Further Studies on Sugar-Beet Autotetraploids

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Tetraploid forms of commercial sugar beet varieties and of certain inbred lines were compared with the respective diploid varieties or lines from which they were originally derived for (1) the economic characters, sugar production, root yield, and sucrose percentage, (2) bolting tendency, and (3) number of true seeds per seed ball.

In the production of tetraploids, colchicine-treated seedlings were selected on the basis of morphological changes induced, stomatal size, or other criteria and were grown to the flowering stage *(1)*[2]. Pollen size was determined for individual plants and was the final basis for selection of plants to produce the forms here designated as tetraploids. Previous cytological studies had determined the pollen grain size associated with doubled chromosome count. A relatively small number of tetraploid individuals (2, 4, or several) of a given variety were isolated and brought to seed. Additional generations were produced by appropriate isolations of the respective cultures.[3] If the diploid parental stock is a mass-selected commercial variety, the tetraploids that were produced necessarily represent a limited segment of the whole genetic complex of the variety. Tetraploids derived from lines inbred for several generations probably more nearly conform in genic composition to the parental 2n lines.

Experimental Work

Productivity of 2n and 4n Forms.—Tetraploids from the mass-selected varieties U. S. 22 and U. S. 23 and from the inbred line U. S. 215 were compared for economic characters with the respective diploid parental varieties in two field tests, one at Beltsville, Md. (table 1), and the other at Ault, Colo.[4] (table 2). The methods used in these tests were those commonly employed in evaluation tests with sugar beets. In the Beltsville, Md., test, sugar production from tetraploids in two out of four comparisons did not differ significantly but did differ significantly in the other two comparisons. In the Ault, Colo. test, sugar production in three out of six 2n vs. 4n comparisons did not differ significantly, but in the three other cases did differ signifi-

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2Italic numbers in parentheses refer to literature cited.

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4The test at Ault. Colo., was. conducted by John O. Gaskill. Acknowledgment is made to him for the contribution of data and for his close observations on effects of freezing on the 2n and 4n forms.

Table 1.—Comparative performance of tetraploid sugar beet strains and their respective diploid parental varieties in a 7 x 7 Latin Square arrangement of plots. Plots were four rows (24 inches) wide, 30 feet long. Test at South Farm, Beltsville, Md, 1942.

			Varietal or strain averages from seven plots†			
			Calc. acre-yield			
Variety or strain	Pedigree number	Chromosome type	Gross sugar	Roots	Sucrose percentage	No. of beets per 100 feet of row
			Pounds	Tons		
U. S. 22	SL 922	2n	3541	14.48	11.66	86
U. S. 22	SL 1-26	4n	3385	14.76	10.84*	83
U. S. 22	SL 1-28	4n	2907*	12.72*	11.83	82
U. S. 23	SL 923	2n	3840	15.10	12.39	89
U. S. 23	SL 1-29	4n	3615	15.56	10.91**	83**
U. S. 215	SP 7-3000-0	2n	4739	17.19	12.83	88
U. S. 215	SP 1-1011A-0	4n	3879**	16.71	11.16**	75**
Difference required for significance:						
Odds, 19:1			707	2.19	0.70	4.03
Odds, 99:1			683	2.95	0.94	5.42

†Values marked * and ** exceed, respectively, the 5-percent or 1-percent point of significance when comparisons between tetraploid strains and the related diploid stocks are made within the three varietal groups, U. S. 22, U. S. 23, and U. S. 215.

cantly. In all cases sugar production from the tetraploids was less than from the diploids.

Tetraploids SL 1-26 from U. S. 22, SL 1-29 from U. S. 23. and SP 1-1011A-0 from U. S. 215 at Beltsville and at Ault, Colo. were about the same in root weight as the diploid parent. Tetraploid SL 1-28 (U. S. 22) was significantly low in yield at both places. Sucrose percentage was usually significantly lower in the tetraploids than in the corresponding diploids. Since the tetraploids did not show outstandingly increased root weights over the diploids, the effects of lower sucrose percentages were not compensated and total sugar produced tended to be low.

