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EFFECT OF COLCHICINE ON CHROMOSOME NUMBER AND CELL SIZE IN SOME HORTICULTURAL PLANTS[1]

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Colchicine is a poisonous, narcotic alkaloid, found in the corms of the autumn crocus *(Colchicum autumnale),* and is related chemically to morphine and codeine. Nebel and Ruttle[2] have shown that it inhibits spindle formation in cell division, and in this way prevents divided chromosomes from separating to form two new nuclei. If the cell survives, the doubled number of chromosomes remains within the one cell, forming a large nucleus. Subsequent cell divisions are normal, and give rise to tissue with double the normal number of chromosomes. Blakeslee and Avery[3] as well as Nebel and Ruttle, and others, have obtained plants or parts of plants with twice the normal number of chromosomes by treating them with colchicine.

Since plants with a doubled chromosome number have larger cells than the normal, the whole plant is usually larger, exceptions occurring when the rate of cell division is much reduced.

Work on the use of colchicine to induce polyploidy was undertaken at the University of Minnesota in February. A number of different horticultural plants were included in the study because the work of others had suggested that different species reacted somewhat differently to colchicine.

In most of the species included in this study too little growth had taken place since treatment for final determinations of chromosome number and cell size, but a report on the methods used and preliminary results obtained are given.

Since asparagus is perennial and can be asexually propagated, it is exceptionally favorable material in which to study the relationship of an autotetraploid to the diploid from which it was obtained. As asparagus also is dioecious, the relationship of chromosome doubling to sex can be investigated. From the prac-

[1] Paper No. 1616 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. The writer is indebted to the Graduate School of the University of Minnesota for a grant which enabled her to undertake this problem.

[2] Nebel, B. R. and M. L. Ruttle. The cytological and genetical significance of colchicine. Journal of Heredity, 29:3–10. 1938 (Illustrated).

[3] Blakeslee, A. F. and A. G. Avery. Methods of inducing doubling of chromosomes in plants by treatment with colchicine. Journal of Heredity, 28:393–412. 1937. (Illustrated).

tical side, there is the possibility that doubling the chromosome number may increase the size and vigor of the plants, giving greater yields, or larger stalks.

Plants were treated at three different stages of growth, (1) the young growing seedling, (2) the two-year-old dormant crown, and (3) the older growing stalk.

Seedlings were grown from an open-pollinated rust-resistant selection. When the stalks were five centimeters tall the plants were dug and washed, and the tops cut back to three centimeters. Ten plants were used for each treatment, the treatments consisting of immersion of the stalks in .02, .1, .5, and 1.0 per cent colchicine for 2, 4, 6, 8, 10, 12, 16, 18, 20, 22, 24, and 26 hours. Checks were immersed in water in the same way for the same lengths of time.

TABLE I. EFFECT OF COLCHICINE ON RATE OF GROWTH OF ASPARAGUS AS INDICATED BY THE PRODUCTION OF SECOND STALKS DURING THE MONTH AFTER TREATMENT
Number of Plants Showing a Second Stalk (10 Plants in Each Treatment)

Number of Hours of Immersion	Percentage of Colchicine					Total
	0	.02	.1	.5	1.0	
2	8	10	9	5	1	33
4	9	10	10	4	1	34
6	10	10	10	9	9	48
8	9	10	3	2	0	24
10	7	7	5	0	0	19
12	10	8	10	2	0	30
16	10	10	8	0	1	29
18	10	10	10	1	1	32
20	10	7	7	0	0	24
22	7	10	7	1	1	26
24	8	10	6	0	2	26
26	10	5	7	0	1	23
Total	108	107	92	24	17	348
Per Cent	89	89	76	20	14	

The first effect was retardation of growth, roughly proportional both to the length of treatment and to the concentration of colchicine, as shown in Table 1, in which the growth of treated plants is compared with that of check plants. This effect was noticeable one week after treatment, at which time the check plants had begun to send out branch shoots while most of the colchicine-treated plants had died back for a short distance from the tips.

The second effect of colchicine was a thickening of the branch shoots and of the second stalk. The number of abnormally thick stalks appearing on treated plants within one month after treatment is compared with the number of slender stalks in Table 2. A later check will probably show a larger number of thick stalks,

as plants treated with .5 and 1.0 per cent colchicine had grown very few second stalks during the first month after treatment.

TABLE II. EFFECT OF COLCHICINE ON PRODUCTION OF THICK SECOND STALKS IN ASPARAGUS DURING THE MONTH AFTER TREATMENT

Number of Plants Showing a Thick Second Stalk (10 Plants in each Treatment)

Number of Hours of Immersion	Percentage of Colchicine					Total
	0	.02	.1	.5	1.0	
2	0	0	1	3	0	4
4	0	1	1	1	0	3
6	0	0	0	1	3	4
8	0	2	2	0	0	4
10	0	4	2	0	0	6
12	0	2	3	1	0	6
16	0	2	3	0	1	6
18	0	2	3	2	1	8
20	0	1	2	0	0	3
22	0	1	5	0	1	7
24	0	1	2	0	1	4
26	0	1	3	0	0	4
Total thick.....	0	17	27	8	7	59
Total Number of Second Stalks.....	108	107	92	24	17	348

Crowns of two-year-old asparagus roots were soaked for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, and 22 hours in .1, .2, .3, .4, and .5 per cent colchicine. Checks were similarly soaked in water. One week after planting many of the plants showed thickened and deformed stalks. In many plants these deformed stalks died before reaching any great height, and normal stalks replaced them.

