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Research Article

Evaluation of Mutant Silkworm Genetic Resources for Important Morphological and Quantitative Characters

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Abstract

Background and Objective: Genetic diversity and variability in the population are pre-requisite for the crop improvement programme. Collection and maintenance of genetic diversity is a fundamental component in long-term management strategies for genetic improvement of silkworm. The silkworms mutant were evaluated for morphological characters and rearing traits during two crop seasons i.e., winter and summer to analyze the performance and to identify better mutant accessions. **Materials and Methods:** Total 23 mutant silkworm accessions were considered for the study, the important morphological characters during egg, larva and cocoon stages were recorded and compared with the catalogue data. Total 12 important silkworm quantitative traits were studied and analysed using multi trait analysis package to identify better accessions. **Results:** Significant amount of variations were observed among different accessions for different traits. Among 23 mutant silkworms 08 were qualified in the cumulative Evaluation Index (EI) (>50). The cluster analysis showed heterogeneity among the silkworm accessions based on the grouping. The Principal Component Analysis (PCA) indicated the grouping of 06 mutants along with commercially important silkworm races Multivoltine Pure Mysore (PM) and bivoltine (CSR-2). **Conclusion:** Since these genotypes were considered most suitable for basic genetic studies rather its usefulness in silk production. The correlation studies using PCA revealed that some of the mutant silkworms conserved in the germplasm showed on par with commercially important silkworm races and can be used to explore the combining ability studies for further commercial exploitation.

Key words: Silkworm, *Bombyx mori*, mutant, evaluation, germplasm, genetic resources, genotypes

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Study about genetic diversity are an important tool that enables breeders to make good selection of parents to ensure genetic variability. Heterosis between genotypes is often enhanced when the two parents are genetically diverse¹. The silkworm *Bombyx mori* L is one of most important economic insect species which is exploited for the production of natural silk. The species has wide distribution found both in temperate as well as tropical regions with greater genetic diversity in morpho-biochemical and biometric characters². It has been estimated that, more than 3000 silkworm strains are available worldwide which developed through breeding^{3,4}. The temperate silkworm strains generally have Uni and bi-voltinism are quantitatively as well as qualitatively superior races compared to tropical polyvoltine silkworm races. On the other hand polyvoltines are better in terms of survival rate, hardiness and resistance to biotic and abiotic challenges⁵. The silkworms has been used as a model for genetic studies because of its large size, ease of rearing in laboratory and short life cycle. The existence of more than hundreds of geographical races and genetically improved strains used for commercial silk production which differ not only qualitative traits but also in quantitative traits such as body size, feeding duration, thermal tolerance and disease resistance. These traits remain to be subjected to systematic analysis using modern genetic tools⁶.

The principal aims of crop improvement is to develop silkworm breeds with superior multiple traits including improved silk productivity, adaptability, disease tolerance and other commercially important characters. Before any breeding tasks, it is imperative to understand the behavior, performance in different life stages and quality and quantity of the silk produced by the silkworm breed/race which is pre-requisite for the selection as parents. The domesticated mulberry silkworm, *Bombyx mori* L. represents itself as various mutants evolved both from spontaneous and induced mutation. These mutants are maintained by fanciers and breeders in the closed line culture system for many years and serve as a basic tool for genetic analyses including phylogenetic, physiological, ethological, biochemical and molecular studies since systematic linkage studies have been successfully carried out⁷. More than 400 mutations have been mapped corresponding to 230 genes with 28 linkage groups⁸⁻¹⁰. The mutant silkworm races shows different phenotypic characters, such as variation in egg color, larval duration, larval marking, cocoon shape, cocoon color and hemolymph colour. Morphological characterization has direct or indirect relation with various quantitative and qualitative traits¹¹. These races also show

wide diversity in the yield, economic parameters and exhibit considerable variations for several heritable characters viz., egg colour, larval markings, cocoon colour and cocoon shape. Further, morphological traits along with correlation parameters help to identify and group similar performing germplasm for effective conservation in the gene bank. As the mutant silkworm genetic stocks, it is possible to use directly in silkworm breeding for evolving new races¹² and characterizations of morphological mutant traits were utilized as a basic tool for genetic analysis and were used to study the genetic diversity and distance among the population. In current research, the performance of mutant silkworm genetic resources conserved in the centre are evaluated for 11 rearing and grainage parameters and also compared with the popular commercially exploited bivoltine silkworm race to examine its potentialities for commercial cocoon production.

MATERIALS AND METHODS

Study area: The present study was conducted during 2018-2019 at Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, Tamilnadu state, India.

Research procedure: Silkworm *Bombyx mori* mutant genetic resources which were collected and maintained at CSGRC, India are selected (Table 1) for the evaluation of important morphological characters, rearing parameters and grainage performance as per the standard descriptor (Table 2, 3). The rearing of 23 silkworm mutant accessions was conducted during June-July and December-January. Three replications of each mutant accession were maintained in a separate 2' x 3' Plastic perforated trays arranged on rearing stand. The rearing was conducted as per the Standard silkworm rearing procedure¹³ and Standard Operational Procedure for conservation of silkworm germplasm. G-2 mulberry variety for chawki rearing and V-1 mulberry variety leaves was used for rearing of late age silkworms. The morphological data was collected based on the physical observation and compared with passport data available at the centre. The data for 12 important rearing and grainage parameters was collected for 5 years (2 crops/year). Mutant silkworm data was compared with commercially important mutivoltine (PM) and bivoltine (CSR-2) silkworm breeds using PCA analysis for better understanding of the possibilities of mutant silkworms for commercial exploitation.

