CERTAIN MORPHOLOGICAL CHANGES INDUCED IN PLANTS OF TWO IPOMOEA SPECIES BY THE COLCHICINE TREATMENT

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CHAPTER I

INTRODUCTION

Most of the species and varieties of Ipomoea cytologically investigated are diploid, with the basic chromosome number 15. The two known exceptions are I. ramoni, a tetraploid with 60 chromosomes and I. batatas, a hexaploid with 6 sets, or 90 chromosomes. Theoretically, the tetraploid form might have arisen from a diploid through mutational chromosome doubling, and the crossing of a diploid and a tetraploid might have given rise to a triploid, which, through chromosome doubling would produce a hexaploid type. Because it is believed that the history of I. batatas was comparatively short, it seems possible to repeat its phylogenic course of development.

For the late 20 years, colchicine had been widely using as a polyploidizing agent with many plant species and therefore it is expected that the drug will have the same effect when applied to members of the genus Ipomoea.

The objectives of this study are to study the morphological variations as well as variations in fertility induced by colchicine treatment so as to gain some knowledge and experience in the use of this chemical on Ipomoea.

CHAPTER II

REVIEW OF LITERATURE

A. THE USE OF COLCHICINE AS SPINDLE POISON

Colchicum is named (9) for the land of Colchis at the Eastern tip of the Black Sea. In this area, the plant is most abundant. Taxonomists recognize 65 species in this genus, and all are limited to the Northern Hemisphere, although none are reported in the Americas. The original Greek name for this plant is Colchicon, as given by Dioscorides, was later changed by Linnaeus into the Latin form of Colchicum, and the binomial name affixed to the autumn crocus as C. autumnale L. in 1753.

The genus Colchicum L. belongs to the family Liliaceae. The genus is divided into two subgenera, namely: (i) Archicolchicum and (ii) Eucolchicum, of the second subgenus is C. autumnale L. belonging to. All species under the genus Colchicum analyzed to date yield colchicine of different concentrations. All species under the second subgenus bloom in autumnand sent leaves and fruits the following spring. Seeds are a rich source of colchicine after maturation, but commonly, the drug is extracted from the underground corms. The corms reach a peak of colchicine content at about June or July and before the flower stalks appear.

In nature, (25) colchicine is of limited significance for the induction of polyploidy. This is explained by the fact that the amounts of colchicine liberated from undamaged plants are limited and also that colchicine may be inactivated rather rapidly in or close to the soil.

Spindle poisons used for chromosome doubling were known long before

colchicine, but none of them was so successful as colchicine. The action of narcotics was first studied on spindles on animal cells. Nemec observed that chloral hydrate arrested mitosis in plant cells which was later demonstrated as being very similiar to colchicine-mitosis. Heat alone can also inactivate the spindle and in fact, this was the method mostly used before treatment with colchicine was known.

The first experiement with plants and colchicine was made by Sir Charles Darwin in 1875, who applied the drug to "insectivorous" and "sensitive" plants. The reactions in leaf movements were tested, but no conclusive results were obtained. Then in 1937, Dustin (9) discovered that colchicine was a polyploidizing agent, and since then, the wide interest that led to "colchicine fad" developed. Future progress in agriculture, medicine, pharmacy, biology and chemistry will obviously be fasciliated much by the possession of such a tool as colchicine.

There are two reasons that all the other methods to induce chromosome doubling are replaced by treatment with colchicine: (i) Colchicine is very effective for making polyploids with many different species, and (ii) The drug is applied easily to young growing plants with very little damage being done to them. Besides, colchicine is highly soluble in water, comparatively less toxic to plant and is effective in concentrations ranging from 1.0 to 0.01 per cent.

B. CHROMOSOME AS A SOIL AMENDMENT

Since 1937, the year the colchicine method was discovered, many experiments have been carrying out with outstanding result. Some of the work that has been done in solving problems in horticulture, genetics, histology, phylogeny etc. are reviewed here:

1. Application in the field of Horticulture

Chromosome doubling does not always result in superior plants. Thus, (2) tetraploid melon and citrus are of inferior quality. In ornamental plants, however, the chances of success appear promising. The treated-plants may have unbidden leaves, distorted stems, and various anatomical malformations, such temporary changes disappear in $1/1$, $1/2$ and later cycles. As a rule, doubling of chromosome number of a diploid plants results in larger flower, thicker petals and generally increased vigor. These changes are transmitted to the next generations.

Emsweller (10) treated the fleshy detached scales of Easter lily with 0.2% colchicine for 2 to 3 hrs. by immersing, and he made tetraploids of four varieties of Lilium longiflorum. He reported the retardation of bulblet formation of treated scales, broader, thicker leaves, and heavier petals. In another paper, Emsweller and Eurdon (11) observed that the tetraploid flowers had an appearance of being less widely open than the diploid flowers and they suggested that this might be due to the thickness of the petal which gave the flower a sturdy structure. Leaves of tetraploid lily plants were significantly longer and considerably thicker. Tetraploid bulbs were lighter and of a greater circumference. They reported the failure of inducing tetraploid by treatment of the growing point of the stem with several concentrations of colchicine prior to

flower buds differentiation.

Pryor (18) in Beltsville, Maryland, treated seedlings of snapdragon (Antirrhinum majus) with 0.2% colchicine solution applied with an eye-dropper to the terminal point of seedlings, or to those in the axil of leaves of larger plants. Both treatments were effective. The autotetraploid Antirrhinum with 16 chromosomes had heavier leaves and larger flowers, in some varieties twice the size of their diploid form. The fertility of these tetraploid plants varied considerably, one being very fertile with a fairly good seed set when hand pollinated.

In the extension breeding program at Beltsville, Maryland (24) many promising seedlings had been obtained which were generally resistant to soil-borne diseases with good color and fragrance, but having small flowers or weak stems. By the treatment with 2% colchicine solution dropped onto the axil of leaves, tetraploid branches were obtained. Some 2,000 disbudded seedlings were raised out of which 50 plants were selected as tetraploids which were sturdy and produced large flowers.

1% lanolin emulsion of colchicine was applied to the dormant lateral buds of petunias (24) as they were starting to grow, thus plants were picked from several branches on tetraploid which gave heavier leaves and the heavier stemmed growth that might improve handling and shipping qualities.

Clyde (4) treated verbena (V. hybrida) by soaking the cuts in colchicine solutions of various concentrations and from 2 to 24 hours. Also 1% emulsion was applied to the growing points of cuts with established plants. Tetraploids were obtained from both treatments. These plants were more vigorous and had larger flowers, the sepals were fleshy and thickened. Diploid verbena was highly self-fertile but neither tetraploid nor triploid was

self-fruitful, this was due either to the high percentage of abortive pollen or the slow growth of pollen tubes. Triploid plants arose from the cross between diploid and tetraploid plants.

2. Application in the field of small fruits and tree fruits

Darrow (6) indicated that it is well known that our best apple varieties are polyploids. Triploid apples and pears are exceptional among fruits in being sufficiently productive to become important varieties, though they develop few seeds, require cross-polination, and are poor pollinators. Tetraploid peach of the Golden Jubilee etc. had been obtained by the colchicine technique. One chance triploid was also reported. The sweet cherries are diploid, the sour cherries tetraploid.

Dermen (7) and Olmo (16) reported that the tetraploid grapes of both Portland and Fredonia varieties had wholly developed cluster and the berries were almost exactly twice the weight of the corresponding diploids. Sekhon (21), in his theses "Colchicine Treatment to Induce Polyploidy in Grapes" concluded that the bud paint treatment in concentrations of 0.6%-1.0% was effective in inducing tetraploidy in grape varieties with less pubescence and thinner leaves.

The application of colchicine to the production of the "seedless" triploid watermelon represents a most specific and outstanding advantage gained from the use of this material. The fact is well-known that seedless fruits in nature are due to certain reproductive failures. Thus an idea rose in H. Kihara's thought that triploid watermelon would be seedless (13). Tetraploid parents were produced by colchicine applied at the seedling stage, and these tetraploid plants became seed parent with the diploid as pollinators to make the triploids. When triploid plants were growing, pollinations must be made by diploids because the pollen of

triploids was not sufficient to induce fruit development. Limiting vegetality of triploid plants crossed the diploid by about twice although special cultivation practices were necessary for triploid interaction.

Other fruits showing polyploidy (6) are strawberry, blackberry, raspberry, blueberry and cranberry. These exhibit the usual polyploid characteristics but varied in their fertility. These species of plants have advanced much in ploidy naturally and the exploitation of artificial production of characters desirable will indicate be a productive field.

3. The use of artificial amphidiploids in plant breeding.

The discovery of a ready technique for making sterile hybrids fertile through colchicine treatment has been a boon to plant breeding.

The objective (17) of making hybrids between Gaillardia aristata and G. pinnatifida was to combine larger outstanding of G. pinnatifida with flower and other fruit characteristics of G. aristata. The two species could be crossed in reciprocal matings, but the F_1 hybrids were discriminated practically by almost complete male sterility. Anthers would not ripen or were small and contain disorganized pollen. The colchicine technique made it possible to stabilize the hybrid of G. aristata and G. pinnatifida known as the self-fertile amphidiploid. Most of the amphidiploid lines showed little or no segregation in early generations but segregated for fruit color, shape, size etc. in the fifth and later generations. The several amphidiploid lines made between various varieties might constitute a potential new species of Gaillardia with economic value.

For treatment with colchicine (17), Jensen et. al. found it most satisfactory to use vigorous rooted seedlings with fully expanded cotyledons. They were inverted over trays of aqueous colchicine solutions so that the cotyledons were immersed. The best results obtained from treatment

with 0.4% aqueous soln. for 48 hrs.

A new species *Ribes nigrolaria* (9) was originated by the use of colchicine and hybridization. The diploid parents were *R. nigrum*, the black currant and *R. grossularia*, the gooseberry.

4. The use of polyploid in histology

When geneticists realized that the treated plants might look like tetraploids yet reproduce as diploids, the significance of periclinal chimeras began to be truly appreciated. However, developmental problems can be traced with closer attention to the origin of tissues, because specific periclinal chimeras should yield certain results in mature organs.

If colchicine changes the cells of the first layer in a primordium to tetraploid while the second layer remains diploid, then the epidermal cells of the mature plant will be tetraploid and the pollen grains diploid because the sporogenous tissue originate from the second layer.

In apple ([illegible]), two groups of naturally occurring diplo-tetraploid chimeras had been discovered, namely: 2-4-2 and 2-2-4. Plants in the first group are seemingly tetraploid and in the second, diploid. Usually, three histogenic layers of the shoot apex contribute to the development of the leaf, L-I gives rise practically always to the single layered epidermal tissue, both L-II and L-III enter into the development of internal leaf tissue. In the area where the mesophyll is 2X, the vein tissue is also 2X and vice versa. In the stem, as a rule, the epidermis is derived from L-I, the hypodermal tissue, consisting one or three layers of cells, is derived from L-II and the rest of tissue, L-III.

Satina (20) reported that in *Datura*, the initiation and development of the leaf, sepal and petal are similar and depend primarily on the

activity of the second germ layer (L-II). The initiation and early development of the stamen depend primarily on L-III. The early stages of development therefore suggest that the stamen is not a modified leaf but a reduced axis.

5. The detection of genotype by polyploids

Janick and Stevenson (12) joined their names in a paper titled "The effects of polyploidy on sex expression in spinach". Tetraploid spinach plants were induced by the treatment of seed with 0.2% colchicine in aqueous soln.. Gene changes or chromosome repatterning (9) through colchicine treatment had never been proved, and (12) as the observed sex ratio of the tetraploid was not dissimilar from that of the diploid line, it was assumed that doubling of chromosome number did not alter their sex type. Triploids were obtained from the cross between tetraploid and diploids, and all three, diploid, triploid and tetraploid intercross readily and are self-fertile. The experiment indicated that the Y factor is male determining because only a single dose of Y is needed to produce the staminate condition even in combination with three doses of X in the tetraploid staminate plants. The hypothesis that monoecious plant is due to an altered balance between X and Y chromosome and that X_yX is monoecious was rejected because the triploid XXY is staminate. Another hypothesis that the monoecious type is due to an alleles of the XY factor as well as modifying genes was accepted.

