Polyploid Sugar Beets—Cytological Study and Methods of Production

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Introduction

It is not quite clear at present whether or not polyploidy could be used to increase the productivity of sugar beets (1, 3, 5)[2]. However, triploids which show heterosis have been developed (2, 3). Polyploidy increases the size of fruits and germs. This property, which counts for little in multigerm beets, can prove very useful in monogerm beets (4). To gain the advantages of polyploidy, it is necessary to develop an easy method of obtaining a sufficient number of tetraploid plants. Such methods must be based upon treatment of the growing point in young seedlings, because the growing point can produce pure tetraploid floral axes. Treatment of the floral axes themselves may cause chimeras or merely islands of 4n tissue. Studies were made to determine the importance of 1. concentration of colchicine solution, 2. duration of treatment and 3. the age of seedlings when the treatment is most effective.

Material and Methods of Treatment

Seedlings of curly-top-resistant strains SL 824 and SL 92, both of which represent selections from the commercial variety U. S. 22/3, were used in the experiment. From both strains tetraploid plants were obtained. Young seedlings were taken from soil, washed and tied in bunches of 50 seedlings each. These bunches were plunged upside down in a crystallizing dish filled with colchicine solution. The cotyledons and the upper part of the hypocotyls were covered by the solution. The roots were wrapped together in wet cheesecloth. A reverse treatment was also carried out with bunches of roots placed in colchicine solution. The crystallizing dishes containing the seedlings were covered by glasses. The temperature during the treatment was maintained at 27° C. The seedlings were placed in a well-lighted room and supplementary light was used at night. Thus, the conditions for growth were optimum and the seedlings grew under the treatment. After treatment the seedlings were rinsed in water and transplanted into pots.

The concentrations of colchicine applied were: 0.05, 0.1, 0.3, 0.4, 0.5 and 1.0 percent. Duration of treatments was 7.5 hours, 16 hours and 24 hours. Seedlings of the same age transplanted without treatment were used as a control.

The number of plants which produced *2n* instead of the normal *n* gametes showed the effectiveness of treatment in each experiment.

About 1,500 seedlings survived after treatment and in 897 of them the gamete formation was examined.

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[2] Numbers in parentheses refer to literature cited.

Experimental Results and Discussion

Effect of Duration of Treatment and Colchicine Concentration

After 7.5 hours of treatment, the number of seedlings which survived in pots outdoors in August was relatively high and was only a little less than the control (Table 1). The mortality of seedlings increased when 1.0 percent colchicine was applied or when the treatment was extended for 16 hours. The survival after 24 hours of treatment was very low, so the experiments were delayed until fall when the seedlings could be transplanted into the greenhouse. In the greenhouse the survival was considerably better. To obtain the highest possible number of well-rootd seedlings it was found necessary 1. to use healthy seedlings, unaffected by root rot, 2. to provide moderate temperatures after transplanting (15° to 25° C.), without sudden changes and with relatively high humidity. The appearance of the first, second and third leaves was slower in treated seedlings than in the control. This indicates a slower rate of growth. After 7.5 hours of treatment the slowest growth was observed with 1.0 percent colchicine. The rate of growth was especially slow after 16 and 24 hours of treatment. Seedlings rooted after treatment were feeble and started to develop leaves slowly. This was the most critical period in their lives. They recovered slowly but after development of the second and third pair of leaves they grew normally.

Table 1.—Effect of Duration of Treatment and Colchicine Concentration.

		Time of Treatment			
		7.5 hours		16 hours	24 hours
Percent colchicine	Untreated control	cotyledons treated	roots treated	cotyledons treated	cotyledons treated
	%	%	%	%	%
			Percent 4n plants		
0.05		5.0	....	....	5.2
0.1		5.7	4.2	16.1	9.0
0.3		10.5	5.1	4.1	7.6
0.4		5.6	....	....	....
0.5		16.6	9.7	6.6	11.2
1.0		0.0	4.1	18.1	10.5
			Percent survival		
0.05		81	....	..	96
0.1		82	....	83	99
0.3		77	....	21	86
0.4		62	..	....	....
0.5		83	....	58	86
1.0		45	....	6.0	60
Untreated control	89				

All treated seedlings were classified as affected or unaffected. Unaffected seedlings grew rapidly and did not develop thick leaves and hypocotyls. The number of unaffected plants was very insignificant when seedlings were treated by plunging in colchicine solution. Only when the low concentrations of colchicine (0.05 and 0.3 percent) were applied about 2 percent of the plants were unaffected. Under heavier concentrations or by longer treatment all seedlings showed thick hypocotyls, cotyledons and leaves, and they developed slowly. Some treated seedlings showed much depression, they were dwarf with small, narrow, irregular leaves and developed extremely slowly. The number of dwarf plants increased with heavier concentrations of colchicine and after prolonged treatments.