Statistical analysis: Mean data of 5 years for 23 mutants from 12 important quantitative characters were considered. The number of rearing conducted during the period was treated

Table 1: List of mutant silkworm germplasm maintained at CSGRC, Hosur, India

Acc. No.	National accession number	Name of the race
BBE-0306	NBAII-CSG---0000306	TMS-12
BBE-0307	NBAII-CSG---0000307	TMS-14
BBE-0308	NBAII-CSG---0000308	TMS-32
BBE-0309	NBAII-CSG---0000309	TMS-33
BBE-0310	NBAII-CSG---0000310	TMS-35
BBE-0311	NBAII-CSG---0000311	TMS-38
BBE-0312	NBAII-CSG---0000312	TMS-61
BBE-0313	NBAII-CSG---0000313	TMS-62
BBE-0314	NBAII-CSG---0000314	TMS-64
BBE-0315	NBAII-CSG---0000315	TMS-65
BBE-0316	NBAII-CSG---0000316	TMS-66
BBE-0317	NBAII-CSG---0000317	TMS-67
BBE-0318	NBAII-CSG---0000318	TMS-75
BBE-0319	NBAII-CSG---0000319	TMS-82
BBE-0320	NBAII-CSG---0000320	TMS-2
BBE-0321	NBAII-CSG---0000321	TMS-17
BBE-0322	NBAII-CSG---0000322	TMS-31
BBE-0323	NBAII-CSG---0000323	TMS-69
BBE-0331	NBAII-CSG---0000331	TMS-34
BBE-0333	NBAII-CSG---0000333	OD-Translucent
BBE-0390	NBAII-CSG---0000390	TMS-04
BBE-0391	NBAII-CSG---0000391	TMS-13
BBE-0392	NBAII-CSG---0000392	TMS-18

BBE: *Bombyx mori* bivoltine exotic, NBAII: National bureau of agriculturally important insect, CSG: Central sericultural germplasm, TMS: Mutant silkworm

Table 2: Parameters for the evaluation of mutant silkworm germplasm during rearing and grainage

Parameters
Fecundity (No.s)
Hatching (%)
Weight of grown larvae (g)
Total larval duration (h)
5th Instar larval duration (h)
Effective Rate of Rearing (ERR) by no.
ERR by wt. (kg)
Pupation rate (%)
Single cocoon weight (g)
Single shell weight (g)
Shell ratio (%)

Table 3: Descriptor for important morphological characters of mutant silkworm germplasm

Egg	Cocoon	Larva
Egg colour	Cocoon colour	Larval markings
Egg Shape	Cocoon shape	Body colour of 5th instar larva
	Nature of constriction	Nature of integument

as replications. Multivariate analysis was conducted to analyze the variability in the parameters among different accession. WINDOWSTAT statistical package was used for ANOVA to compare the performance of the accessions in two different seasons. PAST3 statistical software package was used for Principal Component Analysis (PCA) to compare the mutant accessions with commercially important silkworm races and to generate cluster grouping.

RESULTS AND DISCUSSION

The evaluation of morphological characters of the mutant silkworm genetic resources in the present study was recorded in egg, larva and cocoon stages and compared with the catalogue data (Table 4). The present observations are on par with the catalogue data which is maintained at the Silkworm Germplasm Information System (SGIS). Maintenance of original characters of the silkworm genotypes over the generations is one of the prime objectives of the silkworm germplasm.

Quantitative trait analysis: The data of 23 mutant accessions recorded for 5 years was analyzed for 12 important characters showed significant variations among different mutant genotypes which were evident from the calculation of Coefficient Variation (CV%). The higher variability was recorded with single shell weight, minimum value recorded 0.097 g in BBE-0318 and maximum recorded in BBE-0392 (0.217 g). In contrast, lower variations recorded in total larval duration 533.85 h in BBE-0309 and 566.4 h in BBE-0391 (Table 5). The multiple trait evaluation indices assessment of all the accessions by taking into consideration of all the parameters recorded in Table 5. High variability was observed in the parameters such as fecundity (10.158), larval weight (16.687), survivability /ERR by weight (11.76), cocoon weight (12.47), shell weight (23.49) and SR% (13.17) recorded

Table 4: Morphological characters of mutant silkworm genetic resources

Acc. No.	Name of the race	Egg		Larva			Cocoon		
		Colour	Shape	Marking	Colour	Integument	Colour	Shape	Constriction
BBE-0306	TMS-12	White	Ellipsoidal	Marked	Mixed	Opaque	White	Elongated	Faint
BBE-0307	TMS-14	Creamish white	Ellipsoidal	Plain	Pale red	Opaque	Greenish white	Elongated	Faint
BBE-0308	TMS-32	White	Ellipsoidal	Plain	White	Opaque	White	Elongated	Faint
BBE-0309	TMS-33	White	Ellipsoidal	Marked	Dirty	Opaque	White	Elongated	Faint
BBE-0310	TMS-35	White	Ellipsoidal	Plain	Light yellow	Opaque	White	Elongated	Nil
BBE-0311	TMS-38	Light yellow	Oval	Marked	Brown	Opaque	Flesh	Elongated	Faint
BBE-0312	TMS-61	Brownish red	Ellipsoidal	Faint marked	Light yellow	Opaque	White	Elongated	Faint
BBE-0313	TMS-62	White	Oval	Marked	White	Opaque	Yellow	Elongated	Faint
BBE-0314	TMS-64	White	Ellipsoidal	Marked	Yellow	Translucent	White	Elongated	Nil
BBE-0315	TMS-65	White	Ellipsoidal	Marked	Brown yellow	Translucent	Chrome yellow	Elongated	Faint
BBE-0316	TMS-66	Creamish white	Ellipsoidal	Marked	White	Opaque	Yellow	Elongated	deep
BBE-0317	TMS-67	Creamish white	Ellipsoidal	Plain	Light yellow	Opaque	Flesh	Elongated	Nil
BBE-0318	TMS-75	White with red tint	Ellipsoidal	Plain	White	Semi-translucent	White	Elongated	Faint
BBE-0319	TMS-82	Creamish white	Ellipsoidal	Plain	White	Opaque	White	Elongated	Nil
BBE-0320	TMS-2	White	Ellipsoidal	Marked	White	Opaque	White	Elongated	Faint
BBE-0321	TMS-17	White	Ellipsoidal	Plain	Light yellow	Opaque	White	Elongated	Faint
BBE-0322	TMS-31	Creamish white	Ellipsoidal	Marked	Light yellow	Opaque	Chrome yellow	Elongated	Deep
BBE-0323	TMS-69	White	Ellipsoidal	Marked	Light yellow	Opaque	White	Elongated	Faint
BBE-0331	TMS-34	White	Ellipsoidal	Marked	White	Opaque	White	Elongated	Faint
BBE-0333	OD-Translu	White	Ellipsoidal	Marked	White	Translucent	White	Elongated	Faint
BBE-0390	TMS-04	White	Ellipsoidal	Marked	White	Opaque	White	Elongated	Faint
BBE-0391	TMS-13	White	Ellipsoidal	Marked	White	Opaque	White	Elongated	Faint
BBE-0392	TMS-18	White	Ellipsoidal	Marked	White	Opaque	White	Elongated	deep

