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DEVELOPMENT OF POLYVOLTINE BREEDS / HYBRIDS OF THE SILKWORM *BOMBYX MORI* L. FOR INDIAN CONDITIONS - A REVIEW

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ABSTRACT

India is a key player in the global silk production scenario. But being a tropical country, it has a major share of its silk being of polyvoltine origin. As this is comparatively of inferior quality, concerted efforts have been undertaken by many researchers to develop polyvoltine breeds which are qualitatively and quantitatively superior to the existing ones. The review provides a consolidated account of the literature pertaining to the studies carried out in this direction over a period of many decades.

Key words: *Bombyx mori*, polyvoltine breeds.

INTRODUCTION

The goal of silkworm breeding is to attain maximum productivity of quality produce. This is achieved through a systematic and steady improvement by way of combination of desired genes through breeding and selection. Though, sericulture has been introduced as commercial venture way back in late 17th century, silkworm breeding was initiated only in 1920s. Basically, polyvoltine culture prevailed up to the 50s and indigenous polyvoltine breeds such as Pure Mysore and C.Nichi in South India, Nistari in West Bengal, Sarupat and Moria in the North East were reared. Though these breeds were very well adapted and popular in respective regions, their productivity and quality of silk were strikingly low with very high renditta.

History of Indian silkworm breeds

In India, breeding of silkworms was reported as early as in 1940 (Rangaswamy, 1940). To begin with, two local races, Pure Mysore and Mysore A white were selected for their resistance to hot climate and ability to survive on poor diets. They were crossed with the Russian bivoltine race, Betakokonnaya, selected for its good yield, cocoon

weight, shell weight, filament length and denier (Sidhu, 1967). The resultant newly evolved multivoltine breed, Mysore Princess was noted for the significant improvement in the neatness of silk filament (Narayanan *et al.*, 1969).

Attempts were on in different directions to bring in an overall improvement in the performance of the available breeds. This is quite visible from the reports on studies such as sericin content in cocoons of indigenous silkworm races (*Bombyx mori* L.) (Jolly and Krishnaswami, (1964), effect of genetic diversity on hybrid performance in multivoltine Indian silkworm (Jolly *et al.*, 1965) and analysis of genotype environment interaction in some races (Sengupta, 1969).

Development of polyvoltine breeds in the 70s

The performance of an evolved multivoltine silkworm breed was described (Sidhu *et al.*, 1968). A comparative study of the yield performance of F1 hybrids between four evolved breeds and the local breed Nistari reared in both favourable and unfavourable seasons, was carried out. In F1 combination, the hatching percentage did not vary significantly from the best parent, Nistari. While the

combinations Nistari x D3c, Nistari x D14b and D14b x Nistari were found to be better than the other F1 combinations with regard to effective rate of rearing, all Nistari combinations were superior to non-Nistari combinations in respect of cocoon weight. The single shell weight in the combinations D14b x D3c and D3c x Nistari were better than that of the top parent D14b. It is evident from the F test of absolute silk content in F1 hybrids that D3c x Nistari, D14b x Nistari, MBDIV x Nistari and D14b x D3c were significantly superior to respective top parents and other combinations at 5 per cent level. The results led to a conclusion that F1 hybrid combinations, specially of Nistari are significantly superior to the pure Nistari race and should be used for commercial rearing (Sengupta *et al.*, 1971).

Despite the fact that new foreign (P3) races have been introduced for exploiting hybrid vigour with Pure Mysore as the female parent in Mysore State, the old, long time acclimatized races are still being continued and in fact by a certain section for use in the preparation of crossbreed seed. In order, therefore, to assess the relative merits of different combinations of multivoltine x bivoltine races currently in use in Mysore and to ascertain why the old foreign races like H.S. 6 and C. Nichi are still being continued, a large scale experiment on the performance of twelve different combinations including ten crosses utilizing Pure Mysore as the female parent was conducted at the instance of Research Coordination Committee of the Central Silk Board. The experiments were carried out for eight seasons during 1969 and 1970. The results of the experiment showed that the ten different combinations with Pure Mysore as female parent were not significantly different with regard to all practically important characters. This in other words, would mean that the newly introduced P3 races which have been continuously reared in the plains and perhaps without proper attention to maintenance of their economic characters are in no way superior to the existing races such as C Nichi, H.S.6 or newly evolved races *viz.*, Kolar Gold and N.S.4. (Tikoo *et al.*, 1971).

