A Method for Positive Selection of 4N Sugar Beet Plants in the Vegetative C_0 Generation[1]

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The contingent superiority of polyploid sugar beets over diploids and the possible use of male sterility to facilitate the production of hybrid triploids has created the need to produce tetraploid strains efficiently. It is obvious that a selection criterion that would readily permit selection, within the vegetative C_0 generation, of plants that produce only 4n seed would be of practical value.

Many of the selection criteria found effective in later generations have proven useless in the C_0 generation due to the prevalence of cytochimeras and to colchicine induced morphological variations that are not associated with polyploidy. Savitsky (6) found plant morphology and chloroplast numbers a poor selection criteria in the C_0 generation while Deneuche (1) and Varga (7) found stomata size unreliable. Other criteria including pollen diameter, pollen pore number, and number of nucleoli in resting nuclei of epidermal cells have been used with limited success (2,7).

Kloen and Speckman (5) using the chromosome number of floral heart leaves were only moderately successful in selecting C_0 plants that produced only 4n seed. They used a rapid, non-staining, phase microscopy method which Deneuche (1) later found to be unreliable.

In this study the effect of colchicine treatment on chromosome duplication at various growth stages was determined. This was related to pollen mother cell (PMC) analyses and progeny classification in order to ascertain if certain chromosome number characteristics in the vegetative stage could be used to select C_0 plants that would produce only 4n seeds.

Methods and Materials

The material used was an open pollinated monogerm strain designated as 6210, obtained from the Sugar Beet Breeding Station, Taber, Alberta. The seeds were germinated on damp blotting paper and when 10% of the seeds showed protruding root tips the entire sample was placed in a solution of .3% colchicine for 6 hours at room temperature, washed twice and planted in the greenhouse.

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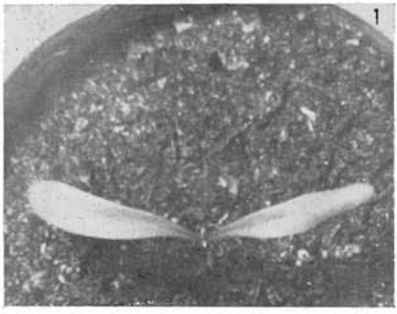
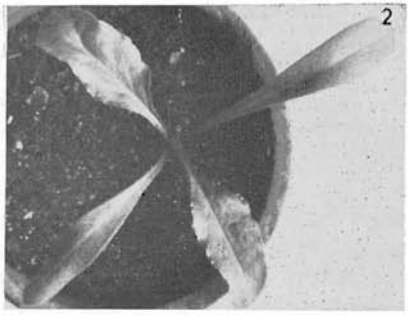
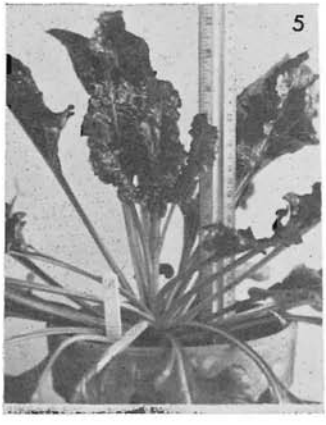






Figures 1-6.—Growth stages from which samples for cytological analyses were collected. 1. two leaf stage, 2. four leaf stage, 3. twelve leaf stage, 4. twenty leaf stage, 5. floral leaf apex stage, 6. inflorescence PMC stage.

As the seedlings grew, an attempt was made to sample each of the 2nd, 4th, 12th, 13th, 19th and 20th heart leaves (Figures 1-4). The heart leaves were excised when they attained a length of four to six millimeters and collected directly into cold water for a 24-hour pretreatment at 0° to 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. Heart leaves then received an 8-minute hydrolysis in 1 normal HCl at 60°C prior to staining in Feulgen. Squash preparations were made using acetocarmine as the counter stain. From each heart leaf two random samples were taken from opposite sides of the leaf. Chromosome counts of twenty-five cells per sample were made to give a total of fifty counts per heart leaf.

