# Polyploidy in Sugar Beets Induced by the Use of Colchicine, Ethyl Mercury Phosphate, and Other Chemicals[1]

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Chemically induced polyploids, particularly those of economic plants, have attracted considerable attention during the past 4 years. Colchicine has been widely used and is now generally accepted as **a** standard chemical for this purpose. Other chemicals and treatments have been investigated, but their application and use has not been as general as the use of colchicine.

This paper presents the results of a detailed greenhouse experiment evaluating the use of colchicine for inducing polyploidy in sugar beets by the seed-soaking method. There are also included the results of numerous preliminary experiments involving the use of ethyl mercury phosphate, sulfanilamide, sulfapyridine, calcium phosphate, and X-ray treatments.

## Colchicine Seed-Soaking Treatments

Our attempts to produce polyploidy in sugar beets during the spring of 1938 by the use of colchicine as suggested by Blakeslee and Avery (3) were not very successful since all of the polyploids produced either reverted to normal or died.[3]

The following experiment was conducted to evaluate the colchicine seed-soaking procedure. Solutions of colchicine, of from 0 to 2.0 percent were utilized and 50 seedballs of a commercial variety of sugar-beet seed were soaked in each of these solutions for from 1 to 6 days at room temperature and then planted. Dry seed was also planted as a check.

The development of the seedlings was closely observed and counts were made on the number of plants produced. The number of polyploids present in each lot was determined by their thickened hypocotyl, and all diploids present were removed. Periodic microscopic examinations of the size of the stomata as suggested by Artschwager (2) were made and counts of the number of plants reverting and of the number dying were maintained for 4 months. The results of this entire experiment are presented in figure 1.

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[3]Figures in parentheses refer to Literature Cited.

In general, the data included in figure 1 show that, as the soaking time or the concentration was increased, the number of plants produced was decreased but the percentage of polyploids obtained was increased. The amount of reversion taking place was not great in most cases but the death rate rapidly increased with the number of polyploids produced, so that at the end of 4 months only a small portion of the induced polyploids remained from any one treatment.

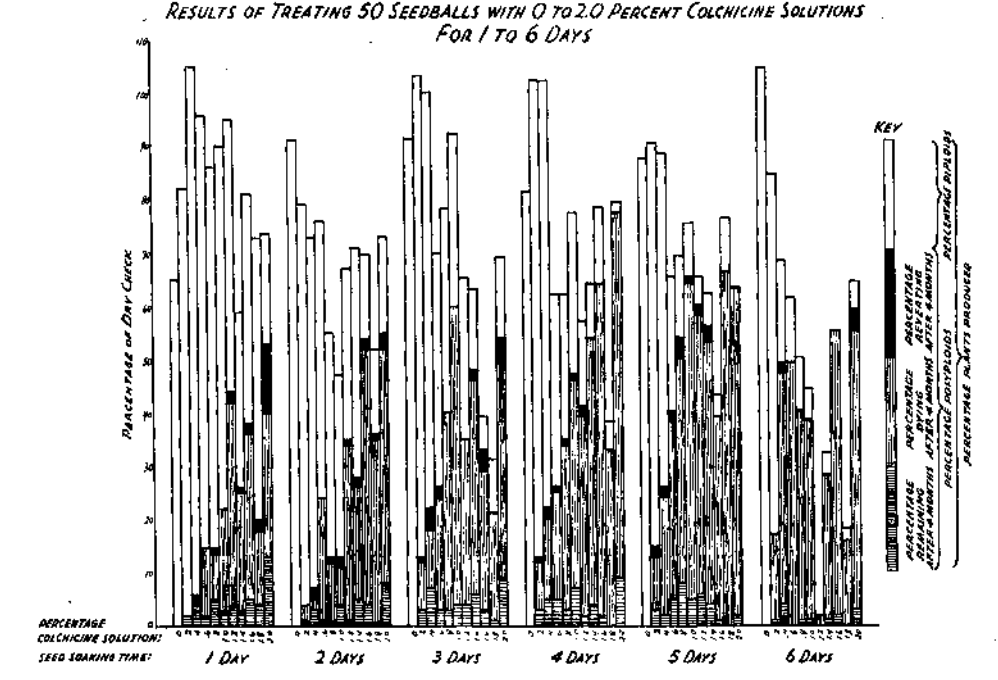
### Miscellaneous Preliminary Experiments

**Hormone Treatment of Colchicine Induced Polyploids.**—It was noticeable in the polyploids induced by soaking the seed in solutions of colchicine that root development was greatly retarded. Six groups of polyploids induced by this method were transplanted and the roots treated with commercial preparations of indolebutyric acid in an attempt to overcome the retarded root development. No apparent value resulted from these hormone treatments.

