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Soluble Proteins and Isoenzymes from Seeds of Diverse Male Steriles of Sorghum, (*Sorghum bicolor* (L.) Moench)

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Summary. A comparison of soluble protein, esterase, GDH and ADH isoenzyme patterns in seeds of different steriles, maintainers and restorer lines exhibited similarities as well as differences. Soluble protein patterns from sterile and maintainer lines differed both qualitatively and quantitatively. Based on the esterase patterns, male steriles with different cytoplasms could be separated into three groups (i) Ck 60A and B; Nagpur A and B, (ii) M 35-1A and 1 B, M 31–2A and 2B, (iii) G1A and B, VZM 2A and 2B. Each group could further be differentiated on the basis of minor differences in esterase isoenzyme patterns within each group. ADH and GDH patterns in general were similar in both sterile and maintainer lines.

Key words: Isoenzyme – Male sterile (A) – Maintainer (B) – Restorer (R) – Apomict – *Sorghum bicolor* (L.) Moench – Gel electrophoresis

Abbreviations

ADH Alcohol dehydrogenase
GDH Glutamate dehydrogenase
NAD Nicotinamide adenine dinucleotide

Introduction

Soluble proteins are the physiologically active fractions which constitute the major bulk of enzymes involved in plant metabolism. Yanofsky and Lawrence (1960) reported the existence of a direct relationship between genes and enzymes. In studying evolutionary mechanisms and species relationships it has been felt that conventional methods such as morphological variations, chromosome behaviour, etc., are not discriminatory enough. Theoretically, proteins which are the primary product of genes probably provide a direct measure of gene homology. Based on this premise attempts have been made by many workers to understand genome relationships using the electrophoretic patterns of proteins and isoenzymes (Alam and Sandal 1969, Schwartz 1966, Siddiq et al. 1972). Payne and Koszykovski (1978) used esterase differences as an aid in identifying soybean cultivars.

In the present study soluble protein and isoenzyme patterns in seeds of sorghum male steriles with different cytoplasms and their maintainer lines have been studied.

Materials and Methods

Mature seeds of six male steriles (Ck 60A, Nagpur A (both *milo*), G1A, VZM 2A, M 35-1A, M 31-2A), their maintainers (B lines), three fertility restorer lines (Nandyal, GM1-5, K.Local) and two apomictic lines (R 473, 302) were used for analysing soluble protein and isoenzymes patterns.

Soluble Protein Extraction

Seeds were ground in chilled pestle mortars with 50 mM Tris-Cl buffer (pH 7.6; 1 : 2.5 w/v) containing 5 mM β-mercaptoethanol and 5 mM EDTA for solubilizing protein and isoenzymes of alcohol dehydrogenase (ADH), glutamate dehydrogenase (GDH) and esterases. All operations were carried out at 4 °C. The cell paste suspension was then centrifuged at 15,000 × g for 30 min at 0 °C. The supernatant obtained was used immediately in gel electrophoresis.

Separation of Proteins

Polyacrylamide gel electrophoresis was used to separate soluble proteins and various isoenzymes. The anionic system of Davis (1964) and Ornstein (1964) was adopted for separation of proteins and isoenzymes. Samples containing 200–225 µg protein were layered above the spacer gel. Electrophoresis was conducted in the cold (about 4 °C). Initially 2 mA were applied (for 15 min); this was increased to 3 mA current per gel tube until the tracking dye (bromophenol blue) entered into the running gel tube. After the completion of electrophoresis, which was indicated by the movement of

tracking dye to the bottom of gels, the gels were stained for 30 min in 0.1% amido black (in 7% acetic acid) and destained by diffusing out excess stain in 7% acetic acid.

Esterase and ADH Isoenzyme Detection

Esterases were detected by incubating gels in 50 ml phosphate buffer (0.05 M; pH 6.0), containing 1 ml of 1% α-naphthyl-acetate in 60% acetone and 25 mg Fast Blue RR, at room temperature for 10–30 min. ADH gels were incubated at 37 °C for 20 min in a reaction mixture containing Tris-Cl, 0.05 M; EtOH, 0.05 ml; phenazine methosulphate (PMS), 1.0 mg; Nitro-bluetetrazolium (NBT), 10.0 mg; NAD, 40 mg; NaCN, 0.002 M, 2.0 ml, with a final volume of 20 ml at pH 8.0.