Table 5: Performance of mutant silkworm accessions for 12 important quantitative characters in mean, range with CV (%)

	Fecundity	Hatching (%)	Larval weight (g)	Tld	Vld	Yld/10000 (No.)	Yld/10000 (Wt)	Pupa (%)	Cocoon wt.	Shell wt.	SR (%)	Coco/100 dfl
BBE-0306												
Average	402.150	94.379	26.462	542.850	124.550	9666.150	11.530	90.912	1.155	0.176	15.257	44.256
Min.-Max.	341-494	87-98.4	19.9-31.9	480-624	90-168	8767-9942	8.4-14.9	71.8-98	0.9-1.3	0.12-0.28	12.01-20.8	26.8-64
CV (%)	13.194	3.440	12.600	8.580	17.900	3.100	17.400	6.700	13.960	23.600	15.400	22.900
BBE-0307												
Average	351.250	92.622	23.459	549.900	131.400	9727.600	11.370	94.038	1.146	0.161	14.082	42.320
Min.-Max.	261-439	80.8-98	16.9-28.6	486-624	90-168	9300-9929	9-15.4	91.05-96.9	0.95-1.37	0.12-0.23	11.8-17.4	33.3-61.65
CV (%)	14.150	4.010	13.800	8.200	18.400	1.900	14.800	1.800	12.300	18.130	9.440	14.600
BBE-0308												
Average	315.925	93.018	21.001	540.350	122.150	9575.250	8.245	90.985	0.928	0.115	12.512	29.351
Min.-Max.	181-450	67.6-98.4	12.3-28.8	480-628	90-168	8233-9925	5.8-11.7	79.3-98.36	0.59-1.23	0.05-0.15	9.45-15.8	14.06-41.2
CV (%)	23.900	6.870	25.700	9.300	21.300	5.200	21.400	6.300	24.050	26.800	11.900	24.200
BBE-0309												
Average	353.350	93.916	21.686	533.850	115.550	9531.900	8.815	92.272	0.942	0.122	13.049	32.759
Min.-Max.	262-438	81.7-97.2	16.5-25.8	456-624	88-168	7967-9960	7-11.01	76.3-98.04	0.69-1.23	0.08-0.15	11.1-14.4	24-43.8
CV (%)	12.150	4.120	13.800	9.500	21.140	5.500	11.060	5.800	16.600	17.300	7.600	15.200
BBE-0310												
Average	267.900	88.706	20.416	553.150	130.550	9561.750	9.945	87.189	0.975	0.122	12.589	35.607
Min.-Max.	116-367	77.4-98	14.9-26.3	480-624	84-168	8867-9960	7.8-13	46.4-97.6	0.813-1.26	0.09-0.15	10.8-14.2	19.8-49.6
CV (%)	25.500	7.500	14.400	7.500	20.300	2.900	14.500	14.900	15.220	15.900	7.240	20.270
BBE-0311												
Average	304.850	90.396	23.254	539.150	121.150	9623.250	9.955	92.480	0.993	0.121	12.281	36.498
Min.-Max.	196-460	71.5-97	17.2-31.2	480-624	84-168	9233-9968	7.2-13.3	85.4-97.4	0.76-1.22	0.08-0.15	10.50-14.7	13.5-55.94
CV (%)	25.300	8.650	17.800	8.600	19.800	2.400	15.100	4.200	14.330	17.160	9.880	25.900
BBE-0312												
Average	354.450	90.496	17.001	537.200	120.650	9605.700	8.070	91.705	0.837	0.103	12.461	29.379
Min.-Max.	177-630	75-99	12.5-24.8	480-624	90-168	8767-9928	6-10.4	86-95.1	0.60-1.17	0.06-0.14	9.60-15.70	19.65-41.57
CV (%)	33.900	7.390	20.000	8.800	18.400	3.300	16.400	3.400	24.040	22.290	12.200	19.050
BBE-0313												
Average	318.000	87.566	21.701	545.050	130.200	9631.900	9.930	92.101	1.007	0.117	11.664	37.258
Min.-Max.	163-449	68.3-65.6	16.7-26.2	486-624	90-168	9000-9957	8.2-13.1	75-98.8	0.82-1.16	0.09-0.14	9.5-13.2	28.52-51.4
CV (%)	25.500	10.400	12.800	7.700	18.400	2.600	14.800	5.800	11.070	14.550	8.300	17.610