On the basis of the chromosome numbers encountered in 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. After the induction period one heart leaf was taken from the floral apex of each plant when the apex was approximately six inches high (Figure 5). Fifty cells per floral heart leaf were counted. Also at this stage one root tip was excised and the chromosome number of 25 cells obtained.

Pollen mother cell analyses were made on all plants that bolted (Figure 6). 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.

Twenty pollen diameters were recorded on each plant that bolted. Also the number of plastids contained in the two guard cells surrounding each of 10 stomata was recorded for ten plants of each group.

Results and Discussion

Pre-bolting Growth

Due to the deliterious effect of colchicine on early plant growth only 10 plants were analyzed at all six growth stages and 22 plants at 5 growth stages. The data from cytological analysis of these 32 plants are regrouped into three growth stages and presented in Table 1. The first stage includes data from all the heart leaves sampled prior to the 12th heart leaf. Stages two and three have the combined data from the 12th and 13th, and 19th and 20th heart leaves, respectively.

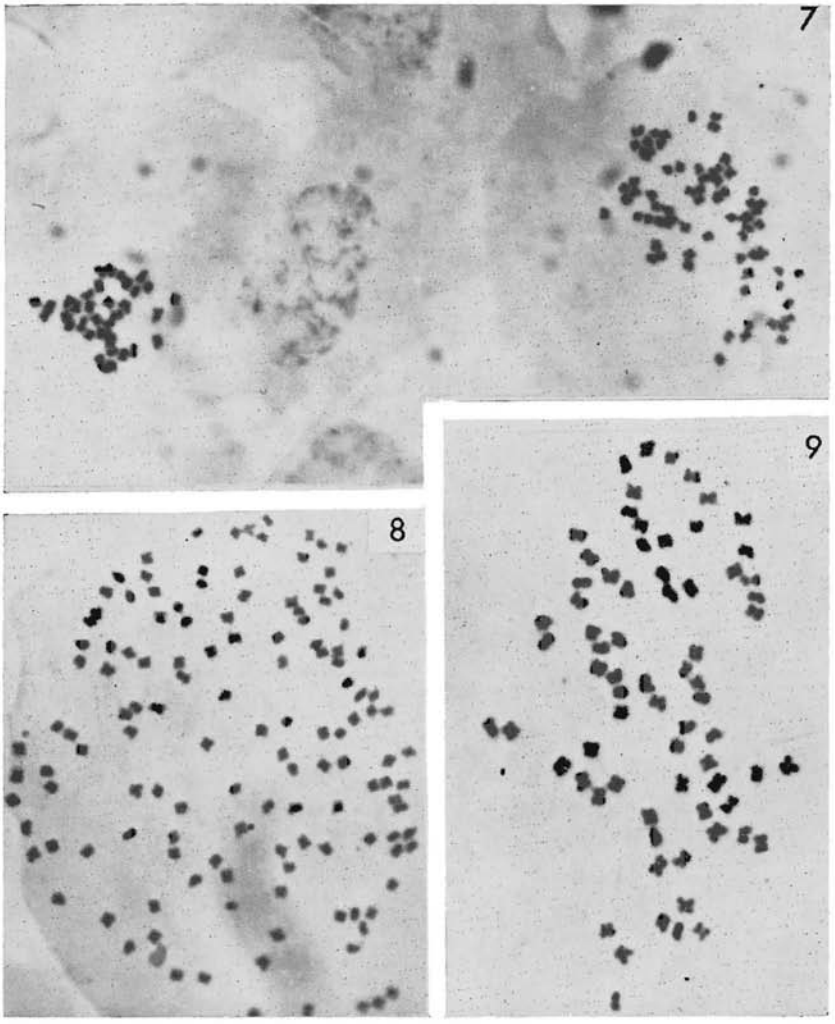
Table 1.—The frequency of 2n, 4n and greater than 4n cells in heart leaves at three growth stages.

