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The Effects of Colchicine on Germination and Polyploid Development of Watermelons

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THE EFFECTS OF COLCHICINE ON GERMINATION AND POLYPLOID DEVELOPMENT OF WATERMELONS

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"The man who classifies facts of any kind whatever, who sees their mutual relationships, and describes their sequences, is applying the scientific method and is a man of science."

-------Pearson

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INTRODUCTION

From the study of the Principles of Genetics[1], it has been determined that certain traits pass as a unit to one group of progeny while some other traits pass as a unit to another group. It must be kept in mind that the only mode of hereditary transmission as determined by the writers referred to, is through the reproductive cells or gametes of the parents. It, also, must be kept in mind that the gametes of the parents differ in something which determines various differences in their progeny. This something which appears to pass through the reproductive cells and which appears to influence a particular trait in the offspring is known as a factor or gene.

It was Robert Browne in 1831 who revealed the fact to the world that cells are made up of definite component parts and that one of these important components is the nucleus. In the continued process of analysis of the cell, it has further been pointed out by O. Hertwig, Van Beneden, Boveri and Wilson,[2,3] expert cytologists, who worked and wrote during the nineteenth and twentieth centuries, that this nucleus is made up of chromosomes and that these chromosomes are the carriers of the genes or factors which cause one individual or organism to vary from another.

The discoveries resulting from the experiments of Gregor Mendel, 1866, made possible the prediction with a considerable degree of accuracy, not only of what the offspring might look like, but also the relative frequency with which contrasting characters brought in from various ancestral lines will appear.

It was Charles Darwin, 1859, who foreshadowed the modern view of the question of variation by recognizing that not all variations were due to a single set of causes and that not all variations were equally heritable.

Variations[1] in individuals were divided into three main groups: (1) those which are due to differences in the environment; (2) those which are due to reappearance and recombination of genetic factors; (3) and those which are due to mutation, the origin of which is not thoroughly understood.

However, it was left for Weismann, 1883,[1] to establish the distinction that many variations caused by the environment are artifically induced.

Probably, it was this distinction that has led modern experimenters [4,5,6] to use a variety of methods to produce chromosome changes which in turn will cause variations in the character of their products.

The alteration of a gene has become known as "gene mutation".[1] Gene mutations that occur in nature or in cultivated plants or animals that have not been treated to produce them are called spontaneous mutations. These changes produced by some treatment are said to be "induced."

These mutations may occur at various stages in the organism's development. They may effect only a single somatic cell which, as in color variation in the endosperm of maize or in the epidermis of flowers or leaves, shows a specific difference from surrounding cells; or they may occur at earlier stages in which case all the descendants of the mutated cell may exhibit the changed condition and thus produce sectors of greater or smaller size which differ from the rest of the individual.[7]

The clearest evidence to this effect is that which Stadler, 1936, has obtained from the irradiation of barley and maize.[7]

Thus, recent experimenters, Beams and King,[8] Sando,[9] Nebel and Ruttle,[10] Brues and Cohen,[11] have been making use of the drug, colchicine, as a stimulus in producing chromosomal changes in individual plants. In some instances, they have been able to produce diploid, haploid, tetraploid and polyploid individuals.

Colchicine is a narcotic alkaloid related chemically to morphine and codeine. It is a very potent and poisonous substance whose immediate effect on growing tissue even in very small concentrations, is to produce stunting and distortion.[12] It offers no "magical royal road" to the production of new varieties of plants and animals. In spite of the discouragement that is shown over-enthusiastic popularizations, the effect which colchicine has on doubling the number of chromosomes in the cells makes it unquestionably one of the most important genetic discoveries of recent times.

Colchicine is said to interfere with the normal cytokinetic processes of a cell and may actually affect the form and structure of the chromosomes.[13] Ludford, 1936, and others noted the following general effects upon chromosomes: abnormalities in size and number, alteration of colchicine affected surface, and fusion and contraction. The administration of colchiin suitable concentrations exerts an inhibitory influence on dividing cells.[4] On the other hand, chromosomes of cells so treated may divide thus giving rise to a polyploid condition. The condition of the cell brought about by colchicine may be considered as an injury by poisoning; and if this poisoning be long continued, it may cause the death of the cell.