6. Polyploids in phylogeny

New species can arise suddenly by interspecific hybridization and doubling of chromosomes. These new species are able to invade new habitats, an invasion not possible by either parent. Colchicine is a useful tool in tracing down certain steps in the origin of the polyploid

species.

Triticum aestivum L, the bread wheat, is hexaploid. Full knowledge of the origin of bread wheat probably will never be obtained, but some phases can be closely inspected by observing the experimentally produced polyploids.

The hypothesis of the Asiatic-American origin of tetraploid cotton Gossypium hirsutum was confirmed by (1) doubling of chromosome number of a sterile hybrid made from an Asiatic diploid and an American wild diploid species.

Very often (26), the chromosome numbers in the various species of a given genus form a more or less complete euploid series. For example, in Chrysanthemun, the various species have 9, 18, 36 and 45 pairs of chromosomes. Series with two or three members are still commoner. Related genera also show related chromosome numbers. Zea mays and certain species of Sorghum have 10 pairs. Perennial species of the latter are tetraploid (22). All these facts suggested that the related plants had some more or less common origin.

The origin of sweetpotato (Ipomoea batatas), which has the somatic chromosome number of 90 (14), had been suggested by Ting and Kehr (27) as an alloploid of the diploid Ipomoea. They observed that secondary associations were common, but multivalents had not existed. As total differenciation of the chromosome had not yet reached the stage where chromosome behaves as diploid, the condition found in many natural polyploids, it was suggested that I. batatas had a comparatively recent origin.

C. CRITERIA FOR JUDGING POLYPLOIDY

1. Fertility

If a sterile species hybrids begins seed production after treatment with colchicine, the evidence is good that polyploidy has been induced. The two parental genomes of a diploid species hybrid are usually incompatible (9) to the extent that no interchange can occur between them. The fact that no gene exchange between the parental sets of chromosomes mean no inter-genomal pairing. Brifly, the diploid hybrid should be entirely sterile until a doubling of chromosome occurs. The chance doubling that might have occured through unreduced gametes is of such low frequency that the effects of colchicine were not obscured by natural or spontaneous doubling. The two examples of Cucurbita and Gossypium given before well illustrated the situation.

Two general methods are used to judge the fertility level of a specific polyploid: (i) percentage of good pollen as demonstrated by microscopic method, (ii) the amount of seed set.

2. Morphological changes

New leaves and stems that grow from treated sectors are usually wrinkled, thicker and darker green, and have coarser texture, as compared with the untreated-plants. By these abnormal appearance, sorting of tetraploids can be made among large populations of treated cultures.

Flowers of tetraploid are larger and more compact than the diploid (4) (7) (17) (18) (24). Emsweller (2) reported that tetraploid flowers of Easter lily were 15% longer and 5.6% broader than the diploids, but some tetraploids showed very little improvement in size (10) over their respective diploids. Sometimes, the leaf shapes are entirely different between the tetraploids and their diploid progenators, such is the case in seasame(23)

(Seasamum orientale L.). The tetraploid leaves were simple and wavy while the corresponding diploid leaves were trilobed. Usually the rate of growth is slower (9), but even the final growth does not produce a plant as tall as the diploid. However, (23) the tetraploid sesame plants were about 1.5 times the size of the diploid plants.

3. Physiological differences

Many tetraploids are physiologically superior over the diploids. Sugar content is increased in triploid watermelon (13) as well as the large root of triploid plants of sugar beet (15).

The colchicine-induced tetraploid plants of Plantago ovata (3) exude a dark brown material from the leaves, and this is associate with the break down of leaf tissues. Up to 97% of the tetraploid plants exude the material but few diploids develop it.

4. Size of stomata

Not only are the leaves broader and thicker in tetraploid plants of P. ovata (3), but the cells are larger. That tetraploid tissue gives large sized cells has been agreed by almost all, and the longitudinal sections of a chimera shoot apex gives a visual picture of the differences. Guard cells of stomata are quite universally chosen to illustrate the increased size of cells in tetraploid tissue because they are less influenced by environmental factors and are symmetrical and most easily measured. Emsweller (11) stated that it is possible to select polyploid lily plants with an accuracy of about 90% by the size of stoma. Chimeral plants may have isolated cells or group of cells that are of the tetraploid size but they are of no significance except when they appear in a crucial position. Also, the distribution of stomata varies, the diploid cells are closer together than the tetraploid.

5. Size of Pollen grain

At 1937, Nakasiko and Avery (2) wrote a paper discussing several methods of inducing polyploidie with colchicine. In which, they wrote: "We are aware of no exception to the rule that within a given form, the size of pollen grain is proportional to the number of chromosomes which they contain". Pollen grains of a tetraploid have 2N chromosomes, their volume is twice that of the N grains of a normal diploid and 1.25 times the diameter of the latter. The correlation between the size of the pollen grain and the number of sets of chromosomes has not been meeting any objection since then. Triploid pollen grains are notable for their irregular dimensions and are useful in separating triploid and tetraploid plants on a field scale basis (9).

6. Chromosome counts

Pollen mother cells undergoing meiosis are quite universally used for counting chromosome and determine the associations between chromosomes during pairing. Also root tips are used in some cases. Acetic-orcein technique had recently been developed in Netherland (23) for the ease production of slides.

CHAPTER III

MATERIAL AND METHODS

A. THE PROGENITORS

Two widely different species of Ipomoea were used in this experiment, namely: (i) I. tricolor, (I. rubro-coerulea Hook, or I. Hokeri Don. and Hort.) a cultivated species and (ii) I. lacunosa*, a native species (local weed) which is a smoothish slender creeping or twining thin leaved annual, leaves entire cordate or hastate. Peduncles short, flowers white, small and about 22m.m. wide and 25m.m. long, one to three in each leaf axil. The stems have a tendency to give laterals and axile leaves. Sepals coriaceous, glabrous and green, anthers purple.

These species are identified as diploids by King and Bamford (5).

B. METHODS OF TREATMENT

The seeds were germinated in vermiculite, and transplanted into 2" pots in soil in the green house when the hypocotyls erected. These plants were then shaded by paper caps until the hypocotyl had become about 6" and 4" in I. tricolor and I. lacunosa respectively. The seedlings were then exposed to full light for two or three days to establish vigorous growth. The seedlings were treated after the epicotyls had begun to elongate but before the first true leaf had expanded. They were inverted into bottles of aqueous colchicine soln. so that the epicotyls were immersed (fig. 1). The plants were rinsed with tap water several times after treatment (17).

*The closest identification possible at this time.

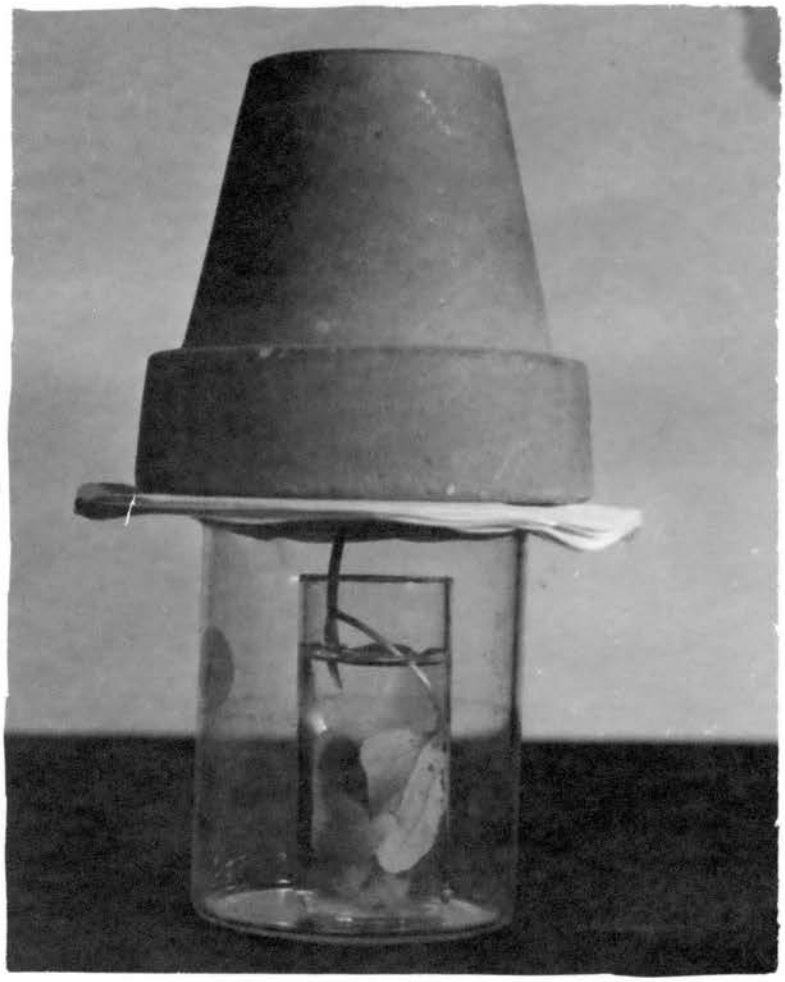


Fig. 1. Illustrating the treatment technique
The growing point of the seedling is inverted into a vial of colchicine. The paper in-between is to retain the soil in the pot.

Six series were arranged by the combinations of three concentrations of colchicine with two periods of time, those were: 0.2, 0.3, and 0.4% by wt. with 24 and 48 hours. Ten plants of each series were treated and the treatment was made at three different dates within 40 days which started from 22nd of February and ended at 27th of March. For each group of plants treated, two plants were left as untreated controls making six control-plants for each species.

C. DATA SECURED FROM THE PLANTS DURING THE PERIOD OF GROWTH

For a considerable length of time, the plants maintained and showed no growth, after growth was resumed and the trailing stems were about 15 cm. long, they were replanted into the 6" pots and one or two bamboo stakes were erected according to the number of branches that arose from the base.

Growth records of the plants were started on the first day that the plants were moved into the 6" pots, and taken later at 10 day intervals. Flowering records were taken daily starting on the day the first flower bloomed. All the records were kept individually.

Hand pollination was made on the treated-plants that showed the most abnormal from the control ones. In I. tricolor, both selfing and the cross, with pollen from untreated-plants, and the treated-plants as seed parents were made.

D. MEASUREMENT OF THE LEAF

Leaf thickness was measured with the help of a caliper. Of the leaves of a single plant that were to be measured, only the largest ten leaves were used and no axile leaf was measured.

With the help of a microscope, guard cells of stomata of individual leaves and pollen grains were studied. To make the preparatory slide of

the stomata, a portion of leaf was cut from the base and a bit of lower epidermis was stripped off from the mesophyll with a pair of forceps. A drop of water was placed on the slide beforehand and the epidermis was put down. A cover glass was then applied and the preparation was ready for observation. Two scrapes were made from each leaf, and all samples were cut from the leaf base. Samples were taken from the lowest leaf upward on the main stem. The microscope was equipped with a camera lucida and all stomata were measured on tracings on paper at a magnification of 270 diameters.

3. MEASUREMENT OF THE REAL SIZES

Anthers from both the control-plants and from flowers on treated-plants after meiotic divisions were studied. The anthers were crushed on the slides and stained with cotton blue in lactophenol (4). A scale was placed by the right side of the microscope and again the camera lucida was assembled. When the slide was under observation, the images of the pollen grains were sketched to the scale and direct measurements were recorded.

In order to transfer the microscopic data to the real size, a factor was obtained through the following procedures.

A micrometer was held on the stage under the microscope, and the image of the division was projected through the camera lucida to ruler held beside the microscope. It was found that 5 divisions on the micrometer equalled 135 m.m. on the ruler. The micrometer was then taken away and examined and it was found that the 5 divisions equalled to 0.5 m.m. and therefore 135 m.m. of the image equalled to 0.5 m.m. of the actual thing and 135÷0.5=270 was the factor. All microscopic data could be transferred to the real size by dividing by 270.

F. COLLECTION OF SEED FOR FERTILITY TEST

At the end of this experiment, seed pods were collected from all plants and their numbers were recorded respectively. With the comparison of the number of seed pods to the number of flowers, percentage of fruit-set was secured.