Plant number	Stage 1			Stage 2	Stage 3
	Heart leaf 2 and 4			Heart leaf 12 and 13	Heart leaf 19 and 20
	% Cells 2n	% Cells 4n	% Cells > 4n	% Cells 4n*	% Cells 4n*
1	2	49	49	100	100
2	0	0	100	100	100
3	0	10	90	75	100
4	0	80	20	100	100
5	0	47	53	100	100
6	0	100	0	100	100
7	32	68	0	95	100
8	12	74	14	100	100
9	0	84	16	100	100
10	24	76	0	30	95
11	33	2	65	61	85
12	0	13	87	96	66
13	5	45	50	28	53
14	0	100	0	85	44
15	26	74	0	4	32
16	40	60	0	23	22
17	0	94	6	25	22
18	46	54	0	71	20
19	0	100	0	46	20
20	5	95	0	8	15
21	31	69	0	0	14
22	2	98	0	66	13
23	0	90	10	20	11
24	63	37	0	20	8
25	66	28	6	26	6
26	0	68	32	21	6
27	0	100	0	6	0
28	23	70	7	0	0
29	19	37	44	0	0
30	68	30	2	0	0
31	88	12	0	0	0
32	89	11	0	0	0

* Cells other than 4n were 2n.

As indicated in Table 1, the heart leaves have a high average chromosome number in early growth due to the occurrence of 8n and 16n cells. The mitotic configuration of a typical 4n - 8n chimera is shown in Figure 7. Figures 8 and 9 show mitotic metaphases of 135 and 72 chromosomes respectively, which presumably have arisen through successive C-mitoses[4]. The occurrence of such cells decreases rapidly with plant maturity and seldom are cells greater than 4n observed beyond the 4th heart leaf. In most cases the chromosome number has stabilized by the 12th heart leaf. In general, the percent 4n cells decreased and the percent 2n cells increased from stage one to stage two,

[4] Chromosome duplication without cell division.



Figures 7-9.—High chromosome number cells in chimeras. 7. thirty-six and seventy-two chromosome cells in close proximity, 8. a 16n cell, 9. a 8n cell.

but occasionally the opposite also occurred. There were some plants (Nos. 3, 12, 14, 17, 19, 23, 26 and 27) which had only 4n or greater than 4n cells in stage 1 that had 2n cells at later stages. Yet there were no instances where stage one had all cells of a lower chromosome number than that subsequently found in stages two and three. Thus it does not seem logical to attribute the observation of cells not previously encountered to a sectorial chimera in the corpus of the apical meristem. The phenomenon is more adequately explained by assuming that the primordia of the heart leaves in stage one were already formed in the embryo at the time of colchicine treatment and thus received a colchicine treatment totally unrelated to that of the plant apex.

Thus the leaves of stage one could have a higher chromosome number due to increased susceptibility to colchicine and the effect of colchicine on the chromosome number of these leaves could be completely different than that of the corpus which later gives rise to the heart leaves of stages two and three.

Table 2.—Data from the analysis of the floral heart leaves, pollen mother cells, root tips, pollen diameter and chloroplast number from 26 plants found to be totally 4n in the last heart leaf counted.