Growing stalks of older plants were immersed for 7 hours in .8 per cent colchicine. Two weeks later they had swelled but had not grown in length. Three weeks after treatment most of the swelled tissue had begun to shrivel and die, but two stalks produced buds on the thickened tissue which grew into shoots with exceptionally long needles, indicating possible chromosome doubling.

Beans, onions, peppers, potatoes, and rhubarb also were treated. Rough, stiff leaves and abnormal buds indicating a possible mixture of diploid and tetraploid tissues have been obtained in some of the colchicine-treated individuals.

The top of the sheath covering an emerging bud of amaryllis (*Hippeastrum* hybrid) was cut off and several drops of .5 per cent colchicine solution were dropped inside the sheath around the three flower buds. The largest flower bud was pricked with a dissecting needle. The effect of the colchicine appeared to extend far below the point of application, as the whole flower stalk, as well as the flowers, was shortened and thickened, as shown in

Table 3. The pedicel, in particular, was much shortened in the treated flowers while the ovary was the least shortened. Anthers were of approximately the same size in treated and check flowers, and the pollen grains were the same size. Although the pollen appeared good and the flowers were hand-pollinated, they dropped off without setting seed.

TABLE III. EFFECT OF COLCHICINE ON GROWTH OF AMARYLLIS FLOWER STALKS

Flower Part	Normal	Colchicine	Ratio of Normal to Treated
Stalk length	47.0 cm.	14.0 cm.	3.4:1
Pedicel length	6.2 cm.	1.1 cm.	5.6:1
Ovary length	1.2 cm.	1.0 cm.	1.2:1
Pistil length	12.0 cm.	3.6 cm.	3.3:1
Filament length	7.4 cm.	1.6 cm.	4.6:1

The tops of large seedlings of annual aster *(Callistephus chinensis)* were immersed for two, three and one-half, and five hours in .1, .2, .3, .4, and .5 per cent colchicine. Checks were immersed in water. Two weeks after treatment most of the plants showed rough new leaves, indicating possible chromosome doubling in part of the new tissues.

Flax *(Linum usitatissimum)* seedlings about three centimeters tall were tied in bundles of five and the tops immersed in .02, .1, .5, and 1.0 per cent colchicine for 2, 4, 6, 8, and 10 hours. Growth was much retarded in all treatments for the first two weeks compared with the water-immersed check plants, and the mortality rate was high in the longer treatments, as shown in Table 4.

TABLE IV. EFFECT OF COLCHICINE ON GROWTH OF *Linum usitatissimum*
Height of Plants in Centimeters One Month after Treatment

Number of Hours	Water	Percentage of Colchicine			
		.02	.1	.5	1.0
2	23	20	18	16	14 (shoots from base)
4	20	18	14	4*	21 (shoots from base)
6	16	13	4*	Dead	Dead
8	11	14	Dead	Dead	Dead
10	15	Dead	Dead	Dead	Dead

* No new growth since treatment.

Plants treated with 1 per cent colchicine for two and four hours, respectively, stopped growing at the tip, but finally sent out exceptionally large, vigorous shoots from the cotyledon nodes. Several of the other treated plants sent out large shoots from the base, while a few sent out similarly large shoots from the tip. The size and vigor of these shoots indicate possible tetraploidy.

All leaves were cut from hollyhock *(Althaea rosea)* seedlings, and the crowns were immersed in .1, .2, .3, .4, and .5 per cent colchicine for 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours. A few days after treatment the 8 and 24-hour treated plants looked wilted, and two weeks later the 24-hour treated plants had not grown any new leaves. The other plants had each put out one new leaf, which was largest in the water-immersed check plants in every case. In many of the colchicine-treated plants the new leaf was rough, indicating possible chromosome doubling in part of the tissue.

Alyssum, Centaurea, Dianthus, Digitalis, Gaillardia, Iris, Delphinium, Matthiola, Nasturtium, Nigella, Petunia, Portulaca, Salpiglossis, Scabiosa, and *Tagetes* also were treated, but too little time has elapsed since treatment for results to show, although in almost every case the type of growth indicates possible chromosome doubling in at least some of the treated plants.

Summary

Horticultural plants belonging to a number of different families were treated with colchicine. Of the vegetables treated, asparagus gave the most definite and the quickest reactions, and it is probable that several plants with larger cells and doubled chromosome number were obtained. Abnormal growth indicated possible chromosome doubling in some of the treated plants in beans, onions, peas, potatoes, and rhubarb.

A number of annual and perennial flowers were treated. Amaryllis flowers were deformed and proved to be sterile. Exceptionally large, vigorous shoots arising from some of the treated plants of flax indicate possible chromosome doubling and larger cell size from colchicine treatment. Rough growth indicating possible chromosome doubling in part of the tissues was found in a number of the plants.

SOME HIGHER FUNGI IN THE VICINITY OF WINONA

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The flora of the State of Minnesota composed as it is of two distinct vegetation regions—forest and prairie, with their diversities of climate, altitude and soil, is proportionally large. In fact, of the quarter of a million classified plants in existence, it is roughly estimated that five per cent may be found within the confines of the state. This proportion does not seem so evident to the inhabitants of the state since, as a result of the geographic, atmospheric,