THE OCCURRENCE AND NATURE OF POLYPLOIDY IN A COLCHICINE TREATED POPULATION OF SUGAR BEETS

A thesis

Submitted to the

Faculty of Graduate Studies and Research

The University of Manitoba

by

Gerald Henry Rank

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

University of Manitoba

April 1964

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. L. E. Evans for the helpful advice and assistance given him during the course of the research and the preparation of the thesis. Thanks are also extended to Dr. A. C. Ferguson and Dr. R. C. McGinnis for their assistance at various times during the preparation of this manuscript and Dr. G. I. Paul for his help with the statistical analysis.

The project was made possible by financial grants from the National Research Council of Canada.

ABSTRACT

Chromosome counts were obtained from heart leaves (2nd, 4th, 12th, 13th, 19th and 20th) of colchicine treated sugar beets plants and correlated with the chromosome counts of pollen mother cells with a view to establishing a method of selecting only 4N inflorescences. It was found that counts of the 12th or a subsequent heart leaf in conjunction with counts of a floral heart leaf were satisfactory indicators of the ploidy level of the inflorescence. The number of chloroplasts per 2 guard cells was not a satisfactory index for selection of 4N inflorescences due to the presence of periclinal chimeras. Pollen diameter was found to be a workable selection criterion for the determination of the ploidy level of the inflorescence.

Root tips were relatively less affected by colchicine than the shoot apex and gave no indication as to the type of inflorescence the plant would produce.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The yield advantage of triploid sugar beets over diploids (30) and the possibility of using male sterility to facilitate the production of hybrid triploids has increased the need for the efficient production of large numbers of tetraploid strains.

Although an effective method for polyploid induction is established, controversy exists as to the best means of identifying tetraploid plants in the Co (treated) generation. Fortunately the type of growth of the sugar beet lends itself to such an investigation. The leaves of the shoot apex grow very vigorously and chromosome counts can easily be obtained on leaves up to 15 mm. in length. Also it is possible to determine the chromosome number of the Co generation by investigation of pollen mother cells (PMC's). Thus one can follow the effect of colchicine on the chromosome number of prefloral growth via cytology of the heart leaves and correlate this with the chromosome number of the C_1 generation.

The "tunica corpus" concept of apical meristem organization visualizes the promeristem to consist of two independent layers (24). Evidence of periclinal chimeras in dicotyledons supports this hypothesis (6). The outer tunica consists of two layers which give rise to the epidermis by anticlinal divisions. The corpus which is enclosed by the peripheral tunica gives rise to the vascular and ground tissue by periclinal and anticlinal divisions. There may be, however, some modifications of this general scheme as the layers of the tunica and corpus sometimes give rise to tissue other than that designated above (7).

Theoretically colchicine could have a range in effectiveness of polyploid induction from complete conversion to no effect. However, even if all of the initials are not converted to the 4N condition, a certain percent conversion may result in a 4N inflorescence if the 4N initials in such a chimera give rise to the floral apex.

Since both the heart leaves and the inflorescence are believed to arise from the corpus, the chromosome "balance" in one should be correlated with the other. One can assume that the leaves are a random sample of the shoot apex since the heart leaves arise from the promeristem in accordance with the phyllotaxis of the shoot (2/5).

The presence of mixoploid or sectorial chimeras could lead to confusing results as the primordia that initiate the heart leaves could come from a different area of the corpus than that which initiates the flowers. Periclinal and mericlinal chimeras could invalidate the use of chloroplast counts of epidermal guard cells as an indicator of chromosome number in the germ plasm. A difference in the rate of division of 2N and 4N cells in the corpus initials would cause the chromosome count at one growth stage to be different than at a subsequent growth stage. Similarly a difference in the rate of division of 2N and 4N cells in the young heart leaf could give a false indication of the chromosome number of the corpus. Another probable source of experimental error is from an inadequate sampling of the heart leaves and inflorescences.

With the above statements in mind a study of the effect of colchicine on the chromosome number of plant growth was made with the object of determining selection criteria that would permit accurate identification of plants in the vegetative state which would produce only tetraploid seed when induced to flower.

