

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.

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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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Materials and Methods

Dry seed was soaked in a 0.4-percent solution of colchicine for a period of 2 days. By selection of 1 he most severely affected seedlings, a high proportion of tetraploids has been obtained in the treated generation.

First-generation tetraploid offspring were obtained by crossing pairs of plants which, on the basis of their pollen size, were judged to have been doubled in chromosome number. The plants from these crosses were characterized by greater leaf-stomala size, larger than normal-sized pollen, and foliage that was altered in both shape and size from diploid types. Tetrasomic inheritance of the *R*—*r* hypocotyl color-factor pair further substantiated the tetraploid nature of the offspring from the above-mentioned crosses.

In 1940. 9 tetraploid ($4n$) strains were compared with the diploid ($2n$) parental stocks for root yield and sucrose percentage in a field plot laid out in an equalized random-block arrangement. Twelve varieties (9 tetraploid strains and 3 parental diploids) were replicated 6 times in 4-row plots. Each plot consisted of 20 plants per variety. Rows and transplants were spaced 24 inches apart. After a season of 4½ months' duration, the clean root yield of the 2 center rows was recorded for each plot, the roots being topped as for mother beets. The average plot weights recorded in table 1 were obtained by summation of weights of individual roots of the respective plots. The total number of roots weighed for each variety was commonly 60, except in a few cases in which the total for a variety was 58 or 59 roots.

In an additional test, other $4n$ strains for which only a small amount, of seed was available were compared for yield with the proper parental varieties in single-row plantings. Twenty-four-inch spacings were used here also. The statistical significance of the average root-yield differences was determined by Student's method from pairs of $2n$ and $4n$ plants, occupying opposite hills in adjacent rows.

The single-row plan used in 1940 was adopted again for the 1941 field test. Rows and transplants within rows were spaced 30 inches apart. The 1941 beets were harvested after a 6-month season.

Sucrose percentages of single roots were determined in both 1940 and 1941. Pulp for the analyses was obtained by use of a boring rasp. Sucrose percentage in the pulp was determined by the Sachs-Le Docte cold-water digestion method. In the 1940 replicated test (table 1) the average varietal sucrose percentage is based on analyses of individual roots. The number of roots analyzed varied from 53 to 60 beets per strain.

During both seasons, lealspot injury was reduced to a minimum by 3 applications of bordeaux spray.

Experimental Results

1. Comparative Root Yields of Tetraploid Strains and Their Respective Parental Diploid Varieties.—In the 1940 replicated test (table 1) the lowest-yielding $4n$ strain averaged 2.51 pounds per beet in contrast with a weight of 3.07 pounds for the comparable diploid variety, IT. S. 22. The highest-yielding $4n$ strain had an average root weight of 3.14 pounds; that of U. S. 215, the related $2n$ parental variety was 2.81 pounds. None of the differences in root weight was significant.

In the 1940 single-row planting (table 2), 95 plants of the $4n$ strain 4302 exceeded the average root weight of the parent stock, U. S. 22, by 13.8 percent. This difference was significant for probabilities between 0.05 and 0.02. Confirmatory evidence that the $4n$ plants had greater root weight than the $2n$ strain was obtained from the additional tests in 1941 (table 3) in which 18 plants of $4n$ progeny 4304, reciprocal of strain 4302, averaged 7.10 pounds per beet as compared with 5.92 pounds for the parental diploid, IT. S. 22. However, strain 4302 and its reciprocal were lower in sucrose percentage than U. S. 22, giving approximately the same sucrose content per beet for both the $2n$ and $4n$ types.

Reference to table 2 shows that 2 tetraploid strains grown in the 1940 single-row plot were markedly and significantly lower in root weight than the parental variety, U. S. 22. Six other $4n$ lots obtained from either U. S. 23 or U. S. 215 gave non-significant differences in root yield. Based on the yield results of all varieties, in the 1940 single-row plantings, the $4n$ strains averaged 2.56 pounds in root weight as compared with an average of 2.79 pounds for the related diploid parental stocks. The difference of 0.23 pound in favor of the diploid variety exceeded the 5-percent point of significance.

