

## Evolution of a Productive Bivoltine Hybrid of Silkworm *Bombyx mori* L. (SD7 × SD12) with Shorter Larval Duration

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With an objective of evolving quantitatively and qualitatively superior bivoltine silkworm hybrid of *Bombyx mori* L. for tropics with shorter larval duration without compromising on productivity traits, a breeding programme was initiated at this institute during 1997 by utilizing breeding resource material from the institutes Germplasm collection. The breed SD7 is characterized with plain larvae spinning white oval cocoons and SD12 is characterized by sex-limited larval markings spinning white dumb-bell cocoons. After fixation, these breeds along with other newly evolved breeds were subjected for hybrid evaluation in the laboratory. Based on the hybrid studies, the hybrid SD7 × SD12 was selected and evaluated for one year comprising three major seasons (6 trials) of tropics viz., Pre-monsoon (characterized by high temperature, low humidity and without any rain fall), Monsoon (characterized by moderate temperature, heavy rain fall with high humidity) and post-monsoon season (characterized by low temperature and low humidity with frequent rain fall) to know the fluctuations in the larval duration. The evaluation studies indicated that the hybrid is having shorter larval duration with productive merits. The hybrid SD7 × SD12 recorded survival percent of 92.74, shell weight of 46.7 cg, shell ratio % of 24.0 and filament length of 1,200 meters with a shorter larval duration of 24 hrs; when compared to productive hybrid CSR2 × CSR5 (control). The breeding methodologies and hybrids usefulness for tropics were discussed.

**Key words:** *Bombyx mori* L., Shorter larval duration, Productivity merits

### Introduction

Indian sericulture which is mostly polyvoltine × bivoltine hybrid oriented (Datta, 1984) has achieved proficiency in increasing the production of quality raw silk to meet the challenges of International market demands by the evolution of productive bivoltine hybrids meant for favourable seasons (Datta *et al.*, 2000). Even though, India occupies second position in the global production of raw silk, the unit production and quality of raw silk produced are very low when compared to China and Japan. The entire commercial rearings of Southern part of India (Andhra Pradesh, Tamil Nadu and Karnataka States) is exclusively polyvoltine × bivoltine hybrid oriented except in the restricted areas where bivoltine hybrid rearing is practiced. But with the introduction of Bivoltine Sericulture Technology Development (BSTD) project under Japan International Collaboration Agency (JICA), few good number of productive bivoltine silkworm hybrids viz., CSR2 × CSR4, CSR2 × CSR5, CSR3 × CSR6, CSR12 × CSR6 and CSR16 × CSR17 were evolved (Datta *et al.*, 2000 a, b, 2001). All these hybrids were authorised by Government of India for commercial production. The first two hybrids were tested in large scale and produced international grade raw silk for the first time in India. The average yield recorded for 1.49 million Disease free laying is around 70 kg/100 Dfls (Nataraju *et al.*, 2002).

The problem of tropical sericulture is aggravated silkworm diseases particularly during final instars resulting in frequent crop losses. The available bivoltine breeds though perform better during early instars, found to be very susceptible to diseases during final instars. These high yielding hybrids showed consistently good performance in the laboratory and field conditions under high input management practices with good hygienic rearing. The feeding of required quantity of qualitatively superior mulberry feed also constraint at farmers level. Keeping

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this in view, a breeding programme was initiated at this institute during 1997 to evolve bivoltine silkworm hybrids with shorter larval duration without compromising on productive merits to save mulberry feed and to avoid contamination during final instars.

## Materials and Methods

The present study was initiated by utilizing productive bivoltine breeds *viz.*, A65, A66, A69, A70 and A71 (Oval type) and NK32, NK33, B20 and B71 (Dumb bell type) from the institutes Germplasm collection as breeding resource material. The initial hybridization was carried out based on larval markings and cocoon shape. The rearing was conducted as per the techniques outlined by Krishnaswami (1978). By the end of F<sub>12</sub>, four lines *viz.*, SD4 and SD7 spinning white oval cocoons characterized with plain larvae and SD11 and SD12 spinning white dumb bell cocoons characterized with sex-limited larval markings were fixed for the desired traits. Hybrids were made among themselves and also by utilizing other productive breeds. Based on the performance and combining ability test results the hybrid SD7 × SD12 was adjudged as best hybrid. For selection of high yielding F<sub>1</sub> hybrids, the following Productivity index was used. Cocoon yield × Raw silk (%) / 5<sup>th</sup> instar larval duration (Gamo, 1976). Hence only SD7 × SD12 hybrid is discussed here with.

