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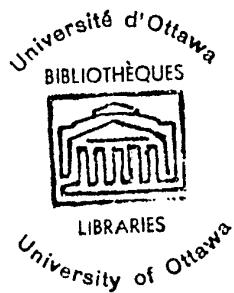
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**EFFECTS OF CENTRIFUGAL FORCE AND PHENOL UPON
THE MUTATION RATE OF BARLEY¹**

by
CHI KANG YU

Submitted in partial fulfillment for the
degree of Doctor of Philosophy in Biology
University of Ottawa

March 1st 1960



Candidate

Supervisor

-
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INTRODUCTION

Inducing permanent and self-reproducing changes in living cells provides much inheritable variability for genetic studies. For well over thirty years, geneticists have been searching for methods to induce mutation. A wide variety of artificial mutagenic agents is known, and it seems to be a useful new approach for breeding work.

Recently, a number of artificial satellites have been blasted into orbit. One of the first of them carried a dog, Laika, as a space traveller, thus opening a new era of space travel the limits of which are entirely speculative. During the blast off period, she was subjected to enormous forces, which nonetheless are smaller than those which will be necessary for the achievement of lunar or interplanetary travel. This leads to new possibilities, for in the future living organisms, especially man, will be space travellers. It is therefore important to learn what effect such forces may have upon the living organism. It might produce genetic changes in the germplasm and hence be inherited by succeeding generations, if acceleration can cause mutations.

Experiments, therefore, were started to find out whether centrifugal force is capable of producing mutations in barley.

Barley (Hordeum sativum), a self-pollinated crop, has been a favorite species for studies on experimentally modified

heredity. It also offers many advantages for cytological studies. It has a small number of chromosomes ($2n = 14$) and its chromosomal characteristics during meiosis can be easily identified. Barley is one of the major crops grown in Canada. Extensive breeding work as well as genetic study is being done with this crop. Barley, then, was used for these experiments because its low chromosome number is advantageous for cytological studies and its phenotypic characters are easily studied genetically. Because of the economic importance of this plant, it is hoped that this research may prove valuable for the plant breeder.

Phenol was also used as a mutagenic agent. It is well known that phenol produces a high mutation frequency in Drosophila (Madorn and Niggli 1946, Niggli 1946), and it was also found to increase the rate of visible mutations in Antirrhinum (Stubbe 1940). To clarify the effect of centrifugal force upon mutation, the effects of phenol were compared with those of centrifugal force.

LITERATURE REVIEW

Centrifugal force has often been applied to various biological problems. Centrifuged materials, however, show physiological or cytological changes, which have not been extensively studied genetically as far as mutation is concerned.

Tanaka and Kawaguchi (1932) centrifuged hybrid eggs of silkworms for 5-10 minutes, but they did not mention the speed of the centrifugation which were used. From this treatment, mosaic worms were obtained, and several abnormal chromosomes were also found during the meiotic stage of the treated hybrids; such as very small mono-chromosomes (probably univalent) and a large triangular chromosome (trivalent?). Kawaguchi (1936, 1938a) obtained triploid, tetraploid and hexaploid silkworms, which were produced by centrifuging the eggs at 3500 r.p.m. for 10 minutes. Among those polyploids, only female tetraploid showed 79.6% fertility, the others were 1.7% to 12% fertile. The number of nucleoli was also associated with the types of polyploidy. Kawaguchi (1938b) also found 2 nucleoli in diploid spermatogonia, 3 in triploid, and 4 in tetraploid, but it was not the same in polyploid oogonia. The number of nucleoli was also directly proportional to the number of Z-chromosomes, which were in all probability Z_{at}-chromosomes.

Kostoff (1937) studied the effect of centrifugation upon the germinated seeds of various plants. The germinated seeds of several spp. were centrifuged repeatedly for short

periods, 15-30 minutes. Centrifugation was done at 1000-4000 r.p.m. Best results were obtained at speeds between 1500-4000 r.p.m. Some morphological abnormalities were obtained, such as variation in leaf shape, semi-sterility, and variegated leaves with chlorophyll deficiency. Centrifuging was also found to interfere with the mitotic processes, during the early metaphase, the transfer of single chromosome from one pole to another caused failure of normal chromosome separation and cell division, and led to formation of monosomic, trisomic, polysomic, tetraploid etc. cells.

Effect of centrifuging upon Tradescantia and Vicia was studied by several workers. Sax (1943) stated that centrifuged Tradescantia microspores, which had been irradiated, were found to have a higher frequency of chromosomal aberration than microspores which were irradiated only. Wolff and von Borstel (1954) found a positive effect of centrifugal force, but they were unable to demonstrate any mutagenic influence produced by centrifugation alone.

Auerbach (1949) summarized the effect of phenol as a mutagenic agent, which had been tested on various organisms by many workers. Phenol was found to increase the rate of visible mutations in Antirrhinum. Applied to excised ovaries of Drosophila, it produced a high frequency of autosomal lethals. Hadorn and Niggli (1946) were successful in using phenol (0.01%) to produce mutation in Drosophila. Of 241 treated ovaries, 44 (=18.3%) had one or more mutations. Niggli (1948)

also reported that the excised ovaries of D. melanogaster soaked in a 0.01% solution of phenol for 15-20 minutes contained 99 recessive lethals, 15 semi-lethals, and 12 recessive visible mutations. Stubbe (1940) exposed the seeds of Antirrhinum to phenol solution, and found that phenol produced an increase of the mutation frequency in A. majus. Levan and Tjio (1948a) studied the effect of phenol on root chromosomes of Allium, and they stated that chromosomal fragmentation could be induced by phenol in a concentration of 0.05%.

Finally, a preliminary report on the present work has been published (Dodson and Yu, 1958).

MATERIALS AND METHODS

This research was done at the University of Ottawa, Ottawa, Ontario, from 1957 to 1959.

Barley of two varieties was used: Hannchen, which has two seed rows and rough awns, and Montcalm, which has six seed rows and smooth awns. Both are well adapted to Canadian growing conditions. The characters of two seed rows and rough awns are dominant over six seed rows and smooth awns respectively.

Seeds were germinated on wet filter paper in Petri dishes at room temperature. After 24 hours, the seeds showed signs of coleoptile growth, and some of them had roots up to one cm long. They were then centrifuged in an International Refrigerated Centrifuge, Model PR-2, fitted with an auxiliary head to permit the use of forces up to 25000g.

Preliminary experiments were made to test the effect of relative centrifugal force upon germination of seeds. The speeds used were 5703r.p.m., 9016r.p.m., 12752r.p.m., 18034r.p.m., and 20163r.p.m. The radius of centrifugation was 5.5 cm., and using the formula $g=0.00001118r(N)^2$, where g is the relative centrifugal force in gravity, r is the radius, and N is the speed of centrifugation. The forces were then calculated and found to be 2000g, 5000g, 10000g, 20000g and 25000g respectively. Again, because some seeds were cold treated at 0°C for the purpose of cutting down the period of dormancy, during centrifugation,

temperature was kept at 0°C for them and for the other seeds in order to provide the same environmental conditions during centrifugation and to eliminate the differences which might arise due to centrifugation at different temperature. Seeds were centrifuged for 3, 6, or 20 hours. After being centrifuged, the seeds were placed in Petri dishes on wet filter paper. For further cytological study, the surviving seeds from the 6 hour-treatment were grown in the greenhouse in 5 inch-pots filled with sterilized soil. Sufficient water was supplied every day. Because of limited greenhouse space, only 40 seeds were planted for each treatment.

Mitosis and meiosis were studied in root tips and in pollen mother cells produced by the surviving plants. For mitotic study, the roots were fixed in Carnoy's acetic alcohol (1:3) over-night, hydrolyzed in 10% hydrochloric acid at 60°C for 10 minutes, and then stained with Feulgen reagent. The most deeply stained part of the root tip was dissected out, cut into several pieces, and squashed between slide and cover-slip. Meiosis was studied in pollen mother cells of florets showing first meiotic division. In sampling, spikes were taken at random from the plants. Ten plants of each treatment were examined. The whole spike of the plant was fixed in Carnoy's solution for more than 24 hours. Anthers were taken from the the florets, stained with iron acetocarmine, cut into fine pieces, and then smeared.

During the summer of 1958, a second experiment was

carried out. After the seeds of the two varieties had been centrifuged at 25000g for 6 hours, they were planted out in the field for further study. The methods described above were also applied to this material. Other seeds were soaked in a 0.00% phenol solution for 24 hours, then rinsed several times in clean water until the smell of phenol was off, then placed in Petri dishes on wet filter paper over night. Surviving seeds were planted in the field.

The combined treatments, either phenol treatment followed by centrifugation or centrifugation followed by phenol treatment, were also used. The various treatments are symbolized as follows:

- Montcalm - Control (M)
- Montcalm - Centrifuged (M-RCF)
- Montcalm - Phenol treated (M-P)
- Montcalm - Centrifuged - Phenol treated (M-RCF-P)
- Montcalm - Phenol treated - Centrifuged (M-P-RCF)
- Hannchen - Control (H)
- Hannchen - Centrifuged (H-RCF)
- Hannchen - Phenol treated (H-P)
- Hannchen - Centrifuged - Phenol treated (H-RCF-P)
- Hannchen - Phenol treated - Centrifuged (H-P-RCF)

Seeds of each group were grown in the field to produce T_1 plants (plants grown from the treated seeds are referred to T_1 , the next generation as T_2 etc.), during the summer of 1958. Only thirty seeds of each T_1 group were grown to produce

T₂ plants in the greenhouse. Cold treatment was used to cut down the period of dormancy, so that the seeds harvested from T₁ plants could be germinated promptly. These T₁ seeds were placed in Petri dishes, which were kept at 0°C in a refrigerator for two days, and then transferred to room temperature. Germination was then normal. Meiosis and phenotypes were studied on both T₁ and T₂ plants.

During the summer of 1958, hybridizations were made between the two varieties as follows:

M x H (control)	H x M (control)
M x H-RCF	H x M-RCF
M x H-P	H x M-P
M x H-RCF-P	H x M-RCF-P
M x H-P-RCF	H x M-P-RCF

Hybrids were grown in the greenhouse during the winter 1958-1959. Meiosis in the hybrids was studied by the methods described above.

Phenotypes of treated plants (T₁ and T₂) and of the hybrids were studied with regard to the following characters:

Texture of the awn,	Seed-row number,
Thickness of the straw,	Height of the plant,
Maturity of the plant,	Fertility of the plant,
Number of tillers,	Pigmentation of the leaf,
Number of leaves.	

EXPERIMENTAL RESULTS

I. EFFECTS OF VARIOUS CENTRIFUGAL FORCES UPON GERMINATION OF MONTCALM BARLEY:

In each test, 100 Montcalm seeds were used. The controls had 98% germination, and the germination was markedly influenced by the centrifugal force (Table 1).

Table 1: Germination percentage of Montcalm seeds obtained from various centrifuging treatments:

Treatment	Total Seeds	Dead Seeds	% of Surv.
Control	100	2	98
2000g - 3 hours	100	2	98
2000g - 6 hours	100	11	89
2000g - 20 hours	100	25	75
5000g - 3 hours	100	20	80
5000g - 6 hours	100	29	71
5000g - 20 hours	100	46	54
10000g - 3 hours	100	31	69
10000g - 6 hours	100	39	61
10000g - 20 hours	100	62	38
20000g - 3 hours	100	38	62
20000g - 6 hours	100	44	56
20000g - 20 hours	100	78	22
25000g - 3 hours	100	60	40
25000g - 6 hours	100	69	31
25000g - 20 hours	100	94	6

The percentage of germination decreased as the severity of the force increased, and also as the duration of treatment increased. Above 20000g, most of the seeds were crushed, and were flat. The lemma and palea were broken. Some of the seeds had a short root before centrifuging. The root then became transparent, and it was sometimes broken off the embryo. Such damage, of course, caused the death of the seed. The relationship of percentage of seed germination to the force used was analyzed by the method of linear regression. The regressions of seed germination on centrifugal force were calculated by the equation, etc. as follows (the resulting regression lines are shown in Figure 1):

a. At 3 hours centrifuging:

Sample equation of regression:-

$$\hat{Y} - \bar{y} = b(X - \bar{x})$$

$$\hat{Y} = 94.6 - 0.002X$$

Note: X = Centrifugal force

Y = % of surviving seed

\bar{x} = Mean of X = 12400

\bar{y} = Mean of Y = 69.8

x = Deviation from mean of X

y = Deviation from mean of Y

b = Sample regression coefficient

$$= \frac{\sum xy}{\sum x^2} = -0.002$$

S_{yx} = Sample standard deviation from

$$\text{regression} = \sqrt{\frac{\sum dyx^2}{d.f.}} = 7.81$$

dyx = Deviation from regression

$$= Y - \hat{Y}$$

S_b = Sample standard deviation of
the regression coefficient

$$= \frac{S_{yx}}{\sqrt{x^2}} = 0.00041$$

$$t = b / S_b = -5.$$

d.f. = degree of freedom = 3

b. At 6 hours centrifuging:

Sample equation of regression:-

$$\hat{Y} = 86.4 - 0.002X$$

Note:

$$\bar{x} = 12400$$

$$\bar{y} = 61.6$$

$$b = -0.002$$

$$S_{yx} = 8.62$$

$$S_b = 0.00041$$

$$t = -5.$$

$$d.f. = 3$$

c. At 20 hours centrifuging:

Sample equation of regression:-

$$\hat{Y} = 63.8 - 0.002X$$

Note:

$$\bar{x} = 12400$$

$$\bar{y} = 39$$

$$b = -0.002$$

$$S_{yx} = 10.46$$

$$S_b = 0.00041$$

$$t = -5.$$

$$d.f. = 3$$

It may therefore be concluded that there is a simple inverse linear relationship between centrifugal force and germination. In view of the low rate of germination at 25000g, it seems probable that this is near the maximum force which barley can survive.