CHAPTER IV

EXPERIMENTAL RESULTS

A. GENERAL APPEARANCE OF TREATED-PLANTS

1. I. tricolor.

Immediately after treatment, many tiny and translucent spots appeared on the lower epidermis of the cotyledons. Sometimes, these spots could be seen through the upper epidermis. Within a day or two, these spots turned yellow, dropped out in the following days and left small holes in the cotyledons. Since further development of the seedlings was temporarily checked, all growth of the cotyledons was restricted and the blades became thick, deep green and in some cases, brittle. Accompanying this enlargement was the thickening of the hypocotyl and the petiole.

The meristem of the epicotyl and the unexpanded first true leaf did not grow for sometime after treatment and in some cases, they wilted for a few days. If they remained alive, the green tissues were soft and dull in color as if they were suffering a drought.

About 30 days thereafter, the meristems of the epicotyl became active. The first leaf unfolded but few showed any enlargement, subsequently they became thick, brittle and looked as if they were covered by a layer of whitish net. The next leaf formed was of the same texture but somewhat lighter green. The place of origin of these two leaves then swelled and gave rise to a cluster of small leaves and leaf-like stalks (which were thick and hard, venations barely showed and had no way to distinguish the petiole and the blade). One or two of these small leaves enlarged in the

subsequent growth, these leaves became very large, sometimes almost like a spoon, sometimes split into two or three leaflets, each of which had its own central vein. The petioles elongated and were mostly deeply grooved. The lowest leaf of the stem was always distorted and the shape abnormal, although the total leaf area was not reduced. The lowest two leaves of the central stem of plant 3-5 were exceptionally large, measuring 10.5 x 11.0 cm. and 9.3 x 10.5 cm. respectively. At higher level, leaves might still show distortion, but for most of the plants they did not.

If the central petioles were externally damaged by the treatment, they generate callus cells at either or both cotyledons and occasionally, they give a cluster of unpaired dissected leaves of two to seven. Some of these leaves were large, pale green and still some were more leaf-stalk, almost needle or with petiole up to 4 cm. long.

Meanwhile, the stem was lengthened, being light or dark and composed of several millimeter internodes. As it grew upward, leaves and branches separated from it, successively, the decapitated stem became a liana after each division and looked at last only the normal single stem.

After the effect of the drug was partially overcome, the main-stem, leaves and laterals were normal, although sometimes additional leaves appeared opposite to the normal ones. The leaves differed in size, some were much larger than the largest on the control-lots (table 5). There were two kinds of flower buds: (i) normal, in which the sepals were linear and thin, (ii) buds in which the sepals were thick and larger, making the young buds look thicker (fig. 2). Not all treated-plants had the latter type bud and sometimes both types were present on one plant or even on the same peduncle. Most of the flowers that developed from the thick buds had thicker tubes, although the limb or the length of the tubes was

not necessarily larger than the normal ones (table 11). The possibility was larger for the deeper blue colored flower to be found on the treated-plants.

2. I. lacunosa

The immediate responses to colchicine were mostly the same in these two species. Often in I. lacunosa, the base that bore the many leaves and branches swelled and occasionally, the leaves were half light green and half deep green, later these leaves became uniform in color.

This species normally produces many laterals. Even in the control-plants, buds in the axils of cotyledons developed into laterals. In treated-plants, the growth of the central stems was retarded and the axile buds started growth and produced a cluster of leaves if not long laterals. Fasciated stems were sometimes produced. Most treated-plants had larger leaves than those of the control-plants, sometimes even twice as large. This was more so in plants that had no laterals and axile leaves were few or none. Only three plants out of all the 55 treated and surviving plants had as dense an appearance as the control-plants.

The flowers of the treated-plants were not generally larger in limb and length but most of them had thicker tubes at least of the first few flowers (Table 12 and fig. 6, 7, and 8).

B. EARLY RESPONSES OF SEEDLINGS TO COLCHICINE TREATMENT

Table 1 and 2 show that the more intensive treatment caused the death of some seedlings and the dying out of the central primordia with the laterals from axil of cotyledons became activated to take their places. Some plants of the species I. lacunosa had the laterals developed along with the central stem and vice versa.

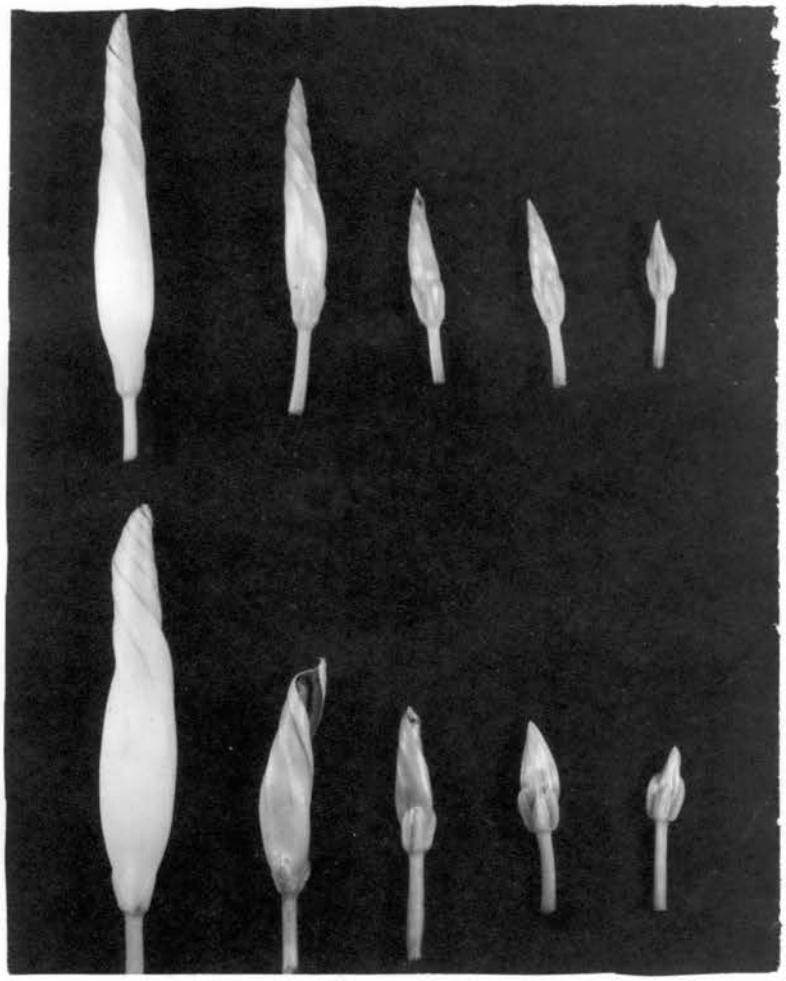


Fig. 2 Comparison of flower buds of different ages showing shorter and thicker sepals of flowers of treated-plants (lower row) in comparison with those of control-plants (at top). (I. tricolor)

Table 1 Early Responses of Seedlings to Treatment Showing The Development of Central Stem or Laterals Only (I. tricolor)

Data obtained	Treatment time and Concentration (%)							
	24 hrs.				48 hrs.			
	0.0	0.2	0.3	0.4	0.0	0.2	0.3	0.4
No. of seedlings treated /or control	3	10	10	10	3	10	10	10
No. of plants survived	3	10	10	10	3	8	8	5
No. of plants with central stem	3	8	8	9	3	4	1	1
No. of plants with lateral only	0	2	2	1	0	4	7	4

Table 2 Early Responses of Seedlings to Treatment Showing The Development of Central Stem or Laterals Only (I. [illegible])

Data obtained	Treatment time and Concentration (%)							
	24 hrs.				48 hrs.			
	0.0	0.2	0.3	0.4	0.0	0.2	0.3	0.4
No. of seedlings treated/or control	3	9	10	10	3	10	10	10
No. of plants survived	3	7	10	10	3	10	9	9
No. of plants with central stem	3	6	6*	5*	3	2	3	0
No. of plants with laterals only	0	1	6*	5*	0	8	6	9

*Both central stem and laterals developed equally in these plants.

C. DAYS FROM SEEDING TO THE FIRST FLOWER

1. I. tricolor

The influence of time of treatment and concentration of colchicine on the time required for the first flower to appear is given in Table 3 and Figure 3. The plants were germinated at three different dates as mentioned.

As indicated by the data there was considerable delay in the flowering date of the treated-plants. The control-plants flowered in about 58 days while the treated-plants required about 22 days longer (80 days). This was an increase of 40% over the time required for control-plants to flower. The delay was directly correlated with the concentration of colchicine and the time the plants were exposed to the chemical. It seemed that the date of treatment also had some influence on the flowering date

and the group that was last treated came to flowering within the shortest period of time. This could most likely to be explained in that the cold winter weather (lower temperatures) retarded the growth of the first two groups of plants.

Two plants treated on the same date and with the same time and concentration would respond differently in resumption of growth, and in extreme cases, a difference of more than one month was recorded. The plant 6-7 of I. tricolor had just started to show buds in the axil of the cotyledons on June 28 following the treatment on Mar. 16. Thus it is looked in that the size or age of plant when exposed to the treatment is an important factor and yet it is difficult to judge and select the seedlings to the very uniformity desired.

2. I. lacunosa

Table 4 and Figure 4 give the results. The two control-plants that germinated last had the shortest date to bloom. Also treatment delayed the flowering date and association between the duration and treatment showed still better here. As shown by the data, the plants treated on Mar. 29 had the shortest period of time to attain flowering. Control-plants flowered in about 36 days while treated-plants required about 22 days longer (58 days). This was an increase of 60% over time required for for the control-plants to flower.

It was noted that the retardation was about the same between these two species although the control-plants of I. lacunosa flowered about 22 days earlier than I. tricolor.

D. LEAF SIZE

Emsweller (10) and others had shown that leaves of polyploid plants were usually larger and thicker, and it was possible to use these factorsas

Table 3

Days from treatment to first flower for plants treated with colchicine of three concentrations and for twenty-four and forty-eight hours. (I. tricolor)

Series No.	Treatment time	conc.	Date treated Feb. 23	Mar. 9	Mar. 28	Ave.
Control	0	0	68 66	51 53	56 56	58.39
1	24	0.2	98 92 89	80 81 78	99 52 66 68	80.3
2	24	0.3	85 83 82 100	79 76	67 70 67 52	78.1
3	24	0.4	84	67 74 82 72 70 70	72 78 68	73.7
4	48	0.2	83 78 86	78 67 84	97 97	83.83
5	48	0.3	86 79 80	85 85 96 82	96	86.12
6	48	0.4	83 92	95 90 90		90
Ave. days to 1st flower from the same treated date			86.25	80.04	74.92	

Table 4

Days from treatment to first flower for plants treated with colchicine of three concentrations and for twenty-four and forty-eight hours. (I. lacunosa)

Series No.	Treatment time	conc.	Mar. 4	Mar. 19	Mar. 29	Ave.
Control	0	0	45 44	34 33	29 29	35.66
1	24	0.2	59	37 40	43 38 49 42	44.07
2	24	0.3	65 58 57 42	40 26 45	39 41 41	48.6
3	24	0.4	46 51	41 64 59 57	42 54 58 38	51.0
4	48	0.2	56 54 60	70 49 70	53 41 58 85	60.0
5	48	0.3	56 86 60	78 66	42 60 68 63	64.42
6	48	0.4	66 55	78 91 61	64 60 64 77	68.53
Ave. days to 1st flower from the same treated date			58.87	58.94	53.47	

Figure 3 Days from treatment to first flower (I. tricolor)

