The Action of Colchicine on the

Induction of Polyploidy in Cereals

A Thesis

Submitted to the
Faculty of Graduate Studies and Research
University of Manitoba

by Didzus Zuzens

In Partial Fulfilment of the Requirements
for the Degree of Master of Science

University of Manitoba

December 1965

Abstract

Colchicine treatment of young barley and F_1 wheat-rye
seedlings was used to elucidate the polyploidizing action
of the drug. A system of response classification of the
treated plants was used to represent the dose applied,
where a product relationship between colchicine concentration
and treatment time was observed. The morphology of survivors
14 days after treatment was related to subsequent shoot
tip ploidy in barley, and to seed set in the otherwise
sterile wheat-rye hybrid. All these effects occurred on a
probability distribution basis within any one sample, and
were related to the dose applied. It was found that plant
morphology after treatment may be used as a criterion of
polyploid induction.

Contents

List of Tables

List of Plates and Figures

Acknowledgements

Introduction

The alkaloid colchicine has been widely used as a
polyploidizing agent for plants since the discovery of
its action by Blakeslee and Avery in 1937 (3).

Various techniques and media have been employed to
secure the drug's contact with meristematic tissue. The
results of most methods have generally been variable,
especially with cereals. While polyploids have frequently
been produced, the degree of success has usually been limited.

Underexposure often fails to produce permanent polyploidy,
while overexposure can quickly result in mortality. The use
of near-lethal doses often ensures the production of
polyploid progeny if large populations are treated and many
individuals are sacrificed. This is a procedure which is not
efficient for smaller populations of intergeneric hybrids.

This study was initiated to obtain information which
would aid in elucidating the action of colchicine on cereals
and thereby attempt to improve the efficiency of polyploid
induction. The approach taken was to study the mode of
action of the dosage components (concentration and time)
of colchicine treatment as they influence the morphological
and cytological responses of cereals, and to investigate
the apparent need to use near-lethal dosage to produce
polyploidy.

Literature Review

In cereals, the use of colchicine as a polyploidizing
agent was first reported in 1939. Muntzing and Runquist
(9) submerged dormant wheat and barley seeds into 0.05% to
0.025% aqueous colchicine for three to six days. No poly-
ploids were produced, and the workers concluded that colchicine
affected tissue in seedlings was replaced by normal tissue
at maturity.

In the same year, Dorsey (7) removed the hulls from
barley and oat seeds, germinated them until the shoots were
0.125 to 0.25 inches long, and immersed the seedlings in
0.25% aqueous colchicine for 20 to 30 minutes. A few polyploids
were produced.

In 1941 Sears (11) used two methods of colchicine treatment
to obtain 18 amphidiploids from 10 species of the Triticinae
with chromosome numbers of 2n=14. One method involved treatment
of very young seedlings. Aqueous concentrations of 0.02%, 0.05%
and 0.10% colchicine for 24 and 48 hours resulted in heavy
mortality and very few polyploids. However, survival improved
when 0.5%, 1.0% and 2.0% by weight of colchicine in lanolin
was applied to the coleoptile bases of young seedlings. After
24 hours the seedlings were transferred to soil without removal
of the colchicine-lanolin mixture. With this mixture the
highest colchicine concentration produced up to 50% polyploidy.
Sears states that the lanolin treatment did not affect growth
of the seedlings as did the aqueous treatment. Effects of the
aqueous method were not described.

The second method reported by Sears (11) involved treat-
ment of potted plants by spreading and packing the tillers
with absorbent cotton saturated with 0.5% aqueous colchicine.
Saturation was repeated twice daily for two to five days. High
humidity during the treatment period was essential for success
with this method. Mixtures of up to 5.0% colchicine in lanolin
were not as effective as saturation with aqueous colchicine.
With the potted plant method survival was high, and up to
two-thirds of the survivors produced polyploid sectors. However,
the polyploid sectors were smaller than with seedling treatment.

In 1944 Armstrong and McLellan (1) employed various
methods, each with different colchicine concentrations and
treatment times, in an effort to double a 4,200 F_1 population
of various _Triticum_ x _Agropyron glaucum_ crosses. Included were
treatments of dry seeds, germinated seeds, plumule immersion,
and treatment of germinated seeds in a partial vacuum.
Concentrations of 0.10% to 0.80% colchicine were employed,
and treatment times varied from 15 minutes to 45 hours.
Success in polyploid induction ranged from 0.0 to 33.3%. The
wheats used as seed parents affected the ease of doubling.
Crosses with tetraploid types were usually more readily
doubled than those utilizing hexaploids. The authors concluded
that this involved "a question of the relative competing
ability of doubled and undoubled cells in the two amphiploid
types."

In 1946 Levan (8) reported a study of thresholds (the
lowest concentration at which colchicine effects are noticed
cytologically) in cereal root tips. He found that the threshold
is lower with increasing time of exposure to colchicine. He
also recognized that growth rates of 2n, 4n and 8n tissues
may differ.

In 1947 Wellensiek (14) reported attempts to obtain
fertile wheat-rye hybrids. He cloned winter types and immersed
the clones in 0.05% aqueous colchicine during four consecutive
days, substituting pure water during the nights. Some polyploids
were obtained.