Close observations on the relative sensitiveness to injury by freezing of the diploid and tetraploid stocks were made by J. O. Gaskill. Tn the test he conducted at Ault, Colo., in 1942, a light freeze occurred September 19 and a heavy one on September 26. The first freeze damaged foliage of the 2n strains to some extent, whereas the 4n strains were considerably damaged. The second freeze caused more severe damage to foliage, especially to the 4n types. Readings on scale 0-8, in which "0" indicates no frost injury, and "8", severe injury, were made and are given in table 2. Tt is clear from these readings that the 4n types are far more sensitive to frost injury than are 2n types.

Table 2.—Comparative performance of tetraploid sugar beet strains and the respective diploid varieties from which they were derived: 9 x 9 Latin Square test. Plots were four rows (20 inches) wide, 28 feet long. Planted May 9, harvested September 28, 1942. Test located on Harry Clark farm, Ault, Colo., and conducted by John O. Gaskill. (Data given as nine-plot averagesf.)

Variety or strain	Pedigree number	Chromosome type	Calc. acre yield		Sucrose percentage	No. of plants per 100 feet of row	Frost injury‡
			Gross sugar	Roots			
			Pounds	Tons			
U. S. 22	SL 922	2n	4,100	15.1	13.6	94	2.9
U. S. 22	SL 1-26	4n	3,700	14.8	12.4*	88**	5.6
U. S. 22	SL 1-27	4n	3,140**	13.4**	11.7**	89*	6.8
U. S. 22	SL 1-28	4n	3,900**	17.5**	13.0	90*	5.8
U. S. 23	SL 923	2n	3,820	13.6	14.0	95	3.0
U. S. 23	SL 1-29	4n	3,520	14.6	12.0**	88**	6.8
U. S. 215	SP 7-3000-0	2n	4,200	14.9	14.1	96	2.1
U. S. 215	SP 1-1011-0	4n	3,820	15.4	12.5**	94	4.6
U. S. 215	SP 1-1013-0	4n	3,740*	14.4	13.0*	87**	6.2
Difference required for significance							
Odds 19:1			440	1.1	1.0	3.9	
Odds 99:1			600	1.4	1.3	5.3	

†Values marked * and ** exceed, respectively, the 5-percent or 1-percent point of significance when comparisons between tetraploid strains and the related diploid stocks are made within the three varietal groups, U. S. 22, U. S. 23, and U. S. 215.

‡Frost injury to foliage was graded on a scale in which "0" indicates no frost injury and "8" very severe foliage injury.

Since the harvest was made 2 days following the heavy freeze, effects on yield and quality of the sugar beet roots probably were small and rather unimportant.

Bolting Tendencies in 2n and 4n Forms.—To compare the relative bolting tendency of the tetraploid with the reading of the corresponding diploid, plants of both types were started from seed at the Beltsville, Md., greenhouse. Seed was planted in 3-inch pots on January 1, 1943, and after relatively rapid growth at about 70° F. the small plants were held in an unheated greenhouse with temperatures approximating 45° F. Throughout the experiment attempt was made to have conditions equalized among varieties and between the tetraploid and diploid forms.

Normal daylight was supplemented by 6 hours of electric light (5 p. m. to 11 p. m.). The varieties were placed on four greenhouse benches, aliquots of each variety occurring at random on each bench. Transplanting to the field was done on April 2, 1943. Cold wet weather prevailed in the early part of April. The varieties, usually assets of 16, were planted in the field so that all plants from one bench constituted a block. Distribution of varieties within the blocks was at random. The summarized data for the test are given in table 3.

Table 3.—Comparative numbers and percentages of bolters of (1) diploid (2n) sugar beet varieties and (2) their related tetraploid (4n) strains. January 1-June 12, 1943, Beltsvillo, Md.