II. COMPARISON OF THE EFFECTS OF CENTRIFUGAL FORCE, PHENOL AND COMBINED TREATMENTS UPON SEED GERMINATION OF MONTCALM BARLEY:

After centrifuging at 25000g for 6 hours, and after 0.06% phenol treatment for 24 hours, the percentage of surviving seeds was 30%.

Combined treatments were much more severe than either one alone. The two kinds of combined treatments were similar in effect, for 11 seeds survived after RCF-P, and 10 seeds survived after P-RCF (Table 2). The mechanism of toxicity of phenol was not investigated, however it penetrates protoplasm rapidly and is known to denature proteins, therefore the effect produced by the combined treatment with phenol and centrifugation would be expected to be additive.

Also, if treatment with phenol or centrifugation should cause mutation, then a combined treatment should result in a greater frequency of mutation than either single treatment.

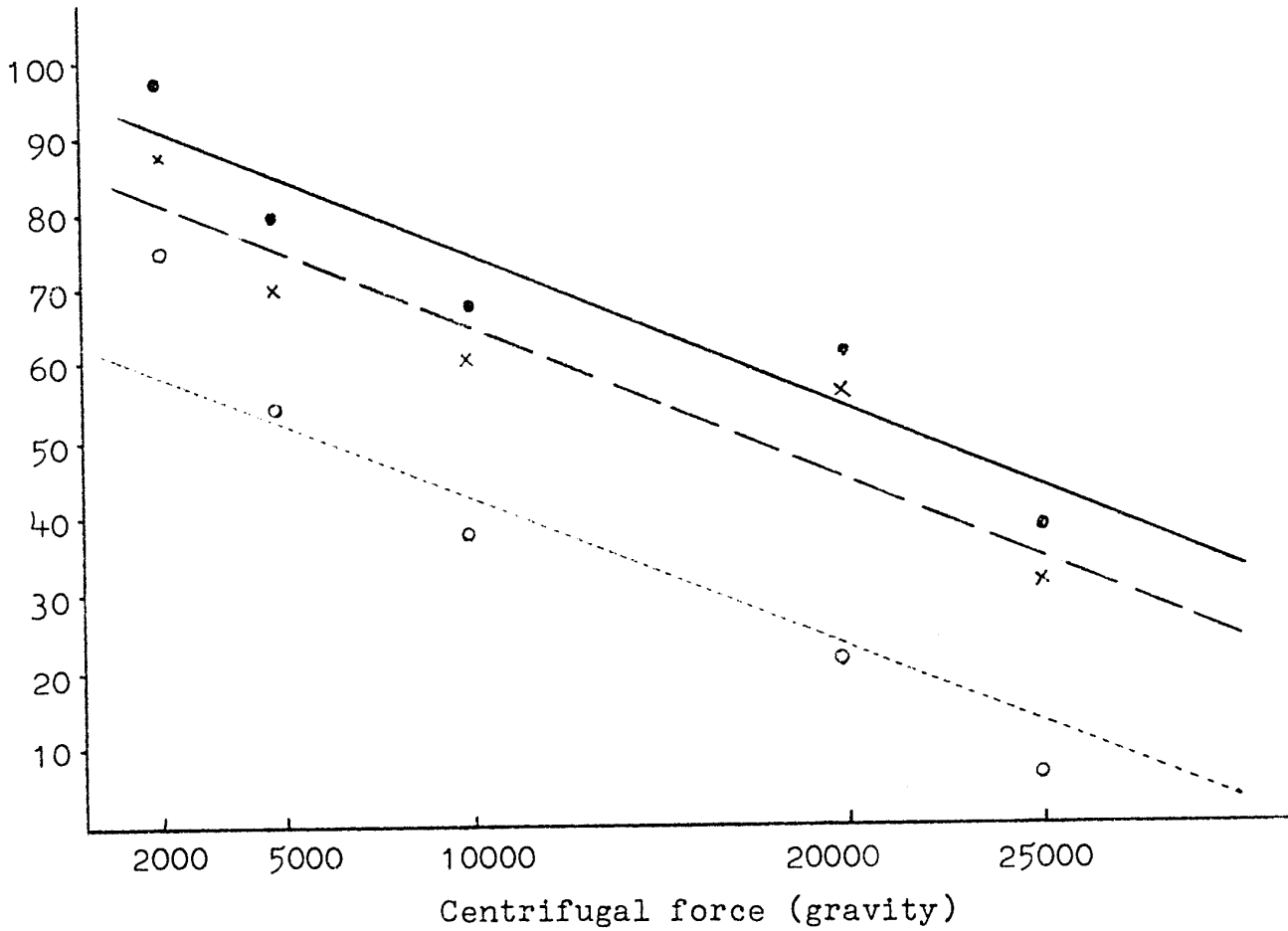


Figure 1: Regression of seed germination

3-hour centrifugation: Solid line, dots.

6-hour centrifugation: Broken line, crosses.

20-hour centrifugation: Dotted line, circles.

Further cytological study of the above aspect is being discussed in the following paragraphs, but only dominant lethals are applicable.

Table 2: Germination percentage of Montcalm seeds obtained in centrifugation, phenol and combined treatments.

Treatment	Total Seeds	Dead Seeds	% of Surv.
Control	100	1	99
Centrifugation	100	70	30
Phenol	100	70	30
Centrifugation - Phenol	100	89	11
Phenol - Centrifugation	100	90	10

III. EFFECT OF CENTRIFUGAL FORCE UPON THE MITOTIC CELLS OF THE ROOT TIPS OF MONTCALM BARLEY:

Cytological analysis was made in ten root tips of germinating seeds which had been centrifuged at forces of 2000g, 5000g, 10000g, 20000g, and 25000g. The force affected the cell in several ways.

The nuclei might be shifted to the centrifugal ends of the cells. In Figure 2, two elongation cells show the nuclei clearly settled to the ends of the cells. This was found in seeds having very short roots and centrifuged at forces of 5000g or higher. At 25000g, an average of 50 nuclei were found to be located at the ends of the cells, which was about 10%



Fig. 2. Root-tip cells of Montcalm variety. Centrifugation - 25000g, 6 hours. Nuclei located at the ends of the cells. X500 Approximately.



Fig. 3. Root-tip cells of Montcalm variety. Centrifugation - 25000g, 6 hours. Nuclei elongated. X2000 Approximately.

of the total elongating cells found in the slide. Approximately 30 nuclei (6% of the elongation cells) were found in such manner at 20000g. At both 10000g and 5000g, only 10 such nuclei were found (about 2%). This was not observed after the treatment at 2000g. This indicates that the difference in specific gravity is not sufficient to permit the separation of nucleus and cytoplasm by a force of this magnitude.

The surviving seeds were kept at room temperature over night, then root tip preparations were made. It was observed that the percentages of centrifugally located nuclei range only up to 10%, hence the majority of nuclei are not strongly displaced. Perhaps this is because the specific gravity of the nuclei is not constant; more probably, they return to the center of the cell after a certain period of restitution; and, also, a possible reason may be that new cells, produced after treatment, are included in the examination.

Centrifugal force not only moves the nucleus, but it elongates it, sometimes to a dumb-bell shape. One side had more nuclear sap, and was denser in appearance than the other side (Fig. 3). In a typical root tip, for example, about 90% of nuclei had the elongated shape (Fig. 4), after treatment at 25000g. But such nuclei were not always found in every root tip after the treatment. Such nuclei were occasionally found at 20000g and 10000g, but never found at

either 5000g or 2000g.

Centrifugal force sometimes interfered with mitosis, for the chromatin clumped, and amitosis occasionally occurred. Some cells showed the central part of the nucleus vacuolated, possibly preparatory to simple direct division -- amitosis (Fig. 5). Such amitotic nuclei might have been formed by continued distension of the dumb-bell shaped nuclei. Such detectable effects were occasionally found even after centrifugation at 2000g, but at higher forces of 20000g and 25000g, they were frequently observed.

The principal mitotic abnormality of chromosomes was chromosomal breakage, which was observed very frequently in the root tips of centrifuged seeds. One or more breaks occurred in one chromosome, or several chromosomes were broken into many fragments. This phenomenon could also be considered as multiple chromosomal breakage, or chromosomal fragmentation (Fig. 6,7). In a few instances, the chromosomes were broken into a series of regularly spaced blocks, suggestive of the large chromosomes of late pachytene stage. Breakage was not localized in a particular region of the chromosome. It might occur in the region of the centromere, or elsewhere in the chromosome. In the present study, about thirty metaphase cells were found in the whole meristematic region of each root tip. On the average, three metaphase cells had at least one chromosome showing breakage, and the chromosome sometimes was broken into many fine fragments. This was observed after the treatments at 20000g and at 25000g.



Fig. 4. Root-tip cells of Montcalm variety. Centrifugation - 25000g, 6 hours. Nuclei elongated. X500 Approximately.



Fig. 5. Root-tip cells of Montcalm variety. Centrifugation - 25000g, 6 hours. Nuclei vacuolated. X2000 Approximately.

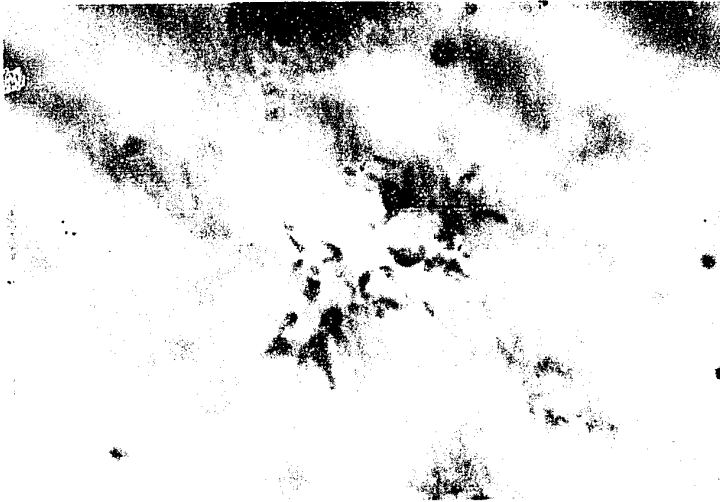


Fig. 6. Root-tip cell of Montcalm variety. Centrifugation - 20000g, 6 hours. Chromosomal breakages arrowed. X1500 Approximately.

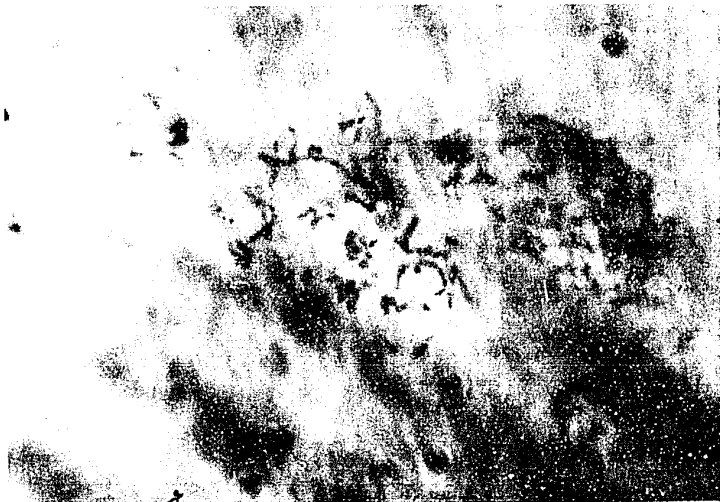


Fig. 7. Root-tip cell of Montcalm variety. Centrifugation - 25000g, 6 hours. Chromosomal breakages arrowed. X1500 Approximately.

At 10000g or 5000g, one or two metaphase cells of each root tip were found to have chromosomal breakage, but such cells had only one or two chromosomes with one or two breaks. No chromosomal breakage was found in the samples centrifuged at 2000g.

