SEGREGATION OF THE ENVIRONMENTALLY INDUCED RELATIVE MOBILITY SHIFTS IN FLAX GENOTROPH PEROXIDASE ISOZYMES

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SUMMARY

Crosses were made in all combinations between the large (L) and small (S) flax genotrophs, and their reciprocal F_1 's. Total main stem peroxidase activity was measured prior to electrophoretic separation; individual anionic isozyme relative mobilities (R_m 's) were obtained from electrophoresis of main stem homogenates. Both activity and R_m were examined in parent, F_1 , F_2 and backcross generations on an individual plant basis. Segregation for isozyme R_m occurred, and indicated control over each isozyme's R_m by a simple system with a dominant allele in L and a recessive in S. For activity, dominance was absent, and there was no evidence of segregation.

1. INTRODUCTION

THE presence of R_m differences between corresponding anionic peroxidase isozymes from the L and S genotrophs of Durrant (1971) has been described by Fieldes and Tyson (1972, 1973*a, b*). All these anionic isozymes displayed IAA oxidase activity (Fieldes, Deal and Tyson, 1977); such activity indicated their possible involvement in the control of growth and morphology through regulation of IAA level within stem tissues. The initial studies, using acrylamide gel electrophoresis of pooled extracts from individual L or S plants, showed that each of the anionic peroxidase isozymes displayed a shift, with the L isozymes all moving slightly faster than their S counterparts. The activities of S isozymes were higher than those of the corresponding L isozymes, and subsequent purification of the L and S isozymes has shown that this reflected increased enzyme production in S compared to L, on a per unit fresh weight basis (Fieldes, Deal and Tyson, 1977). An additional feature of these environmentally induced heritable shifts in isozyme R_m was that subsequent treatments of the same type as those used in the C_0 , or original treatment generation, resulted in further shifts in R_m being superimposed on those shifts already present through induction in the C_0 (Fieldes and Tyson, 1972). Studies of pooled homogenates from L, S and the L by S reciprocal F_1 's showed that the R_m shift was dominant in the hybrid towards L (Fieldes and Tyson, 1973*b*).

The studies mentioned above dealt with information from generation means. The next step, measurement of R_m in individual plants of segregating generations from the L by S cross, is described in detail here. The first two isozymes, *i.e.* the slowest two, were examined; total peroxidase activity of all isozymes in extracts prior to electrophoresis was also measured on an individual plant basis. Evidence was obtained for the first time of simple Mendelian control of the environmentally induced R_m difference between the L and S genotrophs, a control mediated through a dominant and recessive allele.

2. MATERIALS AND METHODS

Under growth chamber conditions, L, S and their reciprocal F_1 's were crossed; the crossing scheme is shown below:

Female parents:	Male parents			
	L $\times$ L (1)	L $\times$ S (2)	S $\times$ L (3)	S $\times$ S (4)
L $\times$ L (1)	11	12	13	14
L $\times$ S (2)	21	22	23	24
S $\times$ L (3)	31	32	33	34
S $\times$ S (4)	41	42	43	44

Types of offspring are identified by the two digit code indicating parentage.

Plants of all 16 types of offspring were grown in vermiculite, using a modified form of the nutrient solution of Murashige and Skoog (1962), under growth chamber conditions. Resultant approximate amounts of N , P and K supplied, on a per plant basis, were respectively 0.13 g, 0.02 g and 0.15 g. Temperature was cycled from 14°C to 22°C on a 24-hour basis; daylength was 20 hour. Each replicate contained one plant of each of the 16 offspring types; 14 complete replicates were available for main stem extract preparation, as detailed by Fieldes and Tyson (1972), 35 days after germination. The 16 extracts from a replicate were run on a single acrylamide gel slab, using the electrophoresis techniques, and subsequent staining and scanning methods described by Hart, Tyson and Bloomberg (1971). Two complete runs were made with the extracts from a replicate; duplicate R_m values for isozyme 1 or 2 from a given offspring type in a replicate were averaged for further analysis. Total peroxidase activity was measured on each extract.

