RECOMBINAGENIC EFFECT OF CERTAIN CHEMICALS APPLIED TO THE GENUS HORDEUM

by

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ABSTRACT

The importance of the manipulation of recombination in both genetics and plant breeding led to an exploration of chemical means of altering recombination in barley. The primary object of the present investigation was to evaluate the effect of diepoxybutane, actinomycin D, 5-fluorodeoxyuridine and para-fluorophenylalanine on genetic recombination of closely linked genes in barley.

Chemical treatments were applied just prior to the onset of meiosis. Two methods were investigated in an attempt to bring the chemicals into intimate contact with sporocyte tissue. Injection into the upper culm and into the leaf whorl near the growing point, using a second needle to release back pressure, was found successful with a wetting agent (Tween 20). A vacuum-immersion method, although it appeared to bring the chemicals in contact with the floret tissues, was less efficient and required larger amounts of the chemicals.

Treatment with diepoxybutane (0.04 percent) resulted in a three fold recombination over the control in the region of the closely linked balanced lethal genes $\underline{x_c}+/+\underline{a_n}$ in chromosome 3. A comparable increase was obtained from treatment with actinomycin D (50 micrograms per ml), in the same region, which is located near the centromere. The antibiotic also affected the frequency of the expression, among progeny

of treated plants, of the gene $\underline{a_n}$. On the other hand, 5-fluorodeoxyuridine (2×10^{-4}M) treatment reduced recombination in the three-marker region uz + al/ + ys + in linkage group 3, to less than half that of the control. The region ys - al was affected to a greater extent than the region uz-ys. The analog was also found to be weakly mutagenic at the concentration used.

Two genes gl and zbc, reported to be closely associated in linkage group 4, showed independent segregation in the control but weak linkage in the treated stocks. When the double recessive (glgl zbczbc) plants were crossed to the line "robust", trisomic for chromosome 4, the gene gl gave trisomic ratios, while the gene zbc gave typical disomic ratios.

From the data obtained, it appeared that gametic selection was a factor in some of the chemical treatments, but further evidence on this effect would be desirable.

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INTRODUCTION

INTRODUCTION

Numerous attempts have been made to evaluate the effect of different physical and chemical agents in altering genetic recombination frequencies in a wide range of organisms. Both mitotic and meiotic crossing-over have been studied, but more rewarding results have been obtained in altering the former. This can be attributed at least in part to greater ease of treatment and better screening techniques available in the study of mitotic crossing-over, especially in the lower organisms. Certain chemicals have shown a specific effect in inducing only recombination and not mutation, which led Holliday (1964) to propose catagorising these chemicals into a separate group called "recombinagens", in contrast to chemicals causing mutations called mutagens.

In higher organisms, intensive work of this nature has been primarily limited to Drosophila and Zea mays. With the availability of different types of genetic markers, inversion heterozygotes, and more numerous genetic markers of linkage maps, studies of this nature have also been extended to barley. Powell and Nilan (1963) have shown the influence of temperature on crossing-over, using an inversion heterozygote of barley. They also indicated the relative advantage of this method over experiments based on F_2 progeny which"require hybridization, the evaluation of 2 generations,

the production of large numbers of plants, and laborious classifications". However, the advantages of inversion heterozygotes are offset by the fact that physical agents like X-rays induce differential degrees of crossing-over in various chromosomal regions (Muller, 1925) and that recombination frequencies are not proportionate to the independent determinations of physical length (Bridges, 1935, McClintock, 1943).

The present investigation was undertaken to determine the effect of certain chemicals on recombination in barley. Four chemicals, namely, diepoxybutane, 5-fluorodeoxyuridine (FUDR), p-fluorophenylalanine and actinomycin D were used in this investigation on the basis of their known effects on biological materials. It was found necessary to explore the methods of treatment before proceeding to determine the relative effects of these chemicals.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The variable nature of the crossing-over phenomenon was realised as early as 1915 when Bridges demonstrated the effect of age on crossing-over in the female flies of Drosophila melanogaster. Since then many external and internal agents have been found to affect recombination. In this regard, Swanson (1961) and Bodmer and Parsons (1962) have given extensive reviews of different factors affecting recombination. In recent years a large number of physical and chemical agents have been reported to influence crossing-over frequencies in a wide range of organisms.

Temperature was one of the first external factors discovered to affect recombination (Plough, 1917). Following this report, a large number of workers showed the effect of temperature on recombination (Plough,1921; Mayor and Svenson 1924b; Stern, 1926; Graubard, 1934; Stern and Rentschier 1936; Smith, 1936; Rifaat, 1959; Hayman and Parsons, 1961; McNelly and Frost, 1963; Towe and Stadler, 1964), chiasma formation (White,1934; Elliot, 1955; Rasmuson,1957; Dowrick, 1957; Wilson, 1959 a,b) and inversion bridges and fragments (Swanson, 1940; Powell and Nilan, 1963) using a wide range of organisms. Studies in both Drosophila and Neurospora indicated that, in general, minimum crossing-over occurs at temperatures between 22°C and 25°C with higher frequencies occurring when the temperature is lowered or raised. Similar effects have also been observed on chiasma frequency

(White, 1934; Dowrick, 1957). Nilan and Powell (1963), working with barley showed that maximum crossing-over occurred at 60°F (16°C), followed by 70°F (21°C), with lowered cross-over values at temperatures below 60°F. Even different plantings of the same cross, in tomato, gave significant differences in recombination values, the highest being in the winter greenhouse and the lowest in the summer greenhouse plantings (Butler, 1958).

In the early twenties of this century, the effects of irradiation on recombination were demonstrated, mostly in Drosophila (Mayor, 1923; Mayor and Svenson, 1924a; Plough 1924; Muller, 1925). Muller (1925) showed that x-rays produced differential effects on crossing-over in different regions of the long autosomes of Drosophila. He found that x-rays induced the greatest increase in crossing-over frequencies in regions adjacent to the centromere. Similar results to those of Muller were produced by Co^{60} gamma rays in Drosophila; the maximum increase of crossing-over frequencies was found to occur in the centromere regions with normal or subnormal frequencies in the most distal regions of the chromosome (Whittinghill,1951). The x-rays and gamma rays were capable of inducing crossing-over in Drosophila males, in which spontaneous crossing-over does not occur, was also demonstrated (Whittinghill, 1955). This phenomenon, of increase in the crossing-over frequency

in the centromere region and decrease in the distal region by different treatments, was characterised by Kikkawa (1934) as the "proximal increase and the compensatory distal decrease". Gamma rays had also been shown effective in raising frequency of chiasmata in Crotalaria (Sybenga,1960).

Herskowitz and Abrahamson (1957) showed a positive relationship between the intensity of irradiation and crossing-over; the more intense treatment produced more cross-overs. The same dose, however, applied over a long period of time was not as effective as an acute treatment. Stage of treatment, as well as dosage, was shown to be important by Schacht (1958), who found the spermatogonial stage of Drosophila males to be the most sensitive for increasing cross-overs by irradiation. X-rays have also been shown to be very effective in inducing mitotic crossing-over in Drosophila (Becker, 1957 cited by Holliday, Abbadessa and Burdick, 1963), in Ustilago (Holliday, 1961b) in Aspergillus (Kafer and Chen, 1964). Briggs and Smith (1965) found x-rays an effective means of inducing intragenic recombination in Zea mays but no effect on intergenic recombination was obtained.

Some negative results have also been reported. Clark (1943) found that x-rays did not affect recombination in Habrobracon. Ives et al (1953) also could not demonstrate any effect of infra-red radiation on recombination, in

Drosophila melanogaster. Even the combined effect of x-rays and infra-red was reported to have a nonsignificant effect on recombination in Drosophila (Yost and Bennevan,1957).

So far most of the studies of ultra-violet light on recombination have been limited to micro-organisms, probably because of the limited penetrating power of these rays. U.V. light has been found to be very effective in increasing mitotic crossing-over frequencies. Holliday (1961b) in Ustilago, Morpurgo (cited by Holliday,1964) in Aspergillus, Wilkie and Lewis (1963) and Kafer and Chen (1964) in yeast, have reported increases in mitotic crossing-over as a result of U.V. treatment. Folsome (1962), while studying the topographical effects of U.V. upon recombination in phage T_4, found locationally differential effects of U.V. on recombination in the ^{II} region. He further indicated that "there appears to be no definite correlation of U.V. recombination sensitive regions with U.V. mutational hot spots".

A number of chemicals have been shown to be effective in altering recombination frequencies. Among the alkylating agents tested, diepoxybutane, a bifunctional agent, has been found to induce mitotic crossing-over as well as mutations, while the monofunctional agents such as ethylene oxide and chlorotri ethylamine induced only mutation and was found ineffective in inducing recombination in Aspergillus

(Morpurgo,1963).

In barley, diepoxybutane has been reported to be weakly mutagenic as compared to ethylene oxide. Ethylene oxide was approximately six times more mutagenic than diepoxybutane (Ehrenberg and Gustafson,1957). Murphy and Patterson (1958) have also indicated that diepoxybutane is a weak mutagen in oats. However, this chemical was found to be a highly effective inducer of endosperm mutants in maize (Kreizinger, 1958).

Sobels and van Steenis (1957) found that formaldehyde increased crossing-over in Drosophila males treated with the chemical, at concentrations known to be mutagenic. They further showed that the most potent chemical mutagens, such as mustard gas, induced fewer cross-overs in relation to induced mutation than did the weaker mutagens, such as, organic peroxide and formaldehyde. Later, Sobels et al (1959) studied the relationship between stage specificity and the induction of lethal mutations and crossing-over by formaldehyde incorporated in the food of Drosophila. Their results suggested that the post-spermatogonial stage was the most sensitive to the mutagenic and recombinagenic action of formaldehyde, based on the groups of identical or complementary cross-over products as the indication of spermatogonial cross-overs. They also found that 0.25 percent formaldehyde was most effective of the concentrations

tried both for inducing lethal mutations and crossing-over. Whittinghill (1955) working with nitrogen mustard found an increase in crossing-over frequencies in the centromere region of treated female Drosophila.

Calcium and magnesium deficiency have been found to cause chromosome fragmentation in meiotic cells of Tradescantia (Steffensen,1953, 1955a,b). Hyde and Paliwal (1958) showed that both excess of calcium and deficiency of calcium and magnesium increased the chiasma frequency of Plantago ovata. On the other hand, Levine (1955) found decreased crossing-over in Drosophila melanogaster as a result of feeding an excess of calcium to young adult females. Later, Eversole and Tatum (1956) showed an increase in crossing-over frequency in cells of biochemically mutant strains of Chlamydomonas reinhardi treated with magnesium chloride. An appreciable increase was found in two chromosome intervals out of three tested. They also demonstrated this effect to be reversible by incubating the treated cells in high concentrations of calcium and magnesium ions. However, Steffensen et al (1956) could not find any difference in maize grown in calcium-deficient quartz sand in comparison with normal nutrition. Hanson (1961) was unable to alter the recombination value of the two closely linked markers in soybeans by treating them with different levels of calcium and phosphorus in nutrient culture. In barley, however, both

deficiency and excess of calcium and magnesium have been shown to increase recombination (Ondrey,1964). Griffing and Langridge (1963) have shown, in tomato, a pronounced decrease in crossing-over by increased sodium ion concentrations. Butler (1958) from his preliminary results, indicated that in F_1 tomato plants, soil pH was inversely related to recombination values. Levine and Ebersold (1957) could not alter recombination frequencies at the arginine 1 and arginine 2 loci in Chlamydomonas through deficiency of calcium and magnesium in the medium.

The chelating agent EDTA (ethylene diamine tetra acetic acid) has been shown in vitro to cause chromosome breakage in Drosophila melanogaster (Mazia, 1954). The chemical has also been found to cause deformational changes in the chromosomes of both animal and plant tissues. Based on cytochemical studies of salivary gland chromosomes of Drosophila and chemical analysis of onion root tips, the involvement of RNA has been indicated in these deformational changes (Kaufmann and McDonald, 1957). They further concluded that "EDTA induced chromosomal changes depend on modification of the general ionic environment of the cell and not merely on the selective removal of specific divalent cations from the fabric of the chromosomes". Hyde and Paliwal (1958) indicated that the structure of sulfur-containing proteins of the nucleus were modified by non-lethal doses

of EDTA. However, Wakonig *et al* (1958) could demonstrate no effect of EDTA on the root tips of *Vicia*. Levine (1955) found EDTA effective in inducing crossing-over in *Drosophila melanogaster* when it was fed during the larval stage, and suggested that crossing-over is sensitive to alteration in the ionic environment of the chromosomes. Kaufmann *et al* (1957) later also found that EDTA increased recombination frequencies only in certain regions of a chromosome, thus indicating the effect of EDTA to be preferential. They also showed that the intensity of the effect of this chemical was concentration dependent. Through genetic analysis in *Neurospora*, Prakash (1964) showed the possibility of changing negative interference to positive chromatid interference by treating hybrids with certain concentrations of EDTA. However, Steffensen *et al* (1956) and Levine and Ebersold (1958a) reported that EDTA had no effect on crossing-over in *Drosophila* and *Chlamydomonas* respectively.

