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Biochemical Basis of Hybrid Vigour. The Genetics of Grain Weight of *Oryza sativa*

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Summary. Four cathodal bands (C_1, C_2, C_3 and C_4) of esterase (E_1, C_1 3.1) were correlated with the grain weight of rice (*Oryza sativa* L.). Zymogram patterns indicated intensity differences among these bands in fine-grain and coarse-grain varieties. Bands C_1 and C_2 were dark in fine grain varieties whereas C_3 and C_4 were dark in coarse grain varieties. These bands were specific to endosperm. Observations on fine-grain (Kalanamak), coarse grain (SR(26)B) varieties and their reciprocal hybrids indicated the presence of 4 esterase loci G_1, G_2, G_3 and G_4, corresponding to bands C_1, C_2, C_3 and C_4, respectively. A possible model for heterosis in grain weight of rice was proposed which supports the dominance theory of heterosis. In hybrid vigour the 4 esterase loci appear to be associated with grain weight and they complemented each other in an additive manner.

Introduction

Isozymes have been used as an efficient tool in interpreting the comlex phenomenon of hybrid vigour at the biochemical level (Beckman et al. 1964); Schwartz and Laughner 1969; Scandalios et al. 1972; Schwartz 1973). Isozyme variations revealed by the zymogram technique also provide built in marker systems for morphological and physiological differences, tissue specificity, developmental and genetic studies of a wide variety of plant species. Non-specific esterases (E_1, C_1 - 3.1) are one of the most extensively studied groups of isozymes. In genus *Oryza* esterase zymograms were observed in 147 strains of *O. perennis* and *O. sativa* by Shahi et al. (1969). The zymogram patterns indicated that there were numerous genes controlling esterase isozymes and some of them might have been substituted in the course of domestication and differentiation into *indica* and *japonica* types. Lin and Li (1971) studied the number and intensity of esterase enzyme in somatic organs of rice.

However, no such studies have been undertaken so far in Indian varieties of rice. Therefore, the present experiment was undertaken to study the variation in esterase isozymes in fine grain (less weight/grain) and coarse-grain (more weight/grain) Indian varieties of rice.

Materials and Methods

Eight varieties of rice (Fig. 1) were taken for the present study. Grain characters and photosensitivity of these varieties are given in Table 1. Two gm. dry and dehusked seeds of each variety were homogenised with 3 ml. distilled water in pre-chilled pestles and mortars. Tissue specificity was studied only in IR 8. Embryos and endosperms were separated. Two gm. of endosperm were homogenised as above, whereas 200 embryos were homogenised in 4 drops of water. Homogenates of hybrid seeds were prepared similarly except that only a few drops of water were added because the quantity of these seeds was less.

The homogenates were centrifuged at 14,000 rpm for 30 minutes at 4°C and the supernatants were used for electrophoresis.

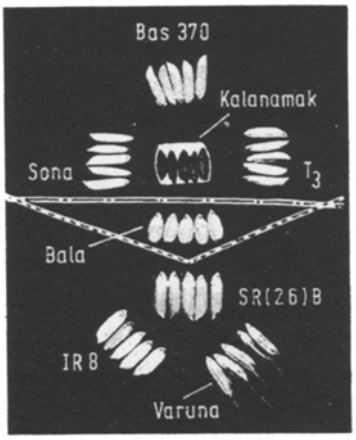

Fig. 1. Different varieties of rice. Note the difference in size, shape and colour

Table 1. Kernel characters und photoperiod sensitivity of different varieties

Grain type and name of the variety	Grain characters				
	Length of kernel mm	Breadth of	Ratio of length/breadth	1000 grain weight gm.	Photoperiod sensitivity
Coarse grain varieties					
IR8	9.10	3.15	2.88	29.00	Insensitive
SR(26)B	9.75	2.80	3.44	28.10	Sensitive
Varuna	9.50	2.70	3.51	25.10	Insensitive
Bala	6.40	3.00	2.13	19.80	Insensitive
Fine grain varieties					
Kalanamak	7.00	2.25	3.11	14.80	Sensitive
Sona	9.50	2.15	4.41	19.75	Insensitive
Bas.370	8.75	2.20	4.43	20.15	Insensitive
T-3	9.20	2.30	4.00	20.26	Insensitive
Hybrids					
Kalanamak × SR(26)B	9.00	2.70	3.33	00.2343*	Not observed
SR(26)B × Kalanamak	10.00	2.81	3.55	00.2879*	Not observed

