Phytochemistry, 1976, Vol. 15, pp. 247–250. Pergamon Press Printed in England.

RELATIVE SHIFTS IN MOBILITY IN ANIONIC PEROXIDASE ISOENZYMES BETWEEN STEM BASE AND APEX OF FLAX GENOTROPHS

MARY ANN FIELDES, HUGH TYSON and NAHID BASHOUR
Biology Department, McGill University, P.O. Box 6070, Station A, Montreal, Quebec, Canada

(*Received* 18 *June* 1975)

Key Word Index—*Linum usitatissimum*: Linaceae; flax genotrophs; anionic peroxidase isoenzymes; R_m shifts.

Abstract—Anionic peroxidase isoenzymes, separated on acrylamide gels, were examined in two flax genotrophs and in their reciprocal F_2 hybrids. Isoenzyme 1 exhibited a significant difference in R_m between stem base and apex and there was a gradient of decreasing R_m and activity between base and apex. Isoenzyme 2 displayed only the activity gradient. The parents differed significantly in the R_m's and activities of isoenzymes 1 and 2, and the F_2's showed complete dominance of the L parent for R_m, with activities being approximately intermediate.

INTRODUCTION

Environmentally induced heritable changes have been produced [1] in an inbreeding higher plant, flax (*Linum usitatissimum L.*). Contrasting large (L) and small (S) genotrophs resulted from the treatment of a single completely inbred flax genotype with high levels of soil supplied nitrogen, phosphorus and potassium (NPK) or with NK respectively. L and S differed markedly in size and weight; the differences were maintained over many generations of progeny grown in uniform environments. The L genotroph also differed from S in that the four anionic peroxidase isoenzymes in its extracts had lower activity, but higher (faster) relative mobility (R_m) than shown by the corresponding four isoenzymes from S [2–4]. In related studies of peroxidase inhibitors in two flax genotypes [5], peroxidase activity was measured in various sections of the stem from the base to the apex. These gross activities, representing the sums of all individual anionic and cationic isoenzyme activities prior to any separation, showed a gradient, decreasing from the base to the apex. The gradient appeared to differ between the two genotypes examined.

The work reported here was undertaken to find if there was a shift in R_m of anionic peroxidase isoenzymes between the basal, central and apical sections of the main stem of the L and S genotrophs and of their reciprocal F_2 progeny. Gross activity was measured in each of the three stem sections. The activities of the individual isoenzymes in each section were also obtained.

RESULTS

Activities and R_m's for the two slowest migrating anionic isoenzymes are shown in Tables 1 and 2. Appropriate covariance analyses of activity and corresponding R_m data for these two isoenzymes confirmed that for each isoenzyme there was no underlying relationship between the two characters; activity and R_m could, therefore, be examined separately and independently. The two isoenzymes are referred to as 1 and 2 in order of their increasing R_m; the activity data are dealt with first. The stem sections are numbered I, II and III, with I being the basal third of the stem above the cotyledons, and III being the apical third.

There were very clear overall activity differences between the two parental genotrophs L and S for both isoenzymes. For isoenzyme 1 there was a significant reciprocal difference; for isoenzyme 2 the mean F_2 activity showed dominance in the direction of the L genotroph. Clear overall differences in stem section activities were also present, with an apparent linear decline from base to apex for both isoenzymes. Within the parents and progenies, however, there were differences in the activity gradients exhibited from base to apex. The reciprocal F_2's resembled the S parent in the case of isoenzyme 2's base to apex activity gradient; the genotroph difference in this gradient was pronounced. Gross activity data (Table 3) indicated a steep decline from base to apex in L, but a much less marked decline in S; the F_2's were somewhat intermediate in their activity gradient. Had this study been carried out with smaller and more numerous sections from the main stem length, the individual isoenzyme or gross activity changes would presumably have appeared smoother and more continuous than they did here. The decreasing isoenzyme activities for each consecutively higher stem section might have been due to steady changes in the rate of synthesis from section to section. Phenolic peroxidase inhibitors, unless directly coupled to the enzyme molecule, would not have been a very likely explanation of the activity gradient, since 'free' phenolic compounds were 88% removed from extracts by the anion resin and dialysis procedure used [6]. Alternatively, continuous modification, in some fashion, of the enzyme itself might have been occurring across the various sections; the possibility of modification leads into R_m data which are shown for isoenzymes 1 and 2 in Tables 1 and 2 respectively.