Variety	Pedigree number	Description	Total No. plants (all material)	Bolters (6-19-1943)		Remarks
				No.	Percent	
U.S. 22/2	SL 97	Parental 2n	103	6	5.8	Curly top resistant parental stock.
U.S. 22/2	F.A.A. 262 R	Derivative 4n	108	2	1.8	First generation 4n strain.
U.S. 22	SL 922	Parental 2n	104	8	7.7	Curly top resistant parental stock.
U.S. 22	SL 1-25	Derivative 4n	48	6	12.5	Second generation 4n strain.
U.S. 22	SL 1-26	do.	107	28	26.1	do.
U.S. 22	SL 1-27	do.	103	3	2.0	do.
U.S. 22	SL 1-28	do.	36	0	0.0	do.
U.S. 22	SL 209	do.	108	0	0.0	do.
U.S. 22/2 (2n) x U.S. 22 (4n)	SL 2100	3n*	188	5	2.6	All red hypoc. color types or identified 3n.
U.S. 23	SL 923	Parental 2n	108	6	5.6	Curly top resistant parental stock.
U.S. 23	SL 1-29	Derivative 4n	104	7	6.7	Second generation 4n strain.
U.S. 23	SL 1-29A	do.	108	20	18.5	do.
U.S. 23	SL 1-29B	do.	108	0	0.0	do.
U.S. 215	SP 7-3000-0	Parental 2n	104	8	2.8	Leaf spot resistant parental strain.
U.S. 215	SP 1-1011A-0	Derivative 4n	108	0	0.0	Second generation 4n strain.
U.S. 215	SP 1-1013A-0	do.	103	0	0.0	do.
U.S. 216	SP 1-16-0	Parental 2n	108	13	12.0	Leaf spot resistant parental strain.
U.S. 216	F.A.A. 264 R	Derivative 4n	108	2	1.8	First generation 4n strain.
1167	F.A.A. 150	Parental 2n	108	31	28.7	6 gener. known inbreeding.
1167	F.A.A. 154	Derivative 4n	84	1	1.2	Third generation 4n strain.

*Derived from U. S. 22/2 as 2n seed parents (r hypocotyl color) x U. S. 22 derivative first generation 4n plants as pollen parents (RR phenotype).

Tn 13 comparisons of the relative amounts of bolting in 2n and 4n types, the tetraploid showed less bolting in 9 eases, approximately the same amount in 1 case, and strikingly more bore bolting in 3 cases. In one of the comparisons the diploid line had been inbred six generations. It showed 28.7 bolters, whereas the tetraploid derived from it (third generation) showed only 1.2 percent. Tetraploids from in-

breds U. S. 215 and U. S. 210, similarly, showed marked drop in bolting. In the test, one triploid was included. It showed a reduced bolting tendency in comparison with the parental stock (U. S. 22). With respect to the three tetraploids showing increased bolting tendency, the notes taken March 21, 1940, when individual plants were selected to produce the tetraploids, show that the majority of the plants producing these three lots were then classed as fast bolters. It would seem plausible to assume that selection had been made toward increased bolting.

Tendency of Seed Balls of 4n Forms to have Single Germs.—In an earlier report *(2)* the senior author called attention to the morphological differences in the seed balls of 2n and 4n forms, and especially that tetraploid seed balls, although larger, contain fewer viable seeds than those from comparable diploids. It seemed desirable to explore this matter further.

Seed balls from field-grown 4n plants were passed over a 12/64 screen in order to retain only the larger seed balls. Seedling production from these seed balls was compared with that from similarly sifted seed from diploid varieties. In all cases the 4n types showed marked reduction of viable seeds per seed ball in comparison with the 2n types. As judged from comparable mature seed balls, the number of flowers that were initiated was approximately the same for the two types. Seed ball size in the tetraploids was larger than in the diploids. The data from the comparisons made with seed screened to include only the large seed balls are given in table 4.