The biological and economic significance of polyploids are well established among agricultural products. So far as we have been able to determine, very little experimental work, comparatively speaking, has been conducted with watermelons. Recently colchicine has been very successfully used in producing polyploids in many plants; such as: Nicotiana species,[14] the pine,[15] ryegrass,[16] wheat,[8] and barley.[8]

During recent months, reports have been accumulating which indicate that the efficiency of the drug, colchicine, in producing polyploid individuals is widespread among higher plants. Observations on the immediate effects of the alkaloid in cells and tissues are, however, not so numerous.[17]

J.G.O'Mara[17] observed the effects of colchicine treatments for different periods and concentrations and recorded the results. The hypertrophy usually associated with the treatment was found not to involve the meristem but the region of elongation. He found that the colchicine affected chromosomes are more accurately measurable than ordinary somatic chromosomes. This may indicate that the drug can be profitably used in studies of chromosome morphology in somatic tissues.

C.F.Poole in his study of the "Genetics of Cultivated Cucurbits"[18] includes data from some watermelon crosses made at Charleston,S.C., not elsewhere reported. It is stated that in 1937 only one half dozen characters in watermelon had been investigated, whereas, today the number has increased to twenty-five qualitative characters determined by fifteen pairs of alleles in which a series of three alleles and thirteen genes determine inheritance of fruit weight in one cross and back cross sufficiently advanced for presentation.

Shimamura[19] soaked tomato seed in a colchicine solution. Elongation of the primary root of the seedling appeared inhibited and stems became thick; a few tetraploids were obtained. A 2 % solution mixed with lanolin placed between the cotyledons before the leaf developed and allowed to act on the growing point, gave still better results.

B. Schmack [20] in showing the effects of colchicine on S. splendens placed eight drops of a 0.5% solution of colchicine on the cotyledon, one drop every three hours. One plant obtained was considered tetraploid since it had larger stomata and pollen grains than normal plants, and altered length and breath ratio of the leaf, protruding styles and a more compact inflorescence. This plant is being propagated vegetatively.

Kihara and Kishimoto [21] found that seedlings produced in colchicine solutions could be transplanted and produced abundantly then studied cytologically. They found that tetraploids are produced abundantly in a .02 % solution. Diploids and mosiacs consisting of diploid and tetraploid cells also occur. They found, also, that the tetraploids so obtained are indistinguishable from normal tetraploids.

Munting and Runquist[22] in "Notes on Some Colchicine Induced Polyploids," treated several different species of plants with colchicine. In most instances, dry seeds were treated with .05% or .025% solutions of colchicine for from three to six days. Chromosome doubling was obtained in some cases.

In showing the effects of colchicine on Petunia, Perak[23] applied four drops of a .5% aqueous solution of of colchicine every three hours. The original material is diploid. Plants which appeared to be polyploid, proved to have larger stomata, broader leaves and larger pollen . Twenty-eight chromosomes were found in the metaphase. Diploid and tetraploid cells were observed in the plants with roughened leaves which showed their myxaploid nature.

Lora Bond of the University of Wisconsin [24] in an article "Colchicine Stimulation of Seed Germination in Petunia Axillaris" found that the germination of Petunia Axillaris seeds was stimulated by colchicine.There was an increase in the number of seeds that germinated as well as a reduction in germination time. The optimum concentration for germination,.04%, was high enough to induce polyploidy. Growth of seedlings was seriously retarded only by colchicine in concentrations of .1% of stronger. In view of these results, it seems that seed treatment using weak solutions of colchicine rather than strong should yield satisfactory results in the practical production of a large number of polyploid plants.

Mendes [25] obtained polyploid cottons through the use of colchicine. Cytological observations were made in Octoploid Gossypium hirsutum.

Mirov and Stockwell[15] in "Colchicine Treatment of Pine Seeds" reported that results on the effects of colchicine treatment on the seeds of Pinus ponderosa revealed a wide variety of effects: particularly in the meristem. Some plants were more affected by the treatment than others. Some of the results indicate that it may be possible to produce stable polyploid plants that can be used in plant breeding.

