Comparison of the Same Sugarbeet F_1 Hybrids as Diploids, Triploids and Tetraploids[1]

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The effect of genome addition into normally diploid sugarbeet (*Beta vulgaris* L.) has not been thoroughly studied even though triploid or polyploid beets are commonly used in many parts of the world. There exists a real need to establish the true effect of genome addition, particularly as regards triploid sugarbeet; tetraploid beets are not directly useful except in developing triploid or polyploid varieties.

The purpose of this study was to compare the performance of the same sugarbeet F_1 hybrids as diploids (2n), triploids (3n), and tetraploids (4n), and to determine if important ploidy level by genotype interactions exist. Comparisons of this nature have not been previously made because homozygous lines have not existed in both the diploid and tetraploid conditions, so that the same hybrids could be developed at 2n, 3n, and 4n levels. In the past decade a limited number of inbred lines have been converted to the tetraploid condition, making possible a study of this nature. It should be emphasized that the experiment being reported is preliminary since not all conditions of the experiment were ideal.

Materials and Methods

The eight inbreds in the study were developed at Sugarbeet Investigations, ARS-USDA, Fort Collins, Colorado, and were inbred at least the equivalent of six generations of selfing. None were closely related. They were phenotypically very uniform within lines. All lines were considerably reduced in vigor. The inbred lines were converted to the tetraploid condition with colchicine. The tetraploids were in the fourth and fifth generations after colchicine treatment when they were used in the production of F_1 hybrids.

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By using root and hypocotyl color marker genes 12 single crosses were made, although all 12 crosses were not achieved at all ploidy levels, as indicated by the missing means in Tables 4 and 5. Several difficulties were encountered in making the crosses and in achieving adequate stands of some of the hybrids: 1) low seed germination of most 3n and 4n populations; 2) high levels of self and sib fertility which reduced the frequency of hybridization in some crossing plots; 3) flowering dates of certain inbreds did not coincide well which also reduced the frequency of hybridization; and 4) contamination of certain tetraploid increase plots by pollen from diploid plants or reversion of certain individuals within the plot to the diploid, triploid, or aneuploid condition. This latter difficulty resulted in the elimination of two triploid hybrids from the analyses of the experiment. The difficulty with spontaneous aneuploidy in tetraploid lines seems to have no solution at this time. Rommel (3)[3] considers 4n varieties or lines to be a mixture of euploid and aneuploid plants, and that, in each 4n variety, a certain level of aneuploidy is maintained by reproduction of aneuploids from euploid plants. Bosemark (1) states that under certain conditions a single generation is sufficient to raise the frequency of aneuploids in tetraploids from 0 to 40%. He further states that 45% of all pollen from 4n plants is chromosomally unbalanced. Bosemark (2) has shown a yield reduction in his polyploid varieties due to aneuploidy. He estimates this to be about 10% *per se* but practically only 3% due to the compensating effects of adjacent euploid plants. If this detrimental effect of aneuploidy was general it would tend to reduce any beneficial effect of triploidy.

The hybrid seed used in the experiment was developed during two summers in spatially isolated plots. As few as 10 parent plants of certain tetraploids were used in these isolations; but more than 50 plants of each inbred were generally used. In the crossing plots pollen diameter was measured on all the tetraploid parents. Those plants with smaller than average pollen were rogued prior to flowering. Seed of all triploid and tetraploid hybrids was germinated and chromosome counts were made from root tip preparations. Chromosome counts were not made with sufficient precision to establish exact aneuploid proportions; but aneuploids appeared to be equally numerous among all 3n and 4n hybrids, most of which had fewer than 27 or 36 chromosomes.

