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Peroxidase Activity in *Linum usitatissimum* L.

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Summary. Crosses were made, in all combinations, between 2 parental genotypes of *Linum* and their reciprocal F_1 hybrids. The parents and progeny obtained were grown in controlled environmental conditions and sampled at 35 and 70 days after germination to determine, on an individual plant basis, total plant fresh weight and peroxidase activity of main stem tissue. Peroxidase activity required transformation to a $\log_{10}$ scale, whereas the original linear scale was satisfactory for plant weight. There was no correlation between plant weight and corresponding peroxidase activity. Pronounced heterosis appeared in the F_1's for both characters at sample 1, but this heterosis had declined at sample 2 and in the F_2's. Heterosis operated in a positive direction for plant weight and in a negative direction for peroxidase activity. No consistent differences were found amongst the variances of segregating or non-segregating generations for either character.

1. Introduction

The occurrence and activity of peroxidase have been investigated in a wide variety of plants. Its precise role is not clear, although it has been implicated in the indole-acetic acid oxidase system, the activity of which was shown by Galston and Dalberg (1954) to be inversely correlated with growth. It may thus regulate growth by controlling the level of indole-acetic acid within the plant. In *Linum usitatissimum* L. wide differences in morphology may be observed between different genotypes (Dillman and Brinsmade, 1938), and also between different genotrophs (Durrant, 1962). This morphological variation concerns in particular the number and size of the basal branches produced from the axils of the cotyledons.

Using controlled environmental conditions, two *Linum* genotypes which differed markedly from one another in degree of basal branching were examined, together with their F_1 hybrids, for peroxidase activity. Distinct differences were found between the peroxidase activities of main stem tissue of the two parental genotypes, and these differences were maintained consistently throughout the growing period (Tyson, 1969). Using similar environmental conditions, these two parental genotypes and the complete set of their F_1's, F_2's and first backcross progeny were grown and assayed for peroxidase at two stages during the growing period. All measurements were made on an individual plant basis.

2. Materials and Methods

The 2 genotypes of *Linum usitatissimum* L. used were the cultivars Royal (*R*) and Mandarin (*M*). After 2 generations in the greenhouse, during which normal complete self pollination took place, a number of plant progenies were obtained; a single plant progeny from each genotype provided the parental plants for the initial crosses. Crosses between and within the 2 genotypes were made in the field; the technique and conditions have been described by Tyson (1969). From the seed produced, plants of the 2 parental genotypes and their reciprocal F_1 hybrids were raised in a growth chamber, for which the temperature, humidity and daylength conditions were also described in the reference above. In the growth chamber the parental genotypes and their F_1's were then crossed in all possible combinations. The progeny of this second set of crosses contained the parental genotypes again, as well as the F_1's, and, in addition, the F_2's and first backcrosses. The complete set of 16 types of progeny is shown in table 1.

These 16 types of progeny were subsequently grown in the growth chamber under the same temperature, humidity and daylength conditions as before, and were arranged in a 3 replicate randomised block design. Individual plants of types 11, 12, 13 . . . 44 were placed at random within the area of each replicate. Each replicate contained 8 plants each of types 11 and 44, 4 plants each of 14 and 41, and 9 plants each of all the remaining (twelve) types. The plants were removed for assay of peroxidase activity 35 days after germination. A second sample, containing the same numbers of each of the 16 types of progeny, was set up in the growth chamber, using the same kind of design as in the first sample, and harvested 70 days after germination. Similar soil was used for both samples; the ratio of soil to peat moss to sand (3:2:1) was identical for both samples, as was the volume of soil used per plant. In the previous experiment (Tyson, 1969) the soil volume used per plant during the second half of the growing period was approximately 50% greater than it was for either sample of the experiment described here. At sampling the plants were cut at soil level and the fresh weights were recorded on an individual plant basis, as were measurements of peroxidase activity made later.

In preparing individual plants for assay of peroxidase activity, leaves were removed from the main (centre) stems, which were then homogenised in distilled water using a 1:20 weight ratio of stem material to water. The part of the main stem used for homogenisation extended from just above the cotyledons to just below the apical bud. Dialysis against distilled water was carried out following homogenisation. Because of very high peroxidase activities in the second sample (70 days), it was found necessary to dilute these homogenates further in a ratio of 1:9 with distilled water. The collection, preparation, dialysis and storage of the samples were otherwise as described by Tyson and Jui (1967). The

technique for measuring peroxidase activity, in which the rate of oxidation of guaiacol following the addition of substrate was tracked spectrophotometrically at a wavelength of 470 μm, was also the same in all essential details. The readings of percent transmission during the reaction were obtained on a Zeiss PMQ 11 spectrophotometer equipped with an automatic cell changer and recorder. Peroxidase activities were expressed as the rates of increase in optical density (O.D.) per minute per unit of fresh weight through the calculation of the linear regressions of O.D. on time.