The effect of colchicine on roots was shown by Dragoni and Crison.[26] Onion and bean plants were cut in two, one half being placed in water and the other half in .1% colchicine. The results indicate that(1) colchicine slows and then completely inhibits root growth; (2) it has a primary stimulating and a secondary inhibitory effect on mitosis; and (3) it causes a disturbance in the post prophase mitotic cycle.

In inducing tetraploidy in perennial rye grass by means of colchicine, Myers[16] placed dry seeds in petri dishes on blotting paper moistened with .1%, 0.2%, or .4% aqueous solutions of colchicine. Sufficient solution was used to provide moisture conditions that seemed optimum for germination. For this purpose about 5.5 c.c. of the solutions were used. Treatment was continued for 5, 24, and 96 hours for each concentration of colchicine, after which the seeds were transferred to blotting paper moistened with tap water. The seed treatment with colchicine delayed germination, reduced the total number of seedlings obtained and caused the production of abnormal seedlings.

With these results in mind, the present investigation was undertaken in an attempt to determine and observe the nature of some of the direct results of the application of the drug, colchicine, to the watermelon seed. The data that is reported in the performance of this experiment came directly as a result of my own observations of the germinating seed, the time required, the percentage of germination and the observation of the components of the affected cells. The observation of the affected cells was made possible by the preparation of slides from the root-tips obtained during the germinating process.

The experiment was carried out in the Research Laboratory of the college during the month of June and the early part of July, 1944.

METHODS AND MATERIALS

There are several methods of using colchicine on plants in order to induce chromosome doubling. The treatment of the seeds with colchicine with a wide range of concentrations is effective in inducing branches with doubled chromosome number.

Methods other than seed treatment include the "Immersion Method" in which flexible stems are immersed in the solution. The effect is greater if the stems or leaves are cut before immersion.

Still other methods are in use such as the method of mixtures with lanolin, capillary string, single drop method, atomizing method and the meristem method.[4]

In a preliminary experiment, watermelon seeds were soaked in a 2% solution of colchicine for 24 hours, 72 hours, 96 hours, 144 hours, 168 hours and 216 hours respectively. Twenty-five seeds in separate covered containers were soaked in 10 c.c. of the colchicine solution. At the expiration of the stated times, the seeds were transferred to moist chambers between two layers of absorbent paper. Seeds soaked in water for corresponding lengths of time were transferred to moist

First Experiment
Started June 13,1944

Showing Concentration of Solution, Hours of Exposure
Observations and Results

Table I

% of Colchicine used	HRS. # of Exposure	No. set to soak on June 13		Date Transferred to moist chamber		Condition of seed when transferred		No. Germinating		Appear. of root-tip		Seedling
		Control	Col	Con.	COl	Con.	Col.	Con.	Col.	Con.	Col.	
2%	24	25	25	June 14	June 14	Normal	Nor.	1	2	small	small	None
2%	72	25	25	June 16	" 16	"	" "	-	2	Small	"	None
2%	96	25	25	17th	17th	"	" "	3	None	small	"	None
2%	144	25	25	19th	9th	molded	Molded	None	None		"	
2%	168	25	25	20th	20th	"	" "	"	"		-"	
2%	216	25	25	22nd	22nd	"	" "	"	"		"	

chambers as a control.

Out of an assortment of two hundred and fifty seeds, only eight showed signs of germination. Four were in the controls and four were from the treated seeds. See Table I

This failure to germinate was probably due to the effects produced by this concentration of colchicine as well as to the fact that the seeds used appeared to be immature.

After further research and consideration, it was decided that in this second trial, weaker solutions would probably give better results. So, .1%, .2% and 1% solutions of colchicine made up with distilled water were used for the seed treatment. Tap water was used for the controls.

In this particular instance, only one method of seed treatment was used. This method was soaking the dry seeds in the colchicine solution and then transferring them to moist chambers between absorbent paper and keeping them at room temperature.

Fifteen hundred seeds were selected for the experiment. They were set apart in lots of fifty.

Fifteen small glass jars were selected and grouped in sets of five. In each of the first five jars , fifty seeds were put to soak in 25 c.c. of a .1% solution of colchicine for 24 hours, 48 hours, 72 hours, 96 hours and 120 hours respectively.