In the 1941 single-row comparisons (table 3) a total of 17 polyploid strains, derived either from IT. S. 22 or IT. S. 23, were tested for yield. One of the strains, numbering 27 plants, was a triploid derived from IJ. S. 23 parentage and showed broader than normal leaves. In this respect, the $3n$ lot resembled closely the broader-leaf character of some tetraploid strains. The average root weight of the triploid strain was not significantly different from that of the related U. S. 23 variety.

Only 1 of the 17 polyploid strains (the previously mentioned $4n$ reciprocal of 4302) gave a moderate increase in root weight as compared with the diploid parental variety. However, 7 of the $4n$ progenies (mostly from the new $4n$ lots not tested in 1940) were markedly and significantly lower in yield than their related diploid varieties.

Table 1.—Continued.

Variance Table

Source of variation	Degrees of freedom	Weight of roots		Percentage sucrose	
		Sum of squares	Mean squares	Sum of squares	Mean squares
Total	71	1290.82		53.12	
Between means of blocks	5	116.82	23.36	5.60	1.16
Between means of columns	5	19.20	3.86	3.62	0.72
Between means of varieties	11	219.24	19.93	32.07	2.92
Remainder (error)	50	884.57	17.69	11.63	0.23
Observed F value for varietal differences ⁴			1.13		12.53**

* Exceeds the 5-percent point of significance.

** Exceeds the 1-percent point of significance. The appropriate comparisons of root weights and sucrose percentages are those between 4w strains and their related parental diploid stocks within each of the three varietal groups U.S. 22, U.S. 23, and U.S. 215.

¹Reciprocal 4n strains tested.

²Two 4n strains pooled.

³Odds 19:1 = 2 x standard error of mean difference. Left blank when F value is not significant.

⁴The required F value for varietal differences to be significant at the 5-percent point is 2.00; for the 1-percent point, 2.00.

Table 2.—Comparison of root yields of tetraploid sugar-beet strains and their respective diploid parental varieties, in single-row plantings at Arlington Experimental Farm, Arlington, Va. 1940

Variety	Chromosome type	Pedigree number	Weight of roots			Value expressed in terms of 2 n
			Roots weighed	Average	Difference	
			number	pounds	pounds	
U.S. 22	2 n	722	20	3.42	+ 1.24**	100
U.S. 22	4 n	4301	20	2.18		63
U.S. 22	2 n	722	95	2.90	-- 0.30*	100
U.S. 22	4 n	4302	95	3.30		114
U.S. 22	2 n	722	60	3.38	+ 1.15**	100
U.S. 22	4 n	4325	60	2.22		65
Total	2 n		175	3.12	+ 0.31*	100
U.S. 22	4 n		175	2.81		90
U.S. 23	2 n	723	57	2.41	-- 0.08	100
U.S. 23	4 n	4201	57	2.49		103
U.S. 23	2 n	723	27	2.51	+ 0.29	100
U.S. 23	4 n	4205	27	2.22		88
U.S. 23	2 n	723	21	2.81	+ 0.24	100
U.S. 23	4 n	4215	21	2.57		91
Total	2 n		105	2.82	+ 0.08	100
U.S. 23	4 n		105	2.44		97
U.S. 215	2 n	7-3000-0	19	2.44	+ 0.28	100
U.S. 215	4 n	4153	19	2.16		80
U.S. 215	2 n	7-3000-0	24	2.37	+ 0.56	100
U.S. 215	4 n	4155	24	1.81		76
U.S. 215	2 n	7-3000-0	20	2.07	-- 0.27	100
U.S. 215	4 n	4160	20	2.34		113
Total	2 n		63	2.20	+ 0.30	100
U.S. 215	4 n		63	2.09		91
Total of all varieties	2 n		343	2.79	+ 0.23*	100
	4 n		343	2.56		92

* Exceeds the 5-percent point of significance.

** Exceeds the 1-percent point of significance.

The statistical significance of the average root-yield difference was determined by Student's method from pairs of 2 n and 4 n plants, occupying opposite hills in adjacent rows.

Table 3.—Comparison of root yields and sucrose percentages of polyploid sugar-beet strains and their respective diploid parental varieties, in single-row plantings at the United States Horticultural Station, Beltsville, Md., 1941.

