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Evaluation of Bivoltine Silkworm Breeds of *Bombyx mori* L. under West Bengal Conditions

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Abstract:
The Indian sericulture industry is beset with many problems. One of the main problems is the inability to produce quality silk of international grade. The quality silk can be produced only form bivoltines. The bulk of silk produced in India is from Multivoltines which are of inferior quality. Therefore, it is highly pertinent to have more productive bivoltine silkworm breeds capable of producing quality silk. However, the hot climatic conditions of India is not conducive to rear productive bivoltines. Hence, there is an urgent need to develop bivoltine breeds which can yield stable crops under the adverse climatic conditions. Accordingly, efforts should be focussed by the silkworm breeders to develop bivoltine breeds with genetic plasticity to buffer against the adverse climatic conditions. The main constraint of the tropical environment is the high temperature coupled with high and low humidity. It is a well established fact that the bivoltines are highly vulnerable to high temperature coupled with high and low humidity especially in the late instars. The hot climatic conditions of tropics prevailing particularly in summer are contributing to the poor performance of the bivoltine breeds and the most important aspect is that many quantitative characters such as viability and cocoon traits decline sharply when temperature is high. Therefore, it is highly pertinent to identify more number of bivotine breeds which can withstand adverse climatic conditions. Accordingly, the present study was carried out to evaluate and identify suitable bivoltine breeds under West Bengal conditions of India.

Keywords: *Bombyx mori* L, bivoltine, evaluation, climatic conditions

1.0 Introduction:
The success of sericulture industry depends upon several factors of which the impact of the environmental factors such as biotic and abiotic factors is of vital importance. Among the abiotic factors, temperature plays a major role on growth and productivity of silkworm, as it is a poikilothermic (cold blooded) insect (Benchamin and Jolly,1986). It is also known that the late age silkworms prefer relatively lower temperature than young age and fluctuation of temperature during different stages of larval development was found to be more favourable for growth and development of larvae than constant temperature. There is ample literature stating that good quality cocoons are produced within a temperature range of 22-27°C and above these levels makes the cocoon quality poorer (Krishnaswami *et al.*, 1973).

The effect of temperature higher than 30°C on silkworm larvae was reported earlier by Takeuchi *et al.,* 1964 and Ohi and Yamashita, 1977. Huang *et al.* 1979 and He and Oshiki, 1984 used survival rate of silkworm as a main characteristic for evaluating thermo-tolerance. The hot climatic conditions of tropics prevailing particularly in summer are contributing to the poor performance of the bivoltine breeds and the most important aspect is that many quantitative characters such as viability and cocoon traits decline sharply when temperature is higher than 28°C (Shibukawa, 1964). The continued efforts for the improvement of cocoon characters of domesticated silkworm were aimed at increased quality silk production. The main objective of silkworm rearing is to produce qualitatively and quantitatively superior cocoons, which in turn will have a direct bearing on the raw silk production. Therefore, it becomes imperative or essential to develop bivoltine breeds/hybrids which can with stand high temperature stress conditions. Sericulture, the viable agro-based industry aptly matches the socio-economic backdrop of rural India. One of the main aims of the breeders is to recommend silkworm breeds/hybrids to farmers that are stable under different environmental conditions and minimize the risk of falling below a certain yield level. Silkworm breeds that are reared over a series of environment exhibiting less variation are considered stable. The climatic conditions prevailing in the tropics are most unpredictable and the problems of tropical sericulture are occurrence of aggravated silkworm diseases, unsuitable mulberry leaf for bivoltine silkworms and lack of sustainable silkworm breeds for effective selection of desirable characters. In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environment. The pre-requisite of summer breeds is healthiness and adaptability to adverse conditions of high temperature, low food quality, relatively higher economic traits, with potential for increased cocoon production.

