Reports

Mutations in Flax Rust Induced by Ultraviolet Radiation

A gene-for-gene relationship between rust reaction in flax and pathogenicity in the flax rust fungus, *Melampsora lini* (Pers.) Lév., has been postulated (*1*). For each gene that conditions the host's reaction, there is a specific gene that conditions pathogenicity in the parasite. Twenty-five pairs of genes have been identified, and a series of flax rust-differentiating varieties, each of which apparently possesses a single unique gene for rust reaction, has been developed (*1, 2*).

The uredospore is the repeating and pathogenic phase of the flax rust fungus; it is dikaryotic. The ability of the rust to attack a flax variety (virulence) that possesses a gene for rust resistance has been inherited as a recessive character except in one case, the ability to attack the variety Williston Brown (*3*). Resistance to flax rust invariably has been dominant. For example, the Dakota variety possesses the genes for resistance *MM* and is not attacked by races either homozygous or heterozygous for the dominant gene for avirulence A_M but is attacked only by races homozygous for the recessive genes $a_M a_M$.

With radiation as the mutagenic agent, a uredospore homozygous for the genes conditioning avirulence on a variety carrying a single gene for resistance could attack that variety only if the identical genes in each of its two nuclei were hit. Probably such occurrences would be rare at sublethal dosages. On the other hand, a uredospore heterozygous for pathogenicity should be able to attack that variety following a single hit that altered its dominant gene. To test this possibility, race 1, which attacks only the varieties Williston Brown and Bison, was crossed with race 22, which attacks all differential varieties except Bombay and Stewart. In an earlier study (*3*) this culture of race 22 had been found to be homozygous for pathogenicity. If race 1 had been homozygous, the F_1 uredospores would have been heterozygous for pathogenicity on all the differential varieties that were resistant to race 1 and susceptible to race 22. However, selfing showed that race 1 was heterozygous for pathogenicity on four of the differential varieties. Each of the five F_1 cultures of race 22 × race 1 that were secured attacked at least two of the varieties for which race 1 was heterozygous. Culture race 22 × race 1-A was selected for study. This culture was homozygous for virulence on Akmolinsk, Bison, Williston Brown, and Victory A and for avirulence on Bombay and Stewart. It was heterozygous for pathogenicity and avirulent on Abyssinian, B. Golden selection, Barnes, Birio, Bowman, Cass, Clay, Dakota, Kenya, Koto, Leona, Ottawa 770B, Pale Blue Crimped, Polk, Towner, and Wilden.

With a camel's hair brush, spores were dusted on sized paper in thin layers. They were then exposed to ultraviolet radiation for 10 minutes at a distance of 4 inches from a 30-watt ultraviolet tube. About 10 percent of the spores survived this treatment.

Approximately 100 vigorously growing 12- to 18-inch plants of each of 22 flax varieties were inoculated by dusting a 1-to-40 dilution of the treated spores and talc. The spores were incubated 24 hours in a moist chamber and were then removed to a greenhouse bench. Uredia that developed on varieties resistant to the F_1 hybrid rust culture were increased either on the variety on which they developed or on Bison, and a pathogenicity determination was made on a complete set of rust-differentiating varieties.

It was estimated that one of the susceptible varieties bore about 40,000 primary uredia. Since at least one viable spore is required to initiate a uredial infection, each differential variety to which the culture was avirulent served as a screen for detecting mutations for virulence to its rust-conditioning gene in at least 40,000 spores.

Mutants were secured that attacked six of the varieties that were resistant to the nonirradiated uredospores. The reaction of these varieties to the mutant rust cultures is given in Table 1. The cultures that attacked Dakota, Cass, and Polk differed from race 22 × race 1-A only in ability to attack each of these varieties. This may be accounted for by the mutation of a single gene in each instance. Although no mutant culture was secured from Birio, the mutants from Barnes and Wilden attacked it. In previous studies on the inheritance of pathogenicity in the flax rust fungus (*1, 3*), virulence to Birio was found to be independently inherited. Both Barnes and Wilden were susceptible to the parent races and to all hybrid cultures in the inheritance studies, so no information was obtained on the linkage relationships of the genes for pathogenicity to Barnes and Wilden with those for pathogenicity to other differential varieties. Each of the varieties Barnes, Birio, and Wilden has been susceptible to races of the flax rust fungus that do not attack the other two. Therefore it is probable that different genes in the parasite condition pathogenicity on each variety. The fact that Barnes, Birio, and Wilden were susceptible to both ultraviolet-induced mutant cultures suggests that the genes for pathogenicity to these varieties are so closely linked that a single hit altered all three genes. The occurrence of four or possibly five other groups of closely linked genes for pathogenicity has been reported (*1*).