### Breeding procedure and selection methods adopted for SD7

The line SD7 was evolved from A71 × A65. The females of A71 are crossed with the males of A65. Mass rearing was conducted from F<sub>1</sub> ~ F<sub>4</sub> and from F<sub>5</sub> onwards cellular rearings were resorted to adopt directional selection for the targeted traits *i.e.*, shorter larval duration and productive merits. The breeding for shorter larval duration is carried out by using the following evaluation index; Productivity per day = Cocoon shell weight / 5<sup>th</sup> instar larval duration (Gamo, 1976). In every generation, early moulters and early spinners were isolated from every bed and mounted separately. Productivity per day was calculated for each bed and the beds showing highest index value were selected and allowed to inbred/ inter-bred during the course of breeding. During the course of breeding, pupation rate (%), larval duration and high productive merits like shell weight, cocoon weight and filament length was considered. The respective control batches were also maintained simultaneously to compare selection effect in every generation. The breed SD7 is having plain larvae spinning white oval cocoons (Fig. 1)

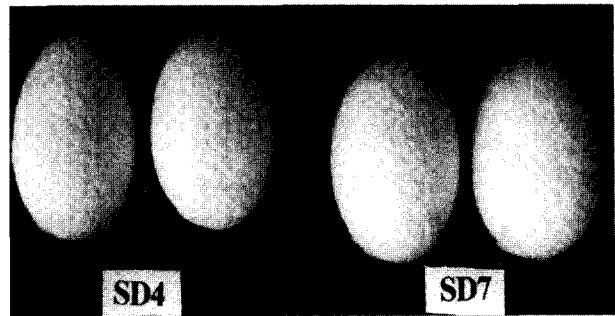


Fig. 1. Newly evolved shorter larval oval breeds SD4 and SD7.

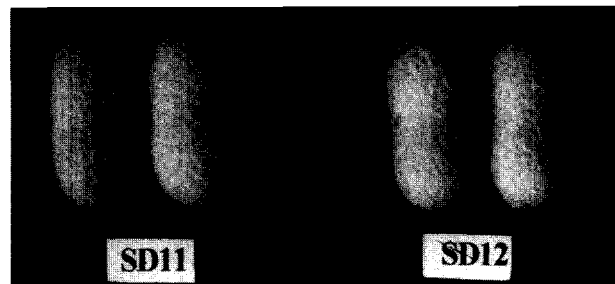


Fig. 2. Newly evolved shorter larval dumb bell breeds SD11 and SD12.

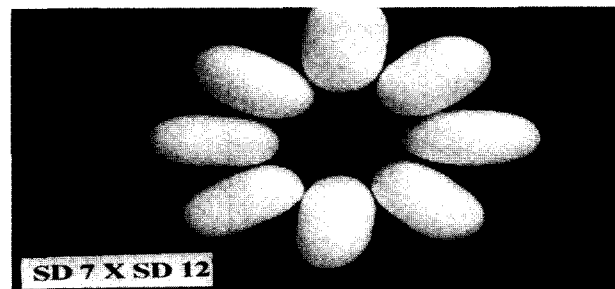


Fig. 3. Newly evolved shorter larval hybrid SD7 × SD12.

### Breeding procedure and selection methods adopted for SD12

The line SD12 was evolved from NK33 × B20. The females of NK33 are crossed with the males of B20. The larvae are having sex-limited larval markings and spinning white dumb bell cocoons (Fig. 2). The same methodology adopted for SD7 was repeated for evolution. After fixation of desired traits by F<sub>12</sub>, these lines were involved in hybrid studies. For selection of high yielding F<sub>1</sub> hybrids, the following productivity index was used. Cocoon yield × Raw silk (%) / 5<sup>th</sup> instar larval duration (Gamo, 1976). Based on the performance and combining ability test results, the hybrid SD7 × SD12 was adjudged as best hybrid.