Table 1. *Key to products of crosses in all combinations between parental and F_1 genotypes*

Genotype:						Code number:
		$R \times R$ = parent 1				1
		$R \times M = F_1$				2
		$M \times R = F_1$				3
		$M \times M$ = parent 2				4
		Male parents:				In the progeny of 1 to 4 crossed in all combinations:
		1	2	3	4	
Female parents:	1	11	12	13	14	11 and 44 = parental genotypes R and M
	2	21	22	23	24	14 and 41 = F_1's
	3	31	32	33	34	22, 23, 32, 33 = F_2's
	4	41	42	43	44	12, 13, 21, 31 = backcrosses to R
						24, 34, 42, 43 = backcrosses to M

3. Results

There were two characters for study, namely, total plant weight and peroxidase activity; both characters displayed continuous variation. The examination of the data was divided into three stages, as follows: (a) the choice of the most appropriate scale for each character, (b) the relationship between total plant weight and peroxidase activity, and (c) the analysis of variance for each character.

(a) In the choice of scale, the procedure used with total plant weight data will be outlined; an essentially similar procedure was used for peroxidase activity. To check the suitability of the scale from the point of view of the criteria put forward by Mather (1949), means and variances were first calculated for all the types, 11 to 44, in each replicate for each sample. There were, therefore, $16 \times 3 \times 2 = 96$ means and the same number of variances; they will be referred to as the within-type means and the within-type variances. In order to determine whether any correlation existed between them, an analysis of covariance was carried out. The result of this analysis is shown in table 2; the value of the correlation coefficient, r, found in the error (GSR) line was not significant. Removal of the segregating generations and recalculation of the covariance analysis yielded the same result. Detailed examination of the means and variances is deferred; at this point it may be noted that, for the means, (x), there was a significant interaction between genotypes and samples (GS). The covariance analyses were, accordingly, carried out within each of the samples; in both analyses the segregating generations were excluded. The results are shown in table 2, and, as with the first analysis including all generation and both samples, gave no evidence in the error lines of correlation. There was no indication, therefore, from this approach that the scale used for total plant weight was unsuitable.

All the covariance analyses showed that, among the genotypes, there were no significant differences in variances. This finding, obtained from the analysis of variance of variances (y), was supported by tests of the homogeneity of the variances of non-segregating generations, carried out with Bartlett's (1937) method. The χ^2 values calculated for data of each replicate in each sample were all non-significant at P 0.05.

Table 2. *Analysis of covariance on within-type means (x) and within-type variances (y) from total plant weight data*

Sums of squares and cross products					
Item	x	xy	y	df	Correlation coefficient, r
Genotype (G)	93.10856	70.85174	973.06460	15	
Sample (s)	3998.32042	5462.18692	7462.00473	1	
Replicate (R)	75.31436	64.22524	76.77779	2	
GS interaction	46.01235	74.35146	1035.73662	15	
GR interaction	30.05778	−35.19551	2582.46826	30	
SR interaction	42.22032	49.74285	65.81747	2	
GSR interaction	36.51544	23.11143	2479.20274	30	0.08

Analysis of covariance on within-type means (x) and within-type variances (y) from total plant weight data; analysis carried out within each sample using non-segregating parental and F_1 generations only

	Item	x	xy	y	df	Correlation coefficient, r
Sample 1	Genotype	16.05090	−6.77534	5.88809	3	
	Replicate	1.70322	1.17558	0.96740	2	
	Error	1.30098	1.37789	10.29954	6	0.38
Sample 2	Genotype	84.79320	187.42050	682.39150	3	
	Replicate	7.78687	55.20927	467.73662	2	
	Error	9.18960	3.68747	1770.39198	6	0.03

A second approach to scaling lay in the comparison of observed and calculated F_2 and backcross generation means. Using the parental and F_1 means within each replicate of each sample, the expected backcross means were calculated from $1/2\ (\bar{P}_1 + \bar{F}_1)$ and $1/2\ (\bar{P}_2 + \bar{F}_1)$, while the expected F_2 was obtained from $1/4\ (2\,\bar{F}_1 + \bar{P}_1 + \bar{P}_2)$. The data for these generations, averaged over replicates within samples, are shown in table 3, together with their standard errors. The standard errors were obtained from analyses of variance utilising the 3 replicates within samples. For example, the F_2 generation within a sample supplied 12 means; these may be symbolised in terms of the table 1 key as follows, with the initial subscript representing replicate:

$$X_{122}\ X_{123}\ X_{222} \ldots X_{323}$$
$$X_{132}\ X_{133}\ X_{232} \ldots X_{333}\ .$$

Replicate as well as reciprocal effects were removed in the analysis of variance and the error mean square provided the appropriate standard error for the F_2 mean. Other generations were treated in a similar manner. The differences between expected and observed means represented Mather's (1949) quantities A, B and C. These differences are shown in table 3, where it can be seen that one backcross in each sample departed significantly from expectation. The differences between the A, B and C values in sample 1 and the corresponding values in sample 2

Table 3. *Generation means, and their standard errors, within each sample for total plant weight data*

