FACTORS AFFECTING CROSSING OVER IN THE TOMATO

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Summary

An experimental technique utilizing two seedling mutants permitted an extensive survey, involving over 129,000 observations, of environmental and genetic factors affecting crossover frequency in the tomato. A significant decrease in crossing over associated with aging was demonstrated for a plant pruned to bear fruit clusters only on the main stem. Aging appeared to be the main cause of significant differences in crossing over detected between data collected for different branches on an unpruned plant.

Although many experiments were made, including treatments with inorganic and organic chemicals, and grafts with other species and genera, no environmental factor gave significantly increased crossing over between the test loci. Pronounced decreases in crossing over were caused by increased sodium ion concentration, certain organic chemicals, and grafting on potatoes.

The genetic background was shown to have a highly significant effect in altering crossover frequency.

I. INTRODUCTION

Linkage can be a very important barrier in certain types of selection programmes which would be greatly facilitated if a means were available for increasing crossover frequency in breeding material. The objective in this study, then, was to search for appropriate chemicals or other means that may aid the plant breeder in minimizing the linkage problem.

It is well known that frequency of crossing over in various organisms can be markedly influenced by such factors as age, genotype, and temperature (see Swanson 1957 for review). More importantly for the present study, there have been reports that the frequency of recombination is strongly influenced by the ionic status of the organism's cells.

The concept that recombination frequency could be modified by adjusting the ionic status had its origin with the publications of Steffensen (1953, 1955) and of Mazia (1954). Steffensen found that the number of chromosomal aberrations was higher in *Tradescantia* plants grown in suboptimal concentrations of calcium or magnesium than in plants grown in optimum concentrations of these elements. Mazia reported the dispersal of chromosomes on treatment with the chelator, ethylenediaminetetraacetic acid (EDTA). He proposed that chromosomes were normally composed of macromolecular units linked together by bridges of the divalent cations calcium and magnesium.

These studies immediately suggested that crossover frequency could be increased by growing organisms in an environment deficient in calcium and magnesium. It

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was thought that a deficiency in these elements could be induced by limiting the amount of the elements in the organisms' culture media, or by supplying EDTA to chelate the calcium and magnesium ions in the cells of the organism.

This hypothesis was soon supported by a considerable amount of experimental evidence. Levine (1955) found that *Drosophila* larvae, fed on normal medium supplemented with 0·01M EDTA, possessed an increased crossover frequency. He also demonstrated that excess calcium fed to young adult females decreased crossing over. Eversole and Tatum (1956) found that crossing over between two loci in *Chlamydomonas reinhardi* was increased by treatment with EDTA or magnesium chloride. The effects of both treatments were reversed by subsequent incubation of treated cells in high concentrations of calcium and magnesium ions. Kaufmann, Gay, and McElderry (1957) reported a significant increase in crossing over in *Drosophila* after treatment with EDTA. Finally, Colwell and Burdick (1959) studied the uptake and effect of EDTA on crossing over and viability of *Drosophila* reared on medium containing various amounts of EDTA. They found crossing over increased at all EDTA levels.

Such experimental evidence strongly supported the EDTA hypothesis as formulated by Mazia, but more recently published data on the matter have been conflicting or directly contradictory to those quoted above.

Kaufmann and McDonald (1957) cast serious doubt on the reality of Mazia's findings, because they found no evidence that EDTA caused fragmentation of chromosomes. They interpreted the EDTA effect on chromosome structure as an alteration in colloidal properties of structural nucleoproteins due to modification of the general ionic environment of the cell rather than to selective removal of specific divalent cations from the chromosome. These authors suggested that any agent capable of altering the normal metabolism of the cell may produce chromosome instability. Levine and Ebersold (1958) failed, in every respect, to duplicate the results of Eversole and Tatum whose experimental procedures they followed as closely as possible. Levine and Ebersold could find no evidence that alteration of an ionic environment either by a chelating agent or a calcium deficiency caused significant alteration in the frequency of crossing over in *C. reinhardi*. Reanalysis of the EDTA data reported by Kaufmann, Gay, and McElderry (1957) by the present authors, using Fisher's (Fisher and Yates 1957) scoring method, suggests that these data are not consistent with the EDTA hypothesis. Finally, Hanson (1961) found no effect on crossing over in the soybean from the adjustment of calcium and phosphorus levels in the growth medium.

