*	TOTAL[1]	*C*	*Su*	*Pr*	TOTAL
2 min.	252	0	0	0	0	3	0	2	5	265	0	1	0	1	2	2	0	3
4 min.	704	4	2	0	6	19	11	12	42	881	0	5	0	5	5	4	1	10
8 min.	99	4	1	0	5	4	1	0	5	371	3	4	3	7	1	4	2	7
16 min.										90	1	0	1	1	3	0	0	3
Total	1055	8	3	0	11	26	12	14	52	1607	4	10	4	13	11	10	3	23
Control	1893	0	0	0	0	1	1	0	2	2543	0	2	1	3	0	3	1	4

[1] *Wx* omitted from total since loss of *C* and *Wx* is usually coincidental.

chimeras (fractionals) is in marked contrast to the effect of x-ray treatment of pollen at the same stage. As previously reported,[7,8] x-ray treatment of ears approximately at the time of fertilization greatly increases the frequency of endosperm chimeras, but x-ray treatment of pollen, though it causes a very large increase in the frequency of deficiencies affecting the entire endosperm, has little or no effect on the frequency of endosperm chimeras.

Deficiencies affecting the embryo instead of the endosperm may be identified, similarly, by the examination of the F_1 plants for plant characters dominant in the pollen parent and recessive in the seed parent. In this series of treatments, the only marker affecting a plant character was *A* in the crosses on the "*a*-recessive" stock, and no losses of *A* were found among the F_1 plants grown. In later trials with better-marked stocks and larger populations, deficiencies affecting plant characters have been found.

These furnish material for the cytological study of deficiency induced by ultra-violet treatment.

Gametophytic Lethals.—Independently of the use of marker genes, deficiencies induced by the treatment may be identified by their effect on pollen development in the F_1 plants. Deficiency at any locus, if it involves genes essential to the normal development of the haploid gametophyte, should cause the abortion or abnormal development of the deficient microspores and megaspores. Although it has been shown that some deficiencies in maize may permit apparently normal functioning of the female gametophyte,[9] no deficiency is known which permits normal development of the pollen. In plants heterozygous for a deficiency half of the pollen is defective, the degree of defectiveness being characteristic of the deficiency concerned. Induced deficiencies, therefore, may be identified *en masse* by examination of the pollen of the F_1 plants.

Segregations for defective pollen may result from genetic alterations other than deficiency. In addition to possible gene mutations affecting pollen development (which cannot be distinguished from short deficiencies), there are certain chromosomal derangements which result in partial pollen abortion. Heterozygous translocations usually have approximately 50 % defective pollen, and heterozygous inversions have defective pollen in proportions determined by the frequency of crossing-over in the inverted segment. Translocations occur frequently as a result of x-ray treatment of pollen, and inversions, though relatively infrequent, are not rare. By analogy with x-ray treatment, it would be expected that the chromosomal derangements identified by the occurrence of defective pollen in the F_1 plants would be made up chiefly of deficiencies and translocations. The frequency of deficiency determined in this way is a minimal value, since only the plants reaching flowering are examined, and deficient plants are more likely to be lost before flowering than non-deficient plants. Plants heterozygous for translocations are normal in development, and their frequency should not be affected by differential mortality.

Deficiencies may be distinguished from translocations by testing transmission of the genetic complex resulting in defective pollen. Translocations are regularly transmitted through both male and female germ cells, while deficiencies producing defective pollen are not transmitted at all through male germ cells, and are transmitted through female germ cells only in exceptional cases (haplo-viable deficiencies). In these trials, since it was desirable to identify and maintain the haplo-viable deficiencies, transmission through female germ cells was determined, and in cases showing transmission a cytological examination of heterozygous plants was made at diakinesis to distinguish between deficiency and translocation.

