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Backcross Breeding and Directional Selection of Two Multivoltines, N^{+p} and N^{p} of Silkworm, *Bombyx mori* L. for Higher Viability and Productivity in Eastern India

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ABSTRACT

A new superior multivoltine breed of *Bombyx mori* L. (D^{+p}) has been developed by crossing between two strains of Nistari Plain (N^{p}) and Nistari Marked (N^{+p}) which are the two most preferred strains of multivoltine breed at the farmer's level of eastern India. Through standard breeding plan this needbased new breed, D^{+p} was developed by adopting three steps: i) Development of Recurrent Backcross Line through backcross breeding for initial eight generations; ii) Improvement of developed RBL following directional selection process for the next twenty generations and ultimately iii) stabilization for next eight generations upto F40 generation at normal environmental conditions. Study of nine quantitative characters related to viability and productivity of the new breed in relation to its parents have been studied statistically and Heterosis%.

Keywords: Silkworm, Multivoltine, RBL, Nistari, Heterosis.

1. Introduction

Eastern India is generally characterized by luxuriant growth of mulberry for its highly fertile soil and rainfall. But, rearing of productive silkworm breed and hybrids is restricted due to highly variable climatic situation which causes poor larval growth, moulting disorder, high mortality due to diseases and low cocoon yield. High temperature along with high humidity acts as a major limiting factor for rearing silkworm during summer seasons but during this time huge quantity of mulberry leaf is available. But during favourable season, optimum quantity of silkworm rearing can't be conducted due to non-availability of quality mulberry leaf. In this region environmental temperature even shoots upto 40 ^{0}C during summer (May-June) and dips as low as 5^{0}C in severe winter (January) and relative humidity ranges between 40% and 95%. In India silkworm breeding efforts for more than 50 years to evolve better breeds than indigenous Pure Mysore and Nistari, have resulted more than 50 new breeds. But, no new multivoltine breed could get established in the field due to the use of bivoltine breed during breeding to improve the quantitative characters of existing multivoltine breed and as a result hibernating eggs in various proportions were obtained. Thus, Nistari are still used for production of commercial cocoons in West Bengal and other eastern states. The two popular hybrids are: i) Multivoltine X Multivoltine based- Nistari X M12W and ii) Multivoltine X Bivoltine based- Nistari X NB4D2. The strength of Nistari is its high adaptability, survival and reproductive efficiency. However, the disadvantage of Nistari is its low productivity.

So, there is need to develop suitable hybrids with higher productivity which can sustain the climatic conditions of this region. If we can improve the quantitative characters of female component (Multivoltine) of this hybrid, then it will meet the demand of hybrids with high survival and productivity. In the present study we tried to improve the productivity of Nistari without compromising its inherent viability by crossing two eco-races of Nistari, N^{p} (Nistari Plain) and N^{+p} (Nistari Marked). After 5 years of breeding experiment, we have developed a new breed, D^{+p} (Debra marked) which has higher viability and productivity in all the seasons of eastern India.

2. Materials and Methods:

2.1. Selection of parents:

The parent, Nistari (both plain and marked) is an original tropical strain of Indian origin and its rearing has been practiced in Gangetic river valley since centuries [1]. It has been demonstrated in various plants and animal species that the lines derived from the same population with different selection histories are known to reveal heterosis when they are crossed [2].

So, for this study the multivoltine race of Nistari i.e. both N^{+p} and N^p race were maintained at Debra Sericulture Complex, Government of West Bengal, Midnapore (west). They were considered as parents. The morphological and physiological traits of them are depicted in Table 1 and Table 2. While comparing the two lines of Nistari, it has been observed that though N^p has better survival rate and cocoon yield, it lacks higher shell weight and shell ratio percent like N^{+p}[3]. Therefore, the aim of the present study is to combine these characters through breeding and to enhance fecundity and other yield parameters through selection consequently.

Table 1: Morphological traits of the selected parents i.e. Nistari plain (N^p) and Nistari marked (N^{+p}) [4].

Traits of N^P	Traits of N^{+p}
a) Egg: colour- yellow, shape-oval, voltinism- polyvoltine	a) Egg: colour- bright yellow, shape-oval, voltinism-polyvoltine
b) Larva: newly hatched- Dark brown covered with black setae, marking and colour- milky white without any marking, shape- long slender, caudal horn- medium and pointed, moultinism-tetramoulter	b) Larva: newly hatched- Deep brown covered with black setae, marking and colour- milky white with two crescent marks on 2nd and 2 star spots on 5th abdominal segment, shape- long slender, caudal horn- short and pointed, moultinism-tetramoulter
c) Cocoon: colour- golden yellow, shape- one end pointed and spindle shaped, grain- course, floss- much flossy	c) Cocoon: colour- golden yellow, shape- one end pointed and spindle shaped, grain- course, floss- much flossy
d) Moth: colour-brownish white, eye colour- black	d) Moth: colour-brownish white, eye colour- black

2.2. Breeding Methods:

The present silkworm breeding strategy was aimed at developing vigorous breed to meet twin demand of high survival and high production of quality silk.

This project work was started with the selection of need base multivoltine breed for better improvement from its parent and accordingly has been divided into three broad categories; as follows (Chart 1):

2.2.1. Development of Recurrent Backcross Line (RBL): Here, breeding was done with N^P X N^{+p} for eight successive generations **(F1–F8)** for the improvement race **(RBL)** of desire characters by giving definite selection pressure of the selected breed.

Backcrossing is generally used to transfer one or two heritable traits into an already superior line. One parent, termed "non-recurrent", is used as the original donor of the one or two characters missing in the other parent. The recurrent parent is involved in repetitive crossing as the recipient of the characters from the non-recurrent parent.

The method of repeated backcrossing among appropriate donor and recipient parents looks appropriate for attaining the quality of commercial traits as was reported by **5, 6 & 7.**

Table 2: Average performance of quantitative traits of parents i.e. Nistari plain (N^p) and Nistari marked (N^{+p}) (Thangavalu *et al.*, 1994).

Sl. No.	Characters	Nistari(N^p)	Nistari(N^{+p})
1	Fecundity(numbers)	426	376
2	Hatching (percentage)	88.80	90.05
3	E.R.R (percentage)/ Survival (percentage)	82.40	77. 30
4	Yield per 100 d.f.ls (kg)	31.5	27.5
5	Pupation rate (percentage)	86.04	81.44
6	Single cocoon weight (g)	0.890	1.042
7	Single shell weight (cg)	10.60	12.75
8	Shell (percentage)	11.91	12.39
9	Filament length (m)	378. 3	405.7

In this breeding programme, crosses were made between N^p (receptor) and N^{+p} (donor) parents to ingress high shell weight and other reeling parameters. In these initial eight generations, mass rearing of five numbers of layings were brushed. After third moult total larvae were divided into in five replications and cocoons were pooled together after assessment.

Female pupae were selected out of those having higher reeling parameters like donor parent and other phenotypic characters having higher yield parameters as receptor parent from the cross (F_1). Thereafter, consecutive back crosses with donor male of N^{+p}, which was reared separately, were conducted up to F_8 generations in a similar fashion. Finally, **Recurrent Backcross Line (RBL)** or **Congenic Line** was developed where maximum homozygocity like receptor parent with high shell weight, Shell percentage and other reeling parameters closer to donor parent were observed.

2.2.2. Directional Selection: Here, selection was done of the improved line (after RBL) from **F9 to F32** where selection pressure was given on need based multivoltine traits for better improvement.

Directional Selection is a mode of selection in which a single phenotype is favored, causing the allele frequency to shift in one direction. Under directional selection, the advantageous allele increase in frequency independently of its dominance relative to other alleles. This is even if the advantageous allele is recessive, it will eventually become fixed.

The selection, though simply is just choosing parents based on ideal phenotypic character, the extent of genetic and end product improvement, however, depends on their variability, level of selection, heritability rate, correlation among the traits chosen; as artificial selection acts only an added force on natural selection [8,9,10].