At anaphase, tetraploid cells were also found in plants centrifuged at 20000g and 25000g (Fig. 8), but the frequency of these was very low. In the sample examined, two tetraploid cells were found after the treatment at 25000g, and only one at 20000g. No tetraploidy was found after other treatments. Tetraploid cells might result from chromosome doubling, or from non-disjunction of chromosomes during the metaphase. If the spindles were severely injured or deformed, chromosomes would remain at the equator, and hence lead to tetraploidy. This may be the reason why tetraploid cells are produced by centrifugation. The centrifugal force may also cause the deformities of spindles and simply drives the entire metaphase chromosome set toward one pole of the cell, thus establishing tetraploidy. If the spindles were slightly injured, then aneuploidy could be produced. Other cytological abnormalities could also be produced by centrifugal force. But, at present, only chromosomal aberration was studied. Future research might eventually lead to a better understanding of this problem.

IV. EFFECTS OF VARIOUS CENTRIFUGAL FORCES UPON THE POLLEN

MOTHER CELLS OF MONTCALM BARLEY:

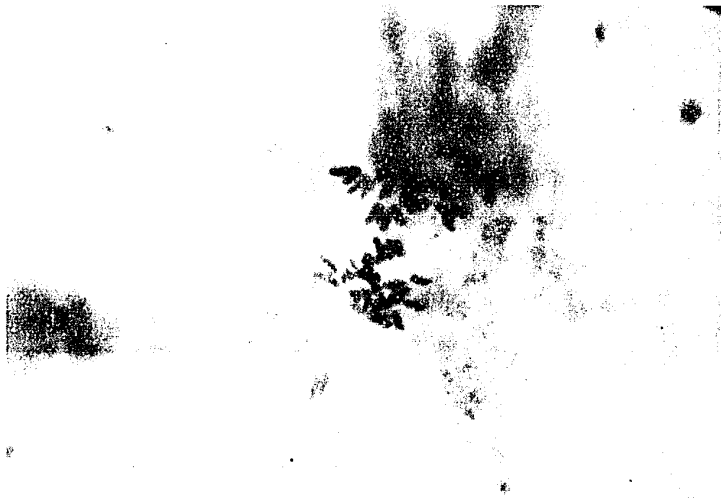


Fig. 8. Root-tip cell of Montcalm variety. Centrifugation - 25000g, 6 hours. Tetraploidy. X1500 Approx.

Montcalm seeds were centrifuged at forces of 2000g, 5000g, 10000g, and 20000g for 6 hours. Pollen mother cells produced by the plants derived from seeds which survived the treatments were studied cytologically. Fragmentation was found as the main chromosomal aberration at metaphase I. In some cases, the chromosome formed several fragments which were completely separated, or, sometimes they were connected together with a fiber (Fig. 9). This was not due to the smearing technique, because the same technique was used on controls, where no such irregularity occurred.

An interesting chromosomal aberration which has not been previously reported was often observed in PMCs (pollen mother cells) of T₁ plants. The metaphase or late prophase chromosomes were drawn out into long, slender fibers simulating leptotene chromosomes, a phenomenon which we call FIBRILLATION. Frequently, a chromosome is partly condensed and partly fibrillated (Fig. 9, 10). Swanson (1958) suggested that heterochromatin is generally more condensed and resistant to change, while euchromatin is more pliable and responsive. Thus it seems probable that, in such partially fibrillated chromosomes, the fibrillated parts are euchromatic and the condensed parts heterochromatic. In some cases, fibrillation is so complete (Fig. 11) that it is difficult to assign the nucleus to a specific meiotic phase. As fibrillation has not been previously reported and it was never observed in controls, it is undoubtedly produced by the treatment, and it may well

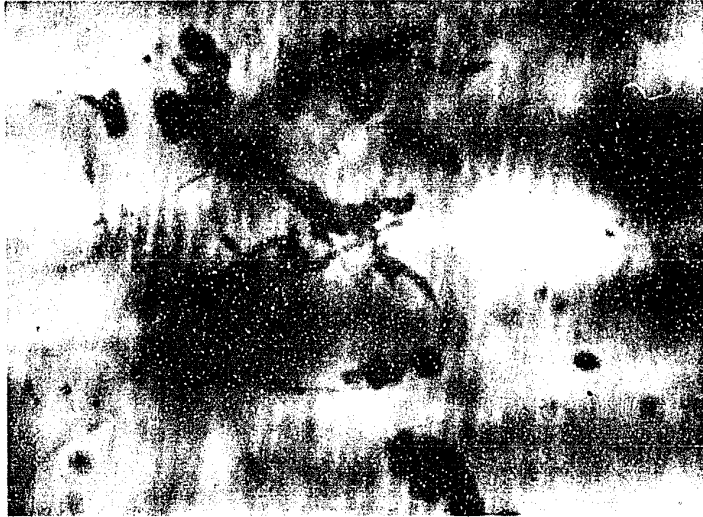


Fig. 9. PMCs of Montcalm variety.
Centrifugation - 20000g, 6 hours.
Chromosomal fragmentation and
fibrillation. X2000 Approximately.

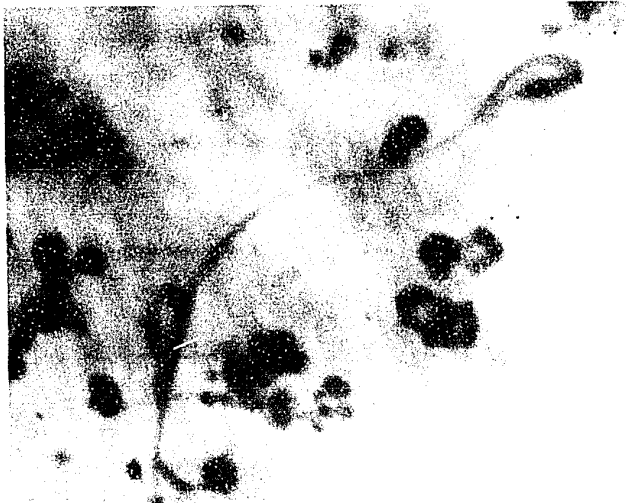


Fig. 10. PMCs of Montcalm variety.
Centrifugation - 20000g, 6 hours.
Chromosomal fibrillation. X2000
Approximately.

be a specific effect of centrifugation.

Inversion was often found, as indicated by a bridge at anaphase I. Of course, only heterozygous, paracentric inversion can be identified in this way (Fig. 12). The acentric fragment, however, was not always seen in the cell. The acentric fragment, being rather small, might have been lost before anaphase or during the preparation of the slide. There were, sometimes, two inversion bridges in one cell; if the inverted regions were not equal in length, one might have been broken earlier than the other (Fig. 13).

Breakage might occur at any region of the chromosome. One, two, or even more breaks could be found in a single chromosome. The broken segments were usually paired with a normal homologous chromosome (Fig. 14).

Chromosomal deficiency was sometimes visible at metaphase. The deleted portion was either interstitial or terminal, and this was clearly observed in meiosis. At metaphase I, for instance, an interstitially deficient chromosome was found as two broken segments which were still paired with the homologue (Fig. 14).

The results of the treatments can be summarized as follows:

1. CENTRIFUGING AT 2000g FOR 6 HOURS:

This mild treatment had little effect on the chromosomes. Fragmentation was the only chromosomal aberration found, and this was observed in six out of ten plants examined. The others

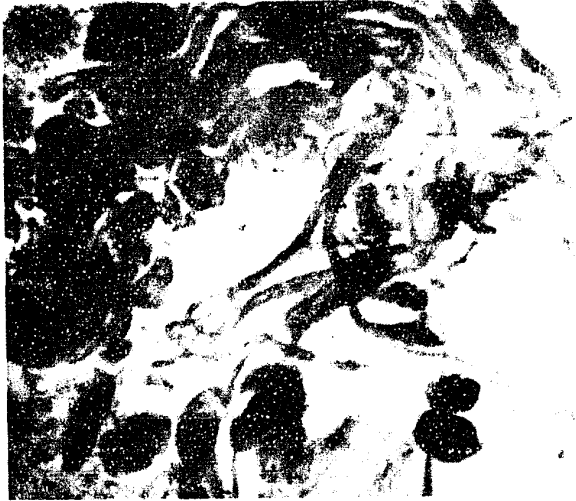


Fig. 11. PMCs of Montcalm variety.
Centrifugation - 20000g, 6 hours.
Chromosomal fibrillation. X2000
Approximately.



Fig. 12. PMC of Montcalm variety.
Centrifugation - 20000g, 6 hours.
Inversion bridge occurred between
two groups of chromosomes at Ana-
phase I. X5000 Approximately.



Fig. 13. PMC of Montcalm variety.
Centrifugation - 20000g, 6 hours.
Two inversion bridges in one cell
at Anaphase I. X5000 Approximately.



Fig. 14. PMC of Montcalm variety.
Centrifugation - 20000g, 6 hours.
Chromosomal breakage also as a
chromosomal deficiency. X5000
Approximately.

seemed to be normal cytologically (Table 3).

Table 3: Pollen mother cells showing chromosomal aberrations in Montcalm barley centrifuged at 2000g for 6 hours.

Aberration	Plant					Number				
	1	2	3	4	5	6	7	8	9	10
Fragmentation(No. of cells)	1	2			1	4			1	4

2. CENTRIFUGING AT 5000g FOR 6 HOURS:

5000g induced much more chromosomal fragmentation than did 2000g. Fibrillation and deficiency were very seldom found after that treatment. Chromosomal breakage and inversion bridge were also observed (Table 4).

Table 4: Pollen mother cells showing chromosomal aberrations in Montcalm barley centrifuged at 5000g for 6 hours.

Aberration	Plant					Number				
	1	2	3	4	5	6	7	8	9	10
No. of cells										
Fragmentation	10	3	1	2	10		1		14	2
Fibrillation				1						
Chromosomal breakage				2						
Inversion							2	1		
Chromosomal deficiency				1						
Total	10	3	2	5	10		3	1	14	2

3. CENTRIFUGING AT 10000g FOR 6 HOURS:

Fewer aberrations were observed after treatment at 10000g (Table 5) than at 5000g. This might be due to the

sampling error, with the sample at 10000g being inadvertently biased in favor of low frequency of aberration.

Table 5: Pollen mother cells showing chromosomal aberrations in Montcalm barley centrifuged at 10000g for 6 hours.

Aberration		Plant					Number				
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation		2		10	4	1	3	1	15	1
	Fibrillation						1				
	Inversion					2					
	Total		2		10	6	2	3	1	15	1

Only fragmentation was frequently observed after this treatment, and plants number 9 and 4 were found to have a considerably higher number of PMCs with fragmentation than others. However, fibrillation and inversion were observed in two different plants, yet they occurred in only one and two cells respectively.

4. CENTRIFUGING AT 20000g FOR 6 HOURS:

A centrifugal force of 20000g seems to be more effective. The number of the cells showing chromosomal aberration was higher than with other forces (Table 6).

After comparing the above four treatments, it is more likely that the higher intensities of centrifugation are more effective than lower intensities in inducing chromosomal aberration. The relationship of aberration to the treatments was analyzed by the linear regression method as shown in Figure 15.

Table 6: Pollen mother cells showing chromosomal aberrations in Montcalm barley centrifuged at 20000g for 6 hours.

	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation	1		17	6	18		2		1	3
	Fibrillation										3
	Inversion	2	4					1		1	
	Total	3	4	17	6	18	1	2	1	4	3

V. MEIOTIC ABERRATIONS IN MONTCALM AND HANNCHEN T₁ PLANTS INDUCED BY CENTRIFUGAL FORCE AT 25000g FOR 6 HOURS, PHENOL AND COMBINED TREATMENTS:

Cytological study was made of the pollen mother cells of ten T₁ plants taken at random from the population. There were eight different kinds of T₁ plants: M-RCF, M-P, M-RCF-P, M-P-RCF, H-RCF, H-P, H-RCF-P, and H-P-RCF. (See Materials and Methods). Normal plants (controls) of the two varieties were also compared with the T₁ plants.

The only chromosomal aberration found in the normal varieties was fragmentation, and this occurred very rarely. Two out of ten Montcalm controls had such abnormality. One of these two plants had only one cell with fragmentation, the other had two. The remainder did not show any sign of peculiarity in meiosis. Of Hannchen controls, four plants had only one such cell each. It appears that fragmentation can be produced spontaneously under normal conditions at very low frequency.

