

Population Genetic Studies on the Total Nitrogen in Sugar Beets (*Beta Vulgaris* L.)^{1, 2}

MERLE G. PAYNE², LEROY POWERS³, AND GRACE W. MAAG²

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The increased use of nitrogen fertilizers in the production of sugar beets has emphasized the importance of chemical genetic studies involving the amount of nitrogen in the "thin juice" obtained during the processing of the sugar beet in the manufacture of sugar. Population genetic studies were conducted on the milligrams of nitrogen per 100 milliliters of thin juice equated to a refractive dry substance of 10. The analyses were made on the thin juice derived from the root of the sugar beet (*Beta vulgaris* L.).

Material, Experimental Design, Methods, and Analyses

Materials

The populations are A54-1, A54-1BB, 50-406, 50-406BB, F₁ hybrid, and 52-307. A54-1 is a commercial variety and A54-1BB resulted from seed harvested from 25 mother beets of A54-1. The 25 mother beets giving rise to population A54-1BB were grown in an isolated seed plot along with 25 mother beets for each of 22 other populations. This isolation plot was composed of 25 rows. One mother beet from each population occurred at random in each row, making a total of 23 mother beets per row. Seed was harvested from all mother beets on an individual plant basis. Seed from each mother beet of A54-1 was bulked and thoroughly mixed to produce the seed lot from which A54-1BB was grown. Seed saved from mother beets of 50-406 and handled in a similar manner produced the population designated as 50-406BB. Populations 50-406 and 52-307 are inbreds and the F₁ hybrid population resulted from crossing them. The inbred 50-406 has green hypocotyls and 52-307 has red hypocotyls. The seed used to produce the F₁ hybrid plants was harvested from mother beets of 50-406, and the plots of the F₁ hybrid population were thinned to red-hypocotyl plants.

There were two treatments, fertilized and non-fertilized. The fertilized plots received a surface application of 100 pounds of

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² Associate Professor of Chemistry and Associate Chemist and Instructor in Chemistry and Research Assistant, respectively, Colorado State University.

³ Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

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N (nitrogen) and 250 pounds of P_2O_5 (phosphorus pentoxide) per acre on April 4, 1956. The fertilizer was cultivated under with a rototiller. The plots were planted on April 10 and 11. On June 26, another 100 pounds of N per acre was drilled in the center of each space between rows of the fertilized plots.

Experimental Design

The sources of variation and number of items are shown in Table 1. There are 40 replications. Treatments are a split plot of replications; the two treatments are randomized within each replication. The six populations are randomized within each treatment and the eight locations are within each plot of each population. Each location within a plot is composed of a single plant. The plots were single rows running from north to south. Hence, any differences due to locations represent a gradient running from north to south. Locations are a split plot of populations and the design is a split-split plot.

Table 1.—Sources of Variation Designated as Main Effects, and Number of Each.

Main Effects	Number
Replications	40
Populations	6
Treatments	2
Locations	8

Every plot was bordered by a row of A54-1. After thinning, each plot had 12 plants and the stand remained excellent during the growing season. Data were taken on only eight plants, the extra plants at each end of the plot being discarded at time of harvest. The rows were spaced 22 inches apart and the plants were thinned to a spacing of 20 inches within the row. Selection was avoided during thinning by saving the plant farthest south in a "blocked group."

Methods

The total nitrogen was determined on thin juice from individual beet roots. The method used was a modified micro-Kjeldahl nesslerization (2)⁵

Micro-Kjeldahl digestion

Frozen juice was thawed and thoroughly mixed. To a pyrex glass digestion tube, marked at 35 and 50 ml, were added 0.5 ml of thin juice, 1.0 ml of oxidizing solution⁶ and four glass beads.

⁵ Numbers in parentheses refer to literature cited.

⁶ Oxidizing agent. Dissolve 10 g of sodium molybdate in 150 ml of distilled water. Add slowly 150 ml of concentrated sulfuric acid. Cool, and add 200 ml of perchloric acid (70-72%). Then mix well. For details of this method see Bolin, King, and Klosterman (1).