Table 5: Continue

	Fecundity	Hatching (%)	Larval weight (g)	Tld	Vld	Yld/10000 (No.)	Yld/10000 (Wt)	Pupa (%)	Cocoon wt.	Shell wt.	SR (%)	Coco/100 dfl
BBE-0314												
Average	353.364	93.990	20.238	550.136	133.227	9512.409	9.569	90.770	0.970	0.121	12.480	35.598
Min.-Max.	248-487	88.6-98	16.2-25.5	486-624	90-168	8267-9915	6.7-16.3	76.6-98	0.8-1.3	0.09-0.18	10.7-14.7	23.4-65.3
CV (%)	18.740	2.900	13.800	7.600	16.900	4.660	22.300	6.100	18.040	19.210	9.500	27.660
BBE-0315												
Average	347.650	95.025	21.210	545.000	129.400	9583.900	9.915	90.418	0.947	0.113	11.905	38.344
Min.-Max.	247-452	88.3-98	14.3-26.2	486-624	90-168	8200-9936	6.3-17.2	78.6-96.3	0.78-1.17	0.08-0.18	10.05-17.4	25.3-68.80
CV (%)	16.480	3.000	16.100	7.600	18.300	3.900	29.500	4.900	14.300	21.770	13.600	31.820
BBE-0316												
Average	291.650	88.249	18.548	552.200	132.100	9585.850	9.065	87.876	0.930	0.102	10.760	34.818
Min.-Max.	184-518	78.3-97	12.1-26.5	486-624	90-168	8933-9942	7.1-11	70.7-97.3	0.73-1.17	0.06-0.16	8.0-15.0	28.5-42.17
CV (%)	28.700	7.800	23.600	8.200	18.300	2.900	13.600	8.100	17.100	33.030	19.090	12.860
BBE-0317												
Average	308.350	91.253	19.188	536.800	120.200	9557.650	7.938	90.242	0.881	0.111	12.586	28.933
Min.-Max.	201-428	78.6-99	15.2-36.3	486-600	90-168	8133-9964	4.8-10.5	79.3-96.7	0.66-1.21	0.08-0.22	10.12-18.5	13.15-38.5
CV (%)	22.900	7.200	23.160	6.300	20.000	4.100	18.300	5.120	19.770	32.400	16.290	22.250
BBE-0318												
Average	296.650	92.094	16.653	538.600	124.400	9657.150	8.430	93.279	0.799	0.097	11.967	30.204
Min.-Max.	208-415	72.1-98	13.0-26.5	480-624	90-168	9233-9955	6.1-11	86.6-97.7	0.57-1.16	0.06-0.19	10.1-18.3	17.5-44.2
CV (%)	23.900	7.800	21.700	8.700	18.200	2.200	18.600	3.500	22.500	33.900	15.960	24.740
BBE-0319												
Average	381.900	94.934	26.119	535.950	119.400	9660.500	9.725	92.661	1.058	0.137	13.015	36.791
Min.-Max.	330-493	90.9-98	18.3-32.3	480-624	90-168	9000-9999	7.5-11.7	84.8-99.5	0.88-1.18	0.1-0.16	10.8-15.6	27.3-46.7
CV (%)	10.940	2.200	13.600	8.800	20.500	2.650	11.200	4.300	8.700	13.330	11.500	16.660
BBE-0320												
Average	343.600	95.201	26.347	535.750	119.200	9660.050	10.215	91.618	1.090	0.149	13.720	39.150
Min.-Max.	266-432	82.6-99	19.4-32.2	480-624	90-168	9133-9946	7.2-14.2	83.7-97	0.86-1.25	0.17-0.21	10.7-17.6	25.4-56.9
CV (%)	14.840	4.100	11.800	8.800	20.700	2.060	16.700	4.050	11.190	18.200	11.260	21.110
BBE-0321												
Average	312.850	88.678	21.296	536.100	119.300	9624.750	9.800	90.656	0.961	0.124	13.025	36.278
Min.-Max.	217-414	56.6-99	16.2-25.8	480-624	84-168	9300-9900	7.2-16.1	80.3-98	0.71-1.19	0.07-0.15	10.2-19.1	24.3-64.3
CV (%)	15.250	15.900	15.320	8.900	20.900	1.800	25.800	4.500	16.600	20.860	15.590	30.800
BBE-0322												
Average	315.150	88.492	19.201	547.600	130.750	9748.250	8.225	93.596	0.874	0.106	12.207	29.768
Min.-Max.	218-405	71.3-98	12.8-24.2	480-624	84-168	9300-9932	5.9-10.4	80.8-97	0.63-1.17	0.06-0.14	9.39-14.2	23.6-36.3
CV (%)	18.900	8.800	17.900	7.900	18.500	1.700	15.700	4.060	18.300	19.800	10.350	11.760
BBE-0323												
Average	367.950	91.863	23.549	541.000	125.400	9672.650	10.570	91.640	1.031	0.136	17.983	39.154
Min.-Max.	230-474	77.2-98	17.3-26.8	480-624	84-168	9200-9967	8.8-12.4	72.7-98	0.51-1.28	0.08-0.19	10.48-13.9	29.8-48
CV (%)	17.610	5.500	10.300	8.900	17.900	2.150	11.600	5.900	18.100	20.670	12.900	11.630
BBE-0331												
Average	276.200	87.113	20.165	545.750	129.550	9551.550	8.370	89.537	0.910	0.112	12.425	30.465
Min.-Max.	151-445	61.4-96	12.2-23.8	480-624	84-168	8533-9965	7-10.7	60-97	0.74-1.19	0.08-0.15	10.01-14.1	19.65-42.7
CV (%)	27.870	11.800	14.300	7.800	19.000	3.800	10.700	11.200	14.700	15.900	8.750	17.180
BBE-0333												
Average	363.150	94.671	24.559	543.500	122.100	9696.950	9.770	94.151	1.047	0.141	13.600	34.952
Min.-Max.	128-530	88.8-99	19.2-28.5	480-624	84-168	9400-9967	7.6-12.3	88.5-98	0.88-1.17	0.10-0.18	8.8-17.8	11.3-49.1
CV (%)	28.630	3.260	12.600	8.100	19.400	2.050	11.600	3.200	10.050	18.730	18.280	25.130
BBE-0390												
Average	348.667	96.348	24.795	536.000	144.000	8914.167	7.950	87.667	1.202	0.178	14.857	27.224
Min.-Max.	300-435	94-98	20.6-31.9	504-576	120-168	8367-9233	5.1-9.8	83.6-92	1.17-1.22	0.15-0.22	12.7-19.2	16.2-30.8
CV (%)	13.260	1.500	22.118	6.100	14.900	3.500	21.200	3.900	1.370	18.000	19.150	20.670
BBE-0391												
Average	356.900	92.924	28.915	566.400	147.600	9367.100	10.405	92.120	1.167	0.189	16.326	34.434
Min.-Max.	306-397	89-95	23-35	504-624	120-168	8933-9935	9.4-12.1	85-98	0.95-1.26	0.15-0.22	12.4-17.9	29.8-38.5
CV (%)	7.815	2.700	16.100	9.100	13.300	3.800	8.200	4.800	10.300	14.900	13.340	8.150
BBE-0392												
Average	341.300	94.148	31.995	542.400	143.900	9354.100	11.350	91.670	1.307	0.217	16.612	35.639
Min.-Max.	315-374	85.3-97.8	24.5-39.35	504-624	108-168	8633-9933	9.4-12.8	83-98	1.2-1.46	0.15-0.26	13.120	30.5-41
CV (%)	4.53	3.9	15.4	8.6	16.2	4.5	11.01	5.2	7.4	15.77	18.426	12.12