In these generations **(F_9 to F_{32}), directional selection** was employed primarily based on fecundity and other yield parameters like Shell percentage, ERR percentage, filament length, cocoon wt., shell wt. etc.

Here, cellular rearing of single laying in five replications was maintained. After harvesting cocoons, best two batches were selected based on ERR percentage and pupation rate percentage. Then the defective cocoons were discarded and remaining cocoons were selected from each batch based on visual parameters like size, shape and uniformity. Then these cocoons were pooled together and cocoons were cut open for pupal sexing. Later, weight of female pupae was taken on electronic mono pan balance and best fifty pupae with more than average weight were selected. Again, fifty numbers of male pupae were selected based on higher shell weight and Shell percentage.

2.2.3. Stabilization Selection: Here, the lines obtained from F32 were reared without giving any selection pressure from generation **F33 to F40**. Thus, total forty generations of rearing were completed in five years.

It is a type of selection in which genetic diversity decreases as the population stabilizes on a particular trait value. It favours the intermediate variants. It reduces phenotypic variation and stabilizes the line.

Here, also cellular rearings were maintained, but no selection at the batch level or individual level was done.

According to **11** the selection pressure must be applied to egg, larva, pupa/cocoon and moth during breeding programme. After initial eight generations of backcross breeding (F-1 to F-8) or during directional selection stage (F-9 to F-32), the selection pressure was applied at all the four stages of development viz. egg, larva, pupa/cocoon and moth to select the parent at every generation.

- **Egg stage:** Only the dfls exhibiting higher number of fecundity was selected. At every generation the replicate showing the highest percentage of hatching was selected in order to improve the percentage of hatching.
- **Larval stage:** After third moult comparatively more active larvae with greenish colour and rough texture exhibiting healthy and uniform growth and medium weight were selected at every generation. Similarly the duration of feeding and moulting of the larvae was recorded and the replicate exhibiting synchrony in growth and development registering highest pupation rate was selected for future generation.
- **Cocoon/pupal stage:** At every generation cocoons were sorted out on the basis of shape and colour discarding the flossy, double, deformed and open end cocoons if there were any. The cocoon and shell weight were recorded and shell percentage was calculated. Female cocoons with higher weight; and male cocoons with higher shell weight and cocoon shell percentage exhibiting uniformity in shape and size were chosen. In addition the replicates exhibiting the higher cocoon yield were selected.
- **Moth stage:** The eggs laid by the moths exhibiting the highest longevity were chosen for next generation. The female moths with proper scales and without any deformed wings; and male moths with flattering wings were selected at every generation.

The aim was to develop a natural environmental condition sustainable race having higher fecundity and other yield parameters. So, rearing was done under prevailing room temperature and relative humidity throughout the year with little manipulation on environmental conditions as available in the farmers house for five years (Average Temperature: Maximum-35.1 ^{0}C and Minimum-16.2 ^{0}C; Average Relative Humidity percentage: High-96.6% and Low-47.5%).

Data collected on different rearing parameters and cocoon yield parameters were statistically analyzed for ANOVA. Analysis of variance **(ANOVA)** was carried out to ascertain the effect of year, season and interaction between year and season on twelve quantitative characters. Critical difference (CD) value at p=0.05 was calculated for those factors which were found to be significant at least at 5% level to compare individual means of respective factor. Although the effects of year, season and year x season were found to be highly significant (P<0.01) for all the characters under study.

2.3. Calculation of Heterosis:

According to **12** heterosis have two general modes of expression viz.

- Increase in size, probably due to faster rate of cell activity and cell division,
- Increase in ‘biological efficiency’ such as reproductive rate and survivality.

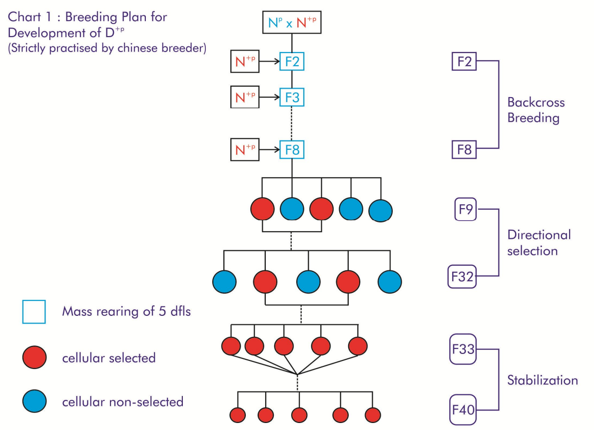
Heterosis over mid parent (MPH) and better parent (BPH) was calculated by using the following formulae:

$$MPH = \frac{D^{+P}V - MPV}{MPV} \times 100 \qquad BPH = \frac{D^{+P}V - BPV}{BPV} \times 100$$

Where, MPV = mid parent value, BPV = better parent value,

D^{+p} V = Value of newly developed breed

3.3.4 Breeding Schemes:



3. Results and Discussion:

With a view to improve the desired quantitative characters of the two selected strains (N^p and N^{+p}) the present study was initiated from 2002 to 2007. Here, we had considered these two strains as parents. N^p and N^{+p} strains were maintained as multivoltine germplasm stock at Debra Sericulture Complex, Government of West Bengal, Paschim Medinipur, and West Bengal.

These breeds were reared at Debra Sericulture Complex with improved S_{1635} mulberry leaves and with improved rearing technology as suggested by **13**. According to **14 & 15** during the process of breeding, it is necessary to provide the rearing strain with a natural environmental condition prevailing throughout the year which will help to express fully the biological characters. Accordingly to understand their phenotypic expression of characters, we had divided every year into three seasons:

Seasons	Generation completed	Duration
Summer	2	Middle of March to 1st week of June
Rainy	3	2nd week of June to 2nd week of October
Winter	3	3rd week of October to Middle of March

We had started our breeding procedure with mass rearing (i.e. five dfls brushed together) after crossing between N^p x N^{+p}. Here, N^p was receptor or mother and N^{+p} was donor or father in the subsequent backcrossing. From generation-1, we had recorded the data on nine quantitative characters. They were fecundity (number of eggs laid by a moth), hatching percentage, survival percentage (Effective Rate of Rearing percentage), cocoon yield (kg) per one hundred disease free layings (dfls), single cocoon weight (g), single shell weight (cg), Shell percentage, pupation rate percentage, filament length (m). Important expression of these nine quantitative characters during silkworm breeding process in relation to four major prevailing environmental factors through forty generations, and their expression of heterosis (percentage) were assessed; which are discussed below:

3.1. Fecundity (numbers):

In course of investigations of quantitative traits, fecundity of female moth had been noted season-wise. First eight generations (F1 to F8) were the process of recurrent backcross line (RBL) breeding for the improvement of race of desired characters by giving selection pressure on fecundity. That is in every generation we had selected the dfls with higher fecundity and then continuing with that eggs for the next successive generations. As a result the data on fecundity which we were recorded are depicted in Table 3a.