* Weight of 10 grains in case of hybrids

Starch gel electrophoresis and staining

Horizontal starch gel electrophoresis (Smithies 1955) using a discontinuous buffer system as modified by Marshall and Allard (1970) was performed using 12 % hydrolysed potato starch (V.P. Chest Institute, New Delhi). The gel was prepared in Tris-Citrate buffer. Supernatants of the homogenates were absorbed on Whatman No. 3 filter paper and inserted 6 cm away from the cathodal end of the gel. The gel was subjected to 8 V/cm current for 1 hr. and then sample strips were removed and a constant current of 10 v/cm was supplied for 4 hr.

After electrophoresis, the gels were sliced and incubated at 37°C in a staining mixture containing 1 % naphthyl acetate in 50 % acetone, 40 mg. of Fast Blue-RR, 40 ml NaH_2PO_4 buffer (pH 4.3, 0.2 m), 10 ml Na_2HPO_4 buffer (pH 9.2, 0.2 m) and 40 ml. water. Esterase bands became clear after 2-3 hr. The stained gels were washed and transferred to 50 % ethanol for storage.

Results and Discussion

Isozyme and allozyme variations associated with grain-weight

A total of 10 esterase bands was seen by starch gel electrophoresis. Of these, 5 moved towards the anode and 5 towards the cathode. All the anodal bands (A_1, A_5, A_6, A_7 & A_8) were present in all 8 varieties and gave more or less similar patterns. This shows that, irrespective of the metabolic rate, these anodal bands are equally important for all the varieties.

The cathodal bands, which were of special interest in the present study, were also five (C_1, C_2, C_3, C_4 and C_6). Variations observed in bands C_1, C_2, C_3 and C_4 were helpful in dividing the varieties into two groups. The differences were quite marked but not absolute, because some bands were present (though very faint) in the types where they are usually absent.

In 'Kalanamak', bands C_1 and C_2 showed greater activity (C_2 being darker than C_1) and C_3 showed very low activity. In 'Sona', 'Bansmati-370'and 'T3', C_1 and C_2 showed high activity (C_2 being darker than C_1) and C_3 and C_4 showed low activity. In 'I.R.8', 'SR(26)B' and 'Varuna', bands C_3 and C_4 showed greater activity (C_4 being darker than C_3), whereas in 'IR 8' band C_2 showed equal activity and, in 'SR(26)B' and 'Varuna', it showed lower activity, when compared with bands C_3 and C_4. In 'IR8', band C_1 was also present with very low activity and, in other coarse-grain 'SR(26)B' and 'Varuna' it was absent. At present we cannot draw absolute conclusions about the role of these isozymes on grain weight, because *in vivo* substrates of esterases are not yet known. But looking at the patterns of bands C_1, C_2, C_3 and C_4 in fine-grain rice, we may conclude that bands C_3 and C_4 are somehow associated with more active metabolism than bands C_1 and C_2. This might be one reason why the varieties where C_3 and C_4 are present are coarse-

grain varieties and where C_1 and C_2 are present are fine-grain varieties.

'Bala'(Fig.3), traditionally classified as a coarse-grain variety, gave the pattern characteristic of fine-grain varieties (Fig.2) in association with lesser grain weight (Table 1). This observation indicates that the 4 cathodal bands are associated with grain weight of rice irrespective of the grain shape.

Cathodal band C_6 (details of which are being studied) was specific to photo-insensitive varieties ('Sona' 'Bansmati-370', 'T3', 'IR8', 'Varuna' and 'Bala') and absent in photo-sensitive varieties ('Kalanamak' and 'SR(26)B').