There were clear overall parental R_m differences for both isoenzymes. The mean F_2 R_m for both isoenzymes

Table 1. Isozyme 1 activities and mobilities in flax genotrophs

Isozyme 1 Activity (Absorbance peak areas in in^2 g fr. wt^{-1})

	Genotroph L			L × S			S × L			Genotroph S		
Section	I	II	III	I	II	III	I	II	III	I	II	III
Rep. A	1·45	1·24	1·06	1·68	1·64	1·49	1·67	1·74	0·53	2·13	1·76	1·71
Rep. B	1·11	0·82	0·96	0·94	1·39	1·31	1·09	0·75	1·46	1·48	1·28	1·07
Means:	1·28	1·03	1·01	1·31	1·52	1·40	1·38	1·25	1·00	1·81	1·52	1·39

L = 1·11; L × S = 1·41; S × L = 1·21; S = 1·57 Section I = 1·45; II = 1·33; III = 1·20

Isozyme 1 R_m values

	Genotroph L			L × S			S × L			Genotroph S		
Section:	I	II	III	I	II	III	I	II	III	I	II	III
Rep. A	0·0717	0·0721	0·0694	0·0725	0·0715	0·0699	0·0721	0·0741	0·0717	0·0670	0·0677	0·0660
Rep. B	0·0823	0·0791	0·0802	0·0818	0·0819	0·0795	0·0805	0·0853	0·0789	0·0731	0·0719	0·0648
Means:	0·0770	0·0756	0·0748	0·0772	0·0767	0·0747	0·0763	0·0797	0·0753	0·0701	0·0698	0·0654

L = 0·0758; L × S = 0·0762; S × L = 0·0771; S = 0·0684
Section I = 0·0752; II = 0·0755; III = 0·0726

showed complete dominance for L, although there was a small, but significant, reciprocal difference for isoenzyme 2. The overall R_m change from base to apex was trivial and inconsistent for isoenzyme 2, but for isoenzyme 1 the overall R_m shift between base and apex was larger and significant. Within each of the parents and the reciprocal F_2's there was a decline in R_m from base to apex; this decline was more or less continuous except in the case of S × L. Comparing the basal to the apical R_m values, there was a consistent fall in R_m for both parents and both reciprocals in each replicate. For isoenzyme 1, then, in contrast to isoenzyme 2, there appeared to be a small, but real shift towards slower R_m in proceeding from the base of the stem to the apex. The centre section R_m values were variable in relation to the mid-points between basal and apical R_m's in each of the parents and progenies in the individual replicates. Evidence for a smooth consistent shift in R_m for isoenzyme 1 was thus inconclusive, although the L, L × S and S centre sections, averaged over replicates, lay

Table 2. Isozyme 2 activities and mobilities in flax genotrophs

Isozyme 2 Activity (Absorbance peak areas in in^2 g fr. wt^{-1})

	Genotroph L			L × S			S × L			Genotroph S		
Section	I	II	III	I	II	III	I	II	III	I	II	III
Rep. A	1·07	0·36	0·40	1·44	0·88	0·39	1·33	1·23	0·30	1·95	2·01	0·90
Rep. B	1·23	0·32	0·30	1·11	1·29	0·61	1·17	1·01	0·52	2·03	1·61	1·48
Means:	1·15	0·34	0·35	1·28	1·09	0·50	1·25	1·12	0·41	1·99	1·81	1·19

L = 0·61; L × S = 0·96; S × L = 0·93; S = 1·66
Section I = 1·42; II = 1·09; III = 0·61

Isozymes R_m values

	Genotroph L			L × S			S × L			Genotroph S		
Section:	I	II	III	I	II	III	I	II	III	I	II	III
Rep. A	0·1608	0·1658	0·1594	0·1599	0·1603	0·1627	0·1614	0·1626	0·1628	0·1440	0·1436	0·1442
Rep. B	0·1841	0·1824	0·1823	0·1809	0·1822	0·1817	0·1813	0·1845	0·1845	0·1674	0·1701	0·1681
Means:	0·1725	0·1739	0·1709	0·1704	0·1713	0·1722	0·1714	0·1736	0·1737	0·1557	0·1569	0·1562