Variety	Chromosome type	Pedigree number	Number of roots compared	Weight of roots		Sucrose		
				Average pounds	Difference pounds	Value expressed in terms of %	Average percent	Difference percent
U. S. 22	2n	022	18	5.92	-1.18	100	12.08	+1.37**
U. S. 22	4n	4303		7.10		129	11.29	
U. S. 22	2n	022	25	7.81	+2.86**	100		
U. S. 22	4n	4304		4.95		63		
U. S. 22	2n	022	26	6.34	+0.18	100	12.18	-0.02**
U. S. 22	4n	4307		6.16		97	13.10	
U. S. 22	2n	022	43	6.95	+1.57**	100		
U. S. 22	4n	4312-3		5.38		77		
U. S. 22	2n	022	13	8.06	-1.26*	100	11.06	+0.09
U. S. 22	4n	4315		6.20		85	10.97	
U. S. 22	2n	022	48	5.85	+0.09	100	11.57	+0.57
U. S. 22	4n	4319		5.16		88	11.00	
U. S. 22	2n	022	106	6.47	+1.43**	100		
U. S. 22	4n	4326-7		5.04		78		
U. S. 22	2n	022	108	5.89	+1.25**	100		
U. S. 22	4n	4328-9		4.64		79		
U. S. 22	2n	022	41	5.62	-0.20	100		
U. S. 22	4n	4332-3		5.52		104		
U. S. 22	2n	022	87	6.27	+0.24	100		
U. S. 22	4n	4334-5		6.03		96		
Total	2n		498	6.31	+0.98**	100	11.86	+0.28
	4n			5.33		84	11.58	
U. S. 23	2n	023	22	5.34	-0.70	100		
U. S. 23	4n	4200		5.44		102		
U. S. 23	2n	023	23	6.01	+1.30*	100		
U. S. 23	4n	4205		4.71		78		
U. S. 23	2n	023	22	5.96	+0.09	100		
U. S. 23	4n	4210		5.87		98		
U. S. 23	2n	023	35	6.62	+3.12**	100		
U. S. 23	4n	4211-2		3.50		53		
U. S. 23	2n	023	24	6.18	+1.00	100	11.00	+1.00**
U. S. 23	4n	4213		5.18		84	10.00	
U. S. 23	2n	023	46	6.31	-0.25	100	10.99	+0.54
U. S. 23	4n	4219-0		6.56		104	10.45	

Table 3.—continued.

Variety	Chromosome type	Pedigree number	Number of roots compared	Weight of roots			Sucrose		
				Average	Difference	Value expressed in terms of 2 n	Average	Difference	
				pounds	pounds	percent- age	percent- age	percent- age	
U. S. 23	2 n	923	27	5.32	-0.44	100	11.33	-0.51	
U. S. 23	3 n	² (4223) (4224)		5.76		108	11.84		
Total	2 n 3 n and 4 n		201	6.04 5.33	+0.71**	100 88	11.09 10.71	+0.38	
Total	2 n 3 n and 4 n		400	6.23 6.33	+0.90**	100 85	11.48 11.16	+0.32*	

* Exceeds the 5-percent point of significance.

** Exceeds the 1-percent point of significance.

The statistical significance of both the average root yield and average sucrose percentage differences was determined by Student's method from pairs of 2 n and 4 n plants, occupying opposite hills in adjacent rows.

¹Reciprocal 4 n strains tested.

²Two 3 n strains pooled.

³The given sucrose percentages were obtained by averaging individual determinations. The total number of 2 n and 4 n roots analyzed for sucrose percentage areas follows:

Parental 2 n variety U. S. 22	103 beets
4 n strains derived from U. S. 22	103 beets
Parental 2 n variety U. S. 23	53 beets
3 n and 4 n strains derived from U. S. 23	98 beets
Total of both 2 n varieties	156 beets
Total of both groups of polyploid strains	201 beets

The reduced number of beets analyzed for parental 2 n variety U. S. 23 was due to the fact that sucrose determinations of the 2 n plants in 1 row served for a comparison with those of 2 different 4 n strains, planted on either side of the diploid row.

Comparing related $2n$ and $4n$ progenies which were evaluated in both 1940 and 1941, respectively, there was, in general, good agreement with respect to the degree of plus or minus deviations in root yield.

