Colchicine Treatments of Sugar Beets and the Yielding Capacity of the Resulting Polyploids

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Since the discovery of the colchicine method of inducing chromosome doubling in plants, a number of workers have tried with varying degrees of success to produce tetraploid sugar beets. Frandsen (3) and Artschwager (2) discussed methods used in attempting to produce tetraploid strains and Rasmusson and Levan (5) and Schwanitz (6) reported the production of tetraploid plants.[2] The senior author in 1938 induced tetraploid sectors in sugar-beet plants and subsequently obtained sufficient triploid seed through cross-pollination with normal haploid pollen to establish a small replicated field trial in 1939. The results, published in 1940 by Peto and Boyes (4), showed that the triploid beets exceeded the diploids in root weight by 12.2 percent, in yield of sugar per beet by 14.9 percent, and in dry top weight by 17.8 percent. The triploids also exceeded the diploids in area index of the leaves by 34.4 percent and in area index of the stomata by 42.6 percent. These results indicated the commercial possibilities in polyploid sugar beets and subsequently sufficient stock of tetraploid and triploid seed was produced at the National Research Laboratory, Ottawa, for further field tests at Buckerfield's Limited, Vancouver, in 1941 and at the Canada and Dominion Sugar Company, Wallaceburg, Ontario.

Colchicine Treatments

The method of treatment consisted of placing a weighed quantity of seed (about 3 grams) on blotting paper in a petri dish, the blotting paper having been moistened with a, measured amount of 0.4 percent colchicine solution (about 6 c. c). It was found to be important to determine accurately the amount of solution necessary for optimum germination conditions. This can be predetermined with a water solution to conserve colchicine. The beet seed was left on the moist blotting paper for periods of from 48 to 96 hours. The most effective duration reduces germination to about 50 percent. At the end of the treatment period the seed was planted in soil contained in greenhouse flats. Shortly after the seedlings emerged, the normal appearing seedlings were discarded, and this process was repeated several times during the seedling stage. The abnormal seedlings were transplanted in pots as soon as possible and eventually transplanted

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[2] Numbers in parentheses refer to Literature Cited.

in the field. The treatments were usually started some time during' March and the plants transplanted in the field late in May. During August, a further elimination of plants was made on the basis of stomatal size which must be determined on strictly comparable, mature leaves. The area index (length and width) of the stomata on the tetraploid sectors should be almost twice as large as the diploid sectors.

At harvest time the final selection of beets which had survived all selections were transferred to the greenhouse and potted. At pollination, a final check on pollen diameter was made and at this time the tetraploid plants could be detected wiith a high degree of accuracy, since the tetraploid pollen was uniformly and unmistakably larger than the diploid pollen. The actual increase in diameter was 25 percent, while the increase in calculated volume would be over 90 percent. The plants which appeared to be definitely polyploid were paired off or isolated in small groups to reduce the risk of contamination with pollen from undetected diploid sectors.

Seed Production

The application of the colchicine method described above resulted in the production from treated plants in 1940 of approximately 11,000 tetraploid seeds of 4 varieties, 3,300 hybrid tetraploid seeds, and 7,000 triploid seeds. The results of the chromosome counts on a portion of this material is shown in table 1. The seedlings produced from 4 of 5 plants thought to be tetraploid proved to be so, the other plant yielded 25 percent triploid seed. The 2 crosses involving diploid plants hand-pollinated with pollen from tetraploid plants yielded 98 and 75 percent triploid seedlings and the remainder diploid. This indicates that tetraploid pollen is highly fertile on diploid plants and that only a low percentage of self-pollination had occurred. It will also be noted in table 1 that the tetraploid seed, and particularly the hybrid tetraploid seed, was much larger than the diploid seed used in the treatments.

The polyploid seed stocks brought from Ottawa to Vancouver in 1940 were multiplied in both the greenhouse and the field during the winter and summer of 1941. The data obtained from this material are shown in table 2. The seed yields of the tetraploids were all very satisfactory in both the greenhouse and the field, the yield of the F_2 of the hybrid tetraploid (7.3 ounces per plant) being particularly heavy. An interesting indication of the relative seeding capacity of diploid and tetraploid plants was afforded where Sandomiersko diploid and Buszczynski tetraploid plants were transplanted to the same room and alternated on the bench to produce the maximum opportunity for cross-pollination. The seed size from the tetraploid plants was approximately double that from the diploid plants whereas the weight of seed per plant was very similar (diploid 1.7

Table 1.—Data on polyploid seeds and seedlings produced in 1940 from cokhicine-treated parents.