LITERATURE REVIEW

Several methods, other than the counting of chromosomes, have been used to predict the ploidy level of sugar beet plants. Due to the frequent occurrence of chimeras in the Co the selection criteria that are suitable in later generations may not be useful in the Co (29).

Artschwager (2) used plant morphology in the Co in conjunction with other selection criteria to evaluate the efficiency of tetraploid induction. Savitsky (29), however, found plant morphology alone to be a poor criterion. Of the plants that appeared to be affected by colchicine in the seedling stage only 6.4 percent produced 4N inflorescences.

Stomata size was used by different workers (1, 2, 3) as a screening devise, but due to the applied nature of their investigations they did not give experimental evidence to show it was a valid criterion. Peto and Hill (21) suggested using the area index of the stomata from comparable leaves as a selection criterion but gave no data to substantiate its value. Deneuche (5), Varga (32) and others (10, 26) indicated that stomatal size is a poor selection criterion.

Evidence has been given (4, 9, 20) that the number of chloroplasts per guard cell is positively correlated with the ploidy level of the plant. Powers and Dudley (22) suggested this as a method for screening Co seedlings in order to eliminate undesirable plants. Savitsky (29), however, found that of 225 Co plants whose inflorescences were shown to be 2N via pollen diameter, 125 (55.5%) had a larger number of chloroplasts than is expected in diploids. Similarily of 361 Co plants whose

inflorescences were shown to be 4N via pollen diameter, 86 (23.6%) had a lower number than is expected in 4N guard cells. Kloen and Speckman (17) and Rosen (26) emphasized that there is a variable environmental effect on the number of chloroplasts per guard cell.

Many workers (1, 2, 11, 12, 27, 31) have indicated that the increased diameter of 4N pollen grains could be used to select for inflorescences with diploid gametes but several workers (10, 17, 26, 32) have found this method unsatisfactory. Kuzdowicz (18) and Walther (33) state that the number of pores per pollen grain can be used to differentiate between haploid and diploid pollen grains. Walther (33) claims that environment produces a smaller variability in pore number than it does on pollen diameter. However, Varga (32) found this method unsatisfactory and Essad and Touvin (10) and Dona'Dalle Rose (8) found it less satisfactory than chloroplast or chromosome counts.

The number of nucleoli in the resting nucleus of epidermal cells has been used by Reitberger (25) and Graf (14) to differentiate between 2N, 3N and 4N plants. Speckman (31) found that the time saved in analysis by this method was not great enough to compensate for the extra time it took to make the preparations. Similarily Varga (32), Essad and Touvin (10) and Dona' Dalle Rose (8) found this method unsatisfactory.

Feltz (11) suggested that higher amounts of abnormal tetrad formation in 4N plants could be used as a selection criterion. However, he stated that the greater amount of non-staining micro pollen grains found among the diploid pollen grains could not be used as an absolute

selection criterion.

Kloen and Speckman (16, 17) found that cytological examination of leaves in the rosette stages was an ineffective method of selecting for 4N inflorescences in the Co. They then examined the cytology of heart leaves of the floral apex from plants previously selected for 4N tissue in the prebolting heart leaves and excised the apices that were not 4N at both sampling times. The progeny (C_1) from plants sampled twice gave 77.3 percent 4N, 20.6 percent 3N and 2.1 percent 2N whereas with only one prebolting cytological examination they got 52.6 percent 4N, 45.6 percent 3N and 1.8 percent 2N. They used a rapid counting method in which there is no staining and the material is observed by aid of phase microscopy. Deneuche (5) using the same method, compared its accuracy in estimating chromosome number with the chloroplast number per guard cell. He made 36 errors out of 142 plants examined using the rapid counting method whereas he made only 3 mistakes using the criteria of chloroplast number per guard cell.

Hammond (15) selected 4N plants based on cytology of the floral apex, that were shown to be highly 4N chimera on the basis of vegetative tissue, and found seed from selected plants to be all 4N.

