ANALYSIS OF PHENOTYPIC DIVERSITY AND PROTEIN POLYMORPHISM IN SOME SILKWORM BREEDS OF *BOMBYX MORI* L.

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ABSTRACT: The assessment of genetic variation is a major aspect in silkworm improvement programmes. It distinguishes different genotypes for designing the breeding programmes and population-genetic analysis. It also estimates the amount of variation within genotypes and between genotypes for predicting potential genetic gain in a breeding programme. Variations in three qualitative traits and nine quantitative traits along with protein polymorphism were studied in ten silkworm genotypes. The results showed significant variation ($P < 0.05$) in the quantitative traits of silkworm genotypes studied. Cluster analysis based on phenotypic variation revealed three clusters separating high, moderate and low silk producers according to the silk producing ability of silkworms. The results of Principle Co-ordinate analysis was in agreement with the dendrogram. Protein profiling divided ten silkworm genotypes into two clusters. The dendrogram deduced from phenotypic data clearly indicates the dominant role of environment on quantitative traits. However, expression of quantitative traits depends on the genotypes and their place of origin. The phenotypic data depicted substantiate amount of diversity among the silkworm genotypes and hence, these breeds can be utilized for improving the silk yielding traits.

KEY WORDS: Genetic diversity, Quantitative traits, Qualitative traits, Protein polymorphism.

The Silkworm (*Bombyx mori*) is the most domesticated lepidoptera with economic significance, attributed to its silk secreting ability. In the long history of domestication, several thousand silkworm strains have been developed and maintained. Currently, more than 4000 strains are available in the germplasm of *B. mori* (Nagaraju, 2002; Kumaresan et al., 2007), which includes uni-, bi- and multi-voltine. Among them, some different genotypes are similar in morphological characters (e.g., larval markings, cocoon shape) although they were collected from different parts of the world revealing morphological divergence between them (Li et al., 2005).

Genetic variability is a prerequisite for an effective selection of any economically important organism and an essential factor in developing high yielding varieties (Akanda et al., 1998). Such genetic diversity studies involving similarities and differences of relationship and genotypes in various pure lines are involved in breeding programs for crossing and hybridization to maximize heterosis. Thus, there must be specified relationships between different pure lines (Nezhad et al., 2010). But the researchers emphasize that the high genetic variation might not give always a high genetic diversity in the inbreeding population, such as silkworm. This is further confirmed that the genetic diversity is not always related with geographical diversity (Rao & Nakada, 1998).

The proteins are the “working horses of the cell”, their expression and abundance leaves a footmark of their functional role in different developmental stages of an organism. The understanding of such variations at protein level not

only helps in the identification of genotypes in the germplasm but also unveil the loci governing the changed phenotype of the pure genotypes (Bakkappa& Subramanya, 2010). The protein polymorphism gives a clue on the heterotic expression for selected traits and can be used as an index in silkworm breeding (Telebi et al., 2011). Perusal of literature indicated haemolymph protein as one of the most extensively studied banding profiles and with wide overlapping substrate specificities and pattern of inhibition and protein polymorphism occurring in numerous forms, which are expressed by distinct gene loci having a high degree of genetic variability (Takasusuki et al., 2006). The importance of similar study relevant to animal and plant breeding (Frey et al., 1983) and conservation of genetic resource (Zeng et al., 2003), genetic variability in mosquitoes (Pushpalatha & Vijayan, 1999) and in silkworms (Moorthy et al., 2007; Doddaswamy & Subramanya, 2007; Akkad et al., 2008; Anuradha et al., 2010) are clearly established.

The selection of best genotypes depends on a number of characters and diversity among them. Therefore, a clear understanding and knowledge of association and contribution of various yield components is essential for any selection programme aimed at yield improvement. Hence, the present study was undertaken to estimate the phenotypic variation and protein polymorphism among ten silkworm genotypes.

MATERIALS AND METHODS

Silkworm genotypes

For the present study ten silkworm genotypes consisting of five bivoltines (CSR2, CSR50, CSR51, BHR3 and SK4C) and five multivoltines (Pure Mysore, ND7, Nistari, Cambodge and L14) were used. Rearing of these silkworm genotypes was conducted by adopting standard technique suggested by Krishnaswamy (1978). At the end of 5th instar, the spinning larvae were collected manually and mounted on plastic collapsible mountages. Data on qualitative characters (morphological) like larval marking, cocoon colour, cocoon shape and data on the economically important quantitative traits, such as fecundity (no), larval weight (g), cocoon yield/ 10000 larvae by number (no.), yield/ 10000 larvae by weight (g), cocoon weight (g), shell weight (g), shell ratio (%), filament length (m) and filament size (d) were collected. The experiment was performed in triplicate with 250 larvae each. Morphological characters of the silkworm genotypes are presented in Table 1.