Table 3a: Performance of mean fecundity (numbers) recorded during rearing of forty generations with specific breeding procedure in five years through different seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	400.40	382.20	469.20	472.20	491.40	443.08
Summer-2	359.80	411.60	456.80	491.80	452.60	434.52
Rainy-1	410.40	428.40	479.00	431.60	475.40	444.96
Rainy-2	467.60	404.20	404.80	490.40	492.40	451.88
Rainy-3	430.40	449.80	449.80	439.80	447.40	443.44
Winter-1	436.00	464.40	462.80	504.80	453.60	464.32
Winter-2	418.20	498.00	475.00	554.60	542.60	497.68
Winter-3	469.80	463.00	505.00	479.60	534.80	490.44
Mean	424.07	436.70	462.80	483.10	486.27	

During this RBL breeding minimum 359.8 eggs/dfl were recorded during summer-2 whereas the maximum 469.8 eggs/dfl were recorded during winter-3 rearing (Figure 1). But during this phase we were able to increase their fecundity in a minor amount through backcrossing method and as such in first year mean value were 424.07 eggs/dfl which was almost closer to their mother (N^p), which had average fecundity of 426.0 eggs/dfl (Table 3a). Again during directional selection i.e. from F-9 generation to F-32 generation, selection pressure was given on fecundity in every generation for better improvement of fecundity (as discussed in materials and methods). Here, we found from year-2 to year-4 that minimum fecundity 382.2 numbers of eggs/dfl was recorded during F-9 generation but maximum 554.6 numbers of eggs/dfl was recorded at F-31 generation. This result indicated that fecundity was increased during directional selection procedure (Table 3a).

The mean result on fecundity in every year was also increased from 436.7 numbers to 483.1 numbers of eggs/dfl. Interestingly during winter-2 season the fecundity showed highest results and the fecundity ranged from 475.0 numbers to 554.6 numbers of eggs/dfl. Although fecundity of the breed at F9, F12 and F20 generation were low ranging between 382.2 numbers to 404.8 numbers due to unfavourable seasons. So, it was observed that during directional selection the fecundity of the new breed had increased considerably from 436.7 numbers to 483.1 numbers of eggs/dfl.

Table 3b: Performance of Analysis of variance on mean fecundity (numbers) during rearing of forty generations along 40 rearing seasons

Source	DF	F	CD at 5%
Year(Y)	4	124.76**	6.862
Season (S)	7	57.02**	8.680
Y x S	28	18.86**	19.409

Finally, during stabilization rearing process i.e. F33 to F40 generations, the recorded data showed that the fecundity characters of the newly developed breed (D^{+p}) ranged from 447.4 (during Rainy-3) to 542.6 (during Winter-2) eggs/dfl (Table 3a).
In case of fecundity, if we consider the results season wise, we will see that the fecundity was more during winter-2 season (average 497.68 eggs/dfl) than other seasons.
If we compare the year-wise mean of fecundity the data obtained were 424.07, 436.70, 462.80, 483.10 and 486.27 in year-1, year-2, year-3, year-4 and year-5 respectively. The year-wise critical difference value (CD) at 5% level was 6.862. Now, the year wise improvement was recorded as 12.63 in year-2, 26.1 in year-3, 20.3 in year-4 and 3.17 in year-5. But, it was not significant in year-5 as the value was less than CD value which confirms the stabilization of the character. Again, the CD value of rearing in different seasons was 8.680 (Table 4b). Here, the lowest value was obtained as 434.52 in summer-2. The next higher values were (in numbers) 443.08, 443.44, 444.96, 451.88, 464.32, 490.44 and 497.68 in summer-1, rainy-3, rainy-1, rainy-2, winter-1, winter-3, and winter-2 respectively and the consecutive improvements were (in numbers) 8.56, 0.36, 1.52, 6.92, 12.44, 26.12, 7.24. Among these values in winter-3 and winter-2 values were found higher than CD value and were significant statistically.

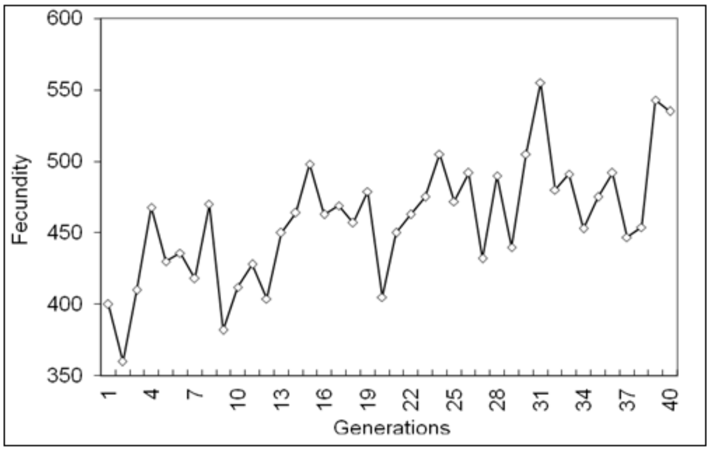


Fig 1: Performance of mean fecundity (numbers) recorded during rearing of forty generations with specific breeding procedure in five years.

In general terms, Heterosis or hybrid vigour can be defined as the "extra vigour" exceeding that of both the parental races which is frequently shown by hybrids from the crossing of different species, breeds and strains [16]. While comparing the new breed with better parent and mid parent values in different economic characters the heterosis was observed; and heterosis over better parent value (BPH) and heterosis over mid parent value(MPH) were calculated. The fecundity was recorded as 426nos, 401nos and 486nos in case of better parent, mid parent and in the new breed, D^{+p} respectively. Thus, the improvement of fecundity was recorded as 13.30% over better parent and it had a gain of 21.20% over mid parent value.

3.2. Hatching of Eggs Percentage:

Hatching percentage of eggs was calculated after the first instar larvae were came out from the egg shell. The unhatched eggs were eggs which remain arrested within the egg shell and looked like black eggs from outside. Again, in unfertilized eggs embryonic development did not occur and as a result looked like yellow eggs.

During recurrent backcross line rearing from generation-1 to generation-8, minimum 88.15% of hatching were recorded during rainy-2 season while maximum 90.05% were calculated during summer-2 and winter3 seasons (Figure 2). If these results were compared with parents (i.e. N^p and N^{+p}) not much improvement was noticed as the hatching percentage of the parents ranged from 88.80% to 90.05% (Table 2).

Table 4a: Performance of mean hatching percentage of eggs recorded during rearing of forty generations with specific breeding procedure in five years through different seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	89.81	89.82	92.95	92.83	90.07	91.10
Summer-2	90.05	89.21	90.10	90.52	91.15	90.21
Rainy-1	92.88	91.13	91.02	89.97	89.82	90.96
Rainy-2	88.15	90.25	90.46	89.52	94.14	90.50
Rainy-3	89.93	92.00	91.75	93.64	90.07	91.48
Winter-1	90.96	94.70	92.10	91.83	92.41	92.40
Winter-2	89.00	92.05	90.26	90.11	92.65	90.81
Winter-3	90.05	93.04	91.99	90.85	90.08	91.20
Mean	90.10	91.53	91.33	91.16	91.30	

Next from 2nd year to 4th year of directional selection studies, (i.e. from generation-9 to generation-32), the mean data on hatching percentage remain varied from 91.16% to 91.53% year-wise with minimum 89.21% of hatching of eggs were recorded on 10th generation (during summer-2) and maximum 94.70% of eggs were hatched out during winter-1 on 14th generation. We gave some selection pressure on hatching percentage (as discussed in materials and methods) but no appreciable improvement was noticed.
From generation-33 to generation-40, (i.e. during 5th year), we reared for stabilization of characters. During this year the hatching of eggs ranged from 89.82% to 94.14% with a mean of 91.30%. Finally, if all forty generation season-wise were considered, we found that winter-1 season favoured the eggs to hatch more (92.40%) in comparison to other seasons (Table 4a).

Table 4b: Performance of analysis of variance of mean hatching percentage of eggs during rearing of forty generations along 40 rearing seasons.