This may stand over night if a large number of samples are being run. It is digested from 20-30 minutes on medium or low heat.^{7, 8}

Nesslerization

The nesslerization process followed the modified techniques of G. W. Plaut (Enzyme Institute, University of Wisconsin, unpublished data). Nessler's reagent was prepared according to Koch and McMeekin (2).

The digestion tube was cooled and 15 ml of distilled water were added. To this tube were added 1 ml of 2% gum ghatti and 8 ml of Nessler's solution. The Nessler's solution was blown directly into the middle of the tube. The mixture was made up to 50 ml with distilled water and rotated for mixing.

Maximum color is reached after about 10 minutes. The optical density of the solution was read at a wave length of 490 millimicrons in a Beckman Model B spectrophotometer. Standard solutions were prepared using ammonium sulfate in the concentration range of 10-500 micrograms per ml. The micrograms of nitrogen per ml were plotted as the ordinate and the optical density as the abscissa. The slope of the straight line was used as a factor with the optical density reading of the sample to determine total nitrogen. The total nitrogen was reported as milligrams of nitrogen per 100 ml of thin juice with an RDS (refractive dry substance) of 10. For example:

$$\frac{10 \text{ RDS}}{13.14 \text{ (RDS of sample)}} \times 42.00 \text{ (mg of nitrogen per 100 ml of sample)} = 32.00 \text{ mg of nitrogen per 100 ml of thin juice with an RDS of 10.}$$

Analyses

The methods used in analyzing the data are those given by Powers (3, 4, 5) and Powers et al. (6, 7, 8, 9); for details of the calculations, the reader is referred to these articles. Primarily the analyses were made by employing the analysis of variance, the partitioning and components of variance methods of genetic analysis, and regression.

Results

The results are discussed under the general headings: means, variances, and frequency distributions. The means and variances are divided further into main effects and interactions. Comparisons made in the discussion of the data involve significant differ-

⁷ No explosions have resulted with this type of heat.

⁸ A study was made of the possible error introduced by the presence of inorganic nitrogen in the samples. It was concluded that the digestion process picks up some inorganic nitrogen in samples of low total nitrogen but in samples of high total nitrogen the inorganic nitrogen had little effect.

ences unless otherwise stated. For sake of clarity in emphasizing the biological deductions, tests of significance will not be given. In this article milligrams of nitrogen per 100 milliliters of thin juice equated to a refractive dry substance of 10 will be termed total nitrogen.

Table 2.—Analysis of Variance for Total Nitrogen, Data Converted to Logarithms.

Source of Variation	Smaller M. S.	Mean Square	Degrees of Freedom	F Value	F Value for	
					5% Level	1% Level
Replications		1.5937b	39	8.27	1.69	2.11
Populations		9.0939a	5	121.90	2.26	3.11
Treatments		129.2414b	1	670.69	4.08	7.31
Locations		0.0338c	7	1.38	2.05	2.73
R X P	a	0.0746d	195	1.06	1.26	1.59
R X T	b	0.1927d	39	2.74	1.45	1.69
P X T	c	0.9981d	5	14.18	2.26	3.11
R X P X T	d	0.0704c	195	2.87	1.17	1.25
Remainder	e	0.0245	3288 ¹			

¹ Degrees of freedom for remainder reduced by 65, the number of plants for which there were no data; values replaced by average of corresponding plot

Means

Analysis of variance

The analysis of variance for total nitrogen is given in Table 2. The smaller mean squares used to calculate the F values are designated by the letters in column 2 and the letters following the mean square values in column 3. The data of individual plants were converted to logarithms. However, since the biologist is primarily interested in the data expressed on the arithmetic rather than the logarithmic scale, the arithmetic means are given. For these data the interpretation and biological conclusions are the same whether the data are expressed on the logarithmic or arithmetic scale. These facts are pointed out because those more interested in statistical procedure may prefer to use the logarithmic scale throughout to interpret the data and draw conclusions. The data were converted to logarithms because the data on the logarithmic scale followed the normal probability integral but did not do so on the arithmetic scale.