In recent years, some of the antibiotics have been shown to influence recombination, and actinomycin D has been one of the drugs investigated. In low concentrations the drug has been found to inhibit DNA directed RNA synthesis but does not prevent DNA replication (Kirk, 1960; Goldberg and Rabinowitz, 1962; Horwitz *et al*, 1962; Reich, 1964). High concentrations of the antibiotic however, have been reported to inhibit protein synthesis, while having little

effect on DNA synthesis (Kirk,1960; Horwitz et al 1962; Reich et al, 1962). Actinomycin D has been found to cause structural changes in the chromosomes, mainly attributable to the inhibition of RNA synthesis. Izawa et al (1963) showed that actinomycin D treatment made the lateral loops of the lampbrush chromosomes, contract and subsequently disappear. They further found that when actinomycin D was added either to isolated or intact amphibian oocytes, first RNA synthesis was inhibited, followed by disappearance of chromosomal loops. Beermann (1965) also demonstrated the size reduction of Balbiani rings of polytene chromosomes in Chironomus, either by feeding the drug to whole larvae, or by incubating the excised salivary gland in low concentrations of the antibiotic. Differential sensitivities of different puffs to the drug were also exhibited. Burdette (1961) found that actinomycin D treatment of irradiated males of Drosophila melanogaster reduced the frequency of lethal mutations. Suzuki (1963,1965a) showed increased crossing-over in Drosophila by actinomycin D treatment. He hypothesized that "the heterochromatin around the centromere is actively involved in the synthesis of m-RNA at the time of crossing-over and that this activity prevents that region from crossing-over. Inhibition of this activity with actinomycin D causes structural change in that region which

permits crossing-over to take place". Another antibiotic reported to be of biological interest is mitomycin C, which has been shown to affect specifically DNA synthesis without affecting RNA and protein synthesis (Shiba, Terawaki, Taguchi and Kawamata,1959). The antibiotic has also been reported to be mutagenic and capable of increasing recombination in some strains of E. coli (Iijima and Hagiwara,1960). The drug also induced phage in lysogenic bacteria, besides increasing mutation and recombination (Otsuji, Sekiguchi, Iijima and Takagi,1959). The effect of mitomycin C has not been limited to micro-organisms but also has been demonstrated in other organisms. Shatkin et al (1962) showed that certain concentrations of mitomycin C caused nuclear fragmentation, partial depolymerization of nuclear DNA and appearance of DNA fragments in the cytoplasm of mammalian cells. Holliday (1964) found the drug to be very powerful in inducing mitotic crossing-over in Ustilago and Sacchromyces while it was non-mutagenic. He also indicated experimental evidence in support of the hypothesis that "an artificially imposed delay in genetic replication in a mitotic cell pushes it towards the meiotic conditions which favor chromosome pairing and crossing-over". Suzuki (1965) showed that mitomycin C treatment increased crossing-over frequencies in the centromere region and two adjacent regions in Drosophila. He further indicated that the effect of the

drug was mainly in the gonial stages of the cell. The drug has also been found to be mutagenic in Drosophila (Mukherjee, 1965).

Among other chemicals tested, ribonuclease has been found to be effective in increasing the crossing-over frequencies in Drosophila, preferentially in certain regions of the chromosome. It has been suggested that this increase is attributable to alterations in the nucleo-protein fabric of the cell and not merely due to direct action on the chromosomes (Kaufmann et al,1957).

The analog 5-fluorodeoxyuridine has been shown to inactivate the enzyme thymidylate synthetase in E. coli (Cohen et al,1958). Taylor et al (1962) reported that FUDR caused chromosomal breakage and inhibition of DNA synthesis in Vicia fabs and postulated that the lesions were due to inhibition of DNA synthesis by FUDR. They also found that thymidine and bromouracil deoxyriboside treatment, of irradiated roots in FUDR blocked condition, was effective in healing of chromosomal breaks; thus indicating the requirement of DNA synthesis for reunion of chromosomes. Bell and Wolf (1964) however, have given experimental evidence, which showed that "chromosome aberrations are induced in cells that are not synthesizing DNA". Bonner and Zeevart (1962) found that 5-FUDR inhibited floral primordial development in cocklebur and also showed this phenomena to be fully reversible by thymidine. Eposito and Holliday (1964) later reported that FUDR treatment inhibited DNA synthesis in

Ustilago maydis and also induced mitotic crossing-over. They suggested that the inhibitors of DNA synthesis cause an unbalanced state of cell growth, which favors chromosome pairing, thus stimulating recombination. Gallant and Spottswood (1965) have shown that thymidylate starvation in E.coli stimulated recombination and have indicated that this effect is associated not only with the inhibition of DNA synthesis but also with "specific physiology of thymine-less death". Phenyl ethyl alcohol has also been shown to cause reversible blockage of bacterial DNA synthesis, but not protein or RNA synthesis (Berrah and Konetzka, 1962). Later, Folsome (1963) showed that the blockage of DNA synthesis by phenyl ethyl alcohol in phage T_4, caused complete inhibition of recombinants and heterozygote formation and concluded that DNA replication is essential for genetic recombination.

The base analogs of thymine and phenylalanine, especially 5-bromouracil and para-fluorophenylalanine, have been shown to be very effective in altering recombination (Maling,1959). In Neurospora, she found that p-fluorophenylalanine increased recombination near the centromere, while 5-bromouracil caused a decrease in the mid-region of the chromosome.

MATERIALS AND METHODS

MATERIALS AND METHODS

The decision was made to utilize known genetic markers exhibiting fairly close linkage, in view of the limitations imposed by the inversion stocks. Genetic markers for chromosomes 3 and 4 were obtained from different sources. For chromosome 3, the genetic markers yellow stripe on the leaves (ys), albino lemma (al) and semibrachytic (uz) were selected for their suitability as seedling markers. The yellow stripe stock was obtained from the University of Alberta, as shown in Table 1. This stock showed a wide variation in the expression of the longitudinal yellow striping on the leaves. In some plants the striping was very heavy while in others the expression was not pronounced. The plants of this stock also showed partial sterility under greenhouse conditions. The stock carrying the genes for albino lemma and semi-brachytic was white in the basal parts of the lower leaf sheath and stem nodes and the plants were short in stature with shortened coleoptiles. Seedling classification was possible for all these characters. This stock had winter growth habit and needed three to four weeks of vernalization to break the winter habit. Crosses were made in the green house reciprocally between the semi-brachytic stock having the genotype uz uz Ys Ys al al and the yellow stripe stock of the genotype Uz Uz ys ys Al Al. Sufficient F_1 seeds were obtained from both reciprocals.

A second stock used in the investigation consisted of a balanced lethal in barley, heterozygous for the two lethal genes $\underline{a}_n$ and $\underline{x}_c$ in repulsion (Table 1). These two genes are closely associated in linkage group 3. So far, there is no report of a recombination value measured directly between these two loci. Based on consideration of tests of each with other markers in linkage group 3, it can be calculated that the map distance from $\underline{x}_c$ to $\underline{a}_n$ is somewhare between 4.70 and 8.25 percent as shown in Table 2. This stock was first grown for two generations to check the segregations of each plant progeny group for green, xantha and albino. Seeds from individual heads off plants chosen on this basis were sown and the green plants from lines showing all three phenotypes were transplanted separately into six inch pots and grown in the growth chamber for subsequent treatment.

The markers zoned leaf and glossy were selected for studies of chromosome 4. These markers were chosen due to their suitability for detecting, in the seedling stage, any decrease or increase in recombination with a relatively small population. Besides, these materials did not require any vernalization. The seed of the zoned leaf (zbc) marker was obtained from the University of Alberta under Accession No.43 (Table 1). The plants of this stock showed a wide variation in expression of the zoned leaf characteristic;

some of the plants were heavily zoned and did not survive till maturity while in a few plants zoning was barely perceptible. Reciprocal crosses were made in the greenhouse between the zoned leaf stock having the genotype G1G1zbczbc and the glossy seedling stock of the genotype g1g1ZbcZbc Sufficient F_1 seeds were produced.

Another two genes, hairy sheath (Hs)and yellow head (yh), shown to be closely associated in linkage group 4, were also selected for the investigation as described in Tables 1 and 2.

The effects on recombination of four chemicals, diepoxybutane, para-fluorophenylalanine, actinomycin D and 5-fluorodeoxyuridine, were studied by treating F_1 plants heterozygous for the marker genes, with solutions of the various chemicals, using methods of treatment to be described.

METHODS OF TREATMENT

Two methods of treatment were used in the present investigation in determining the effect of the different chemicals on recombination.

Injection Method.

A 1 ml tuberculin syringe was used to inject the solutions into the upper culm of the individual tiller of each plant and also into the leaf whorl surrounding the growing point of each culm. Treatment was timed with the objective

of keeping some solution available during the meiotic cell divisions. In order to minimize the expulsion of the solution from the culm due to differential pressure, an injection method was followed as shown in Figure II. Two hypodermic needles were used, one attached to the syringe as usual for injection and another empty needle inserted at the top of the upper hollow culm. Then the solution was injected from the bottom. As soon as the solution reached the top needle, the injecting needle was taken out and the hole plugged with lanolin. A thick layer of lanolin was applied to plug the hole completely, then the top needle was withdrawn and that hole also plugged. It was not possible to control entirely the differential expulsion of the solution from the culms, but extra care was taken to inject equal amounts of solution in each culm. Fresh solutions were always made before injection and a small amount (5 ml/100 ml) of 0.01 percent Tween 20 added to the solution as a wetting agent. Generally the chemical treatment was applied to one or two culms of each plant while one or two culms on the same plant were treated with liquid (distilled water with Tween 20) without the chemical, as control.

Vacuum Method

In this method, one to two main growing tillers were kept immersed in the solution for a certain length of time,

under vacuum pressure, usually 15 minutes per plant. This was accomplished by the following technique.

A hollow glass cylinder of thirty eight mm diameter was taken, one end of which was left wide while the other end was drawn out into a tube and attached to a long rubber hose. A suitable rubber stopper for the wide end was selected and a slit of about 2 cm was made across the cork. A cube was cut from each face of the slit and the space was filled with glued latex foam. The latex foam was used so that damage to the stem of the plant would be kept to a minimum and at the same time leakage of the solution would be prevented. Another hole was made to one side of the slit and a long glass tubing was inserted in it, which was finally attached to the reservoir of chemical solution with a long tube. A conical litre flask was used as reservoir, which was also plugged with a rubber stopper having two holes in it. Two separate glass tubings were inserted in the holes; one was attached to the tube leading to the cylinder with its other end dipped in the solution, while the second glass tube was left open for aeration.

Each separate culm was clamped in the slit of the rubber stopper which was then inserted in the glass tube. The air of the tube was evacuated and the solution was allowed to fill the jar slowly. When the solution came to the top of the plant the rubber tube connecting the reservoir

was plugged with a clamp and then the vacuum tube was closed. The arrangement is shown in Figure I. After keeping each tiller immersed in the solution for 15 minutes, the clamp was opened slowly, the vacuum released, and the solution allowed to drain back into the flask. The solutions were changed after each batch of ten treated plants. Other tillers of the same plant were treated in an identical manner, using distilled water, as a control. It was not possible to use Tween 20 with the vacuum method due to excessive foaming.

Before the actual treatments, preliminary tests were performed to determine whether the solution reached the floral organs within the florets. The dye orange G was dissolved in water and the plants were treated by the vacuum method, keeping them immersed in the dye solution for 15 minutes. After this period the plants were taken out and the floral organs were examined after dissecting the individual tillers. Color adhering to the floral organs indicated that the dye solution went into that part of the plant. These tests with the dye showed the feasibility of treatment by the vacuum method.

The handling of the materials was almost identical in each method of treatment. The F_1 plants were grown in a growth chamber under a controlled temperature of $60^{\circ} \pm 5^{\circ}F$ with approximately 1250 FCP in 16 hours of day light. All the treated and control tillers were tagged, and later each

culm was bagged at the time of heading in order to avoid out-crossing or contamination in pollination. Moreover, only one type of cross was used at a time in the growth chamber. The plants were grown to maturity in the growth chamber and then transferred to the greenhouse bench for drying. Seeds of each treated and control head from different plants were harvested and threshed separately. The F_2 and M_2 segregations were taken in the greenhouse. In the case of the balanced lethal stock, all the green plants of each line were grown to maturity for F_3 and M_3 segregations. This material took one and half extra generations for all the information required to calculate the recombination value as it was not possible to distinguish phenotypically between the two types of albino seedlings having the genotypes $\underline{a}_n\underline{a}_n\underline{x}_c\underline{x}_c$ and $\underline{a}_n\underline{a}_n\ \underline{X}_c^-$ in any generation.

Five separate experiments were performed to test the effect of all the four chemicals by two methods of treatment. Diepoxybutane of 0.01 and 0.04 percent concentrations and para-fluorophenylalanine of 10^{-3}M were applied to the materials heterozygous for markers in linkage groups 3 and 4; using both methods of treatment. Due to shortage of the other two chemicals, it was not possible to use the vacuum method, which required more chemicals than the injection method. Therefore, actinomycin D at a concentration of 50 μg per ml and 5-fluorodeoxyuridine at 2×10^{-4}M were applied with Tween 20 by injection.

The treatments in both the methods were performed in the laboratory at room temperature (70°F approximately) and after each treatment the plants were transferred to the growth chamber at the controlled temperature.

Cytological Study

Preliminary to treatment, floral development was related to the stage of growth of the plant by taking spikes which were fixed in Carnoy's solution (6:3:1 of ethyl alcohol, chloroform and glacial acetic acid respectively) and studying them microscopically after dissection. Some of the spikes were also taken at random after treatment and fixed in Carnoy's solution. Meiosis was studied in PMC's using the acetocarmine smear technique described by Smith (1947). The metaphase and anaphase stages were checked for bridges and fragments. No detailed meiotic study was made as the preliminary observations did not reveal any abnormal chromosome behaviour in either the controls or treated materials.

Fertility Study.

The fertility of each treated and control head was taken by counting seed set in relation to the number of florets, after discarding the first two spikelet groups from the top and bottom of the spike. However, in doing so, whenever there was any seed found in the uppermost or bottom spikelet in the treated material, these were retained

for the experiment though ignored in fertility counts.

Fertility percentage was calculated by:

$$\frac{\text{Number of seeds}}{\text{Total number of florets}} \times 100$$

Besides, this the number of kernels per head was recorded, both from treated and control heads of each plant.

FIGURE I. Arrangement used in the vacuum method of treatment.