GDH Isoenzyme Detection

For GDH detection, gels were incubated at 37 °C until visible bands developed in the reaction mixture containing sodium glutamate, 100 mg; nicotinamide adenine dinucleotide (NAD), 12.0 mg; NBT, 10.0 mg; PMS, 1.0 mg; $MgCl_2$, 8.0 mg, 0.05 M phosphate buffer to a final volume of 20 ml; pH 6.7.

As a control, a blank gel for each enzyme was incubated in the mixture. For dehydrogenases, two control gels were incubated, one in a reaction mixture not containing NAD; the second was a blank gel. In such controls no bands were observed. At least two independent extractions were made for all the material examined. For each group of enzymes, triplicate runs were made. The relative migration (Rm) of each band with respect to the front formed by the tracking dye was calculated. Densitometer tracings of the gels were obtained on a Joyce-Loebl chromoscan.

Results

The soluble protein patterns obtained from different male steriles along with maintainers, restorers and two apomictic lines are shown in Fig. 1. The Rm values for protein bands are given in Table 1. The comparison of protein patterns indicate that patterns in *milo* Ck 60A, Ck 60B, Nagpur A and Nagpur B, are different qualitatively from those of G1A, VZ M-2A, M 35-1A and M 31-2A. In general, the soluble protein pattern of the M 35-1A and M 35-1B lines were similar to those of M 31-2A and M 31-2B. The protein pattern of restorer and apomictic lines differed considerably from other sterile lines and those of maintainer lines. The protein patterns of GM1-5 and K.Local showed considerable similarities. A maximum number of bands were found in Nagpur A while a minimum number were found in GM1-5. In general, restorers and apomictic lines had a fewer number of bands than the A and B lines. Nagpur A, G1A and VZM 2A had more bands compared to their maintainers, whereas the maintainers Ck 60B and M 35-1B had more bands when compared to respective male sterile lines. Bands with Rm 0.01, 0.19, 0.41, 0.45 were common to all male steriles and their maintainers. Bands with Rm 0.29 and 0.57 were common to all except that the former band did not appear in G1 B and the latter did not appear in M 31-2B. Bands with Rm 0.71 were common to only *milo* and were absent in other male steriles. Bands with Rm 0.73 were absent in *milo* and present in other male steriles, as well as in their maintainers.

Characteristic differences were evident between lines of different cytoplasmic origin with respect to protein bands with low electrophoretic mobility. Bands with Rm 0.04 and 0.33 were present in the M 35-1B and M 31-2B lines but absent in other A and R lines with *milo,* G1A and VZM 2A. These protein bands appear to be characteristic of this group (M 35-1B and M 31-2B). Bands with Rm 0.41 and 0.46 were not only common to all male sterile A and B lines but also showed greater intensities in all. Intensity of protein bands with low electrophoretic mobility was greater in Ck 60B, G1B, M 35-1B and M 31-2B.

A Versus B Lines

Comparing protein patterns of Ck 60A with Ck 60B, it was found that bands at Rm 0.25, 0.31, 0.61 and 0.83 were present only in Ck 60B, while bands with Rm 0.11 and 0.51 were present in Ck 60A but absent in Ck 60B. The remainder of the bands were common for both lines. Bands with Rm 0.07, 0.11, 0.13, 0.53 and 0.81, present in Nagpur A, were absent in Nagpur B, and the remainder of the bands were common to both. Protein bands with Rm 0.17 and 0.29 were additional bands in G1A compared to G1B. Most bands are common, except for Rm 0.25 and 0.37 present only in VZM 2A, and Rm 0.63, present in VZM 2B. In the cases of M 35-1A and B, bands with Rm 0.11 and 0.25 were present only in M 35-1A and protein bands with Rm 0.13, 0.15 and 0.27 were present in M 35-1B. Protein patterns from M31-2A and B differed from each other with respect to protein bands with Rm 0.01, 0.09, 0.15, 0.53, 0.57 and 0.67, present in M31-2A and absent in M31-2B. Instead new protein bands with Rm 0.17, 0.51, 0.59, 0.63, and 0.65 were present in M31-2B.