Plant number	Last heart leaf number	Frequencies of 4n:2n Cells			Mean pollen diameter in μ	Mean chloroplast number per 2 guard cells
		Floral heart leaves	Pollen mother cells	Root tips		
1	20	50:0	50:0	0:25	26.64	.....
2	20	50:0	50:0	0:25	26.02	.....
3	20	50:0	50:0	0:25	25.46	.....
4	20	50:0	50:0	.....	25.67	.....
5	20	50:0	50:0	0:25	24.33	.....
6	20	50:0	50:0	0:25	27.00	.....
7	20	50:0	50:0	0:25	27.28	.....
8	20	50:0	50:0	0:25	27.69	.....
9	20	50:0	50:0	0:25	27.36	.....
33	20	50:0	50:0	0:25	28.98	30.25
34	20	50:0	.....	0:25	25.50	.....
35	20	50:0	50:0	.....	25.94	29.90
36	20	50:0	50:0	.....	28.14	29.80
37	20	50:0	50:0	0:25	26.70	20.30
38	13	50:0	50:0	0:25	26.94	24.65
39	20	50:0	50:0	0:25	24.74	31.10
40	20	50:0	50:0	0:25	26.96	.....
41	20	50:0	50:0	0:25	26.23	19.80
42	20	50:0	50:0	0:25	22.84	.....
43	20	50:0	50:0	0:25	27.84	19.30
44	20	50:0	50:0	0:25	27.08	29.00
45	16	50:0	50:0	0:25	24.72	.....
46	11	50:0	50:0	.....	27.05	.....
47	13	50:0	50:0	.....	26.46	.....
48	14	50:0	50:0	.....	26.27	22.80
49	20	10:40	0:50	0:25	.....	.....

Post-bolting Growth

The 32 plants mentioned plus 26 plants that were analyzed at less than 5 growth stages received the photo-thermal induction treatment. On the basis of the last heart leaf sampled 26 plants were classified as 4n, 20 as chimeras and 12 of the 2n plants were included for a control. The data obtained from the floral heart leaves, pollen mother cells, pollen measurements, chloroplast counts and root tip analysis are presented in Tables 2, 3 and 4. The last heart leaf analysis for the chimera group is also included.

The floral heart leaves of 25 of the 26 4n plants were completely 4n (Table 2). Only one plant (No. 49) was a chimera at this stage and it produced a diploid inflorescence. This plant was eliminated from the 4n group before anthesis on the basis of floral leaf cytology. The PMC's of the other 24 plants were all 4n. Seed set on the 4n plants was generally poor and very variable. However, of the 250 progeny analyzed all were at the 4n level, 20% being aneuploids ranging in chromosome number from 34 to 38.

The floral heart leaves of 16 of the 20 chimera plants (Table 3) were chimeral, three were totally 4n (Nos. 10, 26 and 52) and one was diploid (No. 22). PMC's of 16 of these plants were analyzed. One inflorescence was chimeral (No. 24), 12 were 2n

Table 3.—Data from the analysis of the last heart leaves, floral heart leaves, pollen mother cells, root tips, pollen diameter and chloroplast number from 20 plants whose last heart leaf counted was a chimera.

Plant number	Last heart leaf number	Frequencies of 4n:2n Cells				Mean pollen diameter in μ	Mean chloroplast number per 2 guard cells
		Last heart leaf	Floral heart leaf	Pollen mother cell	Root tips		
10	20	45:5	50:0	50:0	0:25	26.58	17.20
11	20	33:17	11:39	0:50	25:0	20.71	22.85
12	20	25:25	37:13	50:0	0:25	26.68	.....
13	20	18:32	10:40	0:50	0:25	21.08	23.45
14	20	18:32	10:40	0:50	0:25	20.98	24.00
15	20	17:33	16:34	0:50		20.40	27.90
16	20	17:33	11:39	0:50	0:25	21.15	15.15
17	20	14:36	9:41	0:50	0:25	21.17	25.80
18	20	9:41	8:42	.....	25:0	19.66	24.60
19	20	9:41	19:31	0:50	0:25	20:55	20.95
20	20	9:41	10:40	0:50	0:25	20.90	25.00
21	20	8:42	6:44	0:50	0:25	21.45	.....
22	20	8:42	0:50	0:50	0:25	20.32	.....
23	20	8:42	5:45	0:50	0:25	23.07	.....
24	20	7:43	6:44	25:25	0:25	20.40	.....
25	20	2:48	7:43	0:50	0:25	21.26	.....
26	20	36:14	50:0	.....	0:25	.....	.....
50	20	27:23	7:43	.....	.....	.....	.....
51	20	8:42	6:44	.....	0:25	.....	.....
52	20	38:5:7	50:0	50:0	0:25	26.46	.....

