THE ACTION AND USE OF COLCHICINE IN THE PRODUCTION OF TETRAPLOID BUCKWHEAT (*FAGOPYRUM ESCULENTUM*).

A Thesis

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Frontispiece

(A tetraploid plant and a diploid plant four weeks after emergence).

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INTRODUCTION

(a) THE ACTION AND USE OF COLCHICINE

The demonstration of the value of colchicine as an agent for chromosome doubling has opened a large reservoir of possibilities in plant breeding work. According to Muntzing (1936), "more than half of all the angiosperms found in nature are polyploids and some others have been produced experimentally." So frequently have these plants been shown to have desirable characteristics associated with their polyploidy (larger fruit, wider distribution, greater adaptability to environmental conditions), that we find full justification for the study of the use of colchicine in inducing polyploidy.

The purpose of this investigation was to study the effects of alkaloid colchicine on diploid Fagopyrum esculentum (Gaertn.) with a view to producing a tetraploid variety.

The beginning of work on colchicine-induced polyploidy in plants may be linked to the work of Lits (1934), a student of A. P. Dustin. Lits studied the general cellular reactions and "lesions" caused in animal tissues. Dustin immediately following the works of Lits, made a study of the action of colchicine on a grafted sarcoma in mice (1934). Lits credits Dixon (1906), and Dixon and Malden (1908), with having done the first cytological work on the effects of colchicine on animal material. They have shown that colchicine usually arrests chromosome development at metaphase in animal cells which after reaching this stage may die. In plants, Blakeslee and Avery (1937), and Nebel and Ruttle (1938) have demonstrated that application of colchicine results in the doubling of the number of chromosomes in cells and have successfully used it in the production of polyploids.

The action of colchicine on mitosis has been investigated by many workers. The following account is based on Levan's work (1939), which is the clearest and most concise. He studied root tips of Allium cepa fixed after immersion in aqueous solution of colchicine, using concentrations ranging from 0.125 to 2.0 per-cent with periods of immersion from seven minutes to seventy-two hours.

In general he stated that colchicine inactivated the spindle mechanism and delayed the division of the centromere. Prophase stages showed no irregularities - the nuclear membrane disappeared but neither spindle nor metaphase plate was formed. As relational coiling disappeared the chromatids tended to repel each other and loops were formed between the centromere and the points where coiling brings the chromatids in contact. These points terminalized as chiasmata do in meiosis, but the ends of the chromatids did not remain in contact. The chromatids were held together only at the centromere. These chromosome structures Levan called c-pairs (colchicine pairs) and they were found only during the first few hours after treatment, showing that the division of the centromere is delayed. After a few hours the division of the centromere took place, but in the absence of a spindle, the daughter chromosomes could not pass to the poles but lay side by side. The chromosomes then passed on to the resting stage and were all included in one polyploid nucleus. Levan showed that the proportion of polyploid cells in treated tissue increased with (i) concentration of colchicine, (ii) length of exposure and (iii) distance behind root tip.

(b) PREVIOUS WORK ON BUCKWHEAT

Three Russian workers, Frolova, Sakharov and Mansurova (1944a, 1944b, 1945, 1946) have recently published several papers on the production of tetraploid buckwheat with colchicine, using several Russian varieties. They report (1946) that after two years of selection the most fertile families of tetraploids were derived from the variety Bolsevik and that even in highly fertile families some plants of low fertility segregate out. A cytological study of this high and low fertility was carried out. In most fertile plants the percentage of abortive pollen was low (two to four percent). Pollen grains with 14, 15, and 16 chromosomes were found to be viable and seed-set was found to be higher than would be expected in an autotetraploid, due to their production of diploid seeds by parthenocarpy. In plants exhibiting low fertility the percentage aborted pollen was zero to eight percent and this low fertility they attributed to the formation of oval pollen grains with an extremely thin exine. They also found empty pollen grains in both diploids and tetraploids of the variety Spanish Silver. They concluded that low fertility due to empty pollen is not caused by tetraploidy. A study was made of pollen development in the diploid but the results were not discussed.

W. J. Sando (1939) reported the production of tetraploid buckwheat by the application of one percent colchicine in a lanolin paste applied to the stems of eight three-weeks old seedlings of tartary buckwheat (Fagopyrum tataricum). In general the tetraploid plants grown outdoors and in the greenhouse during the fall

of 1938 were taller and flowered and matured their seed slightly later than the diploid plants. The main stems of the tetraploids were 33 percent greater in diameter than the diploids. The leaves were more irregular on their margins and surface and deeper green in colour and much greater in diameter than the diploids. Other characters such as anthocyanin in the petioles, leaves, stems and hairs of the stems were greatly accentuated. In these experiments doubling the chromosome number from 16 to 32 increased the size of various plant parts 15 to 63 percent. No mention was made of pollen sterility.

Dr. Hedda Nordenskiöld of Sweden is experimenting with colchicine in the production of tetraploids in several varieties of buckwheat (unpublished). Some of her seed has been sent to this country for field tests.

(c) THE BUCKWHEAT PLANT, FAGOPYRUM ESCULENTUM

The following description is based on Hector (1936).

Roots. Buckwheat possesses a main tap root system which may penetrate to a depth of three or four feet, the lateral roots being comparatively few.

Stems. The stems are erect and succulent, hollow and somewhat angular, downy and swollen at the nodes, green to red in colour, becoming brown with age. Lateral branches are few in number.

Leaves. The leaves are alternate, the lower being about four inches long, the upper becoming much smaller and almost sessile. The ochrea is short and tends to fall early. The blades are hastate, acute, two to four inches long with hairs on the lower surface.

Inflorescences. The inflorescences are terminal or axillary cymes,

erect or drooping, with numerous, often densely clustered flowers.

Flowers. The perianth is five-partite, pink or pinkish-white in colour and persistent, but not enlarging with the fruit. There are eight stamens in two whorls. All have filiform filaments and oblong anthers. Alternating with the filaments are eight small, rounded, yellow nectar glands. The ovary is superior, triangular, one-celled, one-ovuled; the style is tripartite, each branch ending in a knob-like stigma.

Pollination. The flowers are dimorphic. Some plants bear flowers with short stamens and styles about a third as long again (long-styled plants) others have flowers with short-styles and stamens which project some distance above the stigmas (short-styled plants). The pollen grains of short-styled plants are larger than those of long-styled, the ratio of their diameters being about five: four (Stevens 1912b). The gene for short-styles is said to be dominant to that for long-styles, segregating in a three to one ratio (Althausen, 1908, and Egiz, 1925). Short-styled flowers are always found heterozygous in nature, Ss, the long-styled being ss. This is a genetically determined mechanism associated with the differential pollen tube growth factor. Darwin (1859) showed that so-called legitimate pollination was more successful than illegitimate pollination. When both types of pollen were placed on the same plant the legitimate pollen grew faster, effecting fertilization before the illegitimate pollen.

Both Stevens (1912b), and Quisenberry (1927), agree that the chromosome numbers are n=8 and 2n=16. In short-styled flowers the chromosomes have a diameter nearly twice that of the chromosomes of a long-styled

flower. This difference extends to the pollen mother cells themselves, which at diakinesis are definitely larger in short-styled than in long-styled flowers.

At West Virginia Station, Hayes and Garber (1927) stated that selfed-seed is relatively rare and difficult to obtain from varieties such as Japanese, Silver Hull and Grey. In all probability, degrees of self-incompatibility occur.

Fruit and Seed. The fruit is a three-angled, ovate achene five to eight mm. in length by three to four mm. broad. The faces are glabrous and shiny, generally slightly convex, and the angles more or less acutely keeled. The colour is grey or brown, marbled with darker spots and lines; black fruits also occur. With the Silver Hull variety, plants are medium to small, grains are small, non-winged, with a glossy silvery appearance.

PART I

THE EFFECTS OF THE ALKALOID COLCHICINE ON DIPLOID BUCKWHEAT (FAGOPYRUM ESCULENTUM) VARIETY SILVER HULL.

(a) MATERIALS AND METHODS

Buckwheat seed was donated by the Department of Agronomy at McDonald College. An aqueous solution of colchicine 0.4 percent was used in all treatments (this concentration had been used quite successfully on buckwheat by Dr. Boyes). The only variation was in duration and method of treatment.

Method I - Seed-Treatment Method. A hundred dry seeds were placed on paper towelling in a three-inch petri dish and covered with about 25 cc. of a 0.4 percent solution of colchicine. Three such lots each consisting of a hundred seeds were treated, the first for twenty-four, the second for thirty-six and the third for forty-eight hours. After treatment, germination was continued in water until all lots had germinated for a total of three days. Another lot of one hundred dry seeds was germinated in water only for three days under the same conditions to serve as a control.All four lots were kept at thirty degrees Centigrade throughout this three-day germination period. Ten germinated seeds were then chosen at random from each of the four lots and planted in three-inch pots in the greenhouse with number, date and treatment time indicated. (Only ten plants in each lot were planted because of limited space in the greenhouse). Two series of treatments by the above method were undertaken; the first treated series was planted October 19, and the second series November 21, 1949.

Method II - Seedling-Treatment Method. Forty plants in four lots of ten were planted in the greenhouse in three-inch pots on November 21, 1949 to receive special treatment. At the end of a one week period when the plants had attained an average height of eight

centimeters a small ball of absorbent cotton two x two mm. was dipped in 0.4 percent colchicine and placed between the cotyledons against the growing point. Each plant was covered with a six-inch vial lined with a strip of moistened paper towelling to increase moisture content within the tube and to cut down evaporation of the colchicine solution. Three lots of ten plants each were treated by this method for twenty, thirty and forty-four hours respectively. The remaining ten plants, serving as a control lot, received the same treatment except for the substitution of water for colchicine. This same treatment method was repeated on December 14, 1949 for a control lot of ten plants and five lots of ten plants each with treatment times of twenty, twenty-nine, thirty-four, forty-four and seventy-two hours.