		$\bar{P}_1$	$\bar{P}_2$	$\bar{F}_1$	$\bar{F}_2$	$\bar{B}_1$	$\bar{B}_2$
Sample 1	Means:	3.94	3.96	6.23	5.40	5.84	5.26
	St. errors:	±0.32	±0.41	±0.18	±0.16	±0.18	±0.18
Sample 2	Means	14.33	20.07	20.36	18.61	18.03	17.81
	St. errors:	±0.67	±0.24	±0.61	±0.35	±0.32	±0.53

Generation means, and their standard errors, within each sample. Total plant weight data transformed to $\log_{10}$ values

		$\bar{P}_1$	$\bar{P}_2$	$\bar{F}_1$	$\bar{F}_2$	$\bar{B}_1$	$\bar{B}_2$
Sample 1	Means:	0.559	0.553	0.783	0.711	0.750	0.693
	St. errors:	±0.037	±0.044	±0.014	±0.015	±0.013	±0.018
Sample 2	Means:	1.139	1.290	1.295	1.254	1.243	1.229
	St. errors:	±0.024	±0.011	±0.013	±0.009	±0.009	±0.014

Differences between calculated and observed generation means, within each sample, for total plant weight data

	Sample 1	Sample 2	*df*
$A = \overline{2\,B_1} - \bar{P}_1 - \bar{F}_1$	$= 1.51, t = 2.94$*	$= 1.37, t = 1.23$	10
$B = \overline{2\,B_2} - \bar{P}_2 - \bar{F}_1$	$= 0.33, t = 0.57$	$= 4.81, t = 3.86$**	10
$C = \overline{4\,F_2} - \overline{2\,F_1} - \bar{P}_1 - \bar{P}_2$	$= 1.24, t = 1.39$	$= 0.68, t = 0.34$	12

Differences between calculated and observed generation means, within each sample. Total plant weight data transformed to $\log_{10}$ values

	Sample 1	Sample 2	*df*
$A = \overline{2\,B_1} - \bar{P}_1 - \bar{F}_1$	$= 0.158, t = 3.34$**	$= 0.052, t = 1.59$	10
$B = \overline{2\,B_2} - \bar{P}_2 - \bar{F}_1$	$= 0.050, t = 0.85$	$= 0.127, t = 3.88$**	10
$C = \overline{4\,F_2} - \overline{2\,F_1} - \bar{P}_1 - \bar{P}_2$	$= 0.166, t = 1.89$	$= 0.003, t = 0.06$	12

* Significant at probability 0.05. — ** Significant at probability 0.01

Table 4. *Analysis of covariance on within-type means (x) and within-type variances (y) from peroxidase activity data*

Item	*x*	*x y*	*y*	*df*	Correlation coefficient, *r*
GSR	0.00601037	0.00013713	0.00001156	30	0.52*

Analysis of covariance on within-type means (x) and within-type variances (y) from peroxidase activity data transformed to $\log_{10}$ values

Item	*x*	*x y*	*y*	*df*	Correlation coefficient, *r*
Genotype (*G*)	0.70856868	−0.00674215	0.00060050	15	
Sample (*S*)	3.12133544	−0.08189631	0.00214876	1	
Replicate (*R*)	0.02427613	−0.00103249	0.00009411	2	
GS	0.10781760	0.00214778	0.00140586	15	
GR	0.18349814	−0.00711379	0.00211119	30	
SR	0.00707334	−0.00009581	0.00001504	2	
GSR	0.13207950	0.00313154	0.00213019	30	0.19

* Significant at probability 0.05.

were also examined; these differences and their standard errors are shown below:

$A_1 - A_2 = 0.14 \pm 1.22$: not significant
$B_1 - B_2 = 5.14 \pm 1.37$: significant at P 0.005
$C_1 - C_2 = 1.92 \pm 2.18$: not significant .

From this second approach there was some suggestion that the scale, insofar as the removal of non-allelic interaction was concerned, was not entirely suitable, and that the two samples needed, in fact, different scales.

The effect of a $\log_{10}$ transformation of the original, individual weights, and re-calculation of the within-type means and variances, on the values of A, B and C was examined; the results are shown in table 3. This change of scale did not remove the previous discrepancies between observed and expected generation means; at the same time, an analysis of covariance of the type in table 2 showed, again, no significant correlation between means and variances. Since the $\log_{10}$ transformation yielded no improvement, a more complex scale change would have to be envisioned to remove non-allelic interaction in these data. However, this type of disturbance was, at worst, not pronounced in the total plant weight data, and the original linear scale was employed for their further analysis.

The data on peroxidase activity per individual, expressed in terms of the slopes of linear regressions of optical density on time, were treated in essentially the same way as those for total plant weight. The data were first examined with an analysis of covariance to determine whether the within-type means and variances were correlated; this was done both for the 'raw' data as well as the transformed ($\log_{10}$) data. The results are shown in table 4. Transformation to a $\log_{10}$ scale effectively removed the highly significant correlation present in the analysis of the raw data, a finding which was in agreement with the results from a previous experiment, (Tyson, 1969), in which only non-segregating parental and F_1 generations had been examined. Following transformation, the within-type variances (y) appeared, with one exception, to be homogeneous; there were no significant differences among genotypes.