In each of the next five glass jars, fifty seeds were put to soak in 25 c.c. of a .2% solution of colchicine for the same periods of time. Another group of jars each received fifty seeds in 25 c.c. of a 1% solution of colchicine for the same periods of time. For each group of colchicine soaked seeds, fifty seeds were soaked in 25 c.c. of tap water to act as the control. These seeds were placed in small glass staining dishes.

At the expiration of the designated time, each set of seeds was transferred to a moist chamber and placed between four layers of absorbent paper for germination.

These seeds were watched carefully. They were kept moist by watering at least three times daily. The seeds were germinated at room temperature.

The action of these seeds was watched closely. As soon as enough root-tips became about three millimeters long, they were dissected off. These tips were fixed in a modification of Navashin's fluid or fixative. This fixative was suggested by Myers[16] as giving the most satisfactory results when used on the root-tips of rye grass. The watermelon root-tips that had been fixed were finally mounted on slides.

Dehydration and infiltration with paraffin were accomplished by the ethyl-alcohol series. Sections were cut by the use of the microtome. These sections were cut seven microns thick. The sections were then stained with Harris' hematoxylin and double stained with eosin.[27,28]

OBSERVATIONS AND RESULTS

On the basis of work done by such previous investigators as Mendes,[25] Dragon and Crison,[26] Bond,[24] it is to be expected that when watermelon seeds are treated with colchicine, some changes, internal of external, will occur.

In order to detect these changes, careful observations at regular intervals had to be made. The seeds were watched carefully for swollen root-tips, general enlargement of any part, signs of decay or any other abnormal phenomena.

The third day after the seeds had been transferred to the moist chambers, germination began. The seeds treated for 24 hours with a .1%, .2% and 1% solution of colchicine had one, two and three tips respectively. The seeds treated with a .2% solution of colchicine for 48 hours showed no germinations at this time. Those treated with a 1% solution of colchicine had one visible root-tip. Each day the seeds showed a marked increase in the number of seeds germinated. The highest percentage of germination for a period of seven days came from the seeds which had been treated with a

Table II. Showing Concentration of drug, results for 24 hours

% of colchicine	Condition of seed when transferred		Appearance of root-tips		Percentage of germination	
	Control	Colchicine	Control	Colchicine	Control	Colchicine
.1%	Normal	Normal	Long, thin	Short, thick	43%	43%
.2%	"	"	Long, thin, curved	Short thick no root hairs	36%	44%
1%	"	"	Long, thin	Short thick	22%	42%

Table III. Seed Treatment for 48 hours

% of colchicine	Condition of seed when transferred		Appearance of root-tips		Percentage of germination	
	Control	Colchicine	Control	Colchicine	Control	Colchicine
.1%	Normal	Normal	Thin runners	Short, thick	34%	30%
.2%	"	"	"	"	34%	32%
1%	"	"	"	"	26%	32%

Table IV. Seed Treatment for 72 hours & Results

% of colchicine	Condition of seed when transferred		Appearance of root-tips		Percentage of germination	
	Control	Colchicine	Control	Colchicine	Control	Colchicine
.1%	Normal	Normal	Thin	Short, thick	22%	10%
.2%	"	"	Long, thin runners	Short, thick	42%	36%
1%	"	"	"	"	12%	8%

Table V. Seed Treatment for 96 hours & Results

% of colchicine	Condition of seed when transferred		Appearance of root-tips		Percentage of germination	
	Control	Colchicine	Control	Colchicine	Control	Colchicine
.1%	Normal	Normal	Thin, long	Short, Thick	10%	14%
.2%	"	"	"	"	42%	28%
1%	"	"	"	"	6%	6%

