Proceedings of the Iowa Academy of Science

Volume 65 | Annual Issue Article 24

1958

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The Effects of the Chemical Mutagen, Diepoxybutane, on Clintland Oats[1]

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The objective of any mutation breeding program is to create desirable genetic diversity. With the spectrum of mutations from ionizing radiations becoming somewhat defined, several workers have investigated the use of chemical mutagens as a method of broadening the range.

Gibson, Brink, and Stahmann (3) applied mustard gas vapor to corn pollen causing chromosomal derangements most of which appeared to be deficiencies. The number of point mutations was low in relation to sterility-inducing chromosomal derangements. MacKey (11) found that in barley the physical mutagens, x-rays, fast neutrons, and radiophosphates produced more albino type mutations as compared with more viridis types from the chemical mutagens, mustard gas and nitrogen mustard.

True breeding sorghum mutants were produced from colchicine treatment by Ross, Franske, and Schuh (12). The mutants were unchanged in chromosome number and bred true in later generations. Supposedly the mutant genes became homozygous following reduction and subsequent doubling. Harpstead, et al. (4) concluded that the colchicine-induced sorghum variants resulted from gene mutations or cryptic structural changes in the chromatin.

Auerbach and Robson (1) reported mutation rates as high as 24 percent, in *Drosophila melanogaster* Meigen, from treatment with mustard gas. Inversions, large deletions, and translocations were produced, but the frequency of translocations was lower than would have been expected with a comparable dosage of x-rays. The back-mutation rate of the gene adenineless, in *Neurospora crassa* Shear and Dodge, was increased by nitrogen mustard treatment in a study conducted by Kölmark and Westergaard (8).

Jensen, Kölmark, and Westergaard (6) studying other compounds with a chemical reactivity similar to nitrogen mustard found that diazomethane was strongly mutagenic. Kölmark and Westergaard (9) induced back-mutations with ethyleneimine, ethyl oxide,

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[1]Journal paper No. 1274, Purdue University Agr. Exp. Sta. Part of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree.

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and two diepoxides. One of the latter compounds, 1:2,3:4-diepoxy-butane produced more back-mutations than any other previously used mutagen. Those chemicals which gave a strong mutagenic effect were highly reactive compounds which produced free radicals or reactive ions, possessed unstable three-membered rings (such as diepoxybutane), and were able to release free energy. The struc-

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tural formula of diepoxybutane is H_2C-C-C-CH_2. Kölmark (7) dis-

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covered that this compound which induced a high back-mutation rate at the adenineless locus, in *N. crassa,* did not produce comparable effects in reverse mutation tests with inositol dependent mutants.

Likewise, Smith and Srb (14) showed that β-propiolactone was more effective in creating back-mutations in the adenineless strain of *N. crassa* than in the inositolless strain. This compound also caused chromosomal aberrations in *Vicia faba* L.

Since the effects of chemical mutagens on mature plant type mutations has not been investigated the present study was conducted to determine whether the highly reactive compound, diepoxybutane, would cause such mutations in oats.

Materials and Methods

In July, 1956, Clintland oats were planted in buckets and grown under irrigated conditions at the Brookhaven National Laboratory.[3] The tillers of the plants were visually classified, on August 1, into five "tiller classes" on the basis of stage of development. Tiller class 1 represented the most advanced stage of morphological development and tiller class 5 was the least advanced stage. These morphological stages were then related to meiotic development so that meiotic stage at the time of treatment could be related to the data on mutation rates.

On August 2, 1:2,3:4-diepoxybutane solutions, of concentration 0.005, 0.01, and 0.05, were injected into the classified tillers of approximately 60 plants per concentration. Distilled water was injected into the tillers of a fourth group of 45 plants to determine the mechanical effects of injection. The injections were made with a hypodermic syringe, with approximately 1 cc. of the specific solution being injected into each culm near the developing panicle primordia. The seed harvested from these plants was sent to Lafayette, Indiana, for mutation rate studies.

In experiment I (October, 1956) twenty seeds from each dosage-tiller combination for tiller classes 1 through 4 were planted in flats

[3]The authors wish to express their appreciation to Dr. C. F. Konzak and to the Brookhaven National Laboratory for the advice and facilities utilized in the initiation of this study.

in the greenhouse. The experimental design was a randomized block design, with four replications. Seedling heights were measured 9 days after planting and the plants were allowed to mature at which time seed was harvested from each plant separately. The seed from a plant was planted in a four-foot row in the field in 1957 and the progeny rows were examined for agronomic mutations.

Experiment II which included all diepoxybutane tiller classes combinations and was planted in November was conducted in the same manner as experiment I except that the design was replicated five times for all treatments except the check.

Experiment III was planted in January, 1957. Seeds from the check plants only were planted in the greenhouse. Since this experiment was run only to verify seedling height data from Experiments I and II, it was discarded as soon as height measurements had been taken.

Experiment IV was begun in March, using seeds from tiller classes 1-4 for all diepoxybutane dosages. Seedling height measurements were taken 10 days after planting. These plants were transplanted to the field but they were killed by flooding.

Results

Seedling Height

The mean seedling heights for the different concentrations of diepoxybutane for experiments I through IV are given in Table 1. The .005 and .01 percent solutions seemed to have a slight stimulatory effect on seedling height but in neither experiment was the increase significant. The .05 percent solution may have had a depressing effect but it was slight. In all likelihood treating oat plants with solutions of diepoxybutane ranging in concentration up to 0.05 percent had no carry-over effect on the seedling height of the progeny of these plants.