All plants were grown in a soil consisting of a mixture of two parts of garden soil to one part of peat moss - the mixture giving a slightly acid indication when tested. All plants received twelve hours light per day either artificially from three seven hundred and fifty-watt bulbs, or from natural sunlight depending upon weather conditions. At the end of four weeks all plants were transplanted to six-inch pots and fresh soil (the same mixture as before) and tied up to four-foot canes for added support. All plants were randomized and their positions changed weekly so that conditions of light, etc., would be standardized for all lots. A complete record of each plant was kept. The height of each plant above the ground in centimeters was measured each week, for a duration of eight weeks, after the first appearance of the cotyledons. A record

was also kept of the first signs of budding in each plant. At the height of flowering the total number of flowers per plant was counted and the number of abnormal flowers out of the total was determined. In the case of those plants treated by the contact method in which more than one shoot arose from the treatment point the number was recorded.

Recognition of probable tetraploids where treatment had been successful was made on the basis of stomatal and leaf size of diploid and treated plants. A comparison of pollen size was also made as further supporting evidence of successful treatment. Such things as leaf thickness, breadth of leaf, intensity of colour, blossom size, thickness of stalks and petioles, etc., were used as further supporting evidence of probable polyploidy and success of treatment.

All pollinations were carried out within treatment lots and only legitimate pollinations were done (long-style crossed with short-styled plants and the reciprocal cross). Pollinations were done between plants where as far as was ascertainable treatment had been successful. The number of seeds set per hundred pollinations was recorded for each series and also the number of abnormal seeds. All pollinations were done by hand with a paint brush and to prevent possible self-pollination the anthers in all flowers were removed, except those flowers being self-pollinated.

Seed from each plant was packaged separately and the cross indicated. The treatment used was also recorded on the package.

A count of "pollen sterility" was made for each plant based on whether the pollen looked abnormal (thickened walls, distorted shape or empty) using about 25 pollen grains in each case. Five

such counts were completed for each plant within each treatment lot.

Leaf samples of treated and untreated material for determination of chlorophyll content were collected. The chlorophyll was extracted with acetone and the amounts determined by use of the spectrophotometer, using the percentage of light absorbed as a quantitative measure.

Later, when the plants were about seventy cms. in height, nodal cuttings were taken from both diploid and probable-tetraploid plants. The cuttings, ten from the seed-treated group, ten from the seedling-treated group and twenty from control groups were dipped in a mixture of three-indole acetic acid and talcum powder and planted in sand. Later the cuttings which had rooted were repotted in three-inch pots, using the same soil mixture as was used in all previous cases.

All epidermal tissue examined was removed from the under surface of the leaves beside the midvein. Sections were mounted on a slide in ninety-five percent alcohol for three minutes to remove excess chlorophyll. Before all the alcohol had evaporated and the tissue was still moist a drop of glycerine was placed on the tissue and a cover slip mounted. No staining was found necessary to highlight the stomates or epidermal cells.

Photographs of leaves, stems, flowers, roots, seed, etc., as desired for treated and control were taken on Panatomic X film. Camera lucida drawings were made of pollen grains, meiotic and root tip division figures.

(b) RESULTS AND OBSERVATIONS

I. Germination.

The effect of colchicine was seen first in the seeds germinating in colchicine. These seeds were very slow in germinating and the radicles were much shorter and thicker than those of the controls. Swelling was not uncommon on the primary root.

With increased time in colchicine there was a decreased percent germination. (See Table I). After forty-eight hours treatment, only three plants survived, in each of the two seed-treatment series and consequently longer treatment of seed was not attempted. An examination of seeds in these lots showed that they had rotted beneath the soil because of the growth-restricting effects of colchicine, excepting the three plants which had broken the soil and survived.

II. Height Reduction in the Plants from Colchicine-Treated Seed.

It was noted that the treated plants took much longer to break through the soil. The controls, on the average, took a day to come through, the twenty-four hour treated lots took around two days, the thirty-six hour lots an average of five days and the forty-eight hour lots two weeks. Only three plants were obtained in each of the forty-eight hour lots. Figure I and Table II show the growth records for the first series of plants and Figure II and Table III the results for the November 21st series.

III. Height Reduction in Plants Treated by the Contact Method.

The same effect on growth was noticed with this treatment as with the previous one, ie. with increased treatment there was a decreased growth rate. This treatment was more satisfactory since these plants were well established, having attained an average height of eight centimeters before being treated, and all plants survived

Table I. Percentage germination and means and standard errors of radicle lengths in centimeters for seeds treated with 0.4% colchicine.

Series	Lot	Hours in colchicine	Hours in water	Percentage germinated	Radicle lengths
10/19/49.	A	Control	72	94	3.0±0.12
	B	24	48	76	2.0±0.25
	C	36	36	55	1.0±0.12
	D	48	24	40	0.5±0.61
11/21/49.	A	Control	72	89	3.0±0.04
	B	24	48	72	2.0±0.25
	C	36	36	55	1.0±0.13
	D	48	24	36	0.5±0.61

N.B. One hundred seeds in each lot. Mean radicle length calculated by choosing, after three days in solution, ten seedlings at random.

Table II. Means and standard errors of heights in centimeters of plants from treated seed Oct. 21st. planting (ten plants per treatment lot).

Weeks after emergence	Control (A)	Treatment lots (0.4% colchicine). Twenty-four hours (B)	Thirty-six hours (C)	Forty-eight hours (D) *
1	8.6±0.04	2.2±0.07	2.0±0.06	2.0±0.01
2	19.0±0.54	3.9±0.04	3.0±0.06	2.5±0.01
3	35.0±0.31	12.9±0.16	5.0±0.66	2.7±0.01
4	49.0±0.32	22.2±0.62	9.7±0.04	3.0±0.01
5	61.0±0.33	32.2±0.06	16.0±0.70	4.5±0.01
6	70.2±0.05	42.2±0.26	24.0±0.32	6.0±0.01
7	84.2±0.32	52.0±0.98	31.2±0.21	7.2±0.01
8	94.0±0.62	65.0±0.33	37.5±0.11	8.0 (-)

* results based on three plants only.

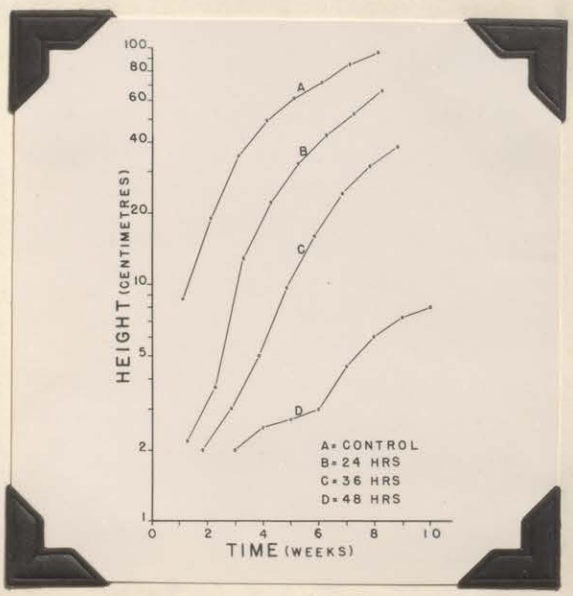

Fig. I- Average height of plants from treated seed, October 21, 1949 planting. Averages for ten plants in each lot except (D) where only three plants survived.

Table III. Means and standard errors of heights in centimeters of plants from treated seed Nov.21 planting (ten plants per treatment lot).

		Treatment lots (0.4% colchicine).		
Weeks after emergence	Control (A)	Twenty-four hours (B)	Thirty-six hours (C)	Forty-eight hours (D) *
1	8±0.34	2.5±0.05	2.2±0.04	2.0± (-)
2	18±0.41	2.7±0.05	3.0±0.04	2.5±0.01
3	34±0.46	12.5±0.28	5.2±0.05	2.7±0.01
4	49±0.48	22.0±0.41	10.0±0.45	3.3±0.04
5	64±0.04	32.0±0.38	17.0±0.23	4.1±0.01
6	70±0.76	43.0±0.59	25.0±0.34	6.1±0.01
7	83±0.53	52.0±0.25	32.0±0.21	7.2±0.01
8	93±0.34	66.0±0.21	38.0±0.48	8.2 ± (-)

* results based on three plants only.

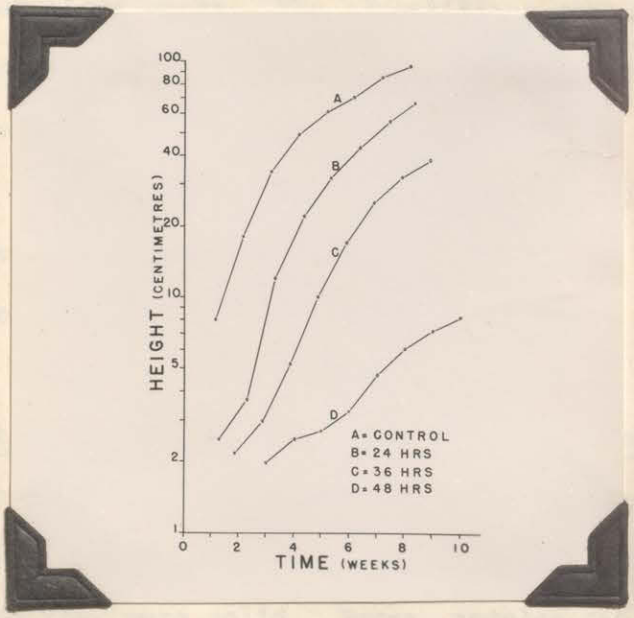

Fig. II- Average height of plants from treated seed, November 21, 1949 planting. Averages for ten plants in each lot except (D) where only three plants survived.

various periods of treatment. Figure III and Table IV show the heights of plants measured each week for lots treated twenty, thirty-four and forty-four hours as well as the control lot.