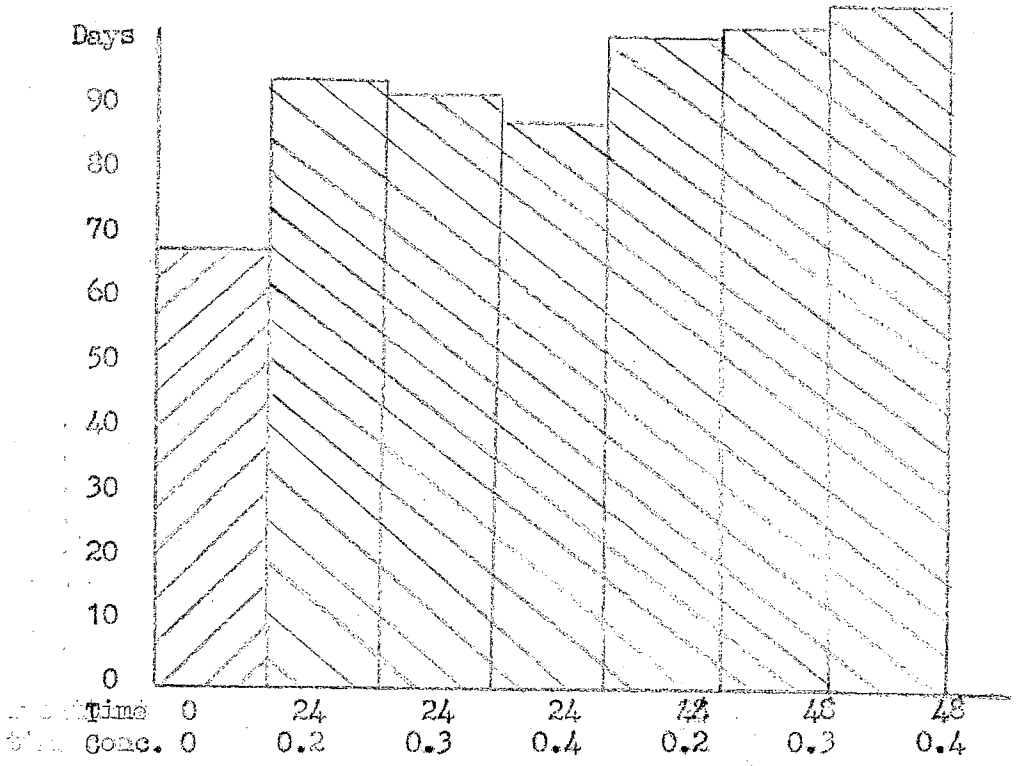
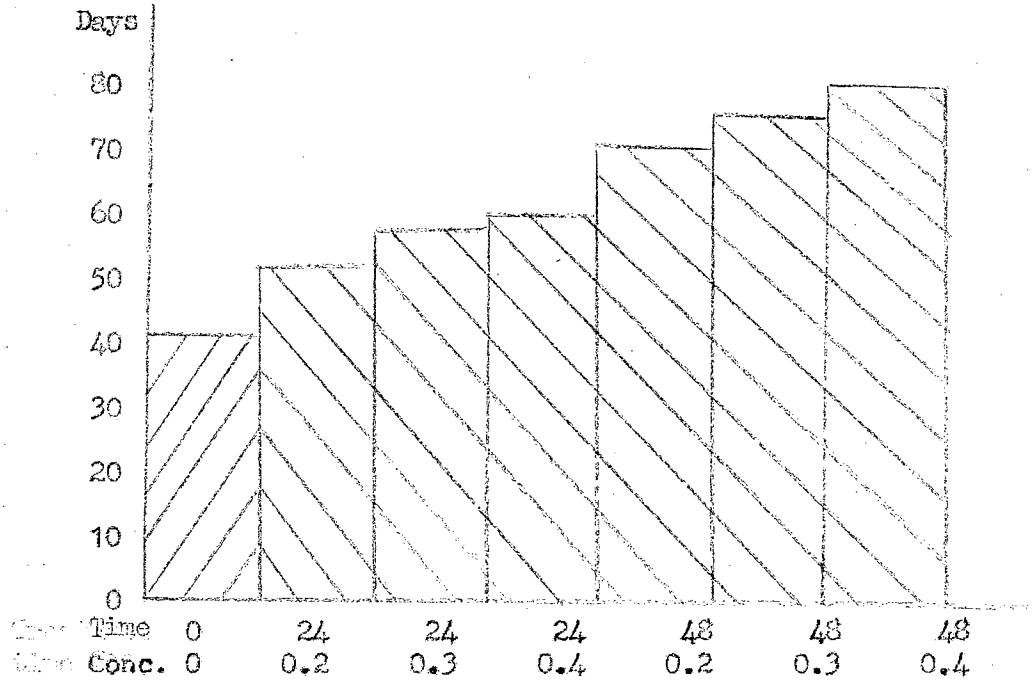


Figure 4 Days from treatment to first flower (I. lacunosa)



criteria to identify polyploidy, our efforts were here paid to measure the leaf size.

1. I. bicolor

Treated-plants were selected for leaf measurements and only those deviating most from the control ones were used. The length and width for the five largest mature leaf blades of each plant were measured. The maximum length and width of the blades of five of the largest and most mature leaves were measured on May 15th.

Table 5 presents the results. They showed that the average of both length and width were larger in the tetraploid-plants than the control ones, yet two plants in the treated group bore leaves of almost the same size as the controls. Furthermore, it was noted that other treated-plants which were not selected for leaf measurement had leaves as large as the controls.

The ratio of length to width was greatest in the control-plants indicating a shortening of the blades of the treated-plants.

2. I. lacunosa

Treated-plants were inspected as before. The average size of the leaves of the treated-plants was decidedly larger than that of the control ones. The ratio of length to width was reduced in the treated-plants as in the previous case. Plant 5-2 was the only one that bore leaves smaller than the control ones, yet this plant grew larger flowers, and larger stamens on some leaves which characterizes it as a possibly tetraploid. (Table 6)

3. MS TRIANGLE OF LIFE

It had been shown by Nebel (19) that the leaves of tetra plant of various ploidy differed in their thickness and it was easy to judge the tetra plants in field just by measuring the leaf thickness. The methods was adopted here in the hope of finding the difference in thickness between

Table 5

Comparison of leaf size of a selected number of Control with that of treated-plants (I. tricolor)

Plant No.	Treatment time	conc.	Average size of 5 leaves* (mm.) length	Width
0-5	0	0	115.8	116.4
0-6	0	0	107.6	94.2
Ave. leaf size of the * control-plant			111.7	105.3
1-1	24	0.2	89.2	93.8
1-4	24	0.2	89.4	91.0
1-6	24	0.2	108.6	121.6
2-2	24	0.3	77.2	92.0
2-3	24	0.3	77.8	84.2
3-1	24	0.4	91.6	91.4
3-5	24	0.4	105.0	103.6
3-6	24	0.4	89.4	91.6
3-7	24	0.4	74.0	75.6
4-3	48	0.2	96.4	99.4
5-2	48	0.3	73.4	68.8
5-4	48	0.3	77.6	85.4
6-3	48	0.4	90.2	90.4
Ave. leaf size of the treated-plant			87.6	91.7

Ratio of length to width Control- - - - - 1.06
Treated - - - - 0.96

*The largest 5 leaves on each plant were measured.

Table 6

Comparison of leaf size of the control with treated-plants (I. lacunosa)

Plant No.	Treatment Time	Treatment conc.	Average size of 8 leaves* (mm.) length	Width
0-3	0	0	46.6	40.2
0-4	0	0	46.6	40.4
0-5	0	0	50.1	46.1
0-6	0	0	50.0	46.1
Average leaf size of the control-plant			47.1	43.9
2-1	24	0.3	50.3	50.4
2-2	24	0.3	53.4	56.3
3-5	24	0.4	51.9	54.4
3-7	24	0.4	69.3	64.5
3-9	24	0.4	56.1	56.8
4-4	48	0.2	56.1	53.7
5-2	48	0.3	40.5	39.3
5-3	48	0.3	50.0	54.5
5-6	48	0.3	60.1	68.4
5-10	48	0.3	58.8	61.9
6-3	48	0.4	52.6	57.9
6-6	48	0.4	55.9	56.3
Average leaf size of the treated-plant			54.6	56.3

Ratio of length to width Control - - - - - - - 1.08
Treated - - - - - - - 0.97

*The largest 8 leaves on each plant were measured.

the treated and control-plants.

1. I. tricolor

Mature leaves of three control-plants and ten treated-plants were measured. The plants were selected from the treated group as the most abnormal as expressed by their comparatively large number of unusual flowers or their large or small leaves.

The largest eight leaves on the plant were selected and the thickness of the basal part of the blade was ascertained. Using care to avoid the main and the secondary veins.

The results are given in Table 7. These data show that there was no marked difference in thickness of leaf between the control and the treated-plants.

2. I. lacunosa

Two control-plants and ten treated-plants were used. The selection of the treated-plants was done on the same basis as for I. tricolor. The data in Table 8 show that the treated-plants had thicker leaves. These measurements were taken late and after some of the largest leaves of the control-plants had dropped. Therefore these data must be viewed with some reservation.

F. SIZE OF STOMATA (LONGITUDINAL DIMENSION).

It was said by Emsweller that it was 90% correct to judge a polyploid by the large sized stomata. Almost all workers agreed that stomata size was exceptionally large in polyploid plants.

1. I. tricolor

The stomata of the control-plants were of uniform size except for a few small ones. In the treated-plants both large and normal sized stomata were encountered and both were measured.

TABLE 7

Comparison of Leaf Thickness of The Control With That of Treated-plants (I. tricolor)

Plant No.	Treatment Time	Treatment Conc.	Average of 8 leaves (mmx100)
0-4	0	0	3.25
0-5	0	0	2.50
0-6	0	0	3.88
Ave. of leaf thickness of the control-plants			3.28
1-1	24	0.2	3.88
1-4	24	0.2	3.88
1-6	24	0.2	3.88
2-2	24	0.3	4.00
2-3	24	0.3	3.38
3-6	24	0.4	3.13
3-7	24	0.4	3.38
4-3	48	0.2	3.50
5-2	48	0.3	4.50
5-4	48	0.3	3.50
Ave. leaf thickness of the treated-plants			3.56

TABLE 8

Comparison of Leaf Thickness of The Control With That of Treated-plants (I. lacunosa)

Plant No.	Treatment Time	Treatment Conc.	Average of 8 leaves (mm x 100)
0-3	0	0	2.75
0-4	0	0	2.37
Ave. leaf thickness of the control-plants			2.56
2-1	24	0.3	3.25
2-2	24	0.3	3.13
3-5	24	0.4	2.63
4-3	48	0.2	3.25
4-4	48	0.2	3.88
5-2	48	0.3	3.13
5-3	48	0.3	2.75
5-6	48	0.3	3.30
6-3	48	0.4	3.50
6-6	48	0.4	2.15
Ave leaf thickness of treated-plants			3.01

Table 9 gives the results. Both large and small sized stomata were found on a single tetraploid-plant and also within a single leaf. The large stomata were always found in the leaves at the lower part of the plant while the leaves in the upper part of the plant had mostly normal sized stomata.

In the control-plants, the size of the leaf in relation to the size of stomata was studied and it was found that larger leaves did not necessarily have larger stomata. Also leaves at various levels on the plant gave stomata of the same size.

2. T. laxumora

Similar data on stomatal size were obtained with this species. The results are given in Table 10. The three plants given as examples here had widely divergent averages. Within the plants studied, only three possessed as large stomata as the plant 5-5 showed. The leaves that bore the largest stomata were characterized by a coarse surface, toothed margins and a deeper green color. The leaves that contained stomata of the size of those on plant 2-1 also were deeper green but showed no variation of either leaf shape or leaf surface.

The average size of stomata for 5 leaves of the control-plant 0-5 was 0.056 mm., for 10 leaves of plant 5-2, it was 0.070 mm. and for plant 5-5, it was 0.10 mm.

Figure 5 is a camera lucida drawing of stomata of the three groups mentioned above.

6. FLOWER SIZE

It has been agreed by all that flower size is increased by the increase of the number of chromosomes. Measurements on flower size were taken in this experiment and the data are presented in the following section.

TABLE 9

Comparison of the Size of Stomata of the Control with That of Treated-plants (I. tricolor)

Size of Stomata Class value (mm x 270)	No. of Stomata Control 0-1	Treated 1-1*
12	5	1
13	3	1
14	13	4
15	18	10
16	17	6
17	7	5
18	7	4
19	5	3
20	2	2
21	2	7
22		4
23		1
24		0
25		5
26		4
27		1
28		0
29		0
30		2
Total No. of Stomata	80	60
Mean	15.7	19.4
Prob. error	±0.17	±0.39
Coef. variability	14%	22.7%

*Plant 1-1 was treated with 24 hrs. and 0.2% conc. combination.