In fact, Genotype environment interactions are of major importance to the silkworm breeders while developing new breeds. The concept of genotype and environment interactions has been well documented in both plants and animal species (Griffing and Zsiros, 1971). Although, it is not necessary to breed a genotype that is adapted to all ecological conditions, breeding methods can be designed towards producing a high yielding one with a considerable degree of general adaptability (Eberhart and Russel, 1966). Further, the methods of management practices, effective disease control through sanitation and raising suitable mulberry leaf under diversified environmental factors prevailing in the tropics plays an important role in the expression of quantitative traits of the silkworm (Benchamin *et al.,* 1983) . Summer breeds are having significant importance in increasing cocoon production through rearing bivoltine hybrids round the year in tropical areas. The advantages of summer hybrids are high pupation rate, adaptabilities to high temperature coupled with high and low humidity and inferior food quality during the rearing. Stable cocoon crop under the bad conditions of high temperature with low quality mulberry leaves are difficult, but summer breeds/hybrids should have the potentiality for increasing production under such un-favourable weather conditions. Keeping this in view, this study has been undertaken to evaluate bivoltine breeds under West Bengal conditions

2. 0 Materials and Methods:

The main objective of the study was to evaluate different bivoltine breeds under West Bengal conditions. Considerable variation exists among the bivoltine silkworm breeds at different rearing conditions, which opens an avenue for selection of suitable bivoltine breeds for tropical conditions Of West Bengal.

2.1 Materials

The following breeds were screened under high temperature conditions to select suitable breeding resource materials.

1) CSR2, 2) CSR4 , 3) CSR6, 4) CSR18, 5) CSR19, 6) CSR26, 7) CSR46, 8) CSR47) 9) Gen3, 10) BHR2, 11) BHR3, 12) SK3, 13) SK4, 14) SK4C, 15) D6PN 16) SK6, 17) SK7, 18) KSO1,19) SP2 20) NP2, 21) Dun6, 22) Dun21, 23) Dun22, 24) ATR16 , 25) ATR29 and 26) NB4D2

2.2. Methods

Silkworm rearing was conducted following the standard method under natural conditions and also at recommended temperature and humidity conditions Plastic collapsible mountages were used for mounting the ripened larvae. After 48 hours of mounting, when the larvae formed hammock, the mountages were turned upside down. Cocoon harvesting was carried out on the 7th day of spinning. The cocoons were deflossed and the defective ones were sorted out. Assessment was carried out on the subsequent day. The survival rate was calculated as the number of live pupae to the number of larvae treated.

2.3. Parameters Studied:

Various quantitative traits such as pupation rate, cocoon weight, cocoon shell weight and cocoon shell percentage were calculated. The characteristic features of the selected breeding resource materials are given below.

3.0 Results and Discussion:

The performance of the bivoltine breeds under optimal temperature (25 ±1°C) and humidity (70 ± 5 %) conditions is presented in Table 1. The fecundity of the 25 breeds considered for the study at 25 ±1°C and 70 ± 5 % RH ranged from 510 to 610 with the lowest of 510 recorded for CSR19 and the highest of 610 recorded for CSR2. The yield/10,000 larvae by number at 25 ±1°C and 70 ± 5 % RH ranged from 8563 to 9154 with the lowest of 8563 recorded for CSR4 and the highest of 9154 recorded for SK6. The yied/10,000 larvae by weight at 25 ±1°C and 70 ± 5 % RH ranged from 13.390 to 15.798 kg with the lowest of 13.390 kg recorded for NB4D2 and the highest of 15.798 kg recorded for Dun6. The cocoon weight at 25 ±1°C and 70 ± 5 % RH ranged from 1.512 to 1.765 g with the lowest of 1.512 g recorded for CSR19 and the highest of 1.765 g recorded for

CSR2. The cocoon shell weight at 25 ±1°C and 70 ± 5 % RH ranged from 0.311 to 0.432 g with the lowest of 0.311 g recorded for BHR3 and the highest of 0.432 g recorded for CSR2. The cocoon shell percentage at 25 ±1°C and 70 ± 5 % RH ranged from 20.4 to 24.5 % with the lowest of 20.4 % recorded for BHR3 and the highest of 24.5% recorded for CSR2.