The generation means and their standard errors were calculated both for raw and transformed data. These, and the values of A, B and C calculated from the generation means are shown in table 5. Back-cross 2 departed significantly from expectation in

Table 5. *Generation means, and their standard errors, within each sample for peroxidase activity data*

		$\overline{P}_1$	$\overline{P}_2$	$\overline{F}_1$	$\overline{F}_2$	$\overline{B}_1$	$\overline{B}_2$
Sample 1	Means:	0.20683	0.11273	0.08378	0.11834	0.14010	0.09853
	St. errors:	±0.00971	±0.00814	±0.00194	±0.00727	±0.00706	±0.00221
Sample 2	Means:	0.06777	0.03987	0.04137	0.05388	0.06093	0.04790
	St. errors:	±0.00784	±0.00349	±0.00321	±0.00208	±0.00250	±0.00155

Generation means, and their standard errors, within each sample. Peroxidase activity data transformed to $\log_{10}$ values

		$\overline{P}_1$	$\overline{P}_2$	$\overline{F}_1$	$\overline{F}_2$	$\overline{B}_1$	$\overline{B}_2$
Sample 1	Means:	−0.70120	−0.95934	−1.09587	−0.94622	−0.86689	−1.01920
	St. errors:	±0.02313	±0.02950	±0.01663	±0.02783	±0.02130	±0.00892
Sample 2	Means:	−1.19057	−1.42463	−1.41831	−1.28899	−1.23819	−1.34779
	St. errors:	±0.04915	±0.02942	±0.03369	±0.01603	±0.01851	±0.01598

Differences between calculated and observed generation means, within each sample, for peroxidase activity data

	Sample 1	Sample 2	*df*
$A = \overline{2B_1} - \overline{P_1} - \overline{F_1}$	$= 0.01041, t = 0.60$	$= 0.01272, t = 1.29$	10
$B = \overline{2B_2} - \overline{P_2} - \overline{F_1}$	$= 0.00055, t = 0.06$	$= 0.01456, t = 2.57$*	10
$C = \overline{4F_2} - \overline{2F_1} - \overline{P_1} - \overline{P_2}$	$= 0.01376, t = 0.43$	$= 0.02514, t = 1.85$	12

Differences between calculated and observed generation means, within each sample. Peroxidase activity data transformed to $\log_{10}$ values

	Sample 1	Sample 2	*df*
$A = \overline{2B_1} - \overline{P_1} - \overline{F_1}$	$= 0.06329, t = 1.23$	$= 0.13250, t = 1.89$	10
$B = \overline{2B_2} - \overline{P_2} - \overline{F_1}$	$= 0.01681, t = 0.44$	$= 0.14736, t = 2.68$*	10
$C = \overline{4F_2} - \overline{2F_1} - \overline{P_1} - \overline{P_2}$	$= 0.06740, t = 0.55$	$= 0.29586, t = 2.71$*	12

* Significant at probability 0.05

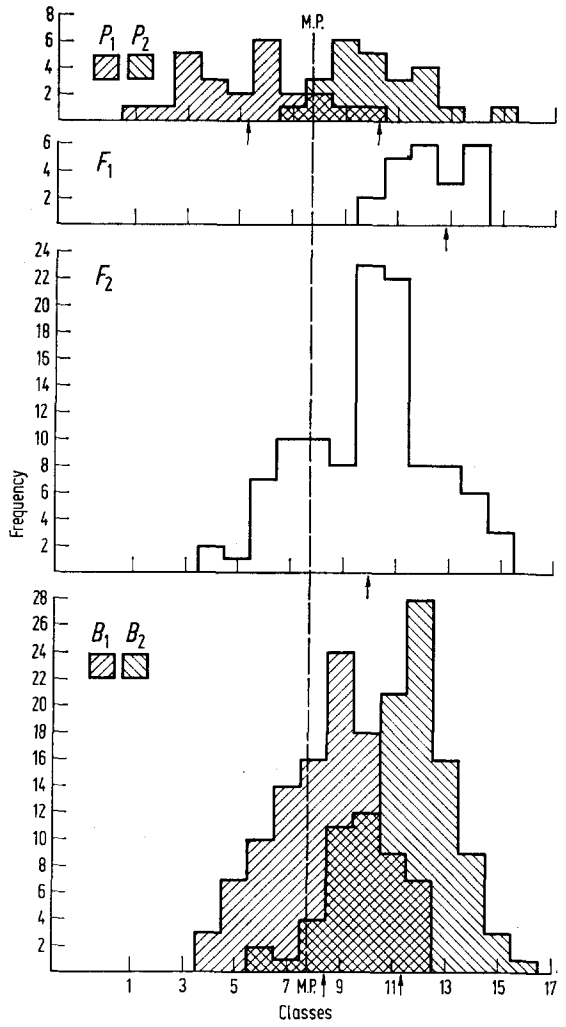

Figure 1. Frequency distributions for $\log_{10}$ peroxidase activity in each generation of sample 1. Class 1 represents highest activity, class 17 lowest. P_1, P_2 represent parents R and M respectively; B_1, B_2 represent backcrosses to parents R and M. M.P. represents midparent activity, while arrows on axes refer to midpoints of each distribution

sample 2, regardless of change to a log scale. The sample 2 value of C was significant following transformation of the data to a log scale. The differences between corresponding A, B and C values in samples 1 and 2 are shown below:

	Raw data	Log data
$A_1 - A_2 =$	0.02313 ± 0.01968	0.06921 ± 0.08688
$B_1 - B_2 =$	0.01400 ± 0.01103	0.13057 ± 0.06698
$C_1 - C_2 =$	0.03890 ± 0.03472	0.22846 ± 0.16382

None of these differences was significant. Although data transformation did not remove the discrepancy for backcross 2, and also introduced an F_2 sample 2 deviation, the change to a log scale was a reasonable preliminary to further analysis of the peroxidase activity data.

The frequency distributions for both plant weight and log peroxidase activity were examined. The distributions of each parental genotype (11 and 44), of the combined F_1's (14, 41), of the combined F_2's (22, 23, 32, 33), of the combined backcross 1's (12, 13, 21, 31), and of the combined backcross 2's (24, 34, 42, 43), were calculated for the data of the 1st and 2nd samples separately. Within a sample the differences between replicate means for any given generation were removed through the addition of suitable constants to the individual data, so that measurements could be pooled over replicates. Where $X_{i/11}$ represents the ith observation in the jth replicate for progeny type 11 in, say, sample 1, the sample mean for this type may be shown as $\bar{X}_{\cdot\cdot 11}$, and the constant added to all X_{i111} data as $(\bar{X}_{\cdot 111} - \bar{X}_{\cdot\cdot 11})$. With suitable constants for data of 11 in replicates 2 and 3, $\bar{X}_{\cdot 111} = \bar{X}_{\cdot 211} = \bar{X}_{\cdot 311}$. The same correction procedure was applied to 44, and to the F_1, F_2, and backcross data. This meant that each of the sample 1 distribu-

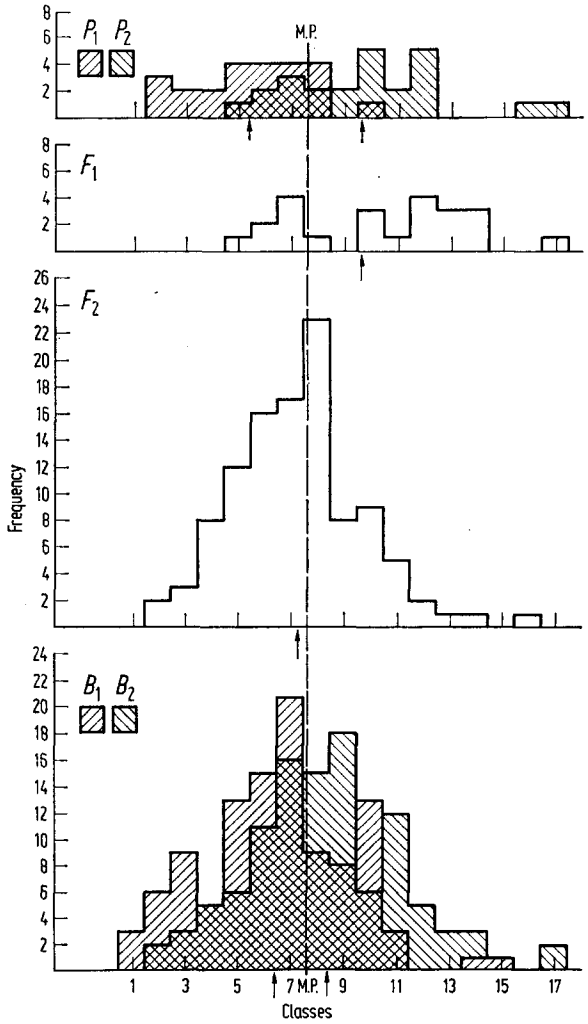

Figure 2. Frequency distributions for $\log_{10}$ peroxidase activity in each generation of sample 2. Symbols and classes as in fig. 1

Table 6. *Analysis of covariance on within-type total plant weight means (x) and within-type peroxidase activity means (y)*

	Item	x	xy	y	df	Correlation coefficient, r
1. y from raw data	*GSR*	36.51543941	−0.02017782	0.00601037	30	−0.04
2. y from $\log_{10}$ data	*GSR*	36.51543941	−0.07069824	0.13207952	30	−0.03

Analysis of covariance on individual plant weights (x) and individual plant peroxidase activities (y)

Sums of squares and cross products

	Item	x	xy	y	df	Correlation coefficient, r
1. y from raw data	Total	48026.40287	−162.57996	1.83057	791	
	Between groups	33922.89651	−160.88069	1.39018	95	
	Within groups	14103.50635	− 1.69927	0.44040	696	−0.02
2. y from $\log_{10}$ data	Total	48026.40287	−865.16426	44.65482	791	
	Between groups	33922.89651	−857.97776	34.25599	95	
	Within groups	14103.50635	− 7.18650	10.39883	696	−0.02

tions for the segregating generations represented 108 data, while non-segregating generation distributions each represented 24 data. Examination of the distributions for plant weight in each sample suggested that the data were distributed in an approximately normal fashion. The same approximate normality held in the case of the log peroxidase activity distributions which are shown in figs. 1 and 2.