Esterase Isoenzyme

Esterase isoenzyme patterns from the seeds of A, B, R and apomictic lines are shown in Fig. 2; Rm values are given in Table 2. Three distinct patterns were evident between A and B lines. The patterns of Ck 60A and B were in general similar to Nagpur A and B. Patterns in G1A and B were similar and resembled closely the isoenzyme patterns found in VZM 2A and B. The patterns of M 35-1A and B showed greater resemblance to isoenzymes in M 31-2A and B.

The comparison of A and B lines indicated some differences in the relative intensity of bands. In the case

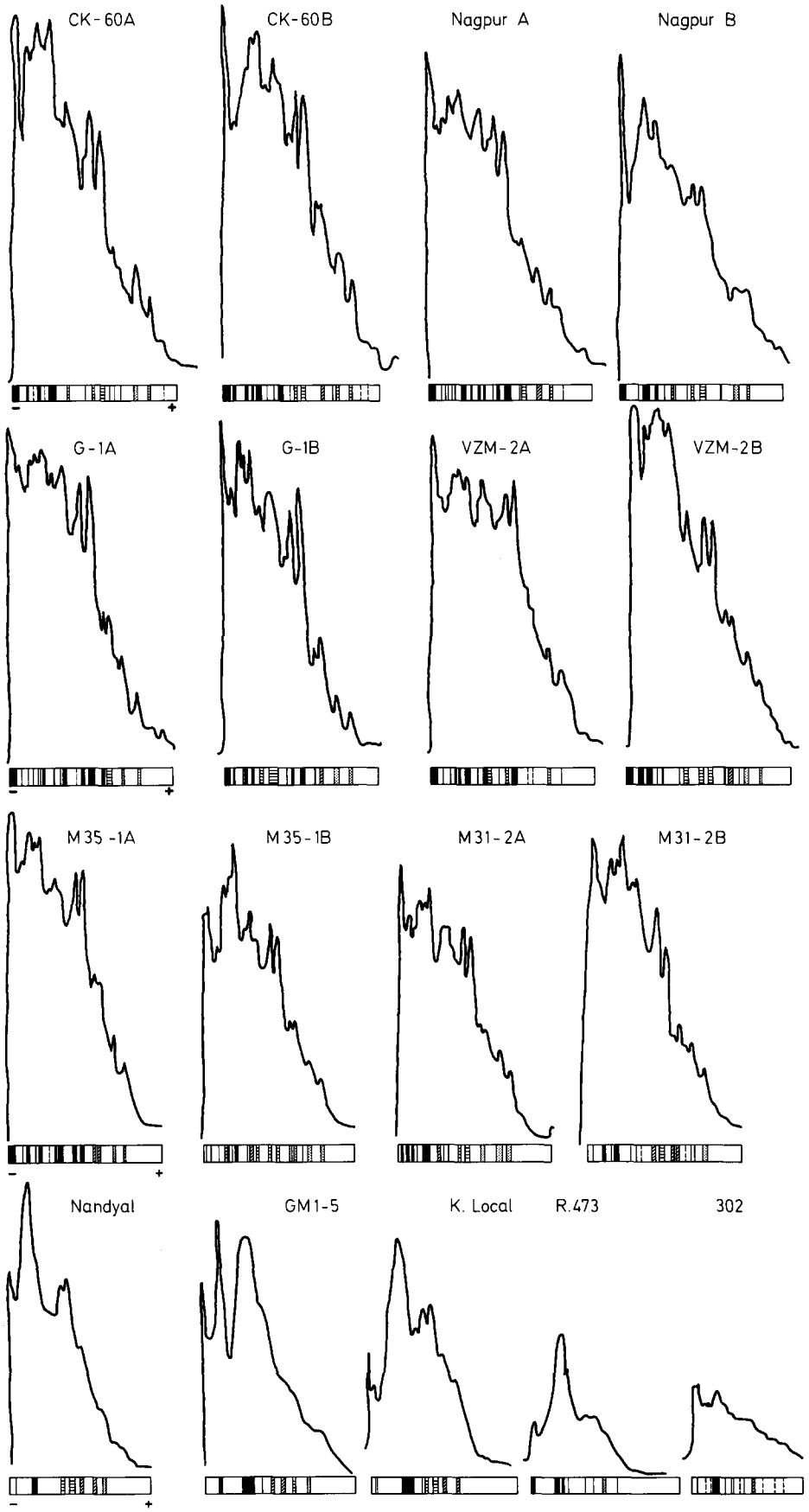

Fig. 1. Densitographs of soluble protein patterns in sorghum seeds

Table 1. Rm values of soluble protein bands found on polyacrylamide gel electrophoresis from seeds of different sorghum strains