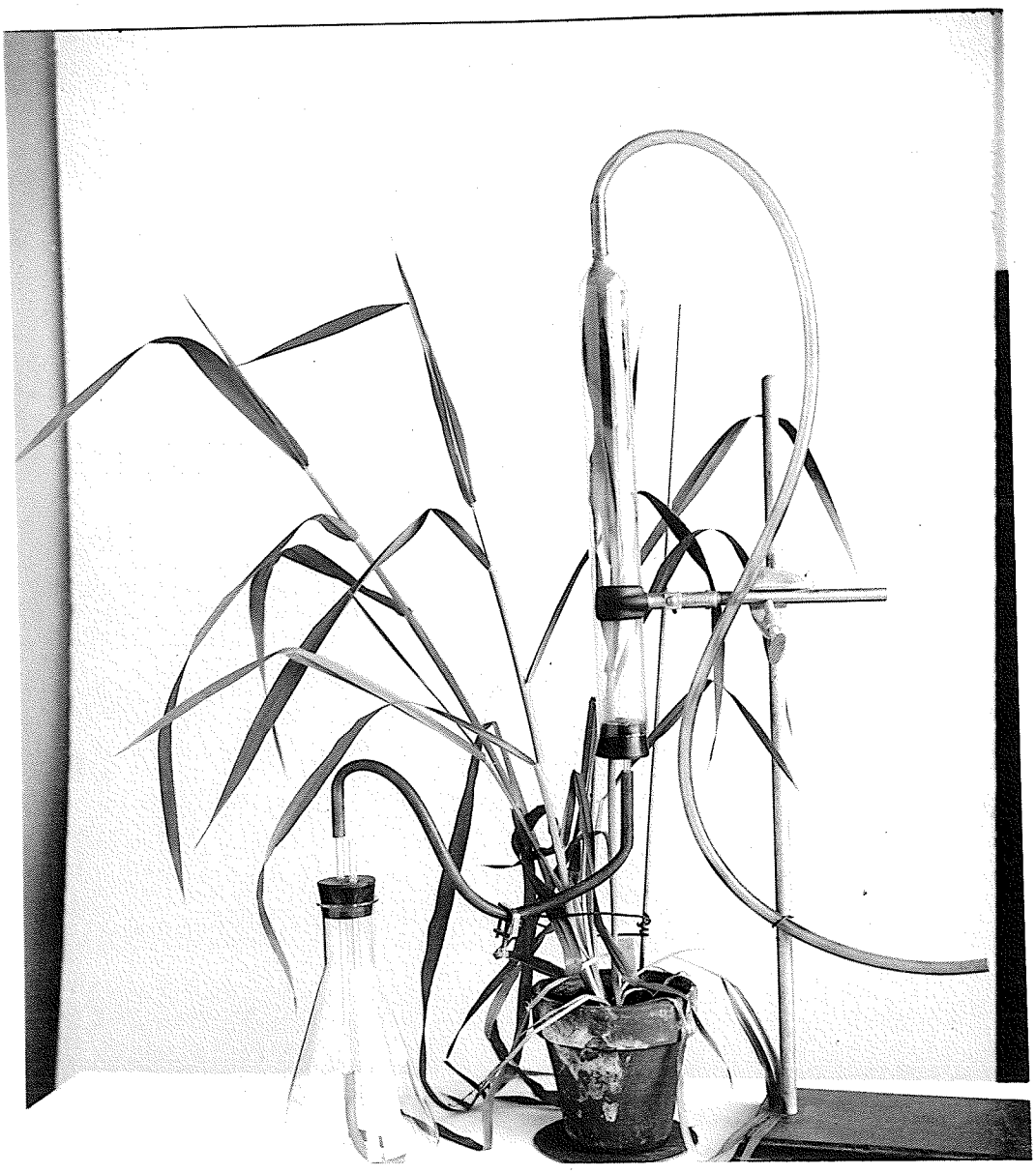

FIGURE II. Arrangement used in the injection method of treatment.

TABLE 1. Descriptions of the different genetic stocks of barley used in the study of the effect of chemicals on recombination.

Genetic stocks	Gene symbol	Description	Linkage Group	Source of Stock	Accession No.
Yellow stripe	ys	yellow longitudinal stripes on the leaves of seedling; stripes persisting till maturity. Most of the plants showed partial sterility in greenhouse	3	Dr.G.W.R.Walker University of Alberta	326
Semibrachytic albino lemma	uz-al	Plants having small stature with shortened coleoptiles. White lower leaf sheath and stem nodes. White (albino)lemma with normal green color compact awns. Heads also small and compact.	3	University of Manitoba	KB2
Balanced lethal heterozygote.	x_c-a_n	Plant progenies segregating for normal green,xantha and albino seedlings. Xantha seedlings, yellow, devoid of normal green chlorophyll surviving for one to two weeks. Albino seedlings white, often having yellowish or greenish stripes on tips of the leaves.	3	Dr.C.R.Burnham University of Minnesota	Line No. 11521 11524 12040 12084

continued

TABLE 1 CONTINUED

Genetic stocks	Gene symbol	Description	Linkage Group	Source of Stock	Accession No.
Zoned leaf	zbc	Banded leaves. Banding in some cases heavy and sensitive to temperature.	4	University of Alberta	43
Glossy seedling	gl	Waxless plants with shiny leaves, this character persists till ripening.	4	University of Manitoba	-
Yellowhead	yh	Yellow head with very small awns, in seedling stage lower leaf sheath and basal nodes devoid of green color.	4	University of Manitoba	KB1
Hairy sheath	Hs	Reported to have lower leaf sheath covered with hairs.	4	University of Manitoba	-

TABLE 2. Recombination values previously reported for the genetic character pairs used in the present study.

Genetic characters	Recommended gene symbol	Percentage recombination	Linkage Phase	Authority
1. Normal vs.Uzu (Uzuz) in relation to:				
Normal vs.yellow stripe.	Ysys	4.2±1.14	Repulsion	Kasha and Walker (1960)
Normal vs.albino lemma	Alal	14.23±3.1788	Repulsion	Takahashi and Hayashi (1959b)
2. Normal vs.Xantha (Xcxc) in relation to:				
Normal vs.albino seedling	Anan	4.70 (derived)		Robertson et al (1965)
Normal vs.albino seedling	Anan	4.75 "		Burnham(1957)
Normal vs.albino seedling	Anan	8.25 "		Takahashi and Hayashi (1959b)
3. Normal vs.Glossy seedling (Glgl) in relation to:				
Normal vs.zoned leaf	Zbczbc	30.0,8.5		Albrechtsen (1957)cited by Nilan (1964)
Normal vs.zoned leaf	Zbczbc	12.5,8.5		Doney(1961) cited by Nilan (1964)
Normal vs.zoned leaf	Zbczbc	14.0		Smith (1953) cited by Nilan (1964).
Normal vs.zoned leaf	Zbczbc	14.0± 5.3 9.3 ± 0.5	Repulsion Coupling	Woodward (1957a)

continued

TABLE 2. CONTINUED

Genetic characters	Recommended gene symbol	Percentage recombination	Linkage Phase	Authority
4. Hairy vs. nonhairy sheath (Hshs) in relation to:				
Normal vs. yellow head	Yhyh	8.5 ± 1.1		Takahashi, Hayashi and Yasuda (1957)

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

EXPERIMENT I:

The effects of the four chemicals para-fluorophenylalanine (conc.10^{-3}M), diepoxybutane (conc. 0.01%), actinomycin D (conc. 50 microgram per ml) and 5-fluorodeoxyuridine (conc. 2 x 10^{-4}M) were studied first on the genotype gl+/+zbc using both the vacuum method and the injection method of treatment.

The para-fluorophenylalanine treatment was applied by the vacuum method only. The F_2 plants were classified, in both seedling and adult stages of growth, into the four appropriate phenotypic classes in each of the treated and control samples. The p-fluorophenylalanine treatment gave disturbed ratios for both the individual gene segregations of Gl:gl and Zbc:zbc, based on the expectation of 3:1 in each of them, (Table 3). The chi-square deviation due to linkage was highly significant, indicating that the two loci involved were linked. The individual gene segregations of the control population gave good fits to 3:1 ratio for each character, and the combined segregations gave a good fit to a 9:3:3:1 ratio. The recombination values for the control and treated samples were calculated by the product method (Immer, 1930 and Immer and Henderson, 1943) and are presented in Table 4. There was an apparent reduction of 13.98 percent in recombination (Table 4) from the p-fluorophenylalanine treatment as

TABLE 3. Observed frequencies of individual gene segregation classes in F_2 of control and parafluorophenylalanine (conc.10^{-3}M) treated populations.

	Genetic traits	Classes* X	Classes* x	Total	χ^2 for 3:1	Prob.	χ^2 L	Prob.
Control	Gl:gl	677	212	889	0.630	0.50-0.25	0.120	0.75-0.50
	Zbc:zbc	687	202	889	2.460	0.25-0.10		
Treated	Gl:gl	672	159	831	15.252	0.005	21.931**	0.005
	Zbc:zbc	593	238	831	5.872	0.025-0.010		

* Dominant class represented by capital and recessive class by small letters (X and x).

** Significant at 1 percent level.

TABLE 4. Effect of para-fluorophenylalanine (conc.10^{-3}M) treatment on the recombination value of two genes reported to be associated in linkage group 4.

		Classes*				Total	Recombination value	S.E.	Diff.	S.E. Diff.
		XY	Xy	xY	xy					
Control	Gl,gl in relation to: Zbc:zbc	521	166	156	46	889	0.4903	0.025		
									0.1398**	0.0390
Treated	Gl,gl in relation to: Zbc:zbc	456	137	216	22	831	0.3505	0.030		

* Dominant class represented by capital and recessive class by small letters (x and y)

** Significant at 1 percent level.

compared to the control.

Diepoxybutane at 0.01 percent concentration gave very similar overall results to those from p-fluorophenylalanine treatment. In this case however, the individual gene segregations of the treated portion as well as those of the control gave good fits to the 3:1 ratio. The control portion again showed independent segregation while the chi-square for linkage in the treated sample was highly significant (Table 5). The recombination value of the treated sample was substantially lower than that of the control (Table 6).

The results of the actinomycin D (50 microgram per ml) treatment are presented in Tables 7 and 8. There was no significant difference between treated and control in any of the values calculated from the data. On the other hand, treatment with 5-fluorodeoxyuridine showed a decrease in recombination value compared to the check (Tables 10).

The consistent independent segregation of the gene pairs gl and zbc in the controls of these experiments (Table 11) suggested the possibility that the loci were in fact independent. In all the previously published work, their linkage has been established with a variable recombination value of 8.5 percent to 30.0 percent (Table 2). Attempts were therefore made to find the cause of this discrepancy. First the double recessives were crossed to

TABLE 5. Observed frequencies of individual gene segregation classes in F_2 of control and diepoxybutane (0.01%) treated populations.

	Genetic traits	Classes* X	Classes* x	Total	χ^2 for 3:1	Prob.	χ^2_L	Prob.
Control	Gl:gl	899	288	1187	1.118	0.50-0.25	0.016	0.900
	Zbc:zbc	887	300	1187	0.474	0.50-0.25		
Treated	Gl:gl	257	75	332	1.028	0.50-0.25	7.331	0.010-0.005
	Zbc:zbc	251	81	332	0.064	0.90-0.75		

* Dominant class represented by capital and recessive class by small letters (X and x).

TABLE 6. Effect of diepoxybutane (conc.0.01%) treatment on recombination of two genes reported to be associated in linkage group 4.

		Classes *				Total	Recombination value	S.E.	Diff.	S.E. Diff.
		XY	Xy	xY	xy					
Control	Gl,gl in relation to:Zbc:zbc	671	228	216	72	1187	0.4980	0.0218		
									0.1392**	0.0521
Treated	Gl,gl in relation to Zbc:zbc	185	72	66	9	332	0.3588	0.0473		

* Dominant class represented by capital and recessive class by small letters (x and y).

** Significant at 1 percent level.

TABLE 7. Observed frequencies of individual gene segregation classes in F_2 of control and actinomycin D (50 µg/ml) treated populations.

	Genetic traits	Classes *		Total	χ^2 for 3:1	Prob.
		X	x			
Control	Gl:gl	673	194	867	3.184	0.100 - 0.05
	Zbc:zbc	642	225	867	0.419	0.75 - 0.50
Treated	Gl:gl	239	60	299	3.881	0.05 - 0.025
	Zbc:zbc	222	77	299	0.090	0.90 - 0.10

* Dominant class represented by capital letter and recessive class by small letter (X and x).

TABLE 8. Effect of actinomycin D (conc. 50 µg/ml) treatment on recombination of two genes reported to be associated in linkage group 4.

		Classes*				Total	Recombination value	S.E.	Diff.	S.E. Diff.
		XY	Xy	xY	xy					
Control	Gl,gl in relation to: Zbc:zbc	498	144	175	50	867	0.498	0.025	0.021	0.051
Treated	Gl,gl in relation to: Zbc:zbc	176	46	63	14	299	0.477	0.044		

* Dominant and recessive classes represented by capital and small letters (x and y) respectively.

TABLE 9. Observed frequencies of individual gene segregation classes in F_2 of control and 5-fluorodeoxyuridine (conc. 2 x 10^{-4} M) treated populations.

	Genetic traits	Classes* X	Classes* x	Total	χ^2 for 3:1	Prob.
Control	Gl:gl	662	209	871	0.469	0.500-0.250
	Zbc:zbc	648	223	871	0.169	0.750-0.500
Treated	Gl:gl	174	51	225	0.653	0.500-0.250
	Zbc:zbc	182	43	225	4.161	0.05 -0.025

* Dominant and recessive classes represented by capital and small letters (X and x) respectively.

TABLE 10. Effect of 5-fluorodeoxyuridine (2×10^{-4} M) on recombination of two genes reported to be associated in linkage group 4.

		Classes*				Total	Recombination value	S.E.	Diff.	S.E. Diff.
		XY	Xy	xY	xy					
Control	Gl:gl in relation to: Zbc:zbc	493	169	155	54	871	0.502	0.025	0.101	0.0604
Treated	Gl:gl in relation to: Zbc:zbc	137	37	45	6	225	0.401	0.055		

* Dominant and recessive classes represented by capital and small letters respectively.

TABLE 11. F_2 segregations of the control samples from different experiments.