The frequency of hybrids was very low in certain crosses. Quantity of seed planted in the experiments was increased for these crosses, but despite this a complete stand of hybrids was not possible in some plots. Plots were thinned to hybrid plants

[3] Numbers in parentheses refer to literature cited.

where possible, otherwise plants of the female parent were left to provide a complete stand. At harvest only plants identified as hybrids by marker genes were retained for analysis. Plot yields were adjusted to an average of 18 roots per 19-foot single-row plot. Due to low levels of competition (inbreds left in the plot to provide a complete stand were less vigorous than hybrid plants), the data for root yield were quite variable. This fact should be kept in mind when reviewing the root yield and recoverable sugar data. This need be only a minor consideration for all other characters.

The experimental materials were planted at both Rocky Ford and Fort Collins, Colorado, in March and April 1967, respectively. Initially there were 48 hybrids in the experiment and a commercial check. In the Rocky Ford test four hybrids were deleted because of low frequency of hybrids and two because of doubtful ploidy level. In the Fort Collins test the same six hybrids and one additional one were deleted from the root yield and sucrose analyses; six additional hybrids (total of 13) were deleted from all other analyses due to insufficient quantities of thin juice. The single-row plots were bordered on each side by a medium vigor competitor (22 inches between rows). The open-pollinated check variety, American #2 Monogerm, was adapted at the Rocky Ford location but was not considered to be adapted in northern Colorado at Fort Collins.

We measured root yield (kilograms adjusted to 18 roots per plot) and percent sucrose at both locations. In addition we measured parts per million of potassium (K), sodium (Na), amino nitrogen (amino N), and an impurity index of the lead defecated sucrose filtrate at Rocky Ford. This impurity index was calculated as follows:

$$\text{Impurity Index} = \frac{3.5\ \text{Na} + 2.5\ \text{K} + 9\ \text{Amino N}}{\%\ \text{sugar on beet}}$$

At Fort Collins the characters determined in thin juice (phosphoric acid method) were apparent purity (%), net recoverable sugar (kilograms per plot, based on root yield, sucrose and purity), potassium, sodium, and total nitrogen (the latter three in mg/100 ml thin juice equated to a refractive dry substance of 10).

Results

The analysis of variance in Table 1 included only those nine hybrids which were represented at all three ploidy levels (2n, 3n, and 4n) at both locations. The 3n data used were means of reciprocal triploids, 4n ♀ × 2n ♂ and 2n ♀ × 4n ♂ [hereafter designated as 3n (4n ♀) and 3n (4n ♂)], if both were present but only one of these if its reciprocal was missing. Only root

Table 1.—Analyses of variance of root yield and percent sucrose for hybrids, ploidy levels, locations, and their interactions.

Source of variation	df	Root yield MS	Sucrose MS
Hybrids	8	13.9220	1.8226*
Ploidy levels	2	0.8405	1.2459
Locations	1	21.1437	118.6074**
H X P	16	3.2314	0.2210
P X L	2	2.1641	0.5836*
H X L	8	21.9133*	0.4032*
Residual	16	8.1857	0.1436
Total	53		

* and ** denote significance at the 5% and 1% level, respectively.

yield and sucrose content were included; these were the only two characters common to both locations. The absence of significant differences between ploidy levels (Table 1) indicated that genome addition did not have a general effect on root yield or sucrose content. The significant ploidy level by location interaction for sucrose indicated that the 2n, 3n, and 4n hybrids did not maintain the same relationship at both locations. The absence of a hybrid by ploidy level interaction for both root yield and sucrose indicated that there was no differential response of genotype to ploidy level, but it must be kept in mind that the 3n data used were actually means of 3n (4n ♀) and 3n (4n ♂) hybrids. It will be noted later, where reciprocal triploids were separated, that there were a few significant differences for root yield and sucrose content between ploidy levels within hybrids within locations. There was a significant hybrid by location interaction for both root yield and sucrose, but this is simply a genotype by environment interaction which has been repeatedly observed and reported.