A summary of the results obtained for root yields of all 28 tetraploid strains of sugar beets tested so far is as follows: (1) In the 1940 tests, a total of 521 diploids (including U. S. 22, U. S. 23, and U. S. 215) averaged 2.82 pounds per beet in root weight as compared with an average of 2.70 pounds based on a total of 879 tetraploids derived from the same varieties. (2) In the 1941 plot, a total of 699 paired yields (plants derived from varieties IT. S. 22 and U. S. 23) gave average root weights of 5.33 and 6.23 pounds per beet for the tetraploid and diploid progenies, respectively. This difference of 0.90 pound was found to be a highly significant, increase for the diploid varieties.

This indicates that, under the conditions of the 2 field tests discussed in the present paper, the average $4n$ yield showed a definite trend towards lower-root weight in comparison with that of the parental diploids. Such a reduction in yield was even more marked with

Table 4.—Effect on root yield of doubling the chromosome number within an inbred line. Single-row plantings at the United States Horticultural Station, Boltsville, Md., 1941.

Pedigree number	Chromosome type	Number of roots compared	Weight of roots		Value expressed in terms of $2n$
			Average pounds	Difference pounds	
B 42	$2n$	40	2.50	+1.03	100
B 36-2	$4n$		1.47		59
(439)		30	2.15	+0.97	100
(440)	$2n$		1.18		55
460	$4n$				
(439)		50	2.81	+1.41	100
(440)	$2n$		1.40		50
461	$4n$				
(439)		58	2.78	+1.35	100
(440)	$2n$		1.43		51
464	$4n$				
Total	$2n$	108	2.62	+1.23	100
	$4n$		1.39		53

¹Seed collected from unbagged branches of plant B 36. This plant was earlier treated with colchicine during a young seedling stage of development. Since B 36 was selected from a highly self-fertile $2n$ line, tracing back to pedigree number 1167, it is assumed that most of the seed set on unbagged branches resulted from self-pollination. Plant B 36 was also the grandparent of the second generation $4n$ progenies 460, 461, and 464, respectively.

4 *n* strains derived from a highly self-fertile inbred diploid stock of beets which traces back to pedigree number 1167. In the 1941 field plot (table 4) a total of 193 tetraploid roots yielded approximately only half as much as the closely related diploid plants.

2. **Comparison of Sucrose Percentage.**—In the 1940 replicated plot, nine 4 *n* strains and the 3 related parental stocks, U. S. 22, U. S. 23, and U. S. 215, were tested for sucrose, from 53 to 60 individuals of each strain being analyzed. Sucrose percentages ranged between 12.56 and 10.19 percent. From the data recorded in table 1 it is evident that 3 of the 4 *n* strains were significantly lower in sucrose percentage than their respective diploid-parental varieties. The differences exceeded that required at the 1-percent point. There were also significant differences in sucrose percentage between 4 *n* strains derived from the same parental stock. However, if root weights are taken into account, the sugar yield of the 12 varieties differed by only small amounts.

Three tetraploid strains planted in the 1941 single-row plot were analyzed for sucrose. Two of these were significantly lower in percentage sucrose than the respective diploid varieties. The other 4 *n* strain, numbering 21 beets, was significantly higher in sucrose percentage, but somewhat lower in root weight than the comparable diploid variety V. S. 23.

A summary of the 1940 sucrose percentage determinations, including those from the single-row planting, is based on a total of 352 diploid beets and 671 tetraploids. The diploids averaged 11.24 in sucrose percentage as compared with an average of 10.94 for the 4 *n* types. The average root weights of the beets analyzed for sucrose, were practically the same, i. e. 2.81 and 2.87 pounds per beet for diploids and tetraploids, respectively.

The 2 *n* and 4 *n* beets tested for sugar from the 1941 plot averaged approximately 6.00 pounds in root weight. The diploid varieties U. S. 22 and U. S. 23 were only slightly higher in average weight per beet than the related tetraploids. Five of the 4 *n* strains in the 1941 plot had been previously tested in 1940. In 1940 these five 4 *n* lots were lower in sucrose percentage than the parental diploid varieties. In 1941 the same 4 *n* strains were again found to be lower in sucrose. The decreases in numerical values for sucrose percentages ranged between 0.5 and 1.4.

