Association of Chemical Characters with Cercospora Leaf Spot Resistance in Sugar Beets[1]

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Introduction

A phenolic compound, 3-hydroxytyramine, identified by Gardner (5)[3], was shown by Harrison et al. (6) to be closely associated in its oxidized form with resistance to *Cercospora beticola* Sacc. (leaf spot) in culture. Polyphenoloxidase may be the oxidizing enzyme activated by some catalyst. Reports in the literature (11,12) indicate that divalent metallic ions may act as catalysts for this oxidizing reaction in various plants. These studies were conducted in an attempt to link certain cations and other chemicals in the leaf and root tissue to leaf spot resistance and to evaluate the interrelations of various chemicals. Copper was of particular interest since it appears as the central atom in the polyphenoloxidase enzyme molecule.

Materials and Experimental Design

The materials used in this experiment were from the sugar beet variety A56-3 and self-pollinated lines of A56-3 (S_1's). This is an open-pollinated commercial variety adapted to the east slope of the Colorado mountains. Four hundred and forty random plants of the A56-3 were self-pollinated in 1964. From these, 180 plants produced enough seed under the bags so that they could be planted in two tests as one row plots. Both tests were planted at Fort Collins, Colorado; the one in the leaf spot nursery was planted May 6, 1965; the one grown under disease free conditions was planted April 8. The field design consisted of 10 blocks of 20 entries each. In each block, 18 entries were S_1 lines and the remaining 2 entries were A56-3. Since A56-3 appeared twice in each block, it provided the opportunity to adjust

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[3] Numbers in parentheses refer to literature cited.

for block differences if these differences were significant. The data were taken on a plot basis from disease free plants. Twelve root samples were taken from each plot. The usual measurements were made for root weight, sucrose and thin juice apparent purity. Chloride, sodium, potassium, total nitrogen and copper determinations were made on thin juice. Dried leaf material prepared from leaves collected and quick frozen August 4 and 5, 1965, was analyzed for copper, calcium and magnesium. Polyphenoloxidase and 3-hydroxytyramine content were determined on leaf extract from the same leaves.

Leaf spot readings were taken from the duplicate planting made in the leaf spot nursery. The standard leaf spot scoring method from 0 to 10 was used; 0 indicating no infection and 10 indicating complete defoliation.[4] It was necessary to grow the plants in separate areas so that the characters other than leaf spot resistance could be determined on healthy plants. The relationships of these characters might be altered if they were obtained from diseased plants.

Complete data were obtained on the twenty A56-3 plots and 173 of the selfed lines.

Experimental Methods

The thin juice was prepared from sugar beet roots by a method developed by Brown and Serro (1) and modified by Carruthers and Oldfield (2). The following characteristics were determined as described by Payne et al. (9,10): percentage sucrose, percent apparent purity of thin juice, mg of Na, K, and N per 100 ml thin juice. Chlorides were determined in meq/l on the thin juice samples with a chloride titrator. The 3-hydroxytyramine and polyphenoloxidase were determined on leaf samples as described by Harrison et al. (7).

The leaf samples were prepared as follows for the determination of calcium, magnesium and copper on an atomic absorption spectrophotometer. About 10 grams of center-cut leaves were dried in an oven at about 82°C and ground in a laboratory mill. One-half gram of the dried, ground leaf was put into a 25 ml digestion tube with 5 milliliters of a digestion mixture and allowed to stand for 24 hours. The digestion mixture was prepared as follows: Solution I—10 g of sodium molybdate and 150 ml H_2O. Solution II—600 ml of concentrated HNO_3, 200 ml of concentrated H_2SO_4, 150 ml of 70% $HClO_4$ and 150 ml of Solution I. The tubes are heated on a rotary digestion apparatus for 20 to 30 minutes until the solution is clear. The mix-

[4] The authors are indebted to J. O. Gaskill for leaf spot scores from his leaf spot nursery at Fort Collins, Colorado.

ture is cooled, diluted to 25 ml with distilled water and mixed well. This solution is used for determination of Ca, Mg and Cu. The values reported for calcium concentrations do not reflect the true calcium levels. There were interferences from phosphate and other ions which were not taken into account. However, since the phosphate interference appears to be relatively small and since constant amounts of other reagents were added to each sample, the calcium results are reported and should still give a measure of relative differences in concentrations. In future analyses lanthanum chloride will be added to mask the phosphate interferences and the procedure will be revised to overcome the other interferences.