Source	DF	F	CD at 5%
Year(Y)	4	14.69**	0.411
Seasons(S)	7	12.78**	0.52
Y x S	28	12.65**	1.162

When we compare the year-wise mean of Hatching percentage the data obtained (in percentage) were 90.10, 91.53, 91.33, 91.16 and 91.30 in year-1, year-2, year-3, year-4 and year-5 respectively. The year-wise critical difference value (CD) at 5% level was 0.411 (Table 4b). Now, the year-wise improvement was recorded as 1.4 in year-2, -0.17 in year-3, -0.17 in year-4 and 0.14 in year-5. So, it was observed that the improvement in year-2 was highly significant, as the values were more than critical value. But, it was not significant in other years as the values were less than CD value. Again, the CD value of rearing in different seasons was 0.52. Here, the lowest value was obtained as 90.21% in summer-2. The next higher values (in percentage) were 90.50, 90.81, 90.96, 91.10, 91.20, 91.48 and 92.40 in rainy-2, winter-2, rainy-1, summer-1, winter-3, rainy-3 and winter-1 respectively and the consecutive

improvements were 0.30, 0.31, 0.15, 0.14, 0.10, 0.28 and 0.92. Among these values in winter-1 value was found higher than CD value and was significant statistically.
According to **17,** the heterosis in silkworm had received considerable attention because of the marked effect of yield improvement and almost all the economic characters of silkworm were shown to express hybrid vigour in the hybrids, exhibiting superiority over the parents. The improvement was observed at the minimum percentage in this character. The hatching percentage was observed as 90.05%, 89.43% and 91.30% in case of better parent, mid parent and D^{+p} respectively. So, the BPH and MPH in the new breed were 1.39% and 2.09% respectively (Table 12).

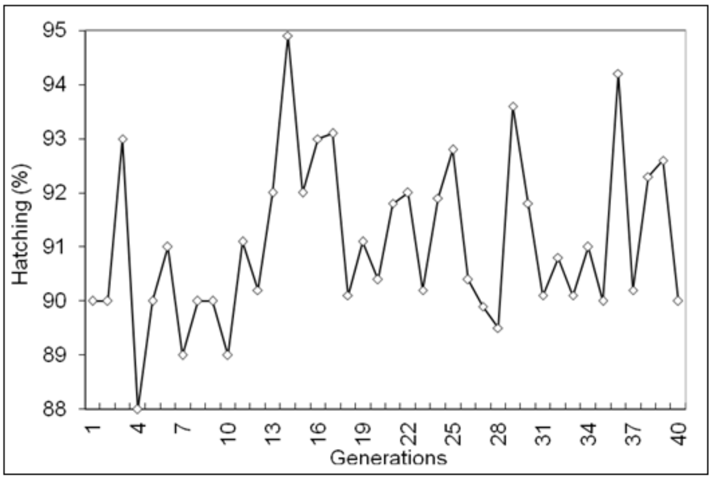


Fig 2: Performance of mean hatching% of eggs recorded during rearing of forty generations with specific breeding procedure in five years through different seasons.

3.3: Effective Rate of Rearing (ERR) percentage or Survival percentage:

ERR percentage or survival percentage is a measure to judge how the rearing of worms has been performed. It is calculated with dividing number of cocoons harvested by number of worms available after 3rd moult and converting it into percentage. The minimum 80.11% and maximum 84.97% of ERR percentage were observed (Table 5a) during Recurrent Backcross Breeding (F1 to F8 generation). Not much improvement was observed in this character during this period (Figure 3), and the mean value was 82.17%, whereas the parents had 82.40% and 77.30% of Effective Rate of Rearing (Table 5a).
However, during directional selection phase (F9 to F32); the data recorded was ranged between 80.11% during F16 to 90.40% during F28 generations. While considering the mean value of the data, the enhancement of ERR percentage was observed during Year-4 with 86.87% whereas 84.10% and 84.51% of survival percentage during Year-2 and Year-3 respectively. But, this value was not sustained during stabilization phase of Year-5 where the mean value was 84.63% with minimum 80.46% during F40 and maximum 86.83% during F35 generations.

Table 5a: Performance of mean ERR percentage recorded during rearing of forty generations with specific breeding procedure in five years through different seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	82.90	83.53	87.82	88.71	86.33	85.86
Summer-2	80.80	84.19	82.65	86.79	85.41	83.97
Rainy-1	80.11	87.02	83.50	89.72	86.83	85.43
Rainy-2	81.08	87.19	88.00	90.40	86.66	86.67
Rainy-3	80.99	84.32	80.30	89.57	82.99	83.63
Winter-1	84.92	85.00	82.37	82.27	85.88	84.09
Winter-2	84.97	81.47	88.11	82.66	82.47	83.94
Winter-3	81.62	80.11	83.36	84.86	80.46	82.08
Mean	82.17	84.10	84.51	86.87	84.63	

The statistical analysis of ANOVA showed that significantly higher ERR percentages was observed in 1st rearing of summer season and 1st and 2nd rearings of rainy season than other seasons (table5b). However, year wise improvement was significant upto 4th year, but during stabilization in 5th year the performance was seen to come down to 3rd year's level.

Table 5b: Performance of analysis of variance of mean ERR percentage during rearing of forty generations along 40 rearing seasons.

Source	**DF**	**F**	**CD at 5%**
Year(Y)	4	163.12**	0.366
Seasons(S)	7	76.74**	0.463
Y x S	28	44.26**	1.036

Again, while considering the seasonal pattern, the rainy season gave better ERR percentage than value than other seasons. The data ranged from minimum 82.08% during Winter-3 and maximum 86.67% during Rainy-2 season.

If we compare the yearwise mean of ERR percentage the data obtained (in percentage) were 82.17, 84.10, 84.51, 86.87 and 84.63 in year-1, year-2, year-3, year-4 and year-5 respectively (Table 7b). The yearwise critical difference value (CD) at 5% level was 0.366. Now, the yearwise improvement was recorded as 1.93 in year-2, 0.41 in year-3, 2.36 in year-4 and -2.24 in year-5. So, it was observed that the improvements in year-2, year-3 and year-4 were highly significant as the values were more than critical value. But, it was not significant in year-5 as the value was less than CD value.

Again, the CD value of rearing in different seasons was 0.463. Here, the lowest value was obtained as 82.08 in winter-3. The next higher values (in percentage) were 83.63, 83.94, 83.97, 84.09, 85.43, 85.86 and 86.64 in rainy-3, winter-2, summer-2, winter-1, rainy-1, summer-1 and rainy-2 respectively and the corresponding improvements were 1.55, 0.31, 0.03, 0.12, 1.34, 0.43 and 0.78. Among these values in rainy-3, rainy-1 and rainy-2 values were found higher than CD value and were significant statistically.
Nistari (both plain and marked) has a great potential to survive in different environment conditions and therefore, left a little scope for improvement of this character. So, the survival percentage of 82.40% in better parent, 79.85% in mid parent and 84.63% in D^{+p} was recorded. Thus, the improvement as per BPH and MPH was 2.71% and 5.99% respectively (Table 12). When we consider the overall performance of the new breed, D^{+p} it was observed that the mean value of ERR percentage at 84.63% which was increased 2.71% as its better parent where N^{p} with 82.40% of ERR percentage (Table 12).

3.4. Cocoon yield/100 dfls (kg):-

During recurrent backcross line rearing from generation-1 to generation-8, minimum 29.08 kg of cocoon production per 100dfls were recorded during summer-2 season while maximum 38.71 kg were calculated during winter-1 season with a mean value of 34.51 kg (Figure 4). If we consider this result with parents (i.e. N^p and N^{+p}) we found much improvement, because the production per 100dfls of the parents ranged from 27.5 kg to 31.5 kg (Table 6a).

However, from 2nd year to 4th year of directional selection studies,(i.e. from generation9 to generation32), the mean data on cocoon yield varied from 36.08 kg to 38.81 kg yearwise with minimum 31.54 kg of cocoon production were recorded on 9th generation (during summer-1) and maximum 42.12 kg of cocoon production were recorded during winter-3 on 32nd generation. From generation33 to generation40, (i.e. during 5th year), we reared for stabilization of characters.