It appears, then, that the Mazian chromosome structure argument is not correct in its simplest form. It also appears that the results of the application of EDTA in increasing recombination frequency are not consistent. This is true even when the same experimental material is used. For example, the experiments of Eversole and Tatum (1956) and Levine and Ebersold (1958) used, not only the same species, *C. reinhardi*, but concentrated attention on the linkage between the same two loci. Also, in all *Drosophila* experiments essentially the same portion of the *X*-chromosome was used.

At the time the present experiments were commenced it seemed quite possible that EDTA could be sprayed on plants in a plant breeder's crossing nursery to increase recombination frequency and thus reduce the effects of linkage. Hence, the earlier experiments are primarily concerned with the effects of EDTA and various concentrations of calcium and magnesium.

Following the published results of Kaufmann and McDonald (1957), which suggested that any agent capable of altering the normal metabolism of the cell could produce chromosomal instability, the programme was expanded to study the effects of a large number of biologically active substances.

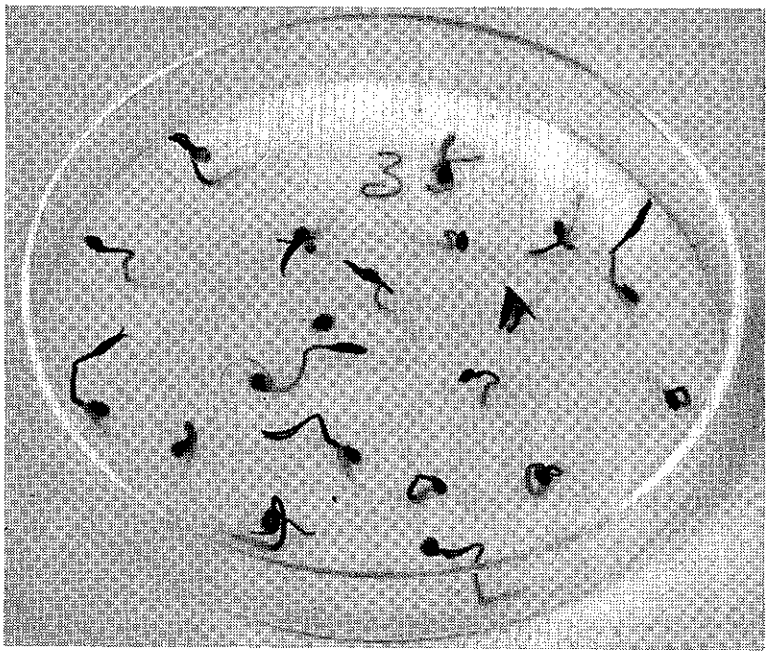

Fig. 1.—Tomato seedlings, 5 days after germination, at the stage at which segregation is scored.

Although the initial aim in this study was to obtain a chemical which could stimulate crossing over, it was decided also to consider other more general aspects of the control and modifiability of recombination frequency. The first aspect of this more general programme concerned the distribution of crossover frequencies as it occurred over the normal growing tomato plant. This is of interest in determining what flower position has the highest average crossover frequency, and is therefore best for purposes of hybridization. From the pattern of crossover frequencies, it is also possible to determine the feasibility of using the same plant for both control and treatment purposes.

Another more general aspect of the problem was the study of the effects of grafting on crossover frequency. It was thought that differences in nutrient uptake and differential transmission of metabolites from stock to scion might cause differences in recombination frequencies as measured in the scion.

Finally, the effects of different genetic backgrounds in changing recombination frequency were studied by transferring the marker loci to different tomato varieties.

II. Materials and Methods

The mutant stock containing two seedling mutations (*a*, anthocyaninless; *hl*, hairless) was kindly donated by Dr. C. M. Rick of the University of California. These marked loci are approximately 20 units apart in linkage group V. The four phenotypic classes are observable in the hypocotyl soon after germination. The procedure was to synthesize the coupling double heterozygote (*a hl*/++) to which the appropriate treatment or series of treatments was applied. Within a week after the fruits were harvested, the seeds were placed on moistened filter paper in petri dishes. These dishes were set in a dark incubator at 25°C for 2 days and then put under continuous light (Fig. 1). Usually the first scoring could be made within a week. Those seeds which had not germinated at the first scoring were covered with activated charcoal. In most cases this application stimulated germination and a second reading, a week later, usually finished the scoring.

