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Isozyme studies in Indian mustard (*Brassica juncea* L.)

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Summary. A comparative study of peroxidase and esterase isozymes was carried out at five developmental stages of siliqua in order to characterize twelve genotypes of Indian mustard. The studies showed nearly the same number of isozyme bands at every stage for peroxidase and a varying number of isozyme bands for esterase. The appearance and disappearance of bands, along with their intensity scores, indicated the role of different isozymes at different stages of siliqua development. It has been ascertained that these patterns, especially the intensity scores, can be successfully used to characterize different Indian mustard genotypes.

Key words: Starch gel electrophoresis – Isozymes – Peroxidase – Esterase – Indian mustard – *Brassica juncea* L.

Introduction

Isozymes serve as unique molecular-genetic markers in a wide range of plant species. Realizing their importance as genetic markers, isozymes have been used to characterize cultivars in a variety of crop plants. The germplasm of Indian mustard has recently been diversified by the development of a number of genetic stocks through the induction of mutations, hybridization and selection. In the present investigation, an attempt has been made to characterize some of these diverse genetic stocks using isozyme studies.

Materials and methods

A set of 12 lines, namely RLM 29, RLM 84, RLM 198, RLM 240, RLM 514, RLM 621, RLC 1005, RL 18, RH 30, RH 7514, P. Rai 34 and Varuna, was analyzed for peroxidase and esterase isozymes at five stages of siliqua development, namely 5(S_1), 10(S_2), 20(S_3), 30(S_4), and 40(S_5) day-old siliquae.

Enzyme extraction

For every stage sampled, 2.0 g of tissue was hand ground in one ml 0.2 M tris citrate buffer (pH 8.65) with a prechilled pestle and mortar. The homogenate was squeezed and centrifuged at low temperature at 10,000 × g for 10 min.

Electrophoresis

Horizontal starch gel electrophoresis (Smithies 1955) was used for studying isozymes. The starch gel was prepared by cooking hydrolysed potato starch (12% w/v) in 0.2 M tris-citrate buffer (pH 8.65). The crude extract was absorbed on 8 × 8 mm filter paper wicks and inserted into the slots made in the middle of gels for peroxidases and towards the cathodal end for esterases. The electrophoresis was carried out at 4 °C using 0.3 M sodium citrate buffer (pH 8.6) for 6 h at 300 V, 30 mA current. Filter paper wicks were removed after 30 min to avoid the trailing effect.

Staining of gels

For peroxidases, one g of benzidine was dissolved by heating it in one l of 0.2 M acetate buffer, pH 5.0. The solution was cooled and filtered. Gels were incubated at room temperature in the following mixture: benzidine solution – 200 ml; 3% H_2O_2 – 5 ml.

After 20 min, blue bands turning brown started appearing slowly; these were zymogramed immediately. Two independent extractions were made for every sample and runs were continued until the desired separation was obtained. The relative position of bands was measured in cm.

For localizing esterases, the method of Harris and Hopkinson (1976) was used.

Naming and scoring of bands

The naming of the bands was done according to the method of Pawar and Gupta (1975). To calculate enzyme activities in terms of intensity scores, the different bads were scored as very light, light, medium, dark and very dark (rating 1 to 5) and the scores allotted were summed.

Results and discussion

For peroxidases, six anodal and four cathodal bands were observed to be present at the different developmental stages (Figs. 1–5). Band A_4, however, was typically absent at S_2 and S_3. Band A_1 was present only at the S_3 stage and band C_1 was altogether absent at S_3, S_4 and S_5. Interestingly, in every genotype, band C_1 had a very high intensity at S_1 and an uniformly low intensity at S_2. This band, therefore, did not help in characterizing the genotypes.