MATERIALS AND METHODS

The material used in this experiment was an open pollinated monogerm strain of sugar beets designated as 6210, obtained from the Sugar Beet Breeding Station, Taber, Alberta. Five hundred seeds were treated with the fungicide, Arasan, and germinated. When 10 percent of the seeds showed protruding root tips the entire sample was placed in a solution of .3 percent colchicine for 6 hours at room temperature, then washed twice in water and planted in the greenhouse.

As the seedlings grew an attempt was made to sample each of the 2nd, 4th, 12th, 13th, 19th and 20th heart leaves (Plate I. Fig. 1-6). However, in the early stages of growth the deliterious effect of colchicine on plant growth made it impossible to always sample the exact heart leaf desired. Thus the first two growth stages were in some instances sampled at the 3rd, and 5th or 6th heart leaf. Since the prime interest of the experiment was not to determine an effective method of polyploid induction but rather to determine the nature of colchicine conversion, seedlings that did not show the morphological effects of colchicine were not sampled and were discarded.

The heart leaves were excised when they attained a length of four to six millimeters. Great care was taken in excising the heart leaves to avoid mechanical damage to the plant apex which would result in abnormal plant growth and thus would not give a true indication of the effect of colchicine on normal plants. However, many plants still had to be discarded because of apical damage which resulted in plants with several apices.