Genotypes tested Xx Yy		F_2 phenotypes XY	Xy	xY	xy	Total	Chi-square for 9:3:3:1	Prob.	Recombination Value	S.E.
GlglZnczbc	1	521	166	156	46	889	3.189	0.50-.25	0.490	0.025
	2	671	228	216	72	1187	0.409	0.90-.75	0.498	0.022
	3	212	61	75	15	363	4.345	0.25-.10	0.449	0.041
	4	498	144	175	50	867	3.619	0.50-.25	0.498	0.025
	5	493	169	155	54	871	0.645	0.90-.75	0.502	0.025
	6	643	216	228	72	1159	0.661	0.90-.75	0.491	0.022
	7	173	68	69	17	327	2.512	0.50-.25	0.434	0.044
	8	364	140	120	28	652	6.485	0.10-.05	0.429	0.032
	Total	3575	1192	1194	354	6315	4.480	0.25-.10	0.483	0.009

the F_1 and the back cross progeny were analyzed. The back cross segregation also gave a good fit to a 1:1:1:1 ratio (Table 12). This was additional evidence in favour of independence. However, it did not give convincing evidence as to their linkage group. The possibility that the loci might be approximately 50 units apart on the same linkage group was suggested by the linkages indicated in some of the treatments. Therefore a further investigation was carried out by trisomic analysis. The double recessive plants were crossed with trisomic plants of robust type, carrying an extra chromosome 4, using trisomic plants as females. The crosses were made in the greenhouse. The F_1 plants were separated into diploid and trisomic types by root-tip counting. The F_2 segregations of the F_1 trisomic plants were again separated into diploid and trisomic types, both by cytological examination and morphological study of the adult plants. The zoned leaf (zbc) character gave a good fit to a 3:1 ratio in both disomic and trisomic classes, (Table 13) but the glossy (gl) character deviated significantly from a 3:1 ratio. There were two plants of glossy phenotype in the disomic population of 53; while none were in 32 trisomic plants. The disomic class showed a good fit to the expected ratio of 8:1 and the overall total of disomic and trisomic frequencies also gave a good fit to a 17:1 ratio expected in this case. This provides ample evidence that the locus of the gene gl is on

TABLE 12. Back cross segregations of F_1 x double recessive (gl gl zbc zbc)

Genotypes tested XxYy	F_2 phenotypes XY	Xy	xY	xy	Total	Chi-square for 1:1:1:1	Prob.
Gl gl Zbc zbc	30	33	21	26	110	2.945	0.500 - 0.250

TABLE 13. Segregation of normal and zoned leaf; normal and glossy characters in F_2 populations of crosses of the zoned leaf-glossy double recessive with "Robust" (chromosome 4) trisomic types.

Trisomic types	Extra chromosome	Class	Normal	Zoned leaf	Total	χ^2 for 3:1 or trisomic Ratio #	Prob.
Robust	4	2n	45	8	53	2.773	0.10-.05
		2n+1	23	9	32	0.166	0.750-.50
		Total	68	17	85	1.133	0.250-.10

Trisomic types	Extra chromosome	Class	Normal	Glossy	Total	χ^2 for 3:1 or trisomic ratio #	Prob.
Robust	4	2n	51	2	53	12.735 2.904#	0.100-.05
		2n+1	32	0	32	-	-
		Total	83	2	85	23.251 2.575#	0.250-.100

chromosome 4 and is close to the centromere, (as they fit ratios based on chromosome segregation); and that zbc is not on this chromosome. This could have been the reason for the unusual behavior of zoned leaf (zbc) reported by Kasha and Walker (1960) in crosses with stock carrying the "long weak basal internode". It is also suggested as the likely cause for conflicting reports of the recombination value between gl and zbc (Albrechtsen,1957; Doney,1961; Smith,1953 as cited by Nilan, 1964), as well as regarding the position of the zbc locus in relation to k, for which values ranging from 0.0 to 35.5 have been reported (Robertson, Wiebe and Shands, 1947; Robertson, 1957).

Based on this trisomic analysis, it is apparent that these genes are not in the same linkage group. This presents some questions when the treated population is taken into consideration; as to whether the quasi-linkage phenomenon shown is due to disturbed segregation of individual genes resulting from some sort of gametic selection or whether it is due to other causes, such as differential fertility or lethality. If the zygotic selection due to differential embryo viability had been in operation, fertility would have been affected. However, difference in the number of kernels per head in the treated and control populations was found to be nonsignificant, indicating that fertility was not affected by treatment. The germination percentage in F_2 of the treated material (81.5 per cent) was even higher than that of the control (67.6 per cent).

Therefore differential germination could not account for the observed change in recombination. These observations suggest that some of the chemicals used had a disturbing effect on the gametic output of treated plants. Para-fluorophenylalanine resulted in an excess of zoned leaf segregations, whereas it reduced the number of glossy progeny obtained. It also showed quasi-linkage between the two loci.

On the other hand the diepoxybutane treatment did not disturb the individual gene segregations but the chi-square for linkage was significant and the recombination value calculated showed a decrease. This indicates that the chemical created an environment favourable to the expression of the quasi-linkage phenomenon.

EXPERIMENT No. II.

The effect on recombination of diepoxybutane at a concentration of 0.01 percent was studied by treating plants heterozygous for three linked genes (uz+al/+ys+) in linkage group 3. The vacuum method of treatment was followed. The phenotype yellow stripe (ys) was expressed very poorly in the F_2 control and treated samples. It was necessary therefore to eliminate this locus from consideration and to classify the material into four phenotypic classes only, involving segregation of the two distal markers which were in coupling. The data for the individual gene segregations of the control and treated population are shown in Table 14. Both gene segregations gave

TABLE 14. Observed frequencies of individual gene segregation classes in F_2 of control and diepoxybutane (0.01 percent) treated populations.

Treatment	Genetic traits	Classes* X	Classes* x	Total	Chi-square for 3:1	Prob.
Control	Uz:uz	1923	627	2550	0.2306	0.750 - 0.500
	Al:al	1902	648	2550	0.2306	0.750 - 0.500
Treated	Uz:uz	868	312	1180	1.3062	0.500 - 0.250
	Al:al	872	308	1180	0.7638	0.500 - 0.250

* Dominant and recessive classes represented by capital and small letters (X and x respectively).

a good fit to the expected ratio of 3:1. The recombination values were then calculated by the maximum likelihood method from control and treated populations (Table 15). Although recombination was greater in the treated group, the difference between the control and treated was statistically non-significant.

EXPERIMENT NO.III.

The effect of 5-fluorodeoxyuridine at a concentration of 2×10^{-4}M was studied on the genotype uz+al/+ys+. The injection method of treatment was followed. The 5-fluorodeoxyuridine treatment gave a high degree of lethality in the M_1 generation. Forty percent of the treated plants failed to head and on examination showed dead heart leaves. Close examination of some of the affected heads showed discoloration and rotting of the growing points. This was taken to indicate that the chemical had penetrated into the cells and probably affected the cell metabolism. Heading was delayed considerably in the treated plants in comparison with those of control. This may have been due to an inhibitory effect of 5-FUDR, as a similar inhibitory effect of this chemical, in cocklebur, has been shown by Bonner and Zeevart (1962). Seed set in the treated (M_1) heads was on the average lower than in the control. The number of kernels per head in the

TABLE 15. Effect of diepoxybutane (0.01 percent) treatment on recombination of two linked genes of linkage group 3.

		Classifications*					Recombination	S.E.	Diff.	S.E. Diff.
		XY	Xy	xY	xy	Total				
Control	Uz,uz in relation to: Al:al	1786	137	116	511	2550	0.1045	0.0065		
									0.0062	0.0117
Treated	Uz,uz in relation to:Al:al	807	61	65	247	1180	0.1107	0.0098		

* Dominant and recessive classes are represented by capital and small letters (x and y).

treated (24 seeds per head) was significantly lower than in the control (36 seeds per head). These observations indicate that there may have been a lethal effect of the chemical both on the chromosomal and cellular levels.

The M_2 plants were grown on a greenhouse bench in sterilized soil after vernalizing the germinating seeds off the M_1 plants for four weeks in a cold chamber. All of the eight possible phenotypic class frequencies were recorded in the seedling as well as the adult stage of plant growth. The data from the M_2 individual gene segregations are shown in Table 16. All of the gene segregations showed a highly satisfactory fit to a 3:1 ratio. The recombination values of both region I (between genes uz and ys) and region II (between genes ys and al) were estimated by Collins (1924) method, as there was no plant of double recessive class in either region I or in region II. The recombination value between the two extreme genes uz and al was calculated by the maximum likelihood method. The recombination values calculated for region I and II were 23.12 ± 49.30 and 0.0 percent respectively, while between the two distal genes uz and al, the value was found to be 5.07 ± 0.99 percent. As the M_2 recombination values were obviously subject to a high error, as is characteristic of close linkage in repulsion, only the combined estimates from M_2 and M_3 were considered in evaluating treatment effects. A genotypic analysis of M_2

TABLE 16. Observed frequencies of individual gene segregation classes in the F_2 of control and 5-fluorodeoxyuridine (2×10^{-4}M) treated populations.

Treatment	Genetic traits	Classes* X	Classes* x	Total	χ^2 for 3:1	Prob.
Control	Uz:uz	5273	1756	7029	0.0012	0.900
	Ys:ys	5325	1704	7029	2.1515	0.250-0.100
	Al:al	5239	1790	7029	0.8138	0.500-0.250
Treated	Uz:uz	374	138	512	1.0417	0.500-0.250
	Ys:ys	394	118	512	1.0417	0.500-0.250
	Al:al	374	138	512	1.0417	0.500-0.250

* Dominant class represented by capital and recessive class by small letters (X and x).

plants was performed by growing and classifying the M_3 lines, and recombination values were calculated from the singly dominant and doubly dominant classes by the maximum likelihood formulae and tables of Immer (1934), Immer and Henderson (1943) and Allard (1956). The combined estimates of recombination values obtained from all the data by weighted average, with weighting based on the information provided by each source of data, are summarized in Table 17.

The progeny of control culms injected with the water-Tween 20 solution and those receiving no treatment were classified separately and the data subjected to a heterogeneity test. As the chi-square test did not indicate significant heterogeneity, the data were pooled. The individual gene segregations for all three genes are in good fit to the expected 3:1 ratio (Table 16). The recombination values were then calculated by the maximum likelihood method for region I, between uz and ys, region II between ys and al and between uz and al and are presented in Table 18. The previously reported recombination value between uz and al alone, in repulsion was 14.23 $\pm$ 3.1788 percent (Takahashi and Hayashi, 1959b), while between uz and ys it was 4.2 $\pm$ 1.14 percent (Kasha and Walker, 1960). The recombination value of the control found in the present investigation with the three point cross, is in very close agreement with

TABLE 17. Class frequencies of F_2 and F_3 and the combined recombination value following treatment with 5-fluorodeoxyuridine of F_1 plants heterozygous for three linkage markers.

Cross	Linkage Between	Linkage Phase*	F_2 phenotypes					F_3 doubly dominant**					F_3 singly dominant #						Combined recombination value and standard error
			a	b	c	d	Total	e	f	g	h+i	Total	j	k	Total	l	m	Total	
uz+al / +ys+	uz-ys	R	256	118	138	-	512	1	3	4	94	102	7	88	95	9	93	102	0.0430 ± 0.0085
uz+al / +ys+	ys-al	R	256	138	118	-	512	0	4	4	94	102	2	99	101	-	92	92	0.0160 ± 0.0063
uz+al / +ys+	uz-al	C	361	13	13	125	512	2	2	3	95	102							0.0481 ± 0.0123

* R = Repulsion ; C = Coupling

** e=XXYY, f=XxYY, g=XXYy, H+i = XxYy

\# j= XXyy, k=Xxyy, l=YYzz, m=Yyzz

TABLE 18. Effect of 5-fluorodeoxyuridine treatment on recombination values of three linked loci (uz+al/+ys+).

Treatment	Classes XY	Xy	xY	xy	Total	Recombination value	S.E.	Difference	S.E. Difference
			Uz,uz in relationto Ys:ys						
Control	3573	1700	1752	4	7029	0.0488	0.0119	0.0058	0.0146
Treated	256	118	138	0	512	0.0430#	0.0085#		
			Ys,ys in relation to Al:al						
Control	3545	1780	1694	10	7029	0.0761	0.0118	0.0601**	0.0134
Treated	256	138	118	0	512	0.0160#	0.0063#		
			Uz,uz in relation to Al:al						
Control	4908	365	331	1425	7029	0.1039	0.0039	0.0558**	0.0129
Treated	361	13	13	125	512	0.0481#	0.0123#		

Combined estimates of recombination values and standard errors,from M_2 and M_3 data.

** Significant at 1 percent level.

the reported value in region I between the genes uz and ys, while the value for the uz - al region was substantially lower. The difference may be mainly due to environmental effects. However, considering the high standard error associated with the recombination value reported by Takahashi and Hayashi (1959b), as well as the linkage phase involved, the value obtained in the present investigation (10.39 ± 0.39 percent) appears to be more reliable. Moreover, the difference between the two recombination values is statistically non-significant as shown below:

	Recombination value	S.E.	Diff.	S.E. Diff.
Previously reported by Takahashi and Hayashi	0.1423	0.031788		
			0.0384	0.03203
Obtained in the present investigation.	0.1039	0.0039		

A comparison of the recombination values obtained from the 5-fluorodeoxyuridine treated population (Table 18) with the values of the control, showed a highly significant decrease in region II as well as between the loci of uz and al. The slight decrease in region I, between uz and ys, was statistically non-significant. The difference in over-all recombination values (uz-al) obtained from the M_2 and M_3 was statistically non-significant, even though the environments

in M_1 and M_2 were different, the latter being the source of the doubly dominant heterozygotes. Thus it is apparent that the decreased recombination values found in M_2 persisted in M_3; which indicates that the variable environment did not seriously affect the modification of recombination by 5-fluorodeoxyuridine treatment.

Applying a chi-square test to class frequencies obtained by recalculating them from the obtained values of recombination, i.e. 4.88 percent in region I, 7.61 percent in region II and 10.39 percent between uz and al gave a good fit to the control frequencies obtained. However, using the recombination values obtained from the treated group, the chi-square showed a significant deviation ($P < 0.005$) for region II, and between uz and al. The various chi-square tests of this nature, on both control and treated groups are summarized in Table 19.

Besides the decrease in recombination frequencies attributable to 5-FUDR, it was also found to produce a weakly mutagenic effect at the concentration used in this investigation. In the M_2 population, three plants were found of two-row intermediate type, as shown in figures III and IV. Both parental stocks used were of six-row true-breeding type as were all the plants in F_1, F_2 and those grown in F_3 for the control. The origin of these two-row intermediate type could be the result of:

FIGURE III. Spikes of two-row intermediate type (centre) obtained in M_2 following 5-fluorodeoxyuridine treatment of previous generation, flanked by six-row parental types.