### Characters of SD7 × SD12 hybrid

The larvae are plain, spinning white cocoons intermediate shaped cocoons between oval and dumb bell (Fig. 3). The

hybrid is having better survival rate, less larval duration, high cocoon shell ratio percentage and better filament length. The percent improvement with regard to seven economic traits of economic importance contributing to the productivity of newly synthesized lines over control breeds/hybrids (CSR2, CSR5 and their hybrid CSR2 × CSR5) were calculated by using the following formulae to determine the

merits/demerits of the newly evolved lines (Table 6).

$$\frac{X-Y}{Y} \times 100$$

Where,

X = the object compared

Y = the compared one

**Table 1.** Generation wise mean values of SD 7 (Oval line)

| Generation      | Fecundity (no) | Pupation rate (%) | Yield (kg) | Cocoon weight (g) | Cocoon shell weight (cg) | Cocoon shell ratio (%) | 5 <sup>th</sup> instar larval duration (hrs) | Filament length (m) | Raw silk (%) |
|-----------------|----------------|-------------------|------------|-------------------|--------------------------|------------------------|--|---------------------|--------------|
| F <sub>1</sub>  | 585            | 90.01             | 14.580     | 1.546             | 34.3                     | 21.75                  | 144  | 942                 | 16.67        |
| F <sub>2</sub>  | 551            | 92.24             | 15.721     | 1.948             | 42.2                     | 21.64                  | 150  | 963                 | 15.83        |
| F <sub>3</sub>  | 542            | 87.81             | 13.990     | 1.598             | 33.5                     | 21.17                  | 136  | 953                 | 12.27        |
| F <sub>4</sub>  | 539            | 93.80             | 14.472     | 1.537             | 33.1                     | 21.53                  | 138  | 1149                | 17.49        |
| F <sub>5</sub>  | 548            | 93.89             | 17.060     | 1.755             | 41.4                     | 23.54                  | 138  | 1116                | 17.30        |
| F <sub>6</sub>  | 570            | 87.14             | 14.116     | 1.621             | 33.6                     | 20.88                  | 150  | 955                 | 15.54        |
| F <sub>7</sub>  | 564            | 90.50             | 14.970     | 1.852             | 45.0                     | 24.20                  | 144  | 1103                | 16.97        |
| F <sub>8</sub>  | 527            | 91.20             | 13.932     | 1.637             | 37.7                     | 23.02                  | 160  | 905                 | 14.63        |
| F <sub>9</sub>  | 483            | 90.67             | 12.730     | 1.507             | 33.4                     | 22.19                  | 130  | 965                 | 16.40        |
| F <sub>10</sub> | 555            | 92.67             | 16.130     | 1.783             | 40.7                     | 22.97                  | 168  | 1003                | 16.95        |
| F <sub>11</sub> | 521            | 82.00             | 12.542     | 1.490             | 33.8                     | 22.69                  | 120  | 984                 | 15.76        |
| F <sub>12</sub> | 471            | 91.47             | 16.073     | 1.796             | 41.1                     | 22.90                  | 142  | 974                 | 15.39        |
| Mean            | 538            | 90.28             | 14.712     | 1.671             | 37.5                     | 22.37                  | 143  | 992                 | 15.93        |
| C.D. 5%         | 43             | 4.33              | 0.99       | 0.077             | 0.020                    | 0.91                   | 10   | 58                  | 0.69         |
| C.V.            | 4.76           | 2.83              | 3.06       | 2.68              | 3.04                     | 2.46                   | 4.00   | 3.47                | 2.52         |

**Table 2.** Generation wise mean values of SD 12 (Dumb bell line)

| Generation      | Fecundity (No) | Pupation rate (%) | Yield (kg) | Cocoon weight (g) | Cocoon shell weight (cg) | Cocoon shell ratio (%) | 5 <sup>th</sup> instar larval duration (hrs) | Filament length (m) | Raw silk (%) |
|-----------------|----------------|-------------------|------------|-------------------|--------------------------|------------------------|--|---------------------|--------------|
| F <sub>1</sub>  | 543            | 95.96             | 14.610     | 1.546             | 32.7                     | 20.96                  | 130  | 953                 | 16.30        |
| F <sub>2</sub>  | 487            | 90.66             | 13.953     | 1.948             | 37.6                     | 21.22                  | 156  | 939                 | 16.25        |
| F <sub>3</sub>  | 541            | 91.77             | 15.212     | 1.598             | 35.2                     | 21.36                  | 130  | 1026                | 16.07        |
| F <sub>4</sub>  | 568            | 92.01             | 14.450     | 1.537             | 34.4                     | 21.56                  | 132  | 948                 | 16.61        |
| F <sub>5</sub>  | 543            | 90.91             | 15.640     | 1.755             | 34.7                     | 20.38                  | 144  | 1015                | 16.70        |
| F <sub>6</sub>  | 547            | 92.47             | 14.110     | 1.621             | 32.2                     | 21.14                  | 144  | 987                 | 17.40        |
| F <sub>7</sub>  | 538            | 88.67             | 15.780     | 1.852             | 38.5                     | 21.20                  | 146  | 971                 | 17.33        |
| F <sub>8</sub>  | 514            | 91.94             | 13.793     | 1.637             | 36.6                     | 21.93                  | 160  | 941                 | 16.64        |
| F <sub>9</sub>  | 415            | 87.00             | 12.771     | 1.507             | 33.7                     | 22.48                  | 130  | 1012                | 17.79        |
| F <sub>10</sub> | 535            | 93.50             | 16.564     | 1.783             | 37.8                     | 21.40                  | 160  | 1042                | 12.80        |
| F <sub>11</sub> | 547            | 82.33             | 12.402     | 1.490             | 32.3                     | 20.97                  | 130  | 986                 | 15.98        |
| F <sub>12</sub> | 564            | 94.00             | 14.840     | 1.796             | 40.7                     | 22.23                  | 126  | 1019                | 16.82        |
| Mean            | 537            | 90.94             | 14.500     | 1.671             | 35.5                     | 21.40                  | 141  | 986                 | 16.38        |
| C.D. 5%         | 39             | 3.19              | 1.40       | 0.077             | 0.015                    | 1.07                   | 3  | 38                  | --           |
| C.V.            | 4.27           | 2.07              | 5.69       | 2.68              | 2.52                     | 2.96                   | 1.06   | 2.25                | 5.39         |

