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Full Length Research Paper

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and random amplified polymorphic DNA (RAPD) based genetic variation studies in eri silkworm (*Samia cynthia ricini* Lepidoptera: Saturniidae)

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Four strains of eri, *Samia cynthia ricini* Lepidoptera: Saturniidae that can be identified morphologically and maintained at North East Institute of Science and Technology, Jorhat were characterized based on their protein profile by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and DNA by random amplified polymorphic DNA (RAPD) technique. Fiber yield was highest in Gs strain. SDS-PAGE profile showed 11 prominent bands in the strains with molecular weight ranging from 35 to 200 kDa together with 28 minor bands with molecular weight ranging from 33 to 210 kDa. Two polypeptides of molecular weight 90 and 110 kDa were absent in both Y and Ys. Eight random primers and one universal primer used for RAPD analysis generated a total of 79 bands, of which 49 were polymorphic. In both SDS-PAGE and RAPD, the UPGMA based dendrogram showed two clusters: cluster 1 included Gs and G, whereas Y and Ys was grouped in cluster 2 by SDS-PAGE analysis but RAPD analysis grouped Ys and G in cluster 1 and Gs and Y in cluster 2. The range of genetic diversity observed among the strains affirms the potentiality of RAPD technique for identification and selection of distant parents for silkworm hybridization for high silk yield.

Key words: Genetic variation, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), random amplified polymorphic DNA (RAPD), silkworm, cocoon.

INTRODUCTION

Silkworm is a domesticated insect having been cultured for a period of over 5000 years (Goldsmith, 1995). It possesses excellent characteristics as an experimental organism. *Samia cynthia ricini* (2n = 28) is predominant in India but also exist in the neighboring countries in a small measure. The species is easy to rear. The primary food plant of this polyphagus insect is castor (*Ricinus communis* L) but it also feeds on a wide range of food plants such as Heteropanax fragrans Seem, Manihot utilissimia Phol, Evodia flaxinifolia Hook, Ailenthus *gradulosa* Roxb, etc (Suryanarayan et al., 2002). There are a number of characters in all stages that are heritable. The morphological characters like body colour, shell weight, cocoon weight, etc has been traditionally used to identify a strain. Lack of assessing genetic diversity in the available strains, unavailability of modern tools to know the genomes at molecular level, environmental disturbances at the time of selection and polygenic control of various traits in silkworm can led to poor selection of parents in breeding programme (Datta, 1984; Datta and Ashwath, 2000). There is limited information about the genetic variability studies in eri silkworm strains. Protein electrophoresis is a powerful tool for resolving taxonomic and evolutionary problems, because

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it offers sensitivity, ease economy, high resolution and ability to analyze a number of protein samples in a short time (Hymowitz and Kaizuma, 1980; Cooke, 1988).

During the last three decades, various PCR based molecular markers has been developed as a powerful tool for such studies. Among them, random amplified polymorphic DNA (RAPD) technique has been efficiently used to study genetic diversity as it gives high degree of polymorphism and thus aid in differentiating even closely related strains. RAPD markers developed during the last two decades have been successfully and largely utilized for taxonomic and systematic classification as well as phylogenic or genetic diversity studies of plants (Rath et al., 1998; Sun et al., 2005; Adiguzel et al., 2006; Meimberg et al., 2006; Hug and Roger, 2007) and in insects like corn borer (Pornkulwat et al., 1998; Krumm et al., 2008), gypsy moth, Lymantia dispar (Reineke et al., 1999) and silkworms (Nagaraja and Nagaraju, 1995; Promboon et al., 1995; Thanananta et al., 1997; Awasthi et al., 2008; Ribeiro et al., 2009). No literature is available on genetic variation of eri silkworm strains through both sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and RAPD technique. This study aims at varietal characterization of four morphologically different strains of eri silkworm through biochemical and molecular techniques.