The average number of seed balls containing a single viable seed per seed ball for four tetraploids derived from U. S. 22 or U. S. 23 was 72 percent as contrasted with 34 percent for diploid TJ. S. 22. Tetraploids U. S. 215, U. S. 216, and TJ.. S. 215 x 216 showed, respectively, 63, 76, and 58 percent seed balls with a single viable seed per ball, as compared with 24 percent for diploid U. S. 215 x 216 used as control.

Attention is called to the fact that these tetraploid seed balls have naturally about the same condition of single germness as is obtained by carefully processing diploid seed to obtain sheared, or segmented, seed.

Conclusions

The following conclusions are drawn from the tests so far conducted. Doubling of chromosomes in sugar beets has not resulted in increased productivity over that of the original diploids. The general effect seems to be production of types that are vegetative and de-

Table 4.—Seedlings per seed ball obtained from 4n and 2n types. Field-grown seed was passed over a 12/64 screen. The large seed balls thus obtained were planted in 3-inch pots filled with sterilized soil, one seed ball per pot. Counts of seedlings per pot were made after 16 days. December 3-20, 1945. Sugar Plant Greenhouse, Beltsville, Md.

Variety	Pedigree number	Chromosome type	From 308 seed balls planted			Classification of seed balls			
			Germination	Viable seed balls	Total seedlings	Seedlings per ball	1-germ	2-germ	3-germ	4-germ, +
			Percent				Percent	Percent	Percent	Percent
U. S. 22	SL 299	4n	82.1	253	380	1.53	55.7	38.0	6.3	0.0
U. S. 22	SL 1-26	4n	86.0	265	418	1.58	53.6	36.2	9.1	1.1
U. S. 22	SL 1-28	4n	39.6	122	133	1.09	91.0	9.0	0.0	0.0
U. S. 23	SL 1-29	4n	63.0	194	214	1.10	89.7	10.3	0.0	0.0
U. S. 22	SL 922	2n	76.6	242	496	2.00	34.3	34.7	23.6	6.6
U. S. 216	SP 3-1041-0	4n	84.7	261	377	1.44	63.6	28.4	8.1	0.0
U. S. 216	SP 3-1042-0	4n	54.6	168	210	1.25	76.2	22.6	1.2	0.0
U. S 215 x 216	SP 4511-0	4n	73.4	226	336	1.49	58.9	34.1	6.6	0.4
U. S 215 x 216	SP 2-1-00	2n	87.0	268	559	2.09	24.6	50.4	16.8	8.2

layed in maturing;. Harvest of tetraploids at the dates customarily used for diploids may, therefore, be disadvantageous to these more vegetative types. Such handicap might be obviated by deferring harvest. However, the greater sensitiveness to frost injury limits the period that harvest of the tetraploids can be extended.

Abegg *(2)* had concluded that, since some of the 4n strains he had studied possessed good vigor, types of value to the plant breeder may be obtainable by wide application of this breeding method. The additional experience with his material confirms the earlier conclusion.

Lessened bolting tendency in tetraploids seems to be indicated by the tests. TetrapJoidy may find some application with a variety that is satisfactory for a given environment except for a pronounced tendency to bolt. It is possible that the tetraploid form of such a variety would show such reduced bolting tendency as to make the variety usable in situations where its use now is precluded.

Of especial interest is the discovery that seed balls of tetraploids are prevailingly single germ in character. The data, based on seed balls screened to large size, show that the tetraploids tested give naturally about the same record as to single germness of seed balls as can be produced artificially by the most careful processing. In the shearing process there is a heavy waste of seed. There is strong possibility that seed of tetraploid forms, if otherwise satisfactory, could be used directly in mechanized operations in growing sugar beets.

Practical application of tetraploidy with sugar beets requires discovery of high yielding strains. The tests so far represent an extremely limited sampling and their outcome is not at all prejudicial to the expectation that valuable material may be produced by this technique. When such high yielding tetraploids are found, they may have additional advantages, namely, reduced bolting tendency and definite tendency toward single germness of seed ball.

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