Similarly, A_5, A_2, C_2 could not be used as distinctly variable bands for the identification of genotypes. Band A_6 was characteristically absent at S_1, S_2 and S_3 in RLM 514, S_1 and S_2 in RLM 29 and RLM 84 and S_1 in RLM 621 and RL 18. The absence of this isozyme (A_6) in the early stages of siliqua development, therefore, seemed to be a characteristic feature of Ludhiana genotypes except in the case of RLM 240 which had as high an activity of peroxidase as observed in the rest of the genotypes. Band A_4 was present at a very low intensity at S_4 and with a maximum of medium intensity in all the genotypes of the final stage (S_5). For band A_3 there was not any typical trend of variation. For band A_3, three Ludhiana genotypes showed a high intensity and five genotypes showed a low intensity at S_3. Compared

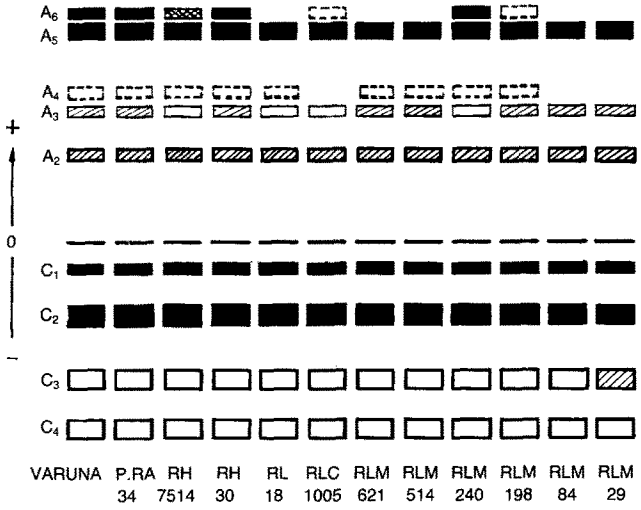

Fig. 1. Zymogram showing peroxidase isozymes of 5-day-old siliqua (S_1). In this and subsequent figures: ⊏⊐ = very light intensity, score = 1; ▭ = light intensity, score = 2; ▨ = medium intensity, score = 3; ▩ = dark intensity, score = 4 and ■ = very dark intensity, score = 5

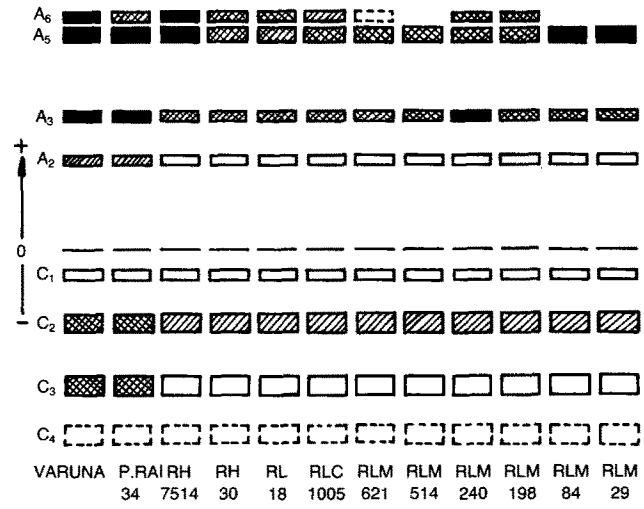

Fig. 2. Zymogram showing peroxidase isozymes of 10-day-old siliqua (S_2)

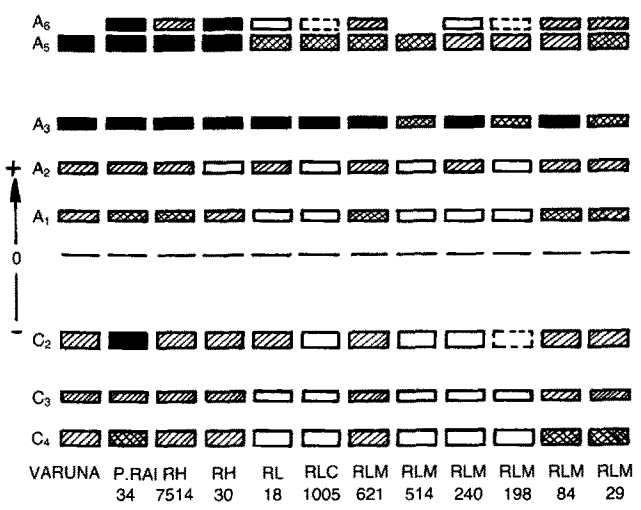

Fig. 3. Zymogram showing peroxidase isozymes of 20-day-old siliqua (S_3)