Seasonal variations were determined by using two-way classification of Kempthorne (1952).

## Results

The generation wise means values of nine economic traits of importance of newly evolved line SD7 were presented in Table 1. The perusal of data of SD7 for the trait fecundity showed highest in F<sub>1</sub> (585). The maximum pupation rate (%) recorded during F<sub>4</sub> (93.80) and cocoon yield/10,000 larvae were observed during F<sub>5</sub> (17.060 kg). The highest cocoon weight and cocoon shell weight was recorded maximum during F<sub>2</sub> (1.948 g and 42.2 cg respectively). The lowest 5<sup>th</sup> instar larval duration was recorded

120 hrs (five days) by F<sub>11</sub>.

The data pertaining to SD12 were presented in Table 2. The perusal of data indicated that the trait fecundity reached 564 at F<sub>12</sub>. The highest pupation rate (%) recorded during F<sub>1</sub> (95.96). The maximum cocoon yield/10,000 larvae recorded during F<sub>10</sub> (16.560 kg). The cocoon weight and cocoon shell weight reached maximum during F<sub>12</sub> (1.832 g and 47.0 cg respectively with a lowest 5<sup>th</sup> instar larval duration of 126 hrs (5.04 days) recorded during F<sub>12</sub>. The seasonal mean values of newly evolved lines over control breeds are summarized in Table 3 and 4. The seasonal studies of these newly evolved breeds SD7 and SD12 along with CSR2 and CSR5 showed significant differences for total larval duration, 5<sup>th</sup> instar larval duration, cocoon weight, shell weight and cocoon shell ratio. The

**Table 3.** Seasonal performance of newly evolved shorter larval breeds (Oval breeds- mean of 6 trials)

| Season         | Breed      | Fecundity (no) | Total larval duration (hrs) | 5 <sup>th</sup> instar larval duration (hrs) | Pupation (%) | Cocoon weight (g) | Cocoon shell weight (Cg) | Cocoon shell ratio (%) |
|----------------|------------|----------------|-----------------------------|--|--------------|-------------------|--------------------------|------------------------|
| Pre-Monsoon    | SD7        | 557            | 514                         | 147  | 89.1         | 1.580             | 37.85                    | 24.0                   |
|                | CSR2(C)    | 530            | 548                         | 180  | 86.7         | 1.654             | 36.81                    | 22.3                   |
|                | C.D. at 5% | --             | 6.86**                      | 4.89**                                       | --           | --                | --                       | 0.38**                 |
| Monsoon        | SD7        | 541            | 505                         | 147  | 92.2         | 1.926             | 45.12                    | 23.4                   |
|                | CSR2       | 572            | 546                         | 174  | 91.8         | 1.844             | 42.53                    | 23.1                   |
|                | C.D. at 5% | --             | 8.99**                      | 6.19**                                       | --           | 0.05**            | 1.57**                   | --                     |
| Post-monsoon   | SD7        | 634            | 528                         | 162  | 91.7         | 1.925             | 45.34                    | 23.6                   |
|                | CSR2       | 585            | 549                         | 175  | 91.3         | 1.946             | 43.87                    | 22.6                   |
|                | C.D. at 5% | 40.62*         | 5.15**                      | 4.00**                                       | --           | 0.08**            | 1.56*                    | 1.86*                  |
| Season × Breed | C.D. at 5% | 35.34*         | 6.31**                      | 5.19**                                       | --           | 0.06**            | 1.58*                    | 0.68*                  |
|                | C.V. %     | 7.00           | 1.35                        | 3.67   | 2.85         | 3.93              | 4.24                     | 3.30                   |

\*\*Significant at 1% level. \*Significant at 5% level. C = Control.