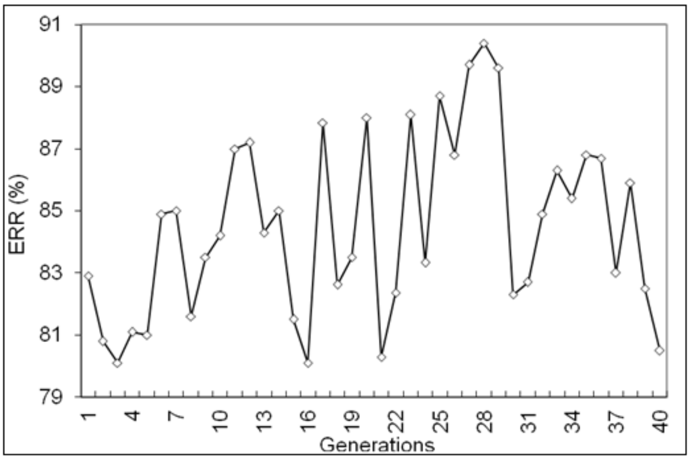


Fig 3: Performance of mean ERR% recorded during rearing of forty generations with specific breeding procedure in five years.

Table 6a: Performance of mean cocoon yield/100 dfls in kg recorded during rearing of forty generations with specific breeding procedure in five years through different Seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	32.26	31.54	33.88	35.00	36.55	33.85
Summer-2	29.08	34.88	38.65	37.96	38.28	35.77
Rainy-1	31.48	33.69	34.48	36.58	35.60	34.37
Rainy-2	36.30	35.38	35.79	37.76	38.99	36.84
Rainy-3	35.22	37.05	38.45	39.00	38.81	37.71
Winter-1	38.71	38.52	39.85	40.87	39.74	39.54
Winter-2	35.31	37.92	38.89	41.69	40.85	38.93
Winter-3	37.71	39.65	40.03	42.12	41.69	40.24
Mean	34.51	36.08	37.50	38.87	38.81	

During this year the cocoon yield ranged from 35.60 kg to 41.69 kg with a mean of 38.81 kg. Finally, if we consider all forty generation season-wise, we will found that winter-3 season favour the cocoon yield more (40.24 kg) in comparison to lower value in summer-1 season (33.85 kg). Significantly higher cocoon yield/100 Dfls was found in all three rearings of winter season. The performance in the 2nd and 3rd rearings in rainy season was significantly better than the remaining three seasons i.e., two summer and 1st rainy. Also there was significant increase in cocoon yield/100 Dfls in each year up to 4th year. However, no significant difference was there between 4th year and 5th year mean values which was in conformity with stabilization of race (Table 6b).

Table 6b: Performance of analysis of variance of mean cocoon yield/100 dfls during rearing of forty generations along 40 rearing seasons.

Source	DF	F	CD at 5%
Year(Y)	4	275.07**	0.703
Seasons(S)	7	112.41**	0.889
Y x S	28	16.32**	1.987

When we compare the yearwise mean of yield/100dfls the data obtained (in kg) were 34.51, 36.08, 37.50 and 38.81 in year-1, year-2, year-3, year-4 and year-5 respectively (Table 8b). The yearwise critical difference value (CD) at 5% level was 0.703. Now, the yearwise improvement was recorded as 1.57 in year-2, 1.42 in year-3, 1.37 in year-4 and -0.06 in year-5. So, it was observed that the improvement upto year-4 was highly significant, as the values were more than critical value. But, it was not significant in year-5 as the values were less than CD value.

Again, the CD value of rearing in different seasons was 0.889. Here, the lowest value was obtained as 33.85 in summer-1. The next higher values (in kg) were 34.37, 35.77, 36.84, 37.71, 38.93, 39.54 and 40.24 in rainy-1, summer-2, rainy-2, rainy-3, winter-2, winter-1 and winter-3 respectively and the significant improvements were 1.92, 2.47, 0.87, 1.83 and 1.31 in summer-2, rainy-2, rainy-3, winter-1 and winter-3 value was found higher than CD value and was significant statistically (Table 6b).

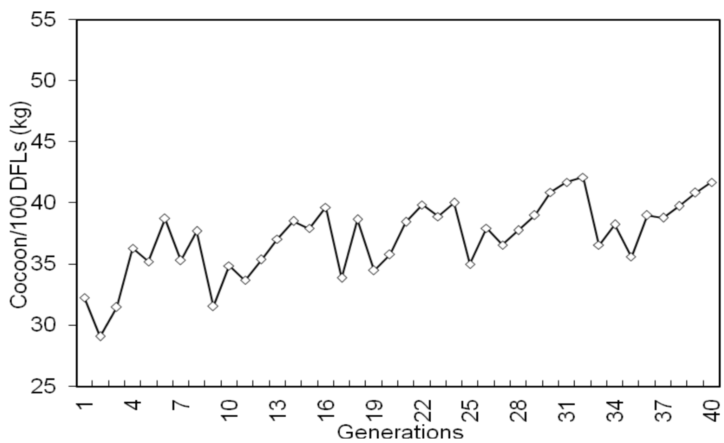


Fig 4: Performance of mean cocoon yield/100 dfls (kg) recorded during rearing of forty generations with specific breeding procedure in five years.

The yield per 100 disease free layings was recorded as 31.50 kg, 29.50 kg and 38.81 kg in case of better parent, mid parent and in the new breed(D^{+p}) respectively. The data recorded for BPH and MPH was 23.21% and 31.56% respectively (Table 12).

3.5. Pupation rate percentage: - This quantitative character is very much important where the cocoons are used for seed or egg preparation. It is calculated by dividing number of cocoons harvested with live pupae by number of worms counted after moult and converting it in to percentage. Cocoons with live pupae are determined by shaking the cocoons. So, aim of the breeder is to enhance the pupation rate percentage in new breed. During RBL breeding process (F1 to F8) we recorded minimum pupation percentage of 79.67% during F-1 and maximum pupation rate percentage of 95.23% during F7 (Figure 5). But, we are unable to increase the mean value over the parents (Table 9a). Next, during directional selection stage (F9 to F32), minimum value of 78.16% was found during F17 generation and maximum value of 97.27% during F30 generation. But the mean value was recorded highest during year-4 with 86.42%; and values of year-2 with 86.29% and with 86.03% during year-3. This was not much improved than the better parent, N^{+p} with 86.04% of pupation rate.

Table 7a: Performance of mean pupation rate percentage recorded during rearing of forty generations with specific breeding procedure in five years through different seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	79.67	82.15	78.16	80.85	82.60	80.68
Summer-2	80.31	83.27	82.19	81.38	80.41	81.51
Rainy-1	83.28	85.35	81.95	83.49	84.35	83.68
Rainy-2	81.09	81.32	82.36	82.17	83.19	82.03
Rainy-3	82.10	83.20	84.89	81.02	81.52	82.55
Winter-1	90.32	91.28	95.26	97.27	96.72	94.17
Winter-2	95.23	93.15	93.09	93.08	93.20	93.55
Winter-3	91.17	90.67	90.33	92.09	91.87	91.23
Mean	85.40	86.29	86.03	86.42	86.73	

Finally, during stabilization process (F33 to F40 generation), the recorded data showed the minimum value of 80.41% and maximum value of 96.72% during F34 and F38 generations respectively. Again, while considering the seasonal variation, the maximum value of 80.68 % was observed during winter-1 and summer-1 seasons respectively. Significantly higher pupation rate percentage was found (Table 9b) in all three rearings of winter season, which was much higher than rainy and summer. It cleared the fact that environment had a great impact on this character. While comparing the year wise improvement, it was observed that in 2nd year it was highest followed by 4th year and no significant improvement was observed in other years.

Table 7b: Performance of analysis of variance of mean pupation rate percentage during rearing of forty generations along 40 rearing seasons.