The performance of the bivoltine breeds under normal temperature (32 ±1°C) and humidity (85 ±5 %) conditions is presented in Table 2. The fecundity of the 25 breeds considered for the study at 32 ±1°C and 85 ± 5 % RH ranged from 387 to 530 with the lowest of 387 recorded for Dun6 and the highest of 530 recorded for NP2. The yield/10,000 larvae by number at 32 ±1°C and 85 ± 5 % RH ranged from 0 to 9187 with the lowest of 0 recorded for CSR2, CSR4, CSR6, CSR26, CSR18, CSR19, CSR46 and CSR47 and the highest of 9187 recorded for SK4C. The yied/10,000 larvae by weight at 32 ±1°C and 85 ± 5 % RH ranged from 0 to 14.230 kg with the lowest of 0 kg recorded for CSR2, CSR4, CSR6, CSR26, CSR18, CSR19, CSR46 and CSR47 and the highest of 14.230 kg recorded for D6PN. The cocoon weight at 32 ±1°C and 85 ± 5 % RH ranged from 0 to 1.547 g with the lowest of 0 g recorded for CSR2, CSR4, CSR6, CSR26, CSR18, CSR19, CSR46 and CSR47 and the highest of 1.557 g recorded for SK7. The cocoon shell weight at 32 ±1°C and 85 ± 5 % RH ranged from 0 to 0.320 g with the lowest of 0 g recorded for CSR2, CSR4, CSR6, CSR26, CSR18, CSR19, CSR46 and CSR47 and the highest of 0.320 g recorded for SK7. The cocoon shell percentage at 32 ±1°C and 85 ± 5 % RH ranged from 0 to 23.9 % with the lowest of 0 % recorded for CSR2, CSR4, CSR6, CSR26, CSR18, CSR19, CSR46 and CSR47 and the highest of 23.9% recorded for Dun6. The yield/10000 larvae by number recorded for all the breeds at two different temperature treatments is depicted in Fig.1.

The breeding of silkworm since long has been aimed towards evolving of superior and hardy breeds either by means of selection alone or by combining out-crossing or backcrossing with selection in the subsequent generations. The final aim of the breeder is primarily to evolve a breed which can give rise to stabilized crops and secondly to improve both quantity and quality of silk (Tazima, 1984). The breeding of silkworm races probably dates back to the beginning of the history of silkworm rearing, but it has made great progress rather recently (Hirobe, 1968). Sericulturally advanced countries like Japan has achieved remarkable progress by executing systematic breeding plans for the development of productive races. In silkworms, studies carried out for various characters have shown that the characters could be changed to suit the breeders choice, since selection for one trait has correlation with genetic change of other characters.

The selection of breeding resource material helps the breeder to successfully amalgamate desired traits. Appropriate experimental design, selection methods employed in fixing the major traits contributing to the improved cocoon yield leads to the success of any breeding programme. Besides, understanding the genetic diversity of parental strains to be utilized in the breeding programme by their systematic evaluation, critical assessment of their quantitative nature which is greatly influenced by the environmental factors such as temperature, light, relative humidity, nutrition and rearing techniques which paves the way for the breeder for effective utilization (Kogure, 1933; Legay, 1958; Ueda and Lizuka, 1962; Suzuki *et al.*, 1962; Yokoyama,1963; Arai and Ito, 1967; Horie *et al*., 1967; Naseema Begum *et al.,* 2001 and Sudhakar Rao *et al.*, 2001. According to Allard and Bradshaw, 1964, performance of the strain itself in a given environment indicates its superiority. While evaluation, emphasis was given on the phenotypic expression of traits of economic importance under different temperature conditions. However, as the objective of the study was for greater viability and high productivity merits, equal importance was given on these two traits while selection of parents. The significant variations observed in the phenotypic manifestation for the traits analyzed can be attributed to the genetic constitution of the breeds and their degree of expression to which they are exposed during their rearing. Such variations in the manifestation of phenotypic traits of the breeds studied can be ascribed to the influence of environmental conditions. Variable gene frequencies at different loci make them to respond differently. The results are in line with the findings of (Watanabe,1928; Hassanein and Sharawy, 1962; Krishnaswami and Narasimhanna, 1874; Ueda *et al*., 1975;Rajanna, 1989;Raju, 1990; Maribashetty, 1991;Kalpana, 1992;Nirmal Kumar,1995; Basavaraja, 1996 and Sudhakar Rao et al., (2001).

