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Research Article

Analysis of genetic variability in some silkworm strains of Bombyx *mori* L. through isoenzyme markers

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ABSTRACT

In this study esterase and acid phosphatase isozyme marker systems were used to study biochemical diversity in 10 silkworm strains. A total of ten isomorphs of esterase and five isomorphs of acid phosphatase were detected. These marker systems generated 15 loci, of which 14 were polymorphic among the silkworm strains with a total polymorphism of 93.33%. The mean observed number of alleles, effective number of alleles and gene diversity based on Nei's coefficient were 1.9444, 1.4016 and 0.2622 respectively. The highest gene diversity of 0.5 was noticed with HEST-6 loci. The multivoltines had more number of bands as compared to bivoltines. Strain specific isomorphs were also shown by both esterase and acid phosphatase. Dendrogram analysis showed five clusters of which ND7 was the most divergent followed by CSR51 and CSR2. The farthest distance was observed between Cambodge and CSR2 (0.69) with an average biochemical distance of 0.33. Based on cluster analysis divergent silkworm strains from different clusters can be utilized as parents in different breeding programs.

Key words: Silkworm, Esterase, Acid phosphatise.

INTRODUCTION

The silkworm is genetically well-characterized Lepidopteran insect with more than 400 described mutations, which have been mapped to more than 200 loci covering 28 linkage groups [1]. About 4000 silkworm strains have been developed and maintained worldwide and is derived from hybridization of different geographical origins, mainly the Japanese, Chinese, European and Indian strains, which have distinct traits.

Silkworm is having more advantages than other insect with commercial interest for farmers and experimental interest for researchers. Due to its importance, diversity study in silkworm has a valuable role in improving commercially important traits through selection of divergent parents. The study of genetic diversity in the silkworm is important for selection of useful parents and specific traits.

Application of isoenzymes and other molecular markers help to estimate genetic diversity much more accurately than that of morphological traits. Electrophoresis identifies variation (alleles) at loci that code for enzymes (usually termed isozymes or allozymes). One advantage of allozyme loci is that they are codominant and heterozygotes can be scored directly. Isozymes are the best suited biochemical markers in revealing inter-strain diversity in silkworm because they are less changeful between individuals [2]. With respect to their genetic structure, enzymes are less changeable between individuals than other biochemical constituents of haemolymph, and other tissues [3]. This characteristic makes them good biochemical markers.

Among the various known isozymes studied, esterase, amylase, acid phosphatase and alkaline phosphatase have been studied extensively because they are the groups of enzymes involved in various metabolic activity, gonadal maturation, maintenance of cell viability, metabolic activities of silk gland and defense functions [4,5,6]. Understanding the genetic constitution of an individual in the population of races and allelic variations through isozyme studies is known to reflect the differential catalytic ability of allelic genes and their significant role in the adaptive strategy of the genotypes [7]. Further, knowledge on

genetic variability is important for effective breeding [8,9] and maintenance of variability within and among population is one of the most important aspects in the conservation of genetic resources. Hence in this study, two isozymes systems viz., esterase (α- EST) and acid phosphatase (ACP) were utilized to study the diversity in 10 silkworm strains.

MATERIAL AND METHODS

Insect rearing

Ten silkworm strains comprising of five bivoltine (CSR2, CSR50, CSR51, BHR3 and SK4C) and five multivoltine (Pure Mysore, ND7, Nistari, Cambodge and L14) strains were reared at Central Sericultural Research and Training Institute (CSRTI), Mysore by following the standard method as suggested by Krishnaswami *et al* [10].

Collection of Haemolymph

Haemolymph was collected from 5th instar 3rd day larvae of each strain (5-6 larvae) in a microcentrifuge tube coated with 0.1M phenylthiourea (to inhibit tyrosinase activity) by cutting the prolegs of silkworms. The haemolymph sample was centrifuged for 10 min at 3000 rpm. The supernatant was transferred to fresh tube and was stored at -80ºC until use.

Qualitative analysis of Isozymes

Esterase (E.C 3.1.1.1) and acid phosphatase (E.C 3.1.3.1) isozyme systems were analyzed to estimate biochemical diversity in the haemolymph of silkworm strains. Separation of haemolymph esterase and acid phosphatase isozymes were carried out on 7.5% polyacrylamide gel following the methodology stated by Harris and Hopkinson [11] and Eguchi *et al.* [12] respectively. The relative mobility of the bands was calculated as follows:

$$\text{Relative mobility (Rm)} = \frac{\text{Migration of isozyme}}{\text{Migration of tracking Dye}} \times \frac{\text{Length of gel before staining}}{\text{Length of gel after staining}}$$

Data scoring and statistical analysis

The isozyme data was analyzed and compared. The isomorphs were scored in a binary code as '1' for presence and '0' for absence. Nei's [13] genetic distance was employed for the isozyme data. The distance matrix was used to construct dendrogram by applying Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Observed no. of alleles (na) and Effective no. of alleles (ne) was also estimated using POPGENE version 1.31[14]. Principal Coordinate Analysis (PCoA) was performed using GenAlEx 6.5 [15].