Source	DF	F	CD at 5%
Year(Y)	4	44.88**	0.411
Seasons(S)	7	961.97**	0.520
Y x S	28	20.74**	1.163

When we compare the yearwise mean of pupation rate percentage the data obtained (in percentage) were 85.40, 86.29, 86.03, 86.42 and 86.73 in year-1, year-2, year-3, year-4 and year-5 respectively. The yearwise critical difference value (CD) at 5% level was 0.411(Table 7b). Now, the yearwise improvement was recorded as 0.89 in year-2, -0.26 in year-3, 0.39 in year-4 and 0.31 in year-5. So, it was observed that the improvement in year-2 and year-4 were highly significant, as the values were more than critical value. But, it was not significant in year-3 and year-5 as the values were less than CD values.

Again, the CD value of rearing in different seasons was 0.520. Here, the lowest value was obtained as 80.68 in summer-1. The next higher values (in percentage) were 81.51, 82.03, 82.55, 83.68, 91.23, 93.55 and 94.17 in summer-2, rainy-2, rainy-3, rainy-1, winter-3, winter-2, and winter-1 respectively and the significant improvements were 0.83, 2.17, 0.52 and 11.62 in summer-2, rainy-1, rainy-3 and winter-1 as the values were more than CD values (Table 7b).

The pupation rate percentage was recorded as 86.04%, 83.74% and 86.74% in case of better parent, mid parent and in the new breed, D^{+p} respectively (Table 12). Here, the improvement was noticed at 0.81% and 3.58% in BPH and MPH respectively (Table 12).

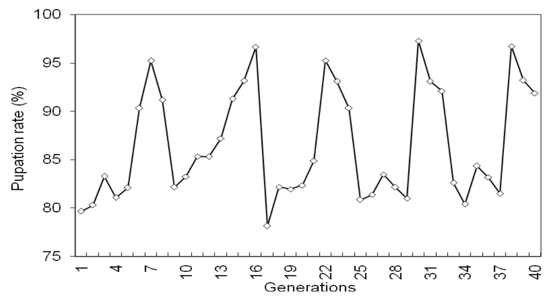


Fig 5: Performance of mean pupation rate (%) recorded during rearing of forty generations with specific breeding procedure in five years.

3.6. Single Cocoon Weight (g):-

Cocoon weight includes both the weight of cocoon shell and pupa. It gives a rough idea about the race and relates to the production of cocoons per unit number of eggs. So, breeders aim to increase the cocoon wt. of a new breed.

During 1st eight generations i.e. during RBL breeding process, minimum single cocoon weight (single cocoon weight) of 0.997g was recorded at F3 generation and maximum of 1.226g at F8 generation (Table 8a).

Here, we observed that the mean value of 1.091 g was better than both its parents with 0.890 g and 1.042 g of single cocoon weight in N^p and N^{+p} respectively (Table 2). Again during F9 to F32 generations, where directional selection pressure was given, the single cocoon weight was recorded minimum value of 1.062 g and max value of 1.267 g in F12 and F30 generations respectively (Table 8a). While comparing the year-wise mean value, a trend of gradual increase of cocoon weight was also recorded at 1.125 g in year-2, 1.139 g in year-3 and 1.163 g in year-4 generations which were better than its parents (Figure 7). Next during F33 to F40 generations i.e. where selection pressure was not given to stabilize the race, the minimum value of 1.084 g and max value 1.206 g of cocoon weight was recorded (Table 8a).

Table 8a: Performance of mean single cocoon weight (g) recorded during rearing of forty generations with specific breeding procedure in five years through different seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	1.068	1.064	1.019	1.072	1.197	1.084
Summer-2	1.121	1.128	1.119	1.058	1.050	1.095
Rainy-1	0.997	1.097	1.083	1.134	1.141	1.090
Rainy-2	1.041	1.062	1.101	1.166	1.121	1.098
Rainy-3	1.080	1.176	1.167	1.179	1.194	1.159
Winter-1	1.123	1.148	1.231	1.267	1.261	1.206
Winter-2	1.076	1.108	1.243	1.249	1.207	1.177
Winter-3	1.226	1.213	1.151	1.178	1.159	1.185
Mean	1.091	1.125	1.139	1.163	1.166	

Table 8b: Performance of analysis of variance of mean Single Cocoon Weight (g) during rearing of forty generations along 40 rearing seasons.

Source	DF	F	CD at 5%
Year(Y)	4	140.93**	0.007
Seasons(S)	7	261.49**	0.008
Y x S	28	62.52**	0.019

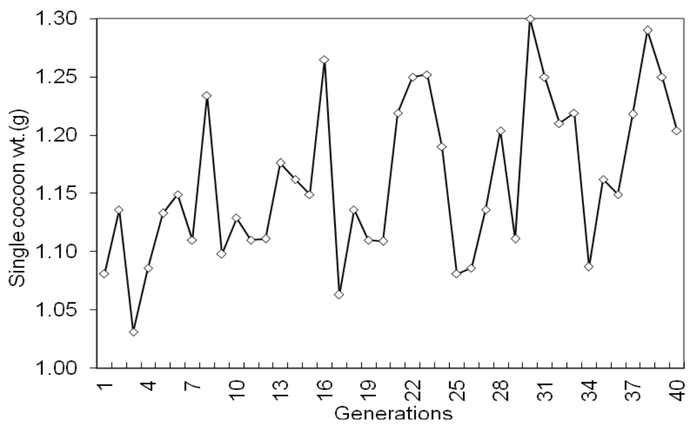


Fig 6: Performance of mean single cocoon weight (g) recorded during rearing of forty generations with specific breeding procedure in five years.

While statistically analyzing the data of table no.10b, it was observed that highly significant improvement of cocoon weight was obtained in 1st winter season than other seasons. Again, the performances of last rainy and all the winter seasons were better than all summer and 1st two rainy seasons. However, highly significant improvement of single cocoon weight was observed in each year up to 4th year.

When we compare the yearwise mean of single cocoon weight the data obtained (in g) were 1.091, 1.125, 1.139, 1.163 and 1.166 in year-1, year-2, year-3, year-4 and year-5 respectively. The yearwise critical difference value (CD) at 5% level was 0.007. Now, the yearwise improvement was recorded as 0.034 in year-2, 0.014 in year-3, 0.024 in year-4 and 0.003 in year-5. So, it was observed that the improvement upto year-4 were highly significant, as the values were more than critical value.

Again, the CD value of rearing in different seasons was 0.008. Here, the lowest value was obtained as 1.084 in summer-1. The next significantly higher values (in g) were 1.095, 1.098, 1.159 and 1.206 in summer-2, rainy-2, rainy-3 and winter-1 respectively and the consecutive improvements were 0.011, 0.003, 0.061, and 0.047. Among these values in rainy-3 and winter-1 were highly significant statistically.

The single cocoon weight was recorded as 1.042 g, 0.966 g and 1.166 g in case of better parent, mid parent and in the new breed, D^{+p} respectively. Here, the percentage of gains in the new breed was 11.90% and 20.70% in case of BPH and MPH values respectively. Both shell weight and pupal weight were contributed in this improvement.

3.7. Single Shell Weight (cg):-

Shell weight is one of the desirable characters for silkworm breeder, because this quantitative character gives an idea of silk content in the cocoons. It is measured by cutting the cocoons at one end and removing the pupa inside. Generally, higher the single shell wt., better the breed as it may give higher filament length.

Table 9a: Performance of mean single shell weight (cg) recorded during rearing of forty generations with specific breeding procedure in five years through different Seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	13.8	13.8	14.1	14.4	15.7	14.4
Summer-2	14.1	13.6	15.1	13.8	14.6	14.2
Rainy-1	12.7	13.7	14.4	15.2	14.9	14.2
Rainy-2	12.7	13.7	15.0	15.6	15.4	14.5
Rainy-3	13.9	14.9	15.1	14.9	16.1	15.0
Winter-1	13.9	14.9	15.8	17.4	17.4	15.9
Winter-2	13.1	14.4	16.2	17.6	16.7	15.6
Winter-3	15.3	14.8	15.1	17.0	16.4	15.7
Mean	13.7	14.2	15.1	15.7	15.9	

Again, during directional selection phase (F9 to F32) this character was further enhanced to maximum 17.6 cg during F31 generation and the minimum value was observed during F10 generation in the tune of 13.6 cg. While comparing mean value from year-2 to year-4 we found that a gradual increase of single shell wt. (e.g. 14.2 cg, 15.1 cg and15.7 cg) was visible (Table 9a).