MATERIALS AND METHODS

Four strains of eri, *Samia cynthia ricini* viz yellow (Y), yellow spotted (Ys), green (G) and green spotted (Gs), which could be distinguished morphologically were collected from experimental farms maintained at NEIST, Jorhat. The shell weight was taken after five days of emergence of moths. Sericin was extracted from the eri silkworm cocoon by the method of Akiyama et al. (1993). The silk fiber analysis was carried out according to Krishnaswami et al. (1987).

SDS-PAGE

Protein was extracted by homogenizing posterior silk gland of the 5th instar larva in 0.05 mM Tris buffer (pH 8) and was estimated by Lowry et al. (1951) method. The protein after quantification was subjected to 10% SDS-PAGE according to Lamelli (1970). The gel was stained with Comassie blue overnight and destained using 40% methanol, 10% acetic acid and 50% distilled water until the bands were clearly visible.

Genomic DNA isolation

From each population, five individual larva at 5th instar was collected and DNA was isolated from the posterior silk gland (Thanananta et al., 1997; Nagaraja and Nagaraju, 1995). About 0.5 g of silk gland obtained after dissecting the larva was ground in liquid nitrogen with 500 µl extraction buffer (2.5% SDS, 1.4 M NaCl, 25 mM EDTA, 100 mM Tris pH 8.0, 0.8% β mercaptoethanol) and incubated at 65°C for 1 h with occasional swirling. The DNA was extracted using chloroform isolamyl alcohol (24:1) twice and the supernatant DNA was precipitated using 2-propanol and resuspensed in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). It was then incubated at 37°C after addition of RNase A (10 $mg.ml^{-1}$). DNA was re-extracted with phenol chloroform isoamyl alcohol (25:24:1) and was precipitated with sodium acetate and absolute alcohol. DNA thus obtained was dissolved in 150 µl of TE buffer and quantified against the known quantity of standard uncut λDNA in 0.8% agarose gel. DNA was diluted to a uniform concentration (25 $ng.\mu l^{-1}$) for RAPD study.

RAPD analysis

A set of 10 mer RAPD primers was obtained from Operon Technologies, USA. A M13 universal primer was also used at an annealing temperature of 50°C. The PCR was performed in a 25 µl reaction mixture containing 2.5 µl of 10 × buffer (with 15 mM $MgCl_2$), 50 µM concentration of dNTP mix, 25 p mol concentration of primer, 0.3 units of Taq polymerase and 50 ng of genomic DNA. Amplification was performed in a thermal cycler (Applied Biosystems) using the following programme: 1 cycle of 94°C for 5 min, 34 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, followed by 72°C for 2 min. PCR products were resolved on 2% agarose gel stained with ethidium bromide (0.5 $\mu g.ml^{-1}$) and run in 1X TBE (100 mM Tris-HCl, pH 8.0, 83 mM boric acid, 1 mM EDTA) at 80 V. Gels were visualized with UV transilluminator and photographed. The DNA fragments amplified by a primer were scored as present (1) or absent (0) for the strain studied.

Data analysis

The DNA fragment amplified by a primer in each gel was analyzed by scoring the bands as present (1) or absent (0). Data matrices were entered into the NTsys version 2.02 K package, a pairwise comparison of germplasm accessions was made and genetic diversity parameters were calculated. Genetic similarities based on Jaccard's similarity coefficient were calculated among all possible pairs, using Simqual option and ordering in a similarity matrix. Based on the data, a dendrogram was prepared by the unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Sneath, 1963) and using a statistical software package "SPSS for MS Windows Release 10.0" to group the individual populations into discrete clusters.