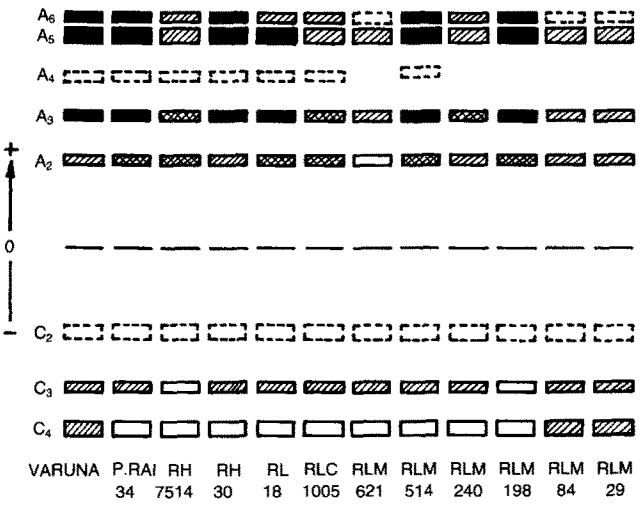

Fig. 4. Zymogram showing peroxidase isozymes of 30-day-old siliqua (S_4)

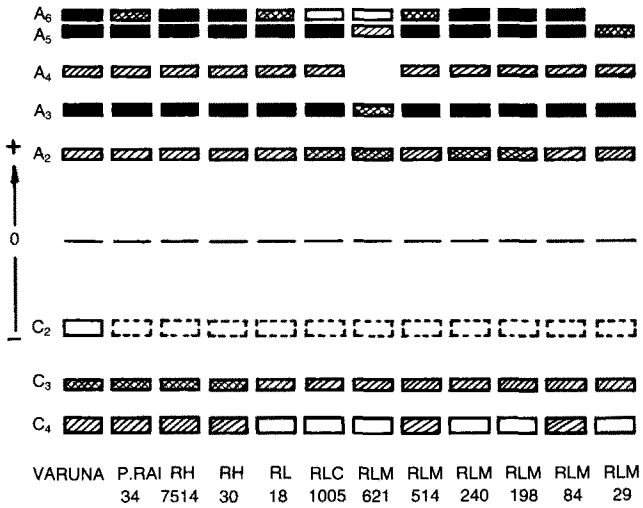

Fig. 5. Zymogram showing peroxidase isozymes of 40-day-old siliqua (S_5)

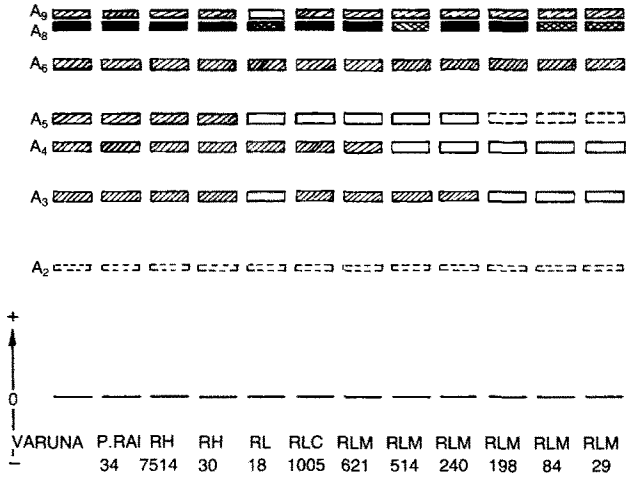

Fig. 6. Zymogram showing esterase isozymes of 5-day-old siliqua (S_1)