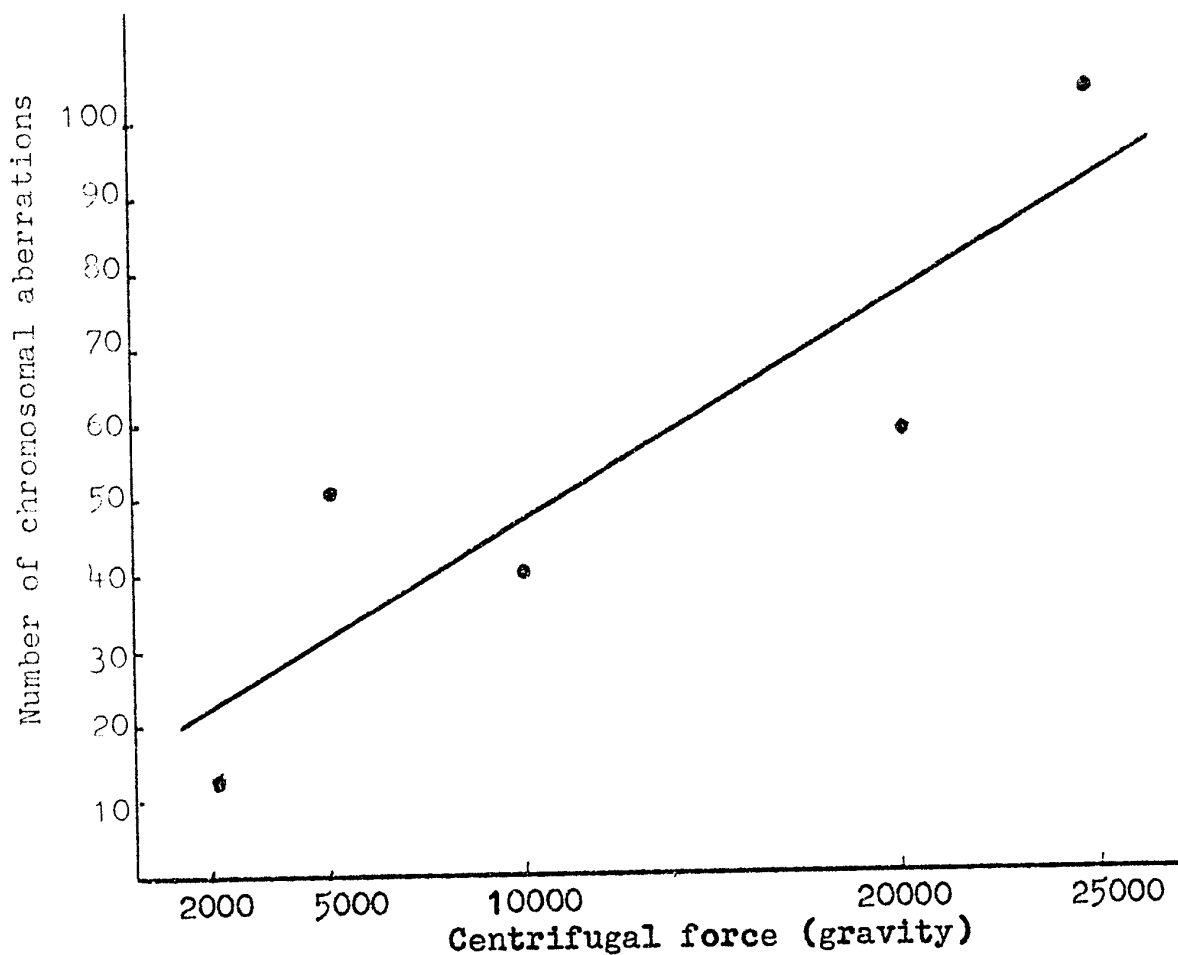


Figure 15. Regression of chromosomal aberrations.

Dots: Chromosomal aberrations induced by various centrifugal forces in Montcalm barley.

Solid line: Relationship of aberrations to the centrifugal force.

$$Y = 16.2 + 0.003X, x=12400, y=53.4, b=0.003.$$

1. M-RCF:

Seven categories of chromosomal aberration were observed after this treatment: fragmentation, fibrillation, inversion, translocation, deficiency, breakage and aneuploidy (Table 7).

Table 7: Pollen mother cells showing meiotic aberrations in M-RCF T₁ plants centrifuged at 25000g for 6 hours.

	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation	2		2	4	2	1		3		
	Fibrillation		2	6	9						
	Inversion			1	2		1				
	Translocation	2	2			5	20		3	2	
	Chromosomal deficiency	1				2	1	6	5	5	
	Chromosomal breakage	10	2								
	Aneuploidy (tetrasomic)	2									
	Total	17	6	11	15	9	2	21	9	8	7

Quadrivalent translocations were usually found. A complex configuration consisting of three pairs of chromosomes arranged as a big ring at diakinesis was also found as a hexavalent translocation (Fig. 16). Another complex configuration consisted of more than three pairs of chromosomes (possibly four). Because the stain was not successful, it could not be photographed.

Cells with fragmented chromosomes were found in six out of ten plants examined, but the frequency in each plant

was low. Figure 17 clearly shows three fragments formed from one pair of chromosomes; these three fragments actually formed from one chromosome of the pair.

Cells with fibrillated chromosomes were found in only three plants, all at diakinesis. Figure 18, for example, shows a cell with several long fibrillated chromosomes, together with normal chromosome rings. The fibers were possibly produced by five pairs of chromosomes. It can be pointed out that, in this figure, at one end of the fibers, three dark particles were connected, possibly representing heterochromatin parts of three pairs of chromosomes. At the other end, two chromosome rings were clearly seen. At the middle of the fibers, a nodule represented another pair of homologues. It is clear then that the fibers were produced by a complex of chromosomes.

Three plants had inversion bridges, however the frequency was low. A double inversion bridge occasionally occurred at anaphase I, and one of the two bridges was broken (Fig. 19). Chromosomal breakage was observed only in two plants. One of them had ten pollen mother cells showing chromosomal breakages, the other had two only. Figure 20 shows a chromosome ring broken into two unequal parts. Two aneuploid cells with eight bivalents were found in one plant, hence they were tetrasomic (Fig. 21). Chromosomal deficiencies were also observed in the pollen mother cells of several plants. They were terminal deficiencies, and the missing segment sometimes was rather big (Fig 22).

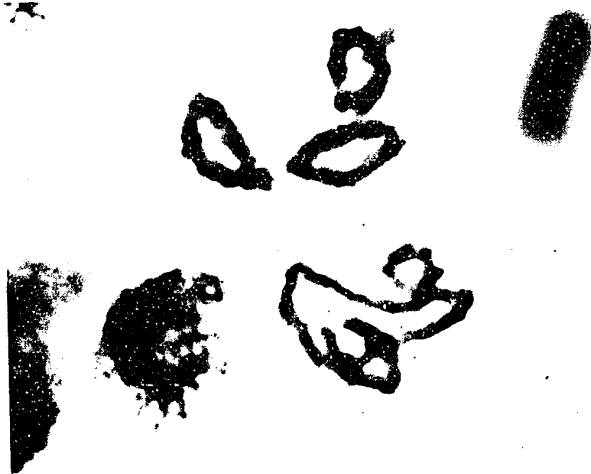


Fig. 16. PMC of M-RCF T₁ plant.
Translocation-hexavalent. X5000
Approximately.

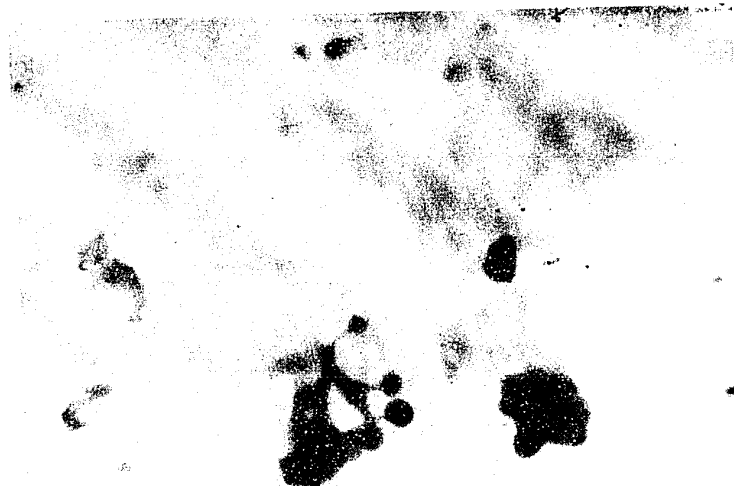


Fig. 17. PMC of M-RCF T₁ plant.
Chromosomal fragmentation. X2000
Approximately.

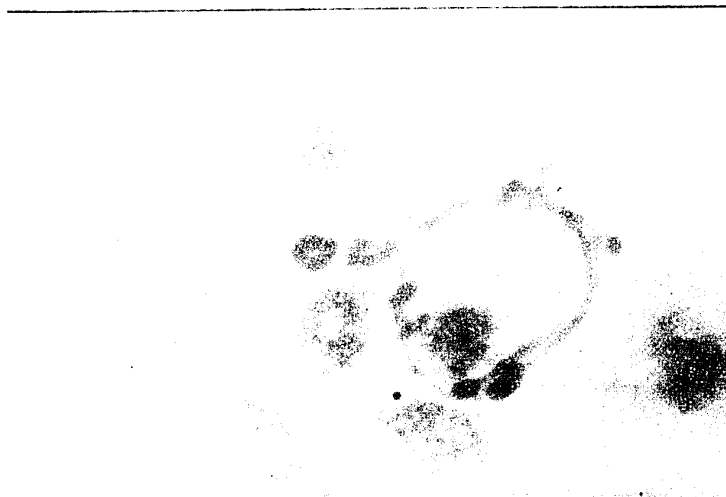


Fig. 18. PMC of M-RCF T₁ plant.
Chromosomal fibrillation. X2000
Approximately.



Fig. 19. PMC of M-RCF T₁ plant.
Inversion bridge. X5000 Approx.



Fig. 20. PMC of H-RCF T₁ plant.
Chromosomal breakage. X5000
Approximately.



Fig. 21. PMC of H-RCF T₁ plant.
Tetrasomic. X5000 Approximately.

2. M-P:

Phenol seemed to have effects similar to those of centrifugal force, but the frequency of most aberrations induced by phenol was somewhat lower (Table 8).

Table 8: Pollen mother cells showing meiotic aberrations in M-P T₁ plants treated with 0.06% phenol for 24 hours.

	Aberration	Plant Number									
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation	2		9	3			3		2	4
	Fibrillation	2		1							
	Inversion	4									
	Translocation				1	1	1	1	2		
	Chromatid deficiency								2	2	
	Chromosomal deficiency							1	5	5	
	Chromosomal breakage			3				3	2		
	Total	8		13	4	1	1	8	11	9	4

Six out of ten plants examined had fragmentation. Fragments could sometimes be assigned to the proper chromosome. In one cell, for instance, there were three fragments produced by one pair of chromosomes, while the other chromosomes paired normally at diakinesis (Fig. 23). Fibrillation was found in two plants, and only one or two cells showed it. It did not always affect the whole chromosome (Fig. 24). Levan and Tjio (1948a,b) observed a thin thread of chromosome in Allium roots, which were treated with phenol. They neither illustrated it nor discussed fully, hence one cannot determine whether they observed fibrillation.

A photograph was taken to show inversion bridge at anaphase. Only one plant out of ten was found to have an inversion (Fig. 25). Translocation was observed in one half of the plants examined, but they occurred in only a few cells. These translocation chromosomes could be identified as quadrivalents consisting of two pairs of chromosomes (Fig. 26). Chromosomal deficiency was present in three plants. Sometimes they could be seen as chromatid deficiency. In one cell, for example, one chromosome had two different arms, one was more slender than the other; this slender arm possibly had a chromatid deficiency (Fig. 27).

3. M-RCF-P:

Only 10% of the seeds survived this combined treatment. It seems that the surviving seeds were more or less resistant to the treatment. A few pollen mother cells of the surviving plants were found to have some chromosomal aberration (Table 9).

Extremely small fragments were found in the pollen mother cells of four plants. In one cell, for example, one chromosome had four fragments; three had one small fragment, each still connected with the mother chromosomes, and the remaining three tetrads were normal (Fig. 28). Sometimes fragmentation occurred with fibrillation in the same cell (Fig. 29). Only three plants appeared to have inversion, as identified by the occurrence of a chromosomal bridge (Fig. 30). Translocation was also observed. A complex configuration of six pairs of chromosomes was arranged nicely as a star (Fig. 31), with the



Fig. 22. PMC of M-RCF T₁ plant.
Chromosomal deficiency. X5000
Approximately.