Collection of Haemolymph

Haemolymph of different silkworm genotypes were collected from the 5^{th} instar 3^{rd} day larvae in a precooled eppendorf tube coated with 0.1M phenylthiourea by cutting the prolegs. The haemolymph samples were centrifuged for 10 min at 3000 rpm and the supernatant was transferred to fresh tubes. The samples were stored at -80^{o}C until use.

Qualitative analysis of haemolymph proteins

A discontinuous gel with 5% of stacking gel and 12% of resolving gel was prepared separately as described by Janarthanan & Vincent (2007). After electrophoresis the gels were removed and stained with coommossie brilliant blue solution for 3 hours and destained with acidic methanol. The destained gels were fixed with 7% acetic acid solution.

Statistical and cluster analysis

The data of quantitative traits were subjected for one way analysis of variance (ANOVA) and cluster analysis using Euclidean distance with complete linkage. Protein bands were scored in a binary code as '1' for presence and '0' for absence. The dendrogram was constructed based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA). All the analysis was performed on Statistical Package for Social Sciences (SPSS) version 10/11.5 and GenAlEx 6.5 (Peakall & Smouse, 2012).

RESULTS AND DISCUSSION

Details of three qualitative traits (morphological) and nine quantitative traits in five bivoltine and five multivoltine silkworm genotypes are shown in Table 1 and 2. Analysis of variance revealed significant differences between the quantitative traits studied. Fecundity, larval weight, shell % and filament length ranged from 563 to 410, 46.41 to 23.28 g, 23 to 14.26 % and 1052 to 352.29 m respectively. CSR2 showed highest fecundity, larval weight, cocoon weight and shell %. CSR50 and SK4C showed highest filament length and filament size, respectively. Nistari showed the lowest fecundity, larval weight, shell % and filament length followed by Pure Mysore. Among multivoltines, L14 had high cocoon weight and shell%. For characters like fecundity, larval weight, shell % and filament length the genotypes ND7 and L14 had moderate values that is higher than the other multivoltines and lower than the bivoltines. Variance component was higher in filament length with 65155.822 followed by fecundity (3050.409), larval weight (71.658) and shell % (10.45 %) (Table 2 & 3).

Phenotypic diversity

The cluster analysis based on phenotypic data clearly indicated that the genotypes are grouped on the basis of their silk yielding ability. Ten silkworm genotypes were grouped into three clusters, of which cluster I contained all the three high silk yielders (CSR2, CSR50 and CSR51) and Cluster II had two bivoltines (BHR3 and SK4C) developed at Eastern India with two multivoltine genotypes (ND7 and L14) developed from Southern India, which were grouped together based on their moderate values for most of the quantitative traits. Cluster III had three multivoltine genotypes namely Nistari, Cambodge and Pure Mysore, which are low silk yielders (Fig. 1).

Phenotypic differences between silkworm genotypes were completely dependent on silk yielding quantitative traits. In cluster II, BHR3 and SK4C were close and shared moderately higher values for all the quantitative traits, that is lesser than other bivoltine genotypes, but greater than multivoltines. The other two multivoltine genotypes, ND7 and L14 were also found close to each other as they shared some of the quantitative traits with intermediate number, especially yield/10000 larvae in weight, cocoon weight, shell % and filament length. Another common factor among them was morphological characters, even though Pure Mysore also shared the qualitative traits with ND7 and L14 (Table 1), but it was grouped with Nistari and Cambodge as their quantitative traits are very similar. It also proves that the base for dendrogram classification was quantitative characters. The relationship between Nistari and Pure Mysore is consistent with the previous reports of Talebi and Subramanya (2009). Nistari and Cambodge may be nearer because of their common biological and developmental performances. These findings are further confirmed by PCoA analysis, which was concurrent with the results of dendrogram by clearly separating the high,

moderate and low silk yielding silkworm genotypes (Fig. 2). Our results demonstrate the dominant role of environmental conditions prevailing in different geographical regions on the variations in the quantitative traits in silkworm as they are varying with the genotypes from different geographical regions (Table 2).

Protein polymorphism in haemolymph

Protein profiling of haemolymph of ten silkworm genotypes under study revealed 15 bands ranging from 11 to 211 kDa, of which one band with molecular weight ~24kDa was polymorphic and it was expressed only in CSR2 and BHR3. Cluster analysis based on protein polymorphism revealed two clusters, cluster I contained eight genotypes and cluster II had two genotypes (Figs. 3 and 4).

Protein analysis was able to generate a minimum level of polymorphism with a single polymorphic protein band and hence, could be used as supplementary criteria for characterization. Approximately, 24kDa protein was expressed in CSR2 and BHR3 genotypes; though the geographic origin is not same for the genotypes, they were able to generate identical protein profiles, indicating a definite role of this protein in their biological and metabolic process.