Table 4.—Data from the analysis of the floral heart leaves, pollen mother cells, root tips, pollen diameter and chloroplast number from 12 plants found to be totally 2n in the last heart leaf counted.

Plant number	Last heart leaf number	Frequencies of 4n:2n Cells			Mean pollen diameter in μ	Mean chloroplast number per 2 guard cells
		Floral heart leaf	Pollen mother cells	Root tips		
27	20	0:50	0:50	0:25	21.85	17.80
28	20	0:50	0:50	0:25	20.72	16.05
29	20	0:50	0:50	0:25	21:56	17.90
30	20	0:50	0:50	0:25	20.81	15.50
31	20	0:50	0:50	0:25	20.46	17.05
32	20	0:50	0:50	0:25	20.44	17.20
53	20	0:50	0:50	0:25	20.56	16.05
54	20	0:50	0:50	0:25	20.45	15.40
55	20	0:50	0:50	0:25	21.42	
56	20	0:50	0:50	0:25	21.24	
57	20	0:50	0:50	0:25	20.54	17.95
58	20	0:50			20.16	16.95

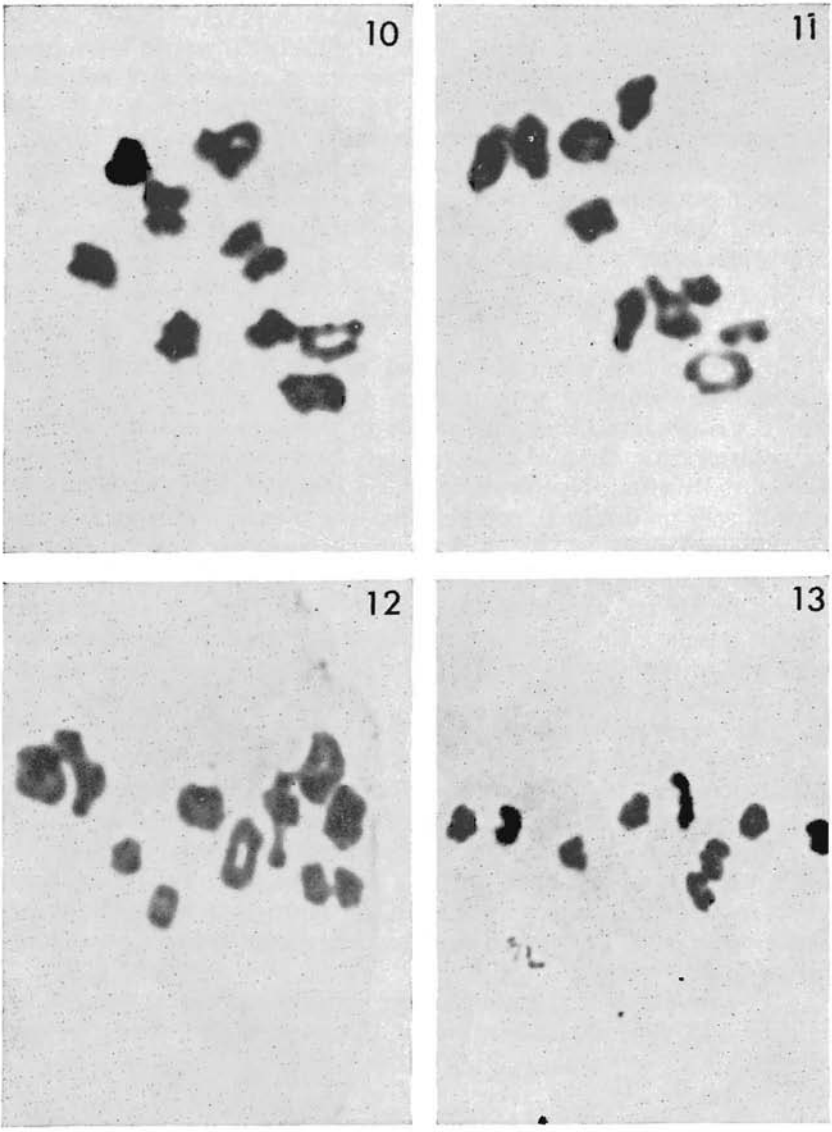
and three were 4n (Nos. 10, 12 and 52). The 12 plants in the diploid class remained diploid in the floral heart leaves and PMC's (Table 4). Only one of the 58 plants analyzed (Table 3, No. 24) was a chimera in the inflorescence as it had one 2n and one 4n floret. None of the individual flowers sampled was a chimera as judged by the PMC's.