The thirty-four hour treated lots seemed to give the most promising results so the experiment was repeated for twenty, twenty-nine, thirty-four, forty-four and seventy-two hours on December 14, 1949. Figure IV and Table V show the heights of plants in centimeters each week for each lot treated at that time.

IV. Effects on Roots of Seed-Treated and Seedling-Treated Plants.

The growth inhibiting effects of colchicine on the radicles (Table I), first noticed among the germinating seeds, was again observed in the seed treated plants. The roots were very stunted and had not penetrated the soil to anywhere near the depth the controls had penetrated at three weeks. The adventitious roots on these plants grew more horizontally than in untreated plants, their main tap roots became swollen and much thickened. Root stunting was not as apparent in the seedling treatment group and no correlation could be drawn between root development and extent of treatment. In all the treated plants aerial roots were quite prevalent, a curiosity not found among the controls.

V. Effect on Stems and Shoots of Seed-Treated and Seedling-Treated Plants.

(a) Stems. The stems of plants, where treatment has been successful, were found to be very brittle and the walls of the stems very much thickened so that sometimes they were solid. Bumps, nodules and striations were not uncommon on the stems.

In the seedling-treated plants a very curious effect was noticed. Tremendous swelling resulted in the formation of a doughnut-shaped mass of tissue around the treatment point. In the centre of the doughnut-

Table IV. Means and standard errors of heights in centimeters of plants from treated seedlings, Nov.21 planting (ten plants per treatment lot)*

Weeks after treatment	Treatment lots (0.4% colchicine). Control (A)	Twenty hours(B)	Thirty-four hours(C)	Forty-four hours(D)
0	8±0.21	8.0±0.21	8±0.45	8.0±0.21
1	19±0.57	12.0±0.23	10±0.45	8.5±0.45
2	35±0.33	17.0±0.34	14±0.31	9.0±0.08
3	50±0.14	28.0±0.45	24±0.36	9.8±0.04
4	62±0.57	37.5±0.27	30±0.25	12.5±0.08
5	70±0.34	47.0±0.39	38±0.36	16.0±0.32
6	84±0.35	56.0±0.40	46±0.50	26.0±0.36
7	95±0.48	65.0±0.29	52±0.70	36.0±0.69

* Forty plants in all were grown, separated into lots of ten, and treated one week after emergence, (ten untreated serving as a control).

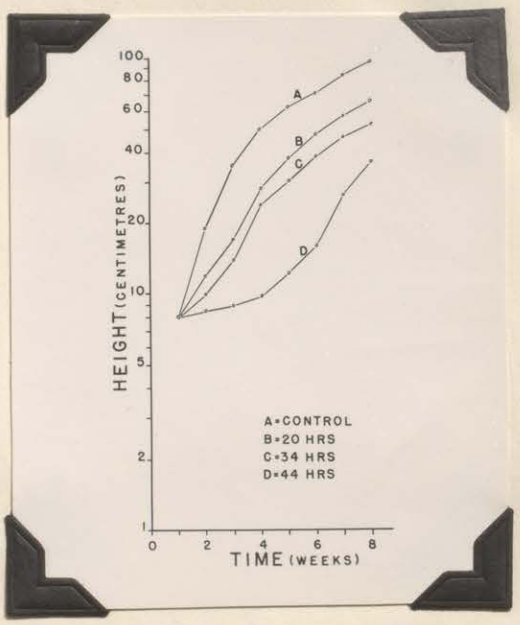

Fig. III- Average height of seedling-treated plants, November 21 planting, based on ten plants in each lot.

Table V. Means and standard errors of heights in centimeters of plants grown from treated seedlings Dec. 14 planting (ten plants per treatment lot)*

Weeks after treatment	Treatment lots (0.4% colchicine).					
	Control (A)	Twenty hours (B)	Twenty-nine hours (C)	Thirty-four hours (D)	Forty-four hours (E)	Seventy-two hours (F)
0	9±0.21	7.0±0.23	7.0±0.26	7±0.25	7.0±0.27	7.0±0.06
1	18±0.47	11.5±0.30	9.5±0.28	9±0.26	8.2±0.29	7.1±0.15
2	35±0.45	14.0±0.29	11.5±0.29	11±0.27	9.0±0.27	7.2±0.23
3	50±0.55	25.0±0.36	21.0±0.33	20±0.34	9.6±0.27	8.0±0.22
4	60±0.56	38.0±0.57	31.0±0.34	30±0.64	12.4±0.28	8.8±0.23
5	71±0.56	47.0±0.24	40.0±0.34	38±0.35	16.3±0.28	9.7±0.21
6	83±0.69	55.0±0.36	47.0±0.32	46±0.31	27.0±0.31	11.0±0.30
7	94±0.70	64.0±0.37	55.0±0.29	53±0.32	37.0±0.34	13.0±0.31

* Sixty plants were grown, separated into lots of ten and treated one week after emergence, (ten untreated serving as a control).

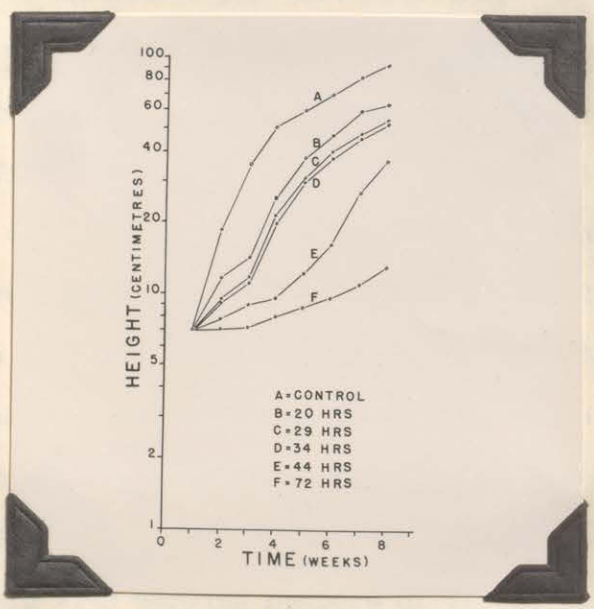

Fig. IV- Average height of seedling-treated plants, December 14 1949 planting, based on ten plants in each lot.

shaped swelling or collar new shoots, both diploid and probable tetraploid (Figure V) arose. With increasing treatment the size of this collar increased until in the seventy-two hour treated lots some were as large as an inch in diameter. Some of this tissue was collected for further study.

Also noticeable was the extreme red colour of the stems of treated plants. At the end of seven weeks the controls had started to turn reddish-brown whereas the treated plants were then bright red in colour. Some of the leaves were also affected in this manner, a condition not noticed in the control plants. This could not be entirely the effect of lighting since the plants were randomized in position each week.

Comparison of stomatal size between diploids and presumed tetraploids indicated that the tetraploid stomates were two to three times as big and fewer in number per unit area (Figures VI and VII).They averaged 1.75 ocular micrometer scale units in area compared to 0.625 for the diploid, based on twenty measurements for each.

No significant difference was found between the stomates of the cotyledons of diploid and probable-tetraploid plants. In the case of seedling-treated plants this was to be expected since treatment was done above the cotyledons. However, in seed-treated plants this was also the case. Presumably the cotyledons were highly developed within the pericarp in the mature seed.

(b) Shoots. In the diploid and seed-treated plants one main stem was the general rule, with varying numbers of shoots or side branches. In the seedling-treated plants varying numbers of shoots arose from the treatment point and accordingly it was not possible to differentiate between the main stem and lateral shoots. Both diploid and polyploid shoots arose from the treatment point. In some of the seventy-two

Fig. V- Shoots arising from treatment point of a seedling-treated plant. (Actual size).

Stomates and epidermal cells for diploid and treated plants

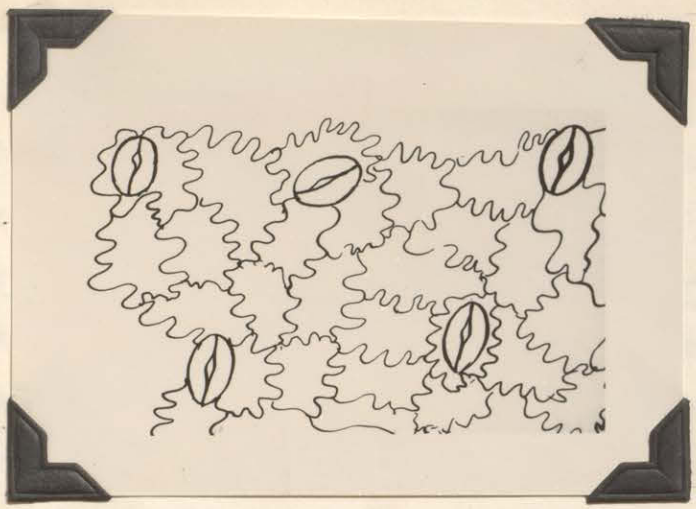

Fig. VI- Stomates and epidermal cells from a normal diploid leaf (Camera lucida drawing X 175).