Table 1

Mean Heights of M_1 Seedlings After Treatment With Different Dosages of Diepoxybutane, in Experiments I and II

Conc. of Solution %	Mean seedling heights (cms)	
	Experiment I	Experiment II
Ck	10.5	10.8
.005	11.0	11.1
.01	10.7	11.2
.05	10.4	10.3

The seedling heights for the different tiller classes grown in experiments I, II and IV are presented in Table 2. The value for each tiller class-experiment combination represents only the data from material which was treated with diepoxybutane originally. The seedlings from tiller classes 1 and 2 were taller than those from the other classes in both the treated and check materials. In experiments I, II, III and the check the seedlings from tiller class 3 were shorter than those from classes 1 and 2 and in all experiments those from classes 4 and 5 were decidedly shorter. Apparently this relationship of seedling height to tiller class is unconfounded with the diepoxybutane treatment since the check showed the same trend as the treated material.

Table 2

Mean Heights of M_1 Seedlings from Tiller Class 1-5, in Experiments I, II, III, and IV

Tiller Class	Mean seedling heights (cms) Exp. I	Exp. II	Exp. IV	Exp. III (ck.)
1	11.0	12.0	12.5	12.5
2	11.1	12.0	12.3	11.9
3	10.7	11.4	12.2	11.7
4	10.1	10.6	11.0	10.9
5	—	8.4	—	9.5
Min. diff. for sig. (Scheffe test)	1.3	1.5	1.3	2.4

Agronomic Mutations

Two distinct types of agronomic mutations were observed. These were several fatuoid and compactoid types. The frequencies of these mutants are shown in Table 3.

Table 3

Agronomic Mutants in Clintland Oats Following Treatment of Different Tiller Classes With Varying Dosages of Diepoxybutane

Dosage (% D.E.B.)	Tiller Class	No. of Treated Gametes Analyzed	Types of Mutation: Fatuoid No. of Mutants	Fatuoid % Mutants	Compactoid No. of Mutants	Compactoid % Mutants
0	1	19	0	0	0	0
	2	17	0	0	0	0
	3	20	0	0	0	0
	4	16	0	0	0	0
	5	10	0	0	0	0
.005	1	73	4	5.5	0	0
	2	77	0	0	0	0
	3	66	0	0	0	0
	4	59	0	0	0	0
	5	25	4	16.0	0	0
.01	1	88	0	0	0	0
	2	68	0	0	1	1.5
	3	73	0	0	0	0
	4	88	0	0	0	0
	5	39	0	0	0	0
.05	1	69	0	0	0	0
	2	80	0	0	0	0
	3	79	0	0	0	0
	4	70	1	1.4	0	0
	5	17	0	0	0	0

Fatuoid types were found in progeny rows in tiller classes 1 and 5 at the 0.005 percent diepoxybutane concentration, and in tiller class 4 at the 0.05 percent application. Both homozygous and heterozygous fatuoid types were observed. This characteristic results from the deletion of a suppressor gene complex which inhibits the expression of fatuoid genes according to Huskins (5). Frey (2) and Konzak (10) have called attention to the fact that fatuoids are very common in progenies resulting from a radiation treatment. If this mutant is due to a deletion effect it would corroborate the evidence of Auerbach and Robson (1), and Gibson, et al. (3), namely that chemical mutagens tend to cause drastic numbers of chromosomal derangements in relation to point mutations.

One plant showing a compact panicle type was found. This plant was also short and late maturing, with a rigid stem. However, the panicles were not as compact as the "Trelle dwarf" (13). It was sterile.

Discussion

Seedling height data from Experiments I, II, and IV strongly indicated reduced seedling growth from grain produced from tillers treated with diepoxybutane in the earlier stages of growth. Experiment III demonstrated that seed from later maturing tillers on the same plant produced seedlings with somewhat reduced vigor as compared to seed from early maturing tillers. While seed weights were not taken, a relation between lower seed weight of the later tillers and reduced vigor of seedlings is presumed. The shorter period of development, characteristic of later tillers, may also be responsible for the reduced growth of their progeny seedlings.

The agronomic mutants reported were quite distinct in appearance. No relation could be detected between the seedling heights in greenhouse experiments and the occurrence of mutations. The mutant types were widely scattered among the dosage and tiller class combinations. Small agronomic variations due to mutations may have been induced, but they could not be distinguished because blue and yellow dwarf diseases introduced considerable height and color differences in the field planting.

The chemical compound 1:2,3:4-diepoxybutane does appear to have a mutagenic effect. The hypodermic method of applying the mutagen seems to be workable at least. Dosage differences would appear to be reflected in seedling height, although substantiating work and higher dosage applications are necessary.

The efficiency of any chemical mutagen is dependent upon the following factors (8): (a) its penetration across cell walls (in plants) and membranes, (b) its diffusion in the cytoplasm of the cell, (c) the rate by which the chemical is destroyed or inactivated in the cell, (d) its ability to penetrate the nuclear membrane, and (e) its reactivity with the genetic material in the cell nucleus. These factors will vary, for any given chemical, with the stage at which the germinal tissues are treated. Therefore, the efficiency of a chemical mutagen is dependent upon the time and method of its application.

Summary

Clintland oats were treated with three concentrations, .005, .01, and .05 percent of 1:2,3:4-diepoxybutane, by hypodermically injecting the chemical into the culms of growing plants. The plant culms were placed in five "tiller classes," based upon their stage of morphological development at the time of treatment.

Progeny seedlings from the later tillers were less vigorous than those from the early ones. There was some indication of reduced seedling height resulting from the heaviest dosage, but the differences were small.

Two mutant types were found, fatuoid and compactoid head types.

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