The demonstration that it is possible by ultraviolet irradiation to produce mutations from avirulence to virulence in the flax rust fungus shows that muta-

Table 1. Reaction of flax varieties on which mutations for pathogenicity were observed to parent races, the F_1 hybrid, and mutants. The accession numbers are those of the Cereal Crops Section; *R*, resistant; *S*, susceptible.

Variety	Accession No.	Parent race		F_1 hybrid (Race 22 × race 1-A)	Mutants				
		1	22		Dakota	Cass	Polk	Wilden	Barnes
Dakota	1071	R	S	R	S	R	R	R	R
Cass	1182	R	S	R	R	S	R	R	R
Polk	1191	R	S	R	R	R	S	R	R
Birio	1085	R	S	R	R	R	R	S	S
Wilden	1193	R	S	R	R	R	R	S	S
Barnes	1190	R	S	R	R	R	R	S	S
No. of mutants having indicated pathogenicity					3	1	1	2	

tions are a factor in the development of new races. Other forms of radiation having greater penetration, such as x-rays and thermal neutrons, may be more effective mutagens. The methods used in these tests are readily adapted to screening large numbers of spores. Therefore it should be possible to determine the relative mutation rates of the genes that condition pathogenicity (*4*).

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References and Notes

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4. This article is a contribution from the Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture, and from the North Dakota Agricultural Experiment Station.

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Effect of Free Radicals on Chromosomes of Barley

Early work on the radiation chemistry of water and aqueous solutions led investigators to conclude that the hydrogen peroxide found in such solutions might also be present in the cells of irradiated biological material and cause at least some of the genetic and physiological effects of radiation (*1*). This contention seems to have gained ground with the findings that organic peroxides and H_2O_2 increased the number of mutations in bacteria (*2*) and that the curves for inactivation of enzymes (carboxypeptidase) and production of H_2O_2 in irradiated aqueous solution were inversely related (*3*). Structural damage to chromosomes in *Tradescantia* microspores that had been irradiated with x-rays was attributed by Gray (*4*) to the effect of H_2O_2.

Recent investigations, however, indicate that hydrogen peroxide per se probably has little or no effect on the biological system and that it is the precursors of H_2O_2, H, OH, and HO_2, that produce the effect sometimes ascribed to H_2O_2. Barron *et al.* (*5*) found the effect of H_2O_2 on cell metabolism, proteins, and enzymes to be negligible unless the concentration was far above that found in the cytoplasm of irradiated cells. They asserted that a large number of cellular oxidations that had previously been traced to H_2O_2 were actually caused by free radicals evolved by the action of catalytic amounts of Cu^+ and Fe^{++} on H_2O_2.

Weiss (*6*) was one of the first workers to ascribe biological significance to radiochemically excited H, OH, and HO_2 radicals, suggesting that such radicals (especially OH) could be considered as the reactive entities in the target theory of mutation and chromosome breakage. Since that time, the role of the various radicals in biological systems has received considerable attention in the literature. Scholes and Weiss (*7*) noted fragmentation of the polynucleotides and increase in titratable acid groups when deoxyribonucleic acid was treated with chemically and radiochemically derived HO_2 radicals. Collinson *et al.* (*8*) showed that OH radicals completely inactivated solutions of ribonuclease.

