



## Research Article

# EVALUATION OF BIVOLTINE SILKWORM GENETIC RESOURCES THROUGH CLUSTER ANALYSIS AND IDENTIFICATION OF BETTER PERFORMING BIVOLTINE ACCESSIONS

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**Abstract:** The evaluation of germplasm is essential for its effective utilization in the silkworm crop improvement and sustainable maintenance of silkworm genetic resources. In the present study, bivoltine silkworm genetic resources comprising of 369 bivoltine silkworm genotypes (209 indigenous and 160 exotic) were evaluated on 11 important quantitative & qualitative parameters. Based on the performance, silkworm genotypes have been grouped into 20 clusters by Ward's minimum variance cluster analysis. High variability was observed in the different genotypes for different parameters studied ranging 3.83 to 14.64% showing highly heterozygous in nature. Further the accessions are assigned the ranking based on the evaluation index of the performance in particular trait.

**Keywords:** *Silkworm germplasm resources, Bivoltine, Conservation, Cluster analysis*

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## Introduction

Conservation of biodiversity and germplasm maintenance is indispensable acts for its sustainable utilization in the future and as resource for breeding, which can be exploited for crop improvement. The serigenetic resources provide a wider genetic variation among the population. The silkworm biodiversity comprises mutants, sex limited races, wild silkworm breeds, evolved races, breeders stock and geographical races etc. forms silkworm gene pool. The main aim of serigermplasm collection and conservation is for utilization to the benefit of the mankind [1]. Evaluation of germplasm is an essential pre-requisite for its effective utilization. As the goals of breeding change rapidly, evaluation needs to be adaptive [2]. Silkworms have been evaluated in many environment and agroclimatic conditions in order to identify the season and region specific breeds, as potential parents for breeding and breeds with specialized traits such as thermo-tolerance, disease or pest tolerance and so on [3]. In India, the Central Sericultural Germplasm Resources Centre (CSGRC) recognized as National Germplasm Active site for silkworms by NBAIR, Bengaluru, conserves 369 bivoltine silkworm Germplasm besides 83 Multivoltine and 23 Mutant silkworm genotypes. The silkworm germplasm is evaluated with important quantitative and qualitative parameters during silkworm rearing on regular basis. This paper depicts the analysis of evaluation data of the 369 bivoltine silkworm accessions conserved at this centre.

## Materials and Methods

### Silkworm breeds and rearing

In the present study, 369 bivoltine silkworm genotypes (209 indigenous and 160 exotic) were evaluated for rearing and grainage parameters. The accessions were reared as per the standard rearing techniques and methodologies [4,5] in three batches viz., June-July, September - October and January-February. Composite disease free layings consisting of about 1000 – 1500 individual eggs were used in each accession for rearing. The important quantitative traits targeted were fecundity, weight of 10 matured larvae (g), total larval duration and V<sup>th</sup> instar larval duration (hrs), cocoon yield/10,000 larvae (No.) and cocoon yield/10,000 larvae (Wt.), single cocoon weight (g), single shell weight (g), shell ratio (%) and

pupation rate (%). The data on the aforesaid parameters were recorded and the mean data obtained for each quantitative trait was analysed for variation among the populations. The top performing accessions were identified through Multiple trait evaluation index method [6] and Sub-ordinate function methods [7]. Based on the average Evaluation Index value in each trait, ranks were assigned to the accessions and the top ranked five accessions in both evaluation methods were considered. All the 369 silkworm germplasm accessions were considered for analysis using Ward's minimum variance cluster analysis method and were grouped based on the degree of variations.

## Results

The analysis of data on performance of 369 bivoltine silkworm accessions for 11 important quantitative parameters during rearing revealed that, based on the CV values higher variations were observed among different accessions for fecundity (14.64%) followed by weight of mature larvae (13.83%). The ERR by number (4.56), ERR by weight (9.32), single cocoon wt.(8.52) and shell ratio (6.07) expressed moderate variation among the accessions. The range of variations among the 369 silkworm germplasm for the quantitative traits is presented in [Table-1]. Multiple trait analysis based on the performance of the bivoltine silkworm accessions for each parameter could rank trait-wise top 10 best performing accessions for each of the 11 economic parameters and is presented in [Table-2].