In silkworm, *Bombyx mori*, the silk yield is contributed by more than 21 traits (Thiagarajan et al., 1993) and there exists an interrelationship between multiple traits in silkworm. Any effort to improve the yield requires consideration of cumulative effect of the major traits which influences the silk yield. It has also been established that selection pressure applied for one character results in correlated changes in other quantitative traits of economic importance (Moorthy et al., 2011). Hence, classification of silkworm genotypes is very important in breeding programs. Commercial rearing of this insect is based on hybrids crossed from pure lines. Due to the presence of many genotypes and continuous production of new lines, furnishing all the possible crosses to obtain the best hybrids and hybrid vigour using heterosis is impossible (Etebari et al., 2005). Hence, phenotypic diversity is of utter importance in silkworm as it is economically important insect; in which most of the commercial traits are quantitative.

These findings are important for breeding programs, as diversity between silkworm genotypes is vital for selection of suitable parents required for successful development of improved variety and hybrids of silkworm that have the potential to adapt to the fluctuating environments and management systems for realising stable and sustainable yields.

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Table 1. Details of the morphological traits in the silkworm genotypes.

Sl No.	Breeds	Voltinism	Larval Marking	Cocoon colour	Cocoon shape
1	Pure Mysore	Multivoltine	Plain	Light greenish yellow	Spindle
2	ND7	Multivoltine	Plain	Light greenish yellow	Elongated oval
3	Nistari	Multivoltine	Marked	Yellow	Spindle
4	Cambodge	Multivoltine	Plain	Yellow	Spindle
5	L14	Multivoltine	Plain	Light greenish yellow	Elongated oval
6	CSR2	Bivoltine	Plain	White	Oval
7	CSR50	Bivoltine	Plain	White	Oval
8	CSR51	Bivoltine	Marked	White	Dumbbell
9	BHR3	Bivoltine	Marked	White	Dumbbell
10	SK4C	Bivoltine	Marked	White	Dumbbell

Table 2. Details of quantitative traits studied in silkworm genotypes.

Genotypes	Fecundity	Larval weight (g)	Yield/100 00 larvae by no.	Yield/100 00 larvae by wt.(kg)	Cocoon weight (g)	Shell weight (g)	Shell (%)	Filament length (m)	Filament size
Pure Mysore	425	23.45	9254	9.427	1.053	0.16	15.11	402	2.1
ND7	422	25.56	9207	13.537	1.456	0.27	18.77	636	2.45
Nistari	410	23.28	9495	12.689	1.182	0.618	14.26	352.29	2.87
Cambodge	450	25.66	9420	9.641	1.23	0.179	14.54	402.16	2.88
L14	510	34.24	9142	13.203	1.446	0.26	17.98	724	2.06
CSR	563	46.41	9077	15.498	1.782	0.41	23	1050	2.82
CSR50	544	44.11	9040	15.757	1.715	0.389	22.6	1052	2.86
CSR51	538	42.45	8847	15.22	1.71	0.367	21.4	975	2.82
BHR3	517	34.33	9226	12.638	1.525	0.297	19.45	622.83	2.93
SK4C	524	34.42	9226	14.362	1.627	0.334	20.51	704.9	2.99

Table 3. ANOVA and summary statistics of the nine quantitative traits measured in 10 silkworm genotypes.

Traits	Mean	Standard Deviation	Range	F-value	*P*-value
Fecundity	490.3	57.42831	410 – 563	1369.280	0.0001
Larval weight	33.391	8.763985	23.28 - 46.41	1099.936	0.0001
Yield/10000 larvae in no.	9193.4	184.995	8847 – 9495	3.113	0.016
Yield/10000 larvae in Wt.	13.1972	2.232143	9.427 - 15.757	496.356	0.0001
Cocoon weight	1.4726	0.248949	1.053 - 1.782	777.019	0.0001
Shell weight	0.3284	0.131434	0.16 - 0.618	258.937	0.0001
Shell %	18.762	3.252704	14.26 -23	247.007	0.0001
Filament length	692.118	264.6746	352.29 – 1052	153383.75	0.0001
Filament size	2.678	0.346404	2.06 - 2.99	25.894	0.0001