Another set of comparisons of interest for all characters was sets of 2n, 3n (4n ♀), 3n (4n ♂), and average of all 3n hybrids within locations. At Fort Collins the eight hybrids noted in Table 2 were the set which had all 2n hybrids and 3n reciprocals present for root yield and sucrose. For the other five characters only five hybrids out of 12 had all 2n hybrids and 3n reciprocals present. The 4n equivalents were excluded from these comparisons, since they were of least interest and their inclusion would have further reduced the completely comparable set. Table 3 shows these same comparisons at Rocky Ford with a set of nine hybrids. Standard errors for the means in Tables 2 and 3 were calculated from the variance within ploidy levels within locations. Individual t tests were made comparing those means of interest. The following observations were made from Tables 2 and 3. At both locations the set of 2n hybrids had a significantly larger root yield than the 3n (4n ♂) hybrids. The set of 3n (4n ♀)

Table 2.—Means of orthogonal sets of single crosses at Fort Collins; for root yield and sucrose the set was hybrids 1, 2, 3, 4, 6, 7, 10, and 11, while for all other characters the set was hybrids 1, 4, 6, 7, and 10 (see Table 4 for hybrid descriptions).

Ploidy class	Root yield (kg/plot)	Sucrose (%)	Recov. suc. (kg/plot)	Purity (%)	Potassium (mg/100ml)	Sodium (mg/100ml)	Total N (mg/100ml)
2n	14.74±0.51	15.35±0.14	1,938±0.091	92.15±0.37	89.26±3.18	41.61±2.54	63.26±2.51
3n (4n ♀)	15.04±0.39	15.26±0.13	2.001±0.066	91.64±0.34	85.54±2.18	45.40±2.71	66.53±2.46
3n (4n ♂)	13.31±0.43	14.57±0.10	1.746±0.060	91.43±0.42	102.78±3.08	49.96±3.10	75.51±2.71
Ave. 3n	14.18±0.30	14.92±0.08	1.874±0.049	91.54±0.30	94.16±1.63	47.69±2.41	71.02±2.17
Am. #2 Mono.	14.00±0.74	14.40±0.25	1.666±0.101	91.43±0.53	86.55±5.11	52.99±5.77	76.78±7.75

Table 3.—Means of an orthogonal set of single crosses at Rocky Ford; for all characters the set was hybrids 1, 2, 3, 4, 5, 6, 7, 10, and 11.

Ploidy class	Root yield (kg/plot)	Sucrose (%)	Amino N (ppm)	Potassium (ppm)	Sodium (ppm)	Impurity index
2n	13.78±0.28	18.06±0.08	2154±76	2015±18	359±10	469±8
3n (4n ♀)	14.53±0.44	17.94±0.11	2294±70	1998±18	395±12	486±9
3n (4n♂)	12.89±0.34	17.91±0.09	2355±115	2011±29	420±13	498±9
Ave. 3n	13.71±0.29	17.92±0.08	2325±80	2004±18	407±9	492±7
Am. #2 Mono.	15.43±0.86	17.56±0.16	2250±20	1889±63	473±36	479±24

hybrids had nonsignificant but larger means at both locations than the 2n set. At both locations the 3n (4n ♀) hybrids had significantly larger root yield than their 3n (4n ♂) reciprocals. For sucrose content the only significant differences occurred at Fort Collins. There the set of 2n hybrids had significantly greater sucrose than the 3n (4n ♂) hybrids. Also, the 3n (4n ♀) hybrids had significantly greater sucrose than their 3 n (4n ♂) reciprocals. There were no differences in thin juice purity. However, the thin juice impurity components potassium, sodium, and total nitrogen at Fort Collins were significantly less in the 2n hybrids than in the 3n (4n ♂) hybrids. The 3n (4n ♀) hybrids had significantly less potassium and total nitrogen than their reciprocal set of 3n hybrids. At Rocky Ford the 2n hybrids had significantly less sodium in the sucrose filtrate than both the 3n (4n ♀) or 3n (4n ♂) hybrids.