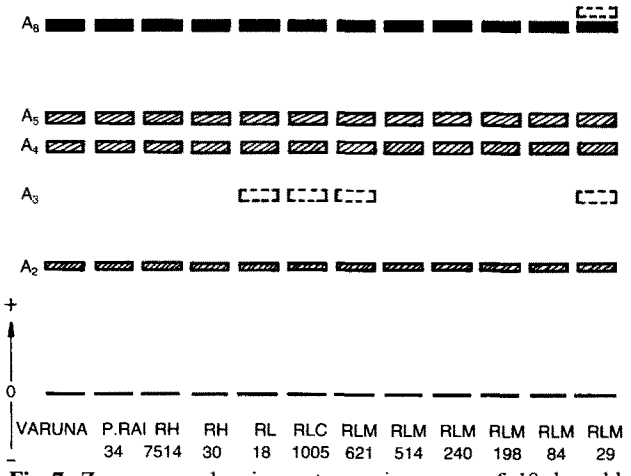

Fig. 7. Zymogram showing esterase isozymes of 10-day-old siliqua (S_2)

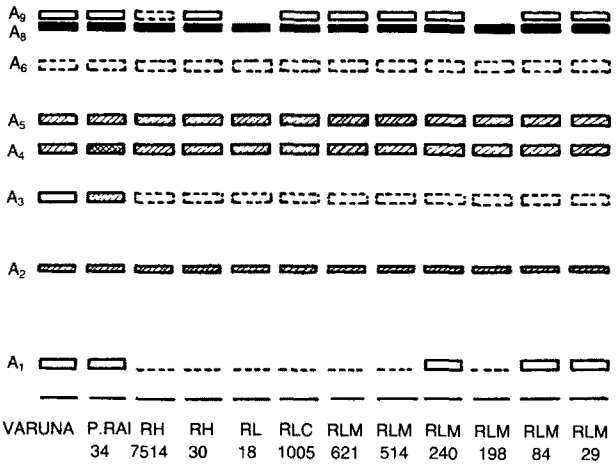

Fig. 8. Zymogram showing esterase isozymes of 20-day-old siliqua (S_3)

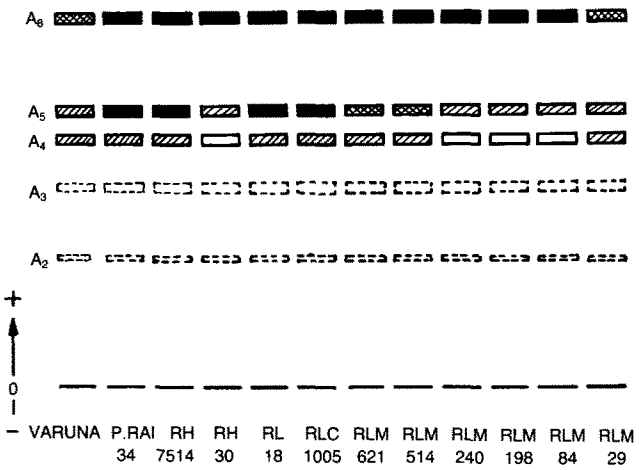

Fig. 9. Zymogram showing esterase isozymes of 30-day-old siliqua (S_4)

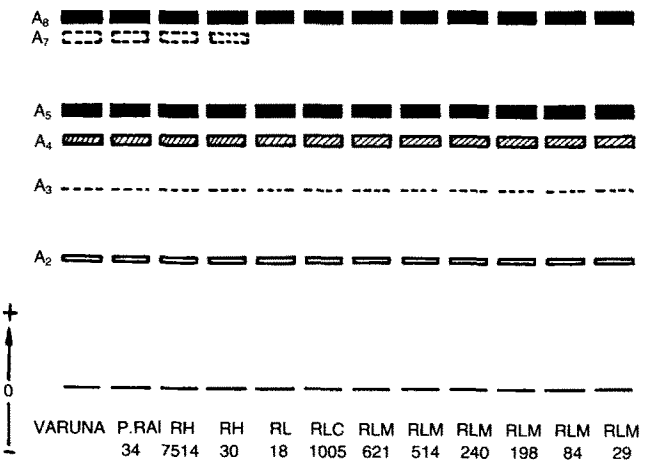

Fig. 10. Zymogram showing esterase isozymes of 40-day-old siliqua (S_5)