RESULTS AND DISCUSSION

Morphological and biochemical parameters studied are presented in Table 1. Among the four different strains studied, the strain Gs was found to be best in terms of high fiber content, with maximum shell weight and sericin content. This strain as shown in Figure 1 can be reared in bulk and can be utilized in breeding programme for high silk production. This strain was followed by G, Y and Ys in terms of fiber content. High shell weight was recorded in G and Y varieties and the values were at par with Gs variety. Similar results were also reported by Vijayan et al. (2006). Total soluble protein was estimated by Lowry's method and analyzed by SDS-PAGE (Figure 2). In the varieties *viz*, Y, Ys, G and Gs, the protein content was found to be 0.6960, 0.9520, 0.8680 and 0.6900 mg/g fresh weight, respectively. SDS-PAGE of total soluble proteins showed 39 polypeptide bands with varying intensity and heterogenous molecular weight. A total of

Table 1. List of morphological and biochemical characters of the four Eri silkworm strains[a].

Character	Y(Sr 01)	Ys(Sr 02)	G(Sr 03)	Gs(Sr 04)
Shell weight (g)	0.3706±0.03	0.3186±0.03	0.3894±0.07	0.4000±0.06
Serecin content (g)	0.0447±0.03	0.0522±0.01	0.0658±0.04	0.0674±0.04
Protein content (mg/g fresh weight)	0.6960±0.05	0.9520±0.03	0.8680±0.02	0.6900±0.05
Fiber content per shell (g)	0.3086±0.02	0.2731±0.10	0.3236±0.04	0.3315±0.03
Larval marking	None	Spotted	None	Spotted

[a]Values are mean of three replicates of 10 silkworm each ± SE.

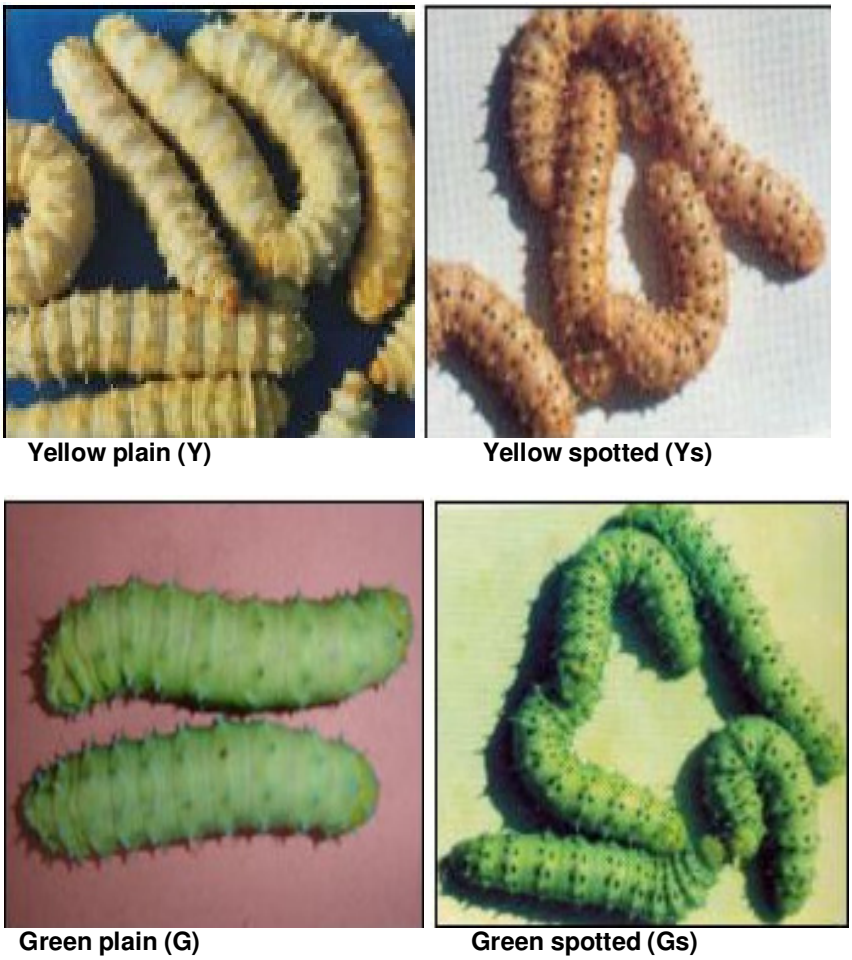

Figure 1. Different strains of eri silkworm used in our analysis.