This study (*9*) was designed primarily to assess the relative effects of H_2O_2 and free radicals on chromosome breakage. Of secondary interest was a comparison of the types of aberrations induced in this experiment with those produced by irradiation on the one hand and by radiomimetic substances on the other. Dormant barley seeds, variety Himalaya, were exposed to H, OH, and HO_2 radicals produced by combining dilute solutions of hydrogen peroxide and ferric sulfate. The seeds were treated for 16 hours and germinated on blotting paper. After fixation in Carnoy III (3 parts ethanol, 4 parts chloroform, and 1 part acetic acid), acetocarmine smears of shoot tips were examined cytologically for chromosome bridges and fragments at late anaphase of the first cycle of mitoses. Photomicrographs of such aberrations may be seen in Caldecott and Smith (*10*). Table 1 presents a condensed summary of numbers of chromosomal aberrations in the four treatments. Three concentrations of H_2O_2 (2, 4, and 6 percent), two concentrations of $Fe_2(SO_4)_3$ (0.01 and 0.1 percent), and the six resultant combinations of H_2O_2 and $Fe_2(SO_4)_3$ (2 percent H_2O_2 and 0.01 percent $Fe_2(SO_4)_3$; 4 percent H_2O_2 and 0.01 percent $Fe_2(SO_4)_3$; and so forth) were used, but since the differences between subtreatments were not significant, they have been combined in Table 1.

As indicated in Table 1, the frequency of chromosome fragments in the seeds treated with hydrogen peroxide and ferric sulfate is 15 to 20 times the frequency found in those treated with either hydrogen peroxide or ferric sulfate alone. It is quite clear that while hydrogen peroxide may play a minor role in chromosome fragmentation [even the small increase in fragment frequency owing to peroxide as compared with the H_2O control might be due to free radicals arising from the splitting of H_2O_2 by endogenous catalysts in the seed (*5*)] the preponderance of bridges and fragments must have been caused by the free radicals derived from the catalytic splitting of the H_2O_2 molecule.

Since H radicals will either combine in pairs to form molecular hydrogen or combine with O_2 to form HO_2 (*11*), it is the OH and HO_2 radicals that must be responsible for the observed effect. Both of these free radicals have been shown to degradate nucleic acids, nucleotides, and nucleosides (*1*).

Table 1. Chromosome bridges and fragments resulting from treatment of barley seeds in H_2O_2, $Fe_2(SO_4)_3$, and H_2O_2 and $Fe_2(SO_4)_3$.

Treatment	Cells (No.)	Bridges (No.)	Fragments (No.)	Fragments/cell
H_2O (control)	400	0	1	0.0025
H_2O_2	1200	0	6	0.005
$Fe_2(SO_4)_3$	800	0	3	0.00375
H_2O_2 and $Fe_2(SO_4)_3$	2400	13	211	0.0875

In order to assess fully the relative frequencies of chromosome and chromatid breaks, one must score cells for types of reunion as seen at metaphase as well as at anaphase. As mentioned in a previous paragraph, chromosome as well as chromatid breakage was estimated from bridge and fragment frequency at anaphase only. But by assuming that paired acentric fragments and paired dicentric bridges reflect a chromosome break, and that single fragments (or single, isolated fragments when there is more than one fragment in a cell) and single bridges indicate origin in a chromatid break, an approximation of the relative frequencies of chromosomes and chromatid breaks can be made.

Based on this assumption, 39 percent of the induced aberrations were of the chromosome type and 61 percent were chromatid breaks. This relationship of chromosome-chromatid breakage is more typical of that induced by x-irradiation than that caused by other radiomimetic chemicals (*3, 12*). Most chemicals have been found to inhibit or upset DNA synthesis, the result becoming manifest as chromosome breaks. The action of x-rays takes place later in interphase of the mitotic cycle after chromosome reduplication and results in a predominance of chromatid breaks (*13*).

This experiment produced two results that seem to be of particular interest. (i) Whereas H_2O_2 per se was shown to be ineffective in causing chromosomal aberrations, the treatment by the free radical precursors of H_2O_2 resulted in chromosome breakage. (ii) Chemically derived free radicals have much the same effect as x-irradiation in the production of chromosome aberrations. This aspect gains importance in the light of recent studies that attribute a major role to organic and inorganic free radicals in the x-ray induction of