These data were adequate to investigate the statistical form of the distribution from several characters which had not been studied previously. A normal distribution is required for most statistical operations and a change of scale was needed for each character which would transform the data to a normal distribution. In addition, the interrelations of the normalized variables and their relation to yield and quality could be studied.

Results and Discussion

The arithmetic means and standard deviations for the various chemical determinations for the controls and inbreds combined, are shown in Table 1. The means and standard deviation of the controls were very similar to those of the A56-3 inbreds and were not statistically different; therefore, the study was concerned only with the combined sample of 193 plots. There were no significant block differences.

Tests for normality using the third and fourth moments about the mean were computed for each character measured on the arithmetic, logarithmic and the square root scales. On the arithmetic scale, weight and purity were found to have a slight positive kurtosis and sucrose a slight negative skewness. None of the transformations attempted improved upon the arithmetic scale for these characters. All of the chemical characters for both thin juice and leaves show significant positive skewness on the arithmetic scale except for polyphenoloxidase which is significantly negatively skewed. Some degree of positive kurtosis is also noted for most characters. Transformation to the log scale made the distribution of all of the thin juice characters acceptably normal but overcorrected the leaf characters from significant positive skewness to significant negative skewness. The square root transformation was tried for the leaf characters and acceptable normality was achieved except that 3-hydroxytyramine retained a slight positive skewness and magnesium retained positive

kurtosis. Polyphenoloxidase presented a special problem as the negative skewness on the arithmetic scale was exaggerated by both the square root and the logarithmic transformations. It was found by trial and error that polyphenoloxidase was normally distributed when transformed to the antilog to the base 10.

Correlation analysis is based on the requirement that a bivariate normal distribution exists. If this is not true, the interpretation of the correlation coefficient is uncertain (3). Changes of scale to achieve approximate normality for each character are essential for a valid study. The changes of scale should also make biological sense and not simply be a mathematical manipulation. Arguments can be presented for the logic of each of the transformations chosen. When the assumption of normality is satisfied, the observed correlation coefficients can be used to test for independence of the two variables involved.

The scales used for correlation analysis as a result of the normality investigations are: arithmetic scale for weight, sucrose and purity; antilog base 10 for polyphenoloxidase; square root for the remaining leaf characters; and log base 10 for the thin juice characters. In order to maintain a familiar scale, the means and standard deviations in Table 1 are shown on the arithmetic scale.

Table 1.—Means and standard deviations of A56-3 and A56-3 sugar beet inbreds.

Character	Mean	Standard deviation
Weight (Kg)	8.012	2.094
Sucrose (%)	14.946	1.192
Thin juice purity (%)	91.80	3.702
Leaf spot (score)	4.010	1.535
3-Hydroxytyramine (mg/100 ml extract)	50.76	31.59
Polyphenoloxidase (optical density after 5 min)	1.186	0.251
Leaf Cu (mg/100gm)	1.394	0.296
Leaf Ca (mg/100 gm)	709.870	237.965
Leaf Mg (mg/100 gm)	625.492	186.268
Thin juice Na (mg/100 ml)	49.248	24.783
Thin juice K (mg/100 ml)	82.575	18.896
Thin juice N (mg/100 ml)	45.109	17.913
Thin juice Cu (mg/100 ml)	0.212	0.082
Thin juice Cl (meq/l)	2.128	0.908

It is interesting to note that all of the chemical determinations made were positively skewed on the arithmetic scale, with the exception of polyphenoloxidase, and that the thin juice characters were log-normal, while the leaf characters were square root-normal. This relation could be a result of the physiologic and/or metabolic systems of the plants. Evaluations such as these

made on only one population are not sufficient for far-reaching conclusions, but it would seem to be a set of relations worthy of further investigation.

The significant simple correlation coefficients of the combined sample of A56-3 and S_1 inbreds are shown in Table 2. There is a small but significant positive correlation between root weight and the thin juice chemical characters sodium, copper and potassium. No significant relation of weight with the leaf chemical characters exists except for polyphenoloxidase which shows a negative association. Sucrose is negatively correlated with weight and positively correlated with purity as is commonly observed.

The thin juice chemical characters which are generally thought of as impurity components are all negatively correlated with sucrose. These correlations are all of similar magnitude. Purity is also significantly correlated negatively with the thin juice chemical characters but to a lesser degree. Even though these relations are of the same magnitude, the sodium, potassium and total nitrogen contribute much more as impurity components than do the chlorides and copper, as is shown by the mean quantity of materials present. The thin juice chemical characters are all significantly positively correlated with each other.