Whether there are significant differences between main effects and whether interactions are statistically significant can be determined by comparing the F values in column 5 with those in columns 6 and 7. There are significant differences for replica-

tions, populations, and treatments. The first order interactions of replications X treatments, and populations X treatments are significant. The second order interaction of replications X populations X treatments is significant.

Table 3.—Means for Total Nitrogen for Populations, Treatments, and Populations X Treatments.

Population	Treatment		Average
	Fertilized	Non-Fertilized	
	mg	mg	mg
A54-1	46.8	18.8	32.8
A54-1BB	44.8	16.9	30.8
Average	45.8	17.8	31.8
50-406BB	33.6	12.6	23.1
50-406	31.2	14.6	22.9
Average	32.4	13.6	23.0
F ₁ hybrid	21.3	9.8	15.6
52-307	18.6	11.1	14.8
Average	20.0	10.4	15.2
Grand average	32.7	14.0	23.3

Main effects

The means of the main effects are listed in Table 3. The F values for populations and treatments compared with their respective F values at the 1 percent and 5 percent levels of significance reveal that there are significant differences between populations and between the two treatments. There are three levels of total nitrogen for populations. Populations A54-1 and A54-1BB compose the high level, 50-406 and 50-406BB the intermediate level, and the F₁ hybrid and 52-307 the low level. The data for 50-406, the F₁ hybrid, and 52-307 furnish evidence as to dominance relations in this cross. The average values for these three populations are 22.9, 15.6, and 14.8, respectively. Clearly in the cross produced by hybridizing the two inbreds (50-406 and 52-307), on an average, lower total nitrogen in the thin juice is nearly if not completely dominant. This deduction is supported to some extent by the values of 23.1 and 22.9 for populations 50-406BB and 50-406 and perhaps to a lesser extent by the values of 32.8 and 30.8 for populations A54-1 and A54-1BB.

If the data from A54-1 and A54-1BB support the complete dominance hypothesis, then on an average the varieties, strains, and inbreds which acted as male parents to produce the A54-1BB population averaged higher in total nitrogen than did A54-1.

First- and second-order interactions

The first order interaction of populations X treatments is shown in Table 3. The differences between populations belonging to different levels of total nitrogen are greater on the fertilized plots than differences involving corresponding comparisons on the non-fertilized plots. For example (45.8-20.0)-(17.8-10.4) giving a value of 18.4 is significantly different from zero. In testing significance t was employed and 18.4 is divided by 2 and the appropriate standard error. Similar comparisons within levels do not approach statistical significance and the differences involved are of such small magnitude, comparatively, as to have little if any practical significance even though the differences were statistically significant. Of considerable practical importance to a breeding program is the fact that the amount of total nitrogen for population A54-1 on the non-fertilized plots is not materially different from the amount of total nitrogen for the F_1 hybrid and 52-307 populations on the fertilized plots. That is, the values of 18.8, 21.3, and 18.6 are not significantly different. Hence, the differences due to treatments and differences due to populations are approximately of the same magnitude.

Such a finding lends considerable encouragement to the plant breeder as it provides evidence that beets can be bred for either low or high nitrogen content. Further it shows that in this study genetic control (represented by populations) of total nitrogen is of about the same magnitude as the environmental control (represented by the two fertilizer treatments).

The replications X populations X treatments means for total nitrogen are listed in Table 4. For clarity in presentation, the data for 8 contiguous replications are grouped and presented as averages. This makes five groups for the 40 replications. The differences in total nitrogen between populations are greatest on the fertilized plots and for the replication group 33-40. In other words the differences between populations are greatest for the replication group and fertilizer treatment having the highest concentration of total nitrogen in the thin juice. Another observation of interest is that on both the fertilized and non-fertilized plots the F_1 hybrid has more nitrogen in the thin juice for those replication groups averaging more than 23 mg of total nitrogen (per 100 ml of thin juice) than does the inbred parent 52-307. The reverse is true for those replication groups averaging less than 23 mg of total nitrogen (see non-fertilized, Table 4). That is, for the replication groups 1-8, 9-16, 17-24, and 25-32 on the non-fertilized plots the F_1 hybrid has less total nitrogen

Table 4.—Means of Total Nitrogen per 100 ml for the Interaction of Replications X Populations X Treatments.