(b) The relationship between total plant weight and corresponding peroxidase activity was examined in two ways. Firstly, the within-type means for total plant weight and peroxidase activity were examined, as x and y respectively, with an analysis of covariance. Both raw and transformed peroxidase activity data were employed as y values. The resultant analyses are summarised in table 6; the linear regressions of activity on weight within the error lines of the covariance analyses were not significant, and indicated that no simple relationship existed between within-type mean weight and within-type mean activity.

In the second approach, individual plant weights and their corresponding peroxidase activities were used in an analysis of covariance. Effects of the main factors, i.e. genotype, replicate and sample, were removed, together with their interactions, through the calculation of a sum of squares for differences amongst types in replicates and samples. There were 96 combinations of type, replicate and sample; the sum of squares for differences among these represented the between-group item of the analysis shown in table 6. The linear regression of activity on weight calculated in the within-group (error) line was not significant; this was true, as before, whether raw or transformed data were employed as y values against plant weights as x. Again, no simple relationship appeared to exist.

The possibility of a curvilinear relationship between weights and activities was investigated in the case of within-type means for weight and log activity. The within-type means for weight were raised to higher powers and included as additional x variables in the analysis of covariance. The multiple linear regression of log activity on weight was then examined in the error line of the covariance analysis. The fitting of first, second, third and fourth order terms for x did not result in the removal of a significant portion of the variation of y, log peroxidase activity. Inclusion of yet higher order terms could be envisioned, but the practicality of extracting such a complex relationship to examine, for example, the effects of genotype on log peroxidase activity over and above any genotype effect on plant weight is questionable. Log peroxidase activity was, therefore, analysed as an entirely separate character upon which plant weight had no influence.

(c) The analysis of variance of the plant weight data utilised the sums of squares for x (within-type means) shown in table 2. A more detailed analysis is shown in table 7, in which the sums of squares for genotype and genotype-sample interaction were each partitioned into 15 orthogonal comparisons. The 15 comparisons made are listed in table 8, in terms of suitable coefficients; the genotype means within each sample are shown in table 9. For the GS interaction, these coefficients were applied to the differences between the corresponding sample 1 and sample 2 genotype means. The analysis of the within-type means showed that there were significant differences among genotypes, samples and replications, together with significant *GS* and *SR* interactions. The partitioning of the genotype sum of squares showed that there was a significant difference between the two parents, and between the mean of the two parents and the mean of the F_1 hybrids; there was no significant reciprocal difference between the F_1's. There was also a significant difference in the backcross to

Table 7. *Analysis of variance of means*[1] *from total plant (fresh) weight data*

Item		*df*	Mean square	*F*
Genotype (*G*)		15	6.21723	5.11***
Comparison	1	1	24.88480	20.44***
	2	1	4.56333	3.75
	3	1	44.45540	36.52***
	4	1	0.25811	—
	5	1	0.33187	—
	6	1	0.03805	—
	7	1	0.38058	—
	8	1	1.61317	1.33
	9	1	3.19416	2.62
	10	1	8.21860	6.75*
	11	1	0.02107	—
	12	1	1.81500	1.49
	13	1	1.94676	1.60
	14	1	0.05691	—
	15	1	1.33076	1.09
Sample (*S*)		1	3998.32042	3284.90***
Replicate (*R*)		2	37.65718	30.94***
GS interaction		15	3.06749	2.52*
Comparison	1	1	24.56582	20.18***
	2	1	1.33333	1.10
	3	1	1.16429	—
	4	1	0.40329	—
	5	1	1.44496	1.19
	6	1	0.34774	—
	7	1	1.24519	1.02
	8	1	1.92667	1.58
	9	1	0.12519	—
	10	1	4.74074	3.89
	11	1	0.12519	—
	12	1	0.63014	—
	13	1	0.36750	—
	14	1	0.69882	—
	15	1	6.89350	5.66**
GR		30	1.00192	—
SR		2	21.11016	17.34***
GSR		30	1.21718	

* Significant at probability 0.05.
** Significant at probability 0.01.
*** Significant at probability 0.001.

[1] Within-type means.

the second parent (4, or *M*) which appeared in the lower progeny weights obtained when 4 was used as a female in contrast to its use as a male in crosses with the F_1 hybrid. The breakdown of the GS interaction showed that the parental difference was significantly larger in sample 2 than in sample 1, and this was also the main reason for the significance of comparison 15.

