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GENETIC DIVERSITY AND ENZYMES AMONG SELECTED SILKWORM RACES OF *BOMBYX MORI (L.)*

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ABSTRACT
Many numbers of geographical races of Silkworm, *Bombyx mori* are being reared in many geographical areas of world and inbred lines. Generally cocoon commercial characters are used to identify silkworm verities and selection of parental strains. However, silkworm verities which are developed from crosses involving many verities cannot be identified by conventional methods. Hence the used the metabolic enzymes profiles to identify the genetic diversity and thermo tolerance among twenty one silkworm races. Using those metabolic enzymes 3 to 4 alleles were identified. Alleles were used to determine the genotypes. Different genotypes were identified using three enzymes viz., Esterase, alkaline phosphotase and acid phosphotase isozymes and eight cluster groups were observed. Origin and parentage were found closely related with cluster groups. CSR-18 (BBI-0293) was identified as a thermo tolerant silkworm breed based on higher protein stability. This isozyme study clearly showed that the main genetic variations can be identified by relating with their morphology and geographical origins.

KEY WORDS: Isozymes, Genotypes, Alleles, silkworm breeds, geographical origin

INTRODUCTION

The Central Sericultural Germplasm Resource Centre, Hosur Tamil Nadu (latitude 12°45'N and longitude 77°5'E, altitude 942M AMSL), is maintaining a large number of silkworm (*Bombyx mori* L.) genetic resources which include both multivoltine (64 accessions) and bivoltine (329 accessions) . Most of the silkworm genetic resources have been characterized for morphological characters and evaluated for all-important economic traits (Thangavelu et al. 1997; 2000). Among the various isozymes, esterases have been studied extensively since they are the group of enzymes involved in metabolic and defense functions and are found in both soluble and membrane bound forms. These have been studied extensively in several insects because of their involvement in resistance/stress to various kinds of insecticides and thermal stresses. (Yoshitake and Eguchi,1965; Yoshitake and Akiyama,1965; Eguchi et al., 1965; Eguchi and Yoshitake,1967) and also studied for their function in the digestion, nutrition and detoxification in insects (Kasim and Ahmed,1978) and reproduction (Richmand et al., 1980).

Allozymes markers are also used in varieties investigation of genes contributing to yield in plants or insects (Tanksley and Rick 1980: Tanksley et al., 1982: Moon and Sole 1983). The research also used biochemical isozymes techniques to a new genetic variability. This research has led to the discovery of many such related proteins in both prokaryotes and eukaryotes cells. In Insects esterases are extensively studied on various kinds of substrates (suddenrudin and Tan 1973) and exhibited high polymorphism and genetic variation..Inhibitory activities for the activation of prophenolyxidase with its activating enzymes were detected in the gel filtration fractions of heamolymph from Bombyx mori, c1-13a, 13b, and 13 c. they inhibited the activation of prophenolyxidase. Extensive use of insecticide on cotton in the mid-south has prompted resistance development in the tarnished plant bug,lygus lineolaris (pliosot de Beauvois).A field population of tarnished plant bugs in Mississippi with 11 – fold higher resistance to malathion was used to examine how gene regulate conferred resistance to this organophosphate insecticide . In laboratory bio assays, synergism by the esterase inhibitors .S, S,S-tributylphosphoro trithionate (DEF) and triphenylphosphate (TPP) effectively abolished resistance and increased Malathion toxicity by more than 80%.

Polyacrylamide gel electrophoresis was used to study the polymorphism of esterase in Allium cepa l. and A.Fistulosum L. two varieties of each species. F1 hybrid of inter species derivatives were analysed for determination of banding patterns upon staining with α and β-naphthyl acetate substrate of esterase complex and pattern were observed. Esterase enzymes provide an additional marker in monitoring introgression of foreign germplasm in inter species onion breeding. Thermo tolerance is the ability of cells of organisms to withstand elevated temperatures and after brief exposure to mild elevated temperature. The ability of organism to tolerate and adjust to varying thermal conditions is a key factor affecting both local habitat selection and geographical distribution (Solitis and Solitis, 1989).