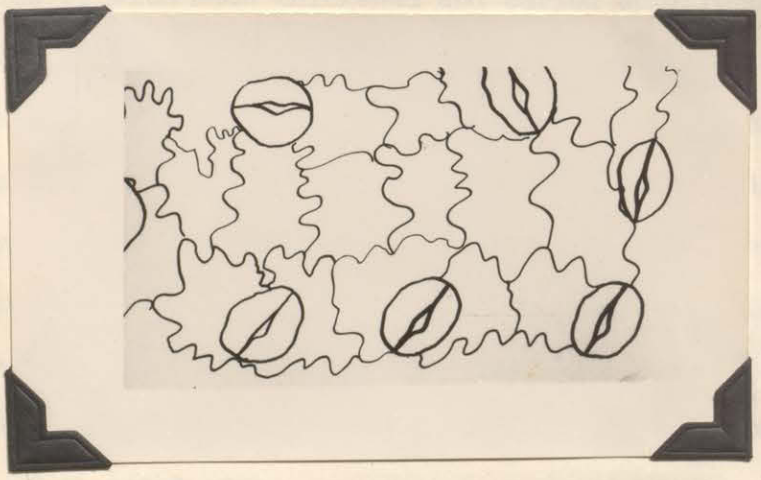

Fig. VII- Stomates and epidermal cells from a leaf from a treated plant (Camera lucida drawing X 175).

hour, seedling-treated plants as many as seventeen shoots arose. On one plant in this lot a completely ablino shoot arose lacking chlorophyll in leaves, stems and buds. Attempts were made to maintain this shoot by feeding into the stem sugar solutions in very weak concentrations but the shoot finally died.

VI. Effects on Leaves of Seed-Treated and Seedling-Treated Plants.

The leaves on the treated plants were much broader and shorter than those of the control plants. They were also much thicker and darker in appearance, although tests as to chlorophyll content showed less chlorophyll per unit area than in the diploids. Perhaps the darker colour was caused by the leaves being much thicker than in the diploids.

Many leaf abnormalities resulted (Figures VIII - X): double leaves on a single petiole, leaves with deep indentations in their edges and leaves with much thickened and curled appearance and extremely thick petioles. On general observation alone it seemed that with increased duration of treatment there was an increasing number of leaf abnormalities. This was most apparent in the seedling-treated group, where at seventy-two hours treatment time the leaves had very large and thick petioles (sometimes a quarter inch in thickness) and look very much like a crumpled ball of paper. They were extremely brittle and it was very hard to take epidermal tissue from them for comparison of stomatal size.

VII. Effect on Inflorescences of Seed-Treated and Seedling-Treated Plants.

Where treatment has been successful and probable-tetraploid plants or shoots were produced the blossoms were very much larger than the diploids. The petals were longer and much broader. Flowers were found bearing two, three, four, six, seven and eight petals, the normal being five (Figures XI - XIV). One plant had only fused flowers consisting

Leaf abnormalities found in treated plants

Fig. VIII.

Fig. IX.

Fig. X.

Fig. VIII- Photograph of leaves from treated plants (half size).
Fig. IX- Photograph of leaves from treated plants (half size). Notice double leaf on a single petiole, and jagged edges.
Fig. X- Photograph of leaves from treated plant (right) and untreated diploid plant (left). Notice the broader and shorter probable-tetraploid leaf.

Flower abnormalities resulting from colchicine treatment

Fig. XI.

Fig. XII.

Fig. XIII.

Fig. XIV.

Figures XI - XIII show flowers, from treated plants, with varying numbers of petals (the norm being five), and note also petals with uneven and jagged edges.

Fig. XIV - shows (upper row) diploid blossoms, and presumably-tetraploid blossoms (bottom row).

(Magnification X2.)

of two blossoms joined together at their bases, having a total of ten petals, sixteen stamens and eight pistils. However, no seed was set on this plant. Even the individual petals had a variety of forms in the treated plants. Some had jagged edges on both sides, some a jagged edge on one side and a smooth edge on the other. Both large and small petals were also found on the same flower giving a very bizarre appearance.

Flower buds on the treated plants were variable in size and shape, some stunted and smaller than in the diploid controls, some very much larger (two to three times as large). In the case of the seventy-two hour seedling-treated plants, some flower buds refused to open and it was not uncommon for the anthers to push through the petals. Time of budding was also affected by various treatments. Generally speaking with increasing treatment time budding was more retarded.

The pistils and stamens of probable-tetraploid flowers appeared to be much longer and thicker than those of the control plants. Numbers of stamens ranging from one to sixteen were found on flowers in the various treatment lots (the normal being eight). In the seventy-two hour seedling-treated lots most anthers seemed unable to shed their pollen and slides had to be made by crushing the whole anther. Stigmata numbers ranged from one to eight (the normal being three) on a single style. Some plants were found with two and three styles fused at the base and bearing various numbers of stigmata.

Another noticeable feature of the petals of buds and flowers as well as the anthers in the treated plants was the high concentration and the greater area covered by the anthocyanin pigment.

At the height of flowering, counts were made of the number of blossoms per plant for controls as well as treated plants. It was

found that with increasing treatment time there was a decreasing number of flowers per plant (Figure XV and Table VI) and an increasing number of floral abnormalities (Figure XVI and Table VII).

VIII. Effects on Pollen of Seed-Treated and Seedling-Treated Plants.

Pollen from the short-styled diploid plant is normally larger than pollen from the long-styled plant. This same rule held true for pollen from probable-tetraploid flowers. On the average pollen from both short - and long-styled probable-tetraploid flowers was two to three times as big as pollen from short - and long-styled diploid plants and nearly spherical in shape. (Calculations of area using ocular micrometer scale units and based on ten pollen grains in each category gave averages of 0.668 and 0.429 for pollen from short - and long-styled presumed tetraploids and 0.228 and 0.125 from corresponding diploids).

A variety of shapes and sizes of pollen was found in the treated plants. Pollen with thick walls, thin walls, fused pollen grains, dwarf pollen grains and giant pollen grains were not uncommon (Figures XVII - XIX).

A determination of "pollen sterility" (by methods described in "Materials and Methods"above) showed that with increased treatment time there was an increasing percentage of pollen sterility (See Figure XX and Table VIII for these results).

IX. Seed Setting on Seed-Treated and Seedling-Treated Plants.

As described under the title of "Materials and Methods," only legitimate pollinations were made. Seed set in the treated lots was rather poor. Results of success in seed-setting was determined in each case by making one hundred hand pollinations of emasculated flowers.

A variety of seeds resulted from these pollinations; some were flat and ovoid, others were normal three-achened and one plant set only four angled seeds, (this plant bore flowers with four stigmata

Table VI. Means and standard errors of normal plus abnormal flowers per plant in different treatment lots of ten plants each.

		Seed-treated lots (0.4% colchicine).		
Series	Control	Twenty-four hours	Thirty-six hours	Forty-eight hours
10/19/49.	190±1.33	180±1.88	116±1.30	22±0.84 *
11/21/49.	188±0.87	174±1.25	110±2.40	20±0.47 *

* results based on three plants only.

		Seedling-treated lots (0.4% colchicine).				
Series	Control	Twenty hours	Twenty-nine hours	Thirty-four hours	Forty-four hours	Seventy-two hours
12/14/49.	190±1.37	148±1.18	118±1.32	98±0.80	70±0.83	10±0.32
11/21/49.	198±1.64	150±1.08	-	100±1.15	71±0.88	-

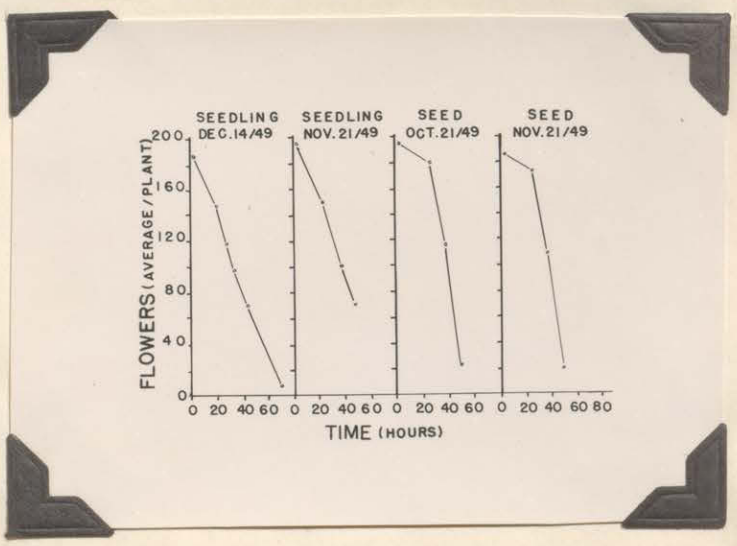

Fig. XV- Graph showing that increased treatment times result in decreasing numbers of flowers per plant. The average number of flowers per plant is based on ten plants for each lot except in the forty-eight hour seed-treated lots where results are based on three plants only. The base line indicates treatment time in hours. The zero mark indicates the control lot in each case. Time of planting of each lot is indicated at the top of the graph.

Table VII.Means and standard errors of percentage aborted flowers per plant in different treatment lots of ten plants each.

		Seed-treated lots (0.4% colchicine).		
Series	Control	Twenty-four hours	Thirty-six hours	Forty-eight hours
10/19/49.	-	10±0.37%	28±0.69%	50±0.72% ✱
11/21/49.	-	10±0.40%	30±0.70%	52±0.64% ✱

✱ results based on three plants only.