**Table 4.** Seasonal performance of newly evolved shorter larval breeds (Dumb bell breeds- mean of 6 trials)

| Season         | Breed      | Fecundity (no) | Total larval duration (hrs) | V age duration (hrs) | Pupation rate (%) | Cocoon weight (g) | Cocoon shell weight (cg) | Cocoon shell ratio (%) |
|----------------|------------|----------------|-----------------------------|----------------------|-------------------|-------------------|--------------------------|------------------------|
| Pre-Monsoon    | SD12       | 570            | 512                         | 140                  | 88.0              | 1.609             | 37.22                    | 23.1                   |
|                | CSR5 (C)   | 540            | 534                         | 156                  | 86.4              | 1.631             | 36.10                    | 22.1                   |
|                | C.D. at 5% | --             | 7.74**                      | 4.04**               | --                | 0.09*             | --                       | --                     |
| Monsoon        | SD12       | 550            | 496                         | 137                  | 93.0              | 1.898             | 44.16                    | 23.3                   |
|                | CSR5       | 528            | 533                         | 174                  | 91.3              | 1.934             | 43.91                    | 22.7                   |
|                | C.D. at 5% | --             | 11.45**                     | 5.45**               | --                | ---               | ---                      | --                     |
| Post-monsoon   | SD12       | 596            | 525                         | 162                  | 91.8              | 1.789             | 42.15                    | 23.6                   |
|                | CSR5       | 552            | 547                         | 174                  | 91.8              | 1.859             | 43.34                    | 23.3                   |
|                | C.D. at 5% | 32.53**        | 9.31**                      | 5.94**               | --                | 0.05**            | --                       | 0.71**                 |
| Season × Breed | C.D. at 5% | ---            | 9.59**                      | 6.23**               | --                | ---               | ---                      | ---                    |
|                | C.V. %     | 6.04           | 2.07                        | 4.46                 | 3.41              | 4.62              | 76.00                    | 95.37                  |

**Table 5.** Seasonal performance of newly evolved shorter larval hybrids (mean of 6 trials)

| Season                 | Hybrid      | Fecundity (no) | Total larval duration (hrs) | 5 <sup>th</sup> instar duration (hrs) | Pupation (%) | Cocoon weight (g) | Cocoon shell weight (cg) | Cocoon shell ratio (%) | Filament length (m) | Raw silk (%) |
|------------------------|-------------|----------------|-----------------------------|---------------------------------------|--------------|-------------------|--------------------------|------------------------|---------------------|--------------|
| Pre-Monsoon (Control)  | SD7 × SD12  | 545            | 516                         | 152                                   | 93.4         | 1.814             | 43.59                    | 24.0                   | 1188                | 19.2         |
|                        | CSR2 × CSR5 | 525            | 528                         | 180                                   | 88.5         | 1.814             | 40.50                    | 22.3                   | 1080                | 17.9         |
|                        | C.D. at 5 % | --             | 6.34**                      | 3.58**                                | 2.03**       | ---               | 1.86**                   | 0.56**                 |                     |              |
| Monsoon (Control)      | SD7 × SD12  | 534            | 501                         | 152                                   | 92.1         | 1.928             | 45.53                    | 23.6                   | 1202                | 17.8         |
|                        | CSR2 × CSR5 | 556            | 534                         | 168                                   | 92.1         | 1.984             | 45.55                    | 22.9                   | 1148                | 17.0         |
|                        | C.D. at 5%  | --             | 10.09**                     | 0.49**                                | --           | ---               | ---                      | --                     |                     |              |
| Post-monsoon (Control) | SD7 × SD12  | 598            | 519                         | 168                                   | 92.7         | 2.086             | 50.72                    | 24.3                   | 1257                | 17.9         |
|                        | CSR2 × CSR5 | 588            | 549                         | 183                                   | 92.0         | 2.095             | 49.21                    | 23.5                   | 1202                | 17.4         |
|                        | C.D. at 5%  |                | 3.29**                      | 1.72**                                | --           | 0.09*             | 2.52*                    |                        |                     |              |