Tld: Total larval duration, Vld: 5th Instar larval duration, SR: Shell Ratio, dfl: Disease free layings, Min: Minimum, Max: Maximum

Table 6: Parameter-wise variability analysis in mean values of 23 mutant silkworm accessions

Parameters	Average	Minimum	Maximum	SD	SE	CV (%)
Fecundity	0333.618	0267.900	0402.150	033.889	07.225	10.158
Hatching (%)	0092.004	0087.113	0096.348	002.792	00.595	03.034
Larval Wt_10	0022.511	0016.653	0031.995	003.757	00.801	16.687
Larval duration total	0543.247	0533.850	0566.400	007.597	01.620	01.398
Larval duration 5th Instar	0127.684	0115.550	0147.600	008.566	01.826	06.709
Yield/ No.)	9568.284	8914.167	9748.250	172.017	36.674	01.798
Yld/10000(Wt)	0009.529	0007.938	0011.530	001.121	00.239	11.765
Pupa %	0091.286	0087.189	0094.151	001.890	00.403	02.071
Cocoon	0001.007	0000.799	0001.307	000.126	00.027	12.470
Shell	0000.133	0000.097	0000.217	000.031	00.007	23.490
SR %	0013.364	0010.760	0017.983	001.760	00.375	13.172
Cocoon/100df	0034.747	0027.224	0044.256	004.441	00.947	12.781

SR: Shell ratio, CV: Cumulative variance

Table 7: Top performing mutant silkworm accessions with cumulative evaluation index value (>50)

Acc. No.	Fec (No.)	Hat (%)	Wt_10 larvae	ERR (No.)	ERR (Wt)	Pupa (%)	Cocoon wt. (g)	Shell wt. (g)	SR (%)	Cocoon yield/100 dfl	CEI	No. of qualified traits	Rank
BBE-0306	70.22	58.51	60.52	55.69	67.85	48.02	61.79	63.57	60.75	71.41	61.83	9.00	I
BBE-0392	52.27	57.68	75.25	37.55	66.25	52.03	73.94	76.63	68.46	52.01	61.21	9.00	II
BBE-0307	55.20	52.22	52.52	59.26	66.43	64.56	61.07	58.69	54.08	67.05	59.11	10.00	III
BBE-0391	56.87	53.30	67.05	38.30	57.82	54.41	62.79	67.80	66.83	49.30	57.45	8.00	IV
BBE-0323	60.13	49.49	52.76	56.07	59.29	51.87	51.89	50.83	76.24	59.92	56.85	9.00	V
BBE-0320	52.95	61.45	60.21	55.33	56.12	51.76	56.63	55.03	52.02	59.91	56.14	10.00	VI
BBE-0319	64.25	60.50	59.60	55.36	51.75	57.27	54.08	51.01	48.02	54.60	55.64	9.00	VII
BBE-0333	58.71	59.55	55.45	57.48	52.15	65.15	53.18	52.24	51.35	50.46	55.57	10.00	VIII

SR: Shell ratio

significant variations among the mutant silkworm genotypes conserved in the germplasm (Table 6). Multiple trait evaluation method is being utilized for testing large number of silkworm germplasm and based on the performance for important economic characters and promising genotypes are selected¹⁴⁻¹⁸. In the present study also the 23 mutant accessions were evaluated based on the multiple trait evaluation to understand the better performing accessions. More than 21 traits contribute to silk yield and there exists an inter-relationship between multiple traits in silkworm¹⁹. The precision of selection of breeds among many numbers of breeds can achieved through the evaluation index method that gives priority to all yield component traits²⁰. Based on the performance of the silkworm genotypes, individual indices were calculated for each of the 10 parameters. Since in larval duration the desirability is lower values, hence this character was not considered for calculation of Evaluation Index (EI). The EI values were calculated for each of the genotype in all the 10 parameters and ranking was assigned based on the qualifying average EI-value>50. It was found that eight accessions such as BBE-0306 (EI = 61.83), BBE-0392, 0307, 0391, 0323, 0320, 0319 and 0333 (EI = 55.57) qualified the EI value >50 with 9-10 qualifying parameters in each accessions. Remaining accessions were also performed better but not qualified the bench mark of EI>50. In the recent past evaluation index method developed by Mano *et al.*²¹ has been utilized for short listing better performing silkworm

genotypes/hybrids for commercial exploitation^{18,22} and the same has been utilized in the present study as well for evaluating 23 mutant silkworm *Bombyx mori* L. genotypes in respect of different traits viz., fecundity, hatching, larval weight, ERR by no. and ERR by Wt., Pupation, cocoon weight, shell weight, shell ratio, cocoon yield by number and by weight. The ranking was assigned to those genotypes which qualifies with Cumulative EI>50 (Table 7). Similar works also reported earlier wherein the identification of top performing silkworm breeds in different seasons was achieved²³. Evaluation index is one such method that increases the precision of selection of breed among an array of breeds by a common index giving due to weight-age to all the yield component traits²⁰.