It is important to measure the phenotypic expression of the major contributing traits of economic importance in the silkworm strains under diversified environmental conditions to understand the genetic endowment pertaining to adaptability and productivity. The balancing of desirable traits during the course of the breeding for varied climatic conditions is a challenging task for the breeder. The choice of parental material is critical and difficult to evaluate all the available silkworm breeds,. However, a few strains of known genetic background, pedigree and specific traits desirable for the new breeding programme have to be taken into consideration while evaluating the breeding material. It is also equally important to understand the traits related to productivity and viability. All the breeds selected for the evaluation are having one or more desirable traits as per the objectives of the present study. In the present study, which envisages to evaluate bivoltine breeds to identify more resistant bivoltines that can give rise to stable cocoon crops with better viability, even though productivity is low compared to the existing productive bivoltine breeds that are currently used in the field. In silkworms, the correlation for some characters is positive and for some it is negative (Gamo and Ichiba, 1971 and Gamo, 1976). Such a negative correlation is observed for the traits productivity and viability and hence the attempt made was to increase the viability of the developed breeds. Moreover as suggested by Lekuthai and Butrachand (1974) and Strunnikov and Strunnikov (1986), the selection parameters were primarily aimed at improving the viability character such as yield by number without sacrificing much of the productivity traits like cocoon weight, cocoon shell weight and yield by weight. In addition, during later generations of inbreeding, selection was applied to select desired genotypes to improve the traits of commercial importance like viability and productivity as suggested by Mano (1993 and 1994) to improve the yield of bivoltines.

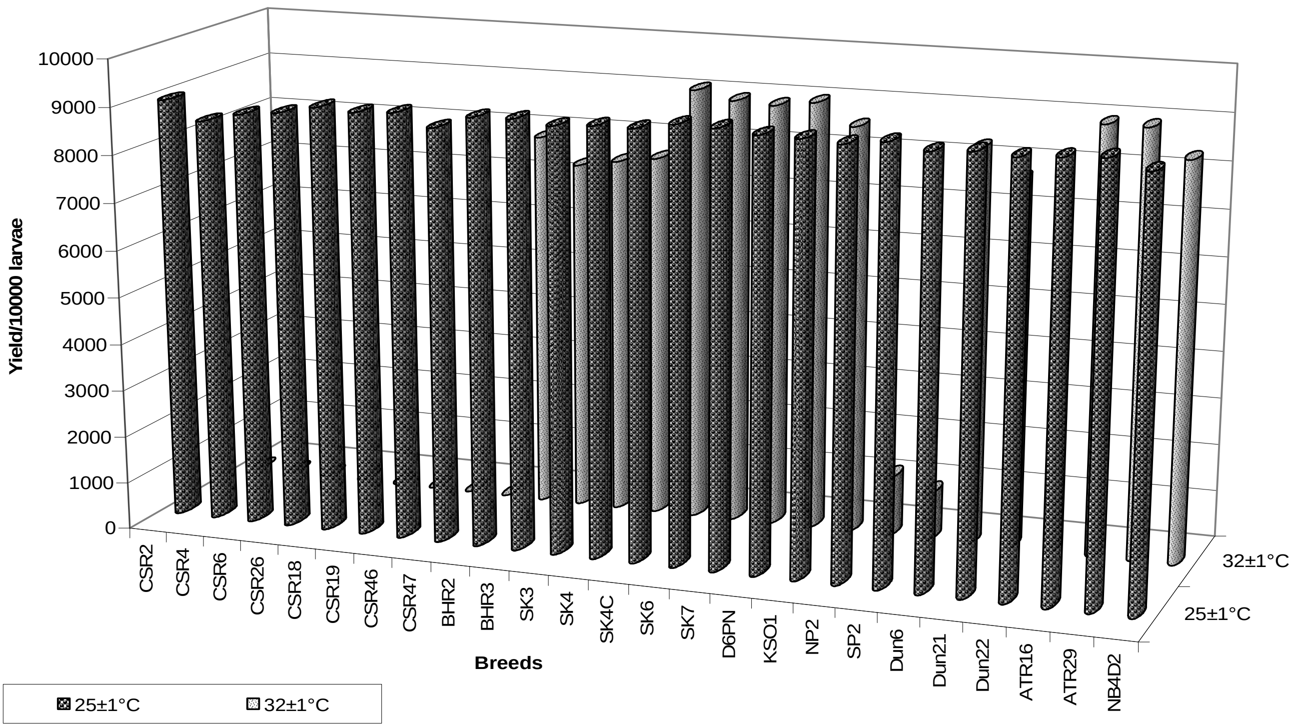
Table 1: Performance of bivoltine breeds under optimal temperature (25 ±1°c) and humidity (70 ±5 %) conditions