The average R_m 's for isozymes 1 and 2 for each of the 14 replicates reflected differences in growth conditions, in electrophoresis procedures, and conceivably in the numbers of L or S segregants in the F_2 , B_1 and B_2 generations for each replicate. Differences in replicate mean R_m 's meant that simple classification into L or S type segregants could have been confused by overlapping of L or S type segregants from various replicates. To obtain a correction for the replicate differences, the regressions of mean R_m for all F_1 , F_2 , B_1 and B_2 progenies in a replicate on either the L or the S parental R_m in that replicate were calculated across all 14 replicates. Isozyme 1 data were examined separately from isozyme 2 data.

There were thus four regressions; each was significant. Neither the L or S regressions for isozyme 1, nor those for isozyme 2, differed significantly in slope. In both cases, therefore, the joint regressions through L and S, using within L and within S sums of squares and cross products, summarised the changes in progenies' mean R_m with parent mean R_m , i.e. $\frac{1}{2}(L+S)$, across replicates. The positions of the joint regression for either isozyme 1 data, or for isozyme 2, were set at $\frac{1}{2}(L+S)$ on the y axis. In other words, b_0 in the equation: $Y = b_0 + b_1(x - \bar{x})$, had the overall mean value for L (11) and S (44) R_m 's. The positions of these lines for isozyme 1 or for isozyme 2 suggested that they could be used (a) to make a distinction between L and S types in the 224 isozyme 1 or 2 values of all five generations examined, and (b), to adjust individual R_m values in all five generations to the same mid-parent R_m value for isozyme 1 or for isozyme 2 data.

Both (a) and (b) were carried out for each isozyme's data. For (a), individuals in all generations were classified as L or S, or as indeterminate, by setting up 95 per cent confidence limits above and below the joint regression line. Those points above the upper limit were classified as L segregants; those below the lower limit were classified as S. Points falling between the two limits, *i.e.* close to the joint regression line, were regarded as indeterminate. This resulted in the tabulation of numbers of L and S types in all five generations for each isozyme. The classification achieved with (a) for isozyme 1 data was compared with that for isozyme 2 data. Individuals classified as, say, L segregants with isozyme 1 data, were checked to see if this was also true for isozyme 2. For (b), adjustment to a common mid-parent value across all replicates was coupled with the histogramming of isozyme 1 or 2 adjusted data, as well as adjusted total peroxidase data.

Finally, analyses of variance of peroxidase activity, isozyme 1 and isozyme 2 data were separately carried out. The orthogonal comparisons made amongst the 16 types of offspring are shown in table 1.

TABLE 1
Orthogonal breakdown of the 15 degrees of freedom for offspring types

	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

3. RESULTS

The 16 offspring type means for peroxidase activity, isozyme 1 and $2R_m$ are shown in table 2. Analyses of variance on each character are summarised in table 3. In the offspring type breakdown of peroxidase activity only the parental ($L-S$) and backcross (B_1-B_2) differences were significant indicating the absence of dominance. Observed and expected generation means corresponded well on this basis. On the other hand, both isozyme 1 and $2R_m$'s displayed dominance since the same breakdown also revealed a significant difference between the midparent R_m and that of the F_1 reciprocal mean. On the basis of the parental and F_1 values, the expected F_2 agreed closely with that observed; other expected generation means for both isozymes showed similar close agreement with those observed.

There was a significant interaction for isozyme 2, which suggested a type of cytoplasmic effect on R_m in a backcross.

When the results from the classification with procedure (a), shown in table 4 were examined, there appeared to be fewer indeterminate points for isozyme 2 data compared to isozyme 1. This stemmed essentially from the shorter migration distance of isozyme 1, and the consequent difficulty of resolving the smaller L to S difference as accurately as with isozyme 2. Although the number of indeterminates was higher for isozyme 1 data, apart from the F_2 ($\chi^2 = 1.1046$, P between 0.2 and 0.3), all other ratios