Treatment and Population	Replication Groups					Average
	1-8	9-16	17-24	25-32	33-40	
Fertilized						
	mg	mg	mg	mg	mg	mg
A54-1	38.53	34.99	38.85	45.90	75.79	46.81
A54-1BB	34.81	37.42	33.87	50.80	67.05	44.79
50-406BB	26.21	25.71	28.01	27.64	60.35	33.58
50-406	26.35	24.25	28.28	30.53	46.80	31.24
F ₁ hybrid	17.41	17.86	18.53	20.32	32.23	21.33
52-307	14.05	16.12	17.36	19.90	25.71	18.63
Average	26.23	26.06	27.53	32.52	51.32	32.73
Non-fertilized						
A54-1	13.78	12.54	11.89	19.77	36.27	18.85
A54-1BB	12.54	13.15	12.50	17.46	29.04	16.94
50-406BB	8.92	10.87	9.31	11.08	22.92	12.62
50-406	10.96	11.29	10.52	14.66	25.39	14.56
F ₁ hybrid	8.49	8.73	7.27	9.84	14.75	9.82
52-307	9.02	10.79	8.43	12.55	14.55	11.07
Average	10.62	11.23	9.99	14.23	23.82	13.98
Grand average	18.43	18.65	18.76	23.38	37.57	23.36

than either inbred parent, 50-406 or 52-307. Thus heterosis for less nitrogen is occurring at the lower fertility levels.

The greatest difference between populations within a fertilizer treatment (75.79-25.71), or 50.08, is greater than the greatest difference between fertilizer treatments within a population (75.79-36.27), or 39.52. The genetic difference represented by populations is larger than the environmental difference represented by treatments. Hence the means for the interaction of replications X populations X treatments support the deduction from the interaction of populations X treatments. These results provide convincing evidence that beets can be bred under the conditions of this experiment for either low or high nitrogen content in the thin juice. This is particularly true for the fertilized plots and for replications in which larger quantities of nitrogen are available to the sugar beet plant.

Variations

Analysis of variance of the within-plot variances

The analysis of variance of the within-plot variances for total nitrogen in the thin juice is given in Table 5. The original data were converted to logarithms. Whether there are significant

Table 5.—Analysis of Variance of the Variances for Total Nitrogen; Data Converted to Logarithms.

Source of Variation	Mean Square	Degrees of Freedom	F Value	F Value for	
				5% Level	1% Level
Replications	0.00046638	39	1.41	1.45	1.69
Populations	0.00632655	5	19.10	2.26	3.11
Treatments	0.00040307	1	1.22	3.89	6.76
R X P	0.00039174	195	1.18	1.26	1.39
R X T	0.00036187	39	1.09	1.45	1.69
P X T	0.00063976	5	1.93	2.26	3.11
R X P X T	0.00033115	195			

differences for main effects can be determined by comparing the F values in column 4 with those in columns 5 and 6. An examination of Table 5 reveals that the only significant differences are between populations and that these differences are highly significant. The large F value for populations could be due to differences between segregating and non-segregating populations. It is desirable to know whether the differences between populations in the homogeneous material are statistically significant. An analysis of variance of the variances involving only the data for homogeneous populations was made. The procedures used in making the analysis are the same as that shown in Table 5 with the exception that the number of populations is 3 instead of 6. The F value for populations in this latter test is 5.88 and that for the 1% level is 4.88. At least some of the differences between the non-segregating populations are statistically significant. This information is useful in deciding whether to use regression in estimating the environmental variances of the heterogeneous populations.