The leaf chemical characters are not so highly and consistently correlated. Calcium and magnesium are very highly positively correlated with each other and both positively correlated to the same degree with polyphenoloxidase. Negative association of these two elements are shown with 3-hydroxytyramine to a higher degree than the positive associations with polyphenoloxidase. Polyphenoloxidase and 3-hydroxytyramine are highly negatively related to each other. Leaf copper is negatively associated with polyphenoloxidase. Leaf copper is negatively associated with calcium and positively associated with 3-hydroxytyramine. Only slight associations are indicated between thin juice characters and leaf characters.

Leaf spot readings are negatively associated with 3-hydroxytyramine and leaf copper, and positively associated with magnesium. Positive relations were also noted between leaf spot and polyphenoloxidase and calcium, but they were not significant.

The general patterns of the relations of leaf spot are consistent with and support the hypothesis that 3-hydroxytyramine oxidixed by polyphenoloxidase effects some measure of control on leaf spot. The oxidation process may be catalyzed by calcium, or magnesium, or both, as they are negatively associated with 3-hydroxytyramine, and leaf copper may be used in formation of polyphenoloxidase although the relations are not all significant.

Table 2.—Significant correlation coefficients between transformed chemical characters.

			Leaf determinations						Thin juice determinations				
Character	Sucrose	Purity	Sq rt Leaf spot	Sq rt 3-hy-t	Anti-log poly	Sq rt Cu	Sq rt Ca	Sq rt Mg	Log Na	Log K	Log N	Log Cu	Log Cl
Weight	—.1801				—.1516				.1819	.2397		.2040	
Sucrose		.3760							—.7606	—.5829	—.5570	—.5096	—.4975
Purity									—.2300	—.2732	—.3621	—.2373	—.2016
Sq rt leaf spot				—.2073		—.2293		.1450					
Leaf determinations													
Sq rt 3-hy-t					—.6562	.1760	—.5059	—.4356					
Antilog poly							.3730	.3695					
Sq rt LCu						—.2152			.1723				
Sq rt Ca								.8355			—.1558		
Sq rt Mg													
Thin juice determinations													
Log Na										.4384	.4442	.4435	.5181
Log K											.5474	.3976	.3334
Log N												.5827	.4188
Log TJCu													.4323

High concentrations of 3-hydroxytyramine have shown correlation with Cercospora disease resistance, but there was a negative correlation between 3-hydroxytyramine and its oxidizing enzyme, polyphenoloxidase. In general, synthetic activity increases markedly around points of injury or infection and these reactions may depend upon 3-hydroxytyramine and polyphenoloxidase.

The progeny-parent regression coefficients for weight, sucrose and 3-hydroxytyramine, which are the narrow sense heritability estimates, are shown in Table 3 along with the progeny-parent correlations.

Table 3.—Progeny-parent correlations and narrow sense heritability ratios for sugar beet estimated from progeny-parent regression.

Character	Correlation coefficient (r)	Heritability h^2
3-Hydroxytyramine	0.2802**	0.57
Percentage sucrose	0.3715**	0.26
Weight	0.1077	0.35

The regression and correlation coefficients were determined from data collected on the S_1 lines in 1965 and from data on the individual parents in 1963. According to Falconer (4), the regression of progeny on mid-parent is a measure of the narrow sense heritability. In self-fertilziation, the value for the parent is the same as the mid-parent in cross-fertilization. Variance due to the additive effects of genes accounted for 57% of the phenotypic variance of 3-hydroxytyramine, 35% for root weight, and 26% for percentage sucrose. The proportion of additive variance in weight has been lower in previous experiments (8); but, as for any heritability estimate, conclusions drawn from these data should be applied only to this particular group of S_1 lines.

The parent and progeny measurements for weight, sucrose and 3-hydroxytyramine were correlated, and it was found that the S_1 lines which had high sucrose had parents that also had high sucrose. The same relationship existed for 3-hydroxytyramine and weight; however, the weight correlation coefficient was not significant. This could be the result of poor stands for some of the S_1 lines, which would directly affect root weight.