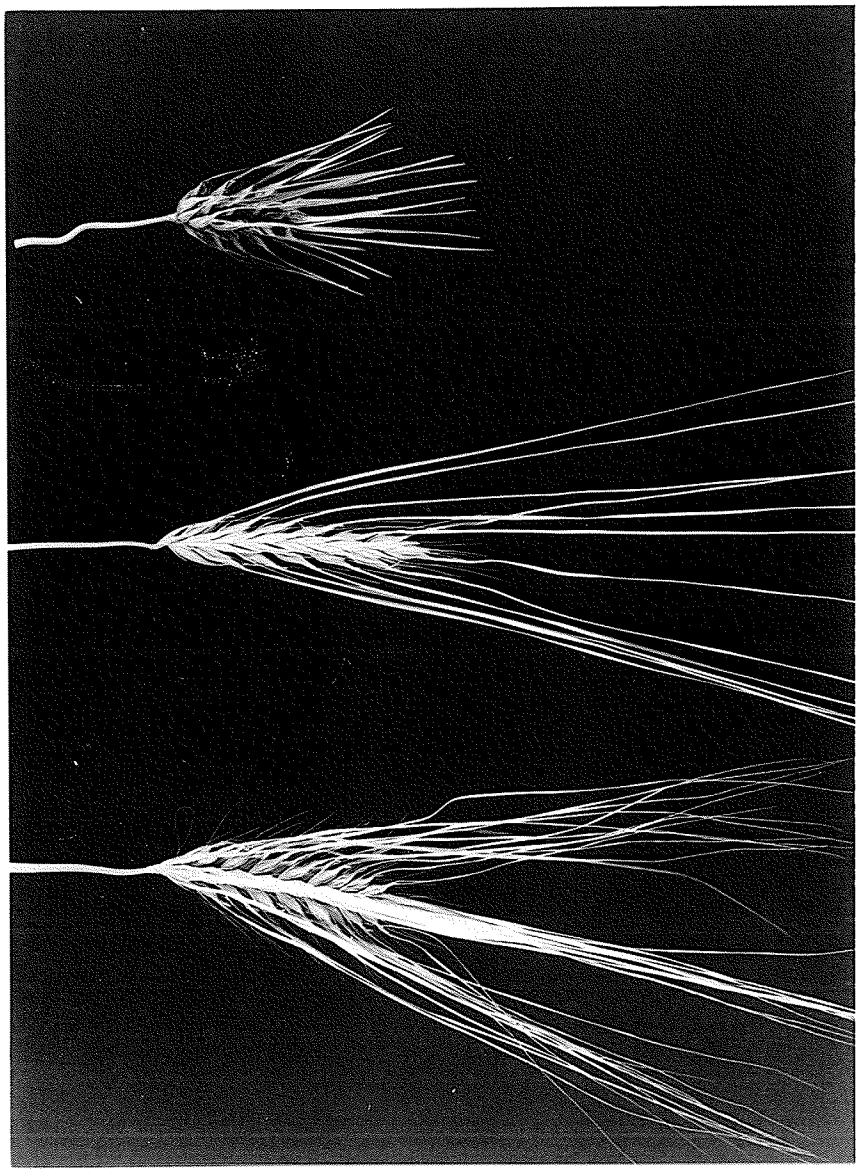

FIGURE IV. Spike of two-row intermediate type (centre) obtained in M_2 following 5-fluorodeoxyuridine treatment of previous generation, flanked by six-row parental types.

TABLE 19. Goodness of fit tests of observed class frequencies based on expected frequencies calculated from recombination values of control and treated groups in each region of a three-point linkage test.

Treatment		Classification XY	Xy	xY	xy	Total	Chi-square	Prob.
Control	Between uz-ys:							
	Observed	3573	1700	1752	4	7029		
	Expected (4.88%*)	3518.70	1753.06	1753.06	4.18	7029	2.453	0.750-0.500
	Expected (4.30%)**	3517.75	1754.00	1754.00	3.25	7029	2.705	0.500-0.250
	Between ys-al:							
	Observed	3545	1780	1694	10	7029		
	Expected (7.61%)*	3524.68	1747.07	1747.07	10.18	7029	2.353	0.500-0.250
	Expected (1.60%)**	3514.95	1756.80	1756.80	0.45	7029	205.480	0.005
	Between uz-al:							
	Observed	4908	365	331	1425	7029		
	Expected (10.39%)*	4925.56	346.19	346.19	1411.06	7029	1.826	0.750-0.500
	Expected (4.81%)**	5106.77	164.98	164.98	1592.27	7029	434.877	0.005
Treated	Between uz-ys:							
	Observed	256	118	138	0	512		
	Expected (4.88%)*	256.30	127.70	127.70	0.30	512	1.868	0.750-0.500
	Expected (4.30%)**	256.24	127.76	127.76	0.24	512	1.406	0.750-0.500
	Between ys-al:							
	Observed	256	138	118	0	512		
	Expected (7.61%)*	256.79	127.21	127.21	0.79	512	2.371	0.500-0.250
	Expected (1.60%)**	256.03	127.97	127.97	0.03	512	1.593	0.750-0.500
	Between uz-al:							
	Observed	361	13	13	125	512		
	Expected (10.39%)*	358.76	25.24	25.24	102.76	512	16.699	0.005
	Expected (4.81%)**	371.98	12.02	12.02	115.98	512	1.185	0.750-0.500

* Control recombination value

** Treated recombination value.

1. Mechanical mixtures or volunteers on the bench,
2. Out-crossing,
3. Mutation.

As segregation would tend to rule out the first alternative, the progenies were grown from each head of the two-row types. They segregated both two-row and six-row types (Figure V) as well as for awn length, and for Uzuz and Alal types. Among the two-row types the fertility of the lateral spikes - varied from 0.0 to 80.0 percent. The differential fertility of the lateral spikes is shown in Figure VI. The fact that segregation was obtained for parental genes under study rules out the possibility of mechanical mixtures and volunteers. As for the question of out-crossing it can be safely ruled out for two reasons. First, the heads were all bagged long before anthesis. Secondly, only six-row types were grown in the growth chamber. Thus the only possibility left, is mutation of the v gene to V. The segregations for awn length and the fertility of lateral spikes may have been due to the association of the complex locus I with its different allelic forms. No attempts however, were made to find out the genotype of the six-row types as regards the involvement of the i gene complex.

While checking the M_2 genotypes by growing M_3 lines, some of the lines showed segregation for xantha seedling

FIGURE V. Head types representing M_3 segregates in the progeny of a two-row intermediate type found in M_2 after treating the previous generation with 5-FUDR.

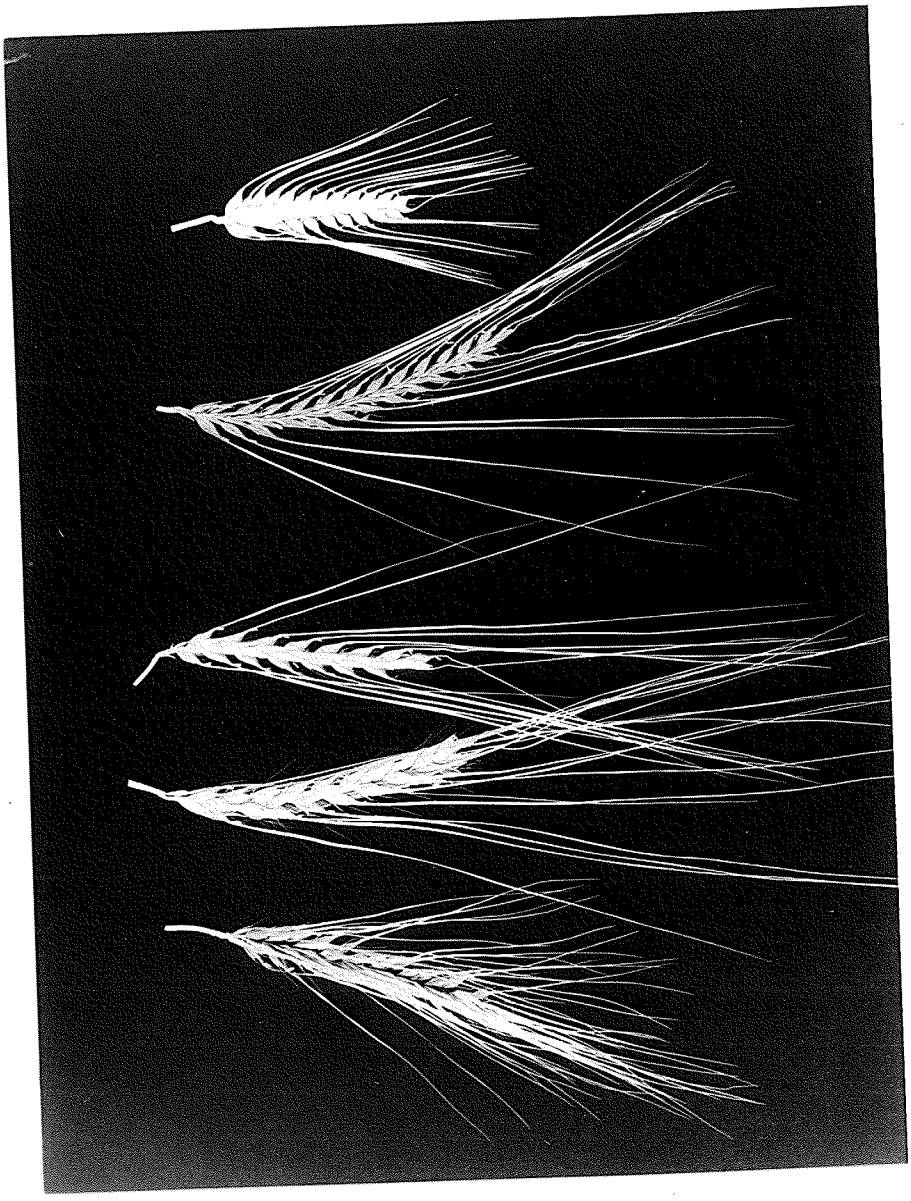

FIGURE VI. Spikes representing extremes in range of fertility of central florets of mutant progeny.

Left: Nil;

Right: 80 percent.

mutants. This may have been due to residual effect of the chemical or may have been of spontaneous origin. The overall mutation frequency on the M_2 plant basis was 0.585 percent. This observation lends support to the evidence, provided by the two-row mutants, that 5-FUDR is a moderately weak mutagen.

EXPERIMENT NO.IV

The effect was studied of diepoxybutane (0.04 percent) applied to plants heterozygous for two closely linked lethal genes in repulsion ($x_c+/+a_n$). The injection method of treatment was used. The F_2 plants were classified in the seedling stage into three appropriate phenotypic classes, i.e. green, xantha and albino, in both the treated and control populations. The individual gene segregations of $\underline{X_c}:\underline{x_c}$ and $\underline{A_n}:\underline{a_n}$ in both F_2 and M_2 generations, were in good fit to the expected ratio of 3:1 (Table 20). The overall ratio of viable green seedlings to nonviable xantha and albino seedlings together, also showed an excellent fit to the expected ratio of 1:1 (actual expected ratio .495 percent green to .505 percent xantha and albino) as shown in Table 21. An estimate of recombination values was made from the control and treated samples by Collins (1924) method, using only two phenotypic classes, green (a) and xantha (b) which indicated an increase in recombination in the diepoxybutane

TABLE 20. Observed frequencies of individual gene segregations in F_2 of control and di-epoxybutane treated populations of balanced lethal heterozygote stock.

	Genetic traits	Classes* X	Classes* x	Total	χ^2 for 3:1	Prob.
Control	$X_c:x_c$	309	89	398	1.4774	0.250 - 0.100
	$A_n:a_n$	299	99	398	0.0033	0.900
Treated	$X_c:x_c$	116	28	144	2.3703	0.250 - 0.100
	$A_n:a_n$	107	37	144	0.0370	0.900 - 0.250

* Dominant and recessive classes represented by capital and small letters (X and x) respectively.

TABLE 21. Observed frequencies of viable and nonviable plants in F_2 of control and diepoxybutane treated populations.

		Classifications* viable	nonviable	Total	χ^2 for 1:1	Prob.
Control	Observed	210	188	398		
	Expected	199	199	398	1.216	0.500-0.250
Treated	Observed	79	65	144		
	Expected	72	72	144	1.361	0.250-0.100

* Classifications represented by viable and nonviable for green seedlings and xantha and albino together.

treated portion. But because of the impossibility of distinguishing $\underline{a_n a_n\ x_c x_c}$ (albino) from $\underline{a_n a_n X_c -}$ (albino), the F_2 recombination percentages of $\underline{X_c x_c}$ with $\underline{A_n a_n}$ (32.71 $\pm$ 12.12) and (46.36 $\pm$ 13.75) for the control and treated respectively, were subject to considerable error. Hence, all the green plants of the F_2 were grown to maturity and a genotypic analysis of the F_2 plants was performed by growing and classifying the F_3 lines. Recombination values were calculated based on the doubly dominant F_2 plants classified into genotypes in F_3, with the help of the formulae and tables of Allard (1956) and Immer and Henderson (1943). Based on these calculations, recombination values of 5.637 $\pm$ 1.122 percent and 16.08 $\pm$ 3.75 percent were obtained in control and treated populations respectively. The final, combined estimates of the recombination values were obtained by calculating the weighted average of F_2 and F_3, based on the amount of information (Immer and Henderson, 1943), each source of data provided. The combined recombination values with their standard errors calculated from F_2 and F_3 data of both control and treated populations are presented in Table 22. All the control data for these markers from different experiments were homogeneous and were therefore pooled.

The chi-square test was made on the following four genotypic classes, based on the expectation recalculated

TABLE 22. Estimates of recombination values between x_c and a_n genes involving chromosome 3 of control and diepoxybutane (0.04 percent) treated populations.