In 1950 Bell (2) reported an extensive experiment on
colchicine methodology with interspecific and intergeneric
crosses involving Triticum. He was successful with tiller
capping (0.10% to 0.30% colchicine for 72 hours); injection
(three times with 0.20% colchicine at 24 hour intervals);
and root immersion (0.05% colchicine for eight hours per
day for four days). Poor results were obtained by applying
cotton wool soaked with 0.05% colchicine to the base of
tillers for five days.

In 1952 Bremer-Reinders and Bremer (4) reported the
treatment of young rye and barley seedlings with shallow
layers of aqueous colchicine solutions in petri dishes.
They found that 0.20% for two hours and 0.10% for three
hours gave approximately equal results, but stated that
it was difficult to give a universally applicable optimum
colchicine treatment. They observed coleoptile thickening,
and obtained 4.2% to 6.5% survivors, and some polyploids.

In 1959 Reinbergs and Shebeski (10) reported an optimum
method for treating germinating barley seedlings in a shallow
layer of 0.10% aqueous colchicine for three hours, which
produced up to 10% tetraploids.

In 1960 Smith (12) reported up to 20% doubling in barley
by treating germinating seedlings in a shallow layer of 0.10%
aqueous colchicine for two hours.

Nearly all previous workers who have reported the use
of colchicine on cereals were concerned with a direct
application of the method to their particular materials, and
few specifically studied the mode of action of colchicine
on both cereal morphology and cytology in relation to the
efficiency of polyploid induction.

From these reports it was also concluded that no treatment
is consistently efficient, and that the percentage of success
in doubling is usually low. Results may vary greatly within a
species, and even within an apparently homogeneous population.
The term "ease of doubling" was encountered, and may indicate
inherent genotypic differences with regard to the response
to colchicine.

Materials and Methods

The objectives of this investigation were to study the
components of colchicine treatment (concentration and time
of exposure) as they affect the morphological and cytological
responses of cereals, and to relate one or both of these
responses to the production of doubled progeny.

Two materials were used during this study: Hordeum
vulgare variety 'Parkland' (2n=14); and the F_1 hybrid of
Triticum aestivum variety 'Chinese Spring' (2n=42) x Secale
cereale variety 'Prolific' (2n=14).

Barley was used for cytological studies due to its low
chromosome number, while the Chinese x Prolific hybrid was
used in the 'production of doubled progeny' study, since the
F_1 of that cross is normally completely sterile, and the
presence of octoploid seeds on treated plants indicates
colchicine induced doubling. Both materials were used to
study treatment components and morphological effects of
colchicine.

Although the stage of development prior to colchicine
treatment, as well as the actual treatment techniques, were
identical for both types of materials, their mode of prepar-
ation differed somewhat.

Untreated barley seeds from a bulk sample were germinated
in vermiculite in shallow metal trays under greenhouse
conditions for approximately three days, or until the most
actively germinating seeds had developed at least three roots,
and the coleoptiles were approximately equal to the length
of the seed. Such seedlings were then separated from the
vermiculite and from those of lesser development by water
floatation.

Most of the vermiculite particles were of a density less than that of water, while the germinated seedlings were found to be only slightly denser than water, and could be suspended with only slight agitation; and hence separated from the ungerminated seeds, which were distinctly denser than water and settled readily.

Seeds of Chinese x Prolific were dusted with the organic seed disinfectant 'Arasan' and placed on moist blotting paper in polyethylene germination boxes under greenhouse conditions. Those seeds which germinated and developed to the stage previously described for barley were removed for treatment. The boxes with the remaining seeds were then stored for seven days at $33^{\circ}F$, then for 24 hours at $80^{\circ}F$, followed by the removal of all seedlings possessing the desired degree of development. Three such cycles resulted in the removal of approximately one half of the population, and further germination was negligible.

Populations of both the Parkland barley and the Chinese x Prolific hybrid were subjected to a number of different colchicine treatments. These treatments were measured in colchicine units (c.u.) as the product of the concentration of colchicine in parts per million (p.p.m.) by weight in aqueous solution, and the duration of treatment time in hours.

In order to test the principle that plant responses
(morphological, cytological and reproductive) to colchicine
were related only to the product of concentration and time,
each dose was formulated separately with each of four concen-
trations (500, 1,000, 1,500 and 2,000 p.p.m.) by adjusting
the treatment times accordingly. For example, a dose of 6,000
c.u. was formulated as follows: 500 p.p.m. for 12 hours;
1,000 p.p.m. for six hours; 1,500 p.p.m. for four hours; and
2,000 p.p.m. for three hours. Doses of 500, 1,000, 1,500, 2,000,
3,000, 4,000, 6,000 and 12,000 c.u. were administered, thus
requiring treatment times ranging from $\frac{1}{4}$ to 24 hours.

Treatment procedure was identical for all populations
of both materials. Seedlings of any one population were
thoroughly mixed before visual division into approximately
equal samples, so as to no more than cover the bottom of a
standard $4\frac{1}{2}$ inch square germination box. Four such samples
in separate germination boxes were utilized for each dose
applied, to correspond with the four concentrations used under
the system described previously. Sample sizes ranged from
less than 30 to nearly 300.