Tissue specificity of isozymes: This experiment was performed to localize the presence of different bands in different seed tissues: husk, embryo and endosperm. 'IR8', which contained all the 10 bands, was used for this purpose. There was no esterase activity in husk of 'IR8'. In the embryo, all the bands except A_1 were present towards the anode, whereas towards the cathode only C_6 was present. In endosperm all the anodal and cathodal bands were present except bands A_5 and A_6 (Fig.4). Zymograms for tissue specificity clearly showed that bands A_5 and A_6 were specific to embryo and A_1, C_1, C_2, C_3 and C_4 were specific to endosperm. The presence of bands

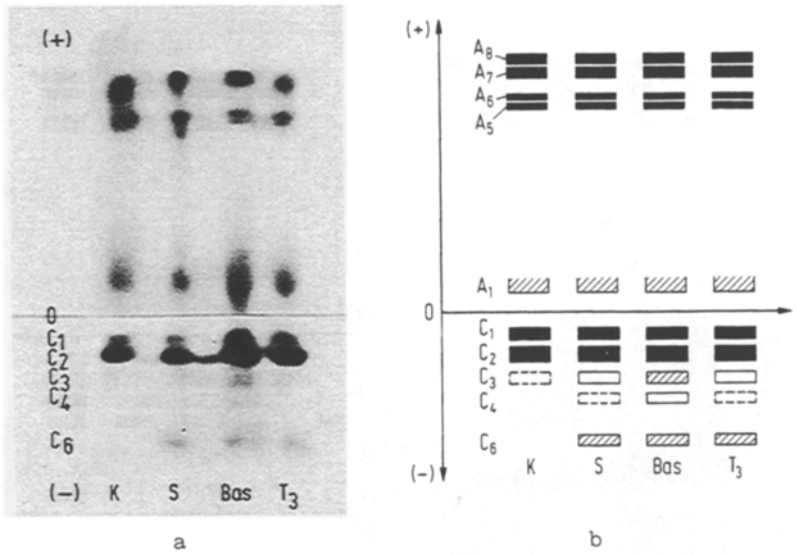

Fig.2a. Zymogram showing the variations in esterase activity in seeds of different fine grain varieties of rice. Kalanamak (K), Sona (S), Basmati (Bas) and T_3
b. Schematic diagram of esterase isozymes in seeds of fine grain varieties of rice. Kalanamak (K), Sone (S), Basmati (Bas) and T_3. Note the bands C_1 and C_2 show greater activity than C_3 and C_4 in these varieties

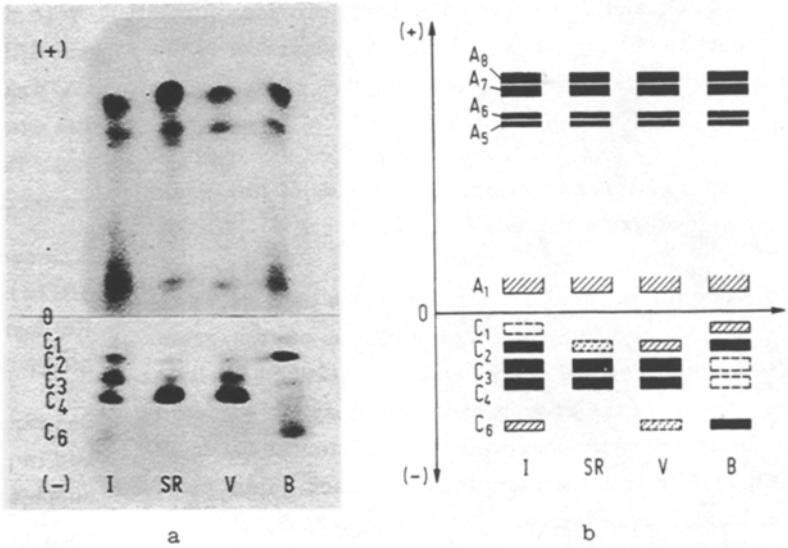

Fig.3a. Zymogram showing the variation in esterase activity in seeds of different coarse grain varieties. IR8 (I), SR(26)B (SR), Varuna (V) and Bala (B). Note that Bala shows banding pattern like fine grain varieties
b. Schematic diagram of esterase activity in seeds of coarse grain varieties of rice. IR8 (I), SR(26)B (SR), Varuna (V) and Bala (B). Note that the bands C_3 and C_4 are darker than C_1 and C_2 in coarse grain varieties