	Plant No,	Weight per 1000 seeds	Germination		No. of seedlings examined eytologically	Polyploidy of seedlings
			No. of seed sown	No. of plants		
		(grams)				
Buszczynski, C.L.E.	Check No. 17	24.1	75	125	**20**	100 percent—2x
Buszczynski, C.L.R.	105 (3x)	23.7	200	255	**72**	2 percent—2x, 98 percent—3x
Buszczynski, C.L.E.	S2 (4x)	33.0	30	68	**60**	100 percent—4x
Buszczynski, C.L.K.	87 (4x)	38.7	35	30	**20**	100 percent—4x
Buszczynski, C.L.R.	89 (4x)	40.2	35	27	**20**	100 percent—4x
Buszczynski, C.L.R,	05 (4x)	41.5	35	40	**20**[1]	25 percent—3x, 75 percent—4x
Sandomiersko, E.	Check No. 15	23.9	75	112	**20**	100 percent—2x
Sandomiersko (2x) x Buszczynski (4x)	40 (3x)	32.2	200	264	**72**	15 percent—2x, 85 percent—3x
Sandomiersko (4x) x Buszczynski (4x)	25 (4x)	02.0	100	221	**20**	100 percent—4x

Table 2.—Multiplication of tetraploid and triploid stock.

Variety	Polyploidy	Location grown	No. of plants	Total weight of seed		Average weight seed per plant	Weight per 1000 clusters	Germination 14-day
				(pounds)	(ounces)	(ounces)	(grams)	
Buszczynski (C.L.R.)	tetraploid	greenhouse	57	8	0		38.40	79
Schreiber S.S'.	tetraploid	greenhouse	10	0	11	1 1	85.25	70
Sandomiersko Type E (4x) x Buszczynski (4x)	hybrid tetraploid	greenhouse	20 (10 each)	2	0	1.6	33.70	09
Sandomiersko Type E (2x) x Buszczynski (4x)	hybrid triploid	greenhouse	(28 2x (35 4 x	3 4	0 0	1.7 1.8	17.75 34.20	SS 74
Schreiber S.S. (4x) x Dobrovice C.Z. (2s)	hybrid triploid	greenhouse	(15 2x (7 4x	0 0	11 **S**	0.7 1.1	23.25 28.80	92 48
Buszczynski (C.L.R.)	tetraploid	field	197	15	0	1.2	38.35	92
Schreiber S.S.	tetraploid	field	G	1	11	4.5	38.70	94
Sandomiersko Type E	tetraploid	field	8	1	7	2.1	39.80	88
Great Western	tetraploid	field	34	3	2	1.5	33.70	58
Sandomiersko Type E (4x) x Buszczynski (4x)	tetraploid F_2	field	77	35	0	7.3	49.55	90
Sandomiersko Type E (4s) x Buszczynski (4x)	tetraploid F_1	field	7	1	8	3.4	44.00	92
Great Western (4x) x Schreiber (4x)	tetraploid F_1	field	9	0	10	4.7	35.35	78
4 commercial samples	diploid	field					23.62	

and tetraploid 1.8 ounces). Consequently the diploid produced almost twice as many seeds of approximately half the weight of the tetraploid. In all instances the weight per 1,000 seedballs of the tetraploid exceeded the average of 4 commercial diploid samples and the F_2 seed of a hybrid tetraploid was over twice as heavy.

The germination of the tetraploid seed was *very* good, particularly that produced in the field, where with 2 exceptions the germination was between 88 and 94 percent. The 2 exceptions both involved the Or cat Western tetraploid which previously had been shown to produce seed of low viability. Consequently some inherent defect must be present in this tetraploid.

The greenhouse-produced tetraploid seed germinated slower and bad a lower percentage of germinable seed than the field-grown tetraploid seed. Tn the attempt 1o produce hybrid triploid seed in the greenhouse by inter-spacing diploid and tetraploid plants, i1 was found that the seed from the tetraploids also germinated slower and exhibited a lower percentage germinal ion than did the seed from the diploids (table 2, hybrid triploids). This comparison is complicated by the unknown proportion of diploid and triploid seed in one lot and triploid and tetraploid in the other. Studies now under way on the chromosome constitution of this material may help explain the differences observed.

In addition to the proved polyploid stocks listed in table 8, a further 8.000 seeds from treated plants of 6 varieties were produced at the National Research Council Laboratories in 1941. These seeds are now available for chromosome counts and these determinations will be made this winter. The varieties treated were Imperial, Cesena, Schreibcr S.S., Sandomiersko "Z," Sandomiersko "E," and Great Western.