TABLE 10

Comparison of the Size of Stomata of the Control with That of Treated-plants (I. lacunosa)

Size of Stomata Class value (mm x 270)	No. of Stomata Control 0-5	5-2*	Treated 5-9*
13	7		
14	12		
15	11	6	
16	10	7	
17	7	18	
18	1	20	
19		8	
20		11	
21		6	
22		4	3
23		2	5
24		0	6
25		1	14
26			14
27			8
28			9
29			8
30			6
31			4
32			2
33			1
34			1
35			0
36			1
Total No. of Stomata	48	83	82
Mean	15.0	18.5	27.0
Prob. error	±0.14	±0.16	±0.22
Coef. variability	9.3%	11.9%	10.7%

*Plants treated with 48 hrs. and 0.3% conc. combination.

1. *L. bicolor*

The flowers measured were taken at random on the control-plants during the earlier stages of the blooming period. Only length of the tube was measured. The limb of the corolla was not measured as the flowers were usually not wholly open on the effectively treated-plants. For the treated-plants, only flowers with thick sepals were measured.

The results are given in Table 11. They show that the average flower size of the treated-plants was larger. One plant treated with the 0.4% concentration (4 hrs.) was not different in flower size from the control-plants.

2. *L. leucanthus*

Only two treated-plants (2-1, 3-2) were selected for measurement as the rest did not continue to produce large flowers and after seeds were set, all flowers became smaller and of uniform size. Another plant, 5-3, also had larger flowers but not as large as the two plants here measured.

Most plants treated gave some flowers that had thicker tubes although neither the diameter of the limb nor the length of the tube was larger in measurement. Both sized flowers might appear on a single branch. Even the plant 3-1 gave some small flowers during its later stage, this could be due to either the external condition or lack of vigor.

Both length of tube and width of limb are given in Table 12. These data show definitely that the flowers of the selected treated-plants were larger than the control ones, this is more clearly shown by Figure 6, 7, and 8 which also illustrates the thick tubes of the large flower.

E. NUMBER OF PETAL

It was noticed that some of the flowers of the species *L. bicolor* have much thicker petal than that of the flowers of the control-plants.

Fig. 6 Comparison of the control (two branches at right) and treated-plants (left) of I. lacunosa at flowering stage.

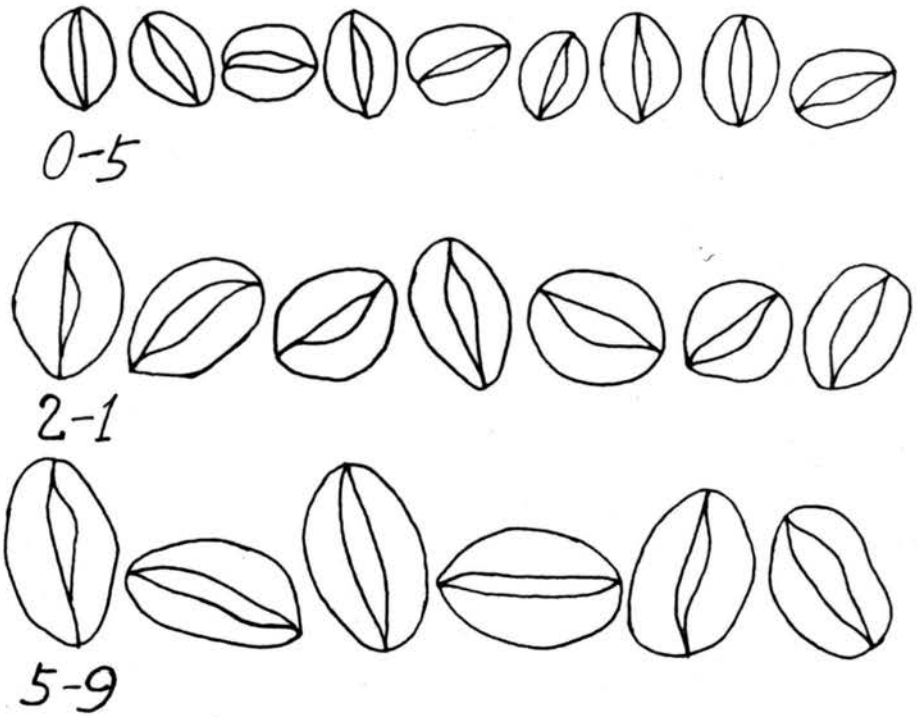


Fig. 5 Comparison of stomata of the control and treated-plants showing the three successively larger size bore on the above numbered three plants.

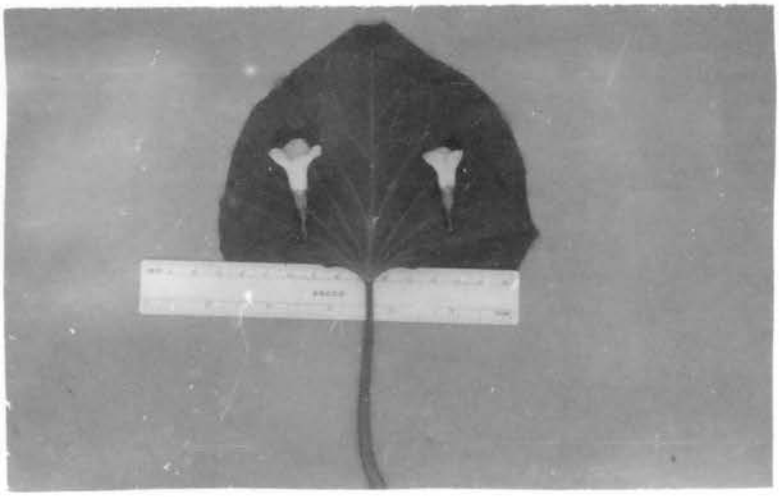


Fig. 7 Comparison of flower of the control (right) and treated-plants (left) of I. lacunosa (side view).

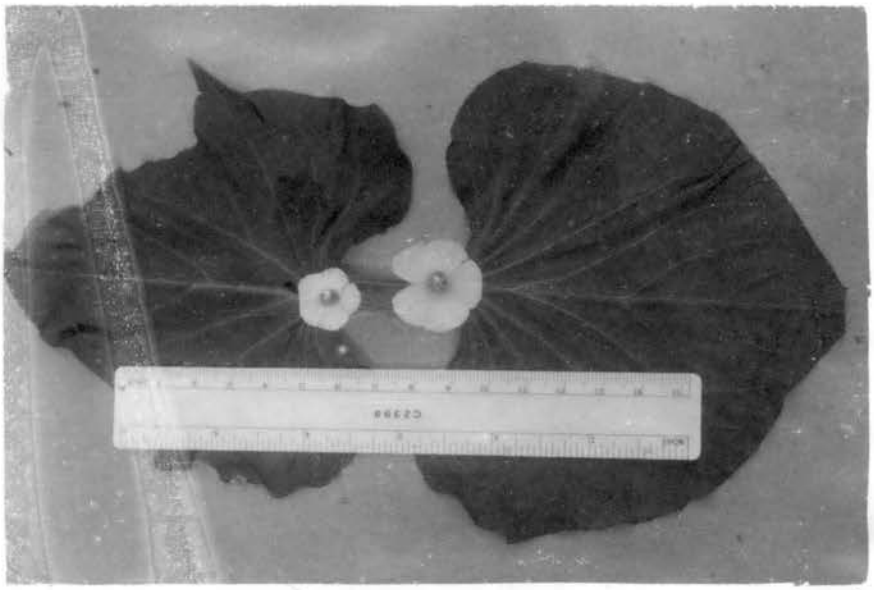


Fig. 8 Comparison of flower of the control (left) and treated-plants (right) of I. lacunosa (vertical view).

TABLE 11

Length of Tube of Flowers of Control in Comparison with That of the Treated-plants (*I. tricolor*)

Length of tube* Class value (mm)	Control	Treated 24 hrs. 0.2	0.3	0.4	48 hrs. 0.2	0.3	0.4
72-73	1						
74-75	1	1					
76-77	0	2					
78-79	4	1					
80-81	3	3	1	1		2	2
82-83	9	3	1	0		0	2
84-85	12	4	1	2		0	0
86-87	7	2	5	0		2	3
88-89	5	2	0	0		1	3
90-91	5	7	4	4		5	
92-93	0	2	0	5		4	
94-95	2	2	6	3		4	
96-97	0	0	1	2		0	
98-99	1	0	1	0		1	
100-101		1		1		1	
102-103				0			
104-105				2			
Average	84.8 mm.	85.9 mm		92.8mm		90.8mm	85.3mm
			90.3mm	3-6			
Plant No.	0-1, 0-2, 0-3, 0-5, 0-6	1-1, 1-4, 1-6,	2-2, 2-8	3-7		5-2, 5-4	6-3

*From lip of limb or bell to base of sepal

(Flowers measured during the period May 13 to June 23, 1957)

TABLE 12

Diameter of Limb and Length of Tube of Flowers of Control in Comparison with That of Treated-plants (*I. lacunosa*)

Diameter of Limb* Class Value (mm)	No. of Flowers Control	No. of Flowers Treated**	Length of Tube* Class Value (mm)	No. of Flowers Control	No. of Flowers Treated**
19	2	0	22	1	0
20	3	0	23	2	0
21	6	0	24	4	0
22	3	8	25	6	0
23	1	3	26	2	1
24	0	5	27	1	10
25	2	6	28	1	10
			29		1
Average	21.4mm	23.5mm		25.0mm	27.5mm
Plant No.	0-1,0-2,0-4	2-1,5-2		0-1,0-2,0-4	2-1,5-2

*Length of tube is from lip of limb or bell to base of sepals, maximum width of flower.

**One plant treated with 0.3 conc. and 24 hrs., the other plant with 0.3 conc. and 48 hrs.

Measurements were made and data are presented in the following table(13).

The thickness of the petiol of the flowers of the treated-plants exceeded that of the controls (Table 13). The same control and treated-plants used for leaf thickness were used here except the plant 3-7. As shown by figure 9, there is a rather distinct difference in diameter of pedicels of the control and treated-plants.

I. SIZE OF POLLEN GRAIN

Size of pollen grains had been used by many to illustrate the polyploid plants and Blakeslee had concluded that the volume of pollen grains doubled with the doubling of chromosome number of which it contained.

1. L. bicolor

Table 14 presents the results. The size of the grains of the treated-plants varied much, but the average fell very close to that of the control-plant. Out of the 3 flowers of the treated-plant, three groups of pollen grains of different sizes can be divided. The first four flowers possessed pollen grains smaller than that of the control-plant, one flower possessed the same sized grains as the control-plant while three other flowers possessed grains distinctly larger than that of the control-plant. These small grains might be explained as having been injured by the chemical, the normal ones as not affected while the larger ones were effectively affected. If this suggestion is true, this plant will experimental result as a chimera. Observations indicated that the anthers in some flowers of both treated and control-plants did not dehisce. A brief study was made to obtain a comparison of pollen grains of dehisced and indehisced anthers of both control-plants and treated-plants. No association could be found in the size of pollen comparing dehiscing and non-dehiscing anthers. The anthers of the affected flowers, that is, the flowers that possessed

TABLE 13

Thickness of Pedicels of Control and Treated-plants Showing The Greater Diameter of the Pedicels of The Treated-plants (I. tricolor)

Plant No.	Treatment Time	Treatment Conc.	Average of 10 Pedicels (mm. x 100)
0-4	0	0	27.7
0-5	0	0	22.0
0-6	0	0	21.8
Ave. thickness of the Control-plants			23.6
1-1	24	0.2	31.3
1-4	24	0.2	31.7
1-6	24	0.2	30.0
2-2	24	0.3	30.0
2-3	24	0.3	32.3
3-6	24	0.4	37.5
4-3	48	0.2	32.5
5-2	48	0.3	32.3
5-4	48	0.4	27.7
Ave. thickness of the Treated-plants			31.4