		Seedling-treated lots (0.4% colchicine).				
Series	Control	Twenty hours	Twenty-nine hours	Thirty-four hours	Forty-four hours	Seventy-two hours
12/14/49.	-	16±0.54%	30±0.59%	32±0.43%	58±0.64%	95±2.60%
11/21/49.	-	14±0.47%	-	36±0.90%	60±0.64%	-

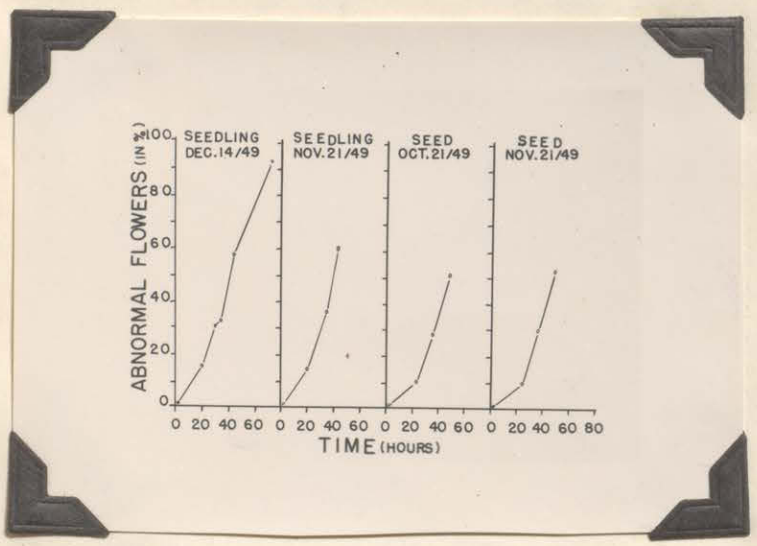

Fig. XVI- Graph showing increased treatment times result in increasing numbers of abnormal flowers per plant. The percentage abnormal flowers per plant is based on ten plants for each lot except in the forty-eight hour seed-treated lots where results are based on three plants only. The base line indicates treatment time in hours. The zero mark indicates the control lot in each case. Time of planting of each lot is indicated at the top of the graph.

Normal and abnormal pollen in treated plants compared with normal pollen from diploid plants

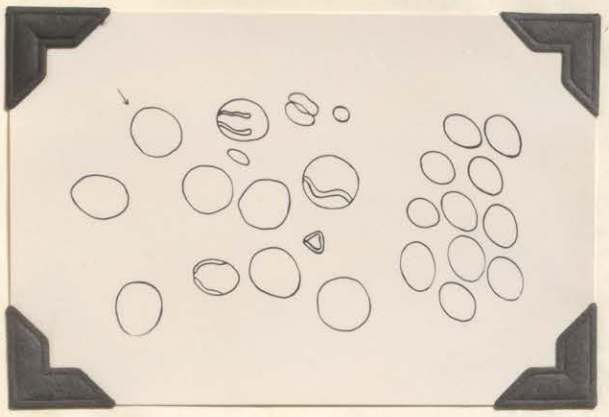

Fig. XVII.

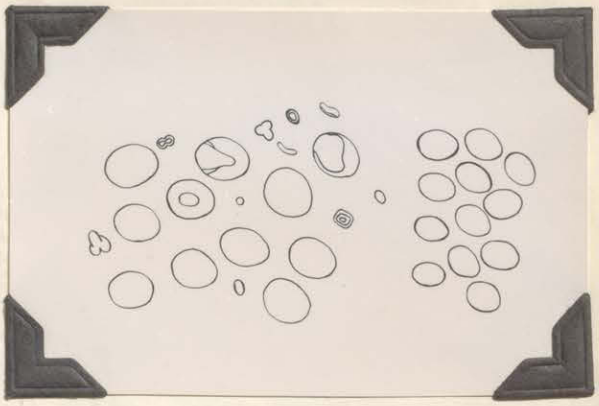

Fig. XVIII.

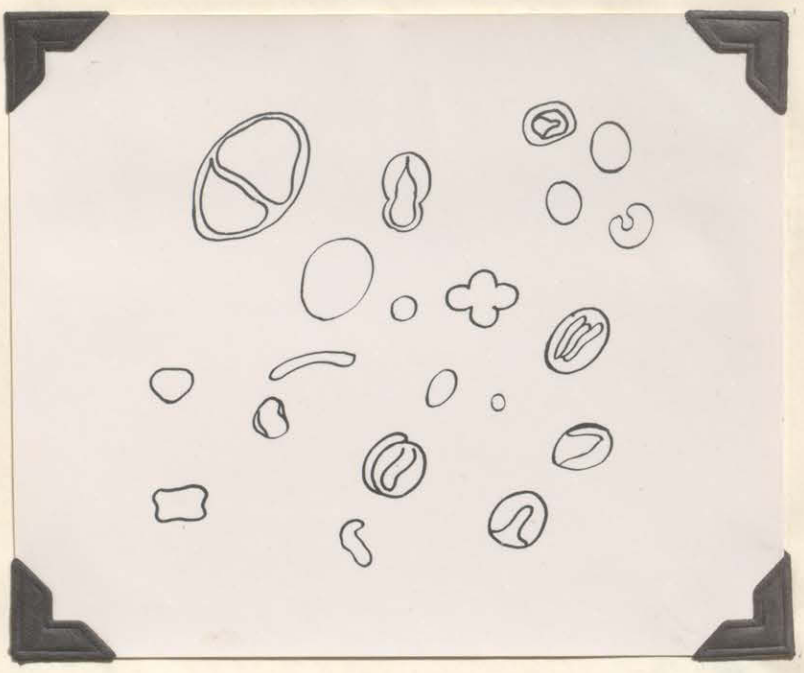

Fig. XIX.

Fig. XVII- Normal and abnormal pollen from a short-styled presumably-tetraploid flower. Arrow indicates normal pollen from a short-styled tetraploid. Pollen to the right is normal pollen from a short-styled diploid flower.

Fig. XVIII- Normal and abnormal pollen from a long-styled presumably-tetraploid flower. Pollen to the right is normal pollen from a long-styled diploid flower.

Fig. XIX- A variety of abnormal pollen from a short-styled presumably-tetraploid flower (treated plant).

(All three photographs are of Camera lucida drawings X 60).

Table VIII. Means and standard errors of percentage "pollen sterility" for different treatment lots of ten plants each.

		Seed-treated (0.4% colchicine).		
Series	Control	Twenty-four hours	Thirty-six hours	Forty-eight hours
10/21/49.	-	8±0.57%	16±0.58%	28.0±0.85% ✱
11/21/49.	-	10±0.95%	17±0.82%	28.5±0.98% ✱

✱ results based on three plants only.

		Seedling treated (0.4% colchicine).				
Series	Control	Twenty hours	Twenty-nine hours	Thirty-four hours	Forty-four hours	Seventy-two hours
12/14/49	-	5±0.66%	8±0.48%	9±0.40%	32.0±1.10%	52.5±1.96%
11/21/49	-	7±0.88%	-	10±0.77%	31.5±7.78%	-

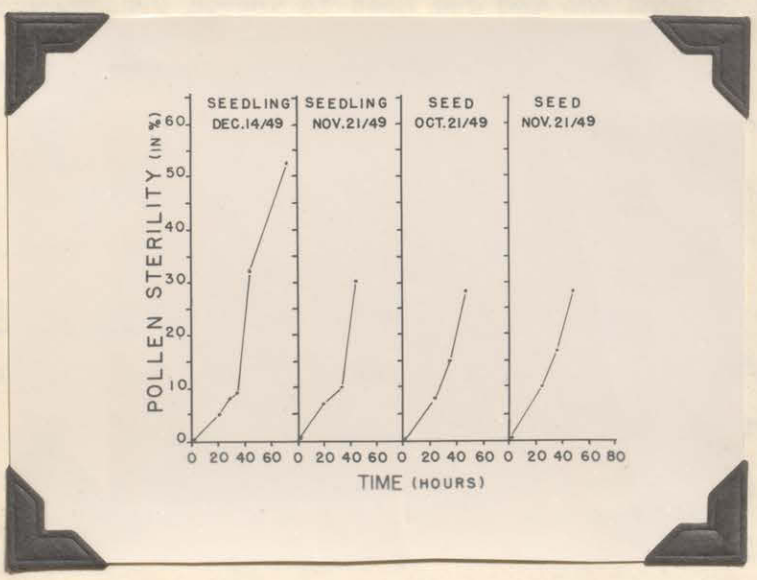

Fig. XX- Graph showing increased treatment times result in increased "pollen sterility." Average pollen sterility in each lot (treated and untreated) based on ten plants (an average of one hundred and twenty-five pollen grains examined for each plant) except the forty-eight hour seed-treated lots where results were based on three plants only. The base lines indicates treatment time in hours for each lot. The zero point indicates the control lot in each case and the times of planting of the lots are indicated at the top of the graph.

throughout). Considerable numbers of the treated plants bore seed with very well developed wings though Silver Hull is described as a non-winged variety (Stevens 1912a). Quite a number of seeds were found with normal well developed pericarps but completely empty except for a small under developed embryo. However, a considerable number of large, full, well developed seeds were produced by some treated plants. No abnormal seed was produced by diploid control plants.

The probable-tetraploid seed was two to three times as large as the diploid seed and ranged in colour from black or dark brown through golden, mottled with various patterns of dark brown spots, to silvery-brown. Figure XXI illustrates these variations in seed types.

Figure XXII and Table IX show that with increased treatment time there was a decreased number of seed set per one hundred pollinations and also an increased proportion of abnormal seed (Figure XXIII and Table IX). In the seventy-two hour seedling treatment group only seven seeds were set for a hundred test pollinations done and all seven failed to germinate in later tests.

X. Diploid and Probable-Tetraploid Nodal Cuttings.

By tests previously mentioned the probable-tetraploid plants and sectors of plants were segregated. Twenty cuttings of presumably-tetraploid nodes and twenty from the diploid control lots (taken at random) were rooted in sand, and repotted in the same soil mixture as was used in previous plantings after they had been in the sand three weeks. Both the diploids and "tetraploids" were very slow in growing and at the end of three months the "tetraploids" were on the average only twenty-five cms. in height as compared to an average of thirty-five cms. for the diploids. Leaves, stems, petioles and flowers

Diploid and tetraploid seeds

Fig. XXI- Photograph of diploid seeds (top row) and presumably-tetraploid seeds (bottom row)X2. The two seeds at the lower right of the photograph were abnormal and did not germinate.