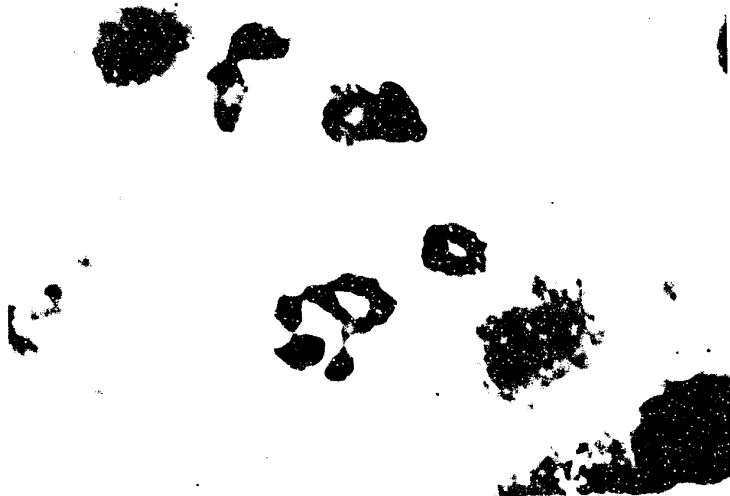


Fig. 23. PMC of M-P T₁ plant.
Chromosomal fragmentation. Two
acentric segments. X2000 Approx.



Fig. 24. PMC of M-P T₁ plant.
Chromosomal fibrillation. X2000
Approximately.



Fig. 25. PMC of M-P T₁ plant.
Inversion bridge. X5000 Approx.

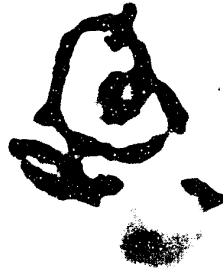


Fig. 26. PMC of M-P T₁ plant.
Translocation - Quadrivalent.
X5000 Approximately.

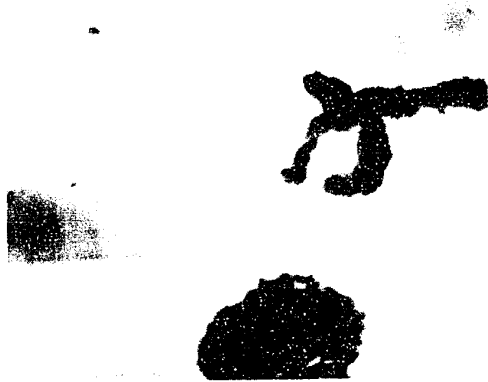


Fig. 27. PMC of M-P T₁ plant.
Chromatid deficiency. X5000
Approximately.

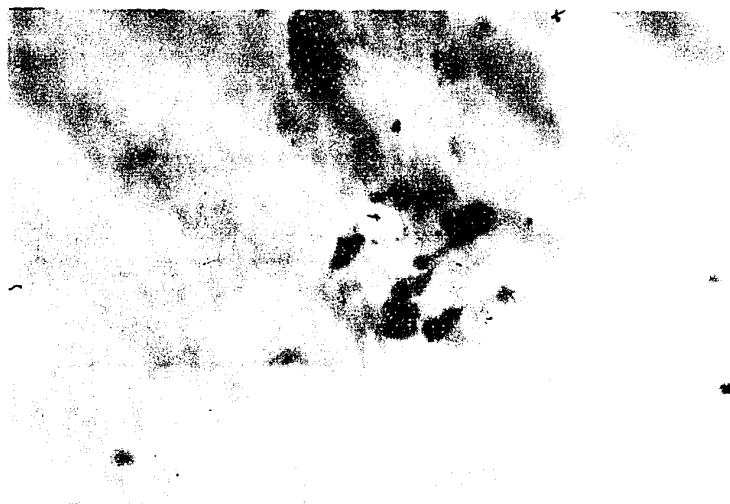


Fig. 28. PMC of M-RCF-P T₁ plant.
Chromosomal fragmentation. X2000
Approximately.



Fig. 29. PMC of M-RCF-P T₁ plant.
Chromosomal fragmentation and
Fibrillation. X2000 Approximately.



Fig. 30. PMC of M-RCF-P T₁ plant.
Inversion bridge. X5000 Approx.

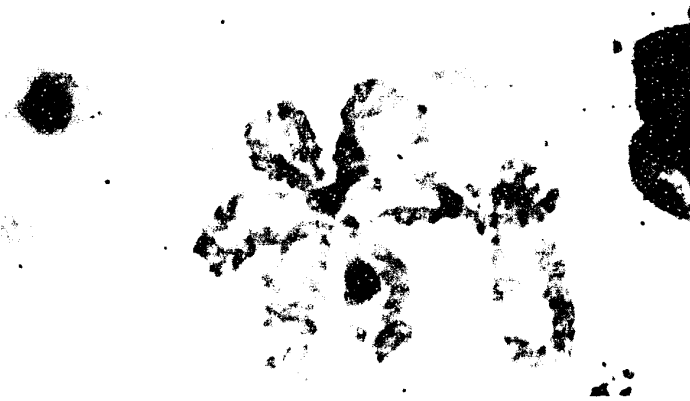


Fig. 31. PMC of M-RCF-P T₁ plant.
Translocation. X5000 Approx.

seventh bivalent appearing normal.

Table 9: Pollen mother cells showing meiotic aberrations in H-RCP-P T₁ plants centrifuged at 25000g for 6 hours and then treated with 0.06% phenol for 24 hours.

	Aberration	Plant Number									
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation	1		3	1	1					
	Fibrillation					1			1	2	5
	Inversion	1	2	1							
	Translocation						1		2	1	1
	Chromosomal deficiency								1	6	
	Aneuploidy (tetrasomic)	1									
	Tetraploidy				1						
	Total	3	2	5	1	2	1		4	9	6

Chromosomal deficiency was visible in some cells, and usually it was terminal. Aneuploidy with sixteen chromosomes was found in one plant. Apparently it was tetrasomic, for there were eight pairs of chromosomes at anaphase I (Fig. 32). A tetraploid cell was also present, the fourteen pairs of chromosomes at metaphase I being clearly visible within the cytoplasm (Fig. 33).

4. H-P-RCP:

In this combined treatment, phenol was applied before the seeds were centrifuged. The results obtained from this treatment are tabulated in Table 10.

Fibrillation and fragmentation were often found in the



Fig. 32. PMC of M-RCP-P T₁ plant.
Tetrasomic. X5000 Approximately.



Fig. 33. PMC of M-RCP-P T₁ plant.
Tetraploidy. X2000 Approximately.

same cells (Fig. 34), and this occurred frequently in two out of ten plants examined. Inversion bridges were found in four plants. In one case, the bridge at anaphase I was heavily beaded (Fig. 35). Heterozygous translocation could sometimes be seen at diplotene as cross-like configurations (Fig. 36). Tetraploid, tetrasomic and double tetrasomic cells were also found but rarely (Fig. 37, 38, 39).

Table 10: Pollen mother cells showing meiotic aberrations in M-P-RCF I, plants treated with 0.06% phenol for 24 hours and then centrifuged at 25000g for 6 hours.

	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation			2	*						*
	Fibrillation				*						*
	Inversion			4		3		1	5		
	Translocation	1	2	1	1						
	Aneuploidy (tetrasomic)										1
	Aneuploidy (double tetrasomic)										1
	Tetraploidy						2				
	Total	1	2	7	*	3	2	1	5	2	*

*: more than thirty.

5. H-RCF:

Fragmentation was the main type of chromosomal irregularity induced by this treatment (Table 11). Each plant examined had this abnormality. Sometimes, several fragments were formed by one chromosome (Fig. 40). Fibrillation, trans-

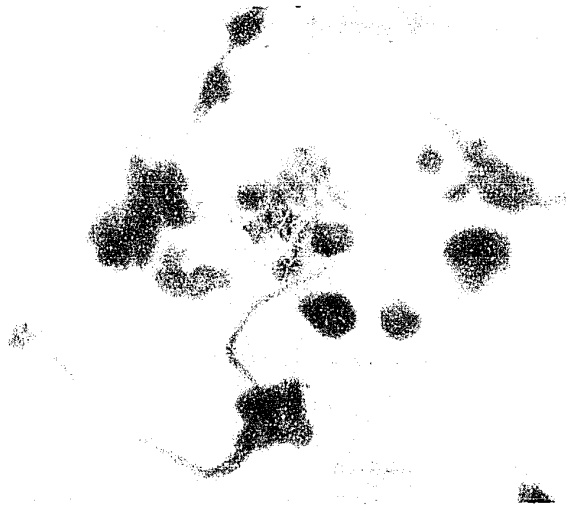


Fig. 34. PMC of M-P-RCF T₁ plant.
Chromosomal fragmentation and
fibrillation. X2000 Approximately.



Fig. 35. PMC of M-P-RCF T₁ plant.
Inversion and fragmentation.
X5000 Approximately.



Fig. 36. PMC of M-P-RCF T₁ plant.
Translocation. X5000 Approximately.



Fig. 37. PMC of M-P-RCF T₁ plant.
Tetraploidy. X5000 Approximately.



Fig. 38. PMC of M-P-RCF T₁ plant.
Tetrasomic. X5000 Approximately.



Fig. 39. PMC of M-P-RCF T₁ plant.
Double tetrasomic. X5000 Approx.

location, chromosomal deficiency and breakage, and inversion, were also induced by this treatment. However, they did not occur as often as fragmentation. The translocations were observed as quadrivalents at diakinesis.

Table 11: Pollen mother cells showing meiotic aberrations in H-BCF T₁ plants centrifuged at 25000g for 6 hours.

	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation	*	5	9	5	21	18	20	16	21	15
	Fibrillation	*	3	1			3	7	4	15	2
	Inversion	1		1						2	
	Translocation	1	1			1					
	Chromosomal deficiency		2			5	8	2		1	
	Chromosomal breakage		7				3	1			
	Total	*	16	11	5	27	32	30	22	37	17

*: more than thirty.

6. H-P:

Six of the ten plants examined had chromosomal aberrations, others were normal (Table 12). There were five kinds of chromosomal aberrations observed; fragmentation, fibrillation, inversion, chromosomal deficiency and breakage. Fragmentation and fibrillation were again the main types of aberration, the others occurred less frequently. The appearance of the aberrations was previously described.

Table 12: Pollen mother cells showing meiotic aberrations in H-P T₁ plants treated with 0.06% phenol for 24 hours.

Number of cells	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
	Fragmentation	3			*	7	*				
	Fibrillation	4	5		12		10				
	Inversion		1	3	1		3				
	Chromosomal deficiency	2									
	Chromosomal breakage	1				2					
	Total	10	6	3	*	9	*				

*: more than thirty.

7. H-RCF-P:

The type and frequency of chromosomal aberration found in the PMC's of H-RCF-P T₁ plants are tabulated in Table 13.

Table 13: Pollen mother cells showing meiotic aberrations in H-RCF-P T₁ plants centrifuged at 25000g for 6 hours and then treated with 0.06% phenol for 24 hours.

Number of cells	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
	Fragmentation	13	1		*	4	2	3	6		1
	Fibrillation	21	4	1	3				1	1	
	Inversion					1					
	Translocation	2	2				1	2	2		
	Chromosomal deficiency	7								1	
	Chromosomal breakage	3	1	1	3			2	1		
	Tetraploidy							1			
	Total	46	8	2	*	5	4	7	11	1	1

*: more than thirty.

Fragmentation and fibrillation were found as usual, often in the same cell. Translocations were mostly quadrivalents. A large chromosomal ring, probably octavalent, was found (Fig. 41). Chromosomal deficiency, inversion, chromosomal breakage and tetraploid cells were also found in several plants.

8. H-P-RCF:

It was not surprising that four plants were found normal cytologically, because the samples were taken at random. Evidently this combined treatment was more effective than either centrifuging or phenol treatment alone, because several of the plants examined had numerous chromosomal aberrations (Table 14).

Table 14: Pollen mother cells showing meiotic aberrations in H-P-RCF T₁ plants treated with 0.06% phenol for 24 hours and then centrifuged at 25000g for 6 hours.

	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
Number of cells	Fragmentation	1	11	26	*	2	*				
	Fibrillation	2	14	3	*		*				
	Inversion			1		1					
	Translocation					2					
	Aneuploidy (tetrasomic)					2					
	Aneuploidy (double tetrasomic)					1					
	Tetraploidy					1					
	Total		3	25	30	*	9	*			

*: more than thirty.

Fragmentation and fibrillation were abundant in some samples, as in plants number 4 and 6. One cell produced more

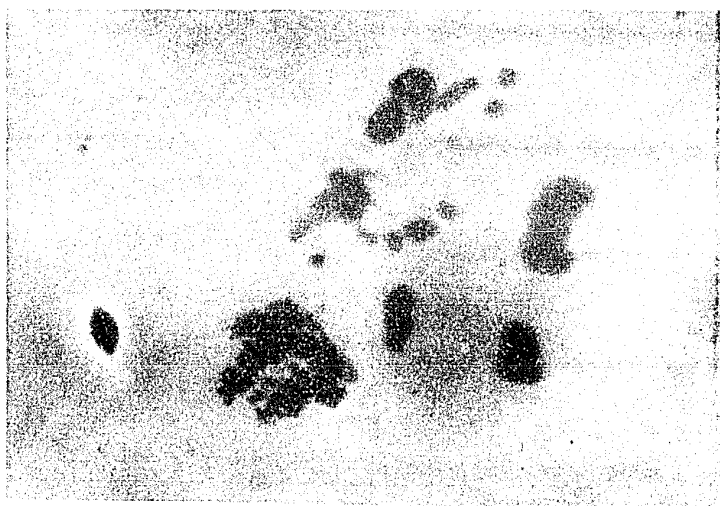


Fig. 40. PMC of H-RCF T₁ plant.
Chromosomal fragmentation. X2000
Approximately.