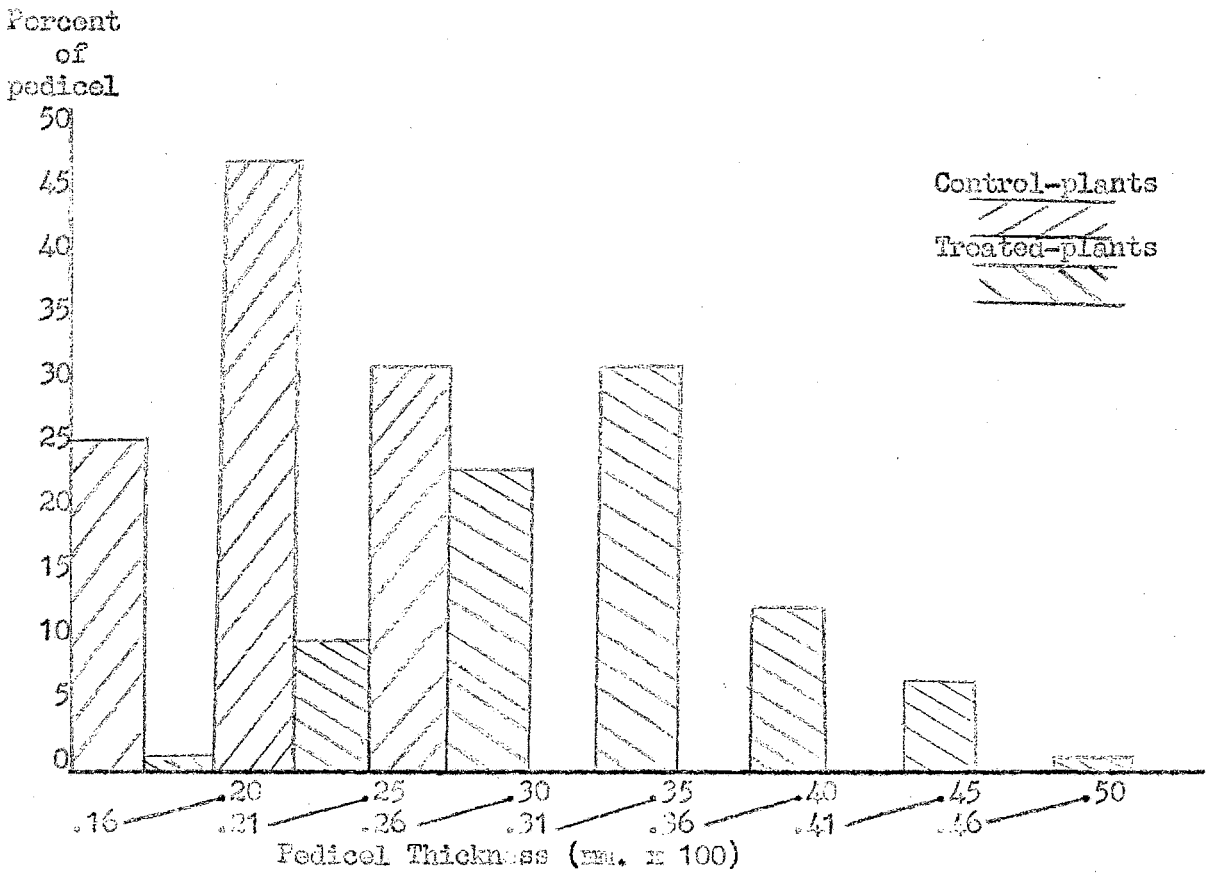


Figure 9 Comparison of Thickness of Pedicel of the Control with That of Treated-plants (I. tricolor)

TABLE 14

Pollen Size for Plants of the Control and Treated series (I. tricolor)

Diameter of Pollen Class Value (mm. x 270)	No. of Pollen grains Control 0-2*	Treated 2-2*	Flower number of the treated-plant 1	1	2	3	4	5	6	7	8
			No. of Pollen grains								
63, 64	2	1	1								
65, 66	0	12	3	5	2	2					
67, 68	0	4	1	1	2	0	1				
69, 70	2	9	3	1	3	1	3				
71, 72	2	6	1	0	0	2	1				
73, 74	3	2	0	0	0	1	2				
75, 76	25	12	1	3	2	2	0	1	1		
77, 78	15	4			1	0	3	1	0	2	
79, 80	10	17				1		5	2	5	
81, 82	1	4				1		1	2	1	
83, 84		2						0	2	0	
85, 86		3						0	2	1	
87, 88		1						1	0	0	
89. 90		2						1	0	1	
91, 92									0		
93, 94									0		
95, 96									1		
Average**	0.28	0.28	0.26	0.26	0.27	0.27	0.28	0.30	0.30	0.30	

*Plant 2-2 was treated with 0.3% colchicine for 24 hrs.. Pollen from 6 flowers of control-plant 0-2 was measured and because of uniformity of size, the data are combined into one frequency distribution.

**Note this is the actual size of the pollen grains in mm..

TABLE 15

Pollen Size for Plants of the Control and Treated Series (I. lacunosa)

Diameter of Pollen Class Value (mm. x 270)		No. of Pollen grains		Flower number of treated-plants									
		Control 0-2*	Treated 2-1*	1	2	3	4	5	6	7	8	9	10
				No. of Pollen grains									
37	38	9											
39	40	11											
41	42	7											
43	44	7											
45	46	14	1	1									
47	48	2	6	2	2	2							
49	50		15	1	3	1	2	4	3	1			
51	52		8	4	1	1	1	0	1	0			
53	54		20	1	0	3	4	3	2	1	5	1	
55	56		27	1	4	3	3	1	3	0	5	2	5
57	58		13					2	1	0		7	3
59	60		6							5			1
61	62		4							3			1
Average**		0.16	0.20	0.19	0.19	0.19	0.19	0.19	0.19	0.22	0.20	0.21	0.20

*Plant 2-1 was treated with 0.3% colchicine and 24 hrs.. Pollens from 5 flowers of control-plant 0-2 was measured and because of uniformity of size, the data are combined into one frequency distribution.

**Note that this is the actual size of pollen grains in mm..

thick sepals and other abnormities, contained much less pollen than that of the control-ones and the percentage of abortive grains was higher. Stigma and style were stained after fixing in lacto-phenol soln. for 30 min. at 140° F, and show weak pollen and no pollen tube on the treated-plant.

2. I. lacunosa

During measuring with this species, only the plump pollen grains were used thus grains of abnormal shape or those staining lightly were discarded.

A comparison of pollen size is given in Table 15. These data show that the pollen of treated-plant 2-1 was consistently larger than that of the control-plant with an average difference of 0.04 mm.. There was some variation in sizes of pollen for individual flower produced on this treated-plant but this appear to be normal. There was little or no tendency for the flower to fall into large and small pollen groups as was true for the I. tricolor plant.

One treated-plant with 0.4% for 48 hrs. (6-1) had many flowers with anthers that did not contain pollen at all.

J. FRUIT-SET

The two methods used to express fertility are percent of fruit-set and percent of abnormal pollen grains. Although polyploids are not necessary highly fertile or exceedingly sterile, yet the percent of fruit-set was calculated here especially because in the species I. tricolor it appeared that there was no tangible factor that seperated the affected and non-affected treated-plants.

1. I. tricolor

The experiment had to be ended by June 23 and before all plants

finished their normal life cycle. The total flowering period up to June 23 as well as the date of the first blossom are given in table 16. Plants that did not have more than ten flowers before the 23rd are not included in the table.

The average fruit-set for 6 control-plants was 20% while that for the treated-plants varied from the high of 38% to the low of 0. As can be seen from the data, flowers blooming late in June probably failed to set fruit due to unfavorable environment, but plants such as 1-1, 2-2 and 5-2 were definitely sterile due to factors within the plant. A high percentage of the flowers of the treated-plant had indehisced anthers, and yet these anthers did have some pollen grains which appeared to be normal. Hand pollinations of flowers on these treated-plants of either self-or by crossing using the pollen grains from the control-plants were made. A total number of forty pollinations of flowers of each was made (during a period from May 30 to June 14) and no fruit-set was obtained.

2. I. lacunosa

The results are given in Table 17. The fruit-set in this species was uniform through out the growing season. Data were not recorded for plants that did not have time to show more than 10 flowers, except for plant 5-9, 5-10 and 6-10.

The average fruit set of the control-plants was 82%, while that for the treated varied widely from 97% to 0. Plants 2-1 and 5-2 set about 26% of its blossoms. It appears most probable that chromosome doubling was obtained in these two plants. Plants 5-9 and 6-10 produced both normal appearing and abnormal branches, no flowers developed on the abnormal stems although flowers were found on the normal branches that divided from the bases of the plants. However, these normal branches were later cut off

leaving the plants in a flowerless condition with no opportunity for pollen measurements. Plant 5-10 had two branches of which one had toothed leaves just as the above plants and the flowers opened were all on the normal appearing branches. Plant 4-3 had had several toothed leaves along with the normal ones.

The mature fruits collected from plant 2-5, 5-2 and also a part from plant 5-3 were apparently larger than the average fruit of the rest of the control and treated-plants. The dry seeds from these large fruits were larger in all three dimensions than the seeds from a normal fruit. As a matter of fact, when the same numbered of seeds were weighed, the seed gathered from plant 2-1 was 21% heavier than seeds from the control-plant.

TABLE 16 Fruit-set of Individual control and treated-plants of *I. tricolor*

Plant No.	Treatment Time	Conc.	No. of flower"	No. of Fruit	%set	Date of 1st Flower	Total flowering period
0-1	0	0	113	17	15	Apr. 22	49 (days)
0-2	0	0	71	15	21	Apr. 20	48
0-3	0	0	66	15	23	Apr. 28	42
0-4	0	0	92	22	24	Apr. 30	40
0-5	0	0	76	13	17	May 22	33
0-6	0	0	48	10	21	May 22	33
Average set of control-plants					20		
1-1***	24	0.2	37	0	0	May 22	33
1-2**	24	0.2	86	6	7.5	May 16	38
1-3**	24	0.2	53	5	9.4	May 13	41
1-4**	24	0.2	55	3	5.5	May 27	28
1-5**	24	0.2	30	1	3.3	May 28	27
1-6***	24	0.2	43	0	0	May 25	29
1-8***	24	0.2	27	0	0	May 18	36
1-9**	24	0.2	30	4	13	June 1	23
1-10*	24	0.2	24	0	0	June 3	20
2-1**	24	0.3	24	1	4.2	May 20	34
2-2***	24	0.3	58	0	0	May 18	36
2-3***	24	0.3	37	2	5.1	May 17	37
2-4**	24	0.3	32	2	6.3	June 3	20
2-5***	24	0.3	53	9	17	May 28	27
2-6*	24	0.3	51	12	24	May 25	30
2-7**	24	0.3	40	1	2.5	June 2	21
2-8**	24	0.3	30	1	3.3	June 5	18
2-9**	24	0.3	33	1	3.0	June 2	21
2-10***	24	0.3	15	0	0	May 22	33
3-1**	24	0.4	73	12	16	May 18	37
3-2**	24	0.4	29	2	6.9	May 16	38
3-3**	24	0.4	47	4	8.5	May 25	30
3-4**	24	0.4	43	2	4.7	May 31	24
3-5**	24	0.4	88	9	10	May 22	33
3-6***	24	0.4	37	1	2.7	May 20	34
3-7***	24	0.4	21	0	0	May 20	34
3-8***	24	0.4	11	0	0	June 13	10
3-9***	24	0.4	45	0	0	June 3	20
3-10**	24	0.4	16	1	6.3	June 7	16
4-1*	48	0.2	33	6	18	May 17	37
4-2*	48	0.2	66	13	20	May 12	42
4-3***	48	0.2	54	15	28	May 24	31
4-4**	48	0.2	19	1	5.3	May 25	30

TABLE 16 Fruit-set of individual control and treated-plants of *I. tricolor* (cont.)

Plant No.	Treatment Time	Conc.	No. of flower	No. of Fruit	%set	Date of 1st Flower		Total flowering period
4-5**	48	0.2	72	2	2.8	May	14	41
4-6**	48	0.2	45	5	11	May	31	24
5-1*	48	0.3	58	14	24	May	20	34
5-2***	48	0.3	30	0	0	May	13	42
5-3*	48	0.3	83	19	23	May	14	41
5-4***	48	0.3	20	0	0	June	1	22
5-5*	48	0.3	28	6	21	June	1	22
5-6***	48	0.3	10	0	0	June	15	8
5-7***	48	0.3	24	0	0	June	1	22
6-1*	48	0.4	32	12	38	May	17	37
6-3***	48	0.4	13	0	0	May	26	29

"Data taken up to June 23rd, 1957.