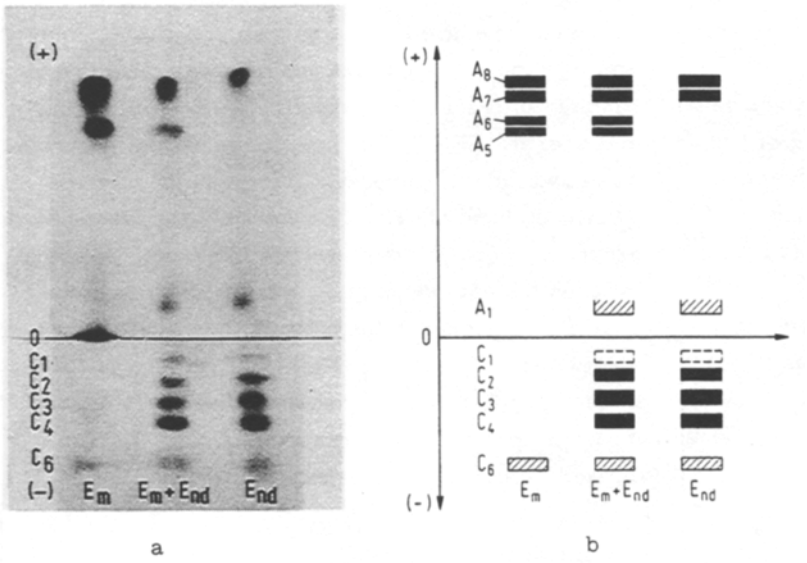

Fig. 4a. Esterase isozymes in different tissues of the seeds of IR8. Embryo (EM) and Endosperm (End) b. Schematic representation of esterase activity in embryo (EM) and endosperm (End). Refer text for details

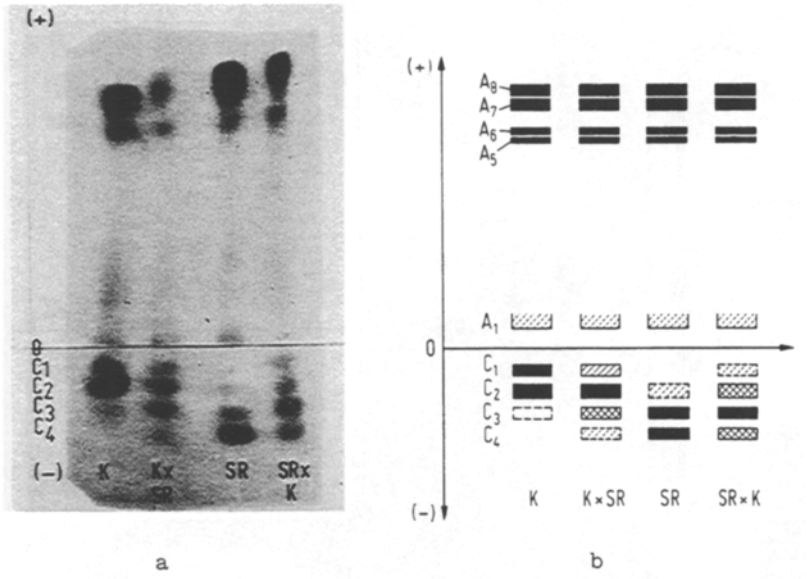

Fig. 5a. Photograph showing esterase isozymes in Kalanamak (K) and SR(26)B (SR) and their reciprocal hybrids b. Schematic diagram of esterase activity in fine grain Kalanamak (K) and coarse grain SR(26)B varieties of rice and their reciprocal hybrids

C_1, C_2, C_3 and C_4 only in endosperm provides further support to our hypothesis, because endosperm is the main constituent of rice grain weight.