In the spring of 1941. seed of Braune. Fredericksen, Dippe "Z," and Kuhn "P" was treated by the senior author at the laboratory of the *H.* C. Sugar Refining Company. Ninety-nine roots believed to be tetraploid were selected in the autumn and potted in 1he greenhouse to produce seed in the spring of 1942.

Preliminary Yield Test of Polyploids at Wallaceburg, 1941

Sufficient polyploid seed was supplied the Canada and Dominion Sugar Company, Chatham, to set out a small unreplicated test at Wallaceburg in 1941. Three-row plots 44 feet long were planted on May 6 by spacing individual seeds at 3-inch intervals. The seedlings were subsequently thinned to 1 foot.

One of the three rows of each plot was harvested September 26 and the remaining two rows were harvested October 21. The results obtained are shown in table 3. Owing to the lack of replication imposed by a shortage of seed and the variable stand obtained, the yield results can only be considered as indicative. However, sufficient interesting: results were obtained to justify inclusion of the data in this report.

Table 3.—Yield teat at Wallaceburg, Ontario, 1941.

Variety	Polyploidy	No. of beets	Feet in row	Percentage sugar	Purity	Weight	Average weight beet	Sugar per beet	Leaf-spot	Uniformity
Harvested September 26, 1941										
						(pounds)				
Buszezynski CLR	diploid	33	44	17.5	88.8	45	1.36	.212	3.0	4/10
Buszezynski CLR	triploid	41	44	18.5	89.6	66½	1.62	.200	1.5	6/10
Buszezynski CLR	totraploid	27	44	17.4	88.1	34½	1.28	.196	1.0	4/10
Great Western x. Schreiber	hybrid tetraploid	35	44	14.9	86.4	52½	1.50	.193	5 A	5/10
Harvested October 21, 1941										
Buszezynski CLR	diploid	77	80	17.2	89.7	102	1.32	.204	2.5	2/10
Buszezynski CLR	triploid	84	96	17.7	90.0	137	1.63	.259	1.5	6/10
Buszezynski CLR	tetraploid	44	86	17.8	89.4	82	1.86	.296	1.5	2/10
Great Western x Schrelber	hybrid tetraploid	54	85	14.5	87.6	116	2.15	.273	5-A	5/10

The susceptible varieties in this field had been severely defoliated by the *Cercospora* leafspot. The hybrid tetraploid was very susceptible as shown by its rating of 5 A. The diploid C.L.R. was intermediate in degree of resistance, but both the triploid C.L.R. and tetraploid C.L.R. were much more resistant. As might be expected, the extreme susceptibility of the hybrid tetraploid depressed greatly the percentage of sugar. The diploid, triploid, and tetraploid strains were very similar in regard to percentage of sugar except that the triploid was 1 percent higher than the others at the first harvest date. At this date the beet weight of the triploid exceeded all others in spite of more competition owing to a larger stand. At the later harvest date the hybrid tetraploid had much the larger beets which may have been the result of recovery from *Cercospora* injury. In regard to sugar per beet, the triploid was definitely superior to the others, but at a later date both tetraploid and the triploid strains were superior to the diploid. Considering stand differences, the comparative yield of the triploid was particularly heavy. The yield results on this test are in agreement with those obtained from a previous test at Ottawa (4) where the triploid outyielded the diploid in root weight and sugar per beet and maintained the percentage of sugar in spite of the increased weight of root.

The C.L.R. triploid and hybrid tetraploid were much more uniform than the diploid and all three polyploids had fewer spraugled roots than the diploid.

Yield Test at Vancouver, British Columbia, 1941

Two parental diploids, a triploid, hybrid triploid, tetraploid, and hybrid tetraploid (table 4) made up the 6 strains included in the yield test. A randomized-block (complete) experiment was laid down, consisting of 5 replications of 3-row plots, 25 feet long. Seeds were hill planted at 1-foot intervals at the rate of 3 seeds per hill.

Ideal conditions prevailed from seeding until thinning time with the result that it was possible, by doing a small amount of transplanting, to have the beets go into the summer with a perfect and uniform stand. The average percentage stands at time of thinning were 93.2, 94.0 and 93.9 for the diploids, triploids, and tetraploids, respectively.

In the seedling stages the thicker stems and more leathery leaves of the tetraploids and triploids made them readily distinguishable from the diploids. At later stages, the polyploids possessed shorter leaf petioles and larger and more crinkly laminae which gave them a more procumbent appearance than the diploids. The hybrid tetraploid exhibited exceptional vigor particularly during the first 2 months of growth. The other tetraploid and the two triploids showed a less-vigorous growth which was, however, decidedly superior to the diploids. As would be expected, the triploids were usually intermediate in morphological characters to the diploids and tetraploids.