Different concentrations of colchicine solution gave approximately the same percentage of tetraploid plants (Table 1). The best concentrations for seedling treatment are considered to be 0.3 to 0.5 percent because these concentrations do not damage the seedlings as much as the heavier concentrations and at the same time they are strong enough to produce tetraploids.

Duration of treatment beyond 7.5 hours was not important. The percentage of 4n plants varied considerably with treatments at different concentrations and sometimes lower concentrations were more effective than the higher concentrations. This indicates that some other factors besides colchicine concentration influenced the results.

Effect of Treatment on Seedlings of Different ages

Seedlings were treated by plunging the cotyledons and the upper part of the hypocotyls into 0.3 and 0.5 percent colchicine solution for 5.5 hours at the following stages of development:

1. When the first pair of leaves had scarcely appeared between the cotyledons. This group represented the oldest seedlings.
2. Before the first leaves appeared. (When one of the cotyledons is torn off a small swelling between cotyledons and two small transparent scales, rudiments of the first pair of leaves, may be seen with the aid of binoculars).
3. Still smaller seedlings. (The length of cotyledons was 0.9 to 1.0 cm. Two very small transparent scales, rudiments of the first pair of leaves, on the swelling may be seen with binoculars. These scales are sometimes absent).
4. Seedlings barely sprouted and above the soil surface. (The length of the partially green cotyledons is 0.5 to 0.6 cm. Some seedlings have curved, yellowish, folded cotyledons. Under binoculars the swellings between cotyledons and the rudiments of the first leaves are absent).
5. Seed balls were forced to germinate on filter paper. When the tip of the radicles had scarcely appeared the seed balls were plunged into colchicine solution for 5.5 hours. The treated seed balls were washed and planted in pots.

Table 2.—Effect of Colcbicine Treatment on Seedlings of Different Ages.

	Colchicine Concentration										
	0.5 percent					0.3 percent					0.1 percent
	Age group					Age group					Age group
	1	2	3	4	5	1	2	3	4	5	5
Percent 4n plants	0	5.0	10.0	12.5	....	2.9	3.7	13.3	29.4	40.0	42.8
Percent survival	72	70	90	38	6.6	80	80	47	34	12.5	38.0

The survival of seedlings and the rate of growth decreased when the youngest seedlings were treated. The youngest seedlings were damaged more than older seedlings at 0.3 percent and 0.5 percent colchicine.

The highest number of dwarf plants was observed in the fourth group. The percentage of seedlings which survived after seed treatment was still lower. The seedlings which survived after the seed treatment (age group 5) appeared still more sensitive and were damaged more by colchicine than a similar treatment of the youngest seedlings. The number of unaffected plants were considerably higher than when seedlings were treated. Part of the plants had very thick hypocotyls and perished in two to three weeks. Such a condition was not observed in treated seedlings (age groups I to 4) .

The percentage of *4n* plants obtained after treatment of seedlings of different ages showed a clear picture with increasing effect of treatment as the age of the seedlings decreased (Table 2) . Treatment of the youngest seedlings produced the highest percentage of 4/z plants, while treatment of germinating seed (age group 5) produced still more tetraploids. The age of seedlings appeared to be a determining factor in colchicine treatment.

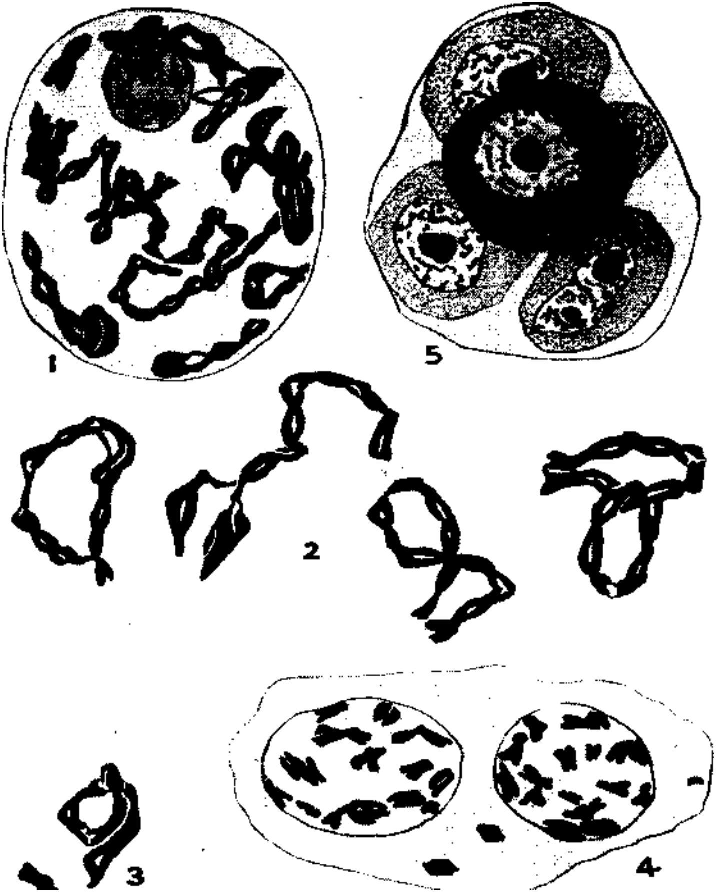
The less the quantity of initial cells which undergo treatment the higher the chances are of obtaining pure tetraploid plants.