11 prominent bands were present in the strains with molecular weight of 35, 37, 39, 40, 43, 56, 68, 75, 80 a, 150 and 200 kDa. Besides, 28 minor bands with molecular weight ranging from 33 to 210 kDa were also observed. Two polypeptides of molecular weight 90 and 110 kDa were absent in both Y and Ys. Cluster analysis (UPGMA) was generated by computing polymorphic as well as monomorphic markers to construct the dendrogram (Figure 4). Jaccard's similarity coefficient values were found to be in the range of 0.923 to 1.00 (Table 3), indicating a narrow genetic base among the strains studied. The analysis grouped the strain into two clusters. Cluster 1 included Gs and G, whereas Y and Ys was grouped in cluster 2. As shown in the data presented in Table 1, both varieties green and spotted green produced higher fiber content and were grouped in cluster 1 and vice versa. Since variations were observed only in minor bands, it is difficult to conclude any relationship among the silkworm strains. This in turn indicated that though the strains used in the study were

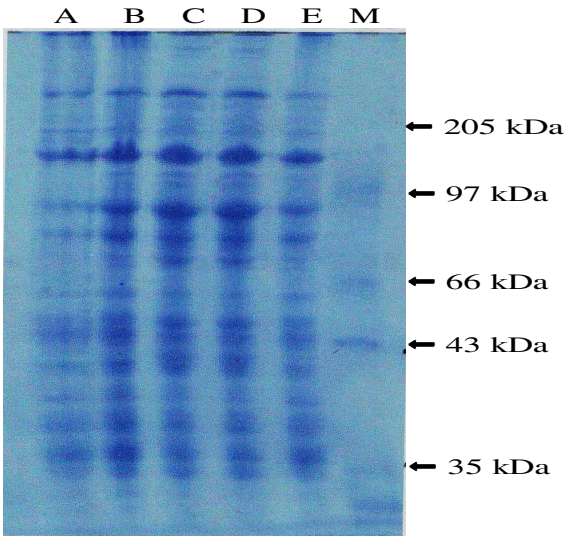

Figure 2. 10% SDS-PAGE of silk gland of eri silkworm. A, Spotted yellow; B, yellow; C, spotted green; D, green: E, yellow; M, marker.