Rm	CK 60A	CK 60B	NagA	NagB	G1A	G1B	VZM 2A	VZM 2B	M 35-1A	M 35-1B	M 31-2A	M 31-2B	Nand	GM 1-5	K.1	R · 473	302
0.01	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–
0.03	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.04	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	–	+
0.07	+	+	+	–	+	+	+	+	–	–	+	+	+	+	+	+	+
0.09	–	–	+	+	–	–	–	–	+	+	+	–	–	+	–	–	F
0.11	F	–	+	–	+	+	+	+	+	–	–	–	–	–	–	–	F
0.13	+	+	+	–	+	+	+	+	–	+	+	+	–	–	–	–	–
0.15	–	–	+	+	+	+	+	+	–	+	+	–	–	–	–	–	+
0.17	F	+	–	–	+	–	–	–	+	+	–	+	+	–	–	–	–
0.19	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
0.21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
0.25	–	+	+	+	+	+	+	–	F	–	–	–	–	–	+	+	+
0.27	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	+
0.29	+	+	+	+	+	–	+	+	+	+	+	+	–	–	–	+	–
0.31	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	F
0.33	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	–	+
0.35	–	–	–	–	–	–	–	–	–	–	+	F	–	–	–	–	–
0.37	+	+	+	+	F	+	+	–	–	–	–	–	+	+	+	–	–
0.39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
0.41	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
0.43	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–
0.46	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	F
0.49	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–
0.51	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–
0.53	+	–	+	–	+	+	F	+	+	+	+	–	–	–	–	–	–
0.55	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
0.57	+	+	+	+	+	+	F	+	+	+	+	–	+	+	–	–	–
0.59	–	–	–	–	–	–	–	–	–	–	–	F	–	–	–	–	F
0.61	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–
0.63	+	+	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–
0.65	–	–	+	+	+	+	+	+	+	+	–	+	+	–	–	–	–
0.67	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–
0.71	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–	–
0.73	–	–	–	–	+	+	+	+	F	+	+	+	–	–	–	–	–
0.79	F	F	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.81	–	–	F	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.83	–	F	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	16	17	18	13	17	15	16	15	15	17	16	16	8	7	8	8	13

+ – band present; – band absent; F – faint band; Nag – Nagpur; Nand – Nandyal; K.1 – K.local

of *milo* cytoplasm bands in sterile lines had greater intensities compared to the corresponding bands in their B lines. The patterns in G1A and B were similar but the intensity of bands in G1B was slightly less compared to those of G1A. Three bands at Rm 0.56, 0.59 and 0.61, present in G1A and B, were similar to bands present in VZM 2A and B. M 35-1A, B and M 31-2A, B showed more isoenzymes than the *milo*, VZM 2 and G1 lines.

The esterase patterns of restorer lines were found to be different from those of the A and B lines. The pattern in Nandyal was similar to that of K.Local but differed from that of GM1-5. Apomictic lines also showed characteristic and distinct isoenzyme patterns which were different from the patterns in R, A and B lines.

Esterase isoenzyme with a Rm 0.56 in Ck 60B had a greater intensity than the other lines. Bands with Rm 0.59 and 0.61 were exclusively present in G1A and B, VZM 2A and B lines. Bands with Rm 0.13, 0.28, 0.43 and 0.49 were present in M 35-1A and B and M 31-2A and B. Bands at Rm 0.60 were common to M 35-1A and B, M 31-2A and B, Nandyal, GM1-5, K.Local and R.473. The band at Rm 0.49 in M 35-1A and B and M 31-2A and B had a greater intensity than any other band.