The technique outlined above has several advantages:

(i) It allows a rapid accumulation of data. Crossing over information can be obtained from a treated plant within 3 weeks after the fruits are harvested.

(ii) It requires only a little effort to accumulate a large number of observations. Since the seedlings can be classified in the germination dishes, thousands may be scored without necessitating a large area of glasshouse or field space.

(iii) By stimulating germination with activated charcoal, a high germination is obtained and every seed may be accounted for. This is particularly important in linkage studies which utilize mutants with reduced viability and which do not include balanced crosses.

F_2 rather than backcross progeny were used exclusively. Since the marker genes are fairly closely linked in coupling phase, F_2 progenies are almost as efficient as backcross progenies. The loss of information per seed is more than compensated for by the increased seed production in selfed, rather than backcrossed, fruits. This is especially true when vibrators are used to ensure good fertilization of selfed flowers. Finally, the use of selfed seed obviates the immense amount of work involved in backcrossing.

The plants used for treatment were F_1's of a cross of *Lycopersicon pimpinellifolium* (Jusl.) Mill. × *L. esculentum* Mill. (tester stock). The wild species *L. pimpinellifolium* was used to introduce a maximum genetic range of quantitative variation so that treatment effects on linked polygenic systems could be studied if desired. However, this aspect of the study was not carried out. The F_1 is fully fertile.

III. Results

(a) *Pattern of Crossing Over in Control Plants*

The first objective was to determine the pattern of crossover frequencies as it occurs over the developing plant. The reasons for this aspect of the study are:

(i) To identify the stage of plant development during which maximum values for crossover frequency can be expected. This is useful from a plant breeding point of view.

(ii) To determine the feasibility of using crossing over data taken from the first few fruits produced by a plant to serve as a control for crossing over measured on the same plant at a later period after some specified treatment.

Both pruned and unpruned plants were examined. In all cases crossover frequencies were determined for individual fruits identified as to their origin in terms of cluster and branch. In Figure 2 the distribution of crossover frequency, based on 16,725 observations, is shown cluster by cluster for a mature plant. In the plant depicted, the crossover frequency for this chromosome segment varied from 6·3 to 20·7%. The notation MC1 is a code designating the first cluster borne on the main stem. Branches initiated above this cluster are designated by the number of nodes above MC1, i.e. B+5 denotes the branch arising from the fifth node above MC1. Similarly the notation, B−1, denotes the branch initiated on the first node below MC1.

The method of scores (see Fisher and Yates 1957) was used to test whether or not data from the various branches were homogeneous. For F_2 coupling data, the scoring function for the kth branch is defined to be

$$S_k = (a)\left[\frac{2(g-1)}{3-2g+g^2}\right]+(b+c)\left[\frac{2(1-g)}{g(2-g)}\right]+(d)\left(\frac{2}{g-1}\right),$$

and the information function is defined to be

$$I_k = n_k\left[\frac{2(3-4g-2g^2)}{g(2-g)(3-2g+g^2)}\right],$$

where a, b, c, and d represent observed numbers for the phenotypic classes A–B–, A–bb, aaB–, and $aabb$, respectively; g is the recombination value and n_k is the total number of observations for the kth branch.

To test the hypothesis that the data are homogeneous, the following chi-square was computed:

$$\chi^2 = \sum_k [S_k(\hat{g})]^2/I_k(\hat{g}),$$

where $S_k(\hat{g})$ is the score for the kth branch evaluated at that value of g which satisfies

$$\sum_k S_k(\hat{g}) \cong 0.$$

It was found that $\chi^2_{15} = 29{\cdot}30$, which implies highly significant differences between branches ($0{\cdot}010 < P < 0{\cdot}025$).

The data were grouped, therefore, into three categories: (i) those from clusters on the main stem, (ii) those from branches above MC1, and (iii) those from branches below MC1. With this classification, differences between categories were not significant, which suggests that the heterogeneity found to exist between branches is not directly due to the position of the branch in the plant structure. It was suspected that an aging effect was at least partly responsible for the divergent results between branches.