TABLE 2
Offspring type means over 14 replicates

Type	Peroxidase activity*	Iso. 1 R_m	Iso. 2 R_m
11	17.07	0.0743	0.1708
12	17.08	0.0743	0.1712
13	18.88	0.0740	0.1697
14	18.93	0.0746	0.1716
21	18.82	0.0742	0.1696
22	20.64	0.0712	0.1613
23	19.30	0.0732	0.1674
24	20.21	0.0718	0.1652
31	19.01	0.0744	0.1700
32	18.42	0.0722	0.1657
33	19.31	0.0729	0.1653
34	20.47	0.0695	0.1578
41	20.28	0.0735	0.1681
42	20.25	0.0708	0.1584
43	20.91	0.0710	0.1608
44	21.99	0.0670	0.1506

* Activity expressed as Δ O.D. $\text{min}^{-1} \text{ml}^{-1}$.

Generation means for each character

$\bar{L}$	17.07	0.0743	0.1708
B_1	18.45	0.0742	0.1701
F_1	19.60	0.0741	0.1699
B_2	20.46	0.0708	0.1606
F_2	19.42	0.0724	0.1649
S	21.99	0.0670	0.1506
$\frac{1}{2}(\bar{L} + \bar{S})$	19.53	0.0707	0.1607

in isozyme 1 data agreed almost perfectly with those based on a simple Mendelian model with two alleles at one locus, where L was homozygous dominant, and S homozygous recessive. The isozyme 2 data were totally in agreement with this model.

A critical point concerned the correlation between the classification of each individual on isozyme 1's data versus its classification with isozyme 2 data. The lower part of table 4 shows the comparison between the two classifications; it is clear that the number of reversals over the total of 224 individuals classified was extremely small (6/224). Amongst the 168 individuals in generations where segregation could occur, five of these six reversals occurred. Such reversals would be either genuine recombination,

or mis-classification through inherent inaccuracies in the classification procedure. The particular techniques used would not distinguish between these possibilities. Only one individual was indeterminate in both its isozyme 1 and 2 data.

The evidence from the (a) procedure and the correlation just described would overwhelmingly support the contention that there is either a single

TABLE 3
Analysis of variance of peroxidase activity

Source	d.f.	Mean squares	F
Total	223		
Between replicates	13	80.72	13.29**
Between offspring types	15	24.73	4.07**
Error	195	6.07	

Offspring type breakdown:

1. ($\bar{L}-S$) $F = 27.87^{**}$
 13. ($\bar{B}_1-\bar{B}_2$) $F = 18.70^{**}$

Analysis of variance of isozyme 1 R_m

Total	223		
Between replicates	13	0.1058×10^{-3}	10.42**
Between offspring types	15	0.0632×10^{-3}	6.23**
Error	194 ^a	0.0102×10^{-3}	

Offspring type breakdown:

1. ($\bar{L}-S$) $F = 36.39^{**}$
 3. ($\bar{L}+\bar{S}$) - $\bar{F}_1$ $F = 16.08^{**}$
 13. ($\bar{B}_1-\bar{B}_2$) $F = 32.87^{**}$

^a One missing value fitted.

Analysis of variance of isozyme 2 data

Total	223		
Between replicates	13	0.0739×10^{-2}	12.02**
Between offspring types	15	0.0498×10^{-2}	8.10**
Error	193 ^b	0.0062×10^{-2}	

Offspring type breakdown:

1. ($\bar{L}-S$) $F = 46.47^{**}$
 3. ($\bar{L}+\bar{S}$) - $\bar{F}_1$ $F = 19.24^{**}$
 12. $F = 5.67^*$
 13. ($\bar{B}_1-\bar{B}_2$) $F = 41.56^{**}$

^b Two missing values fitted.

** Significant at probability 0.01.

* Significant at probability 0.05.

locus, with a dominant and a recessive allele, controlling the R_m shift, or else two loci, each with two such alleles, closely linked in coupling.

The histograms of the adjusted R_m values for isozymes 1 and 2 (figs. 1 and 2) corroborate the classification shown in table 4 from the use of joint regression lines. Isozyme 1 data do not show the distinct segregation found for isozyme 2, but the general trends shown in fig. 1 correspond well with those in fig. 2. A discriminant function calculated from the adjusted

parental L and S R_m 's for 1 and 2 did not improve discrimination over that possible with isozyme 2's data alone.