Table 1. Peroxidase score at different stages of siliqua development

Sr. no.	Genotype	S_1	S_2	S_3	S_4	S_5	C.V.
1.	RLM 29	26	19	28	17	21	26.36
2.	RLM 84	25	19	28	17	28	21.06
3.	RLM 198	27	22	17	24	28	18.39
4.	RLM 240	30	23	20	18	28	20.46
5.	RLM 514	26	18	18	26	27	17.78
6.	RLM 621	26	19	24	15	19	20.96
7.	RLC 1005	25	20	20	20	25	12.99
8.	RL 18	25	22	22	24	27	8.48
9.	RH 30	31	22	29	26	29	11.49
10.	RH 7514	29	24	29	20	29	13.96
11.	P. Rai 34	31	28	34	26	28	10.22
12.	Varuna	31	27	25	26	30	8.80

Table 2. Esterase score at different stages of siliqua development

Sr. no.	Genotype	S_1	S_2	S_3	S_4	S_5	C.V.
1.	RLM 29	16	16	20	12	16	17.68
2.	RLM 84	16	14	20	12	16	19.00
3.	RLM 198	17	14	16	12	16	13.33
4.	RLM 240	19	14	20	12	16	20.66
5.	RLM 514	18	14	18	14	16	12.50
6.	RLM 621	20	15	18	14	16	14.51
7.	RLC 1005	20	15	18	15	16	12.90
8.	RL 18	18	15	16	19	16	7.65
9.	RH 30	21	17	18	12	17	19.06
10.	RH 7514	21	14	17	15	17	15.98
11.	P. Rai 34	21	14	22	15	17	20.00
12.	Varuna	21	14	20	12	17	22.82

to this, other genotypes showed medium to high intensity for band A_1 at this stage. There was a variable pattern for band C_3 and C_4 but the extent of variation was quite less and not ver contrasting.

Since the isozyme banding patterns alone did not serve a very meaningful purpose, peroxidase acitivity calculated in terms of intensity scores was used for characterization. The data are presented in Table 1. These results show that the coefficient of variation at different stages varied from 8 to 26%. The least variation was shown by RL 18 and Varuna, the well-established genotypes. The maximum coefficient of variation was observed in RLM 29. RLM 514 showed typically the lowest score at S_2 and S_3. RLM 29 and RLM 84 showed low peroxidase activity at S_4 and S_5. RH 30 had the highest peroxidase activity at S_1 and S_5. P. Rai 34 had high peroxidase activity at S_1 and S_3 and Varuna at S_1 and S_5.

Thus, the description of genotypes based on scores for total peroxidase activity seem to be more informative than the results on individual banding patterns. Similar results have also been reported by Gupta and Dhiman (1977) in pearl millet.

For esterases, there were nine anodal bands only (Figs. 6–10). Even these isozyme bands did not show as much divergence as otherwise observed for peroxidases. The most informative seemed to be band A_5, because for this band the Ludhiana genotypes had a much lower esterase activity at S_1 and S_2 as compared to the remainder of the genotypes. Intensity scores for esterases are presented in Table 2. Similar to peroxidase, for esterase also there was least co-efficient of variation in RL 18. It may be again emphasized that RL 18 is the oldest well-established genotype. Compared to this, Varuna, another established strain but which has been evolved more recently, was not as consistant for esterase as it was for peroxidase isozymes. Intensity scores for a number of genotypes were identical at the different developmental stages. Intensity scores were high at S_1 and S_5 in the lines that were not of Ludhiana origin. In general, Ludhiana genotypes seemed to have lesser esterase activity as compared to the other genotypes.

It is evident from the results discussed above that appearance and disappearance of bands alongwith their intensities can be effectively used for identification of different genotypes of Indian mustard.

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