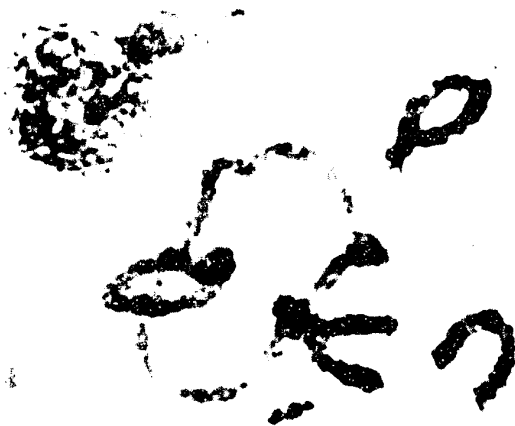


Fig. 41. PMC of H-RCF-P T₁ plant.
Translocation - Octavalent. X5000

than ten fragments (Fig. 42), and a picture showing extensive fibrillation, which might represent more than one cell, also occurred (Fig. 43).

VI. MEIOTIC ABERRATIONS IN MONTCALM AND HANNCHEM T₂ PLANTS INDUCED BY CENTRIFUGAL FORCE AT 25000g FOR 6 HOURS, PHENOL AND COMBINED TREATMENTS:

T₂ plants from the usual eight groups were studied, with normal Montcalm and Hanchen as controls. T₂ plants, were grown from seeds taken at random from the spikes of T₁ plants, which were semi-sterile. Only fragmentation was found in T₂ plants, and this was also observed in both controls (Table 15), though at a lower frequency.

Table 15: Pollen mother cells showing fragmentation in T₂ plants.

	Treatment	Plant										Total
		1	2	3	4	5	6	7	8	9	10	
Number of cells	H(control)			2		1	1			3		7
	H-RCF					1	1		2	4	5	13
	H-P		3						1			4
	H-RCF-P	7		2		4		1	6	1		21
	H-P-RCF		5					8				13
Number of cells	H(control)	3		1	1			2	2		1	10
	H-RCF	3		2	2	4		1	8	5	3	28
	H-P	2		5	3			1				11
	H-RCF-P	11	10		9	5		1		1		37
	H-P-RCF	3	12	4	15	9	8	12	9	4		76



Fig. 42. PMC of H-P-RCF T₁ plant.
Chromosomal fragmentation. X2000
Approximately.



Fig. 43. PMCs of H-P-RCF T₁ plant.
Chromosomal fibrillation. X2000
Approximately.

T₂ plants have a lower frequency of chromosomal aberration than T₁ plants. The explanation for this may be that if T₁ plants have lethal chromosomes, they may be aborted and may not pass into T₂ plants. In other words, the gametes may die off and no seed will be produced by this T₁ plant, or if the zygote is formed, it may die off at the early stage of development. Furthermore, barley is a self-pollinating plant, and hence any lethal genes or aberrations are quite likely to become homozygous, thus eliminating them from the T₂. The semi-sterility of T₁ plant may be due to this. It is also possible that if both gametes carried a slightly damaged chromosome, a viable zygote could also be produced. This might be why only fragmentation was found in T₂ plants.

VII. MEIOTIC ABERRATIONS IN HYBRIDS BETWEEN NORMAL AND TREATED PLANTS:

Ten kinds of hybrids were studied, namely, M x H, M x H-RCP, M x H-P, M x H-RCP-P, M x H-P-RCP, H x M, H x H-RCP, H x M-P, H x H-RCP-P, and H x H-P-RCP.

A number of chromosomal aberrations were observed in the pollen mother cells of the hybrids. On the average, the frequencies of these aberrations were low. Fragmentation and fibrillation were still the principal categories. Some hybrids however were also found to have translocation, inversion, aneuploidy, and chromosomal breakage, but they were found in few cells.

1. M x H:

Fragmentation was found in four control plants (M x H). Three samples had only one such cell, another one had only three. The rest of them had none. An inversion bridge appeared in only one plant. The results are tabulated in Table 16.

Table 16: Pollen mother cells showing meiotic aberrations in the control hybrids of M x H.

		Aberration	Plant					Number			
			1	2	3	4	5	6	7	8	9
Number of cells	Fragmentation	3		1	1			1			
	Inversion		1								
	Total	3	1	1	1			1			

2. M x H-RCF:

Two plants had a large number of cells showing both fragmentation and fibrillation, while five other plants showed these aberrations in low frequency. The rest of the plants examined seemed to be normal (Table 17).

Table 17: Pollen mother cells showing meiotic aberrations in the hybrids of M x H-RCF.

		Aberration	Plant					Number			
			1	2	3	4	5	6	7	8	9
Number of cells	Fragmentation	1		*		1	1	*	1		
	Fibrillation		2	*		7					
	Total	1	2	*		8	1	*	1		

*: more than thirty.

3. M x H-P:

Five different chromosomal aberrations were seen in the meiosis of these hybrids. Fragmentation and fibrillation were the main types. However, inversion bridges, translocation, and chromosomal breakages were also found (Table 18) at very low frequency in a few plants.

Table 18: Pollen mother cells showing meiotic aberrations in the hybrids of M x H-P.

	Aberration	Plant Number									
		1	2	3	4	5	6	7	8	9	10
No. of cells	Fragmentation	5	8	8	*	1	1	1	2		
	Fibrillation			13	1	1					
	Inversion			1							
	Translocation	1	1								
	Chromosomal breakage			8							
	Total	6	9	30	*	2	1	1	2		

*: more than thirty.

4. M x H-RCF-P:

Again, fragmentation was the major chromosomal aberration. It was commonly present in the plants examined (Table 19). Fibrillation was frequent in one plant, and another plant had eleven cells with fibrillation. The others seemed to be normal in this aspect. Fibrillation sometimes could be seen at diplotene. One cell, for example, consisted of seven pairs of chromosomes, four of them being elongated in the central portion to form a fiber (Fig. 44). Five

inversions occurred in one plant, a very high number for a single plant. Translocation occurred twice in one plant.

Table 19: Pollen mother cells showing meiotic aberrations in the hybrids of M x H-RCF-P.

No. of cells	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
	Fragmentation	2	2	2	13	3	1	1	8		
	Fibrillation	11							*		
	Inversion	5									
	Translocation				2						
	Total	13	7	2	15	3	1	1	*		

*: more than thirty.

5. M x H-P-RCF:

Fragmentation, fibrillation, inversion, translocation, and tetrasomic aneuploidy appeared in meiosis (Table 20).

Table 20: Pollen mother cells showing meiotic aberrations in the hybrids of M x H-P-RCF.

No. of cells	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
	Fragmentation	2	1	6	5	*	2	*	11	10	
	Fibrillation					*		*			
	Inversion	1									
	Translocation				1						
	Aneuploidy (tetrasomic)	1									
	Total	4	1	6	6	*	2	*	11	10	

*: more than thirty.

It often happened that one chromosome formed more than one fragment (Fig. 45) Other aberrations: inversion, translocation and aneuploidy, occurred once each.

6. H x H:

Control hybrids of H x H, seemed to be normal upon cytological examination. Although the fragmentations were observed in a half of the hybrids examined, they occurred only in one or two cells. There is no other chromosomal aberration found in the pollen mother cells (Table 21).

Table 21: Pollen mother cells showing meiotic aberrations in the control hybrids of H x H.

Aberration	Plant									
	1	2	3	4	5	6	7	8	9	10
Fragmentation (No. of cells)	1	2				1		1	1	

7. H x M-RCF:

Fragmentation was found in most of the sample examined. In some cases, the chromosomal fragments occurred as big segments connected with a thin chromosomal thread (Fig. 46). Fragmentation was the main chromosomal aberration found in the pollen mother cells of this kind of hybrids, however fibrillation and inversion were also observed, they occurred rarely. The results of the meiotic analysis are tabulated in Table 22.



Fig. 44. PMC of MxH-RCF-P T₁ plant.
Chromosomal fibrillation at pachytene.
X5000 Approximately.

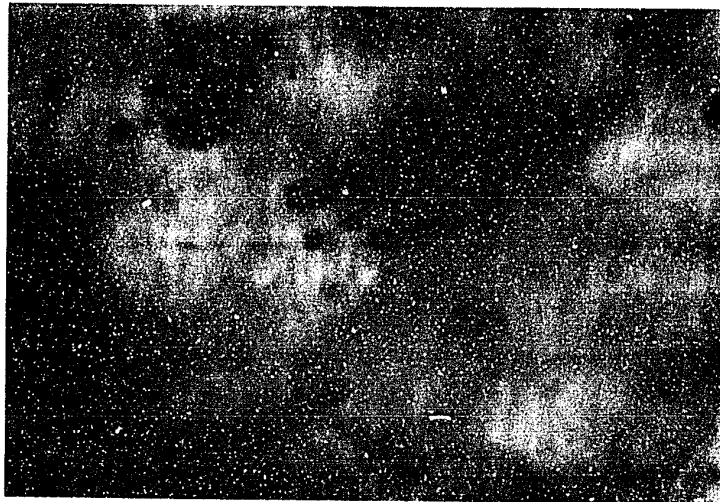


Fig. 45. PMC of MxH-P-RCF T₁ plant.
Chromosomal fragmentation. X2000
Approximately.

Table 22: Pollen mother cells showing meiotic aberrations in the hybrids of H x M-RCF.

Aberration		Plant Number									
		1	2	3	4	5	6	7	8	9	10
No. of cells	Fragmentation	2	13	2	9	*	5	2	7		2
	Fibrillation		1			4					1
	Inversion					1			1		
	Total	2	14	2	9	*	5	2	8	1	2

*: more than thirty.

8. H x M-P:

Only fragmentation and fibrillation were observed in meiosis. They were frequent in several plants (Table 23).

Table 23: Pollen mother cells showing meiotic aberrations in the hybrids of H x M-P.

Aberration		Plant Number									
		1	2	3	4	5	6	7	8	9	10
No. of cells	Fragmentation	3	9	6	3	2	4	*	*	*	
	Fibrillation		5	2				*	*	6	
	Total	3	14	8	3	2	4	*	*	*	

*: more than thirty.

9. H x M-RCF-P:

Half of the hybrids examined showed fragmentation (Table 24). One plant, number 10, showed numerous cells having both fragmentation and fibrillation (Fig. 47). Fibrillation occurred only in that plant.

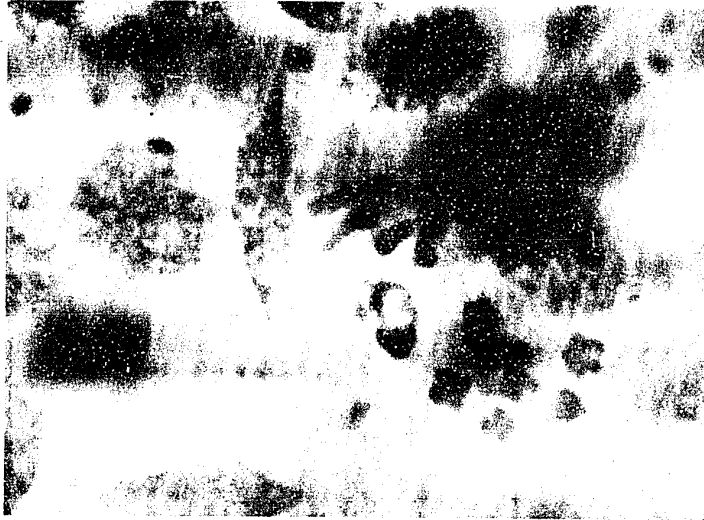


Fig. 46. PMC of HxM-RCP T₁ plant.
Chromosomal fragmentation. X2000
Approximately.

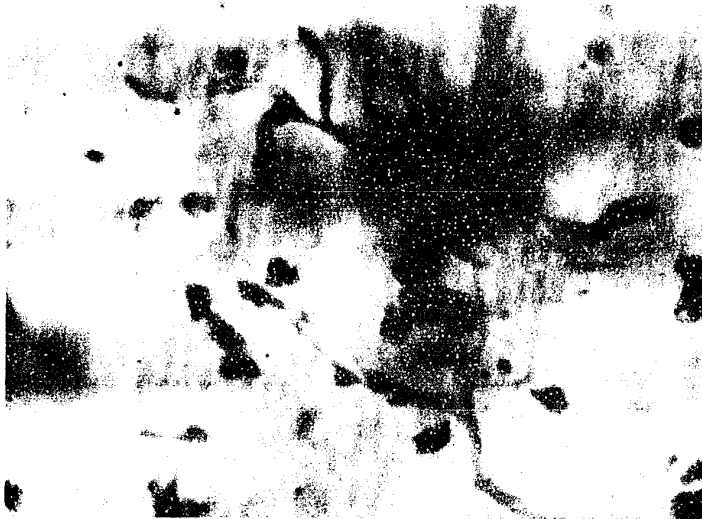


Fig. 47. PMC of HxM-RCP-P T₁ Plant.
Chromosomal fragmentation and fibri-
llation. X2000 Approximately.