Table IX. Total seed set and percentage abnormal seed for different treatments and periods of treatment (ten plants per lot).

Series	Number of hours in 0.4% colchicine	Seed set from a hundred legitimate pollinations	Percentage abnormal seed
Seedling treatment 14/12/49.	Control	36	-
	20	30	25.0
	29	22	31.4
	34	20	35.0
	44	14	61.5
	72	7	100.0
Seedling treatment 21/12/49.	Control	38	-
	20	26	21.5
	34	23	34.8
	44	16	62.4
Seed treatment 10/19/49.	Control	40	-
	24	25	12.0
	36	18	22.2
	48	7	100.0 ✱
Seed treatment 11/21/49.	Control	36	-
	24	28	15.4
	36	18	22.2
	48	6	100.0 ✱

✱ results based on three plants only.

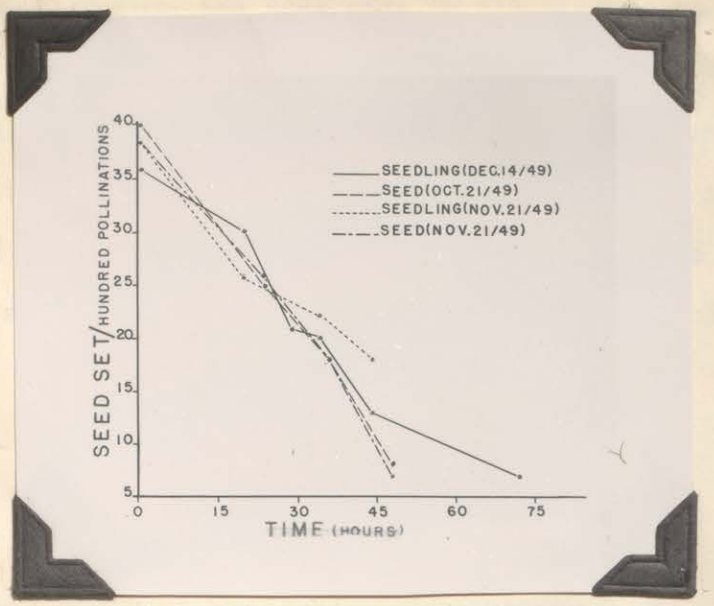

Fig. XXII- Graph showing increased treatment times result in a decreased seed set per plant. The average seed set per hundred pollinations in each lot (treated and untreated) is based on ten plants for each lot except the forty-eight hour seed-treated lots where results are based on three plants only. The zero line indicates the control lot in each case. The base line indicates the treatment time in hours and the times of planting of the lots are indicated at the top of the graph.

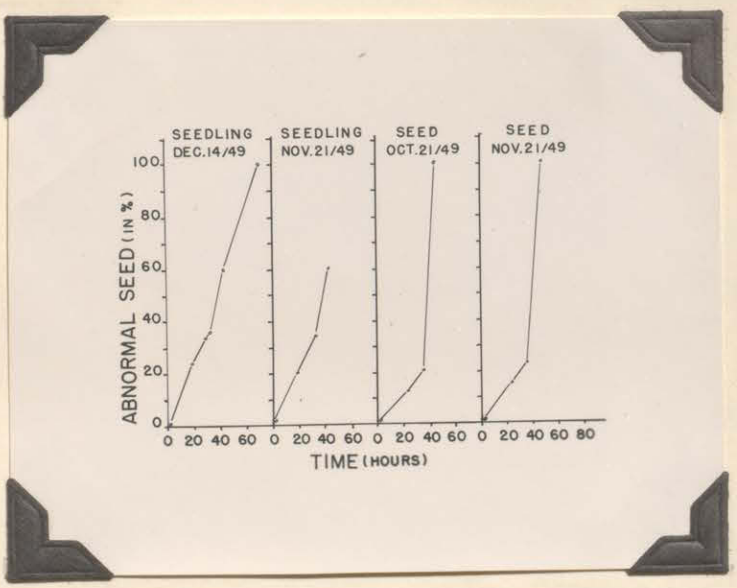

Fig. XXIII- Graph showing increased treatment times result in an increased number of abnormal seeds per plant. Percentage of abnormal seed set of total seed set for each lot (treated and untreated) based on ten plants in each lot except the forty-eight hour seed-treated lots where results are based on three plants only. The zero line indicates the control lot in all cases. The base line indicates treatment time in hours and the times of planting of the lots are indicated at the top of the graph.

as well as pollen were the same size in the diploid and presumed-tetraploid cuttings as in normal diploid and probable-tetraploid plants. Only legitimate pollinations were done between these presumed tetraploids and these yielded on the average nine seeds per hundred pollinations. Among the diploids from the nodal cuttings seed set averaged fifteen seeds for a hundred pollinations.

PART II

A STUDY OF TETRAPLOID BUCKWHEAT (*FAGOPYRUM ESCULENTUM*) VARIETY SILVER HULL

(a) MATERIALS AND METHODS

Two weeks after the seed from the treated plants had been harvested they were germinated. Twenty seeds from each lot were placed in petri dishes (three inch) on moistened germination paper under a sixty-watt bulb and kept at a temperature of thirty degrees Centigrade. Only seeds which were large and full and looked normal were chosen. A suitable control lot of diploid Silver Hull seeds, twenty in number, was germinated also. Time of germination and percentage germination for all lots were carefully recorded.

After three days in water, the germinated seeds were planted in three-inch pots using the same soil mixture as was used in previous experiments. Each plant was tagged to indicate which treatment lot it originated from as well as being dated and numbered. Records were kept of the height of plants each week, first signs of budding and any other noticeable features including number of flowers per plant at the peak flowering period. Root tips from each plant were taken at the end of two weeks, killed and fixed in alcohol-acetic, and stored in the refrigerator. These were used for chromosome counts as final proof of diploidy or tetraploidy.

When suitable buds for meiotic studies had formed samples were taken, killed and fixed in alcohol-acetic and also stored in the refrigerator. These were used in later studies of (1) meiosis in the diploids and tetraploids and (2) meiotic figures in relation to pollen sterility. Squash preparations of meiotic and mitotic figures for both diploid and tetraploid were made in acetocarmine and made permanent by the McClintock method. Camera lucida drawings of meiotic

and mitotic metaphase division figures were done and later photographed.

Epidermal tissue for observation of stomates and pollen was collected from diploids and apparent tetraploids for comparison as further evidence of tetraploidy.

Twenty-seven tetraploid plants were indentified by chromosome counts and repotted in larger, six-inch pots at four weeks, staked, and randomized each week along with the diploid controls. Records were kept of height etc. of these plants and of ten diploid controls during the growth period.

All flowers were emasculated and pollinations done by hand in the greenhouse. Selfing and crossing was done within the tetraploid and diploid control groups as well as all possible crosses between diploids and tetraploids. In pollinating in the tetraploids, plants with the smallest percentage "sterile" pollen were used and those plants with high pollen sterility were placed outside and allowed to pollinate openly. On July 21, 1950, when the plants were eight weeks old, they were all moved outside the greenhouse (after various crosses had been made) and allowed to pollinate openly. By this time, because of the excess heat in the greenhouse, all controls except three had died, and several of the tetraploids were wiped out by an attack of white fly.

Seed collected from various crosses was placed in separate envelopes and labelled to indicate the cross made and the date of seed collection as well as any other facts it seemed necessary to record. Number of seed set, both normal and abnormal, for a hundred pollinations in each self and cross combination was recorded. Diploid and tetraploid seeds were photographed..

(b) RESULTS AND OBSERVATIONS

I. Germination.

The diploid control group of seed showed first signs of germination in sixteen hours and at twenty-five hours eighty percent of the seed had germinated. The tetraploid seed from treated plants showed first signs of germination in twenty-one hours and at thirty-two hours eighty-seven percent had germinated. At forty hours all the tetraploid seed had germinated and ninety-one percent of the control seed had germinated.

The primary roots of the tetraploid seedlings at forty hours were noticeably thicker and shorter than those of the diploid controls, averaging one cm. in length as against an average of one and a half cms. for the control group.

II. Root Development, Cell Size, and Root Tip Cell Chromosome Counts.

Four weeks after planting, root tips were collected from the diploids and tetraploids. Collections made in the morning, just after sunrise, yielded higher numbers of somatic metaphase figures than collections made in late afternoon or evening. This was also true for meiosis. Tetraploid root tip cells were two to three times as large as diploid and all metaphase and anaphase counts gave the expected $4n = 32$ and all the diploids $2n = 16$ chromosomes (Figures XXIV and XXV). No abnormalities in the division figures were seen.

Roots in the tetraploids were much shorter and showed more lateral development than the diploids and the roots were noticeably thicker and larger.

III. Growth in Height and Heat Resistance.

The tetraploids took on the average about three days to come through the soil as against an average time of one day for the controls.

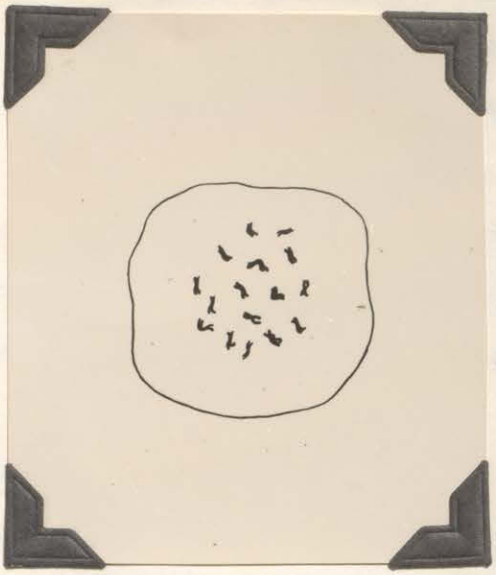

Fig. XXIV- Diploid root tip cell; metaphase (2n-16).
(Camera lucida drawing X 1800).