The extent of genetic variability at isozymes level in plant populations is still largely unknown (Totilieb 1977, 1983). At present, the most powerful method to reveal this sort of variability is thermo stability and pH variation test (Trippa

et al. 1976). Since it is generally known that most structural genes have isoelectrophoretic alleles, which cannot be discovered by electrophoresis (Nei, 1975), the present work is aimed to prepare a biochemical cataloguing based on enzyme profiles and identification of thermo tolerant races based enzyme stability and inhibitions by inhibitors.

MATERIALS AND METHODS

In the present study, twenty one silkworm accessions from bivoltine stock of silkworm germplasm were taken for investigating the genetic variability through three polymorphic isozymes viz., esterase, acid phosphatase and alkaline phosphatase. They were analysed from the haemolymph and gut samples of silkworms in native gel system. The protein bands of them were visualized in the gel. The gels with protein bands of these three isozymes were photographed with the help of Bio-Vis gel instrument. The gels were analysed by biovis gel analyzer and their Rf values were calculated. Based on the Rf values, the different migration mobility was studied. Letters of A for the most anodal band assigned them to B, C, etc in the ascending order. They were analysed through tools for population genetic analysis (TFPGA) to calculate the genetic distances and cluster groups. Haemolymph samples from 4 day old 5th instar larvae of twenty one silkworm races were collected from proleg in pre-cooled Eppendorf tubes containing a pinch of phenyl-thiourea to avoid melanization. The samples were subjected to centrifugation at 5000 rpm for 10 minutes at 4°C to remove hemocytes and debris. The supernatants were stored in –20°C deep freeze in aliquots.

Haemolymph samples (10 µl) were run in 7.5% native PAGE gel using Tris borate buffer (pH 8.2) (Davis, 1964). Samples were run initially at 15 mA for 1 h upto stacking gel for 3h at 150 volts till the dye mark reached the bottom of the gel and the gels were incubated for esterase, alkaline and acid phosphatase substrates for 30 minutes at 37°C. The stained gels were scanned using Biovis gel documentation Instrument for the presence of active bands. For thermo tolerance the native gels after separation of protein were treated under higher temperature for stability and inhibitors for enzyme inhibition studies. Gels were compared using two state characters for the presence or absence of esterase bands. The total number of esterase bands of any two races and the number of bands in common were recorded.

RESULT

The polymorphic locus was identified among the different loci observed through the enzymes. The bands in them were designated as alleles and given letters as a,b,c in the ascending orders from the most anodal bands. A total of 2 to 3 bands were observed in the three enzyme systems. A maximum of three bands were observed in acid phosphatase whereas it was only two bands in esterase and alkaline phosphatase. They were designated as A1, B1, A2, B2 and A3, B3 and C3 in the esterase, acid and alkaline phosphatase isozymes respectively. Further the bands of esterase and alkaline phosphatase enzymes were evaluated for their stability through various inhibitors. Their results are provided in figures 1, 2, 3, and 4. The activity of the bands was identified based on the presence or absence of the bands.

TABLE 1 .Genotypes of 21 BV silkworm accessions based on three isozymes viz., acid phosphatase, alkaline phosphatase and esterase

S.No.	SMGS ACC-No.	Race Name	A1	A2	A3
1	BBI-0064	JAM-124	oo	ab	aa
2	BBI-0065	JAM-125	ab	aa	aa
3	BBI-0066	PAM-101	ab	oo	aa
4	BBI-0067	PAM-102	ab	ab	ab
5	BBI-0068	PAM-103	oo	ab	oo
6	BBI-0069	PAM-104	oo	ab	aa
7	BBI-0070	PAM-105	bb	ab	oo
8	BBI-0071	PAM-106	oo	bb	aa
9	BBI-0072	PAM-107	bc	bb	oo
10	BBI-0073	PAM-108	bc	oo	bb
11	BBI-0074	PAM-109	aa	oo	aa
12	BBI-0075	PAM-110	cd	ab	bb
13	BBI-0076	PAM-111	bb	bb	aa
14	BBI-0077	PAM-112	bd	ab	aa
15	BBI-0078	PAM-113	oo	bb	bb
16	BBI-0079	P5	ab	bb	bb
17	BBI-0080	BL-1	bb	ab	aa
18	BBI-0081	NB-18	ab	oo	bb
19	BBI-0082	NB-7	bc	oo	ab
20	BBI-0083	CC-1	bc	bc	ab
21	BBI-0084	CA-2	bb	bc	aa