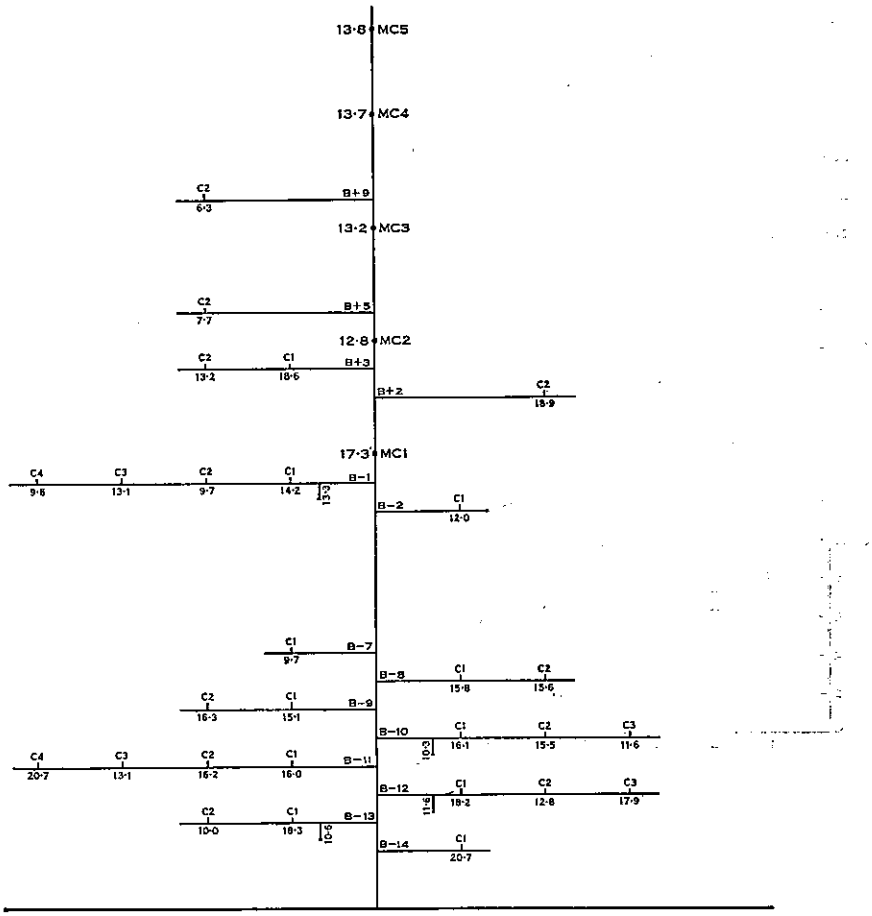

Fig. 2.—Pattern of development of crossover frequency over an unpruned plant.

The aging hypothesis was tested by an examination of pruned plants. In these plants all lateral shoots were pruned, leaving for study only the clusters borne on the main stem. Effect of age on crossing over would be expected to cause a reduction in recombination frequency as the cluster number increases.

Data consisting of approximately 10,000 observations from individual clusters of a single pruned plant were again analysed by the scoring method. A correlation

analysis was used involving cluster number and score variates which are defined as follows:

$$y_k = S_k(\hat{g})/[I_k(\hat{g})]^{\frac{1}{2}} \qquad (k = 1, \ldots 16).$$

It is assumed, under the null hypothesis, that the y_k are normal deviates: i.e. that y_k are normally distributed with zero mean and unit variance.

The correlation coefficient $r(= -0{\cdot}602)$ is highly significant, indicating that aging decreases crossover frequency. The actual trend in recombination values of fruit and cluster means for the 16 clusters is shown in Figure 3. Although a decrease in crossing over occurs, the drop is not great.