The means and within-plot obtained, estimated, and residual variances [(R X L) + L] for total nitrogen are listed in Table 6. That conversion of the original data to logarithms normalized it will be shown later during the analyses of the frequency distributions. Methods of testing the reliability of the residual variances are provided by Powers, Robertson, and Remmenga (8).

Regression was used to estimate the environmental variances of Table 6. Regression was calculated within fertilizer treatments and hence n is 3 instead of 6 as was the case in the article by Powers et al (8). This information is necessary if the reader wishes to calculate the values in column 4 of Table 6.

Table 6.—Means and Within-plot Obtained, Estimated, and Residual Variances $[(R \times L) + L]$ for Total Nitrogen for Treatments and Populations; Data Converted to Logarithms.

Treatments and Populations	Means	Variance		
		Obtained	Estimated	Residual
Fertilized				
A54-1	1.607108	0.037264	0.024402	0.012862
A54-1BB	1.587479	0.037772	0.023768	0.014004
50-406BB	1.445778	0.031687	0.019188	0.012499
50-406	1.453922	0.019888	0.019451	0.000437
F ₁ hybrid	1.291699	0.012581	0.014208	-0.001624
52-307	1.231595	0.013452	0.012965	0.001187
Non-fertilized				
A54-1	1.183687	0.027436	0.023115	0.004321
A54-1BB	1.156908	0.032528	0.022338	0.010190
50-406BB	1.023635	0.026817	0.018473	0.008344
50-406	1.097637	0.020946	0.020619	0.000327
F ₁ hybrid	0.947119	0.016740	0.016255	0.000485
52-307	1.007105	0.017182	0.017994	-0.000812

An examination of the variances and means for populations 50-406, F₁ hybrid, and 52-307 (homogeneous populations) reveals that on the whole there is a positive relation between the means and obtained variances within treatments. For these populations this relation does not hold between treatments. That is, the means are larger on the fertilized plots than on the non-fertilized plots and the reverse is true for the variances. Consequently the within-treatment regressions were used to estimate the environmental variances.

Linear regression of the obtained variances on the means for the non-segregating populations (50-406, F₁ hybrid, 52-307) accounted for 87.5 percent of the variability of the variances on the fertilized plots and 90.9 percent of that on the non-fertilized plots. The use of linear regression to estimate the environmental variances of the segregating generations is justified.

The residual variances listed in column 5 of Table 6 are significantly different from zero for the segregating populations (A54-1, A54-1BB, and 50-406BB), indicating considerable genetic variability in these populations for total nitrogen in thin juice. The residual variances for population A54-1BB are larger than those for A54-1 on both the fertilized and non-fertilized plots. Apparently exposing mother beets of A54-1 to pollination by 22 other varieties or lines has resulted in a broader genetic base.

This finding has a bearing on the interpretation of the data as it indicates that a study of the frequency distributions will yield information of biological importance.

Frequency Distributions

The obtained frequency distributions of populations for total nitrogen in the thin juice expressed in numbers are listed in Table 7. The total number of individuals for each population within a fertilizer treatment is 320. In analyzing the data it is desirable to know whether, on the arithmetic scale, the environmental variability is following the normal probability integral. Goodness-of-fit chi squares were calculated for the non-segregating populations using the arithmetic scale. All the P values fell between 0.01 and 1-infinity. It is evident that the environmental variability is not following the normal probability integral on the arithmetic scale. An examination of Table 7 reveals that for both the segregating and non-segregating populations the data are skewed toward the lower values of the frequency distributions. This suggests that the data may be following the logarithmic scales.

Chi squares for goodness of fit were calculated to determine whether the environmental and genetic variabilities are following the logarithmic scale. For details of the methods employed here and immediately above see Powers (5). The results of the tests to determine whether the environmental variability and both the environmental and genetic variabilities are following the logarithmic scale are given in Table 8.