L = 0·1724; L × S = 0·1713; S × L = 0·1729; S = 0·1563
Section I = 0·1675; II = 0·1689; III = 0·1683

Table 3. Total peroxidase activities in flax genotrophs

	Genotroph L			L × S			S × L			Genotroph S		
Section:	I	II	III	I	II	III	I	II	III	I	II	III
Rep. A	0·2248	0·1154	0·0329	0·2472	0·1663	0·0600	0·2030	0·1274	0·0380	0·2235	·01990	0·1259
Rep. B	0·1983	0·1020	0·0261	0·1920	0·1515	0·0383	0·2167	0·1608	0·0540	0·2453	0·1992	0·1172
Means:	0·2116	0·1087	0·0295	0·2196	0·1589	0·0492	0·2099	0·1441	0·0460	0·2344	0·1991	0·1216

L = 0·1166; L × S = 0·1426; S × L = 0·1333; S = 0·1850
Section I = 0·2189; II = 0·1527; III = 0·0616
* Measured as absorbance min^{-1} g fr. wt^{-1}

between the basal and apical values. The percentage difference between the overall basal section mean R_m and that for the apical stem section amounted to a 3·5% drop in R_m from base to apex. This could be compared to the mean genotroph R_m difference over all sections, which amounted to a 9·8% drop from L to S, or approximately three times the extremely small R_m shift between the basal and apical stem sections detected here for isoenzyme 1.

DISCUSSION

The present results on R_m and activity corroborated earlier findings [2–4] in respect of genotroph differences for individual peroxidase isoenzymes, with L isoenzymes having consistently higher (faster) R_m's and lower activities than their corresponding isoenzymes in S. Complete dominance for R_m towards L and intermediate activity in the L by S hybrids were also again confirmed. More relevant to the results here were the repeated shifts in R_m [2] produced by successive generations of growth in NPK (high nitrogen, phosphorus and potassium) as opposed to NK; a linear relation could be established between the number of generations of NPK and R_m averaged over all four isoenzymes. This environmentally induced shifting of R_m suggested that anionic peroxidase isoenzymes in this species might be continuously modifiable within some finite range of R_m values. For some isoenzymes there was a linear change in R_m with time between the 16th and 36th day after germination [3]; again, the data for the six time points suggested that these particular isoenzyme were being more or less continuously modified. The evidence here from isoenzyme 1's R_m measurements in three zones of the main stem fitted the same theme of modification in the enzyme molecule by very small steps.

If the idea that almost imperceptibly small changes in the enzyme molecule had taken place is correct, it could imply that exceedingly fine adjustment to functional requirements is possible in this enzyme system. The occurrence of different peroxidase isoenzymes through developmental or time changes has been amply documented in a range of plant species; the point arising from the work on flax genotroph isoenzymes is the possibility of smooth and continuous transition from one molecular variant to another.

The mechanism behind such smooth, continuous change is, for the time being, a speculative matter. Peroxidase is a glycoprotein; data [7] have been obtained for the amino acid and carbohydrate composition of horseradish peroxidase isoenzymes. Evidence from horseradish [8] and results from flax genotype and genotroph F_1 hybrids [2,4] have indicated a monomeric structure for the enxyme. However, the finding that a coumarin, scopoletin, might be directly coupled to the peroxidase molecule [9] introduced a third enzyme modification possibility. Thus, amino acid sequence, attached carbohydrate and attached phenolic compounds might be varied by the genotype or genotroph in response to what are presumably varying functional requirements. The multiplicity of peroxidase variants implied by a smooth transition caused, for example, by a series of changing environments, need not necessarily indicate the presence of a very large number of peroxidase structural genes coding for the polypeptide sequence of the enzyme. An array of variants for any one of the four anionic isoenzymes might be accommodated by simultaneous regulation of the genes coding for polypeptide, carbohydrate or phenolic components. Gene regulation [10] would, in any case, be far more plausible as a control mechanism for such isoenzymic variation than an alternative explanation such as direct alteration in the structural gene coding for the polypeptide portion by an environmental influence.

Since the individual anionic peroxidase isoenzymes from flax genotypes and genotrophs can now be purified (Fieldes, unpublished), the variability between corresponding isoenzymes of L and S in terms of amino acid and carbohydrate composition, and possibly attached phenols, may be examined. If differences between L and S in any or all of these features can be detected, an insight will be available into the way in which environmentally induced heritable changes in this species affect an enzyme. The general significance of such changes has been extended by the demonstration of similar effects in another inbreeding species, tobacco (*Nicotiana rustica* L.) [11].