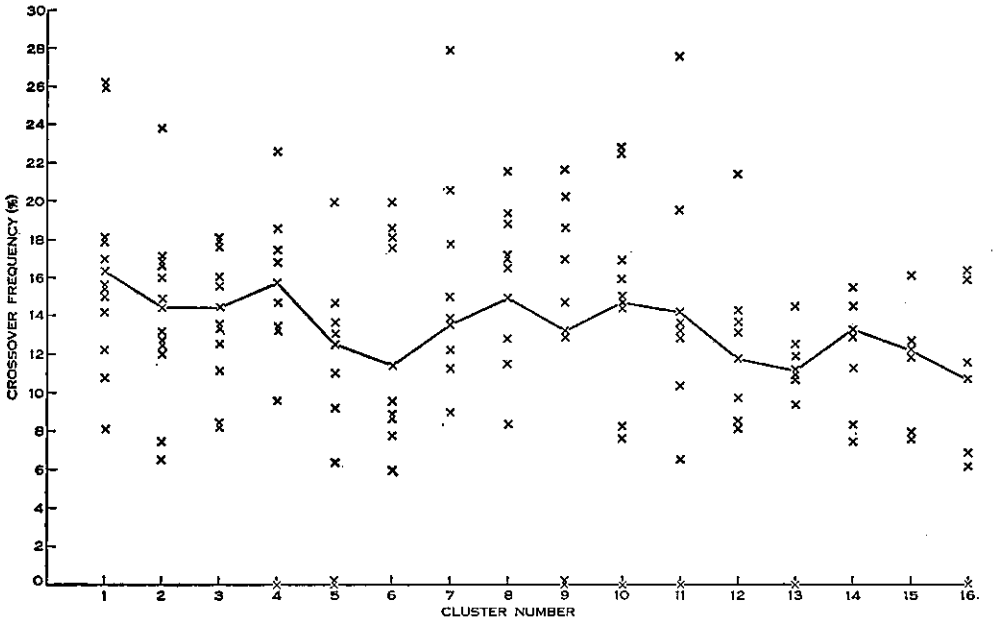

Fig. 3.—Pattern of development of crossover frequency over a plant pruned to the main stem. The points in the graph represent crossover frequencies for individual fruits.

Data from several other pruned and unpruned plants were collected and similar results were obtained. Fertilizer was periodically applied to plants in this experiment and the plants appeared to be in good condition at all times. All plants were grown in a glasshouse with temperature control which provided uniform conditions throughout the growing period. Therefore, it is believed that temperature effects did not contribute to recombination differences.

In summary, the results indicate that crossing with earliest flowers would be the best practice from a plant breeding point of view. However, it was decided that the aging effect would not cause sufficient disturbance to reject the use of built-in controls, if the plants were maintained in good condition. Hence, this procedure was often adopted.

(b) Effect of Inorganic Compounds on Crossing Over

In the following experiments several attempts were made to influence crossing over frequency by altering the ion content of tomato plants. Test plants were variously treated with the chelator EDTA, with foliar sprays of calcium and magnesium, and with nutrient solutions containing different levels of calcium and magnesium. Although over 35,000 seedlings were scored, none of these treatments could be shown to give unequivocal changes in crossover frequency.

In one experiment, the leaves adjacent to the cluster sampled were analysed for calcium, magnesium, potassium, and sodium content and the concentration of each cation plotted against crossover frequency (Fig. 4). There was no correlation

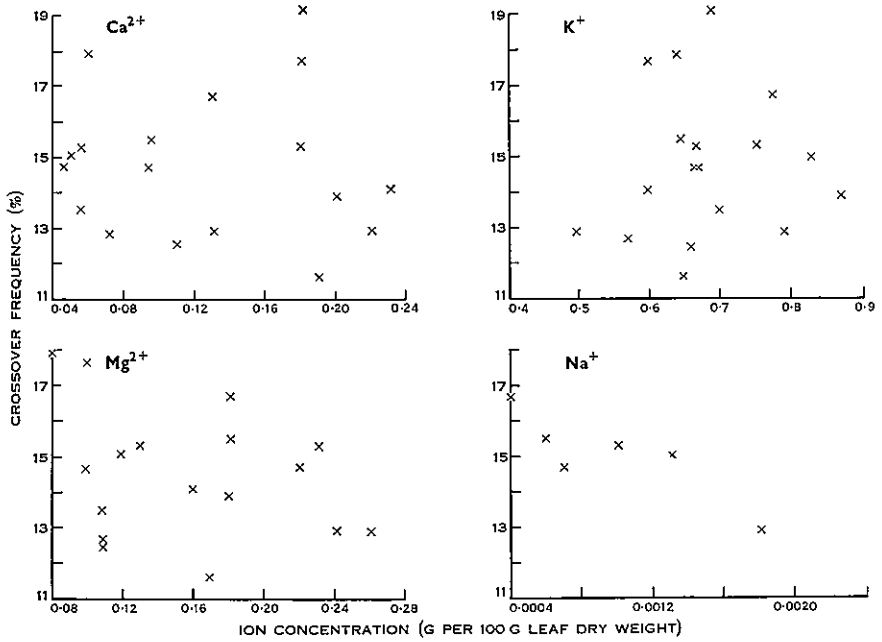

Fig. 4.—Variation in crossover frequency with the calcium, magnesium, potassium, and sodium content of immediately adjacent leaves.

between frequency of crossing over and concentration of calcium, magnesium, and potassium; thus, a nearly five-fold change in the calcium level of the leaf produced no change in recombination frequencies. However, a significant negative association ($r = -0{\cdot}87$) was found between sodium concentration and crossover frequency.