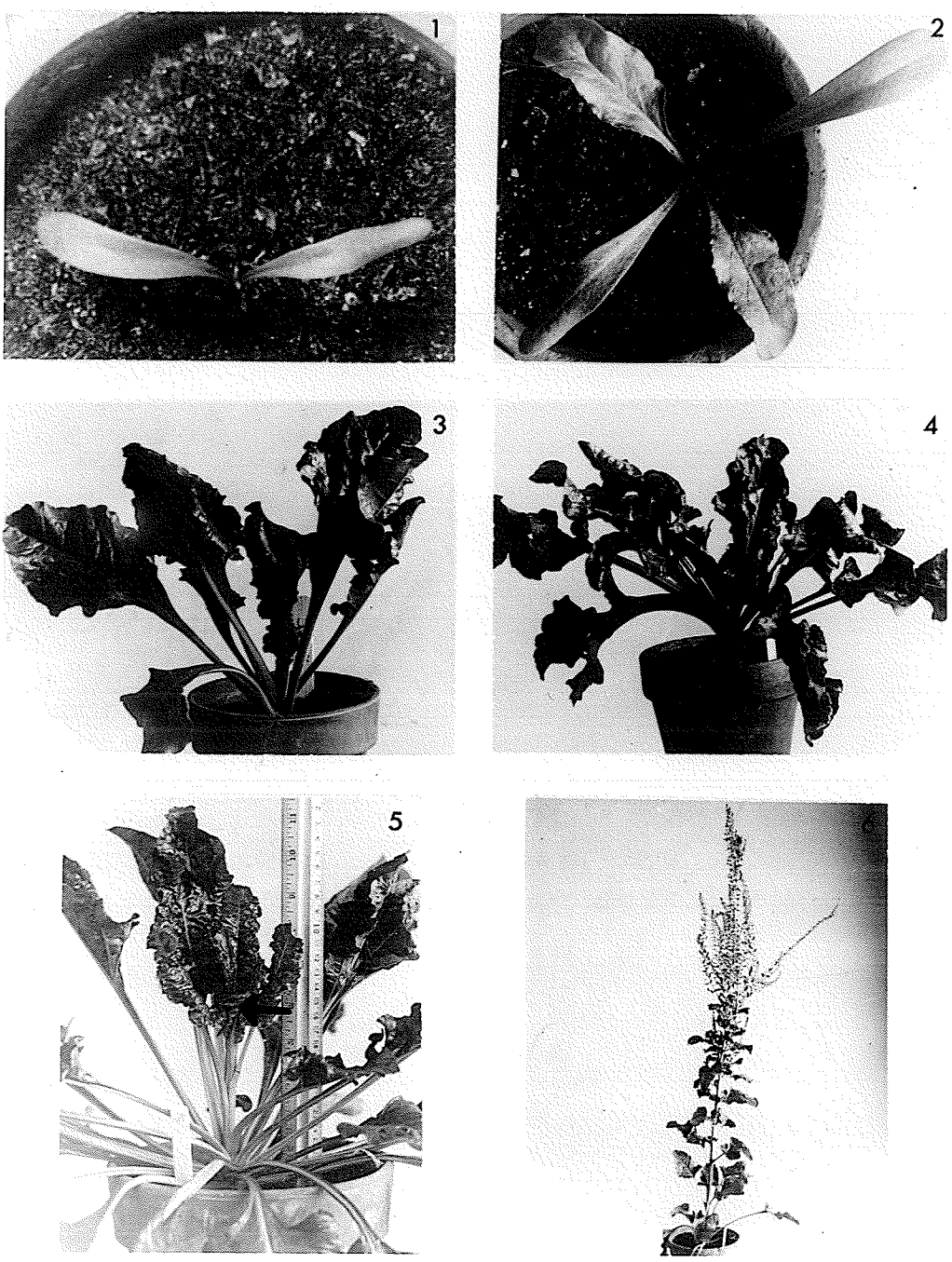

PLATE I - The growth stages from which samples for cytological analysis were taken.

Fig. 1 - two leaf stage

Fig. 2. - four leaf stage

Fig. 3. - 12th leaf stage

Fig. 4. - 20th leaf stage

Fig. 5. - floral apex leaf stage

Fig. 6. - inflorescence - stage from which pollen mother cells were taken.

From each heart leaf collected two random samples were taken from opposite sides of the leaf. This was facilitated by the fact that the periphery of the heart leaf was found to be the area of most active division. Chromosome counts of twenty five cells were made on each sample to give a total of fifty counts per heart leaf.

The heart leaves were collected directly into cold water and received a 24 hour pretreatment at 0° - 2°C. They were then fixed in Farmer's solution (3 parts C_2H_5OH :1 part CH_3COOH) for a minimum of two days. They then received an 8 minute hydrolysis in 1 percent HCl at 60°C. prior to staining in Feulgen. Squash preparations were made using aceto-carmine as the counter-stain.

On the basis of the cytology of the last heart leaf sampled, the plants were divided into 3 groups: diploids, tetraploids and chimeras. These plants then received a photo-thermal induction period of continuous light at 40°F. for three months (13). After the induction period one heart leaf was taken from the floral apex of each plant when the apex was approximately six inches high. Fifty cells per floral heart leaf were counted.

Pollen mother cell counts were made on all plants that bolted. Two random samples were taken from each inflorescence and fixed directly into Carnoy's solution (6 parts C_2H_5OH: 3 parts $CHCl_3$: 1 part CH_3COOH). When a count was made all five stamens were included in the squash preparation. Twenty-five counts were made per slide for a total of fifty counts per inflorescence.

A sample of pollen from one flower of each plant was collected into a solution of 1 part 1 percent aceto-carmine and 1 part glycerol and the diameter of ten viable pollen grains was recorded. The viable pollen grains were defined as those that stained red whereas the inviable remained colorless. It was deemed necessary to do this because the high proportion of aneuploidy in an autotetraploid results in an appreciable amount of inviable pollen grains, of a smaller diameter (11). In addition to this, 2 additional samples of pollen were taken from 2 random flowers from ten plants of the 4N and chimera groups.

Thus a total of three ploidy determinations were taken on all of the inflorescences (2 via PMC's and 1 via pollen diameter), and five determinations on ten plants from each of the 4N and chimera group (2 additional via pollen diameter).

An attempt was also made to count one root tip of each plant that received the photo-thermal induction period. In addition to this a separate population of 106 seeds of strain 6210 were treated with colchicine and root tips were collected from the seedlings. Chromosome counts were obtained by the same technique as described for heart leaves.

The lower epidermis of two leaves (3 inches in length) from ten plants of each of the three groups was stripped into a 1 percent solution of AgCl. The total number of plastids contained in the two guard cells surrounding each of 10 stoma was recorded. Thus there were 200 chloroplast counts per group.

In the case of chimeras an attempt was made to sample prebolting heart leaves at two stages of growth. This was accomplished by excising the leaf tip when the leaf was 4 - 6 mm. long and then excising the remainder of the leaf four days later.