The origin, class, parentage and cocoon shape of the 21 silkworm accessions are furnished in table 1. These 21 silkworm accessions were characterized through 3 enzymes showed different genotypes ranging from 4-7. Esterase isozymes showed 5 distinct genotypes viz.,Est°°,Est^{aa},Est ^{ab},Est ^{bb} , Acid phosphatase genotypes

are Acp ^{00}, Acp^{aa} ,Acp ^{ab},Acp ^{bb}, Acp ^{bc}, Acp ^{bd} and Acp ^{cd}. Whereas only 5 genotypes in alkaline phosphatase. They are Alp^{00}, Alpaa, Alp ^{ab}, Alpbb, Alp^{bc}. The genotypes observed in all the 3 enzymes showed different distribution in their frequency (Fig 1, 2, 3, and 4). Esterase genotypes had frequency distribution in the range from 14.2%-47.6%. In Acid phosphatase it ranges from 4.7% to 28.5%. In Alkaline phosphatase its ranges are from 9.5%to23.8%. Results on the genotype frequency pattern, was predominate as 'aa' in esterase. oo, bb in alkaline phosphatase, and ab in acid phosphatase. The genetic variations noticed through 3 polymorphic enzyme was analyzed through tools for genetic analysis reveal 8 distant cluster groups. A UPGMA tree was constructed from Neil's unbiased genetic distance (Nei,1978).

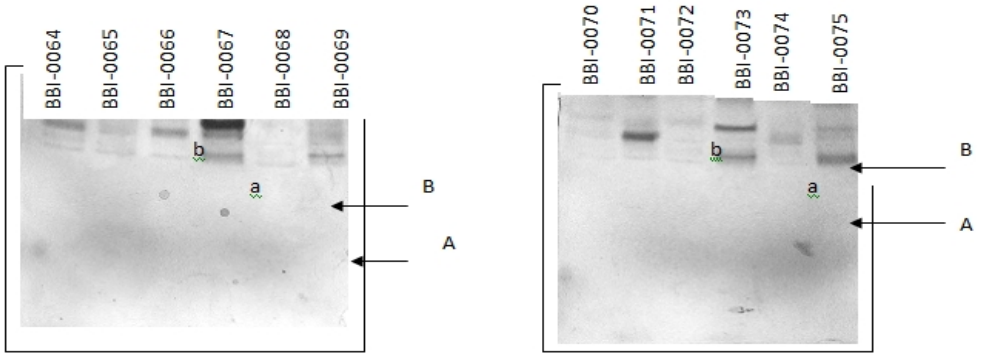

FIGURE .1 Haemolymph Alpha esterase isozyme types in silkworm races. A, B, are alleles of Alpha esterase isozyme

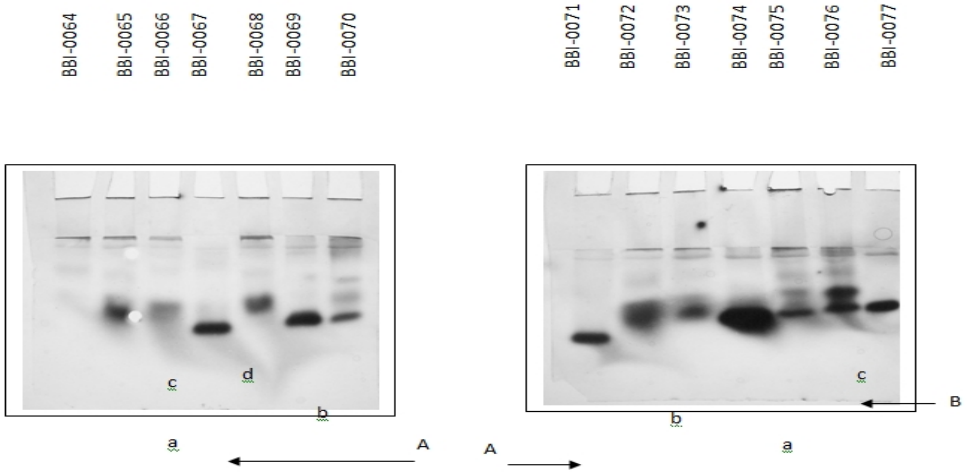

FIGURE 2. Haemolymph Acid phosphatase isozyme types in silkworm races. A, B, C, D are alleles of acid phosphatase isozymes.