Table VI. Seed Treatment for 120 hours & Results

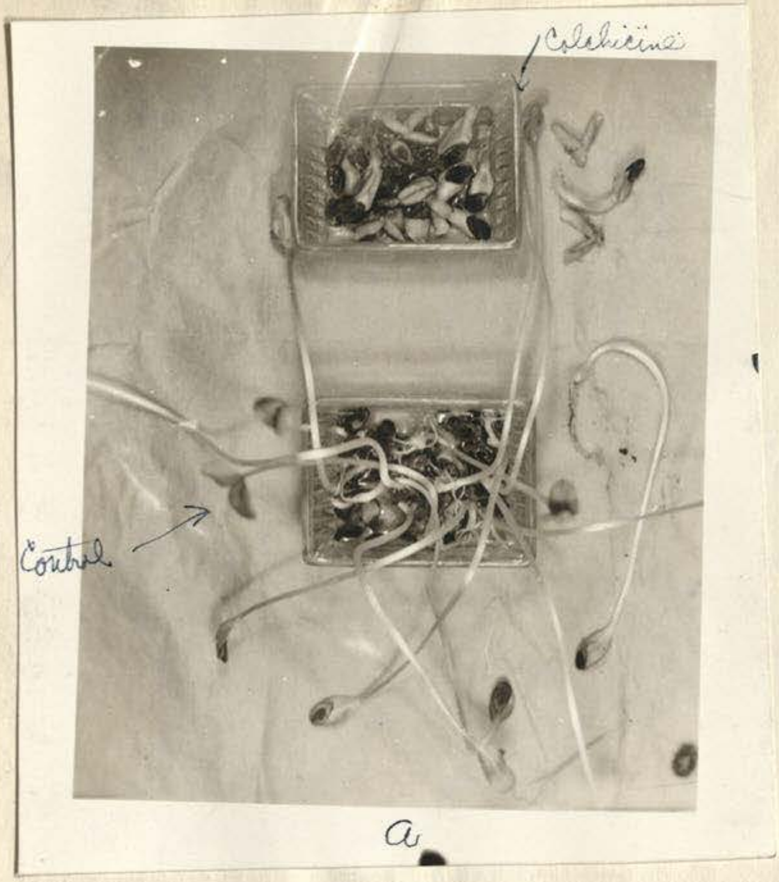
% of colchicine	Condition of seed when transferred		Appearance of root-tips		Percentage of germination	
	Control	Colchicine	Control	Colchicine	Control	Colchicine
.1%	Molded	Molded	Thin	Short thick	12%	8%
.2%	"	"	"	"	20%	16%
1%	"	"	"	"	10%	8%

.1%, .2% and 1% solution of colchicine for 24 hours.

A .2% solution of colchicine in which the seeds were treated for 72 hours, gave four root-tips on the third day after they were transferred to the moist chamber, and eighteen the seventh day afterwards. The same concentration for 96 hours showed no germinations the third day, five germinating seeds the fourth day, and fourteen germinations the seventh day. Tables II, III,IV,V,VI show the variations in the percentage of germination for the different concentrations of colchicine for the five periods of time.

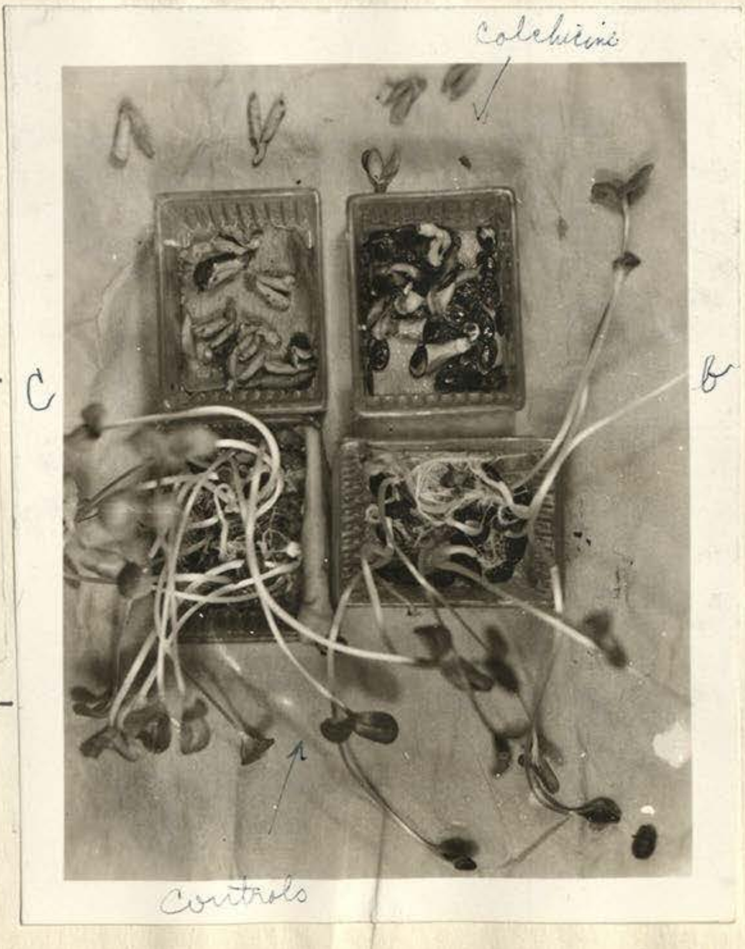
The lowest percentage of germination came from the seeds treated with a .1%, .2% and 1% solution for 96 hours and 120 hours respectively. Following these long treatments, the seeds showed a great tendency to mold. Of the seeds treated for 120 hours, those from the .2% solution gave the highest percentage of germination.