An examination of Table 8 reveals that for the non-segregating populations none of the P values are as low as 0.05 and that the total values for the two fertilizer treatments and grand total have P values falling between 0.20 and 0.10. From these results it seems logical to conclude that the data measuring the environmental variability follow the normal probability integral after conversion to logarithms. Such is not the case for the segregating populations. The only population having P values larger than 0.05 on both the fertilized and non-fertilized plots is A54-1BB. This indicates that the genetic variability for populations A54-1 and 50-406BB are not following the normal probability integral, whereas both the environmental and genetic variabilities are doing so for population A54-1BB. This difference in behavior between populations could be due to the segregation of comparatively few genes conditioning total nitrogen in populations A54-1 and 50-406BB, whereas more genes conditioning total nitrogen are segregating in population A54-1BB.

Table 7.—Frequency Distributions of Treatments and Populations for Total Nitrogen, Expressed in Numbers.

Treatment and Population	Upper Limit of Class, mg of N per 100 ml of Thin Juice																				
	0 to 7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119	126	133	140 and Over	
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
Fertilized																					
A54-1		9	31	41	48	34	32	31	18	18	7	14	8	6	11	4	3		1	1	
A54-1BB		12	29	42	47	48	37	32	12	14	13	7	8	5	6		2	3	1	2	
50-406BB	1	31	80	66	42	25	19	9	12	8	8	1	6	2	5		2	1	1	1	
50-406		11	72	71	75	30	28	13	6	5	4	2	2				1				
F ₁ hybrid	1	64	135	73	22	10	3	6	3	2	1										
52-307	5	94	125	57	22	12	3	2													
Non-fertilized																					
A54-1	21	138	90	31	9	7	4	5	5	1	2	1	2	2		2					
A54-1BB	22	141	92	35	16	2	2	2		2	3			2		1					
50-406BB	55	199	40	7	8	4		1		2	1	1	1	1							
50-406	33	169	76	22	9	1	3	1	3		2		1								
F ₁ hybrid	92	189	23	8	6	1	1														
52-307	55	202	47	14	1	1															

Table 8.—Goodness-of-Fit Chi Squares and P Values to Test Normal Distribution of the Frequencies for Total Nitrogen, Data Converted to Logarithms, Segregating and Non-Segregating Populations.

Segregation Status, Treatment, and Population	Chi Square	Degrees of Freedom	P Lies Between
Segregating			
Fertilized			
A54-1	17.5818	11	0.10 and 0.05
A54-1BB	9.7961	11	0.70 and 0.50
50-406BB	20.0924	9	0.02 and 0.01
Total	47.4703	31	0.05 and 0.02
Non-fertilized			
A54-1	31.6652	6	0.01 and 1—infinity
A54-1BB	5.9551	5	0.50 and 0.30
50-406BB	38.9728	4	0.01 and 1—infinity
Total	76.5931	15	0.01 and 1—infinity
Grand total	124.0634	41	0.01 and 1—infinity
Non-segregating			
Fertilized			
50-406	10.1791	7	0.20 and 0.10
F ₁ hybrid	8.0646	4	0.10 and 0.05
52-307	1.4626	4	0.90 and 0.80
Total	20.0063	15	0.20 and 0.10
Non-fertilized			
50-406	8.2790	4	0.10 and 0.05
F ₁ hybrid	1.4296	2	0.50 and 0.30
52-307	3.1227	3	0.50 and 0.30
Total	12.8313	9	0.20 and 0.10
Grand total	32.8376	24	0.20 and 0.10

The obtained and calculated frequency distributions, differences, and proportions of genetic deviates expected are listed in Table 9. For the method of analyzing the data shown in Table 9, see Powers, Robertson, and Clark (7) and Powers et al (9). The totals for number of genetic deviates expected in the lower classes of Table 9 are listed in the second to last column and those in the higher classes of Table 9 in the last column. The standard error of the binomial distribution may be used to determine whether the populations differ as to number of genetic deviates in the lower classes and the higher classes. Also

Table 9.—Obtained and Calculated Frequency Distributions and Differences and Proportions of Individuals in the Lower Classes and in the Higher Classes Expected to be Genetic Deviates for Total Nitrogen; Data Converted to Logarithms, Fertilized and Non-Fertilized Plots, and Segregating Populations.