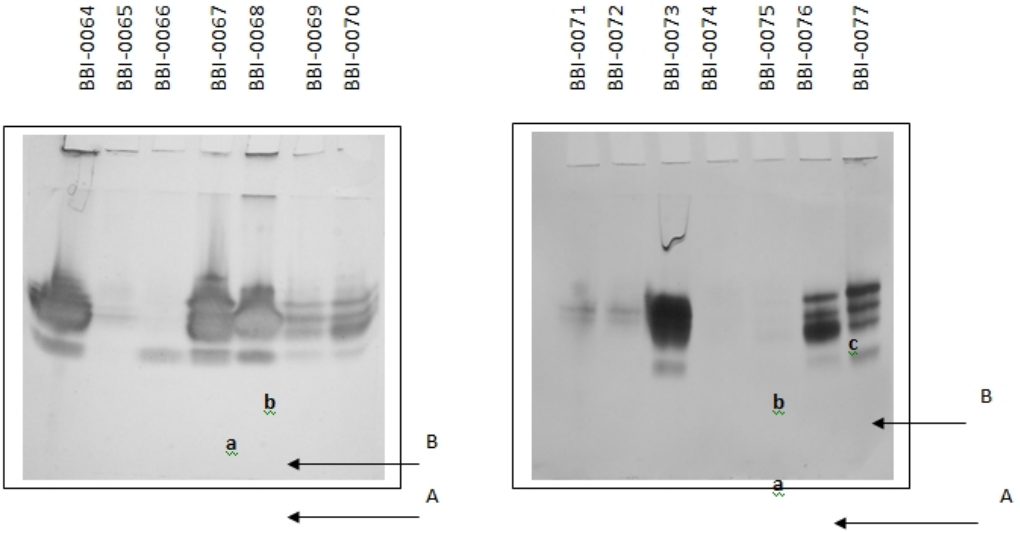

FIGURE 3. Midgut Alkaline phosphatase isozyme types in silkworm races. A, B, C are alleles of alkaline phosphatase isozyme.

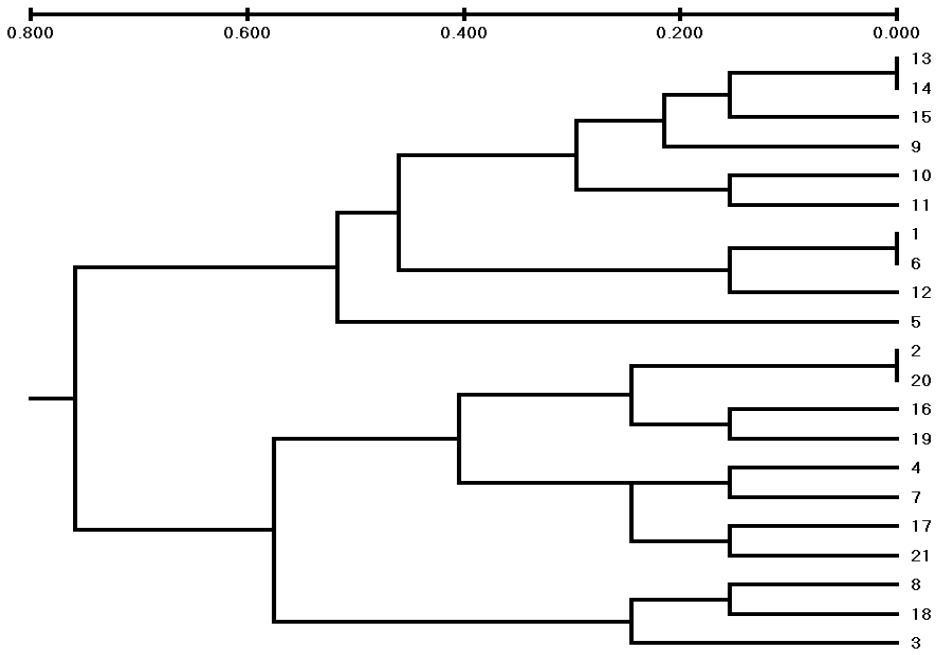

FIGURE 4. Cluster groups of 21 Bivoltine silkworm races based on three isozymes.
Note: The silkworm races numbered from 1 to 21 are given in table 1.

