INDICATIONS OF POLYPLOIDY IN SUGAR BEETS INDUCED BY COLCHICINE

By Ernst Artschwager, Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture.

In attempting to produce polyploidy in sugar beets three types of treatment were employed: (1) soaking of seed, (2) application of colchicine to crown buds, and (3) treatment of mature floral parts. The first had for its object a change of chromosome number in the cells of the vegetative growing point of the mature embryo of germinating seeds. By the second treatment an attempt was made to induce chromosome doubling in meristematic cells of the nascent floral axis of second year seedlings. The third type of treatment sought to influence the fertilized egg or the embryo in its initial stages of development.

To bring about a change in the chromosome complex of cells of the growing point of the mature embryo, the seed itself was subjected to the influence of colchicine. Such treatments have been attempted by practically all who are engaged in genetic research with sugar beets, ever since the discovery of the effects of colchicine on plants. The treatment consisted essentially of soaking the dry seed balls in a weak aqueous solution of colchicine for a given period, usually 24 to 48 hours. The method produced apparent results in that the immediate visible effect was very striking, since many of the young seedlings showed thickened hypocotyls and malformed cotyledons and leaves, and on cytological examination exhibited giant cells with a higher chromosome number. Unfortunately, the effect disappeared sooner or later. Newly developed leaves were of the normal type. 1/

In the writer's experiments, two types of seed treatment were used: (1) Treating dry seed balls with an aqueous solution of colchicine and (2) pre-soaking the seed balls in tap water before treatment with colchicine. Minimum length of treatment was 18 hours without pre-soaking and 10 hours when preceded by 24 hours of pre-soaking. While the strength of the colchicine solution was kept constant (0.5 percent), the length of treatment was varied in the different tests. The longer treatments gave a higher number of malformed seedlings, many of which died in the early stages of development. Of those remaining, some apparently outgrew the effects of colchicine while a few continued to develop tissues with changed chromosomal numbers as attested by cytological examination.

In the second type of treatment, the aim was to influence the meristematic cells of the nascent growing point of the floral axis of second-year seedlings. About 500 plants were decapitated at the advent of seedstalk development, care being taken to leave enough of the embedded leaf primordia to assure new vegetative growth. The stumps left after the removal of the leaves were painted over with one-half percent colchicine agar and, to protect the exposed surface from sunlight and flooding during irrigation, small parchment bags were tied over the stumps and left there until new growth had started. Since the treatment was drastic, a number of treated plants failed to recover and died. Others developed new leaves and normal seedstalks and flowers which, however, produced normal pollen and seed. Less than 10 percent of the treated plants produced new leaves with signs of malformation like those of seedlings from colchicine-treated seeds. Since these plants failed to produce seedstalks,

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1/ Rasmusson, J., and Levan, A. Tetraploid sugar beets from colchicine treatment. Beet Breeding Station Hilleshög, Landskrona, Sweden.

they were transplanted to nursery rows and their further development will be followed this coming spring.

As the vegetative growing point consists of many cells and as only a certain number divide simultaneously even under most advantageous conditions, treatments to influence the fertilized egg or the embryo in its initial stages of development should be more promising than the methods just outlined. With this in view, flowers that had opened the day before and also those of an earlier date of anthesis were treated with colchicine. In one set of experiments the flowers were painted over with colchicine agar, while in another set of treatments, lateral branches of the floral axis from which all unopened flowers had been removed were immersed in test tubes of colchicine solution of one-half and one percent strength, respectively. The length of treatment in the immersion experiment varied from one-half hour to four hours. To keep the agar from drying out too rapidly the treated branches were enclosed in parchment bags for a period of 24 hours. The treated material was left on the plants until harvest time. Each lot was collected separately and planted out in nursery rows during July and August. The development of the young seedlings was followed and all plants that appeared from a study of the lower leaf epidermis to be normal were discarded.

It is a well-known fact that plants with a higher chromosome number have larger stomates, and, since it is time-consuming to make a cytological analysis of all individuals, circumstantial evidence gained from a study of the leaf epidermis is sufficient for making preliminary discards. The leaf epidermis is easily stripped and the length of the stomate measured with an ocular micrometer. By this method about 10,000 seedlings were examined in the course of the summer. All plants with stomates measuring less than 35 microns were discarded and individual records were kept of those remaining in the nursery rows. The plants were tested again after an interval of two months and those that had reverted back to normal were removed. In this manner the number of actual or potential "takes" was narrowed down with final eliminations to be made on actual cytological examination of young floral branches when they appear.

NUCLEAR PHENOMENA IN THE POLLEN TUBE OF SUGAR BEETS

By Ernst Artschwager, Pathologist, Division of Sugar Plant Investigations,
Bureau of Plant Industry, United States Department of Agriculture

Pollen tubes that have developed on nutrient agar are broader than those that grow in the tissue of the style of the beet flower. This fact has been studied in other plants by Wulff (3) [1] who found in certain cases that tubes in culture were from five to six times as broad as those in the style. However, other investigators found no difference in appearance of pollen tubes and their nuclei in artificial and natural media.

Sperm nuclei first appear in the pollen tubes of the sugar beet developing on artificial media after the tubes have attained a length of about 50 microns or approximately the combined diameter of two or three pollen grains. The sperms are not always found close to each other and their position relative to the distal end of the tube also varies. The two sperms are easily recognized. Their shape varies from that of a spindle or shallow crescent to that of a sphere. When emerging from the pollen grain they are practically always elongate but may become round later on. Occasionally extra sperm-like bodies,

1/ Numbers in parenthoses refer to Literature Cited.