Colchicine treatment was done in the germination boxes,
which were closed and placed on a level surface for the duration
of the treatment period. The seedlings were randomly distrib-
uted in the box; a volume of 20 ml. of aqueous colchicine was
applied to each treatment sample; and protruding roots were
pressed into the liquid surface, where they remained by
adhesion.

When the treatment time had expired the treated seedlings were washed three times in tap water. They were then immediately transferred for recovery to a soil-vermiculite mixture in shallow metal trays as follows. A $\frac{1}{2}$ inch thick layer of vermiculite at the bottom of a tray was covered with $\frac{1}{4}$ inch of prepared greenhouse soil. After watering to saturation, treated seedlings were placed on the moist soil layer. A thin vermiculite cover followed, and this was sprinkled with water. Watering continued as required.

The seedlings were removed from the trays for observation and classification 10 to 14 days after treatment. For purposes of morphological classification, a primary consideration was the length of the entire plant above the epicotyl, including the coleoptile and first leaf. Further criteria were the length and thickness of the coleoptile, the presence or absence of a second leaf, and the degree of root development. On this basis all plants of each treatment sample were compared and classified.

Representative plants of each morphological class were bulked from the various treatments within any one population, and retained for cytological or fertility studies. These plants were transplanted to soil in greenhouse beds. Retained Chinese x Prolific plants were all grown to maturity and studied for fertility. With barley, all selected plants were collected for cytological study within six weeks after the date of colchicine treatment as follows.

A method of cytologically determining chromosome number in cereal shoot tips was patterned upon a similar technique for root tip analysis, as described in 1960 by Tsunewaki and Jenkins (13).

In this study shoot tips in barley were located just above
the last well-developed node. With practise, it became possible
to remove young leaf and sheath tissues above the node, and
to expose an embryonic leaf, which enveloped further leaf
primordia and the apical meristem. The bottom of the node was
then cut away. Following such dissection and removal of excess
extraneous tissue, thin slices of nodular tissue with attached
apical meristem were pre-treated at 0° to $2^{\circ}C$ for 24 hours,
before fixing in Farmer's solution (3:1 mixture of ethyl
alcohol and acetic acid, respectively) for a minimum of three
days. Then following acid hydrolysis (1N HCl) for seven minutes
at $60^{\circ}C$, the shoot tips were stained in Feulgen and squashed
in acetocarmine.

After staining and before squashing, further dissection
was done with the unaided eye. Distinguishable embryonic
leaves were discarded. Cytological determinations were gen-
erally made on the youngest primordia. No more than 10 nuclei
were examined per shoot tip, but data from a minimum of three
were retained only if the ploidy was identical.

The main purpose of the cytological work was to attempt
to relate shoot tip ploidy with morphological response to
colchicine as determined at an earlier stage of development.
In this case, shoot tip ploidy was determined at three and
six weeks after colchicine treatment, and related to the
morphological response at 10 to 14 days after treatment.

Two populations of Parkland barley and four of Chinese x
Prolific were treated. The dosages applied, and the disposition
of the populations are described below.

Parkland barley -- Population 1, consisting of 2,972
plants, was treated with 1,000, 2,000, 3,000, 4,000, 6,000
and 12,000 c.u.*, classified, and 208 retained survivors
were collected for cytological analysis three weeks after
treatment. Population 2, consisting of 826 plants, was treated
with 1,000, 2,000, 3,000 and 4,000 c.u., classified, and 182
retained survivors were collected for cytological analysis
six weeks after treatment.

Chinese x Prolific -- Population 1, consisting of 491
plants, was treated with 500, 1,000, 2,000 and 3,000 c.u.,
classified, and discarded. Population 2, consisting of 347
plants, was treated with 1,500 c.u., classified, and 136
retained survivors were grown to maturity. Population 3,
consisting of 993 plants, was treated with 2,000 c.u., class-
ified, and 24 retained survivors were grown to maturity.
Population 4, consisting of approximately 150 plants, was
treated with 1,750 c.u., and 10 specifically retained surviv-
ors were grown to maturity.

* Three additional control samples were concurrently
treated with tap water for 24 hours.

Results

Both Parkland barley and Chinese x Prolific seedlings showed a number of morphological responses to colchicine as described below. These responses were similar for the two materials, and were not present in tap water control samples. Not all plants within a sample were affected to the same extent. This applied especially to the milder treatments, where a considerable proportion of plants within the same sample appeared morphologically unaffected.

Thickening and shortening of the coleoptiles was the most noticeable morphological response. Such hypertrophy has been reported by Dermen (6). In this study the condition was often associated with retarded development of the first leaf, or the complete absence of such development in extreme cases. Root tips were similarly thickened, and their development often arrested. Such severely affected plants, either in root or coleoptile characteristics, or both, often failed to survive.

Between this extreme, and that of virtually no visible colchicine effect, a complete range of intermediate types was observed. Intermediates were distinguished by two main characteristics: (1) the length of the first leaf; and (2) the presence and degree of development of a second leaf, which was indicative of the continuing physiological growth and development of the treated plant. Failure of the development of a second leaf was observed to be synonymous with subsequent necrosis of the plant, and eventual death.

As a result of these observations, colchicine treated
plants were classified into three primary categories:
A (normal); B (affected); and C (non-survivors).