Since, the rearing of mutant silkworms were conducted in two different seasons. The comparative analysis of the performance of these silkworms was made by ANOVA, which revealed significant difference between the seasons, between the accessions and between the accessions and the seasons. Highly significant values were recorded in the important commercial characters of the silkworm such as fecundity ($p<0.01$), survivability ($p<0.01$), cocoon weight ($p<0.01$), shell weight ($p<0.05$) and total cocoon yield ($p<0.01$). However, there was no significant difference in values were also recorded with larval duration, larval hatching and in shell ratio during the two seasons. However, the analysis of data indicates that all the genotypes utilized in the study vary

Table 8: Mean performance of silkworm mutant accessions during winter season (December-January)

Acc. No.	Fec (No.)	Hat (%)	Wt_10		Tld (h)	Vld (h)	Yld/10000	Yld/10000	Pupa (%)	Cocoon wt. (g)	Shell wt. (g)	SR (%)	Coco/100 dfl
			larvae	larvae			(No.)	(Wt)					
BBE-0306	406.2	94.64	25.922	575	130	9698	10.91	91.65	1.080	0.168	15.519	43.73	
BBE-0307	357.0	93.34	23.834	584	139	9693	11.11	93.51	1.113	0.160	14.358	41.24	
BBE-0308	300.9	91.13	19.483	577	132	9767	7.78	93.47	0.863	0.114	13.300	26.77	
BBE-0309	354.5	93.50	20.272	570	124	9734	8.57	94.95	0.905	0.120	13.337	32.15	
BBE-0310	262.6	88.54	19.666	581	136	9544	9.49	90.05	0.963	0.124	12.927	34.69	
BBE-0311	306.7	92.17	22.012	573	128	9586	9.34	92.31	0.939	0.121	12.918	35.79	
BBE-0312	402.6	90.74	16.771	573	128	9660	7.41	92.16	0.764	0.098	12.946	27.89	
BBE-0313	323.2	87.10	21.133	577	135	9649	9.73	91.50	0.955	0.113	11.976	37.19	
BBE-0314	350.9	93.41	19.830	580	137	9619	8.90	92.46	0.963	0.129	13.224	33.03	
BBE-0315	347.9	94.63	20.072	576	136	9649	8.45	91.03	0.903	0.111	12.233	32.58	
BBE-0316	329.5	89.78	17.910	586	134	9608	8.71	87.58	0.890	0.093	10.212	34.57	
BBE-0317	338.5	91.62	17.678	562	122	9643	7.83	90.99	0.853	0.119	13.747	28.37	
BBE-0318	300.9	93.28	16.916	572	128	9656	7.28	93.60	0.783	0.094	12.051	25.91	
BBE-0319	385.1	95.45	25.554	571	126	9684	9.32	92.39	1.074	0.141	13.291	35.62	
BBE-0320	368.7	96.37	24.931	571	126	9707	9.50	91.80	1.049	0.144	13.749	37.59	
BBE-0321	304.8	89.95	20.665	572	127	9627	8.35	89.59	0.958	0.132	14.087	30.43	
BBE-0322	311.8	87.56	19.370	582	137	9794	8.15	94.15	0.843	0.108	12.879	29.35	
BBE-0323	407.9	92.59	22.685	578	134	9734	10.36	92.76	0.999	0.131	13.228	39.90	
BBE-0331	295.7	91.10	19.517	577	136	9645	8.15	93.35	0.900	0.116	12.942	29.88	
BBE-0333	408.2	94.56	24.019	572	132	9718	9.51	94.69	1.035	0.145	14.236	35.96	
BBE-0390	396.5	95.20	31.846	576	144	8863	7.60	86.50	1.215	0.158	12.960	30.70	
BBE-0391	367	92.81	30.325	588	156	9605	10.00	93.84	1.089	0.193	17.726	33.94	
BBE-0392	351.7	92.92	32.600	588	156	9791	10.75	95.40	1.263	0.220	17.393	35.01	
Mean	346.9	92.28	22.305	577	134	9638	9.01	92.16	0.974	0.133	13.532	33.58	
Range	262.6-408.2	87.10-96.37	16.771-32.600	562-588	122-156	8862-9794	7.28-11.11	86.50-95.40	0.764-1.263	0.093-0.220	10.212-17.726	25.92-43.73	
SD	41.891	2.501	4.515	6.363	8.763	180.772	1.134	2.201	0.127	0.031	1.622	4.612	
CV	12.076	2.710	20.240	1.104	6.542	1.876	12.585	2.388	13.036	23.385	11.985	13.734	

SR: Shell ratio

Table 9: Mean performance of silkworm mutant accessions during summer season (June-July)