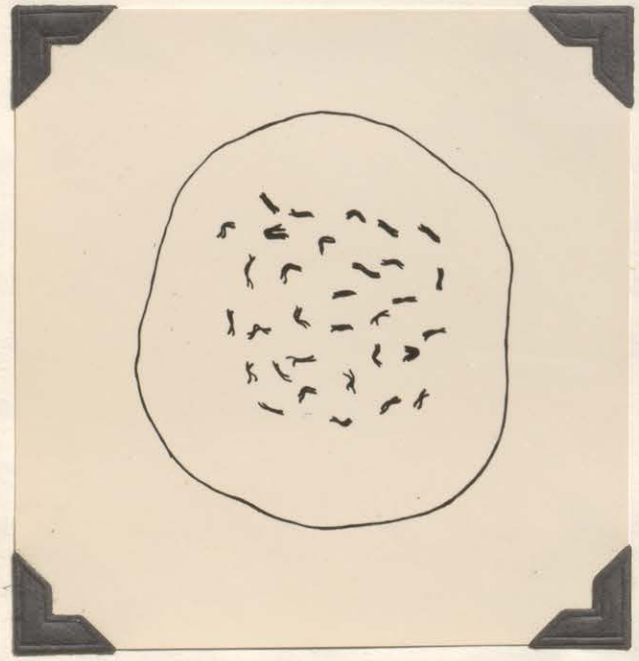

Fig. XXV- Tetraploid root tip cell; metaphase (4n-32).
(Camera lucida drawing X 1800).

Up to five weeks after emergence the diploids surpassed the tetraploids in height. At the end of the next three weeks the tetraploids had surpassed the controls in height(Figure XXVI and Table X).

The extremely hot and dry conditions in the greenhouse adversely affected the controls so that all but three plants had died by the end of seven weeks. The tetraploids, however, seemed to suffer less from these conditions and did not show signs of wilting to the same degree as the controls.

IV. Leaves and Stems.

Tetraploid stems, were on the average thicker, less brittle (unlike those of treated plants) and less swollen at the internodes than the diploids. The diploids tended to be more constricted at the nodes. The tetraploid stems at three weeks were very dark red in colour and even the veins on the leaves were dark red, whereas diploid leaves and stems at this time were still light yellowish-green. The tetraploid leaves were much thicker, broader and contained less chlorophyll per unit area than did the diploid. Their darker colour may have resulted from their greater thickness. The diploid leaves had a smooth, symetrically curved edge, whereas the tetraploid leaves were notched and indented. The cotyledons of the tetraploids were larger than those of the diploids. The stomates of all tetraploid leaves were larger and fewer in number per unit area than in the diploid.

V. Buds and Flowers.

Tetraploid plants usually budded a few days earlier than the diploids. Their buds were well formed, larger and darker pinkish-white than the diploid buds. Tetraploid flowers were larger in all respects than those of the diploids. The petals were longer and wider and pistils and stamens were much larger and better developed than those

Table X. Means and standard errors of heights in centimeters of diploid and tetraploid plants planted May 15/50.

Weeks after emergence	Diploid (10 plants)	Tetraploids (27 plants)
1	8±0.23	6±0.16
2	15±0.25	12±0.63
3	28±0.25	25±0.18
4	38±0.27	35±0.18
5	48±0.34	48±0.17
6	54±0.36	57±0.18
7	58±0.33	67±0.21
8	64±0.31*	75±0.21

* results based on three surviving plants.

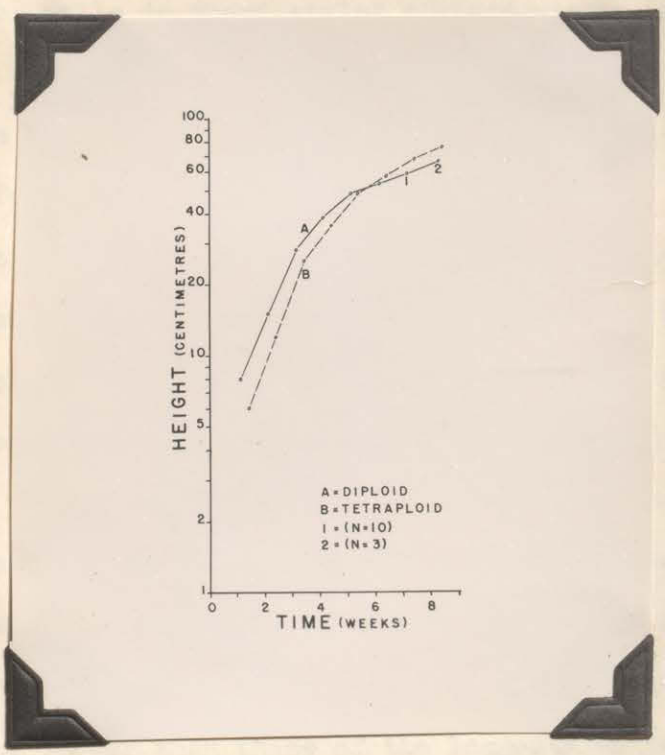

Fig. XXVI - Heights of diploid and tetraploid plants. Curve A - calculations based on ten plants except between (1) and (2) where results were based on three surviving plants. Curve B - calculations based on twenty-seven tetraploid plants.

in the diploid. Very few abnormal blossoms were seen among these tetraploids. However an occasional flower with six petals did occur on some plants. Also the tetraploid plants on the average produced fewer flowers than the diploids. The average for tetraploids was one hundred and fifteen per plant with a range from fifty-five to one hundred and sixty-five. The diploids produced on the average one hundred and sixty-five per plant with a range of eighty-five to two hundred and ten.

VI. Meiosis and "Pollen Sterility."

Studies of meiotic pairing and chromosome counts were done for both diploid n= 8 and tetraploid n= 16. No abnormalities were seen in the control group in which pairing in diplotene, diakinesis and metephase I was completely bivalent (Figure XXVII). Chromosomes and pollen mother cells in short-styled diploid plants were found to be larger than those of long-styled plants as stated by other workers. Also, counts done at Metaphase II in the diploids gave eight chromosomes in each of four cells.

As mentioned above, in the first division in the diploids eight bivalents were regularly present. On a theoretical basis in the tetraploids one should expect to find eight quadrivalent associations. However division figures at Metaphase I (Figure XXVIII) in the tetraploids showed that pairing varied tremendously from plant to plant. Those with high percentages of sterile pollen had higher numbers of univalents. Pentavalents, quadrivalents, trivalents, bivalents, and univalents were seen (Table XI). Pollen mother cells in the short-styled tetraploids were slightly larger than those of the long-styled tetraploids; the chromosomes themselves were also larger.

Pollen sterility was quite low in the tetraploids, ranging from

Fig. XXVII- Diploid metaphase I, short-styled plant, showing only bivalent associations (2n=16). (Camera lucida drawing X 1800).

Fig. XXVIII- Tetraploid metaphase I, short-styled plant, showing mostly bivalent associations (4n=32). The arrow indicates a quadrivalent association. (Camera lucida drawing X 1800).

Table XI. Chromosomes associations at metaphase I.

	Number of cells	The valency of the configurations.					Total count
		I	II	III	IV	V	
Diploid							
ss	3	0	8	0	0	0	16
ls	3	0	8	0	0	0	16
Tetraploid							
ss	1	2	13	0	1	0	32
ss	2	1	12	1	1	0	32
ss	2	0	11	2	1	0	32
ss	1	2	10	2	1	0	32
ss	1	1	10	1	2	0	32
ss	1	2	9	1	1	1	32
ss	1	1	9	3	1	0	32
ss	1	2	8	2	2	0	32
ls	1	1	10	1	2	0	32
ls	1	0	9	2	2	0	32
ls	1	2	8	2	2	0	32
ls	1	1	7	3	2	0	32
ls	2	4	7	2	2	0	32
ls	1	3	6	3	2	0	32
ls	1	2	6	2	3	0	32
ls	2	0	5	3	2	1	32
Total	26						

ss - short-styled; ls - long-styled.

one to ten percent with an average of four percent. The pollen of tetraploids was found to be more nearly spherical and much larger than that from diploids, as was indicated in Part One. Pollen of short-styled plants, both diploid and tetraploid, was slightly larger than that of the long-styled plants.

VII. Pollinations.

A total of twenty-seven tetraploids made up of twenty-four long-styled plants and three short-styled plants was grown.

All possible ways of crossing diploid and tetraploid plants failed, both legitimate and illegitimate. In some legitimate crosses of 2X x 4X plants using the 2X as both male and female, abnormal seed was set. The pericarp developed but the embryo did not and the seed shrivelled up and died. No seed was obtained at all from these crosses. Test pollinations so far would indicate that diploids and tetraploids will not cross normally and perhaps should be considered as separate species. A total of three hundred pollinations were done to test out this fact.

Both long and short-styled plants were selfed. Among the short-styled plants continued selfing of both diploids and tetraploids yielded no seed nor did selfing of long-styled diploids yield seed. A total of one hundred pollinations in each of these cases yielded nothing. However, certain of the long-styled tetraploids when selfed yielded normal seed at an average rate of sixteen per one hundred pollinations. With certain other long-styled plants continued selfing produced no seed.