Type B was divided into three sub-classes: B1, B2 and B3.
Type B1 closely resembled type A, while type B3 resembled
type C, except for the development of a second leaf, and
subsequent survival. Type B2 was intermediate.

Plate I shows representatives of the characteristic
morphological response types as they occurred in a sample of
colchicine treated Parkland barley 12 days after treatment.

A sixth morphological response type occurred when a
plant with type B3 coleoptile and first leaf morphology
failed to show second leaf development, and was therefore
classified as a type C. When transferred to soil in the
greenhouse for a period of two or even three weeks, such
plants sometimes recovered, and developed a second leaf.
These individuals, which then resembled type B3, were given
the designation of type B3X.

Plate I

Characteristic representatives of the morphological
response types of Parkland barley seedlings 12 days after
treatment with aqueous colchicine.

Shown left to right: type A, equivalent to a tap water
control; type B1; type B2; type B3; and type C. The stage
of development immediately before treatment is represented
by the germinated seedling at the upper-right.

As described in the section under Materials and Methods, Parkland barley and Chinese x Prolific seedlings were treated with various colchicine doses (in c.u.), each dose being formulated with four different colchicine concentrations, and with one treatment sample per concentration.

Using the morphological criteria described previously, treated seedlings from each sample were classified into the following categories: (1) type A; (2) types B1 and B2; (3) type B3; and (4) type C. From the total number of plants in each sample, the percentage of plants in each of the three primary groups, consisting of general types A, B and C was calculated. These percentages were then arranged in the order A--B--C to form a response formula for each treated sample. The above data for the colchicine treated Parkland barley populations are shown in Table 1 and Table 2.

Both Table 1 and Table 2 data indicate that all three primary response types (A, B and C) often occur in the same sample. This fact is recorded in the response formulae, which also indicate the general consistency of response within any dose group which has been formulated with four different concentrations.

With increasing severity of colchicine dose, a brief predominance of type A changes rapidly to a predominance of type C. The frequency of type A soon falls to zero; type B demonstrates a brief maximum before its frequency tapers off; and type C eventually constitutes close to 100% of the population.

Table 1

The response classification of Parkland barley seedlings
of population 1 treated with 1,000 to 12,000 c.u.

Response types

Conc.,	Time,	Number of plants					Response formula
p.p.m.	hrs.	A	B1 & B2	B3	C	Total	Types A--B--C
Dose product -- 1,000 c.u.							
500	2.00	105	13	0	15	133	78.9--9.8--11.3
1,000	1.00	94	33	3	6	136	69.1--26.5--4.4
1,500	0.67	71	29	8	12	120	59.1--30.9--10.0
2,000	0.50	105	14	3	6	128	82.1--13.2--4.7
2,000 c.u.							
500	4.00	17	18	16	55	106	16.0--32.1--51.9
1,000	2.00	24	30	13	58	125	19.2--34.4--46.4
1,500	1.33	16	28	12	87	143	11.2--28.0--60.8
2,000	1.00	19	26	12	61	118	16.1--32.2--51.7
3,000 c.u.							
500	6.00	2	11	8	105	125	1.6--15.1--83.3
1,000	3.00	3	19	10	103	134	2.2--21.7--76.1
1,500	2.00	4	14	14	97	129	3.1--21.8--75.1
2,000	1.50	4	24	10	73	111	3.6--30.6--65.8
4,000 c.u.							
500	8.00	0	26	17	84	127	0--33.9--66.1
1,000	4.00	0	3	2	104	109	0--4.6--95.4
1,500	2.67	1	10	9	100	120	0.80--15.8--83.4
2,000	2.00	1	8	7	100	116	0.90--12.9--86.2
6,000 c.u.							
500	12.00	0	5	9	122	136	0--10.3--89.7
1,000	6.00	0	1	5	127	133	0--4.6--95.4
1,500	4.00	0	2	5	103	110	0--6.3--93.7
2,000	3.00	0	4	4	129	137	0--5.8--94.2
12,000 c.u.							
500	24.00	0	0	0	132	132	0--0--100
1,000	12.00	0	0	0	104	104	0--0--100
1,500	8.00	0	1	2	120	123	0--2.4--97.6
2,000	6.00	0	0	0	117	117	0--0--100

Table 2

The response classification of Parkland barley seedlings of population 2 treated with 1,000 to 4,000 c.u.

Conc., p.p.m.	Time, hrs.	Number of plants					Response formula Types A--B--C
		A	B1 & B2	B3	C	Total	
			1,000 c.u.				
500	2.00	21	15	5	20	61	34.4--32.8--32.8
1,000	1.00	8	10	0	22	40	20.0--25.0--55.0
1,500	0.67	16	7	2	18	43	37.2--20.9--41.9
2,000	0.50	15	9	2	24	50	30.0--22.0--48.0
			2,000 c.u.				
500	4.00	5	13	5	29	52	9.6--34.6--55.8
1,000	2.00	10	32	9	16	67	14.9--61.2--23.9
1,500	1.33	2	13	6	33	54	3.7--35.2--61.1
2,000	1.00	7	12	7	27	53	13.2--35.8--51.0
			3,000 c.u.				
500	6.00	5	23	4	24	56	8.9--48.2--42.9
1,000	3.00	2	14	11	27	54	3.7--46.3--50.0
1,500	2.00	1	8	3	43	55	1.8--20.0--78.2
2,000	1.50	4	15	6	22	47	8.5--44.7--46.8
			4,000 c.u.				
500	8.00	4	11	10	23	48	8.3--43.8--47.9
1,000	4.00	3	4	6	34	47	6.4--21.3--72.3
1,500	2.67	2	2	11	31	46	4.3--28.2--67.5
2,000	2.00	3	9	4	37	53	5.7--24.5--69.8