**Table 6.** Comparative mean values and percent improvement/of newly evolved breeds/hybrids over control breeds

| Fecundity (no) | Total larval duration (hrs) | 5 <sup>th</sup> duration (hrs) | Pupation rate (%) | Cocoon weight (g) | Cocoon shell weight (cg) | Cocoon shell ratio (%) |
|----------------|-----------------------------|--------------------------------|-------------------|-------------------|--------------------------|------------------------|
| SD7 vs.        | 577                         | 516                            | 91.00             | 1.810             | 42.77                    | 23.62                  |
| CSR2 at        | 562                         | 548                            | 89.93             | 1.814             | 41.07                    | 22.64                  |
| % improved     | 2.66                        | -5.83                          | -13.63            | 1.18              | -0.22                    | 4.32                   |
| SD12 vs.       | 572                         | 511                            | 90.93             | 1.765             | 41.13                    | 23.30                  |
| CSR5 at        | 540                         | 538                            | 89.83             | 1.808             | 41.12                    | 22.74                  |
| % improved     | 5.92                        | -5.01                          | -13.09            | 1.78              | -23.78                   | 0.02                   |
| SD7 × SD12 vs. | 559                         | 512                            | 92.73             | 1.942             | 46.70                    | 23.96                  |
| CSR2 × CSR5    | 556                         | 537                            | 90.86             | 1.964             | 45.00                    | 22.90                  |
| at % improved  | 0.53                        | -4.65                          | -13.25            | 2.05              | -0.22                    | 3.77                   |

seasonal mean values of the newly evolve hybrid SD7 × SD12 over control hybrid CSR2 × CSR5 are presented in Table 5. The hybrid SD7 × SD12 recorded a mean value of fecundity 559, pupation rate (%) of 92.73, cocoon weight of 1.942 g, shell weight of 46.7 cg shell ratio (%) of 24.00, raw silk (%) of 18.3, filament length of 1,215 meters and filament neatness of 94 P. The total larval duration recorded 512 hrs (21.10 days) with a 5<sup>th</sup> instar larval duration of 157 hrs (6.12 days). On the other hand control hybrid CSR2 × CSR5 (Productive hybrid) recorded fecundity of 556, pupation rate (%) of 90.86. Cocoon weight of 1.964 g, shell weight of 45.0 cg, shell ratio (%) of 22.90, filament length 1,143 meters and raw silk (%) of 17.4. The total larval duration recorded 537 hrs (22.06 days) with a 5<sup>th</sup> instar larval duration of 181 hrs (7.13 days). The percent improvement over control breeds was presented in Table 6.

The percent improvement indicated not much improvement over control breeds, but the values for total larval duration and 5<sup>th</sup> instar larval duration have shown negative values which indicates their shorter larval duration. Only slight improvement was noticed in pupation rate, cocoon shell weight and cocoon shell ratio percentage.

## Discussion

Synthesis of new genotypes by conventional breeding method is one of the powerful tool for exploiting heterosis phenomenon in the silkworm *Bombyx mori* L. (Harada, 1961). Though the nature of silkworm healthiness is not known clearly, different breeding methods have been adopted for the improvement of survival. The breed that has shown shorter larval duration has less chance to get infected with diseases. It could be seen from the Table 5 that the hybrid SD7 × SD12 has shown comparatively more pupation rate over control hybrid during all the major seasons confirming the validation of more healthiness and shorter larval duration. Generally, F<sub>1</sub> hybrids are more resistant, shorter in larval duration and more productive due to the presence of high magnitude of heterosis. But shorter larval duration, productive merits and higher survival are also reflected in newly evolved pure lines (Table 3 and 4) over control breeds indicate the superiority of newly evolved lines. These studies assume significance that maturity genes control larval duration, which has close correlation with larval body weight,

cocoon weight and cocoon shell weight. In silkworm, the selection for one character is found to result in correlated changes in other quantitative characters of economic importance (Kobari and Fujimoto, 1966). Though the hybrid is having shorter larval duration, productivity traits such as cocoon weight, shell weight, raw silk percentage and filament length were maintained on par with control hybrid, which is productive, indicates the effectiveness of selection methods adopted during the course of breeding. It is concluded that by utilizing above selection indices, the productivity of silk per day of silkworm breeds and cocoon yield can be increased considerably by breeding shorter larval duration breeds for tropics.

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