Attempts were made to outcross short-styled diploid plants to other short-styled diploid plants and the same was done for long-styled plants. One hundred pollinations in each case yielded no

seed. The same resulted with the short-styled tetraploids - no seed was set. However, crosses between certain long-styled tetraploid plants yielded both normal and abnormal seeds with an average seed set of thirteen per one hundred pollinations. Other combinations of long-styled x long-styled tetraploids yielded no seed.

In the legitimate cross (long-styled x short-styled and the reciprocal) within both diploid and tetraploid groups thirty-two seeds per one hundred pollinations were obtained for the diploids whereas sixteen seeds per hundred pollinations were obtained in the case of tetraploids (See Table XII).

Normal tetraploid seed was found to be larger (two to three times) than the diploid and ranged in colour from black to mottled-brown to silver as was the case with the seed produced by the treated plants.

Table XII. Summary of pollinations carried out on diploids and induced autotetraploids.

Plant number	Cross	Pollinations	Seed set per hundred Pollinations
Diploid			
9 ss	selfed	100	0
10 ls	"	100	0
10 ls x 8 ls	reciprocal	100	0
9 ss x 7 ss	"	100	0
4 ss x 5 ls	"	300	32✱
Tetraploid			
10 ss	selfed	100	0
9 ls	"	100	16
10 ss x 8 ss	reciprocal	100	1
9 ls x 7 ls	"	200	13✱ ✱
9 ls x 10 ss	"	300	16✱
Tetraploid x diploid			
6 t, ls x 10 d, ls	"	100	0^P
10 t, ss x 9 d, ss	"	100	0^P
6 t, ls 9 d, ss	"	100	0^P

0^P, pericarp showed signs of developing, however no embryos were found developing.; t, tetraploid; d, diploid; ss, short-styled; ls, long-styled.

✱ Mean of three separate sets of readings.
✱ ✱ Mean of two separate sets of readings.

DISCUSSION

After carefully considering both the seed - and seedling-treatment methods the latter treatment method seemed preferable. In the case of seed-treatment many seedlings were so slowed down in growth that they rotted beneath the soil. Seed-treatment beyond thirty-six hours did not produce satisfactory results and the few plants surviving the treatment time of forty-eight hours were small, stunted, produced few flowers and frequently had high percentages of pollen sterility. The seed set in these forty-eight hour seed-treated plants was low and mostly all abnormal. Seed treated twenty-four hours with colchicine yielded only three tetraploid plants. By increasing the treatment time to thirty-six hours nine out of ten plants proved to be tetraploid, seed set was fairly good, and the number of abnormal seeds was low.

With the seedling-treatment method all plants, within the treated lot, survived the lengthy treatment time of seventy-two hours. Treating seedlings after emergence made it possible to study the effects of prolonged treatment with colchicine which was not possible in the seed-treated group. With as short a duration of treatment as twenty hours tetraploid shoots did develop along with diploid shoots in the same plant. A treatment time of about thirty hours seemed to give the best results. Following this treatment few diploid shoots were present, flowers were numerous, percentage of abnormal flowers low, pollen sterility low, and seed set good with few resulting abnormal seeds.

Taking nodal cuttings from probable-tetraploid plants and rooting them seems quite an effective way of increasing the number of tetraploid plants. True, the number of flowers produced by each plant is low, but when the total number of flowers for each plant produced by taking nodal

cuttings from a single plant is considered the overall total number of flowers is in excess of that produced on the average by a single plant. (Nodal cuttings were not confined to any one treatment lot but were taken at random).

In general with increasing duration of treatment more polyploid cells are apparently produced to a point where diploid cells are in the minority and the tissue to all intents and purposes is polyploid.

At thirty-six hours in the seed-treated lots and around thirty hours in the seedling-treated lots a threshold is reached above which the toxic or poisonous effects of colchicine give increased physiological disturbances leading to production of mixaploid tissue (apparently) and manifold abnormalities such as decreased flowering, increased pollen sterility, etc. Below this threshold level, it is true, polyploid cells are produced but the balance weighs in favour of rapidly dividing diploid cells which apparently have a selective advantage over the slowly dividing polyploid cells. At the threshold level, however, the balance apparently tips in favour of establishment of polyploid cells and polyploid tissue can compete successfully.

A lower threshold level in treatment time in the seedling-treated lots (circa thirty hours giving most favourable results) may be explainable on the basis that the colchicine is applied closer to the tissue that is dividing (the growing point emerging from between the cotyledons) and consequently effects are more direct, whereas with the seed-treatment method the colchicine has first to penetrate several layers of tissue (pericarp, etc.) before producing its effects upon dividing cells.

The problems of self-incompatibility, pollen sterility and heterostyly warrant a few words of discussion. In the diploid plants

cross pollination is favoured by heterostyly, a character apparently controlled by a single gene pair Ss. The heterozygotes of the constitution Ss have short-styles and the recessive homozygotes (ss) long-styles. In the first generation from treated plants obtained by cross pollination (both long-styled x short-styled and reciprocally) of tetraploid flowers produced through colchicine action the ratio shifted to a marked preponderance of short-styled plants, since the S factor was dominant to three s factors and thus the short-style character was expressed in tetraploids of either the genotypes SSss or Ssss. This observation has also been reported by Sakharov (1946a) who states, "the normal ratio of 1:1 is restored, however, towards the third, fourth, and fifth generations as a result of the fact that crossing could only occur between short and long-styled plants and plants of the original constitution SSss are replaced by Ssss plants."

In my first generation (tetraploids grown from seed collected from treated plants) twenty-four long-styled plants and only three short-styled plants were obtained. Since more short-styled plants were expected on the basis of the above genetic mechanism of determination of style length, these results are peculiar and merit further study.

Diploid-tetraploid crosses were not successful. In crosses made in both directions all seed set was abnormal. In some cases pericarp development was initiated but no embryos developed. Sakharov (1946b) also reported that diploid x tetraploid crosses were unsuccessful. The difficulty may lie in the inability of triploid embryos to survive when the ratio of genomes in embryo and endosperm is changed, in as much as no embryo development was seen in any of the abnormal seeds.

As far as incompatibility is concerned there is more here than meets the eye. Selfed seed is difficult to obtain from the variety Silver Hull. No seed was set on long-styled or short-styled diploid

plants by selfing although numerous pollinations were done. However, three of my long-styled tetraploid plants selfed quite readily and were able to cross among themselves. It is possible that in the tetraploid there is a breakdown of incompatibility factors, a fact not incompatible with other workers' findings with autotetraploids. Lundquist (1941). found that in rye the inbred tetraploid strains were as self-sterile as the original diploids, but incompatibility is less pronounced and some selfed seed is set in certain plants.

To insure crossability and to maintain heterosis selection in the past must have been responsible for the build up of incompatibility factors. It therefore may be possible in these long-styled plants, which set seed, by continued selfing, to select for self-compatibility and select out self-incompatibility. In the autotetraploids with four sets of genomes many more combinations than in diploids are possible and selection should prove interesting.

With the short-styled diploids and tetraploids no seed was set by selfing or crossing between them excepting in one case in two short-styled tetraploids which when crossed yielded one seed which might have resulted from contamination. A total of one hundred pollinations had been done in this case. If contamination was not involved it would seem to follow that there is also some breakdown of incompatibility factors in the short-styled plants, but not to the same degree as in the long-styled tetraploids. It is also possible that in the short-styled tetraploids certain types of these "S" factors may be conducive to seed set. Also other combinations of factors are possible in the tetraploids which are not possible in the diploids.

Pollen sterility in the tetraploids was surprisingly low, averaging around four percent. Division figures of meiosis were quite

difficult to obtain in these tetraploids and only twenty cells were analysed at Metaphase I. At this stage mostly bivalent associations with few multiple associations were found. In those plants where the amount of sterile pollen was very low, the highest numbers of bivalents were recorded (twelve on the average) with few multiple associations and few univalents. In two plants where pollen sterility was quite high (around ten percent) few bivalents were seen (six on the average) and multiple associations (pentavalents, etc.) and univalents were numerous. After looking at and studying various divisions it was surprising that the percentage of pollen sterility was not higher in these plants. Frolova, Sakharov and Mansurova (1946) reported that pollen grains with fourteen and fifteen chromosomes were viable. As regards whether pollen with more or less than sixteen chromosomes is capable of fertilization, he points out that the occasional occurrence of diploid seeds on fertile tetraploids suggests that "parthenocarpy" may occur. Also he claims that hypoploid and hyperploid egg cells were more frequent in plants forming a large amount of abortive pollen, and rare in highly fertile plants.

Whether induced autopolyploids improve in fertility and regularity in meiotic behaviour after many generations is a significant question now being studied. Should they do so and become distinct new types with regular bivalent formation, it is possible that they might have greater genetic stability than their ancestral diploid types, for the reason that a new recessive mutation would remain hidden longer and affect the phenotype only in those rare individuals having all the increased number of controlling factors in the recessive state.

At this point it would be difficult to say whether the tetraploids

will prove to be of economic value or not but if fertility and seed set can be brought up to the diploid level they should prove their worth on the basis of seed size alone. Only field tests on selected lines will provide an answer to this question.

SUMMARY

Two methods of treatment were used in this study (i) seed treatment method and, (ii) a contact method for seedling treatment. Treatment times of thirty-six hours in the seed-treated group and thirty hours in the seedling-treatment group gave the most satisfactory results.

Twenty-seven tetraploid plants made up of twenty-four long-styled and three short-styled plants were obtained. These plants exhibited the "gigas" characteristics of tetraploid plants, larger and thicker leaves and stems, larger flowers and seed and increased pollen sterility over the diploids. A short study of meiotic figures was done and multivalent associations were seen.

Further field tests will be conducted in the future to test the worth of the tetraploids over their diploid ancestors.

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