From these observations, we noted that the long periods of treatment yielded the lowest percentage of germination. From two to three seeds of each lot treated with a .2% solution of colchicine for 72, 96 and 120 hours, showed a tendency to swell and split the seed coat. This was not noticeable among the seeds treated



a. Showing the seedlings from seeds treated in a .1% solution of colchicine.

Lower view shows seedlings from untreated seeds



c. Top view shows seedlings from seeds treated with a .2% solution of colchicine.

Bottom shows seedlings from untreated seeds.

b. Top view shows seedlings treated with a 1% solution of colchicine.

Bottom shows untreated seedlings.

with other concentrations of the drug for shorter periods.

The seedlings from the treated seeds lacked the vitality shown by the seedlings from untreated seeds. They were never erect nor were they able to push out from under their moist paper cover. On the other hand, each control of untreated seeds produced seedlings that grew erect and raised themselves several inches above the sides of their containers. These plants appeared to be turgid, green and well developed.

Of the seeds treated in the various concentrations of colchicine, none showed a tendency to decay. However, those seeds that were treated with the different solutions of colchicine for 120 hours, showed a marked tendency to mold. To help the situation as much as possible, all of the seeds were washed carefully in tap water before being transferred to the moist chamber.

Root-tips were taken from the seedlings that developed from seeds treated with a .2% solution for 24 hours, 48 hours, 72 hours and 96 hours. These tips made more than six hundred sections for slides.

A study of these root-tips by means of the microscope, showed that the cells in the treated root-tips were smaller and that their cell walls were less distinct than those of the untreated root-tips. Slides prepared from the root-tips of the untreated seeds soaked for 24 and 48 hours, respectively, in tap water, showed large and distinct cells in which the chromosomes could be distinguished. On the other hand, colchicine treated root-tips in each concentration showed in all instances an increase in the chromosomes and a decrease in the size of the cells. Apparently, these cells were crowded together or fused. Their cell walls could be distinctly seen only under high power or in oil immersion.

The normal number of chromosomes in watermelon is eleven pairs or twenty-two chromosomes.[12] A study of thirty-six slides and several hundred sections showed, as well as the writer could determine, in all instances, an increased number of chromosomes.

DISCUSSION OF RESULTS

Previous workers (Smith,[14] Myers,[16] Dorsey[6]) with colchicine have reported the occurrence of abnormal growth types as a result of treatments with this drug, and have found that these abnormalities were related to the presence of tissue with doubled or increased chromosome number.[16]

It has previously been pointed out that too much eannot be expected from the use of colchicine. al-though it is a very potent and a very poisonous sub-stance[12] that is capable of producing stunting and distortion, it will also cause an increase in the num-ber of chromosomes in a cell.[14,15]

To further explain the presence of an increased number of chromosomes, one might add that there is evi-dence that frequently in the history of organic life,[29] a cell too feeble to maintain itself alone under exist-ing conditions, has been able to survive by merging its resources with those of another individual, thus making of the two cells one, with the double number of chro-mosomes. Occasionally, however, in the higher plant, a cell in the diploid state may function as a gamete uniting with an ordinary gamete to form a triploid individual. The point to note is that whatever else might happen, this action on the part of cells increases the number of chromosomes.