Two additional polyploid strains tested for the first time in 1941 (table 3) were from 0.5 to 0.9 percent, higher in sucrose percentage than the parent stocks. Only the latter difference, referring to 4 *n* strain 4307, was a significant increase.

The average sucrose percentage of the 201 tetraploids analyzed for sugar in 1941 was 11.16 as compared with an average of 11.48 percent sucrose for the diploids. This difference in sucrose per-

centage between the $2n$ and $4n$ chromosome types was significant, exceeding the 5-percent point.

From the combined results of sugar analyses in 1940 and 1941, it is evident that the more vigorous $4n$ strains of sugar beets selected for testing may vary in either a plus or minus direction when compared to the proper parental varieties. In both years, under the conditions of these experiments, the tetraploids were characterized by a somewhat lower average sucrose percentage than their comparable diploid varieties.

So far, 3 tetraploid strains have exceeded the root yield of their related diploid stocks by approximately 15 percent. However, these $4n$ strains tested from 0.7 to 1.5 percent lower in sucrose. Therefore, the average sucrose yield of this group of vigorous tetraploids was estimated to be nearly the same as that of the diploids.

3. **Morphological Differences.**—In the following, a brief summary is presented of the chief comparative morphological differences between diploids and related tetraploids:

The leaves of some $4n$ strains were observed to be larger, broader, and sometimes rounder in shape than those of the parental diploid varieties. Other $4n$ strains were not as strikingly modified in leaf size or shape.

The leaf stomata of the plants from several $4n$ strains were found to be larger than those of comparable diploids.

Root shape of $4n$ beets, judging chiefly from transplants, was not obviously modified.

Haploid or n pollen produced by normal diploids has been found to vary in diameter from 19.5μ to 22.8μ . Diploid pollen produced by comparable tetraploids varied between 25.2μ and 29.1μ in diameter. The difference in pollen size has served as a good criterion in the identification of tetraploid plants.

With respect to size and weight of seedballs, the most critical comparison available is that between the highly self-fertile inbred diploid strain 1167 and the second-generation $4n$ offspring derived therefrom. Two groups of 6 plants each were isolated in separate greenhouse units. Based on the combined seed yield of 1 group of diploid plants, the weight of 100 seedballs averaged 0.97 gram. The average weight of 100 seedballs from 4 representative tetraploid plants in the second isolation group was 1.39 grams. It was also observed from greenhouse plantings that the seedling stand of tetraploid strains was markedly reduced in comparison with that of the diploid lines. This indicates that the seedballs formed by tetraploid plants contained fewer viable seeds than those from comparable diploids.

4. Fertility of Tetraploid Plants.—A majority of greenhouse-grown tetraploid plants derived from the highly self-fertile 1167 diploid strain produced only a small amount of seed from self-pollinations enforced by the use of kraft paper sacks. Comparative seed yields from greenhouse group isolations of the diploid and $4n$ types, respectively, indicated that the reduction in fertility of the tetraploids was still quite evident but not as marked as occurs when kraft sacks are used as isolators.

Under greenhouse-bagging conditions, most of 1 lie diploid plants from varieties U. S. 22, U. S. 23, and U. S. 215 were found to be relatively low in self-fertility. From limited bagging trials it was found that $4n$ plants derived from the economic varieties were not markedly altered with respect, to the degree of self-fertility.

It was evident that a doubling of the chromosome complement of several inbred strains, related to pedigree number 1167, resulted in striking modification of yield and fertility. However, the same degree of change may not necessarily apply to most $4n$ strains derived from heterozygous sugar-beet stocks. This result is similar to that noted for maize by Randolph (2) who found that a marked decrease in vigor and fertility accompanied chromosome doubling in inbred strains. Tetraploids from maize stocks not inbred were as vigorous or more vigorous than the diploid parents, and were highly fertile.

The experimental production of polyploid strains of sugar beets has increased the scope of genetic problems, particularly those relating to cytology, tetraploid inheritance, and fertility characters.

In so far as the work has progressed, it may be concluded that doubling of the chromosome number in sugar beets has not generally resulted in greater productivity as compared with the original diploid varieties. On the other hand, the occurrence of a few $4n$ strains which possess good vigor brings up the question whether, by discriminating selection and breeding, tetraploids may not be found which will eventually prove to be of value to the plant breeder.

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