EXPERIMENTAL

Plant material, growing conditions and extraction. Three generations of the L and S genotrophs were produced in growth chambers prior to this experiment; during these generations the reciprocal F_1's and F_2's between L and S were made. Reciprocal F_2's were used in the present study. Growth chamber conditions were identical with those already detailed

[2]. The plants in the present study were again grown in vermiculite, to which inorganic nutrients were added at intervals in a modified form of a nutrient solution [12]. One pot each of L and S and their reciprocal F_2's was grown in each of two replicates (Rep.'s A and B). Thirty days after germination, the plants were cut for preparation of extracts; the approximately 20 plants in each pot were pooled. Main stem tissue between the cotyledons and apices was subdivided into three sections of equal length; all plants were vertually identical in height at sampling, and numbers also were almost all identical throughout the eight experimental pots. Twenty-four tissue extracts (2 replicates × (2 parents + 2 progenies) × 3 stem sections) were made by homogenising stem material in 0·1 M Pi buffer at pH 8·0, in a ratio of 1·0 g stem tissue to 4·0 ml buffer. Dowex anion exchange resin (1 × 8, 200–400 mesh) was incorporated in the homogenisation mixture (20% w/v, resin/buffer) to remove phenolic peroxidase inhibitors [6]. Following homogenisation, each extract was strained through nylon mesh, frozen, thawed and then centrifuged at 45000*g* for 20 min. Each extract's supernatant was dialysed against phosphate buffer (0·1 M, pH 8·0) for 18 hr.; storage of the dialysed extract supernatants was at −10°. The technique for the electrophoresis of these isoenzymes has been given [13]. Duplicates of each extract in a replicate were run simultaneously, using a constant 400 V. Two runs were made of all extracts in a replicate; there were thus 4 gels of each of the 24 extracts. Extract positions within the electrophoresis tank were randomised for each run. Isoenzyme reaction with a hydrogen peroxide–guaiacol system at pH 7·0 was recorded by scanning each gel at 295 nm. Peroxidase activity prior to electrophoresis (gross activity) was assayed in each extract with the same system. Attention was focussed on the two slowest migrating anionic isoenzymes for R_m and activity measurements, since their absorbance (A) peaks of the scans were exceptionally sharp. A chart recorder speed was used which effectively increased the gel tracks' total lengths by a factor of 1·7 over the original approximately 13·5 cm. Standard analysis of variance and covariance techniques were applied to the activity and R_m data.

Acknowledgements—Thanks are extended to the National Research Council of Canada whose financial assistance made this work possible, and to Dr. Alan Durrant, Aberystwyth, U.K., who very kindly provided seed of the L and S genotrophs.

REFERENCES

1. Durrant, A. (1971) *Heredity* **27,** 277.
2. Fieldes, M. A. and Tyson, H. (1972) *Can. J. Genet. Cytol.* **14,** 625.
3. Fieldes, M. A. and Tyson, H. (1973) *Can J. Genet. Cytol.* **15,** 731.
4. Fieldes, M. A. and Tyson, H. (1973) *Can. J. Genet. Cytol.* **15,** 745.
5. Tyson, H. and Fieldes, M. A. (1972) *Z. Pflanzenphysiol.* **66,** 385.
6. Fieldes, M. A. and Tyson, H. (1973) *Phytochemistry* **12,** 2133.
7. Shannon, L. M., Kay E. and Lew, J. Y. (1966) *J. Biol. Chem.* **241,** 2166.
8. Shih, J. H. C., Shannon, L. M., Kay, E. and Lew, J. Y. (1971) *J. Biol. Chem.* **246,** 4546.
9. Sirois, J. C. and Miller, R. W. (1972) *Plant Physiol.* **49,** 1012.
10. Bussey, H. and Fieldes, M. A. (1974) *Nature* **251,** 708.
11. Perkins, J. M., Eglington, E. G. and Jinks, J. L. *Heredity* **27,** 441.
12. Murashige, T. and Skoog, F. (1962) *Physiol. Plantarum* **15,** 473.
13. Hart, M. A., Tyson, H. and Bloomberg, R. (1971) *Can. J. Botany* **49,** 2129.