Table 9b: Performance of analysis of variance of mean single shell weight (cg) during rearing of forty generations along 40 rearing seasons.

Source	DF	F	CD at 5%
Year(Y)	4	318.57**	0.101
Seasons(S)	7	111.43**	0.205
Y x S	28	21.00**	0.409

Finally, during F33 to F40 generation (stabilization phase), the recorded data showed that the single shell wt. of newly developed breed, D^{+p} ranged from 14.6 cg to 17.4 cg. The season-wise data depicted that lower mean value was of 14.2 cg during summer-2 and rainy-1 and higher mean value was of 15.9 cg during winter-1 season.

Statistically it can be interpreted that (Table 9b) performance of last four seasons i.e. 3rd rainy and all winter seasons were significantly higher than 1st four seasons i.e. two summer and 1st two rainy seasons. However, there was a significant improvement of shell weight in each year up to 4th year.

When we compare the yearwise mean of single shell weight the data obtained (in cg) were 13.7, 14.2, 15.1, 15.7 and 15.9 in year-1, year-2, year-3, year-4 and year-5 respectively (Table 9b). The yearwise critical difference value (CD) at 5% level was 0.101. Now, the yearwise improvement was recorded as 0.5 in year-2, 0.9 in year-3, 0.6 in year-4 and 0.2 in year-5. So, it was observed that the improvement upto year-5 were highly significant, as the values were more than critical value. Again, the CD value of rearing in different seasons was 0.205. Here, the lowest value was obtained as 14.2 in summer-2 and rainy-1 seasons. The next higher values (in cg) were 14.5, 15.0 and 15.9 in rainy-2, rainy-3, and winter-1 respectively and the consecutive improvements were 0.3, 0.5 and 0.4. Among these values in rainy-3 and winter-1 were highly significant statistically as the values are more than CD value (Table 9b). It is one of the important economic parameters. The single shell weight was recorded as 12.90 cg, 11.75 cg and 15.90 cg in case of better parent, mid parent and in the new breed, D^{+p} respectively. The improvement was in the tune of 24.71% and 36.13% in respect of BPH and MPH (Table 12).

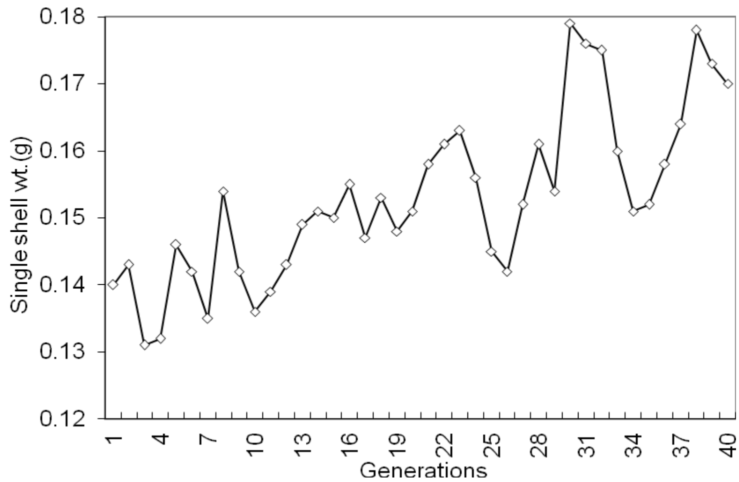


Fig 7: Performance of mean single shell weight (cg) recorded during rearing of forty generations with specific breeding procedure in five years.

3.8. Shell percentage:-

Shell percentage is determined by dividing shell weight by cocoon weight and converting it into percentage. This parameter is also important as far as the breeder is concerned because this data gives an idea of silk content in the cocoons and higher shell percentage is a preferred criterion.

Table 10a: Performance of mean Shell percentage recorded during rearing of forty generations with specific breeding procedure in five years through different Seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	12.95	12.93	13.83	13.40	13.12	13.24
Summer-2	12.59	12.05	13.47	13.07	13.89	13.01
Rainy-1	12.71	12.51	13.33	13.38	13.08	13.00
Rainy-2	12.15	12.87	13.61	13.37	13.75	13.15
Rainy-3	12.89	12.67	12.95	13.85	13.46	13.16
Winter-1	12.36	12.99	12.88	13.76	13.79	13.16
Winter-2	12.16	13.04	13.02	14.08	13.83	13.23
Winter-3	12.47	12.24	13.10	14.45	14.12	13.28
Mean	12.53	12.66	13.27	13.67	13.63	

One of the considerations of RBL development was to improve the breed with higher Shell percentage by repeated backcrossing with N^{+p} followed by selection. During this period we recorded minimum Shell percentage of 12.15% in F4 generation and maximum Shell percentage of 12.95% in F1 generation which was somewhat better than N^{p} and N^{+p} having Shell percentage of 11.91% and 12.39% respectively (Table10a).

Table 10b: Performance of analysis of variance of mean Shell percentage during rearing of forty generations along 40 rearing seasons.

Source	DF	F	CD at 5%
Year(Y)	4	214.25**	0.102
Seasons(S)	7	4.93**	0.129
Y x S	28	15.04**	0.288

During directional selection procedure selection pressure was given on this character for maximizing the Shell percentage and in every consecutive generation cocoons with better Shell percentage was selected till F32 generation (Figure 8). Data recorded showed that minimum Shell percentage of 12.05% during F10 generation and higher Shell percentage of 14.45% during F32 generation. During this period the mean value was also increased from 12.66% during Year-2 to 13.67% during Year-4. This result indicated that Shell percentage increased during directional selection procedure.

As far as stabilization phase (F33 to F40) was concerned, minimum value of 13.08% during F35 and maximum value of 14.12% during F40 were observed with a mean value of the year in the tune of 13.63%.

Seasonal variation of the data was not much distinct which ranged from 13.00% during Rainy-1 and 13.28% during Winter-3 season.

The statistical analysis of the data showed that as far as the performance of shell percentage was concerned (table no.10b), seasonal influence was not much profound and there was no significant difference. However, there was a significant improvement of shell percentage in each year up to 4th year.

When we compare the yearwise mean of shell percentage the data obtained (in percentage) were 12.53, 12.66, 13.27, 13.67 and 13.63 in year-1, year-2, year-3, year-4 and year-5 respectively. The yearwise critical difference value (CD) at 5% level was 0.102. Now, the yearwise improvement was recorded as 0.13 in year-2, 0.61 in year-3, 0.40 in year-4 and -0.04 in year-5. So, it was observed that the improvement upto year-4 were highly significant, as the values were more than critical value except in year-5 (Table 10b).

Again, the CD value of rearing in different seasons was 0.129. Here, the lowest value was obtained as 13.00 in rainy-1. The next noticeable higher values (in percentage) were 13.15, 13.23, 13.24, and 13.28 in rainy-2, winter-2, summer-1 and winter-3 respectively and the consecutive improvements were 0.15, 0.08, 0.01and 0.04. Among these value in rainy-2 was highly significant statistically as the values are more than CD value.

Shell percentage gives us a hint about the quantity of silk which can be obtained in a particular breed. The shell percentage was recorded as 12.39%, 12.15% and 13.63% in case of better parent, mid parent and in the new breed, D^{+p} respectively (Table 12). Here, improvement was a noticeable one, to the extent of 10.01% and 12.18% in cases of BPH and MPH respectively.