Sl. No.	Breed	Fecundity	Yield/10000 larvae		Cocoon weight	Cocoon Shell weight	Cocoon Shell Percentage
			By No.	By Wt			
1	CSR2	610	8956	15.785	1.765	0.432	24.5
2	CSR4	542	8563	13.859	1.627	0.366	22.5
3	CSR6	524	8758	13.885	1.589	0.356	22.4
4	CSR26	516	8843	13.857	1.574	0.357	22.7
5	CSR18	508	9012	13.725	1.523	0.332	21.8
6	CSR19	510	8958	13.544	1.512	0.321	21.2
7	CSR46	527	9008	14.285	1.589	0.362	22.8
8	CSR47	536	8755	14.112	1.612	0.356	22.1
9	BHR2	535	9023	13.910	1.542	0.321	20.8
10	BHR3	545	9043	13.780	1.525	0.311	20.4
11	SK3	524	8956	13.810	1.543	0.316	20.5
12	SK4	521	9012	13.925	1.546	0.320	20.7
13	SK4C	532	9016	13.942	1.547	0.322	20.8
14	SK6	554	9154	13.950	1.525	0.318	20.9
15	SK7	543	9136	14.905	1.543	0.319	20.7
16	D6PN	538	9053	13.992	1.546	0.322	20.8
17	KSO1	546	9034	13.935	1.543	0.335	21.7
18	NP2	523	8987	14.065	1.565	0.335	21.4
19	SP2	528	9087	14.012	1.542	0.327	21.2
20	Dun6	568	8956	15.798	1.764	0.427	24.2
21	Dun21	576	9012	15.220	1.689	0.410	24.3
22	Dun22	565	8965	15.014	1.675	0.404	24.1
23	ATR16	548	9015	14.126	1.567	0.368	23.5
24	ATR29	554	9065	14.255	1.573	0.373	23.7
25	NB4D2	528	8865	13.490	1.522	0.327	21.5

Table 2: Performance of bivoltine breeds under normal temperature (32 ±1°C) and humidity (85 ±5 %) conditions

Sl. No.	Breed	Fecundity	Yield/10000 larvae		Cocoon weight	Cocoon Shell weight	Cocoon Shell Percentage
			By No.	By Wt			
1	CSR2	525	0	0	0	0	0
2	CSR4	515	0	0	0	0	0
3	CSR6	485	0	0	0	0	0
4	CSR26	475	0	0	0	0	0
5	CSR18	452	0	0	0	0	0
6	CSR19	425	0	0	0	0	0
7	CSR46	445	0	0	0	0	0
8	CSR47	438	0	0	0	0	0
9	BHR2	430	7970	10.090	1.266	0.210	16.6
10	BHR3	517	7430	9.400	1.266	0.259	20.4
11	SK3	512	7568	10.568	1.265	0.257	20.3
12	SK4	480	7687	11.258	1.289	0.265	20.6
13	SK4C	417	9187	12.580	1.273	0.221	17.4
14	SK6	496	9020	13.030	1.445	0.295	20.4
15	SK7	415	8970	13.880	1.547	0.320	20.7
16	D6PN	455	9085	14.230	1.465	0.276	18.9
17	KSO1	431	8640	8.450	1.317	0.262	20.0
18	NP2	530	1260	1.400	1.096	0.206	18.8
19	SP2	486	1000	1.180	1.164	0.211	18.1
20	Dun6	387	8370	10.700	1.278	0.306	23.9
21	Dun21	404	7790	10.520	1.351	0.311	23.0
22	Dun22	414	7910	10.590	1.340	0.308	22.9
23	ATR16	410	9020	13.120	1.365	0.275	20.5
24	ATR29	499	9012	12.730	1.341	0.272	20.3
25	NB4D2	397	8420	10.930	1.297	0.248	19.2

Fig.1 Yield/10000 larvae by number at two different temperature treatment



Thermal acclimatization and stress have been studied during different developmental stages of insects (larvae, pupae and adults) as well as different physiological conditions (Melanby, 1954; Lee et al., 1988) . Surviving a thermal stress depends in part upon the individual organism's stress history. Brief exposure to a mild stress can protect an organism against a normally lethal stress. Brief exposure to a mild temperature than the optimum temperature induces tolerance (Thermo-tolerance) in insects. Once thermo-tolerance has been induced, it may persist for days (Carretero et al., 1991; Yocum and Denlinger, 1992). Some stresses will even increase an organism's sensitivity to a future stress. Survival of high temperature stress appears to be energy dependent in insects and the loss should be compensated by nutrition (Haveman and Hahn, 1981; James et al., 1986).