DISCUSSION

Studies conducted on Isozymes variations in silkworm germplasm of *Bombyx mori* L. showed 8 clusters with 3 enzymes. Dendrogram representing the relationship between the 20 manihot esculenta cultivars based on UPMGA cluster analysis of the esterase isozymes defected with substrate using Jaccard's similarity coefficient (Adriana et al. 2004), the first cluster groups comprises 4 silkworm accessions viz., 9, 13, 14, 15. These four accessions of major cluster groups belong to the cocoon shape EFC (Elongated with faint construction). The cocoon shape majority second and third cluster groups are the oval and the cluster four is completely different from the 2 and 3 and this group belongs to the cocoon shape of elongated oval. Whereas in the fifth. 2, 20 accessions are completely oval the 16, 19 accessions are elongated oval. The cluster sixth, accessions 4, 7 are belonging to the cocoon shape of EFC. The cluster seventh, accessions 17, 21 indicate the cocoon shape as oval, while the last cluster 8th is of EFC and the accession 18 is oval in nature. The study indicates the clustering is closely associated with cocoon shape. This study coincides with the earlier studies (Somasundaran et al, 2004) who also revealed close relationship with origin and banding pattern in different selected silkworm races.

Results on thermo stability for esterase isozymes showed that the thermo morphs are more stable at the temperature of 80^{o}C in the race 293- 80%,(85%).while such a stability was not found in the race 290 as it was only 14.8% (Fig 5). The thermo stability in alkaline phosphatase isozymes showed that the thermo morphs are more stable at the temperature of 80^{o}C in the race 293- 80% (85%). While such a stability was not found in the race 290 since it was 60% (57.14%).The ability of organism to tolerate and adjust to variety of thermal conditions is a key factor affecting both local habitat selection and geographical distribution (Spotila et al. 1989).

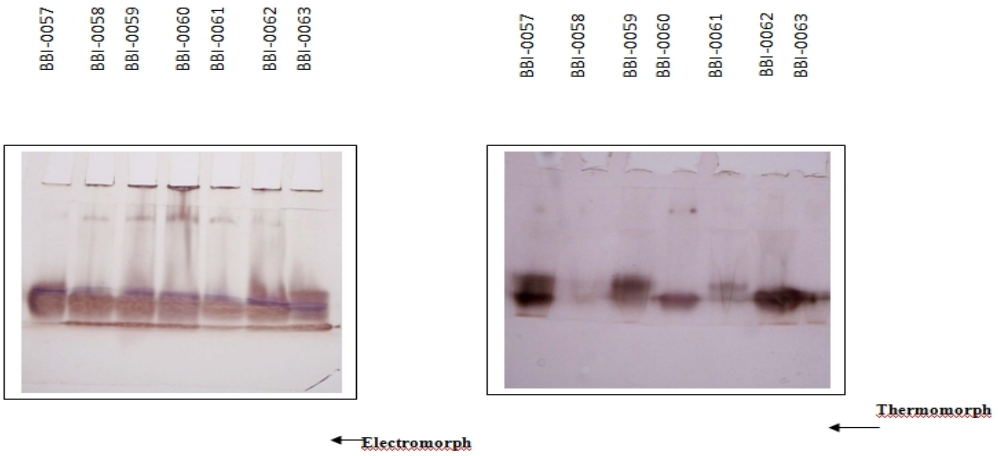

FIGURE 5. Alkaline phosphatase isozyme pattern, Figures A, B are the gels treated at 80^{0}c for 10 minutes and stained for alkaline phosphates isoenzymes.