Cytological data for the various response types at
three and six weeks following colchicine treatment are
shown in Table 3. Shoot tip countability ranged from over
80% for type A to less than 40% for type C. Countability
generally was slightly lower at three weeks than at six weeks
following colchicine treatment. Shoot tip analysis indicated
that polyploidy had been induced, sometimes to rather high
levels; that a considerable proportion of the polyploidy
existed in chimeral form; and that some aneuploidy was also
present.

Specifically, at three weeks following colchicine
treatment, plants of original morphological response type A
possessed mainly diploidshoot tip nuclei, and no plants were
purely tetraploid. Type B1 resembled type A. Type B3 however,
had no purely diploid shoot tips, but some were entirely
tetraploid. Type C had no shoot tips with diploid nuclei,
and both types B3 and C possessed nuclei with very high
polyploidy (8n and 16n).

This wide range of chimeral polyploidy was reduced at
six weeks following colchicine treatment. Diploid nuclei
predominated for morphological response types A, B1 and B2.
Pure tetraploids predominated for type B3. However, diploid-
tetraploid chimeras were still present, except for type A.
Higher polyploids were entirely absent.

Table 3

Shoot tip ploidy of Parkland barley response types at three and six weeks after colchicine treatment.

Type of ploidy	Response types, number of plants							
	3 weeks				6 weeks			
	A	B1	B3	C	A	B1	B2	B3
1n*, 2n	0	0	0	0	5	0	0	0
2n	21	11	0	0	32	48	15	4
2n, 4n	15	6	17	0	0	3	11	7
4n	0	2	13	3	0	0	3	19
4n, 8n	0	0	4	9	0	0	0	0
8n	0	0	2	5	0	0	0	0
16n	0	0	1	2	0	0	0	0
2n,4n,8n	3	2	0	0	0	0	0	0
1n*,2n,4n	5	2	0	0	0	0	0	0
2n,3n*,4n	2	0	1	0	0	0	0	0
Totals	46	23	38	19	37	51	29	30
Not countable	9	14	27	32	5	14	7	9

* Sometimes aneuploid, with modal chromosome number as indicated.

Simple doubling was of paramount interest during this investigation. Therefore the major shoot tip ploidy changes affecting the induction of tetraploidy from three to six weeks after colchicine treatment were calculated for the two populations as percentages of the total in each morphological response type sample. These data are shown in Table 4.

Table 4 data indicate that the frequency of shoot tips with only diploid nuclei increases for all the response types A, B1 and B3 between three and six weeks after colchicine treatment. The frequency of diploid-tetraploid chimeras decreases for all three types. Pure tetraploids are important mainly for type B3, where their frequency increases from one third of the total at three weeks, to almost two thirds at six weeks. With this increase there appeared to be a proportional decline of 2n-4n chimeras, and a disappearance of 4n-8n chimeras, for the B3 category.

Fertility studies of colchicine treated survivors were carried out with Chinese x Prolific grown to maturity. As indicated earlier, these originated from the treatment of a number of populations in a manner identical to that described for Parkland barley. Initial morphological classification of the treated Chinese x Prolific was also carried out as described for barley. These results were recorded, and are shown in Table 5.

The behavior of Chinese x Prolific under colchicine treatment was similar to that of Parkland barley, as indicated by the response formulae. The major difference was that Chinese x Prolific hybrids appeared to be somewhat more sensitive to colchicine, as type A disappeared and type C began to predominate at a lower dose level than was the case for barley.

Table 4

Major shoot tip ploidy changes affecting the frequency
of tetraploids in Parkland barley response types between
three and six weeks following colchicine treatment.

Type of ploidy	Survivor response type	Per cent of total, 3 weeks	sample size	Per cent of total, 6 weeks	sample size
	A	45.7	21	86.5	32
2n	B1	47.8	11	94.1	48
	B3	0	--	13.3	4
	A	32.6	15	0	--
2n, 4n	B1	26.1	6	5.9	3
	B3	44.8	17	23.3	7
	A	0	--	--	--
4n	B1	8.7	2	0	--
	B3	34.2	13	63.4	19
	A	0	--	--	--
4n, 8n	B1	0	--	--	--
	B3	10.5	4	0	--

Table 5

The response classification of Chinese x Prolific seedlings treated with colchicine, from populations 1, 2 and 3.