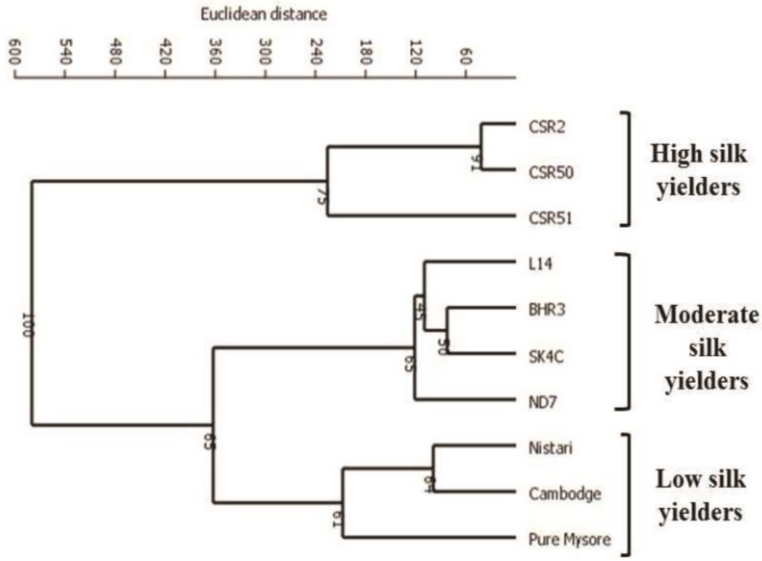

Figure 1. Dendrogram based on the phenotypic data of ten silkworm genotypes elucidated by using Euclidean distance with complete linkage.

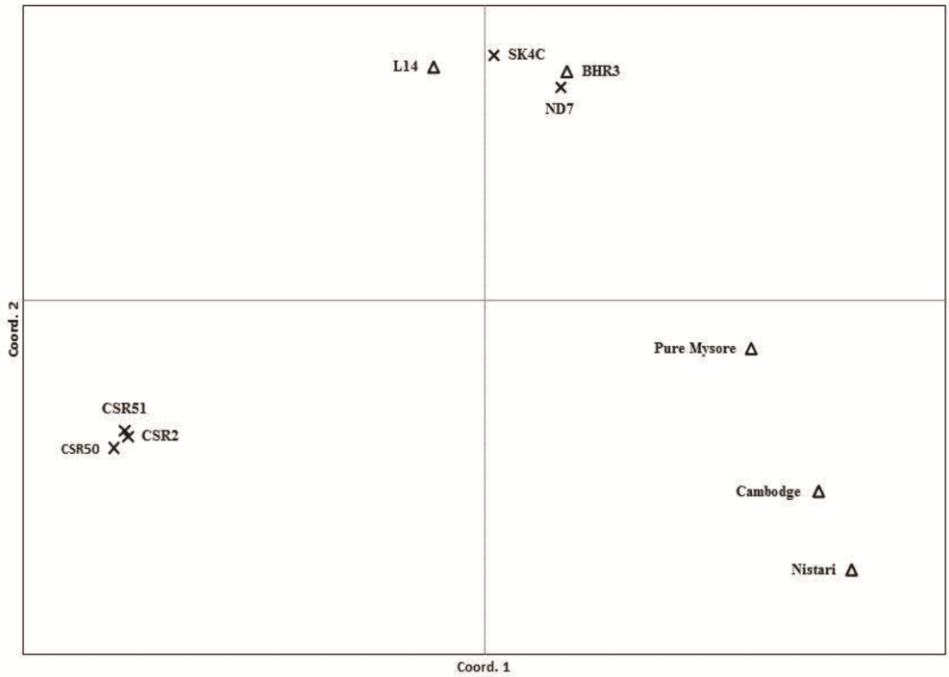

Figure 2. PCoA based on phenotypic data of ten silkworm genotypes.

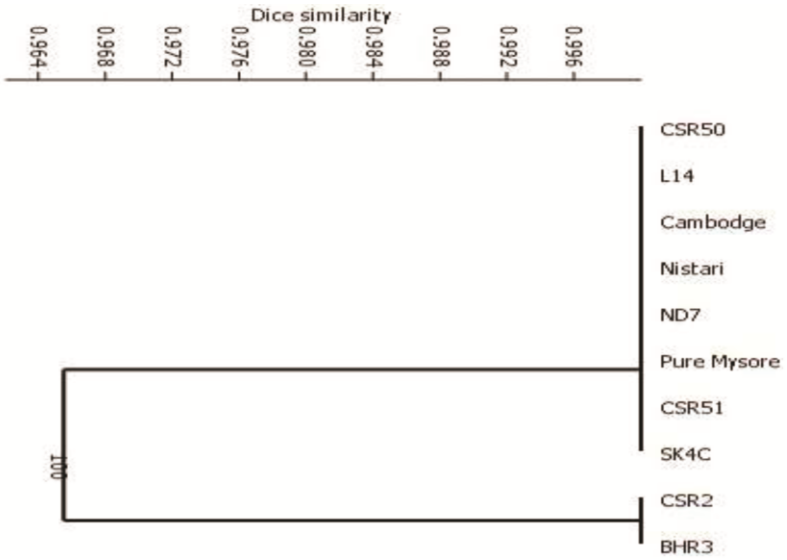

Figure 3. Dendrogram based haemolymph proteins of ten silkworm genotypes elucidated by using Dice similarity.