RESULTS

Esterase and acid phosphatase isozyme marker systems were used to study biochemical diversity in 10 silkworm strains. A total of ten isomorphs of esterase and five isomorphs of acid phosphatase were detected. Nomenclature of isomorphs was done from number 1 with prefix HEST for esterase and HACP for acid phosphatase from the anodal end of the zymograph. The multivoltines had more number of bands as compared to bivoltines. Among the 10 isomorph of esterase noticed, HEST-2 (Rm-0.47), HEST-5 (Rm-0.42) were strain specific present only in CSR2 and CSR51 respectively, whereas, HEST-6 (Rm-0.33) was specific for multivoltines. In case of acid phosphatase, HACP-2 (Rm-0.47) and HACP-1 (Rm-0.48) were specific to BHR3 and ND7 respectively. Together, these marker systems generated 15 loci of which 14 were polymorphic among the silkworm strains with a total polymorphism of 93.33%.

The mean observed number of alleles, effective number of alleles and gene diversity based on Nei's coefficient were 1.9444, 1.4016 and 0.2622. The highest gene diversity of 0.5 was noticed with HEST-6 loci.

Dendrogram analysis showed five clusters of which ND7 was the most divergent group followed by CSR51 and CSR2. The farthest distance observed between Cambodge and CSR2 (0.69) with an average biochemical distance of 0.33. Another cluster was formed by bivoltines with exception to L14, which is a multivoltine. CSR50 was close to L14; BHR3 and SK4C were close to each other. Three multivoltines (Pure Mysore, Nistari and Cambodge) were grouped to form another cluster (Figure 1). The PCoA analysis was in agreement with the results of the dendrogram (Figure 2). The first two principal components explained a total of 82.87% of the biochemical variation.

DISCUSSION

Isozymes like esterase, acid phosphatase, alkaline phosphatase, amylase, phosphoglucomutase, aspartataminotransferase, malate dehydrogenase, glucose 6 phosphate dehydrogenase and carbonic anhydrase have been used by various authors to study diversity in silkworm genotypes [16,17,18,19,20,21]. Among the different isoenzymes analyzed esterase was most preferred because of its diverse substrate specificity and polymorphic expression followed by acid phosphatase [17,22,23].

In this study also only two isozymes were used, but, they were potential enough to reveal diversity by generating a total of 15 isomorphs with 93.33%

polymorphism. The isozyme diversity revealed through dendrogram showed that the low productive multivoltine strains like Nistari, Pure Mysore and Cambodge were grouped together indicating their genetic similarity and closeness of genetic architecture between them. Further, BHR3 and SK4C were in same arm demonstrating their closeness. Though both are bivoltine and known for medium productivity nature and tolerant to high temperature [24]. But L14 and ND7 though both are multivoltine, but distanced from other multivoltine (Nistari, Pure Mysore and cambodge) and nearer to bivoltine strains. These two strains produce quality silk better than conventional multivoltine and slightly lower than bivoltine justifying their existence in different arms. HEST-2 (Rm-0.47) and HEST-5 (Rm-0.42) were strain specific present only in CSR2 and CSR51 respectively, which may be resulted in the allocation of separate clusters for these strains in the phylogenetic tree. ND7 was out grouped in the tree; this is because of expression of low number of esterase (four) and HACP-2 (Rm-0.48) band, which was specific to ND7. The biochemical markers utilized in this study resulted in grouping of some strains with different origin in one group and also strains with the same origin in different groups which may be due to the changes in their biological and developmental process and their adaptation to environmental conditions [3,25].

CONCLUSION

Our study has showed the potentiality of esterase and acid phosphatase in revealing genetic diversity between silkworm strains. The isozyme systems used were further able to reveal substantial amount of diversity in terms of high polymorphism percentage, high number of genotype-specific isomorphs and average genetic distance. Based on all these factors the isozymes were able to differentiate ten silkworm strains into five clusters, hence the silkworm strains from different clusters can be utilized in crossing to manifest high heterosis.