		Response types					
		Number of plants					Response formula
Conc., p.p.m.	Time, hrs.	A	B1 & B2	B3	C	Total	Types A--B--C
Population 1 -- 500 c.u.							
500	1.00	15	6	1	3	25	60.0--28.0--12.0
1,000	0.50	18	8	2	7	35	51.4--28.6--20.0
1,500	0.33	14	12	2	4	32	43.8--43.7--12.5
2,000	0.25	13	12	6	10	41	31.7--43.9--24.4
1,000 c.u.							
500	2.00	5	7	6	7	25	20.0--52.0--28.0
1,000	1.00	6	13	2	5	26	23.1--57.7--19.2
1,500	0.67	11	15	4	8	38	29.0--50.0--21.0
2,000	0.50	5	7	2	4	18	27.8--50.0--22.2
2,000 c.u.							
500	4.00	2	11	7	18	38	5.3--47.3--47.4
1,000	2.00	1	7	7	12	27	3.7--51.8--44.5
1,500	1.33	1	12	5	10	28	3.6--60.8--35.6
2,000	1.00	1	5	4	22	32	3.1--28.1--68.8
3,000 c.u.							
500	6.00	0	4	3	27	34	0--20.6--79.4
1,000	3.00	0	5	3	30	38	0--21.1--78.9
1,500	2.00	0	3	2	26	31	0--16.2--83.8
2,000	1.50	0	0	2	21	23	0--8.7--91.3
Population 2 -- 1,500 c.u.							
500	3.00	9	36	33	4	82	11.0--84.2--4.8
1,000	1.50	20	36	35	5	96	20.8--74.0--5.2
1,500	1.00	17	35	32	6	90	18.9--74.4--6.7
2,000	0.75	17	31	27	4	79	21.5--73.4--5.1
Population 3 -- 2,000 c.u.							
500	4.00	1	20	42	171	234	0.4--26.5--73.1
1,000	2.00	0	8	22	225	255	0--11.8--88.2
1,500	1.33	0	3	10	263	276	0--4.8--95.2
2,000	1.00	0	11	17	200	228	0--12.3--87.7

As outlined in the section under Materials and Methods, no plants of Chinese x Prolific were retained from population 1. Representative survivors from both populations 2 and 3 were grown to maturity in the greenhouse. Only a small number of type B3X plants (described previously at the beginning of this section) were retained from population 4. All survivors were studied for seed set, and the results are shown in Table 6.

A large number of plants were fertile. A total of 28 plants from four families were subjected to cytological root tip analysis. All were octoploids. It was concluded that fertility represented colchicine induced doubling, since in this experiment all untreated control plants were sterile.

Only one type A individual was fertile. Most type B categories had some fertile plants. This fertility ranged from one to 37 seeds per spike. Except for the type B3X plants of population 3, each plant was represented by one spike. There was a steady, though slight increase in the percentage of fertile plants through types B2, to B3, to B3X.

Table 6

Fertility of retained Chinese x Prolific colchicine treated survivors from populations 2, 3 and 4.

Response type	Survivors in sample	Fertile plants	% plants fertile	Seeds per plant	Total seeds
		Population 2			
A	22	1	4.5	21*	21
B1	22	0	--	--	--
B2	47	3	6.4	1,2,5	8
B3	45	5	11.1	1,4,6,7,32*	50
		Population 3			
B2	9	2	22.2	7,7	14
B3	12	3	25.0	4,9,37*	50
B3X	3	1	33.3	7	7
		Population 4			
B3X	10	4	40.0	2,4,13,23*	42

* Root tips of seven plants from each of these families were checked cytologically to confirm a modal chromosome number of 56.

Discussion and Conclusions

It is apparent that colchicine treatment of cereal
seedlings may result in a variety of morphologically
distinct response types, the detailed classification and
description of which has hitherto not been available.

The occurrence of more than one, or all of these
morphological types within a single, even relatively small
sample of treated plants is noteworthy, but not surprising,
since this mixture of types merely indicates that each type
has a distinct probability of occurrence after a colchicine
dose of any given severity.

The reasons for the occurrence of mixtures of response
types remain obscure, but may include the effects of residual
dormancy and growth rate factors. (Random orientation of the
seedlings during treatment is not a factor, since samples of
uniform geotropic orientation invariably produce mixtures,
at least with the materials used in this study.) In any event,
this aspect is of little practical importance where sufficiently
large populations are available for treatment, since with
increasing population most or all of the response types
would be expected to be present.

It is significant that the severity of a colchicine dose
may be indicated by a response formula based on the system
of morphological classification. On this basis it would
appear that doses of equal severity, or of any required
severity, may be formulated at will by adjusting any combin-
ation of colchicine concentration and treatment time in the
product relationship of colchicine units (c.u.), at least
within the ranges utilized during the course of this study.

Several minor aspects deserve clarification. One is that a deviation from optimum environmental conditions during the recovery period of colchicine treated seedlings may contribute to inconsistencies between populations with respect to response from the same colchicine dose, as may be observed when comparing, for example, the data of Tables 1 and 2. Favourable conditions will tend to reduce excess mortality above that attributable to the effects of colchicine. However, this condition does not account for differences between populations with respect to the general severity of a treatment, as measured by the persistence of apparently unaffected plants. Fortunately this aspect has relatively little practical significance.

Another deviation involves the apparent cytological observation of nuclei with abnormal chromosome numbers. These may in part be due to the squash technique; but other workers (10) have also reported aneuploidy in barley after treatment with colchicine. The actions of multipolar spindles may also be involved.

Finally, minor variations in frequency of certain types of ploidy between three and six weeks after colchicine treatment most probably were due to sampling differences in the two separate populations, and the possible failure of very high and other unusual types of ploidy, such as aneuploids, to survive, especially in competition with normal diploid tissue for six weeks after treatment.