The adjusted values of activity have been histogrammed in fig. 3, and showed no evidence of segregation.

TABLE 4

Classification of all individuals in five generations into L, S or indeterminate through regressions of figs. 1 and 2

	Isozyme 1			Isozyme 2		
	L	S	Indeterminate	L	S	Indeterminate
L	14	0	0	14	0	0
F ₁	23	0	5	26	2	0
B ₁	47	2	7	54	1	1
B ₂	25	25	6	27	28	1
F ₂	35	16	5	40	15	1
S	1	12	1	0	13	1

Comparison of classifications made for isozyme 1 with that for isozyme 2

1. Classified as L in both isozymes 1 and 2 = 140
2. Classified as S in both isozymes 1 and 2 = 51
3. Indeterminate in both isozymes 1 and 2 = 1
4. Indeterminate for isozyme 2, classified for isozyme 1 = 3 (2 L's, 1 S)
5. Indeterminate for isozyme 1, classified for isozyme 2 = 23 (19 L's, 4 S's)
6. Classified as L for isozyme 1, S for isozyme 2 = 3
7. Classified as S for isozyme 1, L for isozyme 2 = 3

Total = 224

4. DISCUSSION

Our findings showed that R_m 's of two anionic stem peroxidase isozymes in L and S were controlled by dominant and recessive alleles. L was homozygous dominant. Either one single locus was involved in controlling both isozymes' R_m 's, or else there were two loci closely linked in coupling. Extension of the R_m measurements to the remaining isozymes separated will help to resolve the number of loci involved.

In relation to the molecular differences which exist between corresponding peroxidase isozymes of L and S, the relatively high percentage carbohydrate (CHO) content in them (Fieldes, Deal and Tyson, 1977) must be taken into consideration. Isozymes 1 and 2 have similar molecular weights (loc. cit.), but the percentage CHO in isozyme 1 was significantly higher than that of isozyme 2 in both genotrophs; estimates of the order of 24 per cent and 14 per cent respectively were obtained. These figures, agreeing in magnitude with those of Shannon, Kay and Lew (1966) from horseradish peroxidases, emphasise the large fraction of the peroxidase molecule which must be affected by (a) genes coding for enzymes involved in CHO synthesis, and, (b) genes coding for enzymes involved in the assembly of the effective peroxidase molecule. Inclusion of the polypeptide portion suggests that at least three systems of structural genes have roles in producing peroxidase.

The R_m changes displaying segregation here were sharply demarcated. The size of the R_m differences between corresponding isozymes from L and S, as in the data here, contrasts with the smaller R_m shifts superimposed by subsequent inducing treatments in the C_1 and C_2 generations on the

FIG. 1.—Isozyme 1 adjusted R_m for each generation. L and S positions indicated by arrows on x axis.

original L and S differences of the C_0 (Fieldes and Tyson, 1972), and with the yet smaller R_m shifts detectable between isozymes from the stem base and apex (Fieldes, Tyson and Bashour, 1976). The range of R_m differences represented in these three cases may reflect a range of degree of modification in the peroxidase molecule. In the context of very small R_m changes,

it is known that micro-heterogeneity (micro-banding) revealed through gel electrophoresis of apparently pure glycoprotein samples can originate in the variation possibilities of the CHO moiety (Gibbons, 1972).

The extent to which the CHO moiety of this glycoprotein may be

FIG. 2.—Isozyme 2 adjusted R_m data for each generation. L and S positions indicated by arrows on x axis.

responsible for the R_m differences between corresponding L and S isozymes must obviously be examined along with the search for any polypeptide differences between corresponding isozymes. The likely connection of the peroxidase isozymes in the genotrophs to a physiological role in controlling IAA level, through their dual catalytic functions, gives further impetus to the detailed dissection of the L and S peroxidase isozymes. Peptide mapping

techniques for peroxidase (Welinder, 1976) should help to clarify the differences underlying the R_m shifts, and provide clues as to the origin and mechanism of the genotroph changes.

FIG. 3.—Peroxidase activity adjusted data for each generation. L and S positions indicated by arrows on x axis.

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