Growth conditions were very satisfactory for the first 2 months after planting. However, the months of July and August brought unusually hot, dry weather and as it was not practical to irrigate, the beets suffered from lack of moisture. With the onset of damp

Tabic 4.—Yield test Vancouver, British Columbia, 1941.

				Means of		
S-train	Polyploidy	Root weight	Percentage sugar	sugar per acre	tons per acre	suscep-tibility
Buszczynski C.L.R.	diploid	23.92	**15.1**	4081	13.53	2.2 '
Sandomiersko B.	diploid	30.80	14.3	4997	17.42	2.6
Buszczynski C.L.R.	tetraploid	19.78	13.3	2980	11.19	2-7
Sandomiersko x Buszczynski	hybrid tetraploid	29.65	12.8	4294	16.77	2.3
Buszczynski	triploid	24.05	13.6	3691	13.60	1.7
Sandomierski (2x) x Buszczynski (4x)	hybrid triploid	22.18	**11.6**	2909	12.54	3.1
Calculated F value		10.9	6.5	8.66		
Significant F value odds 99 to 1.		4.10	4.10	4.10		
Difference necessary for significance	Odds: 19 to 1	3.84	1.39	**801**		
	Odds: 99 to 1	5.21	1.89	1087		

weather in September, *Ccrcoxpora* leafspot became very prevalent and no doubt this disease bad a deleterious effect on the sugar content of some of the strains. On October 11 a study was made on the susceptibilities of the various strains to this pathogen. Each beet in all the plots was graded by applying a numerical rating from 0 to 5, 0 being complete immunity and 5 being highly susceptible to the extent that all leaves on the plant were affected. The tabulated results for each variety are shown in table 4.

At time of harvest all roots were graded as to general uniformity of type and also as to neck characteristics. A system of rating from 0 to 5 was used, 5 being a highly desirable type. By this method all diploids had an average rating of 2.9 in general uniformity, triploids 3.1, and tetraploids 3.4. Jn neck characteristics the averages were as follows: Diploids *'2.7* triploids 2.7, and tetraploids 3.5. It is worthy of note that the hybrid tetrapioid, which was conspicuous in its uniformity during the growing season, graded 4.0 in general type and 4.2 in neck characteristics.

Beets were harvested on October 30 and analyzed for sugar by the hot-water digestion method. The yield results are presented in table 4.

These results are not closely corroborative of tests made at Ottawa (4) and Wallaceburg (discussed above), but it is thought that the unfavorable environment may have suppressed the inherent advantages of some of the strains. The performance of the hybrid tetraploid, however, was very good in comparison with the other polyploids and this fact would seem to suggest the economic possibilities of polyploidy coupled with heterosis.

In yield of sugar per acre the hybrid tetraploid and the C.Li.Il. diploid were significantly better than the C.Lf.R. tetraploid and the hybrid triploid. The poor performance of the latter strain is no doubt partly attributable to its severe defoliation by *Cercospora.* Otherwise no obvious relationship seemed to exist between polyploidy and disease resistance. Some of the polyploid plants exhibited an. apparent immunity, consequently it should be possible to produce highly resistant strains of these polyploids through selection.

It is generally conceded that after roots have reached a certain size, their sugar percentage tends to decrease with a further increase in root weight. A previous test (4) showed that this decline was less rapid in the triploids than in the diploids. In the present test, however, all roots were abnormally small and it is questionable if the advantage previously demonstrated could be expressed.

Although the Vancouver yield results are not highly conclusive in themselves, the general data accumulated on polyploids together with the results obtained on the three yield tests can be summarized as follows:

Summary

1. Tetraploid sugar beets of a number of varieties have been produced by means of colchicine treatments.

2. Triploids have been produced in abundance through pollination of diploid plants with pollen from tetraploids.

3. Excellent seed yields of highly germinable seed were obtained from field-grown tetraploids.

4. In seed size the tetraploids exceeded the diploids but in number of seeds per plant the reverse was observed.

5. Tetraploid plants were morphologically distinguishable from diploids at all stages of development. The most striking differences were in (a) seed size, (b) petiole length, (c) lamina size and texture, (d) stomatal size, (e) seedstalk length and diameter, (f) pollen size.

6. In two out of three environments certain triploids and tetraploids exceeded diploids in root weight and sugar per beet.

7. Heterosis combined with polyploidy appeared to have economic possibilities.

8. Triploids and tetraploids were usually much more uniform than the diploids from which they were derived.

9. The agronomic qualities of both triploids and tetraploids in limited tests to date are sufficiently promising to justify increased emphasis on this new method of plant breeding.

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