*Plants not affected.

**Plants partially affected.

***Plants more seriously affected.

TABLE 17 Fruit-set of individual control and treated-plants of *I. lacunosa*

Plant No.	Treatment Time	Conc.	No. of flower"	No. of fruit	% fruit-set
0-2	0	0	69	56	81
0-3	0	0	69	54	78
0-4	0	0	72	64	89
0-5	0	0	61	51	84
0-6	0	0	42	32	76
			Total Average		81.6
1-3	24	0.2	43	28	65
1-4	24	0.2	58	55	95
1-6	24	0.2	70	66	94
1-7	24	0.2	78	69	88
1-8	24	0.2	54	46	85
1-9	24	0.2	86	79	92
1-10	24	0.2	36	27	75
			Total Average		84
2-1**	24	0.3	77	19	25
2-2*	24	0.3	74	18	24
2-3***	24	0.3	33	17	52
2-4	24	0.3	90	75	83
2-5	24	0.3	30	13	43
2-6	24	0.3	52	49	94
2-7	24	0.3	57	50	88
2-8	24	0.3	70	66	94
2-9	24	0.3	96	90	94
2-10	24	0.3	70	68	97
			Total Average		69
3-1	24	0.4	96	82	12
3-2	24	0.4	76	56	74
3-3	24	0.4	120	35	29
3-4*	24	0.4	62	51	82
3-5	24	0.4	65	35	54
3-6	24	0.4	83	63	76
3-7	24	0.4	97	81	84
3-8	24	0.4	70	68	97
3-9	24	0.4	36	18	50
3-10	24	0.4	95	36	38
			Total Average		60
4-1	48	0.2	70	37	53
4-2	48	0.2	74	28	38

TABLE 17 Fruit-set of individual control and treated-plants of I. lacunosa (cont.)

Plant No.	Treatment Time	Conc.	No. of flower	No. of fruit	% fruit-set
4-3*	48	0.2	34	2	5.9
4-4*	48	0.2	27	12	44
4-5	48	0.2	97	71	73
4-6	48	0.2	28	13	46
4-7	48	0.2	16	1	6.3
4-8	48	0.2	81	63	78
4-9	48	0.2	13	1	7.7
			Total Average		40
5-1	48	0.3	61	37	61
5-2**	48	0.3	15	4	27
5-3**	48	0.3	79	6	7.6
5-4	48	0.3	16	9	56
5-6	48	0.3	41	1	2.4
5-7	48	0.3	74	66	89
5-8	48	0.3	37	22	59
5-9***	48	0.3	1	0	0
5-10***	48	0.3	14	0	0
			Total Average		34
6-1	48	0.4	35	3	8.6
6-3*	48	0.4	87	5	5.7
6-4	48	0.4	35	33	94
6-6	48	0.4	88	57	65
6-7	48	0.4	10	9	90
6-8	48	0.4	20	18	90
6-10***	48	0.4	2	0	0
			Total Average		51
			Average set % of the treated		56.3%

*Data taken up to June 23rd, 1957

*Plants most likely to be chimera
**The possibly tetraploid plant
***The possibly octaploid plant and the flowers recorded were produced from the normal appearing branch.

CHAPTER V

DISCUSSION AND CONCLUSION

A. GENERAL APPEARANCE OF TREATED-PLANTS

The general appearance of colchicine treated-plants seemed common to all species. The following is a quote from Blakeslee's paper on Datura: "When seeds are heavily treated, plumule growth is checked, shoots develop from the axil of cotyledons, and buds are abnormally arranged. Sometimes even the buds in axil of cotyledons are inhibited and no growth takes place beyond the cotyledon stage". Reports of other investigators turn-out the literature on this subject gave more or less the same description.

Colchicine in a wide range of concentrations appeared to be effective in this experiment, as has been reported by many investigators. For I. Incurva, the best results appeared to come from the treatment of 0.3% and 0.4% colchicine combined with 48 hrs., and also possibly the 0.2%-48 hrs. combination. These concentrations are within the range reported to be effective on other plant species.

The growth of treated-plants was delayed for some time after treatments were applied. As expressed by the date of the first flower the average is about 22 days late for either species. The number of days required to attain flowering became progressively greater as the concentration of the colchicine increased.

Control-plants and treated-plants germinated March 21 flowered in a shorter period than those of a period from Feb. 29 and March 19. The influence of this environmental factor was more apparent in the control-

plants than in the treated-plants.

B. MEASUREMENT IN DETAIL

The ratio of length to width of leaves was lower in plants of treated-group as compared to the control-group. This was true in both species. Numerous workers have reported that the triploid and tetraploid leaves were usually more rounded and shorter.

As described by Sick in tomato plants, the thickness of leaves increased with the increase of the level of ploidy. However, the data secured from the present experiment did not conform with his result as the leaves of species Lacunosa was somewhat thicker in the treated-plant than those of the control-plant, and the leaves of species bricolor did not have any difference in thickness between the control and treated-plants.

Large stomata were found in the leaves of treated I. bricolor. Within the plants studied, however, these large stomata were intermingling with those of normal size, furthermore, some of these leaves enveyed very much and this might be another result of the distribution of unequal sized cells in the tissues. The range of size of stomata of the treated-plants was much wider than that of the control-plants. The former extended from 12 to 30 while those of the control-plants were within a range of 12 to 21 (mm. x 270). The coefficient of variability was larger in the treated-plants (22.7%) as comparing to 14% of the control. As suggested by the small probable error, the size difference of stomata from the leaves of control and treated plants was significant. Since both malformation of leaves and the large stomata disappeared at a higher level in the plant, the phenomenon was assumed to be a temporary effect of the drug which became ineffective when it was much diluted by growth or it was destroyed by chemical processes within the plant.

Stamia of three different sizes were noticed in plants of the species Lagunae. The basal leaves were crowded and distorted while the leaves at the higher level were normal in outline. Most of the leaves that have large stamia were deeper green in color. With these results accompanied by the larger size of flowers and pollen grains, it seemed safe to assume that these affected plants are diploid in chromosome number. The plant suspected to be at the highest level is badly grown as tall as usual but the leaf cells were devoid of internal branches, cells larger and fewer in number. These features are the basis for identifying polyploids as shown by numerous investigators.

In this experiment, the shape and size of flowers of I. tricolor were the most obscure question. The flowers of the tetraploid-plants were not much larger than the flowers of the control-plants, but were still have much thicker tube of deeper blue color and thick and shorter corolla. There were branches on the tetraploid-plants that gave normal looking flowers and good for harvest, but also many of them showed looking flowers but imperfect anthers and poor pollen contents. On a branch which gave mostly flowers that were affected in appearance, were two or three flowers did not deviate much from the normal and hand-cut was secured from them. Of the anthers of the affected flowers, some'dehisced without dehiscing, but most dehisced late in the afternoon. The only conclusion that likely could be drawn is that the flowers which had thick corolla and a thick corolla tube, and did not fully expand and the anthers could not dehisce into and there for the flowers were sterile and set no fruit.

The first few flowers of tetraploid-plants of I. lacunae usually appeared to be larger due to the thick tube, but neither the limb of corolla nor the length of tube of flowers in most of the plants (two excepted) were

any larger in comparison with the control-plants . The flowers of both the control-plants and the treated-plants declined in size very fast as soon as fruits-set started and when the fruit-set percentage was high. As a matter of fact, less than ten flowers were produced on each control-plant and also on most of the treated-plants before the decrease in size was apparent.

Some flowers from the treated-plants of I. tricolor had normal sized pollen grains, in some, there was anincrease of 7%. The percentage of abortive pollen grains was high in the treated-plants and especially in the affected flowers.

In lacunosa, some flowers showed definitely large pollen grains while the flowers in between the large and normal size gave either larger or normal sized pollen grains. This was believed to indicate either periclinal or sectorial chimera and both kinds were likely present. The average size of pollen grain of the control-plants given was 0.16 mm. cross, and according to Blakeslee, the 2X pollen grain should therefore be 0.16 x 1.25 or 0.21 mm. cross. The average pollen grains obtained was 0.20 mm., however, two individual flowers had the averages of 0.21 and 0.22 respectively. The results here therefore conform to Blakeslee's ratio of 1:1.25. (1x:2x)

C. FRUIT-SET

The set percentage of the control-plants of I. tricolor might be lower than usual because of a mite infestation which prevailed at the later growing season. The low setting percentage of the treated-plants was due primarily to the fact that some of the flowers had thick sepals of the type that were largely sterile. The difficulty was that there were degrees of difference in sepal thickness and other abnormalities and no clear line can be drawn between flowers badly affected and sterile or moderately

affected with a low fruit-set potential. In the treated-group, plants that gave a high set percentage are those that produced normal appearing flowers except plant 4-3 that had had three thick-sepalled flowers and a distorted flower bud.

The possible reasons for the sterile flowers are: (i) the non-dehisced anthers that made the pollen grains unavailable, (ii) the high percentage of abortive pollen from the anthers of treated-plants, (iii) the stigma was not receptive, (iv) the abnormality or abortion of the ovules, and (v) the slow germination of the pollen grains. Since hand pollination did not help the situation, the first reason is eliminated. In one observation pollen grains were abortive (small and poorly staining), but viable pollen from control-plants was applied to these abnormal flowers also failed to produce fruit-set. It is also possible that the flowers were more sensitive to the poison of the colchicine drug than were the leaves, and therefore the abnormal flowers extended to the end of the flowering period while the later-coming leaves were normal.

The tetraploid-like plants of I. lacunosa had lower fruit-set and hand pollination was apparently helpful. It was noticed that flowers on plants 2-1 and 5-2 had shorter styles and the anthers were high above the stigma, and this could be one of the reasons that caused the lower fruit-set, and after the first few flowers of these two plants failed to set the remaining were hand pollinated and fruit-set was obtained.

The high set of some of the treated-plants could be a consequence of the simpler plant structure since the colchicine treatment reducing the vegetative growth. Plant 4-3 was a possible chimera involving the presence of the normal and the highest level of ploidy secured from this treatment. Plant 5-3 was assumed to be one at the same level of ploidy

as plant 2-1 and 5-2. Both of these plants had fruit-set lower than ten percent.

At this time, it is believed that chromosome doubling had occurred in plants 2-1, 5-2 and possibly 5-3. The plants produced larger flowers, leaves with large stomata and large pollen grains. The percentage of fruit-set was reduced in these plants and they were single in growth with fewer laterals than the control-plants.

A total of 29 fruits were gathered from the three plants 2-1, 5-2 and 5-3. The seed from these fruits were much larger in size in comparison with the seeds of the control-plants and the plants that were not effectively treated. Forty-four seeds from plant 2-1 weighed 636 mg. and the same numbered seeds from the control-plants weighed 492 mg., indicating increase of 21%. Although no correlation has been reported between the size of seed and the level of ploidy of a plant, yet it can well be said that these large sized seeds were the result of doubling or redoubling of the chromosomes.

Plant 3-9, 5-10 and 6-11 are so atypical in many respects that they are thought to be at the highest ploidy level. Each had one branch that bore toothed leaves and initiate no flower buds. The size of stomata in the plant was found to extremely large as shown by Fig. 5.

Many of the plants exhibited chimeral characteristics. The plants 4-3 and 6-3 had had several toothed leaves with extra large stomata and plants 3-4, 4-4 and many others had large flowers, large pollen grains and large stomata as in typically tetraploids.

For this species, it was concluded that treatment for 48 hrs. combined with 0.3 percent of colchicine gave best results. However, 0.2 and 0.4% in the 48 hrs. treatment were reasonably effective according to the

criteria for judging the plans available at this time.

CHAPTER VI

SUMMARY

1. Two diploid species of Ipomoea: I. tricolor and I. lacunosa were treated with aqueous colchicine, applied to seedling plants in three concentrations and for two time intervals. Observations were made on the various reactions of the plants to these treatments.