Table-1 Variability statistics of 369 Bivoltine silkworm genetic resources

Traits	Mean	Min	Max	SD	CV%
Fecundity (Nos.)	396	235	591	57.91	14.64
Hatching (%)	93.93	75.22	98.65	3.59	3.82
Wt. of grown larvae(g)	35.89	18.454	46.3	4.96	13.83
Total larval duration(h)	576	504	624	38.93	6.75
V <sup>th</sup> age larval duration(h)	153	96	168	19.04	12.48
ERR. by no.	9399	7200	9917	428.41	4.56
ERR.by wt.(kg)	12.93	7.75	15.7	1.21	9.32
Pupation rate(%)	90.02	60.16	98	5.70	6.33
Single cocoon weight(g)	1.461	1.006	1.766	0.12	8.52
Single shell weight(g)	0.264	0.123	0.342	0.03	11.87
Shell ratio	18.12	12.25	21.07	1.10	6.07

Table-2 Trait-wise top 10 best performing accessions

Traits	Range	Top ten accessions
Fecundity(No.)	591-500	BBE-0030, BBE-0031, BBI-0048, BBE-0019, BBI-0279, BBI-0335, BBI-0325, BBI-0063, BBI-0047, BBI-0085
Hatching %	98.65-98.02	BBI-0387, BBI-0389, BBI-0114, BBE-0181, BBE-0191, BBI-0369, BBI-0361, BBE-0260, BBI-0283, BBE-0193
Wt. of grown larvae(g)	46.3-43.6	BBE-0039, BBI-0285, BBE-0003, BBI-0328, BBE-0024, BBE-0013, BBE-0040, BBE-0035, BBE-0280, BBI-0284
Total larval duration(h)	504-504	BBE-0018, BBI-0044, BBI-0045, BBE-0031, BBE-0051, BBE-0025, BBE-0024, BBE-0023, BBE-0022, BBE-0019
V <sup>th</sup> age larval duration(h)	96-96	BBE-0017, BBE-0006, BBE-0007, BBE-0008, BBE-0009, BBE-0010, BBE-0011, BBE-0012, BBE-0013, BBE-0014
ERR. by no.	9916-9866	BBI-0092, BBI-0325, BBI-0110, BBI-0295, BBE-0177, BBI-0324, BBI-0140, BBI-0354, BBI-0330, BBI-0084
ERR. by wt.(kg)	15.7-15.4	BBI-0291, BBI-0282, BBE-0014, BBI-0328, BBE-0199, BBI-0285, BBE-0002, BBE-0003, BBI-0287, BBI-0293
Pupation rate (%)	98-97	BBE-0180, BBE-0177, BBI-0330, BBI-0243, BBI-0344, BBI-0285, BBI-0346, BBI-0354, BBI-0336, BBI-0325
Single cocoon weight(g)	1.77-1.67	BBE-0039, BBI-0044, BBE-0014, BBE-0038, BBE-0034, BBI-0377, BBI-0291, BBE-0023, BBE-0003, BBI-0133
Single shell weight(g)	0.34-0.33	BBE-0014, BBI-0044, BBE-0007, BBI-0290, BBI-0328, BBE-0038, BBE-0039, BBE-0034, BBI-0324, BBI-0291
Shell ratio (%)	21.07-20.16	BBE-0186, BBI-0358, BBI-0326, BBI-0081, BBI-0325, BBE-0188, BBI-0290, BBE-0007, BBI-0278, BBI-0055

Table-3 Cluster groups showing Bivoltine genotypes

Cluster	No. of genotypes	Accessions numbers
I	13	1 49 50 4 57 338 53 55 7 44 39 34 38
II	11	32 145 369 40 43 33 24 45 51 46 35
III	13	17 106 52 193 204 19 202 42 136 209 116 126 29
IV	7	31 48 41 47 36 37 23
V	30	5 8 64 71 22 271 267 88 160 155 105 87 112 90 91 86 357 9 16 54 266 355 358 356 344 12 365 143 360 13
VI	25	56 342 6 192 327 329 354 339 144 300 125 336 347 102 302 328 318 345 346 364 148 361 362
VII	22	124 134 110 205 350 127 195 319 128 265 138 317 130 137 191 132 322 169 225 194 172 189
VIII	30	10 251 15 175 206 212 199 200 211 98 104 122 133 190 209 203 222 231 241 248 263 201 219 188 119 11 25 26 114 20
IX	36	58 303 113 352 224 111 264 165 220 275 223 276 228 258 259 121 246 213 287 252 182 216 274 210 237 99 269 96 107 118 261 295 115 218 183 184
X	27	89 296 103 157 101 100 320 142 270 154 95 316 331 272 166 273 268 108 146 349 109 294 311 291 298 304 340
XI	12	214 226 129 221 229 167 312 324 321 325 326 315
XII	15	97 351 299 233 234 117 238 243 69 21 242 260 297 150 179
XIII	45	27 215 68 323 61 62 280 334 73 314 168 60 123 63 67 120 30 28 363 75 76 94 313 83 131 301 366 343 353 147 149 162 151 158 171 152 341 159 77 72 78 74 161 359 337
XIV	30	65 308 66 279 80 330 185 307 135 153 196 197 173 186 176 277 306 285 333 82 84 18 85 310 278 174 178 262 181 198
XV	16	70 163 164 240 156 348 59 332 282 293 170 335 177 180 187 81
XVI	13	79 283 3 281 286 2 284 289 309 290 292 305 14
XVII	4	93 141 139 140
XVIII	7	227 247 217 92 236 249 208
XIX	12	230 256 244 245 239 232 255 257 253 254 250 235
XX	1	288