**X-Ray Seed Treatment.**—Three X-ray exposures were made of 8 lots of beet seed ranging from 0 to 36 seconds. One hundred seedballs were planted of each lot after each exposure, making a total of 24 lots containing X-ray exposures of 0 to 108 seconds. Good germination was obtained in every case. The development of the seedlings was closely observed for the appearance of polyploids, but they appeared to be normal in every respect.

**Ethyl-Mercury-Phosphate Seed Treatment.**—A series of experiments indicated that a commercial seed-treating dust containing 5-percent ethyl mercury phosphate would induce polyploidy in sugar beets. Beet seed soaked for 48 hours in solutions of this dust containing from .00001 to .001-percent ethyl mercury phosphate produced from 2 to 42 percent polyploids. A number of comparisons were made of a .001-percent ethyl-mercury-phosphate solution and a 1-percent colchicine solution, soaking the beet seed for 24, 30, and 48 hours. It was noticeable that the polyploids induced by the ethyl-mercury-phosphate treatments were more vigorous than those induced by colchicine and the death loss was considerably less. However, a larger percentage of these plants reverted to normal so that the final percentage of polyploids remaining after 4 months was approximately the same for the 2 chemicals.

**Ethyl-Mercury-Phosphate Soil Treatment.**—A series of experiments was conducted applying a commercial dust containing 5-percent ethyl mercury phosphate to the soil. Applications were made of from .005 to .1 gram of ethyl mercury phosphate per row foot directly below the seed, with the seed, directly above the seed, and on top of the soil surface. Polyploids were obtained in all experiments and further experiments showed that polyploids may be produced in abun-


RESULTS OF TREATING 50 SEEDBALLS WITH 0 TO 2.0 PERCENT COLCHICINE SOLUTIONS FOR 1 TO 6 DAYS
PERCENTAGE OF DAY CHECK
KEY
PERCENTAGE REMAINING AFTER 4 MONTHS
PERCENTAGE DYING AFTER 4 MONTHS
PERCENTAGE REVERTING AFTER 4 MONTHS
PERCENTAGE POSTPLOIDS
PERCENTAGE DIPLOIDS
PERCENTAGE PLANTS PRODUCED
PERCENTAGE COLCHICINE SOLUTION:
SEED SOAKING TIME:
1 DAY
2 DAYS
3 DAYS
4 DAYS
5 DAYS
6 DAYS

dance by applying .05 gram per row foot on top of, the soil directly above the seed and scratching it into the soil sufficiently to prevent washing. The number of polyploids obtained is dependent on the variety of beet seed used, varying as much as 50 percent between varieties.

Calcium phosphate was also investigated by this procedure but no polyploids were obtained.

**Sulfa Compounds and Other Irrigation Treatments.**—A series of experiments was conducted in the greenhouse in which sugar beets were planted in pots and irrigated continuously for 2 months with solutions of .005-percent sulfanilamide, .0077-percent sulfapyridine, .02-percent colchicine, and .00005-percent ethyl mercury phosphate. Polyploids were obtained from all treatments but continual contact of the colchicine and the ethyl-mercury-phosphate solutions proved to be toxic to young seedlings and they died. The growth of the plants in the sulfa treatments was very slow and at the age of 4 months, after treatments had been discontinued for 2 months, these plants were only 1/4 to 1/2 inch in diameter.

**Colchicine-Agar Crown and Branch Treatment.**—Colchicine in concentrations of from .5 to 2.0 percent added to 1-percent agar as suggested by Artschwager (2) was applied with a brush to the crowns of stecklings to produce seed-bearing polyploid tissue. The crown buds were retarded and the plants began dying after a few days of growth. This loss continued until only a few plants survived to shed pollen and these failed to produce seed.

Colchicine agar was also applied to open flowers and tips of branches of seed beets and viable seed was obtained from all treatments in about equal amounts. Three times as many polyploid plants were obtained from the seed of the treated tips as from the seed of the treated open flowers. Twice as many plants were obtained from the 1.5-pereent concentration as from the other treatments.