2. The growth of the plants was very much checked after treatment and they started to develop only after a period of inactivity. This period as indicated by time of flowering was about 30 to 40 days.

3. The more intensive treatments (as those from 0.2%-24 hrs. to 0.4%-48 hrs.) prohibited the development of the central stem in some plants and then only lateral branches developed.

4. The ratio of length to width of leaf of both species was changed from about 1.07 to 0.96, indicating that the leaves of the treated-plants were broader.

5. Leaves of the treated-plants of I. lacunosa were about 17% thicker than those of the control-plant according to the data obtained in this study. No difference in leaf thickness was noticed between the control and treated-plants of I. tricolor.

6. Treated-plants of both species had larger stomata. These large stomata was interspersed with normal ones in I. tricolor but all the stomata were large in I. lacunosa. There were three kinds of plants with stomata of succeedingly larger size in the latter species. These are 15 for the control and 18.5 and 27 for treated-plants. This phenomenon led to the

conjecture of the presence of three levels of ploidy within this species.

7. Flowers were larger in some treated-plants of the species I. lacunosa, especially plants 2-1 and 5-2. Branches which were thought to be at a high ploidy level as indicated by stomatal size failed to bloom. Various abnormalities were found in flowers of I. tricolor such as thick pedicels, large flowers, half-opened corolla and non-dehisced anthers.

8. Large pollen grains were found in some of the flowers of treated-plants of both species. The greatest increase in size of pollen found was 7% in favor of the flower from the treated-plant of I. tricolor. The diameter of pollen of flowers from treated-plants of lacunosa was as much as 24% larger than that for pollen from the controls. This is approximately equal to the ratio predicted by Blakeslee (1:1.25).

9. The percentage of fruit-set of treated-plants of both species varied from 0 to levels higher than or equal to that of the control-plant. Pollen abortion was noted in flowers of the severly treated-plants of I. tricolor.

10. Seeds from plant 2-1, 5-2, 5-3 and from the larger flowers of some of the other treated-plants were noticably larger than the seed from the control and the rest plants. An over-weight of 21% was secured in favor of seeds from plant 2-1 over seeds from the control-plants.

11. The best treatment for I. lacunosa is 48 hrs. with colchicine of 0.3%.

12. The optimum treatment for I. tricolor was probably 24 hrs. with concentrations of 0.2, 0.3 or 0.4% of colchicine.

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APPENDIX

TABLE A.

Data of Treatment and Flowering Date of *I. tricolor* of Individual Plants

Plant No.	Treatment Time	Conc.	Date Treated	Date of 1st Flower
0-1	0	0	Feb. 23	Apr. 22
0-2	do		do	Apr. 20
0-3	do		Mar. 8	Apr. 28
0-4	do		do	Apr. 30
0-5	do		Mar. 27	May 22
0-6	do		do	do
1-1***	24 hrs.	0.2%	Feb. 23	May 22
1-2**	do		do	May 16
1-3**	do		do	May 13
1-4**	do		Mar. 8	May 27
1-5**	do		do	May 28
1-6***	do		do	May 25
1-7**	do		Mar. 27	June 24
1-8***	do		do	May 18
1-9**	do		do	June 1
1-10***	do		Mar. 28	June 3
2-1**	24 hrs.	0.3%	Feb. 23	May 20
2-2***	do		do	May 18
2-3***	do		do	May 17
2-4**	do		do	June 3
2-5***	do		Mar. 10	May 28
2-6*	do		do	May 25
2-7**	do		Mar. 27	June 2
2-8**	do		do	June 5
2-9**	do		do	June 2
2-10***			Mar. 31	May 22
3-1**	24 hrs.	0.4%	Feb. 23	May 18
3-2**	do		Mar. 10	May 16
3-3**	do		Mar. 12	May 25
3-4**	do		Mar. 11	May 31
3-5**	do		do	May 22
3-6***	do		do	May 20
3-7***	do		do	do
3-8***	do		Mar. 27	June 13
3-9***	do		do	June 3
3-10**	do		do	June 7

TABLE A. cont.

Plant No.	Treatment Time-Conc.	Date Treated	Date of 1st Flower
4-1*	48 hrs. 0.2%	Feb. 23	May 17
4-2*	do	do	May 12
4-3***	do	do	May 24
4-4**	do	Mar. 8	May 25
4-5**	do	do	May 14
4-6**	do	do	May 31
4-8**	do	Mar. 27	June 25
4-10**	do	do	do
5-1*	48 hrs. 0.3%	Feb. 23	May 20
5-2***	do	do	May 13
5-3*	do	do	May 14
5-4***	do	Mar. 8	June 1
5-5*	do	do	do
5-6***	do	Mar. 11	June 15
5-7***	do	do	June 1
5-8**	do	Mar. 27	June 24
6-1*	48 hrs. 0.4%	Feb. 23	May 17
6-5***	do	do	May 26
6-4**	do	Mar. 8	June 11
6-5**	do	Mar. 11	June 19
6-6**	do	Mar. 16	June 14

* Plants Not Affected
** Plants Partially Affected
*** Plants More Seriously Affected

APPENDIX

TABLE B.

Detailed Measurement of Individual Plants of *I. tricolor*

Plant No.	Leaf Size (Length-Width)	Leaf Thickness	Stomata Size	Flower Size	Pollen Size
0-1			16	87	
0-2				85	76
0-3				87	
0-4		0.0325		88	
0-5	116-116	0.025	16	84	66
0-6	108-94	0.0388		81	
1-1***	89-94	0.0388	19	81	63
1-2**			18	85	72
1-3**			17	85	70
1-4**	89-91	0.0388	17	88	68
1-5**				87	
1-6***	108-121	0.0388	19	88	68
1-7**					
1-8***				85	
1-9**				84	
1-10***			20	82	
2-1**				80	
2-2***	77-92	0.04	17	93	75
2-3***	78-84	0.0338		85	
2-4**				82	
2-5***			20	84	
2-6*		0.0313		85	
2-7**				83	
2-8**			18	87	60
2-9**				84	
2-10***				83	
3-1**	92-91		17	86	
3-2**				84	
3-3**				83	
3-4**				84	
3-5**	105-104			84	
3-6***	89-92			92	
3-7***	74-76	0.0338	16	94	65
3-8***			21	85	70
3-9***				86	72
3-10**				85	

TABLE B. cont.

Plant No.	Leaf Size (Length-Width)	Leaf Thickness	Stomata Size	Flower Size	Pollen Size
4-1*				86	
4-2*			17	83	
4-3***	96-99	0.035		86	
4-4**				85	
4-5**				84	
4-6**				82	
4-8**				85	
4-10**				85	
5-1*				83	
5-2***	73-69	0.045	18	90	72
5-3*			17	85	
5-4***	78-85	0.035	22	92	71
5-5*				84	
5-6**				84	
5-7**				85	
5-8**				86	
6-1*			18	85	
6-3***	90-90			85	
6-4**				80	
6-5**				83	
6-6**				85	

* Plants Not Affected
** Plants Partially Affected
*** Plants More Seriously Affected

APPENDIX

TABLE C.

Data of Treatment and Flowering Date of Individual Plants of *I. lacunosa*

Plant No.	Treatment Time-Conc.	Date Treated	Date of 1st Flower
0-1	0 0	Mar. 2	Apr. 16
0-2	do	do	Apr. 15
0-3	do	Mar. 19	Apr. 22
0-4	do	do	Apr. 21
0-5	do	Mar. 29	Apr. 27
0-6	do	do	do
1-3	24 hrs. 0.2%	Mar. 2	Apr. 30
1-4	do	Mar. 19	Apr. 27
1-6	do	do	Apr. 30
1-7	do	do	May 10
1-8	do	do	May 11
1-9	do	do	May 6
1-10	do	do	May 17
2-1*	24 hrs. 0.3%	Mar. 2	May 8
2-2***	do	do	Apr. 28
2-3***	do	do	Apr. 27
2-4	do	do	Apr. 12
2-5	do	Mar. 19	Mar. 28
2-6	do	Mar. 22	Apr. 17
2-7	do	Mar. 29	May 9
2-8	do	do	May 7
2-9	do	do	May 9
2-10	do	Mar. 14	Apr. 28
3-1	24 hrs. 0.4%	Mar. 2	Apr. 16
3-2	do	Mar. 14	Apr. 22
3-3	do	Mar. 7	Apr. 27
3-4***	do	Mar. 13	May 9
3-5	do	do	May 11
3-6	do	do	May 9
3-7	do	Mar. 29	May 10
3-8	do	do	May 22
3-9	do	do	May 26
3-10	do	do	May 6

TABLE C. cont.

Plant No.	Treatment Time-Conc.	Date Treated	Date of 1st Flower
4-1	48 hrs. 0.2%	Mar. 2	Apr. 27
4-2	do	do	Apr. 25
4-3***	do	do	May 1
4-4***	do	Mar. 19	May 28
4-5	do	do	Mar. 7
4-6	do	Mar. 18	May 27
4-7	do	Mar. 29	May 31
4-8	do	do	May 9
4-9	do	Mar. 30	May 27
4-10	do	do	June 22
5-1	48 hrs. 0.3%	Mar. 2	Apr. 27
5-2*	do	do	May 27
5-3*	do	do	Apr. 30
5-4	do	Mar. 13	May 30
5-6	do	Mar. 14	May 18
5-7	do	Mar. 29	May 9
5-8	do	do	May 27
5-9**	do	do	June 5
5-10**	do	do	May 31
6-1	48 hrs. 0.4%	Mar. 2	May 7
6-3***	do	do	Apr. 26
6-4	do	Mar. 11	May 28
6-5	do	do	June 10
6-6	do	Mar. 13	May 13
6-7	do	Mar. 29	June 1
6-8**	do	do	May 28
6-9	do	do	June 1
6-10	do	do	June 14

* The Possibly Tetraploid Plant
** The Possibly Octaploid Plant
*** Plants Most Likely to be Chimera

APPENDIX

TABLE D.

Detailed Measurements of Individual Plants of *I. lacunosa*

Plant No.	Leaf Size (Length-Width)	Leaf Thickness	Stomata Size	Flower Size (Limb-Tube)	Pollen Size
0-1			15	21-24	
0-2		0.025		22-26	43
0-3	4.6-4.0	0.024		21-25	
0-4	4.6-4.0		15	21-24	
0-5	5.0-4.6				46
0-6	5.0-4.6				
1-3				21-25	
1-4					
1-6				21-25	
1-7					
1-8					
1-9					
1-10		0.033	18	20-26	
2-1*	5.0-5.0	0.031		24-27	54
2-2***	5.3-5.6			22-26	50
2-3***				20-27	53
2-4				21-25	50
2-5					
2-6					
2-7				20-22	
2-8					
2-9					
2-1-			17	22-27	
3-1			18	22-26	
3-2				21-24	49
3-3				22-25	
3-4***		0.026	19	21-24	53
3-5	5.6-5.4			21-27	52
3-6				22-26	
3-7	6.9-6.5				
3-8					
3-9	5.6-5.7			20-25	
3-10					

TABLE D. cont.

Plant No.	Leaf Size (Length-Width)	Leaf Thickness	Stomata Size	Flower Size (Limb-Tube)	Pollen Size
4-1			20	22-26	50
4-2		0.033		20-25	52
4-3***		0.039	23	21-27	52
4-4***	5.6-5.4			20-27	54
4-5					
4-6				21-24	55
4-7				20-25	
4-8					
4-9			19	20-25	54
4-10					
5-1				22-27	
5-2*	4.0-3.9	0.0313	19	22-28	55
5-3*	5.0-5.5	0.025	19	21-28	53
5-4					
5-5	6.0-6.8	0.03		21-26	
5-7					
5-8				20-25	
5-9***			27		
5-10***	5.9-6.2		26		
6-1					
6-3***	5.3-5.8	0.035	23	23-37	
6-4				18-20	
6-5				18-24	
6-6	5.6-5.6	0.022		20-25	
6-7					
6-8					
6-9					
6-10***			25	20-26	

* The Possibly Tetraploid Plant
** The Possibly Octaploid Plant
*** Plants Most Likely to be Chimera

VITA

FEN-WEN WOO

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