The fact that poor nutrition may affect crossing over was indicated by periodic examinations of a test plant which was kept growing for more than a year. The crossover frequency dropped from a value of over 17% to 6% in 6 months during which time fertilizer was not applied. Fertilizer was then added to the pot in which the plant was growing and the crossover frequency soon returned to 12%.

In a further test involving 7500 observations, foliar spraying with complete mineral solutions did not cause the treated plants to differ significantly from the untreated controls. However, the plants so treated were not so old as to have their crossover frequency much reduced below that of young plants.

(c) *Effect of Chemicals on Crossing Over*

The effect on crossing over of 21 chemicals when sprayed on test plants was assessed in a study involving over 27,000 observations. These chemicals (mutagens, hormones, anti-metabolites, metabolic inhibitors, etc.) were chosen for their known pronounced biological activities. Solutions of the chemicals were prepared in concentrations known to be active on other systems, adjusted to pH 6·0, and sprayed at weekly intervals on test plants for 3 weeks. The effects of the chemicals on recombination frequency are shown in Figure 5. A slight, but not significant, increase was caused by 3-indolylacetic acid and benzthiazoleoxyacetic acid. Barbituric acid, streptomycin, and ribonucleic acid apparently lowered crossover frequency. However, as decreased crossing over was not of interest, these substances were not re-tested.

(d) *Effect of Grafting on Crossing Over*

In an attempt to determine the influence of the root system on crossing over, a series of grafting experiments were made which yielded a total of 13,000 observations. The test plants were grafted on another tomato variety (Suttons Best of All), on other *Lycopersicon* species (*L. pimpinellifolium*, *L. peruvianum*, and *L. hirsutum*), and on related genera (*Capsicum annuum*, *Solanum tuberosum*, and *Datura stramonium*). Grafted and ungrafted controls were included. Grafting on *S. tuberosum* produced the only significant result which was a pronounced decrease in recombination frequency.

(e) *Genetic Modification of Crossing Over*

Influence of the genetic background on frequency of crossing over between the two test loci was examined by a series of backcrosses to eight different parental stocks including *L. pimpinellifolium*, several cherry fruit size ecotypes of *L. esculentum*, and several commercial varieties (*L. esculentum*). The results reported here pertain to stocks which have been backcrossed to the parental line three times.

The crossover frequencies were recorded for 20 plants, i.e. an average of 2·5 plants per line. The total number of progeny scored was 19,671, approximating the goal of 1000 observations for each plant. The mean recombination values given in Table 1 range from 9·7% in *L. pimpinellifolium* to 21·0% in the Ponderosa variety.

The statistical analysis was based on normal deviates derived from the scoring system as indicated earlier. Thus the variate

$$y_{ij} = S_{ij}(\hat{g})[I_{ij}(\hat{g})]^{-\frac{1}{2}},$$

is defined for the jth plant in the ith stock, where $\hat{g}$ is that value which satisfies

$$\sum_{i,j} S_{ij}(\hat{g}) \cong 0.$$

The analysis of variance for the data is also given in Table 1. It is clear that the different genetic backgrounds cause highly significant differences in crossover frequency. The analysis also suggests that differences among plants within lines persist.