Individual hybrid means, within ploidy levels, for root yield are shown in Table 4. Of particular interest was triploid hybrid 1, (4n ♀), at Fort Collins and triploid hybrid 6, (both 4n ♀ and ♂), at Rocky Ford both of which had significantly larger root yield than their comparable diploids. There were no significant differences in root yield between the individual 3n reciprocal hybrids at Fort Collins, but two such reciprocal differences occurred at Rocky Ford, hybrids 5 and 11, where the 3n (4n ♀) was superior. Significant inferiority of 3n hybrids to 2n occurred in two cases at Rocky Ford; no cases occurred at Fort Collins.

The individual hybrid means for sucrose content are tabulated in Table 5. There were a few significant differences between different ploidy levels within particular hybrids; these did not tend to be the same hybrids at both locations. In hybrid 11 at Fort Collins the 3n (both 4n ♀ and 4n ♂) had significantly greater sucrose than the 2n. In hybrid 3 at Rocky Ford the 3n (4n ♂) had significantly greater sucrose than the 2n. Inferiority of one or the other 3n to its comparable 2n occurred in hybrids 2, 4, and 10 at Fort Collins and in hybrids 6 and 10 at Rocky Ford. There were reciprocal 3n differences at both locations. Four 2n hybrids had significantly greater sucrose than their 4n equivalents at Fort Collins. In no case at either location was the 4n significantly greater in sucrose than the 2n.

Individual hybrid means were not tabulated for the other characters. There were very few differences between individual 2n and 3n hybrids for these other characters.

All possible combinations of simple correlation coefficients were calculated within ploidy levels within locations. At Fort Collins genome substitution appeared to have been a factor

Table 4.—Root yield means (kg per plot) of F_1 hybrids at the different ploidy levels and check at Fort Collins and Rocky Ford. (Duncan's multiple range tests are between ploidy levels, within hybrids, within locations; means followed by the same letter were not significantly different at the 5% level. LSD values allow comparison of 2n hybrids with the check.)

Population and No.	Fort Collins				Rocky Ford			
	2n	3n (4n ♀)	3n (4n ♂)	4n	2n	3n (4n ♀)	3n (4n ♂)	4n
1. 50-406 X 52-408	14.05bc	19.58a	15.23ab	9.30c	15.26a	14.94a	13.88a	17.31a
2. 52-430 X 54-520	11.98a	11.42a	8.11a	12.08a	11.59a	11.75a	13.71a	11.75a
3. 50-406 X 52-407	14.14a	15.27a	13.80a		14.69a	15.51a	12.16a	
4. 50-406 X 54-520	12.28a	13.40a	10.16a	12.14a	13.39a	13.88a	14.12a	12.82a
5. 52-430 X 54-480	16.06a	13.41a			15.18a	15.75a	10.45b	
6. 52-430 X 52-307	15.25ab	15.13ab	13.13b	19.88a	12.08b	17.39a	16.24a	16.16a
7. 34 X 54-520	18.48a	17.03a	18.63a	15.24a	12.90a	11.92a	13.39a	11.92a
8. 52-430 X 52-408	16.03a		12.72a	14.04a	13.96a		14.78a	13.96a
9. 34 X 52-307	19.54a	18.04a		17.61a	12.98a	12.65a		10.45a
10. 50-406 X 52-307	14.68a	13.14a	14.91a	11.25a	12.98b	13.31b	13.47b	20.73a
11. 50-406 X 54-480	17.04a	14.72a	12.49a		15.84a	16.24a	8.57b	
12. 52-430 X 52-407	15.71ab		19.80a	13.92b	13.63a		12.33a	13.71a
13. Am. #2 Mono. (check)	14.00				15.43			
LSD (0.05)	4.38				3.83			

Table 5.—Sucrose means (%) of F_1 hybrids at the different ploidy levels and check at Fort Collins and Rocky Ford. (Duncan's multiple range tests are between ploidy levels, within hybrids, within locations; means followed by the same letter were not significantly different at the 5% level. LSD values allow comparison of 2n hybrids with the check.)