Isozymic patterns in reciprocal hybrids of fine-grain and coarse-grain varieties

To study the mode of inheritance of the cathodal bands C_1, C_2, C_3 and C_4, reciprocal crosses of fine-grain ('Kalanamak') and coarse-grain 'SR(26)B' varieties were made in the greenhouse. The isozymic variations in the two parents occurred only in cathodal bands (Fig. 5). In both the parents, 2 dark and 1 faint cathodal bands were visible. In 'Kalanamak', C_1 and C_2 were dark, C_3 was faint, and C_4 was absent, whereas in 'SR(26)B', C_3 and C_4 were dark, C_2 was faint and C_1 was absent. In hybrids all the 4 bands were present with the intensity difference in reciprocal crosses. The intensity of these bands in parents and their reciprocal hybrids, in descending order, was

Kalanamak : $C_2 > C_1 > C_3$ (very faint)
SR(26)B : $C_4 > C_3 > C_2$ (very faint)
Kalanamak ♀ × SR(26)B♂ : $C_2 > C_3 \geqslant C_1 > C_4$
SR(26)B♀ × Kalanamak♂ : $C_3 > C_4 \geqslant C_2 > C_1$

Schwartz et al. (1965) observed that esterase occurred in plants as a dimer, composed of homologous subunits. However, in homozygotes there is no indication of dimerization with other nonallelic esterase.

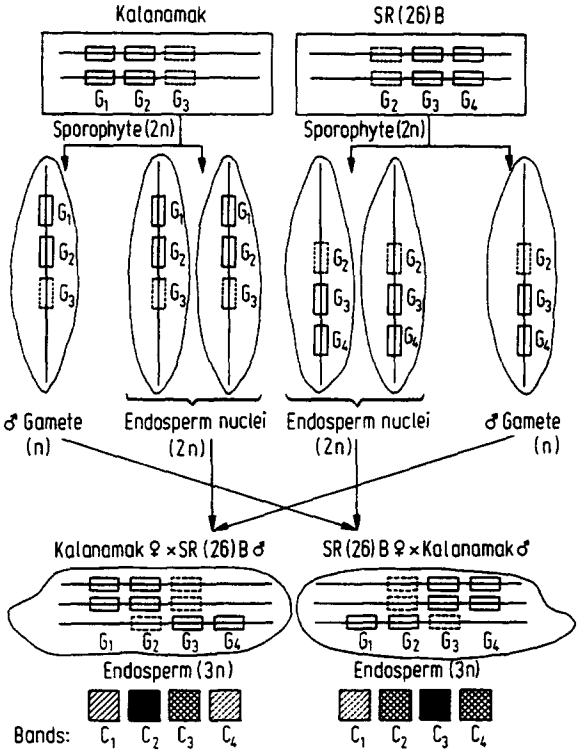

Fig.6. Model to explain the differences in intensity of different bands (esterase activity) in the parents-Kalanamak and SR(26)B and their reciprocal hybrids. Refer text for details

Therefore, in homozygotes each band represents one gene locus.

As we have already assumed, isozymes C_3 and C_4 are active metabolically when compared with C_1 and C_2; the reciprocal differences (maternal effects) observed in grain weight could be ascribed to different gene dosage (present in the endosperm to which these 4 bands are specific Fig.4). These bands are contributed by the genome of two parents for different loci.

In rice varieties (which are homozygous for most of the genes because of the inbreeding nature of the crop) we suppose that there are 4 esterase loci giving rise to C_1, C_2, C_3 and C_4 cathodal bands. We further assume that the presence or absence, and the differences in the intensities, of these bands indicate the differential amount of ultimate product of corresponding genes (Scandalions 1974).

The heterozygotes of fine-grain and course-grain varieties, where both the types of genomes were present, gave rise to all the 4 bands but with different intensities. The results obtained with hybrids can be explained with the help of a hypothetical model, as given in Fig.6. We have denoted the 4 loci G_1, G_2, G_3 and G_4, corresponding to C_1, C_2, C_3 and C_4 bands, respectively.