These data indicate that the floral heart leaves of the plant will have the same chromosome number as its pre-bolting heart leaf. Also if the floral heart leaf is 2n or 4n then the inflorescence will be 2n or 4n respectively. However, if the floral heart leaf is chimera then the inflorescence will likely be completely 2n although occasionally it may be 4n or a chimera.

During the analysis of PMC's a range from complete bivalent to complete quadrivalent chromosome association was observed. Generally the 4n plants had 2 to 4 quadrivalents at metaphase but some individual plants had a very high number of multivalent associations (Figures 10-12).

It is evident from the data on root tip analyses in Tables 2, 3 and 4, that there is no correlation between the effect of colchicine on the root apex and the floral apex. Of the 49 plants whose root tips were analyzed all were totally 2n except 2 which were 4n.

There was only one 4n plant (No. 42) whose pollen diameter was smaller than the largest 2n diameter. In general pollen diameter is an adequate selection criterion of the ploidy level of the inflorescence as one could discard the few plants whose pollen measurements were intermediate between the two extremes. However, plants selected during anthesis may interpollinate before they can be isolated.



Figures 10-13.—Meiotic metaphase configurations in C_0 sugar beets. 10. a 4n cell with 9 quadrivalents, 11. 8 quadrivalents plus 2 bivalents, 12. 7 quadrivalents and 4 bivalents, 13. a 2n cell with 9 bivalents included for comparison purposes.

On the basis of data given by other workers (3,4,6), some plants with 4n inflorescences (Nos. 37,41,43,48) would have been classified as 2n if one used the chloroplast counts as a selection criterion. Also four plants (Nos. 14,15,17,20) which had 2n inflorescences would have been classified as 4n on the basis of chloroplast counts. Thus it is obvious that the mean chloroplast number per two guard cells is not a good selection criterion for ploidy number of the inflorescence in the C_0 generation due to the presence of periclinal chimeras.

Summary

Colchicine had a variable effect on the type of chimera and chromosome number produced in different plants.

It was observed that 2n initials in the corpus usually divided at a faster rate than the 4n initials but the opposite was also occasionally true. However, in many instances the chimeral condition was maintained at the time the floral leaf was sampled but the majority of the inflorescences produced were entirely 2n with a few 4n and very few chimeral. Heart leaves of entirely 2n or 4n always produced 2n and 4n inflorescences respectively. Thus to select for C_0 plants with totally 4n inflorescences only plants with totally 4n cells at the 20th heart leaf stage should be induced to bolt.

The chloroplast counts per two guard cells in conjunction with cytology of PMC's indicates that periclinal chimeras were often present which invalidates the use of chloroplast counts as a selection criterion for 4n inflorescences.

The ploidy of the inflorescence could in most cases be determined by the pollen diameter. However, it could be expected that in many instances such identification could not be made sufficiently early to remove undesirable plants from a population prior to flowering.

The root tips of the plants were seldom converted to a higher ploidy and gave no indication of the type of inflorescence produced.

Acknowledgments

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