Cluster analysis based on 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 variations [8,9]. Among the 20 clusters, Cluster XIII revealed maximum number of genotypes (45) followed by cluster IX (36) and clusters V, VIII, XIV (30), while, those having less than 30 genotypes stood as separate cluster [Table-3].

**Discussion**

The silkworm *Bombyx mori* L has many breeds based on geographical areas (rearing area) and breeding lines, which differ from each other in many quantitative traits [10]. The most important economic traits linked with silk production include the weight of one cocoon (g), the weight of one shell (g), the per cent of shell, cocoon efficiency (g) for every 10,000 larvae, fertility (per cent), the cost of pupa establishment (percent), the length of larvae period (hour), the average length of fibre (m), unbroken fibre length (m), denier (g), spinning capability (per cent) and raw silk % [11]. Evaluation of silkworm genetic resources is the most important aspect of germplasm management, which is pre-requisite for their further use in silkworm crop/ race improvement [12]. The germplasm stocks can be utilized directly as a local breeds or as a parent material, whereas the international need focuses towards germplasm systems that emphasize the use and employment of materials rather than mere acquisition and storage [13]. Multiple trait evaluation method is being utilized for testing large number of silkworm germplasm and based on the performance for important economic

characters, promising genotypes are selected [14,15,16,17,18]. 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 traits [19]. More than 21 traits contribute to silk yield [20] 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 impartially. To judge the superiority of the silkworm breeds, a common index method is required. This is also an established fact as observed by that superiority of one or a couple of characters may not reflect the overall merit of the breed. Since the comprehensive merit of the breed over a range of traits depends on relative superiority of many individual traits, selection needs to be based on multiple traits contributing to overall silk output [21,22]. In general, the silkworm ingests same amount of mulberry leaves, it shows significant difference in its ability to digest, absorb and convert the leaves to silk [23,24]. Fecundity is determined based on the genotype of maternal parent and environmental conditions prevailing at the time of oviposition and it is one of the fitness components reflecting on the productivity. Reports reveal that, genotypes also differ from one another in the total number of eggs laid by a single female moth. The trait, yield per 10,000 larvae by number which showed variations among the bivoltines silkworm genotypes can be attributed to the genetic characters, influence of environmental factors and interaction of alleles. As the cocoon yield is related to pupation rate and cocoon weight, increase could be achieved by emphasizing on these traits.

Pupation rate is an important parameter which reflects the viability of the breed and the fluctuations observed among various genotypes could be partially attributed to the interaction of alleles responsible for expression of the trait [25]. The cocoon weight is considered to reflect the vigour of the silkworm breed and similarly the cocoon shell weight showed wider variations in different silkworm accessions. Of the 20 distinct clusters observed through cluster analysis of the 369 bivoltine genotypes based on the Ward's minimum variance revealed that, Cluster XIII consisted of maximum number of genotypes which infers the low degree of variance for maximum genotypes[26].

### Conclusion

The present study has helped in understanding the variability that existed among the 369 bivoltine silkworm genotypes on evaluation of important quantitative traits which is useful information. Further, identification of better performing genotypes by multiple trait analysis of vast silkworm germplasm, can be recommended for further exploitation in the silkworm breeding programmes. The cluster analysis to study the degree of variance and grouping of genotypes based on genetic relatedness will reassure for selection of parents. Since the variability is the basis for selection of parents in the crop improvement and is desirable due to increased gene frequency and is essential to maintain minimal degree of heterozygosity in the population and is a requisite for better conservation of silkworm germplasm.

**Application of research:** The research help to utilize the better performing silkworm accessions to evolve the high yielding as well as disease resistant hybrids.

**Research Category:** Bivoltine Germplasm

**Abbreviations:** ERR by no.: Effective rate of rearing/ number,  
ERR by wt.: Effective rate of rearing by weight (kg)

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**Study area / Sample Collection:** Central Sericultural Germplasm Resources Centre, Hosur, Pincode-635109, Krishnagiri dt. Tamil Nadu.

**Cultivar / Variety / Breed name:** Bivoltine germplasm

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.  
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