Approximately three-fourths of the seed produced by the use of treated pollen and about one-fifth of the seed from the control ears were planted,

and in all plants which reached the flowering stage, pollen was examined for defective pollen segregations. Thirty-eight segregating plants were found among the 1065 plants from treated pollen examined, and 3 segregating plants among 632 control plants examined. Each segregating plant was back-crossed with the multiple recessive parent and in most cases also was self-fertilized. In several cases the ratios of the marker genes used indicated a deficiency affecting a specific chromosome. In one instance a close association of sugary endosperm and aleurone color in the back-cross progeny indicated a translocation involving chromosome 4 and chromosome 9 or 10.

Preliminary tests of transmission of the defective-pollen complex through female germ cells have been made in 29 of the 38 cases. Transmission was found in 15 cases. In each of these, cytological examination of segregating plants at diakinesis showed 10 bivalents. The absence of rings or chains involving two or more pairs of chromosomes indicates that translocation was not responsible for the defective pollen in any of these cases. Thus, except for the genetic indication of translocation in one F_1 plant (not yet confirmed cytologically), there was no indication of any effect of ultra-violet treatment of pollen on the frequency of translocation. The frequency of haplo-viable deficiencies indicated by the proportion of pollen defects transmitted is surprisingly high.

Mutations.—All of the F_1 plants were self-fertilized and the resulting F_2 ears examined for the occurrence of mutations affecting seed characters. A sample of 25 seeds was planted from each ear and the resulting seedling progenies examined for mutations affecting seedling characters. Among 830 F_2 ears from treated pollen, 31 recessive mutations were found, including 19 affecting seed characters and 12 affecting seedling characters. Among the 557 F_2 ears of the control, 6 recessive mutations were found, 3 affecting the seed and 3 the seedling. The mutations affecting seed characters included a variety of defective types, such as "small," "scarred," "rudimentary," "miniature," "aborted," "germless" and "gnarled," and one distinctive new aleurone variant designated "aleurone-spot." The seedling mutations included "white," "virescent," "yellow-green," "speckled," "glossy," "rolled," "dwarf" and "corrugated." Many of these mutants resemble recessives previously known, but no tests of genetic identity have yet been made.

A striking feature of the mutation results was the occurrence of three cases of association of two or more mutants in a single F_2 progeny, indicating the occurrence of two or more mutations in the same treated sperm. One F_2 ear showed segregation of both miniature seeds and glossy seedlings, another germless seeds and yellow-green seedlings, a third rudimentary seeds and both yellow-green and virescent-white seedlings. In each case the mutants were unlinked, or at any rate not linked closely enough to show

significant association in populations of 100 to 300 individuals. The endosperm mutant "aleurone-spot" and the seedling mutant "corrugated" occurred in the same F_2 progeny, but showed complete linkage in the population grown, and therefore may represent two effects of the same mutant gene. In addition, three of the mutants (small seed, miniature seed, white-tip seedling) were found in progenies of F_1 plants segregating for defective pollen.

The number of cases of apparently independent genetic variations occurring in the same treated gamete is much higher than that expected from chance coincidence. But not enough is known as yet of the absorption of ultra-violet radiation in pollen to make possible even a rough estimate of the proportion of the incident energy which reaches the sperm nucleus. It is possible that the sperm nuclei receive an effective dose only in the most favorably oriented pollen grains. If so, coincidences would be expected in larger numbers. However, if this is the explanation of the coincidences observed, the frequency of induced mutation in effectively treated nuclei must be extremely high.

Summary.—1. Ultra-violet radiation applied to the pollen of maize greatly increased the frequency of both entire and fractional endosperm deficiencies. The average size of the fraction showing deficiency was approximately one-half of the endosperm.

2. Deficiencies affecting the F_1 plants also were induced by the treatment. Many of these were haplo-viable.

3. There was no significant increase in the frequency of translocation.

4. Numerous point mutations affecting seed and seedling characters were induced by the treatment. Several of these occurred in treated germ cells in which apparently unrelated mutations or other germinal alterations also occurred.

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