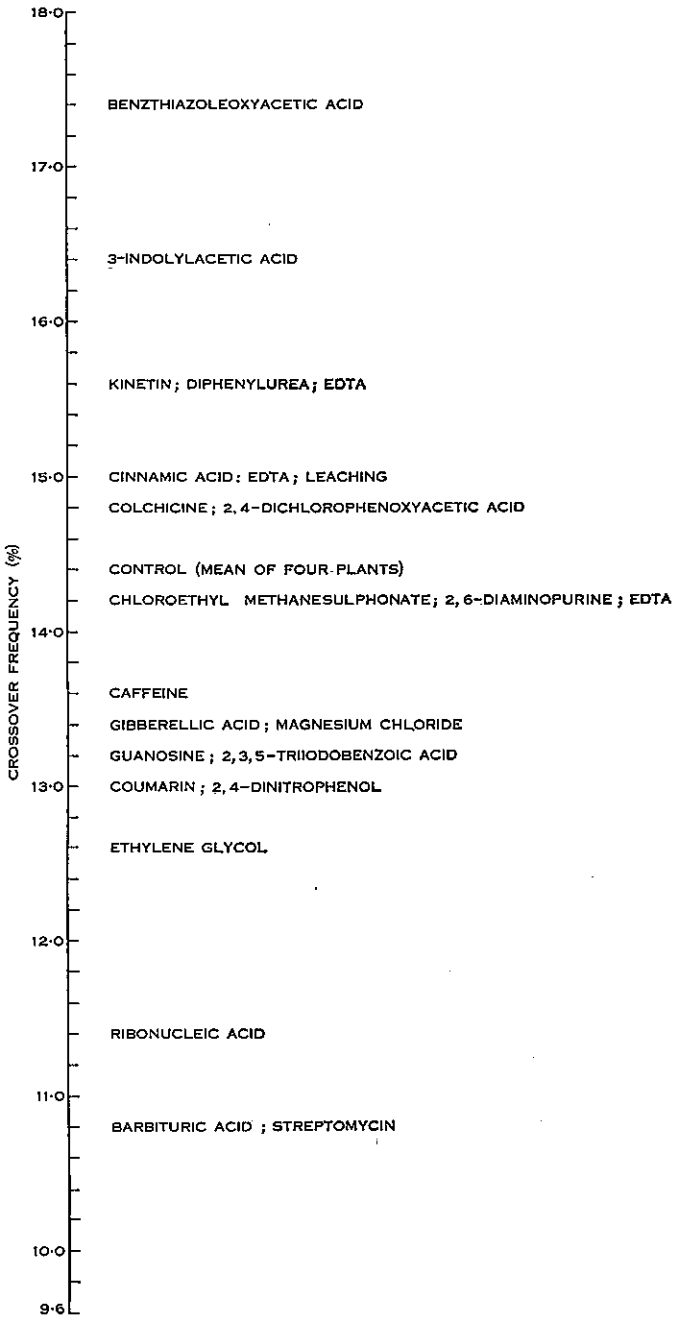

Fig. 5.—Effect of chemicals on crossing over shown by their position on a scale of recombination frequency.

IV. Discussion

From the experimental data obtained, it is evident that the problem of increasing crossing over in plants by the manipulation of environmental stimuli remains unsolved. The maximum crossover frequency in the tomato appears to be a characteristic of the genotype brought about by apparently unknown and uncontrollable factors. Such treatments as did have a significant effect decreased crossover frequency. The depressing agents discovered included sodium ions, certain chemicals, and grafting on potatoes. The only treatment successful in raising the level of crossing over was the addition of nutrients to the soil when the crossover frequency had first been depressed by age.

Table 1

CROSSOVER FREQUENCIES FOR DIFFERENT TOMATO STOCKS INTO WHICH THE TEST LOCI HAVE BEEN BACKCROSSED

Tomato Stock	Plant Number				Mean Crossover Frequency (%)
	1	2	3	4	
L. pimpinellifolium	9·7	9·7			9·7
Florida cherry	14·8	16·6			15·7
Phillipine cherry	20·8	18·0			19·4
Red cherry	14·6	15·3			15·0
2n-ex-haploid	19·3	18·3	18·2		18·6
Goldball	15·9	19·0	16·7	17·0	17·2
Suttons Best of All	13·8	18·3			16·1
Ponderosa	18·6	23·3	21·2		21·0

Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square	Probability
Between stocks	7	15·230	$P < 0·005$
Between plants within stocks	12	1·588	$0·05 < P < 0·1$

With respect to the tomato plant, the results suggest that an upper limit to crossing over is set by the genotype, is reached in young healthy plants, and cannot be further increased. Tomato breeders should, therefore, maintain crossing material in good nutrition and use flowers from the first clusters where possible.

The upper limit of crossing over may, however, be changed by altering the genetic background. This fact suggests that it may be wise to screen tomato breeding stocks for recombination frequency, and, in the long run, to select for increased

frequency. As a corollary to the postulate of a genetically fixed maximum crossover frequency, experimental treatments are likely to be effective only when crossing over has dropped below this maximum. This prediction is yet to be properly tested.

V. Acknowledgment

We are indebted to Mr. J. R. Twine for the inorganic analyses.

VI. References

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