A conservative method of estimating broad sense heritability was used for all characters. Since the data obtained from A56-3, as well as the S_1 lines, were measured on the plot basis and not as individual plants, the A56-3 variance of plot means should have little genetic variance. Therefore, plot measurements on A56-3 can be used to estimate the environmental variance which

is subtracted from the total variance for the S_1 lines, leaving an estimate of their total genetic variance. This ratio of total genetic variance to total variance estimates broad sense heritability and indicates the expected progress from selection if one is using a breeding method which capitalizes on both additive and non-additive gene action. These calculations are summarized in Table 4. The broad sense heritability estimates for weight and sucrose are 0.62 and 0.48, respectively. When these estimates are compared with estimates from previous experiments with similar material they appear reasonable (8). Therefore, this method should give a reasonable estimate for chemical characters. The estimate for 3-hydroxytyramine was 0.39, which implies that some progress could be made among these S_1 lines when selecting for high 3-hydroxytyramine, provided the proper breeding method was used, but not as much as for weight and sucrose. When the narrow sense heritability estimate from 3-hydroxytyramine shown

Table 4.—Broad sense heritability estimates of the S_1 lines of sugar beet.

Character	Total variance	Estimated environmental variance	Genetic variance	Heritability ratio (broad sense)
Weight	4.66463	1.78632	2.87831	0.62
Sucrose	1.48726	0.77882	0.70844	0.48
Purity	14.22721	8.71432	5.51289	0.39
Sq rt leaf spot	0.15554	0.05954	0.09600	0.62
Leaf determinations				
Sq rt 3-hy-t	4.64815	2.79937	1.84878	0.39
Antilog poly	68.36030	19.07317	49.28713	0.72
Sq rt Cu	0.01573	0.01372	0.00201	0.13
Sq rt Ca	0.20236	0.24129		
Sq rt Mg	0.12760	0.27397		
Thin juice determinations				
Log Na	0.17392	0.05570	0.11822	0.68
Log K	0.05103	0.03445	0.01658	0.32
Log N	0.15257	0.17671		
Log Cu	0.13362	0.15196		
Log Cl	0.17670	0.13730	0.03940	0.22

in Table 3 is compared with the broad sense heritability estimate, it appears that most of the genetic variability is due to additive genes. A larger proportion is indicated than in the case of weight or sucrose. This indicates that mass selection within these S_1 lines should be an effective means for selecting lines having high concentrations of 3-hydroxytyramine. The broad sense heritability coefficients for both leaf spot resistance and polyphenoloxidase were quite high, being 0.62 and 0.72. This indicates that genetic shifts could be made in breeding for these characters within this population of S_1 lines, provided all types

of gene action were utilized. Narrow sense heritability estimates were not available for these characters. The broad sense heritability estimates for purity, sodium, potassium, leaf copper and chlorides were 0.39, 0.68, 0.32, 0.13 and 0.22. Estimates for the heritability of nitrogen, thin juice copper, calcium and magnesium were zero, possibly due to the conservative nature of this estimation method.

Summary and Conclusions

The purpose of this study was to determine if certain chemical components found in the leaf and root tissue of sugar beets are correlated to Cercospora leaf spot resistance. Also an evaluation was made on the interrelation of these chemical characters.

The materials used were from the sugar beet variety A56-3 and self-pollinated lines of A56-3 (S_1's). Sodium, potassium, copper, chloride and total nitrogen analyses were made on the thin juice as well as root weight, sucrose and apparent purity. Dried leaf material was analyzed for the divalent metallic ions, copper, calcium and magnesium. Determinations for the phenolic compound, 3-hydroxytyramine and its oxidizing enzyme, polyphenoloxidase, were made on fresh frozen leaves.

From the statistical study of the data the following conclusions were drawn:

1. High 3-hydroxytyramine in disease-free plants is associated with high Cercospora leaf spot resistance.

2. In this study, involving one heterogeneous population and S_1 lines derived from it, the thin juice chemical characters were found to have a log-normal distribution, and the leaf chemical characters were found to have a square root-normal distribution, with the exception of polyphenoloxidase which was normal as the antilog to the base 10.

3. Thin juice impurity components (sodium, potassium, total nitrogen, copper and chlorides) were all positively correlated with each other and negatively correlated with sucrose and purity. However, based on the means, copper and chlorides contribute little to impurity.

4. Leaf chemical characters are not so highly and consistently correlated. The general pattern of the relations is consistent but the correlations are weak. It is possible that certain divalent metallic ions could catalyze the oxidation of 3-hydroxytyramine by polyphenoloxidase leading to some measure of control of leaf spot; at least the relations do not conflict with this hypothesis. However, this is only theory until more is known of the reactions in the plants.

5. This experiment indicates that a considerable portion of the 3-hydroxytyramine variance was due to additive genes which

means that mass selection should be an effective method of selecting for high 3-hydroxytyramine lines. Polyphenoloxidase would very likely respond to the same selection technique.

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