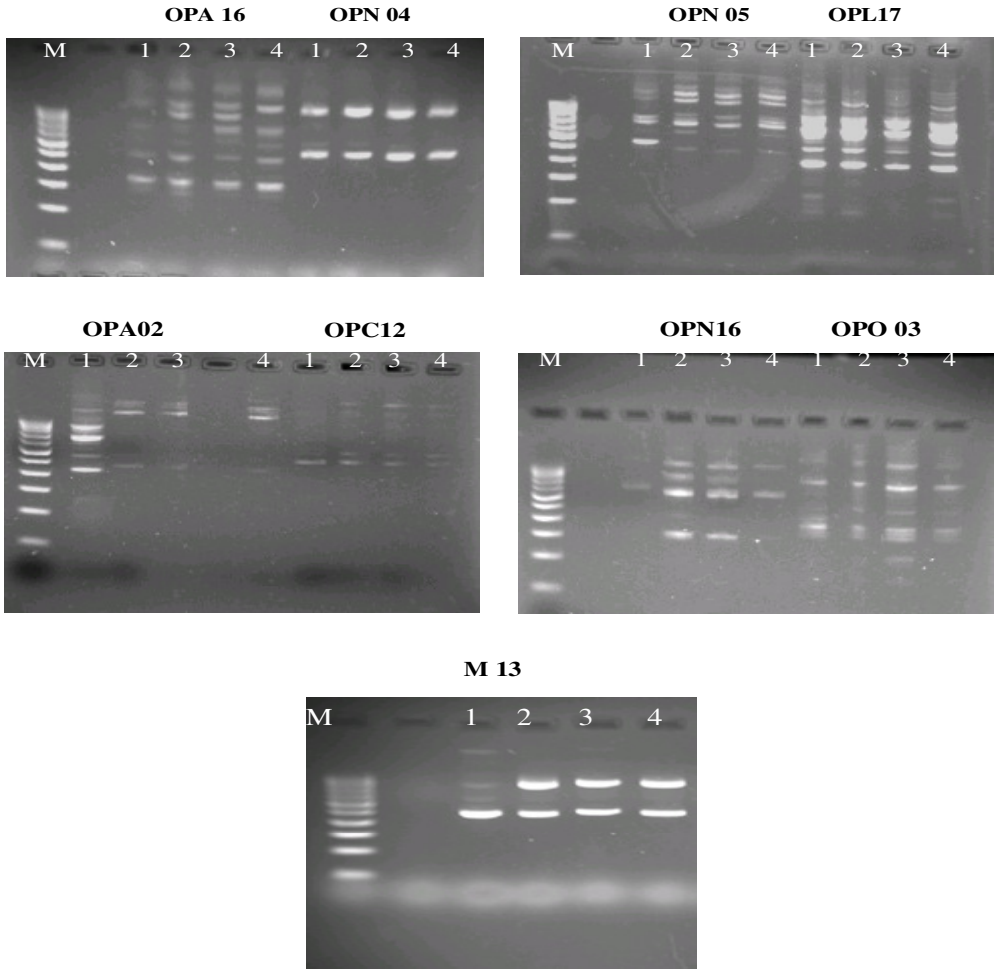

Figure 3. RAPD banding pattern of eri silkworm strains. M, 100 bp DNA ladder, varieties in the order; 1, spotted green, 2, yellow; 3, spotted yellow; 4, green.

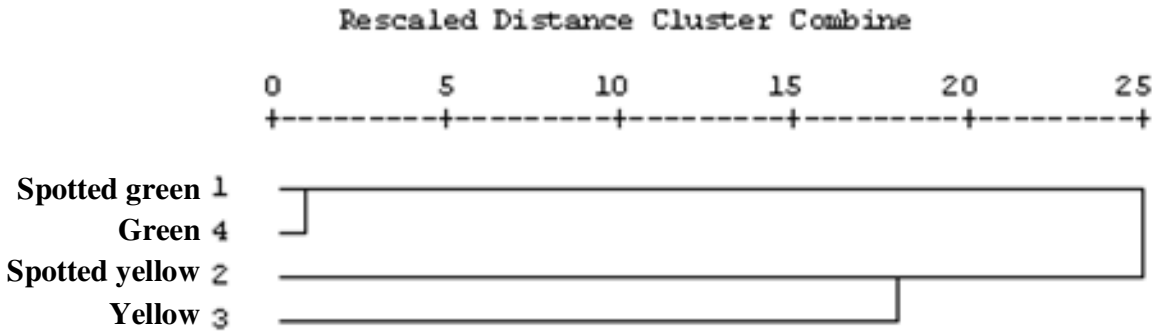

Figure 4. Dendrogram of eri silkworm varieties based on Jaccard's similarity coefficient of SDS-PAGE banding pattern.

Table 2. Banding profile produced by selected RAPD primers.

S/N	Primer	Total band	Polymorphic band	PPB	Range of fragment size (bp)
1	OPA 02	10	8	80	250 - >1000
2	OPC 12	7	7	100	450 - >1000
3	OPL 17	16	6	37.5	150 - >1000
4	OPN 16	6	6	100	300 - >1000
5	OPO 03	9	5	55.56	150 - >1000
6	OPA 16	10	6	60	250 - >1000
7	OPN 04	4	2	50	500 - >1000
8	OPN 05	10	4	40	250 - >1000
9	M 13	7	5	71.43	500 - >1000
Total		79	49		
Average		8.78	5.45	66.05	

PPB = Proportion of polymorphic bands.