The analysis of variance of the within-type variances, for which the sums of squares are also shown in table 2, indicated that only between the samples was there a significant difference. The breakdown of the genotype and genotype-sample interaction sums of squares in the same way as above showed that there were no significant differences for any of the 15 comparisons made.

The analysis of variance of the $\log_{10}$ peroxidase activity is shown in table 10, and the genotype means within each sample are shown in Table 11. There were significant differences among genotypes and sample,

Table 9. *Total plant weight: mean weight (gms) per plant for each genotype within samples, and averaged over samples. Key to genotypes as in table 1*

		1	2	3	4
Sample 1	1	3.94	5.47	6.00	6.52
	2	6.26	4.93	5.59	5.33
	3	5.62	5.38	5.71	5.47
	4	5.95	5.27	4.96	3.96
		1	2	3	4
Sample 2	1	14.33	17.41	19.37	21.31
	2	17.57	19.13	18.78	18.30
	3	17.79	18.11	18.40	19.37
	4	19.41	17.11	16.44	20.07
		1	2	3	4
Means over Samples	1	9.14	11.44	12.69	13.91
	2	11.92	12.03	12.19	11.81
	3	11.70	11.74	12.06	12.42
	4	12.68	11.19	10.70	12.01

Table 8. *Orthogonal breakdown of the 15 degrees of freedom for genotypes*

Genotypes:																
Comparison:	11	12	13	14	21	22	23	24	31	32	33	34	41	42	43	44
1	+1															−1
2				+1									−1			
3	+1			−1									−1			+1
4						+1	+1			−1	−1					
5						+1	−1			+1	−1					
6						+1	−1			−1	+1					
7		+1	+1		−1				−1							
8		+1	−1		+1				−1							
9		+1	−1		−1				+1							
10								+1				+1		−1	−1	
11								+1				−1		+1	−1	
12								+1				−1		−1	+1	
13		+1	+1		+1			−1	+1			−1		−1	−1	
14	+1			+1		−1	−1			−1	−1		+1			+1
15	+1	−1	−1	+1	−1	+1	+1	−1	−1	+1	+1	−1	+1	−1	−1	+1

Table 10. *Analysis of variance of means[1] from $\log_{10}$ peroxidase activity data*

Item		*df*	Mean Square	*F*
Genotypes (*G*)		15	0.04723791	10.73***
Comparison	1	1	0.18169317	41.23***
	2	1	0.00037147	—
	3	1	0.21241396	48.25***
	4	1	0.00002820	—
	5	1	0.02850240	6.47*
	6	1	0.00152598	—
	7	1	0.00035632	—
	8	1	0.00728320	1.65
	9	1	0.00012573	—
	10	1	0.01164833	2.65
	11	1	0.00038543	—
	12	1	0.02177608	4.95*
	13	1	0.20579220	46.74***
	14	1	0.02473946	5.62**
	15	1	0.01192674	2.71
Sample (*S*)		1	3.12133544	709.40***
Replicate (*R*)		2	0.01213807	2.76
GS interaction		15	0.00718784	1.63
Comparison	1	1	0.00043489	—
	2	1	0.02508143	5.70*
	3	1	0.03598514	8.17**
	4	1	0.00341291	—
	5	1	0.00024879	—
	6	1	0.00125431	—
	7	1	0.00033052	—
	8	1	0.00868271	1.97
	9	1	0.00100367	—
	10	1	0.00365101	—
	11	1	0.00773062	1.76
	12	1	0.00199587	—
	13	1	0.00547529	1.24
	14	1	0.00978623	2.22
	15	1	0.00274510	—
GR		30	0.00611660	1.39
SR		2	0.00353667	—
GSR		30	0.00440265	

* Significant at probability 0.05.
** Significant at probability 0.01.
*** Significant at probability 0.001.
[1] Within-type means.

but not among replicates; there were no significant first order interactions between the main factors. Partitioning of the genotype sum of squares showed that the difference between the parents was significant, as was the difference between the mean of the parents and the F_1. There was no significant reciprocal difference between the F_1's. Among the F_2's there was a significant effect of the $F_1R \times M$ (i.e. 2) as a male parent, in comparison to the use of $M \times R$ (i.e. 3) as a male. In the backcross to the second parent there was a significant effect of the use of $R \times M$ and $M \times R$ respectively as male and female parents, versus the converse. The partitioning of the *GS* interaction showed that in sample 2 there was a significant reversal in the reciprocal difference between the F_1 hybrids, and a significant decrease in the departure of the F_1 mean from the parental mean.

The analysis of variance of the within-type variances, for which the sums of squares are shown in table 4, indicated that, as with the total plant weight data, the only significant effect was that due to sample. The breakdown of the genotype and genotype-sample interaction sums of squares revealed no significant differences for any of the comparisons.