In the estimation of the writer, this offers an explanation for the apparent increase in the number of chromosomes in the cells of the colchicine treated root tips.

The colchicine treated seeds in our experiment which were subjected to the drug in a weak concentration for 72, 96 and 120 hours, showed, in most cases, no roots. This may be accounted for from the fact that colchicine being a poison, produces on the seeds a toxic effect. In other words, the toxin present in the drug tends to slow up action; therefore, the root-growth is partially or completely inhibited. [26]

This inhibitory influence of colchicine may be noted from the fact that seedlings from seeds so treated were lethargic in that they lacked energy and vitality.

There was better growth in weak solutions because in such solutions the strength of the toxin had been decreased. This in turn decreased the toxic effects on the cell tissue in the same proportion.

The first group of seeds which we attempted to use in the performance of this experiment, failed to germinate. Failure to germinate, according to Oran Raber[30], may be due to any of six causes when water, air, the proper temperature and all favorable external conditions are supplied. They are:

1. The seed coats are too hard for the growing plant to open.

2. The seeds are impermeable to oxygen.

3. The seeds are impermeable to water and therefore do not swell quickly.

4. The embryos are not mature.

5. The embryos require a period of "after ripening."

6. Secondary dormancy has set in.

Apparently this failure to germinate should be attributed to the immaturity of the embryos rather than to the strength of the colchicine solution used, since the untreated seeds failed to germinate also.

The apparent swelling and enlargement noted in the root-tips of the treated seeds complies with the results obtained by many former workers (Myers,[16] Dragoniand Crison[26]) who have explained this appearance as being due to the presence of an increased number of chromosomes in the cells of those regions.

The counting of the chromosomes of the cells in order to determine the changes in number that had occurred in the cells, proved rather difficult in spite of the fact that the cells were examined under high power and in oil immersion.

It is hoped that future experimenters will be able to employ better methods along with better equipment, in order to carry forward the work of this nature.

Babcock and Clausen[31] have observed that fragmentary as is the present knowledge of the various ways in which the internal organizations of chromosomes may become altered, there is reason to doubt that many and diverse methods are involved in the instances which have come under observation. In most cases, the adequate study of these rare types of mutation necessitates a more extensive knowledge of the normal genetic situation than is available at the present in most forms. It is therefore well nigh certain that as information about genetics of species is extended, numerous phenomena belonging in this category will come to light and will be subjected to accurate experimental analysis. There is reason to believe that some of the obscure phenomena of inheritance exhibited by certain horticultural forms will not be satisfactorily analyzed until methods of dealing with these phenomena shall have been perfected.

SUMMARY

1. The root-tips of seeds treated in colchicine were abnormally thich and short.

2. There was a greater percentage of germination from seeds treated with a weak solution of colchicine for a short time than from seeds soaked in concentrations of the same strength for long periods of time.

3. Plants from the treated seeds were less active and less energetic.

4. Plants from treated seeds appeared stunted and distorted.

5. Cells of treated root-tips are smaller and are crowded to the point of fusion.

6. Colchicine treated cells showed an increased number of chromosomes.

7. Further work will have to be done to determine the effects of the colchicine treatment on the development of the fruit.

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BIOGRAPHY

The writer, a native of Marlin, Falls County, Texas, is the teacher of science and the assistant teacher of music in the Booker T. Washington High School of that city. She received her elementary and junior high school training in the local school and continued this training in Prairie View Normal and Industrial College, Prairie View, Texas. After graduating from this institution, she entered Fisk University, Nashville, Tenn. where she continued her training on the college level. Later, she received her Bachelor's Degree from Wiley College, Marshall, Texas.

She is interested in the advancement of her group and does many things to promote this advancement. She participates in the religious, civic and social activities of her locality.

At present she is serving as Vice-President of the Texas Federation of Colored Women's Club and she is also a member of Theta Chapter of the Zeta Phi Beta Sorority.