Acc. No.	Fec (No.)	Hat (%)	Wt_10		Tld (h)	Vld (h)	Yld/10000	Yld/10000	Pupa (%)	Cocoon wt (g)	Shell wt (g)	SR (%)	Cocoon/100 dfl
			larvae	larvae			(No.)	(Wt)					
BBE-0306	398.1	94.12	27.00	510	119	9635	12.15	90.18	1.230	0.184	14.994	44.779	
BBE-0307	345.5	91.91	23.08	516	124	9762	11.63	94.57	1.178	0.161	13.806	43.395	
BBE-0308	330.9	94.91	22.52	504	112	9384	8.71	88.50	0.993	0.117	11.724	31.935	
BBE-0309	352.2	94.33	23.10	498	108	9330	9.06	89.59	0.978	0.123	12.760	33.372	
BBE-0310	273.2	88.87	21.17	526	125	9579	10.40	84.32	0.986	0.120	12.251	36.529	
BBE-0311	303.0	88.62	24.50	505	114	9661	10.57	92.65	1.047	0.122	11.644	37.207	
BBE-0312	306.3	90.25	17.23	501	113	9551	8.73	91.25	0.910	0.109	11.975	30.869	
BBE-0313	312.8	88.03	22.27	513	126	9615	10.13	92.70	1.060	0.120	11.353	37.327	
BBE-0314	356.3	94.68	20.73	515	129	9385	10.37	88.74	0.979	0.112	11.587	38.677	
BBE-0315	347.4	95.42	22.35	514	123	9519	11.38	89.80	0.991	0.115	11.576	44.106	
BBE-0316	253.8	86.72	19.19	518	131	9564	9.42	88.17	0.971	0.111	11.308	35.066	
BBE-0317	278.2	90.89	20.70	512	119	9472	8.05	89.50	0.909	0.103	11.426	29.492	
BBE-0318	292.4	90.90	16.39	505	121	9658	9.58	92.96	0.815	0.099	11.884	34.494	
BBE-0319	378.7	94.42	26.68	501	113	9637	10.13	92.93	1.042	0.133	12.739	37.962	
BBE-0320	318.5	94.04	27.76	501	113	9613	10.93	91.44	1.131	0.155	13.691	40.709	
BBE-0321	320.9	87.41	21.93	500	111	9623	11.25	91.72	0.964	0.116	11.962	42.128	
BBE-0322	318.5	89.42	19.03	514	125	9703	8.299	93.04	0.905	0.104	11.534	30.189	
BBE-0323	328.0	91.13	24.41	504	117	9612	10.78	90.52	1.062	0.141	22.737	38.409	
BBE-0331	256.7	83.12	20.81	514	123	9458	8.59	85.73	0.920	0.109	11.908	31.054	
BBE-0333	318.1	94.78	25.10	515	112	9676	10.03	93.61	1.059	0.136	12.965	33.938	
BBE-0390	324.8	96.92	21.27	516	144	8940	8.125	88.25	1.196	0.189	15.805	25.486	
BBE-0391	350.2	93.00	27.97	552	142	9209	10.675	90.97	1.220	0.187	15.392	34.761	
BBE-0392	334.3	94.97	31.59	512	136	9063	11.75	89.18	1.337	0.215	16.092	36.060	
Mean	321.68	91.69	22.90	512	122	9506	10.03	90.45	1.038	0.134	13.179	35.997	
Range	253.80-398.10	83.12-96.92	16.39-31.59	498-552	107-144	8940-9761	8.05-12.15	84.32-94.57	0.815-1.337	0.099-0.215	11.308-22.737	25.486-44.779	
SD	35.791	3.467	3.634	11.281	9.790	206.917	1.229	2.521	0.127	0.032	2.570	4.988	
CV	11.126	3.781	15.865	2.205	8.045	2.177	12.253	2.787	12.193	24.198	19.499	13.856	

SR: Shell ratio

significantly with respect to most of the parameters studied during winter and summer seasons ($p < 0.001$, Confidence Distribution (CD) value 40.73). Higher mean values were recorded for all the parameters during winter season (Table 8) compared to the multi-trait values recorded in the summer season (Table 9). This shows the favorable

season for better expression of quantitative traits in mutant silkworm accessions. Since, these mutant silkworms are temperate in origin, the silkworm rearing performance always better due to congenial and less fluctuations in the environmental temperature during winter season (Table 10)²⁴.

Table 10: ANOVA of seasonal variation in the performance of mutant silkworm accessions for different parameters

Acc. No.	Fec	Hat (%)	Wt_10 larvae	Tld	Vld	Yld/10000 (No.)
Season	96208.16***	67.34 ^{NS}	73.77*	458074.46***	17868.89***	1817433.46***
Accession	14510.09***	86.99***	235.19***	553.29 ^{NS}	536.06 ^{NS}	598906.67***
Season x Accn	12239.27***	52.35 ^{NS}	35.85***	663.11 ^{NS}	203.07 ^{NS}	252284.11***
Error	4293.44	37.70	13.58	494.99	386.82	86795.71
CD (S)	12.0111	1.125	0.675	4.078	3.605	54.007
CD (A)	40.733	3.817	2.291	13.831	12.227	183.147
CD (S X A)	57.606	5.398	3.239	19.559	17.291	259.009
Acc.No.	Yld/10000(Wt)	Pupa (%)	Cocoon wt	Shell wt	SR (%)	Cocoon/100df
Season	153.63***	166.03*	0.73***	0.004*	2.678 ^{NS}	669.48***
Accession	16.52***	37.76 ^{NS}	0.33***	0.018***	51.28**	242.94***
Season x Accn	6.60***	71.78***	0.04*	0.002***	27.96 ^{NS}	115.969***
Error	2.47	28.49	0.02	0.001	25.05	48.05
CD (S)	0.288	0.978	0.027	0.005	0.917	1.271
CD (A)	0.976	3.318	0.091	0.017	3.111	4.309
CD (S X A)	1.381	4.692	0.129	0.024	4.400	6.094

Significance level: *p<0.05, **p<0.01, ***p<0.001, CD: Confidence distribution

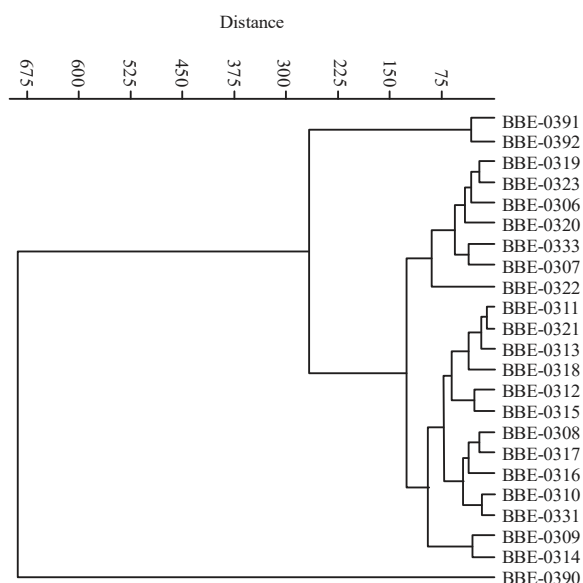


Fig. 1: Cluster analysis based on all 12 parameters for 23 silkworm mutant accessions according to grouping from multivariate PAST