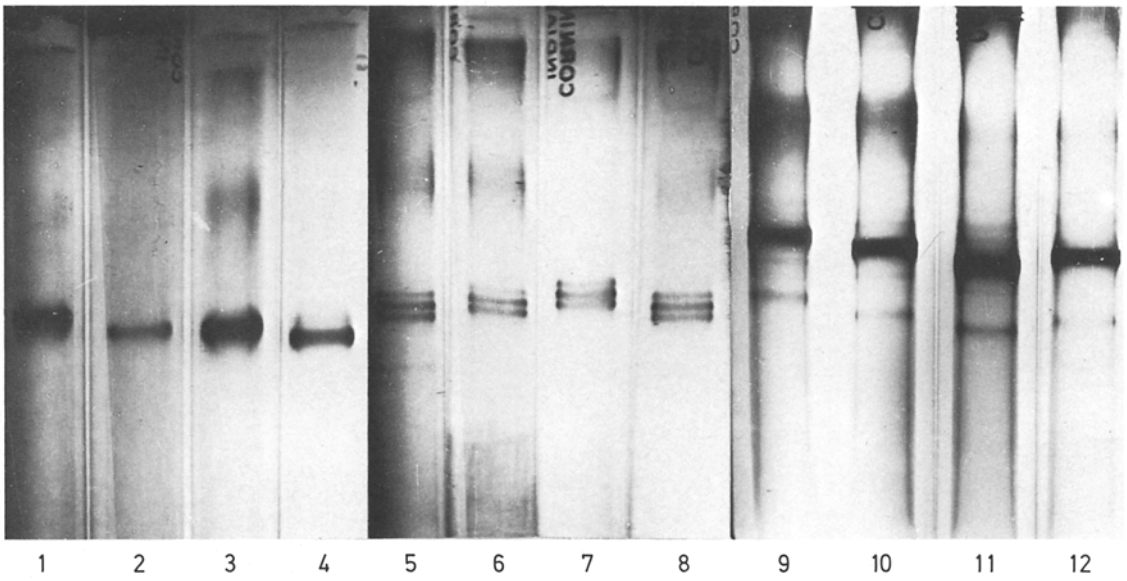

Fig. 2. Esterase isoenzyme from sorghum seeds. *1* Ck 60A; *2* Ck 60B; *3* Nagpur A; *4* Nagpur B; *5* G1A; *6* G1B; *7* VZM 2A; *8* VZM 2B; *9* M 35-1A; *10* M 35-1B; *11* M 31-2A; *12* M 31-2B

GDH Isoenzyme

Two GDH isoenzymes (Rm 0.14 and 0.26) were present in male steriles of Indian origin (M 35-1A & B, M 31-2A and B, VZM 2A and B and G1A and B) whereas one band at Rm 0.16 was detected in Ck 60B and Nagpur A and B. Ck 60A also had major band at Rm 0.16 in addition to a faint band at Rm 0.30.

ADH Isoenzyme

Three ADH isoenzymes were present in all A and B lines. Bands in all A and B lines, except for M 35-1A and B, had similar enzyme patterns with Rm values of 0.27, 0.29, and 0.31. In M 35-1A and B, the Rm of the isoenzymes was 0.25, 0.27 and 0.29. The intensity of ADH isoenzymes was greater in Ck 60A, Nagpur A and M 31-2A in comparison to their maintainer lines. Isoenzymes in remaining strains showed similar intensities. The R lines Nandyal, GM1-5, K.Local and apomictic line R 473 also showed an ADH isoenzyme pattern similar to that of the *milo* lines.

Discussion

Proteins which are the primary product of genes (Yanofsky et al. 1960) are a direct measure of gene homology. Based on this premise, various workers (Alam and Sandal 1969; Schwartz 1966; Siddiq et al. 1972) haved tried to understand genome relationships by using electrophoretic patterns of proteins and isoenzymes. In the present study biochemical differences among sorghum strains with diverse cytoplasmic and nuclear factors have been studied by examining soluble protein and isoenzyme patterns of esterase, ADH and GDH from seeds, employing the gel electrophoresis technique. Soluble protein patterns from diverse cytoplasmic sources differed qualitatively and quantitatively. General comparisons of protein patterns show some basic similarities and differences among male sterile lines. The *milo* cytoplasmic steriles CK 60A and B and Nagpur A and B pattern differed qualitatively from that of G1A, VZM 2A, M 35-1A and M 31-2A. In general, a similarity in soluble protein pattern of M 35-1A and B to that M 31-2A and B indicates some common genomic relationship between these groups. A protein band with Rm 0.71 was present only in *milo* and absent in other male steriles, whereas a protein band with Rm 0.73 was absent in *milo* and present in other male steriles as well as their maintainers. Protein bands with Rm 0.04 and 0.33 were present only in M 35-1B and M 31-2B.