4. Discussion

Analysis of variance of the within-type means for plant weight revealed a significant difference between the 2 parents at sample 2; for log peroxidase activity the parental difference was also significant, and consistent at both samples. F_1 — midparent departures were highly significant for both characters, but had opposite signs, as can be seen from tables 9 and 11. The results from this experiment may be compared with those obtained in a previous experiment (Tyson, 1969) in terms of the midparent values for the 2 characters at approximately the same time point. For example, the midparent plant weight here 70 days after germination (sample 2) was 17.2 *gms.*; in the previous experiment, with the same parental genotypes, midparent plant weight at 75 days was 44.8 *gms.*, and the F_1-midparent departure was in the direction of the lower parent. The comparison may be summarised as shown below:

1969 data:	Mean weight per plant at 75 days(*gms.*)				
	R	*M*	mid-parent	F_1	average dominance (potence ratio)
	49.4	40.2	44.8	39.6	1.13
Data here: (70 days)	14.3	20.1	17.2	20.4	1.10

Table 11. *$\log_{10}$ peroxidase activity: mean activity per plant for each genotype within samples, and averaged over samples. Key to genotypes as in table 1*

		1	2	3	4
Sample 1	1	−0.70120	−0.86126	−0.87281	−1.14715
	2	−0.86933	−0.98827	−0.91279	−1.00856
	3	−0.86417	−0.94926	−0.93459	−1.01046
	4	−1.04459	−0.98595	−1.07187	−0.95934
		1	2	3	4
Sample 2	1	−1.19057	−1.29095	−1.20056	−1.37816
	2	−1.25831	−1.33400	−1.23973	−1.36661
	3	−1.20294	−1.33677	−1.24549	−1.26024
	4	−1.45847	−1.35685	−1.40745	−1.42463
		1	2	3	4
Means over Samples	1	−0.94589	−1.07611	−1.03669	−1.26266
	2	−1.06382	−1.16113	−1.07626	−1.18758
	3	−1.03356	−1.14301	−1.09004	−1.13535
	4	−1.25153	−1.17140	−1.23966	−1.19199

There was clearly, a switch in the relative positions of the parents, and in the direction of dominance between the 2 experiments, a switch which underlined the interdependence of stage of development and genetical analysis. The reason for the higher midparent plant weight in the 1969 data could be traced to the use of a greater soil volume per plant (approximately 50% greater) during the 30 to 75 day period, in comparison to the volume used per plant for the equivalent period in the experiment here. Genotype-environment interaction was thus occurring with plant weight; similar changes, although much less pronounced and involving average dominance and midparent activity without any switch in the general relationship of R to M, were noted for log peroxidase activity. The comparison in the case of log peroxidase activity is summarised below:

1969 data:	Mean log peroxidase activity per plant at 75 days				
	R	M	midparent	F_1	average dominance
	−1.08346	−1.19326	−1.13836	−1.16059	0.40
Data here: (70 days)	−1.19057	−1.42463	−1.30760	−1.41832	0.95

The direction of dominance remained the same in both experiments, but the F_1 was closer to the lower parent (M) in the data here.

The degree of genotype-environment interaction displayed by these 2 characters differed markedly. A more adequate specification of the interaction of R, M and the F_1 in terms of plant weight and peroxidase activity will be made by setting up a controlled range of environments in which these genotypes could be grown for more than one generation. This approach, in which different levels of soil nitrogen represent the range of environments, may also reveal, in the case of R and M, after-effects of previous generations' environments. Such after-effects have been documented for *Linum* by Durrant (1962). Genotype-environment interaction may in part be due to differential expression of after-effects among a group of genotypes, where, as in the work reported here, the plants of one experiment, or environment, were the parents of plants in the succeeding, environmentally different, experiment(s). To what extent such after-effects might have been operating over the 2 experiments compared here must remain conjecture, as must their possible involvement, for example, in the significant F_1 male parent influence in the F_2 means for log peroxidase activity (comparison 5, table 10), an influence which could otherwise be classified as nuclear-cytoplasmic interaction.

Recent work with electrophoretic techniques in the separation of variant forms of peroxidase in a wide variety of plants has suggested that the detailed investigation of the activity and relative mobility of such isoenzymes, should they exist in the genotypes used in this study, might clarify the behaviour and distribution of peroxidase activity reported here. It might also supply an estimate of the number of loci responsible for peroxidase synthesis in the 2 genotypes R and M.

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References

1. Bartlett, M. S.: Supplement to the Journal of the Royal Statistical Society **4**, 137 (1937). — 2. Dillman, A. C., Brinsmade, J. C.: The effect of spacing on the development of the Flax plant. J. Amer. Soc. Agron. **30**, 267–278 (1938). — 3. Durrant, A.: The environmental induction of heritable change in *Linum*. Heredity **17**, 27–61 (1962). — 4. Galston, A. W., Dalberg, L. Y.: The adaptive formation and physiological significance of indoleacetic acid oxidase. Am. J. Bot. **41**, 373–380 (1954). — 5. Mather, K.: Biometrical Genetics. London: Methuen and Co. Ltd. 1949. — 6. Tyson, H., Jui, P. Y.: Peroxidase activity in *Linum usitatissimum* L. Annals of Botany **31**, 489–495 (1967). — 7. Tyson, H.: Peroxidase activity in *Linum usitatissimum* L. Annals of Botany **33**, 45–54 (1969).

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