A detailed analysis was undertaken to test the efficacy of hierarchical agglomerative clustering (UPGMA method) in grouping the different mutant silkworm accessions based on quantitative trait analysis (Fig. 1). The results indicate that all the mutant accessions were grouped together in two major groups. Further, sub-grouping under these 2 groups highlights genetically different association with the differentiation of various groups. The cluster analysis provides scope for adopting a re-combinational breeding program using distant cluster members. Thus, the sub-grouping of high yielding bivoltine strains offers an opportunity to exploit the genetic differences between high yielding strains^{25,26}. The

clustering also indicates the possibility for recombining low and high-yielding members from genetically distant clusters. The results presented here establish its usefulness in realizing a better projection of the genetic difference between silkworm strains of different yield potentials²⁷. Recently the cluster analysis of 369 bivoltine silkworm accessions was done using Ward's minimum variance cluster analysis method for 11 economic traits revealed the diversity among the bivoltine genotypes that were grouped into 20 clusters based on the degree of variations²⁸. The maintenance of heterogeneity in the germplasm is important and is necessary for better maintenance without any inbreeding depression²⁹. The inclusion of genotypes of the same origin in different clusters clearly indicates the presence of considerable genetic diversity among the population used in this study.

The PCA was conducted for multivariate traits and analyzed for correlation of the performance of mutant silkworm accessions along with the commercially exploited silkworm races such as Bivoltine CSR-2 (BBI-0290) and Multivoltine Pure Mysore (PM) (BMI-0001) indicated that the performance of mutant accessions viz., BBE-0306, 0307, 0319, 0320, 0323 and 0333 are positively correlated with the performance of BBI-290 (CSR-2) and BMI-0001 (PM) and grouped under one group (Fig. 2). Whereas, accessions such as BBE-0308, 0310, 0314, 0316 and BBE-0317 are negatively correlated compared to the BBI-0290 and BMI-0001 and grouped separately. The grouping of silkworm genotypes indicates the higher similarity with respect to multiple traits³⁰. Similarly, 6 mutant accessions showed similarity with CSR-2 and PM silkworm races for some of the commercially important characters. This indicates the possibilities of using mutant silkworm genetic resources for commercial exploitation through crop improvement programme.

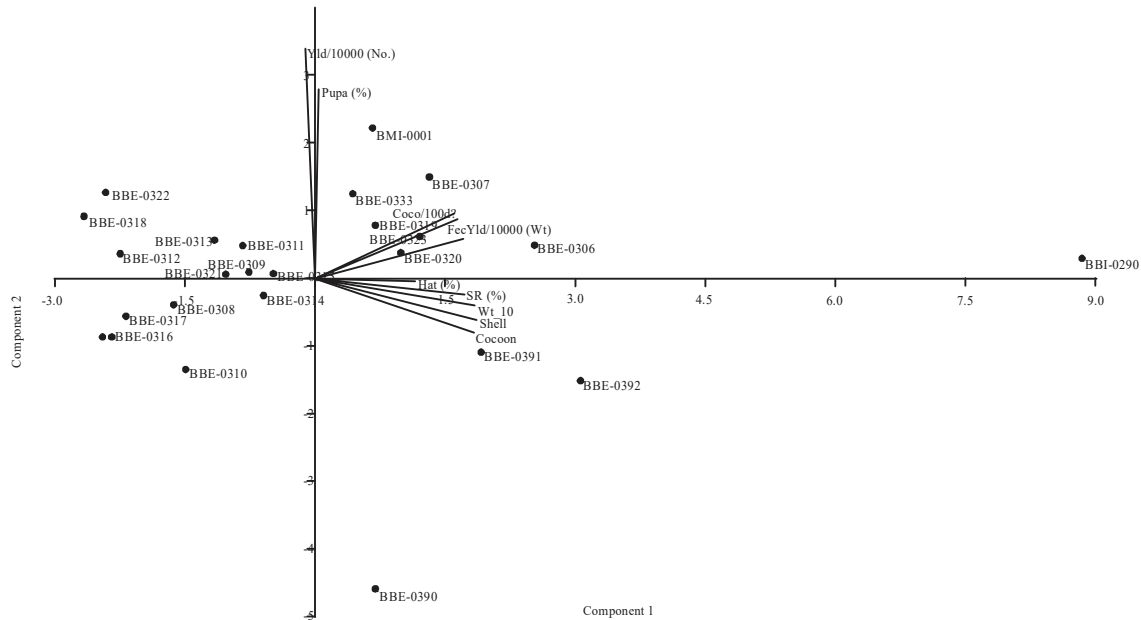


Fig. 2: Comparative (PCA) analysis of 23 mutant silkworm accessions with commercially important silkworm races

CONCLUSION

The extent of variability among different genotypes is essential and is basis for the germplasm collection for their effective utilization in the future. The concerted efforts are utmost necessary for the characterization, evaluation and identification of trait specific genotypes using standard and reliable methods. The present study is an attempt to showcase the potentialities of the mutant silkworm accessions for their utilization in the commercial exploitation. Since these genotypes were considered most suitable for basic genetic studies rather its usefulness in silk production. The correlation studies using PCA with commercially important silkworm races PM and CSR-2 revealed that some of the mutant silkworms conserved in the germplasm can be used to explore the combining ability studies for further commercial exploitation.

SIGNIFICANCE STATEMENT

This study emphasizes the importance of the mutant silkworm germplasm and its proper maintenance for their sustainable utilization in the future. This study showcases the genetic potentialities of the mutant silkworm genetic resources which can be utilized by the silkworm breeders to develop trait specific breeds. Also, this study give a light on the efficient management of silkworm germplasm and also their evaluation to understand the genetic potentialities of silkworm genetic resources to exploit for commercial silk production through appropriate silkworm breeding strategies.

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