It is very important to discard all seedlings unaffected by colchicine and to leave for further work only affected ones. But we cannot count much on attempts to select tetraploids according to their morphological appearance, or investigation of vegetative tissues. From 72 plants showing superficial tetraploid characteristics (thickened, dark green leaves) selected among treated seedlings, only 6.4 percent were found to be true tetraploids with diploid gametes. A large group of other plants which had the general appearance of ordinary diploids produced 8.2 percent of tetraploid plants. A group of 70 plants with large stomata was selected; only 11 of them produced diploid gametes. Nearly 100 percent of the seedlings in all of the experiments were affected. Probably all of them developed chimeral tissue, but only certain ones produced diploid gametes.

It is more satisfactory to select tetraploid plants after treatment according to the size of pollen grains, because this is the only character which indicates the kind of progeny which will be produced by the given plant. It is difficult to agree with the opinion that the considerable variation in the size of pollen grains makes this character uncertain for recognizing tetraploids (6) . The pollen must be taken for measurement only when the anthers are dehiscing, *i.e.,* at the time when pollen grains reach their full size. For the majority of diploid plants the size of pollen grains at this time is 18.7 to 21.8u, and in tetraploids the size of pollen grains reaches 28.0 to 31.2u. Variation *in* size of some pollen grains may occur, but they cannot obscure the general picture of the kind of pollen produced by the given plant.

Tetraploid plants often produce some small defective pollen grains, but a large percentage of large diploid pollen shows without doubt that it is a tetraploid plant. If a plant produces only a few large diploid pollen grains and the size of the remaining pollen is comparable in size with haploid pollen grains, it is highly probable that such a plant is a chimera and must be discarded.

The progeny obtained from selected tetraploid plants must be checked as to chromosome number.



Figures 1 through 5.—Meiosis in 4w Plants.
(Descriptions of these figures appear on page 475)

Considerable irregularity was observed in meiosis in tetraploid plants. Chromosomes for the most part were associated in pairs (bivalents). Some chromosomes formed quadrivalents and trivalents (Figures 1, 2, 3). During the first metaphase certain chromosomes were forced out into plasma. The nuclei after the first division contained at interkinesis 18, 17, 16, 15 and 14 chromosomes (Figure 4). Besides the diploid gametes containing 18 chromosomes, the tetraploid beets produced gametes with different numbers of chromosomes. Many of the tetrads were normal and contained four nuclei, but some tetrads contained five, six and seven nuclei (Figure 5). A considerable number of small degenerating pollen grains side by side with normal large diploid pollen grains were formed. These small pollen grains contain fewer chromosomes than the diploid set and were not viable. Therefore, in spite of considerable irregularity in meiosis, sugar-beet tetraploids produce for the most part tetraploid progeny, but some aneuploids can always be expected among them.

Summary

Studies were made to determine the importance of colchicine concentration, duration of treatment and the age of seedlings at which the treatment was the most effective. The best concentration for seedling treatment was considered to be 0.3 to 0.5 percent colchicine. Increased concentration and duration of treatment beyond 7.55 hours were not important. The age of seedlings appeared to be a determining factor in colchicine treatment. The youngest seedlings developed the highest percentage of tetraploid plants. Size of pollen grains was considered better than investigation of vegetative tissues for recognizing tetraploid plants after treatment.

Irregularities in meiosis in tetraploids may lead to appearance of some aneuploids in their progeny.

Meiosis in 4n Plants

Following are descriptions of Figures 1 through 5, which appear on page 474.

Figure 1.—Diakinesis with three quadrivalents and 12 bivalents. x 7,500.

Figure 2.—Quadrivalent associations, x 8,000.

Figure 3.—Trivalent association plus univalent, x 8,000.

Figure 4.—Interphase-nuclei with 18 and 16 chromosomes, two chromosomes and fragment in plasma, x 7,500.

Figure 5.—Tetrad containing six cells, x 3,180.

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