No major differences were observed in the ADH and GDH isoenzyme patterns of seeds from A, B and R lines. The esterase isoenzyme pattern from sorghum seeds with different cytoplasm showed characteristic and distinct differences among *milo* steriles, G1A and B, VZM 2A and B, M 35-1A and B. Nagpur A and B had greater homology with Ck 60A and B while the G1A and B esterase patterns resembled more closely those of VZM 2A and B. M 35-A and B patterns also resembled closely that of M 31-2A and B. Esterase patterns not only showed differences with different cytoplasmic background but also showed minor but con-

Table 2. Rm values and intensity of esterase isoenzyme bands found on polyacrylamide gel electrophoresis from seeds of different sorghum strains

Rm	CK 60A	CK 60A	NagA	NagB	GlA	GlB	VZM2A	VZM2B	M35-1A	M35-1B	M31-2A	M31-2B	Nand	GM 1-5	K.l	R · 473	302
0.04	–	–	–	–	–	–	–	–	+	+	+	+	–	+	–	+	+
0.06	–	–	+	–	+	+	+	+	–	–	–	+	+	–	+	–	–
0.08	–	–	–	–	–	–	–	–	+	+	+	–	–	+	–	–	–
0.10	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
0.13	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–
0.17	+	–	–	–	–	–	F	F	–	–	–	–	–	–	–	–	+
0.23	–	–	–	–	–	–	–	–	–	+	–	–	–	F	–	–	–
0.25	–	–	–	–	–	–	–	–	+	+	–	+	–	–	–	–	–
0.28	–	–	–	–	–	–	–	–	+	+	+	F	–	–	–	+	–
0.31	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–
0.33	+	–	F	–	+	+	–	F	–	+	–	F	–	–	–	–	–
0.43	–	–	–	–	–	–	–	–	F	F	+	+	–	–	–	–	–
0.48	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
0.49	–	–	–	–	–	–	–	–	+ + + +	+ + + +	+ + + +	+ + + +	–	–	–	–	–
0.50	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+
0.53	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
0.54	–	–	–	+	–	–	–	–	–	–	–	–	+	+	+	–	+
0.55	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
0.56	+ + + +	+ + + +	+ + + +	+ + + +	+	+	+	+	–	–	–	–	–	–	+	+	–
0.59	–	–	–	–	+ +	+ +	+ +	+ +	–	–	–	–	–	–	–	–	–
0.60	–	–	–	–	–	–	–	–	+ +	+ +	+ +	+ +	+	+	+	+	–
0.61	–	–	–	–	+ +	+ +	+ +	+ +	–	–	–	–	–	–	–	–	–
0.67	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
0.69	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
0.71	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Total	3	1	3	2	6	5	5	6	12	14	10	10	4	7	4	6	5

+ band present; – band absent; F – Faint band; Nag – Nagpur; Nand – Nandyal; K.l – K.local
NB: Increasing no. of + indicate increasing intensities

sistent differences between A and B lines. In general, intensity of esterase isoenzymes in B lines was less when compared to A lines in *milo* steriles. VZM 2A and B differed from G1A and B with respect to low and medium electrophoretic mobility (Rm 0.17, 0.33) bands. Similarly, M 31-2A and B esterase isoenzymes with low electrophoretic mobility had lesser intensities than corresponding bands in M 35-1A and B. Based on esterase isoenzyme patterns, the different sorghum lines studied could be classified into three major groups: first, represented by Ck 60A and B, Nagpur A and B; second, represented by G1A and B, VZM 2A and B, and third, represented by M 35-1A and B, M 31-2A and B. These individual classes could further be subdivided into two classes, each representing A and B lines, by comparing intensities and minor isoenzyme band patterns. Esterase isoenzyme band patterns from Nandyal and K.Local were similar while that of GM1-5 and two apomictic lines differed. The esterase patterns are clearly distinguishable in different cytoplasms.

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