Reciprocal differences due to the maternal genome

'Kalanamak' × 'SR(26)B' showed heterosis in grain weight (0.2343 g/10 grains) over the mid-parental value (21.45 g/100 grain). The heterotic effect observed could be attributed to the presence of all the cathodal bands in the hybrid genotype. The intensity, in descending order, was $C_2 > C_3 \geqslant C_1 > C_4$. Band C_2 was most intense in this hybrid because it was produced by 2 highly active dosages of the G_2 locus, contributed by endosperm nuclei, and 1 less active dose of G_2 contributed by the male gamete nucleus. The next most intense band was C_3 because two less active dosages of C_3 and one less active dose were present. Band C_1 had an intensity equal to C_3. This band was the product of 2 active dosages of G_1 and an inactive third dose. Band C_4 was the least intense, which was the product of only one highly active dose of G_4 (Fig.6).

'SR(26)B' × 'Kalanamak' showed heterosis in grain weight (.2879 g/10 grain) over the better parent (28.10 g/100 grain). Hybrid vigour in this case can also be attributed to the presence of all the 4 cathodal bands, but with different intensity order, viz. $C_3 > C_4 \geqslant C_2 > C_1$. This order can be explained in the same manner as for the cross 'Kalanamak' × 'SR(26)B'.

It is clear that the intensity of the bands was dependent not only on the activity of the genes but also on the gene dosage by which the particular band was produced. Our observations are in accordance with those observed and explained by Beckman et al. (1964) and Scandalios (1969).

While observing the grain weight of parents and hybrids (Table 1) with their isozymic pattern (Fig.5,6), it appears that 4 esterase loci, G_1, G_2, G_3 and G_4, complement each other in an additive manner to bring about the hybrid vigour in grain weight. 'Kalanamak' × 'SR(26)B' (Fig.6) hybrid received four highly active genes - G_1 and G_2 from the female parent and G_3 and G_4 from the male parent. The reciprocal hybrid also received the same number, but in reverse order.

The reciprocal differences observed in grain weight of hybrids, which were also consistent in their isozymic patterns, give further support to our hypothesis that 4 esterase loci are associated with grain weight of rice and they complement each other in additive manner.

Detailed ontogenetic variations are to be worked out to explore the pleiotropic effect of these loci on other yield components, if any.

Our results may be helpful in screening desirable parents for grain weight in the hybridization programme. According to our conclusions one can expect heterosis in at least grain weight in a cross of coars × fine grain varieties, rather than in crosses fine × fine or coarse × coarse varieties.

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Literature

Beckman, L.; Scandalios, J.G.; Brewbaker, J.L.: Catalase hybrid enzymes in maize. Science 146, 1174-1175 (1964)

Lin, Wu; Li, H.W.: Esterase isozyme patterns in rice somatic organs and 2, 4-D induced callus tissues. Bot. Bull. Acad. Sci. (Acad) II, 113-117 (1971)

Marshall, D.R.; Allard, R.W.: Isozyme polymorphism in natural populations of *Avena falula* and *Avena barbata*. Heredity 25, 373-382 (1970)

Scandalios, J.G.: Genetic control of multiple forms of enzymes in plants. Biochem. Genet. 3, 37-79 (1969)

Scandalios, J.G.: Isozymes in development and differentiation. Ann. Rev. Plant Physiol. 25, 225-258 (1974)

Scandalios, J.G.; Liu, E.; Campeau, M.A.: The effect of intragenic and intergenic complementation on catalase structure and function in maize. A molecular approach to heterosis. Arch. Biochem. Biophys. 153, 695-705 (1972)

Schwartz, D.: Single gene heterosis for alcohol dehydrogenase in maize. The nature of subunit interaction. Theor. Appl. Genet. 43, 117-120 (1973)

Schwartz, D.; Laughner, W.J.: A molecular basis for heterosis. Science 166, 626-627 (1969)

Schwartz, D.; Fuchsman, L.; McGrath, K.H.: Allelic isozymes of the pH, 7.5 esterase in maize. Genetics 52, 1265-1268 (1965)

Shahi, B.B.; Masishima, H.; Oka, H.I.: Survey of variation in peroxidase, acid phosphatase and esterase isozymes of wild and cultivated *Oryza* species. Japan J. Genetics 44, 303-320 (1969)

Smithies, O.: Zone electrophoresis in starch gel: Group variation in serum proteins of normal human adults. Biochem. Jour. 61, 629-641 (1955)

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