Population and No.	Fort Collins				Rocky Ford			
	2n	3n (4n ♀)	3n (4n ♂)	4n	2n	3n (4n ♀)	3n (4n ♂)	4n
1. 50-406 X 52-408	15.73a	15.83a	14.93a	14.08b	18.19a	18.69a	18.50a	18.39a
2. 52-430 X 54-520	15.77a	14.82a	14.02b	14.77a	18.30a	17.75a	17.99a	17.80a
3. 50-406 X 52-407	15.72a	15.28a	14.70a		17.99b	18.04b	19.29a	
4. 50-406 X 54-520	15.07a	16.07a	14.58b	14.52b	18.39a	17.74a	17.59a	18.14a
5. 52-430 X 54-480	14.07a	14.90a			17.45a	18.02a	17.40a	
6. 52-430 X 52-307	15.60a	14.80a	15.43a	15.87a	18.45a	17.00b	17.89a	18.50a
7. 34 X 54-520	15.03a	14.48a	14.20a	13.93a	17.50a	16.87a	17.27a	16.75a
8. 52-430 X 52-408	15.30a		15.15a	14.97a	17.92a		17.70a	18.35a
9. 34 X 52-307	14.95a	14.32a		13.42b	16.40a	16.34a		15.90a
10. 50-406 X 52-307	16.12a	15.65a	14.62b	14.10b	18.50a	19.14a	17.45b	18.69a
11. 50-406 X 54-480	13.78b	15.15a	14.08ab		17.82a	18.27a	17.82a	
12. 52-430 X 52-407	14.08a		13.60a	14.57a	17.95a		17.62a	17.97a
13. Am. #2 Mono.	14.40				17.56			
LSD (0.05)	1.10				0.81			

only in the correlation of purity with potassium, sodium, and total nitrogen. Purity was highly significantly correlated with each of these characters in all ploidy levels except 3n (4n ♂) where the three correlations were not significantly different from zero. At Rocky Ford there were a few correlations quite different among hybrid groups, but these differences in correlation were sporatic and did not appear to be related to genome addition or substitution.

Discussion

A primary objective of the experiment was to detect a general or specific effect of genome addition as exhibited by triploid hybrids. When reciprocal triploids were averaged for the entire experiment, there was no general effect on root yield or sucrose content due to genome addition. The significant ploidy level by location interaction for sucrose indicated that a particular ploidy level that is best in one environment may not be best in other environments. The absence of a hybrid by ploidy level interaction indicated that when the means of reciprocal triploids were compared with diploids and tetraploids the ploidy levels ranked essentially the same in all genotypes tested. However, this was not the case when reciprocal triploids were considered separately within locations. In this latter case there was evidence that 2n and 3n (4n ♀) hybrids were superior for root yield and sucrose to the 3n (4n ♂) hybrids. There were also indications of lower quantities of impurities in the 2n and 3n (4n ♀) hybrids than in the 3n (4n ♂) hybrids. These differences resulted primarily from the effect of these ploidy levels within specific hybrids.

There was one hybrid at each location where the diploid root yield was exceeded by that of the 3n (4n ♀) hybrid. For sucrose there was one triploid hybrid at each location, a 3n (4n ♀) in one case and a 3n (4n ♂) in the other, that exceeded its diploid equivalent. There was a possibility that the root yield differences could have partly resulted from adjustment of yield data to a complete stand.

There did not appear to be any consistent differences in correlations of the various characters due to genome addition.

Even though this experiment indicated that there was no general beneficial or detrimental effect from the addition of one genome into F_1 hybrid genotypes, there may be specific genotypes which respond either in the desirable or undesirable direction when one genome is added. There was some evidence that this response may depend on the parent from which this additional genome came, since there were differences between specific 3n reciprocals with the 3n (4n ♀) hybrids usually being superior.