Following the procedure suggested by Peto (4), capsules containing 1.0 and 1.5-percent colchicine agar were placed on decapitated branches. Pollen examinations as suggested by Abegg (1) were made and from 2 to 3 dozen polyploid seedballs per plant were obtained from the new growth adjacent to each capsule.

Capsules containing a .001-percent ethyl-mercury-phosphate agar were also used, but the tips were killed and the new growth arising lower down on the stems was diploid.

**Colchicine Crown Injection Treatment.**—Three mm. of a 0.2-percent solution of colchicine were injected into the crowns of steckiings. The results were similar to the agar treatment inasmuch as the plants soon began dying off; although a few plants survived to produce pollen, no seed was obtained.

**Colchicine Branch Immersion Treatment.**—Ten cc. portions of from 0.5 to 2.0-percent colchicine solutions were placed in test tubes and branches of seedstalks immersed for from 1 to 48 hours. Seed was obtained from all treatments. The 16 and 24-hour treatments produced about 8 times as many plants as other treatments and the 16-hour treatment produced 5 times as many polyploids as other treatments. The 1.5-percent concentration produced twice as many polyploids as the other treatments.

A similar experiment using from .0005 to .001-percent ethyl-mercury-phosphate solutions was conducted but no polyploids were obtained.

**Colchicine Branch Spray Treatment.**—An excess of solutions of colchicine of from 1.5 to 5.0 percent was sprayed on to branches of seedstalks by atomizers and about equal amounts of seed were obtained from all treatments, while 1.0 and 2.0-percent concentrations produced 3 times as many polyploids as other concentrations. Seed from treated tips produced 2.5 times as many polyploids as the seed from treated open flowers. Further experiments comparing the use of 1-percent colchicine and .0005-percent ethyl mercury phosphate for spraying entire plants on 181 plants showed that 10 percent of the plants produced polyploid pollen after 2 sprayings with colchicine as compared to 8 percent for ethyl mercury phosphate after 4 sprayings.

## Summary

The results of 66 treatments of sugar-beet seed in colchicine solutions are presented showing the number of polyploids obtained after 4 months to be quite limited from all treatments. Hormone treatments failed to overcome retarded root development of the polyploids. Twenty-four X-ray seed treatments gave negative results. Seed-soaking treatments of concentrations of ethyl mercury phosphate also induced polyploids. Calcium phosphate was not effective. Irrigation treatments of sulfapyridine and sulfanilamide solutions induced polyploids. Crown injections and crown agar treatments of colchicine induced polyploid tissue in the seed-bearing generation. Comparisons oF concentrations of colchicine agar applied to the tips of branches indicated that the 1.5-percent concentration is the most effective. Polyploid seed was obtained from the use of 1.0 and 1.5-percent colchicine-agar capsules; 0.001-percent ethyl-mercury-phosphate agar used in capsules killed the tissue. The results of immersing tips of branches in concentrations of colchicine solutions indicated that a 1.5-percent solution for 16 hours is the most effective. Ethyl-mercury-phosphate capsules did not induce polyploidy.

### Conclusions

In the treatment of seed with colchicine, ethyl mercury phosphate, or other chemicals to produce polyploids the mortality rate in the early seedling stage is very great. The root development of the seedlings is limited and they do not survive this early period of growth very well. Those seedlings which do survive have all the first season in which they may revert to normal. If they survive the first season there is opportunity for losses in storage and subsequent transplanting. The production of polyploid seed directly through the treatment of the inflorescence appears to be the most promising procedure for obtaining polyploid sugar beets.

### Literature Cited

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# Evaluation of Polyploid Strains Derived From Curly-Top Resistant and Leafspot-Resistant Sugar-Beet Varieties

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With the discovery that polyploid types of plants can be produced readily by use of colchicine, great interest has attached to the application of this new technique to various economic plants. Methods of inducing polyploidy in sugar beets have been previously described.[2] (1). It is now possible to report results from 2 years of comparative yield tests with 4 *n* strains derived from the important diploid varieties II. S. 22, U. S. 23, and U. S. 215.

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[2]The writer wishes to express his appreciation to Dr. G. H. Coons, Principal Pathologist, Division of Sugar Plant Investigations, U. S. Department of Agriculture, for suggestions and criticisms during the progress of this work and in connection with the preparation of the manuscript.