Table 24: Pollen mother cells showing meiotic aberrations in the hybrids of H x M-RCP-P.

No. of cells	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
	Fragmentation	4				3	4	18			*
	Fibrillation										*
	Total	4				3	4	18			*

*: more than thirty.

10. H x M-P-RCP:

Approximately the same results were obtained as with the hybrids of H x M-RCP-P (Table 25). Again, one plant, number 10, showed numerous pollen mother cells having both fragmentation and fibrillation; inversion also occurred in the same plant.

Table 25: Pollen mother cells showing meiotic aberrations in the hybrids of H x M-P-RCP.

No. of cells	Aberration	Plant					Number				
		1	2	3	4	5	6	7	8	9	10
	Fragmentation	15	10			1				9	*
	Fibrillation		1								*
	Inversion										1
	Total	15	11			1				9	*

*: more than thirty.

VIII. PHENOTYPIC EFFECTS OBSERVED IN T_1 AND T_2 PLANTS,
AND IN HYBRIDS:

Phenotypes of T_1 and T_2 plants, and of hybrids were examined. The results are described as follows:

1. Seed rows and texture of awn:

Hannchen barley has two-seed rows and rough awns, while Montcalm has six-seed rows and smooth awns. The Hannchen characters are dominant over those of Montcalm.

One plant derived from the cross of M x H-RCF, had six-seed rows and rough awns. A spike of another plant, derived from a similar cross, had two-seed rows plus an incomplete third row consisting of only three seeds. Another plant derived from the cross of M x H-P-RCF had six-seed rows and smooth awns (Fig. 48). Two plants derived from the same cross of M x H-P-RCF were smooth awned and two seed rowed. Thus it must be due to gene mutation which is either mutated or deleted from the treated male parent, hence permitting pseudo-dominance.

2. Fertility of seed set:

Partial or complete sterility of seed set frequently resulted from the treatments. T_1 plants of both varieties derived from the treatments with centrifugation or phenol, or from the combined treatments (either centrifuged first and then treated with phenol or vice versa) often showed this effect. In other words, the partially sterile spike had only

one or two, sometimes up to five seeds; the completely sterile spike had no seed at all (on the average, Montcalm-control had 78 seeds, while Hannchen-control had 24 seeds). After each treatment, four or five out of thirty T_1 plants (about 15%) were partially sterile; three plants (10%), on the average, were completely sterile. This was never found in T_2 and hybrid plants. The cause of sterility is of course due to the lethality of chromosomal aberrations previously described.

3. Maturation of plants:

From May 10th to 12th, 1958, treated Hannchen seeds and controls were sown in the field. One week later, from May 17th to 19th, the treated and the control seeds of Montcalm were planted. Both varieties started to mature in the first week of August, 1958, and by August 15th, both varieties had ripened. In other words, T_1 plants and the controls of Montcalm variety took about 90 days to ripen while the Hannchen variety took about 100 days. Several M-RCF T_1 plants delayed their maturity for about three weeks, until September 1st to 6th, 1958. This means that they took 110 days to ripen.

Seeds of T_1 plants and controls of the two varieties were sown in the greenhouse on September 10, 1958. T_2 plants and controls of Montcalm matured during the second week of January, 1959, which was about 120 days of maturation. Hannchen matured about two weeks later. 8 M-RCF T_2 plants, which



Fig. 48. A mutant with six-seed rows and smooth awns derived from a cross of M x H-P-RCF.

were originally derived from the later maturing M-RCF T₁ plants, ripened in the second week of February, 1959, that is, they had grown for about 150 days. The delay of maturation however is not exactly known, but it is probably also due to the effect of the treatment.

4. Thickness of straw:

The late maturing T₁ plants of M-RCF, as previously described, had thicker straw than did the Montcalm controls. The width of the stem of the Montcalm controls and later maturing M-RCF T₁ plants was measured in centimeters. Ten plants from each group were taken at random for measurement, which was made at the second internode below the stem apex of these plants. The width of these stem and their statistical analysis follow:

Plant No.	Montcalm Controls			Late Maturing T ₁ M-RCF Plants		
	Width (cm.)	d _a	d _a ²	Width (cm.)	d _a	d _a ²
1	0.4	0.02	0.0004	0.5	0.1	0.01
2	0.4	0.02	0.0004	0.5	0.1	0.01
3	0.5	0.08	0.0064	0.6	0.0	0.00
4	0.4	0.02	0.0004	0.5	0.1	0.01
5	0.5	0.08	0.0064	0.6	0.0	0.00
6	0.4	0.02	0.0004	0.7	0.1	0.01
7	0.4	0.02	0.0004	0.6	0.00	0.00
8	0.4	0.02	0.0004	0.7	0.1	0.01
9	0.4	0.02	0.0004	0.6	0.0	0.00
10	0.4	0.02	0.0004	0.7	0.1	0.01
			0.0160			0.06

\bar{m}_a = mean of Montcalm controls = 0.4

\bar{m}_b = mean of late maturing T₁ M-RCF plants = 0.6

$\bar{m}_{diff.}$ = mean difference = 0.18

Variance of the distribution of the mean of each group:

$$V_a = 0.00017, \quad V_b = 0.00066$$

$$S_{\bar{m}} = \text{Standard error} = 0.0288$$

$$t = 6.251$$

Thus, a 't' value of 6.251 for 18 degrees of freedom has a probability of less than 0.001, which shows that the difference between the two groups is highly significant. This is probably caused by difference in genotypes resulting from the treatment.

5. Height of the plant:

One plant derived from a cross of H x H-RCP, was taller than the control of H x H by about $1 \frac{3}{4}$ ft. On the average, the controls of H x H were about two feet high under the greenhouse conditions. This taller plant of H x H-RCP was about $3 \frac{3}{4}$ ft. in height (Fig. 49). Others were about the same height as the controls, hence the tall plant may have resulted from mutations affecting this trait. Unfortunately, the seed was lost, and so this possibility could not be checked.

6. Number of tillers:

Regarding the number of tillers, no significant difference could be shown among the various treatments. The average number of tillers of ten samples of each group taken at random was calculated and tabulated as follows:

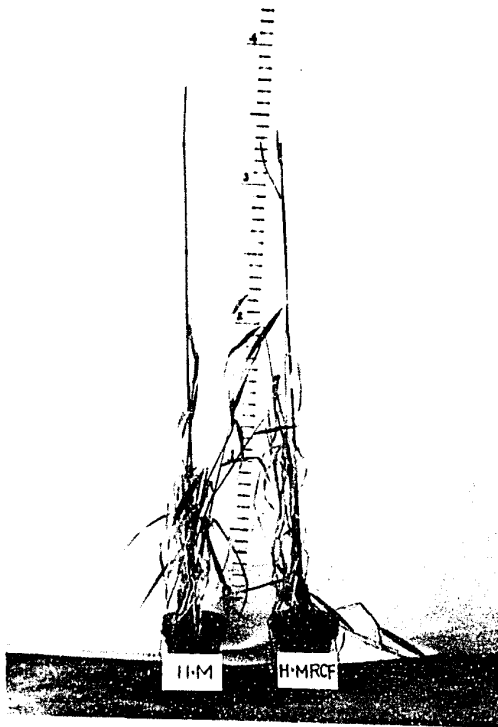


Fig. 49. A tall plant derived from a cross of H x M-RCF.

Table 26: Number of tillers of T₁ and hybrid plants.

T ₁ Plant	Average Tillers	Hybrids	Average Tillers	T ₁ Plant	Average Tillers	Hybrids	Average Tillers
H(cont.)	7.5	HxH(cont.)	3.8	M(cont.)	3.9	HxH(cont.)	3.7
H-RCF	7.5	HxH-RCF	3.5	M-RCF	3.6	HxH-RCF	3.9
H-P	7.6	HxH-P	3.3	M-P	4.2	HxH-P	3.4
H-RCF-P	7.3	HxH-RCF-P	3.6	M-RCF-P	3.1	HxH-RCF-P	3.3
H-P-RCF	7.9	HxH-P-RCF	3.2	M-P-RCF	3.2	HxH-P-RCF	3.8

Montcalm had a smaller number of tillers than Hannchen. From the above data, the smaller number of tillers appears to be a dominant character.

7. Number and pigmentation of leaves:

Again, there was no remarkable difference in the number of leaves or chlorophyll content. They seemed normal in various treatments.

DISCUSSION

The purpose of this research is to assess centrifugal force as a mutagenic agent. The data presented in the preceding parts of this study show convincingly that centrifugal force can produce a great diversity of chromosomal aberrations as well as some phenotypic changes in barley. The chromosomal aberrations, for examples, inversion, deletion, translocation, fragmentation, fibrillation etc. certainly have mutational effects which are, however, mostly harmful. Deletion can be the result of chromosomal fragmentation. The loss of a fragment of a chromosome is not likely to be reversible. If this deletion causes a change in phenotype, it will be inherited by the succeeding generations. On the other hand, chromosomal breaks may cause a rearrangement of a chromosome, such as inversion, translocation, deficiency, or duplication. These very frequently have phenotypic effects, whether because of point mutations near the break, or because of position effects due to changed spatial relationships with the chromosome. Goldschmidt (1955) claimed that the position effect is not the correct term for such phenomenon, because in many cases where the chromosomal break is far away from the affected locus, the locus still remained in its normal chromosome neighbourhood, but it nevertheless changed its action because of a break at some distance. He, therefore, considered "rearrangement effect" to be a more accurate term. Whatever

the terminology, it is generally agreed that chromosomal rearrangements are an important source of hereditary variability.

The percentage of seed germination was markedly influenced by centrifugal force and decreased as the severity of the force increased. The duration of centrifugation also affected germination, the percentage of germinating seeds decreasing as the duration of centrifugation increased (Fig. 1). Centrifugal force also produces mechanical damage to the seeds, such as the breaking of the embryo and of the coleoptile. Such damage, of course, leads to the death of the seeds. The percentage of seed germination was also very low after the phenol treatment. 0.06% phenol treatment for 24 hours was compared with centrifugal force at 25000g for 6 hours. Both phenol and centrifugal force, used as described, gave thirty percent germination. The combined treatments, in which the seeds were either treated with phenol first and then centrifuged or vice versa, were additive in effect; for 11 out of 100 seeds survived in the treatment RCF-P, and 10 out of 100 seeds survived in P-RCF. It is a well known fact that phenol acts on proteins by forming insoluble proteinates which result in the precipitation of protein in the protoplasm and even in the destruction of the cell (Salle, 1954). As a result, the effectiveness of phenol in inducing a heritable mutation is due to the sub-lethal reactions of phenol with cell components, particularly the

chromosomes. However, all known mutagenic agents are destructive, and their severity is proportional to their abilities to penetrate and to act within the cell. Thus, if a mutagen acts either on some part of a chromosome to produce a change in the chromosome constitution, or induces some chromosomal aberration in a cell, it will have a mutational effect.

A cytological study of the mitotic cells of root tips showed that the mechanical action of centrifugal force might shift their nuclei to the centrifugal poles of the cells, elongate the nuclei, interfere with mitosis, break the chromosomes into fragments, or induce tetraploidy depending upon the intensity of centrifugation. Not all of the nuclei in this experiment occupied the centrifugal position. As often happens, the nucleus may return to its original position in the cell after a period of restitution. In addition to simple displacement, the formation of a dumb-bell shaped nucleus is quite common. In the case of such disturbances in the nucleus, amitosis might have been produced by the continued distension of the dumb-bell shaped nuclei. If it is a dividing cell, the centrifugal force may interfere with the process of cell division. As a result, the chromosomes may be clumped or entangled together, spindles deformed or injured, and, tetraploid and aneuploid cells induced. The poly-, or aneu-, ploidy which occasionally occurred in the centrifugation treatment might

have been produced by such disturbance of the chromosome. Consider, for instance, the formation of tetraploid and aneuploid cells. Usually, tetraploid cells are caused either by chromosome doubling or non-disjunction of chromosomes at metaphase. If the spindles are seriously injured or deformed, or, if they were smashed or mixed together with the chromosomes toward the centrifugal end, the sister chromosomes will not separate at anaphase, and a tetraploid cell will be produced as a result of these deformities. If, on the other hand, the spindle is slightly injured or a few spindles damaged, a single chromosome may remain at the equator or go to the same pole with its homologue, and, as a result, an aneuploid cell will be formed.