Source of data	Cross	Linkage between	Linkage phase*	F_2 phenotypes a	b	c	d	Total	F_2 recombination value	S.E.	F_3 Doubly dominant e	f	g	h+i	Total	F_3 recombination value	S.E.	Combined recombination value and standard error
Control (pooled)	$x_c+/+a_n$	x_c-a_n	R	443	186	213**	-	842	0.3360	0.0812	1	0	12	210	232	0.0509	0.0113	0.0564 ± 0.0112
Treated	$x_c/+a_n$	x_c-a_n	R	79	28	37**	-	144	0.4636	0.1375	2	4	4	38	48	0.1364	0.0391	0.1608 ± 0.0375

* R = Repulsion

** Classes c and d could not be distinguished in the seedling lethals.

from the recombination values of control and treated groups.

Class	Genotype	Breeding behaviour
1.	++++	true breeding green plants
2.	+++a_n	segregating for green and albino
3.	+x_c++	segregating for green and xantha
4.	+x_c+a_n	segregating for all three

The genotype of the first class is made up of both cross-over gametes; the second and third classes are constituted of one cross-over and one non-cross-over gamete; while the third class is made up of two non-cross-over gametes. The chi-square test on the class frequencies of the control population gave a good fit to the expected, based on the 5.6 percent recombination value obtained from the pooled data, while the test based on frequencies calculated from a value of 16 percent the recombination value obtained from the treated population, showed a highly significant ($P < .005$). deviation from expectation. All the chi-square values calculated are summarized in Table 24.

The recombination values between $\underline{X_c x_c}$ and $\underline{A_n a_n}$, obtained from the control populations were consistently very close to the value reported by Burnham (1957) as well as to the value derived by interpolation from the data based on a multiple cross, as reported by Robertson et al (1965). Hence, the recombination value obtained in the present investigation appears to be quite reliable.

The recombination value obtained from the treated population compared to the value obtained from the control (Table 23) shows a highly significant ($P < 0.001$) increase from the diepoxybutane treatment. The direct increase from the treatment was 10.44 percent. Diepoxybutane has been shown to induce recombination and mutation in Aspergillus (Morpurgo,1963). Mutagenic action of the chemical has also been reported in barley, oats and maize (Ehrenberg and Gustafson,1957; Murphy and Patterson,1958; Kreizinger,1958). It is interesting to note, that the chemical is weakly mutagenic in barley and oats while a powerful inducer of somatic mutation in maize. In the present investigation, however, diepoxybutane treatment did not result in detectable mutation. The preliminary cytological examination of a random sample of treated heads did not show any chromosomal aberrations. Furthermore, the satisfactory fit to a 3:1 ratio of the individual gene segregations shows that the expected frequencies of both lethal genes are unaffected by the chemical. Thus the main effect of diepoxybutane was an increase in recombination; while neither affecting the transmission of pre-existing lethal genes, nor inducing any mutations or chromosomal aberrations. This investigation has produced evidence, therefore, that diepoxybutane, at the concentration used, had a powerful recombinagenic effect without inducing mutation.

TABLE 23. Effect of diepoxybutane (0.04percent) treatment on recombination value of two linked genes.

	Classifications				Total	Recombination value*	S.E.	Diff.	S.E. Diff.
	XY	Xy	xY	xy					
	X_c, x_c in relation to A_n, a_n								
Control	443	186	213**		842	0.0564	0.0112		
								0.1044	0.0391#
Treated	79	28	37**		144	0.1608	0.0375		

* Estimates of combined recombination value from F_2 and F_3 data.

** Classes xY and xy could not be distinguished in seedling lethal.

\# Significant at 1 percent level.

TABLE 24. Goodness of fit tests of observed genotypic class frequencies based on the expected frequencies calculated from recombination values of control and treated groups.

Treatment		Classes $X_cX_cA_nA_n$	$X_cx_cA_nA_n$	$X_cX_cA_na_n$	$X_cx_cA_na_n$	Total	Chi-square	Prob.
Control	Observed	1	12	9	210	232		
	Expected (5.6%)*	0.70	12.30	12.30	206.70	232	1.073	0.90-0.75
	Expected (16.0%)**	5.90	31.20	31.20	163.70	232	44.775	0.005
Treated	Observed	2	4	4	38	48		
	Expected (5.6%)*	0.15	2.54	2.54	42.77	48	25.027	0.005
	Expected (16.0%)**	1.23	6.45	6.45	33.87	48	2.847	0.50-0.25

* Recombination value obtained from control group.

** Recombination value obtained from treated group.

EXPERIMENT NO.V.

The effect of actinomycin D (50 µg/ml) was studied on two closely linked loci in repulsion phase ($+a_n/x_c+$) of linkage group 3. The drug was administered by the injection method of treatment. The treatment was lethal to 43.33 percent of the M_1 plants, while 7 percent of the water injected control plants died. The death in the control was likely due to mechanical damage caused during injection. Taking this into consideration, the remaining 36 percent of death in the treated population was attributable to the action of actinomycin D. This indicated that the drug was being absorbed by the cells in which it interfered with cellular metabolism thus causing death. This interpretation was indicated by the fact that cytological examination of the treated heads did not reveal any bridges and fragments showing that the drug did not cause any chromosomal aberrations.

The F_2 plants were classified into three appropriate phenotypic classes of green, xantha and albino in each of the control and the treated populations. In the control, the individual gene segregations, based on the expectation of a 3:1 ratio, gave a good fit for both $\underline{X_c}:x_c$ and $\underline{A_n}:a_n$ (Table 25) whereas in the treated population, the segregation for $\underline{X_c}:x_c$ was in good fit to the expected 3:1 but $\underline{A_n}:a_n$

TABLE 25. Observed frequencies of individual gene segregation classes in F_2 of control and actinomycin D treated populations.

	Genetic traits	Classes# X	Classes# x	Total	χ^2 for 3:1	Prob.
Control	$X_c : x_c$	347	97	444	1.453	0.250 - 0.100
	$A_n : a_n$	330	114	444	0.108	0.750-0.500
Treated	$X_c : x_c$	259	77	336	0.778	0.500-0.250
	$A_n : a_n$	272	64	336	6.349*	0.025-0.010

Dominants and recessive represented by capital and small letters (X and x) respectively.

* Significant at 5 percent.

deviated significantly from 3:1. The overall ratio of viable green seedlings to nonviable xantha and albino seedlings also fitted well to the expected ratio of 1:1 in the control, while in the treated population this ratio deviated significantly ($P < 0.005$) from expected 1:1, as shown in Table 26. In the treated population there was a significantly higher proportion of green plants than of the total of xantha and albino ones.

The recombination value was first estimated by Collins (1924) method from only two phenotypic classes, green (a) and xantha (b), in both control and treated. However, these values are subject to considerable error. All the green plants were therefore grown to maturity and an F_2 genotype analysis was performed by growing F_3 lines of both the control and treated groups. The recombination values were then calculated from doubly dominant F_2 plants classified into appropriate genotypes, with the help of the formulae and tables of Allard (1956) and Immer and Henderson (1943) and these are presented in Table 27 for the pooled control and treated groups. The combined estimates of F_2 and F_3 were obtained by weighted average, based on the total information provided by each source of data.

The chi-square tests were performed on the observed genotypic class frequencies, based on the expectation calculated from the recombination values obtained, using the

TABLE 26. Observed frequencies of viable and nonviable plants in F_2 of control and actinomycin D treated populations.

		Classifications		Total	χ^2 for 1:1	Prob.
		Viable	Nonviable			
Control	observed	233	211	444		
	expected	222	222	444	1.090	0.500-0.250
Treated	observed	195	141	336		
	expected	168	168	336	8.678	$<$0.005

TABLE 27. Estimates of recombination values between x_c and a_n genes in chromosome 3 of control and actinomycin D (50 micrograms per ml) treated populations.

Source of data	Cross	Linkage Between	Linkage Phase	F_2 phenotypes a	b	c	d	Total	F_2 Recombination value	S.E.	F_3 doubly dominant e	f	g	h+i	Total	F_3 Recombination value	S.E.	Combined recombination value and standard error
Control (pooled)	$x_c+/+a_n$	x_c-a_n	R*	443	186	213**	-	842	0.3360	0.0812	1	9	12	210	232	0.0509	0.0113	0.0564 ± 0.0112
Treated	$x_c+/+a_n$	x_c-a_n	R	195	77	64**	-	336	0.3882	0.1055	3	5	12	73	93	0.134	0.029	0.1505 ± 0.0269

*R = Repulsion

** Classes c and d could not be distinguished in seedling lethals.

classification described in the experiment No.IV. The chi-square values calculated from the control genotypic classes indicated a satisfactory fit to the expected frequencies, based on the 5.6 percent recombination values obtained from the pooled F_2 control data. On the other hand, the chi-square value obtained from the frequencies based on the 15.0 percent recombination value of the treated groups showed a highly significant deviation from the expectation ($P < 0.005$). Similarly the chi-square calculated from the observed frequencies of the treated population, based on expectation calculated using a recombination value of 15 percent, showed a good fit, while recalculating the expected classes using the recombination value obtained from the control groups produced a highly significant deviation in each case. The results of these tests are shown in Table 28.

Comparison of the recombination value of the control with that of the treated (Table 29) shows a highly significant difference. The value obtained shows that the chemical treatment resulted in recombination frequency more than two and a half times that in the control. It is unlikely that the difference in the proportion of viable and nonviable plants in the treated group compared with that in the control, was due to selective elimination of cross-over and non-cross-over gametes carrying the $\underline{a_n}$ gene. The chi-square test made on the three phenotypic classes of the M_2 population,

TABLE 28. Goodness of fit tests of observed genotypic class frequencies based on the expected frequencies calculated from recombination values of control and treated groups.

Treatment		Classes $X_cX_cA_nA_n$	$X_cx_cA_nA_n$	$X_cX_cA_na_n$	$X_cx_cA_na_n$	Total	Chi-square	Prob.
Control	Observed	1	12	9	210	232		
	Expected* (5.6%)	0.70	12.30	12.30	206.70	232	1.073	0.90 - 0.75
	Expected** (15.0%)	5.22	29.58	29.58	167.62	232	38.890	0.005
Treated	Observed	3	5	12	73	93		
	Expected* (5.6%)	0.29	4.92	4.92	82.87	93	36.688	0.005
	Expected** (15.0%)	2.09	11.86	11.86	67.19	93	4.868	0.25 - 0.10

* Recombination value obtained from control group.

** Recombination value obtained from treated group.

TABLE 29. Effect of actinomycin D (50 µg/ml) treatment on recombination value of two linked genes.

Treatment	Classifications				Total	Recombination value*	S.E.	Diff.	S.E. Diff.
	XY	Xy	xY	xy					
	X_c, x_c in relation to A_n, a_n								
Control	443	186	213**		842	0.0564	0.0112		
								0.0941	0.0291#
Treated	195	77	64**		336	0.1505	0.0269		

* estimates of combined recombination value from F_2 and F_3 data.

** classes xY and xy could not be distinguished in seedling lethal.

\# significant at 1 percent level.

based on the expected frequencies from F_2 control also showed a significant deviation ($P < 0.05$). This suggests that the deviation from the 1:1 ratio in the treated portion is real, and is attributable to the direct effect of actinomycin D and not due to disturbed individual gene segregation *per se*. However, that does not rule out the possibility that the drug may have reduced the frequency of gametes carrying the lethal gene a_n, as it has already been demonstrated that actinomycin D reduced, the frequency of lethal mutations in *Drosophila melanogaster* (Burdette, 1961). If this is so, then the action of actinomycin D must be considered gene specific, as it did not affect the frequency of the other lethal gene, x_c, involved in the present investigation.

GENERAL DISCUSSION

GENERAL DISCUSSION

The manipulation of recombination by any agent is an important area of research in genetics and plant breeding alike. In the field of genetics, the alteration of recombination by chemicals of known mode of action could lead to a better understanding of the mechanism or mechanisms involved in the process of recombination on both the cellular and molecular levels. In plant breeding, the importance of altering recombination has many aspects, but is especially important from the point of view of exploiting the potential of a cross more fully. Hanson (1961) has shown the importance of an increase in recombination by demonstrating mathematically that, in a self fertilized crop, a one and a half-fold increase in recombination is equivalent to intercrossing all of the F_2 progenies. It may also be desirable to reduce the recombination frequency in order to increase the rate of return to homozygosity in a heterozygous population.

COMPARISON BETWEEN TWO METHODS OF TREATMENT

Two methods of treatment, a vacuum immersion method and an injection method, were used in this investigation. In the vacuum method of treatment, it was possible to keep the growing points of the plants in contact with the chemicals for any length of time desired, while there are obvious difficulties in accomplishing this with the injection method

of treatment. Thus it appeared that by the vacuum method, plants could be treated without difficulty with any chemical solution at the desired stage of development. However, certain inherent drawbacks were found to apply with the vacuum method of treatment. First of all, it requires an evacuating mechanism and large, expensive glass tubes of proper length and shape. Therefore, its use is limited to places where vacuum pipes or some small evacuating pumps are available. Secondly, it takes a great deal of time for treatment of a substantial number of culms, especially when only one glass tube is used for both the treatment and control as was done in the present investigation. Thirdly, it was found difficult to use any wetting agent in this method because of foaming on evacuation. However, there are ways of improving the procedure. The method can be made portable by using small pumps run by an internal combustion power unit so that it can be used anywhere at any time. The actual time of treatment can be minimised by using a large number of glass tubes so that more than one plant can be treated at one time. Modification can also be made to achieve a fairly air tight system which can hold the solution with a wetting agent without making much foam.

On the other hand, the injection method of treatment can be used any where without any extra equipment. It also takes relatively less time to treat a large number of plants,

though mechanical injuries are more likely to occur than with the vacuum procedure. In the injection method of treatment, attempts were also made to keep the meiotic cells in contact with the chemical solution by filling the spaces around the sporocyte tissues at the apex of the culm enclosed in the leaf whorl a few hours before meiosis. In this way the injection method of treatment proved successful in three experiments of the present investigation. The vacuum and injection methods of treatment gave comparatively similar results in altering the recombination frequencies. The net effect of any chemical will depend more on the penetrating power of the chemical and stage of treatment than on the method of treatment as such. The injection method of treatment also seems superior to treatment of F_1 resting seeds, where the treatments are made many cell divisions prior to meiosis. By either of the methods employed in the present investigation, it was possible to treat the cells during meiosis or just prior to this stage, bearing in mind that the nature of cell division in plants is such that synchronous division can never be obtained in all the sporocytes of a single head.