The presence and absence of isozyme alleles was analysed through the software programme of analysis. Different cluster groups were identified. The cluster groups thus identified are given in dendrogram figure1. There are about 8 distinct cluster groups. Esterase isozyme exhibited higher-level of polymorphism in vertebrates and invertebrates (Selander, 1976 and Singh, 1976). Gillespie and Kojima (1968) demonstrated a relationship between level of polymorphism and metabolic enzymes such as esterase, in which are not involved in glycolysis and the citric acid cycle, than other enzymes involved in energy metabolism. High level of allozyme diversity for esterases may also be related to greater variability of substrates and many of which are of external origin (Kojima *et al*., 1970).

CONCLUSIONS

This isozyme study clearly showed that the main genetic variations can be identified by relating with their morphology and geographical origins. Based on the thermo stability of metabolic enzymes, the race, CSR-18 (BBI-0293) is identified that it has the geographical origin of Kashmir. On the whole, metabolic enzymatic profiles are useful in producing reliable estimates of genetic diversity for selection of parents for the development of elite hybrids.

REFERENCES

Adriana Gazoli resende, Pedco Soares Vidigal Filho, Maria de Fatima, P. S. Machado I. (2004.) Esterase polymorphism marking cultivars of *Mainhot esculenta*, crantz. Beaz, Arch biotechnol 47, Curitiba July.

Eguchi, M., Yoshitake, N and Kai, H.(1965) Types and inheritance of the blood esterase in the silkworm, *Bombyx mori* L. J.Genet 40: 15-19.

Eguchi, M. and Yoshitake, N (1967) Interrelation of non-specific esterase among various tissues in the silkworm, *Bombyx mori* L Jpn. J. Seri.,36: 193-198.

Kasim M, A. and ahmad, S (1980) Esterase in larval tissues of gypsy moth, *Lymantria dispar* (L) Optimum assay condition, quantification and characterization. Insect Biochem 10 : 331-337.

Laemmli U K (1970) Cleavage of structural proteins of the head of bacteriphage T4Nature, 227: 680.

Nei. M (1975) Molecular population Genetics and Evolution.Front Biol 40:1-288.

Richmond , R. C., Gilbert D G, Sheehan K B, Gromko M H and Butterworth F M (1980) Esterase and reproductions in *Drosophila melanogaster.* Science 207: 1483-1485.

Soltis D.C. and Soltis P.S (1989). Isozymes in plant biology In: Advance in plant sciences series (ed T.R. Dudley) discorides press, port land, Oregon pp 268.

Sudderuddin, K. I. and Tan, K. H.(1973). Some hydrolases and their involvement in insecticide resistance. Pest Articl News Summaries 19:24-35.

Thangavelu, K,. Mukherjee, P., Sinha R,K., Mahadevamurthy, T. S., Sunita ,M. Sahani, N K, Kumaresan, P, Rajarajan, P A,, Mohan, B. and Sekar, S.(1997) Catalogue on silkworm (*Bomyx mori* L). Germplasm, Silkworm and Mulberry germplasm station, Hosur, Tamil Nadu, India 1: 1-138.

Thangavelu, K, Sinha R. K, Mahadevamurthy, T. S, Kumaresan P, Mohan B, Rayaradder, F. R. and Sekar S (2000) Catalogue on silkworm (*Bomyx mori* L). Germplasm, Central Sericultural Germplasm Resources Centre, Hosur, Tamil Nadu, India 2 : 1-138.

Tanksley S.D., Medina F.H., Rich C.M., (1982) Use of naturally occurring enzyme variation to defect and map genes controlling quantitative traits in an inter- specific backcross of tomato. Heredity 49:11.25.

Tanksley.S.D. and C.M. Rick. (1980). Genetics of esterase in species of Lycopersicum theor. Appl. Genet 56: 209 – 219.

Trippa. G. Catamo, A. Lombardozzi and Cicchetti. R, (1978) A simple approach for discovering common non-electrophoretic enzyme variability. A heat denaturation study in drosophila melanogaster. Biochem Gent 16: 299-305.

Yoshitake, N. and Akiyama, M (1965) Genetic aspect on the esterase activities of the egg in the silkworm *Bombyx mori* L (in Japanese). Japan Seri Sci 34 : 327-332.

Yoshitake, N. and Eguchi, M (1965) Distribution of blood esterase types in various strains of the silkworm, *Bombyx mori* L (in Japanese) Japan. Seri Sci 34 : 95-98.