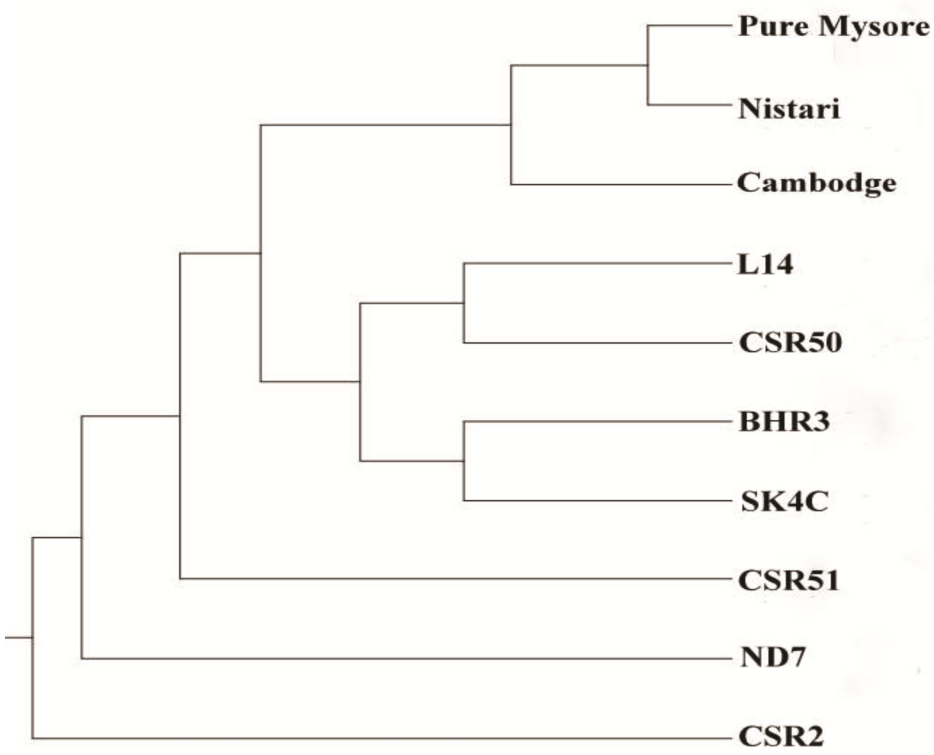

Figure 1: UPGMA dendrogram of silkworm strains generated from isozyme data

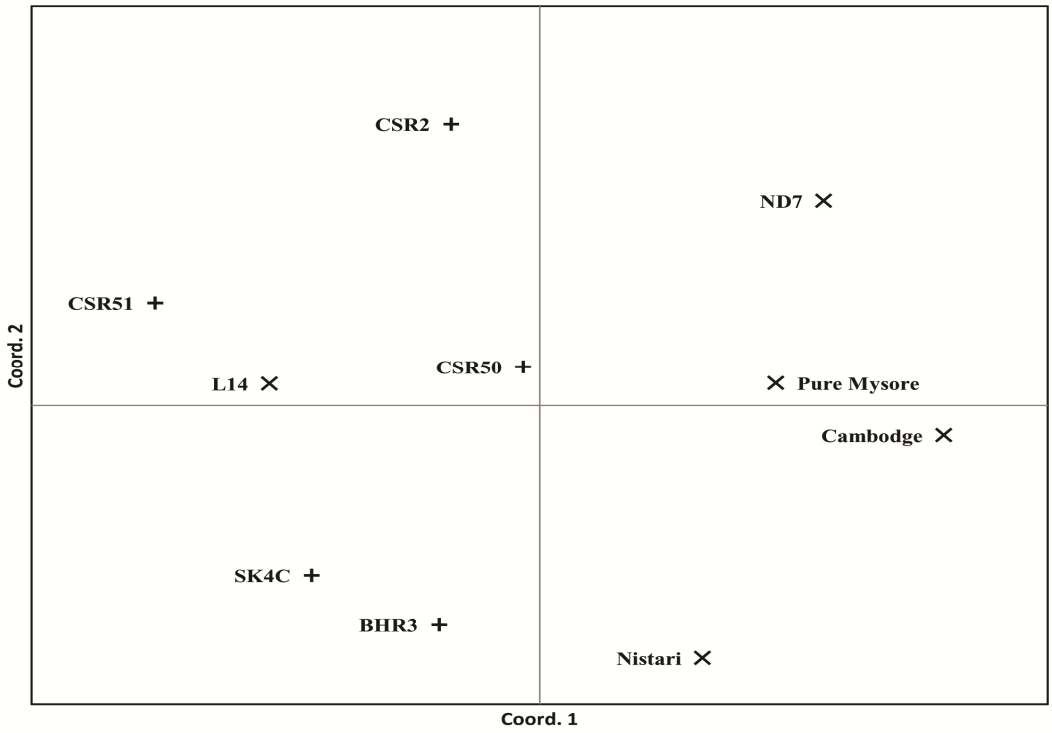

Figure 2: PCoA plot of silkworm strains based on isozyme data

+ - Bivoltine; x - Multivoltine

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