Chromosomal aberrations, such as inversion, translocation, deficiency, breakage, fragmentation and fibrillation, induced by phenol and centrifugation, were often encountered in this experiment. Meiotic results obtained in the various centrifuging treatments indicated that the production of chromosomal aberrations depends upon the magnitude of the forces used. At 2000g only fragmentation was found, and its frequency was very low. Six out of ten plants examined had one to four cells with chromosomal fragmentation. Centrifugal force at higher speed is more effective than lower speed in producing chromosomal aberration. At 5000g, for example, five kinds of chromosomal aberrations were found in the ten samples examined: they were fragmentation, fibrillation, breakage, inversion and deficiency. Fragmentation

was found in nine plants in PMCs, in frequencies of one to fourteen, but other chromosomal aberrations as above described were rarely found. At 10000g and 20000g, the variety of chromosomal aberrations observed, however, was not as great as that observed in the treatment at 5000g, but the total frequency of the aberrations was higher. At 25000g, seven kinds of chromosomal aberrations were observed, and they occurred very often. Every plant examined had chromosomal aberrations, however, different aberrations occurred in different plants. It is clear from the results that the rate of production of chromosomal aberrations is linearly proportional to the force applied, this has been shown in Figure 15.

Phenol treatment was found less effective than centrifugation in inducing chromosomal aberrations. The number of pollen mother cells showing chromosomal aberrations in T₁ plants was higher after centrifuging than after phenol treatment. On the average, it was twice as high as phenol treatment; however, the variety of aberrations was the same after both treatments.

One might expect that combined treatment would cause a higher mutation rate than single treatment. Comparing the data of Tables 7-14, the total number of pollen mother cells showing chromosomal aberrations after combined treatment was sometimes higher than after single treatment. Because of

combined treatment, a large number of seeds were killed, only 10% of the seeds surviving (single treatment, either centrifuging or phenol, gave 30% surviving seeds). As combined treatment was more toxic than single treatment, the surviving seeds after combined treatment were the most resistant, and this might explain why the results were not always more severe than after centrifuging alone.

The effect of several known chemicals and ionizing radiations on dividing or metabolic cells can be assessed in a variety of ways. For instance, mutagenic action of a number of mustard gases both of the sulphur and the nitrogen type may act either on protein of the chromosomes, or on enzymes concerned with chromosome metabolism, or it may act directly on the amino acids of the gene proteins (Auerbach, 1951). Guyer and Claus (1947) pointed out that urethane is a powerful poison which produces mutation by its inhibitory action on mitosis. Colchicine is well known as a mitotic poison which produces a general blockage of mitosis through the destruction or deformation of spindles. Each of these substances is capable of producing aberrations in different ways and hence mutation can be produced. The number of application of a mutagen, therefore, depends on the type of substance used and the type of organism and tissue to be tested. The same mutagen may have different effects in different organisms. All mutagens need not necessarily act by one and the same ultimate mechanism.

Concerning the genetic effects produced by radiation, radiation not only produces chromatid and chromosomal breaks by its action in terms of 'hit', but a large part of information gained has given us a better understanding of how radiation affects the living cells. Radiation damage to a cell can be assessed by a variety of means, e.g. inhibiting the synthesis of DNA, depression of mitotic activity etc. Howard and Pelc (1953) demonstrated that radiation inhibits the synthesis of DNA in Vicia root tips and brings about mitotic delay. In dividing cells the general effect of radiation is the appearance of clumped chromosomes at metaphase and an irregular separation of chromatids at anaphase, therefore the chromosomes appear to be pycnotic or sticky (Marquardt, 1938). This also appears to be an effect on the nucleic acid and may be due to the non-sticky polymerized nucleic acid coating on the condensing and spiralizing chromosome being broken down by the irradiation to a fluid non-polymerized state in which parts of different chromosomes become attached to one another (Darlington, 1942). Darlington (1953) has suggested that the stickiness arises from the DNA on the surface of the chromosome which has been depolymerized and hence rendered more fluid by the radiation. Apparently all authors agree that the ionizing radiations, such as alpha, beta and gamma rays, and X-rays, etc. produce their effects as the result of paths of ioniza-

tion. The biological action of radiation is most plausibly attributed to chemical change resulting from ionization. Since the chemical bonds which hold a molecule together are constituted by electrons shared between the two atoms joined by the bonds, the removal of a bonding electron is likely to bring their dissociation or other chemical change. The energy involved in ionization greatly exceeds that required to remove an atom from the molecule (Catcheside, 1948). From the above statements, it may be understood that radiation can be assessed as a chemical agent. The action of centrifugal force on the cell appears to be a simple physical action capable of inducing meiotic and mitotic aberrations. Thus, while other agents for experimental mutation appear to act principally through chemical pathways, centrifugal force appears to have a simple physical action by distension and breakage of the chromosomes. Yet it can not be denied that centrifugal force might promote chemical reactions by having more intensive contact among the reactants. This, however, is purely speculative. So far, the chromosome appears to be a dual structure made up of a great portion of DNA-basic protein (histone or protamine) and a small amount of RNA-protein (containing tryptophane and tyrosine) (Swanson, 1957). The former appears more likely to fulfill the role of the genetic material of the cell, and also provides for the diversity of genetic function. The great likelihood of DNA possessing a major genic role has recently been studied (Watson and Crick, 1953, Beadle, 1955).

The latter RNA group is necessary for protein synthesis (Swift, 1953). However, the exact constitution of the chromosome is not known. In the case of centrifugation, DNA-, and RNA-, groups can be broken or otherwise interfered with, thus permitting a genetic change through a chemical reaction. Future research may help to interpret this point further.

A very important question is that of whether the genetic effects produced by centrifugation are similar to those of such mutagens as mustard gas and X-radiation. Auerbach (1949) compared the genetic effects produced by mustard gas and X-ray and found a striking similarity. She pointed out that in Drosophila, lethals are scattered along the X-chromosome in a similar way by both agents. Large rearrangements are less frequent after mustard treatment than after a dose of X-radiation which produces the same frequency of sex-linked lethals. The sex-linked lethals induced by mustard gas comprised from 2 to 25% of the sample treated. Slizynska and Slizynski (1947) obtained 89 sex-linked lethals in Drosophila, effecting 7% of a population treated with mustard gas. These did not seem to differ from those produced by X-radiation in either relative frequency or type. Smith (1950) obtained 138 mutants in barley (5.4%) from 2550 seeds X-rayed at 16000r. In a meiotic examination of those mutants, it was found that 12 out of 76 spikes showed a tetravalent

ring (16%). Moh and Nilan (1956) found 8 mutant barley lines (less than 10%) induced by the same amount of X-radiation which Smith used. In the present study, the chromosomal aberrations (rearrangements) obtained in the pollen mother cells of Montcalm T_1 plants after centrifugation occurred in 1 to 10% of the cells examined, and in 2 to 16% of Hannchen T_1 plants. Visible mutations, for example changes in seed rows or texture of awns after centrifugation, was about 3%. The genetic effect resulting from centrifugation is in linear proportion to the dose used (Fig. 15). But the above results do not permit a clear decision on whether centrifugal force has mutagenic effects similar to those of mustard gas and X-radiation. Different material will offer different results, and even the same mutagen may have different effects in different organisms as already stated, therefore, the same material and method should be used for testing this point. Future research of this kind will be helpful in solving this problem.

A greater frequency of meiotic aberrations was observed in the pollen mother cells of T_1 plants than in those of T_2 plants. In T_2 plants, only fragmentation was found in the pollen mother cells, and its frequency was very low. The number of pollen mother cells showing fragmentation was 0 to 3 in both Montcalm and Hannchen controls, 0 to 8 in Montcalm T_2 plants, and 0 to 15 in Hannchen T_2 plants. But in T_1 plants, there were from 0 to more than thirty pollen mother cells

found to have fragmentation in Both Montcalm and Hannchen. The degree of lethality produced by chromosomal aberrations in T_1 plants is very high. This may cause the death of the gametes before fertilization, or the zygote may be formed only to die in the early stages of development. For a self-pollinating plant with a high frequency of abnormal chromosomes, both male and the female gametes have an equal chance of receiving them. Hence the probability of any recessive lethal becoming homozygous is great. Barley is a such self-pollinating plant, therefore it is not surprising that the sterility of the seeds was high in T_1 plants. On the other hand, if a self-pollinating plant has a low frequency of chromosomal aberrations, both the male and female gametes produced will have a good chance of being normal and, as a result, a normal zygote will be produced. Following the same line of reasoning, one can explain the occurrence of chromosomal aberrations in pollen mother cells of the hybrids. The hybrid of this experiment is produced by a cross of normal female plant (where the ovum is normal), with a treated male parent plant. If the male gamete carries some slightly damaged chromosomes, a zygote could be produced and some chromosomal aberrations could also be observed in the pollen mother cells of the hybrids. In this case the degree of aberration and its frequency, in comparison with that found in T_1 plants, will be low, and this was exactly what was observed in this experiment.

Hybrids were also made for the purpose of determining the effect of centrifugal force on the specific gene loci. One plant derived from the cross of M x H-RCF had six-seed rows and rough awns. This can be explained either by gene mutation from two-rowed to six-rowed, or by pseudo-dominance based on deletion of this locus from the treated male parent. Another plant derived from the same cross had two rows plus an incomplete third row consisting of only three seeds. This might also have been a mutant. Two plants derived from the cross of M x H-P-RCF were smooth-awned and two-rowed. Again, it is possible that either the rough-awn locus was mutated in the treated male parent, or that it had been deleted, thus permitting pseudo-dominance. A plant derived from the same cross of M x H-P-RCF had six-seed rows and smooth awns. Thus it could be inferred that both gene loci (two-seed rows and rough awns) were either mutated or deleted in the treated male parent.

The late maturing plants with thicker straw obtained in this experiment might also be mutants due to the effect of centrifugal force. One plant derived from a cross of H x M-RCF was taller than the control of H x M by about $1 \frac{3}{4}$ ft. that is, the taller plant of H x M-RCF was about $3 \frac{3}{4}$ ft. in height. Under the environmental conditions in the greenhouse, on the average, the height of H x M controls was about 2 ft. The multiple gene loci for plant height had perhaps mutated, and thus a taller plant was produced.

In conclusion, through the evidence derived from this research, it is possible to state that centrifugal force can be considered as an agent useful in the production of mutation, both chromosomal aberrations and phenotypic changes. It also seems to be a useful new approach, in addition to X-radiation and mustard gas, for breeding work as a new source for production of genetic variation. But the method of centrifuging at 25000g for 6 hours used in this experiment may not be useful for other organisms. The degree of force as well as the duration of centrifuging to be used will depend upon the type of organism to be tested. The same force may have different effects in different organisms.

Finally, one must discuss the implications of the present research for space travel. Although centrifugal force, as observed in this research, does produce both chromosomal aberrations and phenotypic changes, the forces used here were very great, up to 25000g. Artificial satellites, as now used, involve very much smaller forces. They encircle the world at a speed of about 18000 miles per hour, but, because of their great orbital radius, the centrifugal force produced is inconsequential, only a fraction of one g. Much more critical is the blast off force. This averages about 7 to 10g, with a maximum on the order of twice these figures. These are still inconsequential forces by comparison to those used in the present research. What the magnitude of forces may be if interplanetary travel ever becomes a reality is unpredictable. At present,

however, it appears that the dangers of space travel will derived from other causes than mutagenic effects of the blast off forces.

SUMMARY

Effects of centrifugal force and phenol were studied on two barley varieties: Montcalm and Hannchen.

Various centrifugal forces; 2000g, 5000g, 10000g, 20000g and 25000g were used. 0.06% phenol was also used.

The percentage of seed germination was markedly influenced by centrifugal force, decreasing as the intensity of the force and the duration of centrifuging were increased.

A cytological study of root tips showed that centrifugal force might shift the nuclei to the centrifugal ends of the cells, elongate the nuclei, break the chromosomes into fragments, induce tetraploid cells. Numerous chromosomal aberrations were found to occur in the pollen mother cells of the plants centrifuged, such as fragmentation, fibrillation, inversion, translocation, deficiency, tetraploidy and aneuploidy. But the rate of production of chromosomal aberrations is linearly proportional to the force applied. T_1 plants were produced by the seeds which had been either centrifuged at 25000g for 6 hours or treated with 0.06% phenol for 24 hours, or had received the combined treatments -- either one of above two treatments used first. Again, an examination of these T_1 plants at the meiotic stages showed that centrifugal force was more effective than phenol in inducing chromosomal aberrations.

Hybrids were made between the two varieties. One of the untreated varieties was used as the female parent, and the

various treated plants (T₁ plants) used as the male parents. A few hybrid plants showed that the specific gene loci of two-seed row as well as rough awn were either mutated to six-seed row and smooth awn respectively, or deleted. A few plants of M-RCF showed late maturity with thicker stem; they were possible mutants too.

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