From previously reported experiments, under both *in vivo* and *in vitro* conditions, it is apparent that the four chemicals used in the present investigation, are of quite different nature in their action on biological materials.

Diepoxybutane is an alkylating agent, the nature of which is to readily esterify anions, mainly the phosphate groups of nucleic acids are involved (Alexander,1952). The mutagenic action of this chemical has already been shown both in micro-organisms and plants (Kolmark and Westergaard,1953; Morpurgo,1963; Ehrenberg and Gustafson,1957; Murphy and Patterson,1958, and Kreizinger,1958). Diepoxybutane, a bifunctional agent, has been found to be more effective than monofunctional agents in inducing mitotic crossing-over and mutation in Aspergillus (Morpurgo,1963). However, so far as mutagenic action is concerned, alkylating agents, in general, are powerful mutagens irrespective of their functional groups (Auerbach,1958). It is interesting to note that diepoxybutane has been found to be a comparatively weak mutagen in barley and oats while a strong inducer of endosperm mutants in maize. In the present investigation, diepoxybutane produced a more than three-fold increase in recombination while giving no evidence of being mutagenic. Thus it appears that by esterifying the phosphate groups of nucleic acid, (involved in the formation of the nucleo-protein complex) the chemical may have caused structural changes in the chromosome thus stimulating recombination. Sobels and van Steenis (1957) have also shown, in Drosophila, that some of the most potent chemical mutagens, such as mustard gas, induced fewer cross-overs in relation to induced

mutation than weaker mutagens like organic peroxide and formaldehyde. Keeping in view the three-fold increase in recombination caused by diepoxybutane without inducing any visible mutation and considerations based on the findings with regard to other alkylating agents by Sobels and van Steenis (1957) and Morpurgo (1963), in other organisms, it appears that the mechanism or mechanisms involved in recombination may be different from those involved in the induction of mutation.

Kaufmann et al (1962) have made suggestions with regard to the essential role played by RNA in crossing-over. Actinomycin D, an inhibitor of DNA-dependent RNA synthesis, has been demonstrated to produce a four-fold increase in recombination in Drosophila melanogaster (Suzuki,1963,1965). The important role of RNA in the production of structural change in chromosomes has been demonstrated by Izawa, Allefrey and Mirsky (1963). They have suggested, on the basis of their experimental results, that "the morphology of an active chromosome site is not only closely related to its capacity to synthesize RNA, but is dependent upon it". In the present investigation the antibiotic produced a three-fold increase in recombination. It is also evident that the chromosome region involved in this study (x_c-a_n) is located very close to the centromere (Kasha and Walker,1960). If this evidence can be accepted as valid, there is a basic

similarity between the effect obtained in this region with actinomycin D and those obtained with the same chemical on a region close to the centromere of chromosome 3 in Drosophila by Suzuki (1963,1965),although the location of the centromere in barley is not as well defined as in Drosophila. Thus, based on consideration of these findings it appears most probable, as suggested by Suzuki (1963) that actinomycin D, by inhibiting DNA-dependent RNA synthesis may have caused structural changes in the chromosome region and in doing so stimulated recombination. However, the inhibition of RNA synthesis may not be the sole basis for the stimulatory effect on recombination, as diepoxybutane, a bifunctional alkylating agent, has given a similar increase in recombination in the same region of the chromosome. So far,there is no evidence that diepoxybutane affects m-RNA synthesis in a manner similar to that of actinomycin D.

A specific inhibitor of enzyme thymidylate synthetase in E. coli (Cohen et al 1958) 5-fluorodeoxyuridine, which blocks the conversion of deoxyuridylate to thymidylate (Taylor, Haut and Tung,1962), was used in one of the experiments of the present investigation to observe the genetic consequences of the inhibition of DNA synthesis on recombination. This analog reduced the overall recombination value of three linked loci under study to less than half that of the control. There was a differential effect on the two

chromosome regions involved, resulting in a much more pronounced decrease in one region of the chromosome than the other. As regards the location of these genes with respect to the position of the centromere, it appears that uz-ys is further away from the centromere than ys-al (Kasha and Walker, 1960). However, Kasha and Burnham (1965) have shown that the gene ys is close to the centromere. In any event ys-al which responded more than the other region uz-ys, to FUDR treatment appears to be closer to the probable location of the centromere.

Bonner and Zeevart (1962) using 5-fluorodeoxyuridine at 2 x 10^{-} M, the concentration used in the present investigation, reported 94 percent inhibition of DNA synthesis and 0 percent inhibition of the rate of RNA synthesis in developing influorescence primordia of cocklebur. Such a sharp decrease in DNA synthesis could be expected to have a depressing effect on a process such as recombination. High concentrations of FUDR have also been shown to cause high frequencies of chromosomal fragments with low frequencies of exchanges in the mitotic cells of Vicia faba (Taylor, Haut and Tung, 1962). Thus it appears that high frequencies of fragments with low frequencies of exchanges (reciprocal) would result in a decrease in genetic recombination.

For recombination to take place, two events have to follow. The first is synapsis of homologous chromosome and

the second is the crossing-over event. Recombination can be altered by affecting either or both events directly or indirectly. However, there is no way of differentiating between the two events. Taylor et al (1962) have given experimental evidence for the fact that "at least part of the linear axis of the chromosome is DNA". They have further indicated that the broken chromatids fail to rejoin in the absence of thymidylate.

Evidence of DNA synthesis at pachytene comes from the work of Hotta and Stern (1961), who found a small rise in thymidine phosphorylation during pachynema in Lilium and Trillium. Further evidence of DNA synthesis at pachytene have been given by Prensky (1962) and Wimber and Prensky (1963) in both plants and animals by autoradiographic study using tritiated thymidine. Since meiotic chromosome pairing occurs in pachytene(from zygonema to pachynema), any inhibition of DNA synthesis would disrupt the pairing or the rejoining of the broken chromatids and hence affect recombination. Thus it would appear that inhibitors of DNA synthesis would tend to decrease recombination. Phenylethyl alcohol, a specific inhibitor of DNA synthesis in bacteria, has been shown to cause inhibition of recombinants in phage T_4 (Folsome,1963). Based on consideration of these studies it seems reasonable to conceive that the decrease in recombination of FUDR treated groups in the present investigation

could be mainly associated with the inhibition of DNA synthesis. If this is so,it leads to the obvious conclusion that DNA replication is required for genetic recombination. However, Taylor et al (1962) indicated that "recombination in higher forms would not necessarily be limited to the principal DNA synthetic interval. It could occur before, during, and after this stage if homologous DNA helices were close enough together to rejoin with each other when broken.

Powell and Nilan (1963) have suggested the use of inversion heterozygotes in barley for screening new agents such as chemicals for their effect on recombination, based on the criteria of the number of bridges and fragments formed. However, chemicals causing chromosomal aberrations and also affecting recombination might lead to erroneous conclusions about the separate effect on recombination. This could occur in the case of 5-fluorodeoxyuridine, which has been found to cause high frequencies of chromosomal fragments and a moderate number of bridges in Vicia faba (Taylor, Haut and Tung, 1962) and has also been found to affect recombination adversely, as shown in the present investigation. Such fragments without bridges could be interpreted as an indication of crossing-over according to the criteria of Powell and Nilan (1963) e.g. two fragments with no bridge might be classed as a triple cross-over, indicating

increased crossing-over, which may not be the case. Thus, although an inversion heterozygote would be expected to give reliable estimates of recombination for certain chemical treatments, the utility of this technique as a screening tool is limited because of the diverse effects which have shown to be produced by different agents. However, the disadvantages of the inversion heterozygote are offset to some extent by precision with limited sample size, a factor which is a problem in closely linked genes in repulsion, as found in the present investigation.

SUMMARY

SUMMARY

Five experiments were conducted to study the effect on genetic recombination of four chemicals applied to barley at a stage just prior to meiosis. Three of the chemicals, namely 5-fluorodeoxyuridine, actinomycin D and para fluorophenylalanine were specific inhibitors of DNA synthesis, DNA dependent RNA synthesis and protein synthesis, respectively, while the fourth one was diepoxybutane, a bifunctional alkylating agent.

Two methods of applying the chemicals were tested, a vacuum-immersion procedure and hypodermic injection into the upper internode and the growing-point region. The latter was found more efficient in treating large numbers in a controlled environment.

Genes gl and zbc, reported to be closely linked on chromosome 4, were studied. These genes showed independent segregation in F_2 and backcross progenies. Trisomic analysis showed the locus gl to be on chromosome 4, probably close to the centromere based on a good fit to chromosome ratio, whereas zbc was shown not to be on this chromosome.

Plants heterozygous for the genes gl and zbc, treated with parafluorophenylalanine (10^{-3}M) gave disturbed individual gene segregations for both genes as well as showing quasi-linkage between genes involved. Diepoxybutane (0.01 percent) and 5-FUDR (2×10^{-4}M) treatments, on the other hand, showed quasi-linkage without giving disturbed individual gene segregations.

Diepoxybutane (0.04 percent) was found to induce about a three-fold increase in recombination between genes $\underline{x}_c$ and $\underline{a}_n$ on linkage group 3, as compared to 5.6 percent recombination found in the control portion.

Acintomycin D (50 microgram per ml) gave an increase in recombination approaching the three-fold level in the same chromosomal region ($\underline{x}_c$-$\underline{a}_n$). It also affected the frequency of expression of the lethal gene $\underline{a}_n$.

The effect of 5-fluorodeoxyuridine (2×10^{-4}M) was studied by treating plants heterozygous for three linked genes (uz+al/+ys+) in linkage group 3. The analog gave a highly significant over-all reduction in recombination between uz-al. The chemical treatment showed a differential effect on the two regions involved. A more pronounced effect was obtained in region II between ys-al than in region I between uz-ys. As these were in the repulsion phase, the realiability of the extent of the differential effect remains in doubt.

The importance of the manipulation of recombination by chemical treatments and the probable mechanisms involved were discussed.

LITERATURE CITED

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Abbadessa, R. and A.B. Burdick. 1963. The effect of X-irradiation on somatic crossing-over in Drosophila melanogaster. Genetics 48: 1345-1356.

Alexander, P. 1952. Interference with the formation of a nucleoprotein complex by radiomimetic compounds. Nature, 160: 226-228.

Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24: 235-278.

Auerbach, C. 1958. Mutagenic effects of alkylating agents. Ann.N.Y. Acad. Sci. 68: 731-736.

Beermann, W. 1965. Structure and function of interphase chromosomes. Genetics Today. Proc. XI Internat. Cong. Genetics. Vol. 2: 375-385.

Bell, S. and S. Wolff. 1964. Studies on the mechanism of the effect of fluorodeoxyuridine on chromosomes. Proc. Natl. Acad. Sci. (U.S.). 51: 195-202.

Berrah, G. and W.A. Konetzka. 1962. Selective and reversible inhibition of the synthesis of bacterial deoxyribonucleic acid by phenethyl alcohol. Jour. Bacteriol. 83: 738-744.

Bodmer, W.R. and P.A. Persons. 1962. Linkage and recombination in evolution. Adv. Genet. 11: 1-100.

Bonner, J. and J.A.D. Zeevart. 1962. Ribonucleic acid synthesis in the bud an essential component of floral induction in Xanthium. Plant.Phys. 37: 43-49.

Bridges, C.B. 1915. A linkage variation in Drosophila. J. Exp.Zool. 19: 1-21.

Bridges, C.B. 1935. Salivary gland chromosome maps. J. Hered. 26: 60-64.

Briggs, R.W. and H.H. Smith. 1965. Effects of X -radiation on intracistronic recombination at the waxy locus in maize. J. Hered. 56: 157-162.

Burdette, W.J. 1961. Alteration of mutation frequency by treatment with actinomycin D. Science. 133: 40.

Butler, L. 1958. Heterogeneity of recombination values in the tomato. Proc. X. Inter. Congr. Genet. Vol.II:39.

Clark, A.M. 1943. Linkage relations in Habrobracon and the effects of temperature, X-radiation and age on crossing-over. Proc.Pennsylvania. Acad.Sci. (U.S.) 17: 47-64.

Cohen, S.S., J.G. Flanks, H.D. Barner, M.R. Loeb., and J.Lichtenstein. 1958. The mode of action of 5-fluoro uracil and its derivatives. Proc.Nat.Acad.Sci.(U.S.) 44: 1004-1012.

Collins, G.H. 1924. Measurement of linkage values. J.Agr. Res. 27: 881-891.

Dowrick, G.J. 1957. The influence of temperature on meiosis. Hereditas. 11: 37-49.

Ehrenberg, L. and A. Gustafsson. 1957. On the mutagenic action of Ethylene Oxide and Diepoxybutane in barley. Hereditas. 43: 595-602.

Elliot, C.G. 1955. The effect of temperature on chiasma frequency. Heredity. 9: 385-398.

Eversole, R.A. and E.L. Tatum. 1956. Chemical alteration of crossing-over on Chlamydomonas. Proc.Nat.Acad.Sci. (U.S.) 42: 68-73.

Esposito, R. and R. Holliday. 1964. The effect of 5-fluoro-deoxyuridine on genetic replication and somatic recombination in synchronously dividing cultures of Ustilago maydis. Genetics 50: 1007-1017.

Folsome, C.E.1962. Topographical effects of ultra violet irradiation upon recombination. Chrift. fur. Vererb. 93: 404-416.

Folsome, C.E. 1963. Inhibition of recombination and heterozygosis in Phenyl Ethyl alcohol treated phage T4. E. coli B. complexes. Biochem. and Biophys. Res. Comm. 11: 97-101.

Gallant, J. and T. Spottswood. 1965. The recombinagenic effect of thymidylate starvation in E. coli. Genetics. 52: 107-118.

Goldberg, I.H.and M. Rabinowitz. 1962. Actinomycin D inhibition of deoxyribonucleic acid-dependent synthesis of RNA. Science. 136: 315-316.

Graubard, M.A. 1934. Temperature effect on interference and crossing-over. Genetics. 19: 83-94.

Griffing, B. and J. Langridge. 1963. Factors affecting crossing-over in the tomato. Australian. J. Biol. Sci. 16: 826-837.