Table 3. Similarity matrix for Jaccard's coefficient based on SDS-PAGE banding pattern for Eri silkworm strains.

	Gs	Y	Ys	G
Gs	1.000	0.923	0.923	0.999
Y		1.000	0.946	0.923
Ys			1.000	0.923
G				1.000

genetically different, their expression of genes appeared almost identical for which no clear distinction could be made among the four strains based on SDS-PAGE (Rathi et al., 2004).

Genetic diversity among the four eri silkworm strains was investigated by RAPD analysis. RAPD profiles of the four strains generated with RAPD primer OPL 17, OPA 02, OPC 12, OPL 17, OPN 16, OPO 03, OPA 16, OPN 04, OPN 05 and M 13 universal primer is shown in Figure 3. The total number of DNA fragments amplified and the number of polymorphic bands from each primer is shown in Table 2. Only one primer (OPL 06) did not show amplification profiles among the four strains and hence was not considered for further analysis. A total of 79 scorable fragments ranging from 150 to >1000 bp were generated by nine primers, of which 49 were polymorphic as shown in Table 2. The similarity coefficients ranged from 0.720 to 0.778 (Table 4). Associations among the four strains revealed by UPGMA cluster analysis based on RAPD are presented in Figure 5 and it grouped the strains into 2 clusters. Cluster 1 included Ys and G, whereas Gs and Y was grouped in cluster 2. Since RAPD's are random selection of DNA sequence, it was apparent in the study that RAPD technique was sensitive

Table 4. Similarity matrix for Jaccard's coefficient based on RAPD banding pattern for eri silkworm strains.

	Gs	Y	Ys	G
Gs	1.000	0.759	0.720	0.743
Y		1.000	0.747	0.747
Ys			1.000	0.778
G				1.000

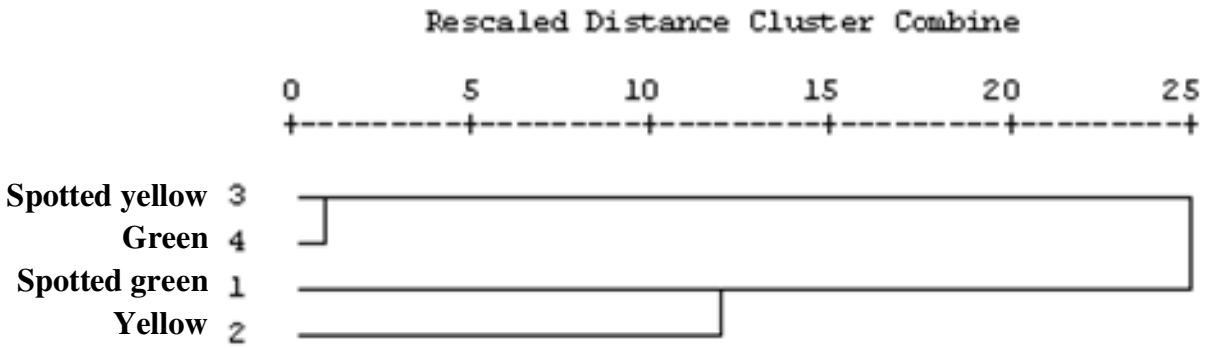

Figure 5. Dendrogram of eri silkworm varieties based on Jaccard's similarity coefficient of RAPD banding pattern.

enough to detect differences between strains of eri silkworm in which differentiation is not always possible morphologically. In this study, an average of 66.05% PPB demonstrated the potential of the method in evaluating genetic diversity. Considering the diversity, the genotypes belonging to different groups will constitute promising parents for hybridization in silk improvement programme. For example a cross between Sg and Y, and G and Ys is likely to generate promising recombinants. Thus, this study along with the analysis of their rearing performance is essential to understand the genetic relationship among the four strains for use in breeding programme. To sustain the silk industry, the utilization of hybrids in silkworm breeding programme is very much necessary to develop a farmer preferred high yielding variety (Murthy et al., 2006).

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