The fluctuations noticed in the pupation rate among the bivoltine breeds considered for this study can be partially attributed to the influence of environmental factors and the interaction of alleles responsible for the expression of the trait. The fecundity is largely dependent on the genotype of mother moth and environmental conditions prevailing at the time of oviposition.. The phenomenon of cocoon yield is correlated to cocoon weight and because of high temperature stresses it was drastically reduced. It was observed that under optimal conditions it reached to maximum and under stress conditions it was minimum. The overall picture shows that cocoon weight was low in the high temperature treated batches when compared to room temperature batches. Generally as the temperature increases, larval weight decreases. High temperature did not favour the productivity and tends to the withering of mulberry leaves which were fed to the silkworms, thereby reducing the feeding quantum. But high temperature accelerates the growth rate leading to poor cocoon quality (Pillai and Krishnaswami, 1980). This may be due to the fact that high temperature will cause leaf withering leading to low rate of ingestion by the silkworm. During this situation the silkworm larvae cannot eat more time on provided leaf and it leads to decline in the cocoon weight (Gangawar *et al.,* 1993). Further, the analysis of variance indicating significant ($P < 0.001$) differences can be attributed to differential selection response.

Similarly, cocoon shell weight which was found to exhibit variation during the process of selection indicating interesting results. The cocoon shell weight which was known as moderately heritable (Tsuchiya and Kurashima, 1959; Gamo and Hirabayashi, 1983) showed positive selection response in both the temperature conditions. Application of selection for the trait not only increased the cocoon shell weight but also concomitant increase for most of the productivity traits as suggested by Kobayashi (1962). With regards to cocoon shell percentage, the results indicated positive selection response. It has been pointed out by many Japanese breeders that the trait is very difficult to increase by selection (Gamo and Hirabayashi, 1983; Mano et al., 1988). However, an attempt made in the present experiment indicates that the response was slow at high temperature and varied response was observed whereas at room temperature, lower values were observed. Thus cocoon shell percentage was significantly improved under optimum conditions than high temperature conditions. These findings are in agreement with Shivakumar *et al.,* (1997). Silkworm breed which are reared over a series of environments exhibiting less variation are considered stable. One of the objectives of the breeder is to recommend stable breeds to the farmers for rearing under different environmental conditions. Effect of high temperature and high humidity in terms of cocoon crop depends on several factors that operate within and outside the body of the silkworm. In the present study, it was observed that apart from the temperature, humidity also influences the productivity pattern in the silkworm and is in agreement with Krishnaswami (1986) and Sudhakar Rao (2003). It was also reported that the cocoon yield/10000 larvae, cocoon weight, cocoon shell weight and cocoon shell percentage were also low in the high temperature treated batches when compared to the batches reared under optimum rearing conditions (Suresh Kumar and Harjeet Singh,2011; Suresh Kumar *et al.,* 2011) and which corroborates the findings of the present study.

4.0 Conclusion:

Eastern India is generally characterized by luxuriant growth of mulberry for its highly fertile soil and rainfall. But, rearing of productive silkworm breeds and hybrids are restricted due to highly variable climatic situation, which causes poor larval growth, moulting disorder and severe mortality of silkworm caused by diseases and ultimately leading to low cocoon yield. High temperature coupled with high humidity act as the major limiting factor for rearing silkworm particularly during the seasons falling between May and September when huge leaf biomass is available.

Low temperature with low humidity during December and January severely affects the performance of P1 rearing. Besides, due to low production of mulberry leaf in low temperature regions, which affects the growth of silkworm resulting in poor performance of bivoltine P1 rearing. Though, February-March season is suitable for raising bivoltine as a P1 but subsequent commercial rearing for multivoltine x bivoltine is unpredictable due to prevalence of high temperature during late age rearing. Therefore, the silkworm egg producers have to depend on other parts of the country for bivoltine cocoons as P1 material. In West Bengal the bivoltine parent rearing was found to be difficult during unfavourable season due to the prevalence of high temperature and high humidity. Further bivoltine seed crop stabilization, which is bottleneck for the development of sericulture in the region, can also be solved by the development of sustainable bivoltine breeds suitable to the West Bengal conditions.

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