Hanson, W.D. 1961. Effect of calcium and phosphorus nutrition on genetic recombination in the Soybean. Crop. Sci. 1: 384.

Hayman, D.L. and P.A. Parsons. 1961. The effect of temperature, age and an inversion on recombination values and interference in the X-chromosome of Drosophila melanogaster. Genetics. 32: 74-88.

Herskowitz, J.H. and S. Abrahamson. 1957. Induced changes in female germ cells of Drosophila. IV. Dependence of induced crossing-over like exchanges in oocytes and oogonia upon X-ray intensity. Genetics. 42: 444-453.

Herwitz, J., J.J. Furth, M. Malamy, and M. Alexander. 1962. The role of deoxyribonucleic acid in ribonucleic acid synthesis. III. The inhibition of enzymatic synthesis of ribonucleic acid by actinomycin D and proflavin. Proc.Nat.Acad.Sci. (U.S.) 48: 1222-1230.

Holliday, R. 1961.a. Induced mitotic crossing-over in Ustilago maydis. Genet.Res. 2: 231-248.

Holliday, R. 1961.b. The induction of mitotic recombination by mitomycin C in Ustilago and Saccharomyces. Genetics. 50: 323.335.

Hotta, Y. and H.Stern. 1961. Transient phosphoxylation of deoxyribosides and regulation of deoxyribonucleic acid synthesis. J. Biophys.Biochem.Cytol. 11: 311-319.

Hyde, B.B. and R.L. Paliwal. 1958. Studies on the role of cations in the structure and behaviour of plant chromosomes. Am.J.Bot. 45: 433-438.

Ijiima, T. and A. Hagiwara. 1960. Mutagenic action of mitomycin C on Escherichia coli. Nature. 185:395-396.

Immer, F.R. 1930. Formulae and tables for calculating linkage intensities. Genetics. 15: 81-98.

Immer, F.R. 1934. Calculating linkage intensities from F_3 data. Genetics. 19: 119-136.

Immer, F.R. and M.T. Henderson. 1943. Linkage studies in barley. Genetics. 28: 419-440.

Ives, P.T., B.S. Fenton, H.T. Yost, Jr. and R.P. Levine. 1953. The effects of infra red radiation and dessication on crossing-over in Drosophila melanogaster. Proc. Nat. Acad. Sci.(U.S.). 39: 1134-1141.

Izawa, M., V.G. Allefrey,and A.E. Mirsky. 1963. The relationship between RNA synthesis and loop structure in lampbrush chromosomes. Proc.Nat.Acad.Sci. (U.S.) 40: 544-551.

Kafer, E. and A. Chen. 1964. U.V. induced mutations and mitotic crossing-over in dormant and germinating conidia of Aspergillus. Microbial.Gen.Bull.20: 8-9.

Kasha, K.J. and G.W.R. Walker. 1960. Several recent barley mutants and their linkages. Canad. J. Genet. Cytol. 2: 307-415.

Kasha, K.J. and C.R. Burnham. 1965. The location of interchange breakpoints in barley. I. Linkage studies and map orientation. Can.J.Genet.Cytol. 7: 62-77.

Kaufmann, B.P. and M.R. McDonald. 1957. The nature of the changes effected in chromosomal materials by the chelating agent EDTA. Proc.Nat.Acad.Sci.(U.S.) 43: 262-270.

Kaufmann, B.P., H.Gray, and M.J.McElderry. 1957. Effect of ribonuclease on crossing over in Drosophila. Proc.Nat.Acad. Sci.(U.S.) 43: 255-261.

Kikkawa, H.1934. Studies on non-inherited variation in crossing-over in Drosophila. J.Genet. 28: 329-248.

Kirk, J.M. 1960. The mode of action of actinomycin D. Biochem. Biophys.Acta. 42: 167-169.

Kolmark, G. and M.Westergaard. 1953. Further studies on chemically induced reversions at adenine locus of Neurospora. Hereditas. 39: 208-224.

Kreizinger, J.D.1958. Diepoxybutane as a chemical mutagen in maize. Dissert.Abstr.Cornell Univ.Ithaca, New York. (L.C. Card No.58-5880).

Levine, R.P. 1955. Chromosome structure and the mechanism of crossing over. Proc.Nat.Acad.Sci. (U.S.).41: 727-730.

Levine, R.P. and W.T. Ebersold. 1957. Studies on crossing over in Chlamydomonas reinhardi (Abstr.) Rec.Genet. Soc.Amer. 26: 383.

Levine, R.P. and W.T. Ebersold. 1958a. The relation of calcium and magnesium to crossing over in Chlamydomonas reinhardi. Z. Verb. 89: 631-635.

Maling, B.A.D. 1959. The effect of environmental factors on crossing over in Neurospora crassa. Dissert.Abstr. Stanford Univ. (L.C. Card.No.39.Misc.39-3712).

Mayor, J.W. 1923. An effect of X-rays on linkage crossing over in Drosophila. Proc.Soc.Exp.Biol.and Med.20: 335-338.

Mayor, J.W. and H.K. Svenson. 1924a. An effect of X-rays on linkage of Mendelian characters in the second chromosome of Drosophila melanogaster. Genetics. 9: 70-89.

Mayor, J.W. and H.K. Svenson.1924b. A comparison of the effects of X-rays and temperature on linkage and fertility in Drosophila. Genetics. 9: 588-608.

Mazia, D.1954. The particulate organization of the chromosome. Proc.Nat.Acad.Sci.(U.S.). 40: 521-527.

McClintock, B.1943. Maize Genetics. Carnegie.Inst.Wash.Year book. 42: 148-152.

McNelly, C.A. and L.C. Frost. 1963. The effect of temperature on the frequency of recombination in Neurospora crassa. Genetics. 48: 900 (Abstr.)

Morpurgo, G.1963. Induction of mitotic crossing over in Aspergillus nidulans by bifunctional aklylating agents. Genetics. 48: 1259-1263.

Mucherjee, R.1965. Mutagenic action of mitomycin C on Drosophila melanogaster. Genetics.51: 947-951.

Muller, H.J. 1925. The regionally differential effect of X-rays on crossing over in autosomes of Drosophila. Genetics.10: 470-507.

Murphy, C.F. and F.L. Patterson.1958. The effects of chemical mutagens Diepoxybutane on cultivated oats. Proc.Iowa Acad.Sci.165: 184-189.

Nilan, R.A. 1964. The cytology and Genetics of Barley.1951-1962. Monographic Supplement No.3.Research Studies Wash. State Univ.

Ondrej, M.1964. Changes in crossing over frequency in barley caused by different concentrations of calcium magnesium.Biol.Plant.Acad.Sci.Bohemoslov 6 (3): 171-184.

Otsuji, N.M., M.Sekiguchi, T.Tjiima and Y.Takagi,1959. Induction of phage formation in the lysogenic Escherichia coli. K12 by mitomycin C. Nature.184: 1079-1080.

Powell, J.B. and R.A. Nilan.1963. Influence of temperature on crossing over in an inversion heterozygote in barley.Crop.Sci.3: 11-13.

Prakash, V.1964. Chromatid interference in Neurospora crassa. Genetics. 50: 297-321.

Prensky, W.1962. Uptake of thymine-methyl-H^3 by pachytene chromosomes of Lilium longiflosum. Genetics.47: 977.

Plough, H.H. 1917. The effect of temperature on crossing over in Drosophila. J.Exp.Zool. 24: 147-209.

Plough, H.H. 1921. Further studies on the effect of temperature on crossing over. J.Exp.Zool. 32: 187-202.

Plough, H.H. 1924. Radium radiation and crossing over. Am.Nat. 58: 85-87.

Reich, E., R.M. Franklin, A.J. Shatkin and E.L. Tatum.1961. Effect of actinomycin D on the cellular nucleic acid synthesis and virus production. Science.134: 556-557.

Reich, E.R., M.Franklin, A.J. Shatkin and E.L.Tatum.1962. Action of actinomycin D on animal cells and viruses. Proc.Nat.Acad.Sci.(U.S.) 48: 1238-1245.

Reich,E.1964. Actinomycin:Correlation of structure and function of its complexes with purine and DNA.Science.143: 684-689.

Rasmuson, M.1957. Unequal crossing over at the bar region of Drosophila melanogaster. I. High temperature as a means of inducing the frequency. Hereditas.43: 589-594.

Rasmuson, M.1961. Unequal crossing over in the bar region of Drosophila melanogaster. II. Influence of temperature, X-rays and EDTA. Hereditas.47: 358-378.

Rifaat, O.M. 1959. Effect of temperature on crossing over in Neurospora crassa. Genetics. 30: 312-23.

Robertson, D.W., G.A. Wiebe, R.G.Shands, and A.Hagberg.1965. A summary of linkage studies in cultivated barley. Hordeum species:Supplement III, 1954-1963. Crop Sci. 5: 33-43.

Robertson, D.W. 1957. Summary of linkage studies in barley 1953-1956. Abstracts of third barley improvement conference. Mimeographed, pp.37-46. Fort Collins, Colorado.

Robertson, D.W., G.A. Wiebe, and R.G.Shands.1947. A summary of linkage studies in barley:Supplement I.1940-1946. J.Amer.Soc.Agron.39: 464-473.

Schacht. L.E. 1958. The time of X-ray induction of crossing over and translocation in Drosophila melanogaster males. Genetics 43: 665-678.

Shatkin, A.J., R.M.Franklin and E.L. Tatum.1962. Effect of mitomycin C. on mammalian cells in culture. Biochim. Biophys.Acta.55: 277-289.

Shiba, S.A., T.Terawaki, T.Taguchi, and J.Kawamata. 1959. Selective inhibition of deoxyribonucleic acid in Escherichia coli by mitomycin C. Nature. 183: 1056-1957.

Smith, H.F.1936. Influence of temperature on crossing over in Drosophila. Nature. 138: 329-330.

Smith, L.1947. The aceto-carmine smear technique. Stain Tech. 22: 17-31.

Sobels, F.H. and H.Van Steenis. 1957. Chemical induction of crossing over in Drosophila males. Nature. 79: 29-31.

Sobels, F.H., D.Bootsma and A.D. Tates. 1959. The induction of crossing over and lethal mutations by formaldehyde food in relation to stage specificity. Notes and News. DIS. 33: 161-162.

Steffensen, D.1953. Induction of chromosome breakage at meiosis by magnesium deficiency in Tradescantia. Proc.Nat.Acad. Sci.(U.S.). 39: 613-620.

Steffensen, D.1955a. Breakage of chromosomes in Tradescantia with calcium deficiency. Proc.Nat.Acad.Sci.(U.S.) 41: 155-160.

Steffensen, D.1955b. Interaction of effects of metal ions in the production of spontaneous chromosomal aberrations in calcium deficient Tradescantia.(Abstr.)

Steffensen, D., L.E. Anderson and S.Kasa. 1956. Crossing over studies with Drosophila and maize.(Abstr.) Genetics.41: 663.

Stern, C.1926. The effect of temperature and age on crossing over in the first chromosome of Drosophila melanogaster. Proc.Nat.Acad.Sci.(U.S.).12: 530-532.

Stern, C. and V.Rentschler.1936. The effect of temperature on the frequency of crossing over in Drosophila melanogaster. Proc.Nat.Acad.Sci.(U.S.) 22: 451-453.

Suzuki, D.T. 1963. Studies on the chemical nature of crossing over on Drosophila melanogaster. I. Preliminary results on the effects of actinomycin D. Canad.J.Genet.Cytol. 4: 482-489.

Suzuki, D.T.1965a. Effects of actinomycin D on crossing over in Drosophila melanogaster.Genetics.51: 11-21.

Suzuki, D.T.1965b. Effects of mitomycin C on crossing over in Drosophila melanogaster. Genetics. 51: 635-640.

Swanson, C.P.1940. The distribution of inversions in Tradescantia. Genetics.25: 438-465.

Swanson, C.P.1961. Cytology and Cytogenetics. Englewood Cliffs, N.J. Prentice Hall Inc., Fourth printing Feb. 1961.

Sybenga, J.1960. Theeffect of gamma rays on frequency of localization of chiasmata in Crotalaria intermedia. (Abstr.) Radiation Res.12: 478.

Takahashi, R. and J. Hayashi.1959b. Linkage study of albino lemma character in barley. Ber.Ohara Inst.Landwirtsch. Biol.11: 132-140.

Taylor, J.H., W.F. Haut, and J. Tung.1962. Effects of fluorodeoxyuridine on DNA replication, chromosome breakage and reunion. Proc.Nat.Acad.Sci.(U.S.).48: 190-198.

Towe, A.M. and D.R.Stadler.1964. Effects of temperature on crossing over in Neurospora. Genetics.49: 577-583.

Wakonig, R. and T.J. Arnason. 1958. Some effects of the chelating agent EDTA on plant chromosome. Proc.Genet. Soc.Can. 3: 37-40.

White, M.J.D. 1934. The influence of temperature on chiasma frequency. J. Genet. 29: 114-129.

Whittinghill, M.1937. Induced crossing over in Drosophila males and its probable nature. Genetics. 22: 114-129.

Whittinghill, M. 1951. Some effects of gamma rays on recombination and crossing over in Drosophila melanogaster. Genetics. 36: 332-355.

Whittinghill, M. 1955. Cross over variability and induced crossing over. Symp.Genet.Recomb.J.Cell. Comp. Physiol.(Suppl.2) 45: 189-220.

Whittinghill, M. and B.M. Lewis. 1961. Clustered crossing overs from male Drosophila raised on formaldehyde media Genetics. 46: 459-462.

Wilkie, D. and D. Lewis. 1963. The effect of ultra violet light on recombination in yeast. Genetics. 48: 1701-1716.

Wilson, J.Y. 1959a. Temperature effect of chiasma frequency in bluebell, Endymion nonscriptus. Chromosoma.10: 337-354.

Wilson, J.Y. 1959b. Chiasma frequency in relation to temperature, Genetics. 29: 290-303.

Wimber, D.E. and W. Prensky. 1963. Autoradiography with meiotic chromosomes of the male newt (Triturus viridescens) using H^3 thymidine. Genetics.48: 1731-1738.

Yost, H.T. Jr. and R.N.Benneyan. 1957. The effects of combined radiation on crossing over in Drosophila melanogaster. Genetics 42: 147-160.