Treatment, Population, and Distribution	Upper Limit of Class (mg per 100 ml) and Logarithms																			Genetic Deviates			
	(0 to 7)	(14)	(21)	(28)	(35)	(42)	(49)	(56)	(63)	(70)	(77)	(84)	(91)	(98)	(105)	(112)	(119)	(126)	(133)	(140)	Lower Values	Higher Values	
	0 to 0.8451	1.1461	1.3222	1.4472	1.5441	1.6232	1.6902	1.7482	1.7993	1.8451	1.8865	1.9243	1.9590	1.9912	2.0212	2.0492	2.0755	2.1004	2.1239	2.1461			
Fertilized	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
A54-1																							
Obtained		9	31	44	48	34	32	31	18	18	7	14	8	6	11	4	3		1	1	84	73	
Calculated		1	11	38	61	62	52	36	24	14	9	5	3	2	1	1	0	0	0	0	50	35	
Difference		8	20	6						4	-2	9	5	4	10	3	3		1	1	34	38	
Proportion		0.89	0.65	0.14						0.22	0.64	0.62	0.67	0.91	0.75	1.00		1.00	1.00	0.40	0.52		
A54-1BB																							
Obtained		12	29	42	47	48	37	32	12	14	13	7	8	5	6		2	3	1	2	41	61	
Calculated		1	13	44	67	64	50	33	21	12	7	4	2	1	1		0	0	0	0	14	27	
Difference		11	16							2	6	3	6	4	5		2	3	1	2	27	34	
Proportion		0.92	0.55							0.14	0.46	0.43	0.75	0.80	0.83		1.00	1.00	1.00	1.00	0.66	0.56	
50-406BB																							
Obtained		1	31	80	66	42	25	19	9	12	8	8	1	6	2	5		2	1	1	1	112	56
Calculated		0	5	55	101	82	44	20	8	3	1	1	0	0	0	0		0	0	0	0	60	15
Difference		1	26	25					1	9	7	7	1	6	2	5		2	1	1	1	52	43
Proportion		1.00	0.84	0.31					0.11	0.75	0.88	0.88	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00	0.46	0.77
Non-fertilized																							
A54-1																							
Obtained		21	138	90	31	9	7	4	5	5	1	2	1	2	2						159	31	
Calculated		4	124	134	45	10	2	1	0	0	0	0	0	0	0						128	3	
Difference		17	14				5	3	5	5	1	2	1	2	2						31	28	
Proportion		0.81	0.10				0.71	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00						0.19	0.90	
A54-1BB																							
Obtained		22	141	92	35	16	2	2	2		2	3			2						22	30	
Calculated		6	145	126	34	7	2	0	0		0	0			0						6	9	
Difference		16				9	0	2	2		2	3			2						16	21	
Proportion		0.73				0.56	0	1.00	1.00		1.00	1.00			1.00						0.73	0.70	
50-406BB																							
Obtained		55	199	40	7	8	4		1		2	1	1	1	1						55	26	
Calculated		30	231	55	4	0	0		0		0	0	0	0	0						30	4	
Difference		25			3	8	4		1		2	1	1	1	1						25	22	
Proportion		0.45			0.43	1.00	1.00		1.00		1.00	1.00	1.00	1.00	1.00						0.45	0.85	

homogeneity chi square can be employed for this purpose (Powers 3, 4) and perhaps is both easier to apply and more comprehensive.

There are no significant differences between populations with regard to the number of genetic deviates in the higher classes (last column of Table 9). However, the numbers of genetic deviates in the higher classes for both treatments and all populations are significantly different from zero.