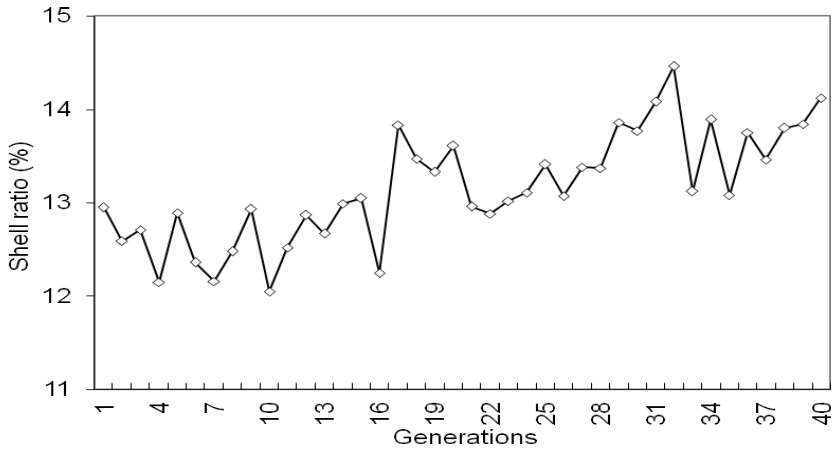


Fig 8: Performance of mean Shell% recorded during rearing of forty generations with specific breeding procedure in five years

3.9. Filament Length (m):-

Filament length (in meters or m) can be determined by unwinding the silk from cocoons during reeling procedure. Enhancement of silk filament length is one of the aims of a breeder which gives higher production of silk.

Table 11a: Performance of mean filament length (m) recorded during rearing of forty generations with specific breeding procedure in five years through different Seasons.

Seasons	Year-1	Year-2	Year-3	Year-4	Year-5	Mean
Summer-1	330.80	346.80	352.20	416.60	412.00	371.68
Summer-2	323.60	361.80	366.60	411.80	400.40	372.84
Rainy-1	345.40	352.80	374.40	393.60	404.60	374.16
Rainy-2	355.60	356.80	387.40	400.40	413.80	382.80
Rainy-3	341.40	343.20	387.60	418.40	433.40	384.80
Winter-1	367.80	370.20	402.00	428.60	445.40	402.80
Winter-2	356.60	385.80	413.60	451.00	459.40	413.28
Winter-3	349.20	373.00	425.20	463.20	467.80	415.68
Mean	346.30	361.30	388.62	422.95	429.60	

During RBL breeding, filament length ranged from 323.60 m during F2 generation to 367.80 m during F6 generation with mean value of 346.30 m which was lower than its parents with 378.3 m and 405.7 m of filament length respectively (Table 11a). However, during directional selection procedure where selection pressure was given this data ranged from 343.20 m in F13 to 463.20 m in F32 generations respectively and its mean value ranged from 361.30 m in year-2 to 422.95 m in year-4 respectively (Figure 9). While comparing these data with parents some improvement of filament length was observed than its parents (N^p and N^{+p}) (Table 11a). Finally, during stabilization rearing process (F33 to F40) the recorded data showed that the filament length ranged from 400.40 m during F34 to 467.80 m during F40 generation with mean value 429.60 m (Table 11a).

Table 11b: Performance of analysis of variance of mean filament length (m) during rearing of forty generations along 40 rearing seasons.

Source	DF	F	CD at 5%
Year(Y)	4	189.74**	7.123
Seasons(S)	7	25.94**	9.009
Y x S	28	2.68**	20.146

Season-wise variations were also observed in this data which varied from 371.68 m during Summer-1 to 415.68 m during Winter-3. Statistically significant higher filament length was found in rainy-2 (382.80 m) and winter-2 (413.28 m) seasons (table 13b). There were not any significant differences in all other seasons as far as the filament length was concerned. However, there was significant increase of filament length in each year.

When we compare the year-wise mean of filament length, the data obtained were 346.30 m, 361.30 m, 388.62 m, 422.95 m and 429.60 m in year-1, year-2, year-3, year-4 and year-5 respectively. The year-wise critical difference value (CD) at 5% level was 7.123(Table 13b). Now, the year-wise improvement was recorded as 15.00 in year-2, 27.32 in year-3, 34.33 in year-4 and 6.65 in year-5. So, it was observed that the improvement upto year-4 were highly significant, as the values were more than critical value except in year-5 which signified the stabilization of character. Again, the CD value of rearing in different seasons was 9.009. Here, the lowest value was obtained as 371.68 m in summer-1. The next higher values (in meters) were 372.84, 374.16, 382.80, 384.80, 402.80, 413.28, and 415.68 in summer-2, rainy-1, rainy-2, rainy-3, winter-1, winter-2, and winter-3 respectively and the consecutive improvements (in meters) were 1.16, 1.32, 8.64, 2.00, 18.00, 10.48 and 2.4 (Table 13b). Among these values in rainy-2, winter-1 and winter-2 were highly significant statistically as the values were more than CD value.

Filament length is another important economic parameter which determines the quantity of silk present in a particular breed. In the present study the filament length was recorded as 405.7 m, 392.0 m and 429.6 m in case of better parent, mid parent and in the new breed, D^{+p} respectively (Table 12). The improvement of 5.89% and 9.59% in cases of BPH and MPH respectively in the new breed was observed in this character.

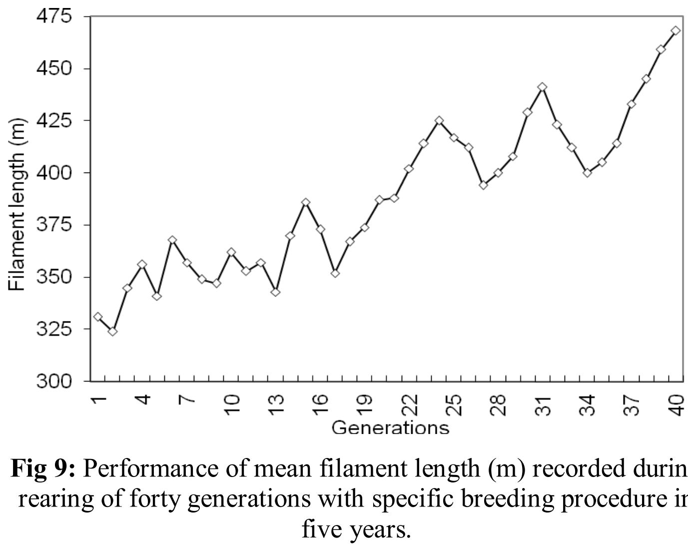


Fig 9: Performance of mean filament length (m) recorded during rearing of forty generations with specific breeding procedure in five years.

Table 12: Heterosis percentage over mid and better parent values (MPH & BPH) of newly developed breed (D^{+p}).

Sl.No	Traits	MPV	BPV	D^{+p}	MPH	BPH
1	Fecundity(numbers)	401	426	486.27	21.20	13.30
2	Hatching (percentage)	89.43	90.05	91.30	2.09	1.39
3	E.R.R/Survival (percentage)	79.85	82.40	84.63	5.99	2.71
4	Yield/100 dfls (kg)	29.50	31.50	38.81	31.56	23.21
5	Pupation rate (percentage)	83.74	86.04	86.73	3.58	0.81
6	Single Cocoon wt.(g)	0.966	1.042	1.166	20.70	11.90
7	Single School wt. (cg)	11.68	12.75	15.90	36.13	24.71
8	Shell (percentage)	12.15	12.39	13.63	12.18	10.01
9	Filament length (m)	392	405.7	429.6	9.59	5.89

Note: BPV-Better parent values, MPV- Mid parent values

Thus, an improved multivoltine breed, D^{+p} suitable for eastern Indian climatic conditions was developed having higher single shell weight, yield/100 dfls, fecundity, single cocoon weight, shell percentage and filament length. In addition, genetic variability between parents and the new breed was studied through investigating the electrophoretic profiles of esterases and acid phosphatase [18].

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