There appears to be a definite relationship between the morphological response to colchicine as observed 10 to 14 days following treatment, and the subsequent shoot tip ploidy (in Parkland barley), and the fertility of an otherwise sterile hybrid (Chinese x Prolific).

It is this relationship which constitutes the major
practical applications of the findings of this investigation,
since it should facilitate the selection and retention of
the most polyploidogenous (capable of producing polyploids)
survivors from a population of treated plants. The relation-
ship appears to be somewhat less critical for species which
are readily doubled, such as the Chinese x Prolific hybrid
in this study, where all response types were represented by
at least one fertile individual, and the general level of
fertility was fairly high. In other cases, such as that of
Parkland barley, there exists a more precise relationship
between early morphology and subsequent shoot tip ploidy,
which may be projected to represent expected progeny to a
substantial extent. Here only the most severely affected
survivors are at all polyploidogenous, and remain so to a
significant extent.

One cytological aspect which differs in this group from
that of less severely affected plants, is the behaviour of
diploid-tetraploid chimeras in shoot tips. This aspect bears
re-emphasis. In this study it is highly significant that an
increase in the category of pure tetraploids occurs in only
the most severely affected surviving group: type B3. This is
consistent with the observation that for other groups virtually
all stabilization of 2n-4n chimeras is towards diploidy,
whereas a considerable proportion of such stabilization in
type B3, and only for this type, is towards tetraploidy. This
is also true for the 4n-8n chimeras, which occur mainly in
type B3 meristems. All of the latter which survive, stabilize
at the tetraploid level, thereby also increasing the propor-
tion of doubled plants. Other workers (5) have reported diploid
progeny from colchicine treated barley plants producing
tetraploid pollen mother cells.

It can be concluded therefore that morphological response type B3 is always the most polyploidogenous. Hence it is of interest to examine the frequency of its occurrence with increasing colchicine dose. One set of treatment means for each of Parkland barley (from Table 3) and Chinese x Prolific (population 1, Table 5) is presented in Table 7. The barley data are represented graphically in Figure 1.

These data indicate that with increasing colchicine dose, the frequency of type B3 follows approximately that of general type B, with the peak for type B3 occurring slightly after that of general type B. However, the percentage of type B3 within the general type B category increases steadily until it becomes conceivable that at extremely high doses, virtually all survivors should be of type B3 or type B3X. Naturally, the frequency of such survivors would then be quite low, and probably far too low for efficient polyploid induction. Such a situation would then be far from an optimum one.

Table 7

Means of response classification data for one lot of
each of Parkland barley and Chinese x Prolific, indicating
the behaviour of the most polyploidogenous type with increasing
colchicine dose.

Dose product, c.u.	Total plants	Mean % of totals for response types			% type B3	% type B3 within type B
		A	B	C		
Parkland barley -- Table 1 data						
1,000	517	72.6	19.9	7.5	2.7	13.6
2,000	492	15.4	31.5	53.1	10.8	34.3
3,000	500	2.6	22.0	75.4	8.4	38.2
4,000	472	0.4	17.4	82.2	7.4	42.5
6,000	516	0.0	6.8	93.2	4.5	66.2
12,000	476	0.0	0.6	99.4	0.4	66.7
Chinese x Prolific -- Table 5 data, population 1						
500	133	45.1	36.9	18.0	8.3	22.5
1,000	107	25.2	52.4	22.4	13.1	25.0
2,000	125	4.0	46.4	49.6	18.4	39.7
3,000	126	0.0	17.4	82.6	7.9	45.4

Figure 1

The graphical representation of Table 7 data for Parkland barley, showing changes in the frequencies of the three major morphological response types with increasing colchicine dose. The frequency of type B3 is indicated separately by a broken line.