Turning to a consideration of differences between populations for the lower classes (second to the last column of Table 9) there are significant differences between fertilizer treatments and between populations for both fertilizer treatments. The non-fertilized plots are lower in number of genetic deviates. These data furnish some evidence that breeding for low nitrogen at the higher fertility level would be more effective. A54-1BB is lower in genetic deviates than either of the other two populations. Again all of the numbers of genetic deviates are significantly different from zero for all populations in both treatments.

From these considerations breeding within these three populations for either low, intermediate, or high nitrogen would be expected to result in some genetic improvement and would be more effective at the higher fertility level.

The proportions of genetic deviates in the different classes should be considered in selecting individuals for further breeding. This is particularly true if selection is employed as the breeding method. Those classes having 100 percent genetic deviates would be of particular interest as they allow the identification (with reasonable limits) of the genetic deviates. A progeny test is required to identify the genetic deviates selected from those classes having less than 100 percent genetic deviates. Of course those individuals selected from classes showing 100 percent genetic deviates should be progeny tested also as occasionally chance environmental deviates would be expected to occur among them.

An examination of the frequency distribution of the genetic deviates for population A54-1 reveals that this frequency distribution has a mode among the lower deviates and another among the higher deviates. These modes have upper class limits of 21 and 105, respectively. Such being the case the mean total nitrogen for each of these two groups can be estimated. The procedure will be illustrated for the lower classes. The class centers may be obtained by dividing the class interval

7 by 2 (equals 3.5) and adding this value to the value for the immediately preceding class. By so doing the class centers are 10.5, 17.5, and 24.5. The frequencies for these classes taken from Table 9 opposite row heading differences are 8, 20, and 6, respectively. Multiplying the class centers by their respective frequencies and summing we have $[(8 \times 10.5) + (20 \times 17.5) + (6 \times 24.5)]$ and this divided by 34 equals 17, the mean for the lower classes. Similarly the mean for the higher classes is estimated as 94. It should be possible to obtain by breeding, a population having a mean of 17 and another population having a mean of 94. The latter population would have 5 times as much total nitrogen as the former. The mean of population A54-1 for the fertilized plots as shown in Table 3 is 46.8. The decrease in total nitrogen for the first population is 275 percent and the increase for the second population is 201 percent.

Summary

1. The unit of measurement is milligrams of nitrogen per 100 milliliters of thin juice equated to a refractive dry substance of 10.

2. Significant differences in amounts of total nitrogen were found between populations and between the two fertilizer treatments. Populations represent three levels of total nitrogen: A54-1 and A54-1BB, 31.8; 50-406BB and 50-406, 23.0; and F₁ hybrid and 52-307, 15.2.

3. Nearly complete dominance of smaller amounts of total nitrogen was found for those replication groups in which the total nitrogen averaged over 23 whereas heterosis for smaller amounts of nitrogen was found for those replication groups in which the total nitrogen averaged less than 23.

4. The amount of total nitrogen for population A54-1 (18.8) on the non-fertilized plots is not materially different from the amount of total nitrogen for 52-307 (18.6) and F₁ (21.3) on the fertilized plots. Hence in this study genetic control of total nitrogen represented by differences between populations is of about the same magnitude as environmental control represented by differences between fertilizer treatments.

5. The differences between populations in amount of total nitrogen are greatest for the replication group 33-40 which had the greatest amount of nitrate nitrogen in the petioles (see Powers et al. 9). This is substantiated also by the fertilizer treatments as the range for populations for the fertilized plots was 18.6 to 46.8 and for non-fertilized plots was 9.8 to 18.8.

6. Such findings lend considerable encouragement to the plant breeder, as they provide evidence that beets can be bred for either low or high nitrogen content. Furthermore, the accomplishments are expected to be more marked at the higher fertility level, for which improved varieties are much needed.

7. The residual variances for populations A54-1, A54-1BB, and 50-406BB were greatly different from zero, indicating that considerable genetic variability existed in these populations. The partitioning method of analyzing the frequency distributions confirmed this deduction and showed that breeding within these populations and between the six populations should be effective. It was found that breeding within A54-1 should result in the production of populations differing by five times in the amount of total nitrogen.

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