If this is the case triploid hybrid breeding programs will have to involve specific test crosses to determine which genotypes responded beneficially to genome addition. This would be little different than single cross tests for specific combining ability; in fact, a beneficial response due to genome addition could be classed as a form of heterosis. Not only would test crosses for triploid effect have to be made, but also reciprocal triploid test crosses. With $\frac{n(n-1)}{2}$ single crosses possible from n inbreds, excluding reciprocals, n (n-1) triploid single crosses would be possible.

If one then projects this to the double cross hybrid level, eight inbred lines in both the 2n and 4n conditions would lead, excluding reciprocal single crosses, to n (n-1) (n-2) (n-3)/4 = 420 double cross triploid hybrids instead of the n (n-1) (n-2) (n-3)/8 = 210 possible diploid double cross hybrids. This examination of single-cross and double-cross combination would be extremely laborious and expensive. Whether or not some sort of "top-cross" test might be developed to detect genotypes which do particularly well at the triploid level cannot be predicted at this time; however, such a possibility should be investigated. A triploid hybrid breeding procedure, then, would depend on the relative benefit of hybrid vigor and triploid effect. If heterosis was most important, then breeding lines with high general combining ability might next be tested for high specific combining ability and specific triploid combining capacity. Combinations chosen for hybrids would then be a function of the relative advantage of heterosis to triploid effect. This experiment provides no evidence of this relationship.

The genotype by triploid interaction in this study could possibly be related to a general or chromosome specific detrimental effect of aneuploidy, if the frequency of aneuploidy differs among genotypes. However, in this study there appeared to be no great difference in aneuploid frequency among reciprocal 3n and 4n genotypes.

Further research is needed on several problems in this area, namely: 1) establish more clearly whether the general condition of addition of one genome is universally beneficial, detrimental, or immaterial; 2) establish whether genotype by triploid interactions are primarily or solely important with respect to beneficial effects of addition of one genome; 3) determine the frequency of useful genotype by triploid interactions; 4) if specific beneficial effects of triploidy are significant, determine whether or not they are in any way related to combining ability or heterosis at the 2n level; 5) determine if the frequency of aneuploids is different among triploid genotypes and if differences

affect performance; 6) establish more clearly whether reciprocal 3n hybrids are different, and if so, which parent should be tetraploid; 7) determine how genotypes, which result in a beneficial genotype by triploid interaction, can be most efficiently identified; and 8) determine the type of genetic action or enzyme relationships which lead to a favorable interaction. Answers to these questions await synthesis of numerous tetraploid genotypes, some from cytoplasmic male sterile females, so that external problems in developing the necessary populations will be eliminated.

Summary

An experiment was conducted for 1 year at two locations to compare the same F_1 hybrid sugarbeets as diploids (2n), reciprocal triploids (3n), and tetraploids (4n). Eight inbred lines were converted to the 4n condition and 12 F_1 hybrid combinations were made using seedling marker genes; some of the 12 4n hybrids and some of the 24 reciprocal 3n hybrids were not included in the analyses, due to low frequency of hybrids. Root yield data were quite variable due to adjustment for stand, necessitated by the low frequency of hybrids in some crosses and poor germination of certain 3n and 4n hybrids. This factor should have had little effect on sucrose content, quality measurements, and impurity components.

The experiment indicated that there was no general beneficial or detrimental effect on root yield or sucrose content from addition of one genome into F_1 hybrid genotypes. However, for root yield and sucrose content there were specific genotypes which responded favorably to the addition of a genome in specific environments. Significant differences between reciprocal 3n hybrids indicated that this response may depend on the parent from which this additional genome came. Most instances of triploid advantage occurred in those hybrids where the 4n parent was used as the female. The effect of genome addition on quality and impurity components was detrimental or immaterial. Any advantage due to triploidy would most likely be genotype specific and result from an increase in root yield and possibly sucrose, accompanied by the possibility of a detrimental effect on quality. Those genotypes which respond to genome addition would have to be identified in some type of combining test. This would add greatly to the complexity of a triploid hybrid program unless some direct relationship between triploid effect and specific combining ability could be found.

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