c.u. (thousands)
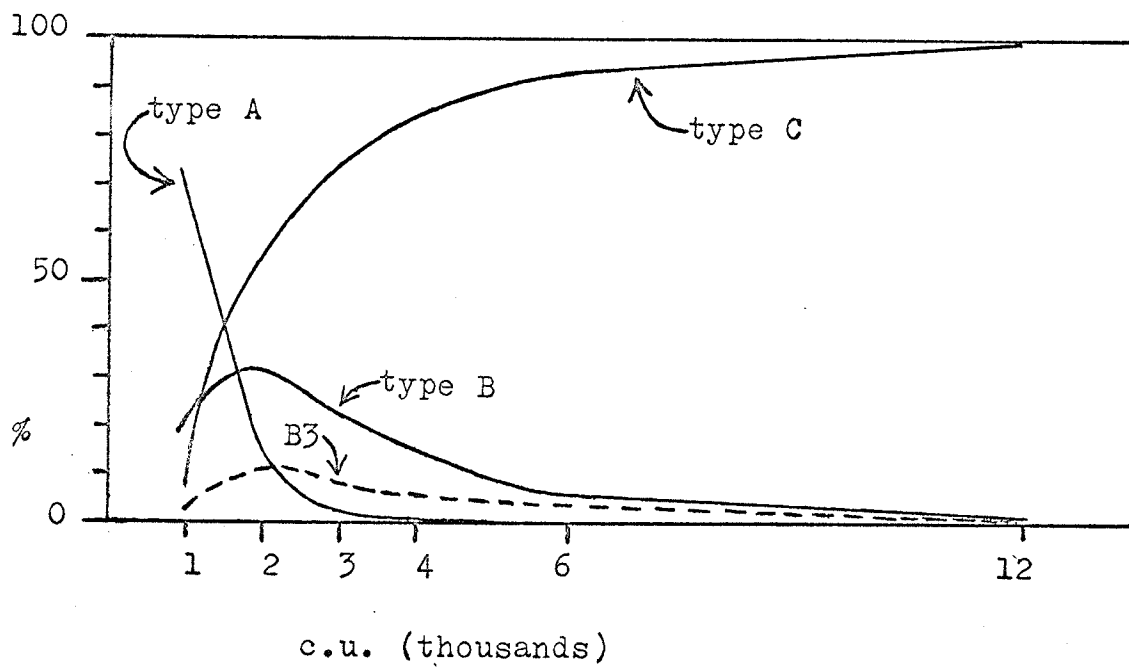
Summary

The mode of action of colchicine on polyploid induction, as uncovered by this investigation, may be summarized as follows.

The dose of colchicine is represented by plant response, and is indicated through the relative frequency of occurrence of resulting distinct morphological response types.

Doses can be formulated as required, by adjusting certain concentrations and times in a product relationship.

These requirements of dose are such that a maximum of polyploidogenous types are obtained after treatment. This maximum usually coincides with the point where virtually all of the population demonstrates symptoms of being affected through contact with colchicine.

The most polyploidogenous types can be recognized morphologically by their extreme hypertrophy, which borders on lethality, but is always accompanied by the development of a second leaf and new roots, this being indicative of continued growth, and hence survival.

The retention of such survivors should always increase the probability that polyploid progeny will be obtained.

By having verified the foregoing, this investigation
could serve to enhance the efficiency of the retention of
the most polyploidogenous survivors following colchicine
treatment, as well as clarify their mode of production. In
addition, an extension of these methods towards greater
precision is also possible, especially from the standpoint
of increasing the percentage of polyploidogenous plants in
any treated sample. This could be accomplished by treating
at an earlier stage of seedling development; by employing
lower concentrations and longer treatment times; and by using
coleoptile morphology as the sole visual criterion of colchi-
cine effect on the apical meristem. Essentially this would
involve a shift in emphasis from a sample of plants, to
seedlings as individuals.

To a limited extent, this approach has already been found
to be feasible. By applying small agar blocks exposed to
aqueous colchicine to the bases of coleoptiles of germinated
seeds, the characteristic morphological changes induced by
colchicine can be observed in the coleoptiles. Using only
coleoptile morphology as the visual criterion of colchicine
effect on the apical meristem, the precise concentration of
colchicine in the agar can be ignored, since treatment time
and possibly mass of the agar block would then determine the
dose applied. Treatment for any individual plant can be
stopped by removing the agar when the required coleoptile
morphology is produced.

References

1. Armstrong, J.M., and McLellan, H.A. 1944. Amphidiploidy in Triticum-Agropyron hybrids. Sci. Agr. 24: 285-298.

2. Bell, G.D.H. 1950. Investigations in the Triticinae. I. Colchicine techniques for chromosome doubling in interspecific and intergeneric hybridizations. J. Agr. Sci. 40: 9-18.

3. Blakeslee, A.F., and Avery, A.G. 1937. Methods of inducing chromosome doubling in plants by treatment with colchicine. Science 86: 408.

4. Bremer-Reinders, D.E., and Bremer, G. 1952. Methods used for producing polyploid agricultural plants. Euphytica 1: 87-94.

5. Chen, S., Shen, S., and Tang, P.S. 1945. Studies on colchicine-induced autotetraploid barley. I and II. Cytological and morphological observations. Am. J. Bot. 32: 103-106.

6. Dermen, H. 1940. Colchicine polyploidy and technique. Bot. Rev. 6: 599-635.

7. Dorsey, E. 1939. Chromosome doubling in cereals. J. Hered. 30: 393-395.

8. Levan, A. 1946. The thresholds of colchicine action in barley, rye, diploid oat and their artificial tetraploids. Hereditas 32: 294-295.

9. Muntzing, A., and Runquist, E. 1939. Note on some colchicine induced polyploids. Hereditas 25: 491-495.

10. Reinbergs, E., and Shebeski, L.H. 1959. Fertility of barley autotetraploids. I. Fertility in successive generations of four autotetraploid barley varieties and the effect of selection for fertility in the O.A.C. 21 autotetraploid. Can. J. Plant Sci. 39: 98-107.

11. Sears, E.R. 1941. Amphidiploids in the seven-chromosome Triticinae. University of Missouri Agricultural Experiment Station Research Bulletin 336.

12. Smith, W.E. 1960. Fertility of autotetraploid varieties and hybrids of barley. Can. J. Plant Sci. 40: 434-442.

13. Tsunewaki, K., and Jenkins, B.C. 1960. A comparative study of various methods of root tip preparation in screening wheat aneuploids. Cytologia 25: 373-380.

14. Wellensiek, S.J. 1947. Methods of producing triticales. J. Hered. 38: 167-173.