Sex-limited counterparts of three races, C-Nichi-1,

H.S.6 and Pure Mysore were studied for the effect of hyperploidy on the possible differences in survival and yield. All the three sex-limited races showed a significant gender based difference in viability, females being less viable. But the difference was the lowest in C-Nichi-1 and the highest in Pure Mysore. The data suggest that the deleterious effect of the introduction of the sex limited markings is not severe in these races, and the large advantage compensate for the slight difference in viability (Iyengar *et al.*, 1975).

Development of polyvoltine breeds in the 80s

Eight breeds of silkworm *i.e.*, CSRTI - I to VIII which were multivoltine in nature and bred true, were developed under NSPS-plan No.2 (modified) using two exotic breeds, Belakokonaya and Tashkashi and local Pure Mysore race. The breeds gave reasonably expected yield, significantly longer filament than that of the local multivoltine breed and certain quantitative characteristics equivalent to that of the exotic parental breeds. These breeds were evolved for commercial exploitation (Sidhu, 1984).

In order to evolve disease resistant multivoltine races of silkworm *Bombyx mori* suitable to tropical climates, an attempt was made utilising polyvoltine Pure Mysore race and bivoltine KA and NB18 races by conventional breeding. The progenies obtained after reciprocal matings of these races resulted in varied cocoon colours in the F2 and F3 generations exhibiting the desirable qualities of both the parents. The analysis of the rearing performance of some of these lines following inbreeding revealed a marked improvement in terms of the effective rate of rearing and hatchability over the parents. Raju and Krishnamurthy (1984) discussed the possible mechanism of inheritance of the said traits.

At present, multivoltine Mysore race is being used as female parent in the cross breeding programme with bivoltine race as male parent for the production of commercial seed. Sex separation of the parents at the time of eclosion, is a time consuming and uneconomical

process, in the large scale production of hybrids seeds. An investigation was taken up to transfer w⁺ chromosome with a translocated piece of 3rd chromosome from the bivoltine race (Zepere) where the female larvae carry Zebra markings, into the chromosomal complement of Mysore race. The hybrids of these parents were inbred up to 9 generations. In every generation, segregation with respect to the colour of the cocoon and hibernation features of the eggs were observed, in addition to the larval markings. By continuous selection, it was possible to fix this trait in one of the sub-lines with multivoltine features which also shows a marked improvement in the commercial qualities such as cocoon weight, shell weight, shell percentage, denier and renditta, over the traditional Pure Mysore race. The advantages of the large scale multiplication of this strain in commercial rearing are discussed (Sreerama Reddy and Ashwathnarayana, 1984).

The combining ability effects of nine multivoltine races of *B. mori* L. were analyzed in a 9 x 9 diallel cross for cocoon yield, fecundity, shorter larval duration, pupation rate, shell percentage and filament length for three seasons. The quantities for both general (GCA) and specific (SCA) combining ability were significant for all the traits. The GCA quantities indicated that Mysore Princess followed by Kollegal Jawan are good general combiners. Most of the combinations of Hosa Mysore with indigenous and exotic multivoltine races proved good specific combiners. The positive reciprocal effects in Hosa Mysore hybrids indicate the accumulation of cytoplasmic genes. The selective utilization of these races in future breeding programme are discussed (Pershad *et al.*, 1986).

Nine indigenous, exotic and evolved multivoltine races maintained at CSRTI, Mysore were reared during four distinct rearing seasons at Mysore. Their performance for six independent characters, valuable to silkworm breeding, was statistically analyzed. Mysore princess was found superior with respect to fecundity, cocoon yield, shell percentage and filament length whereas, Diazo was superior for shorter larval duration

and Nistari for pupation rate during all the rearing seasons. Analysis of variance indicated that races and seasons registered significant differences for all characters, while interactions of races and seasons had significant variations for fecundity and pupation rate during all seasons (Pershad *et al.*, 1986).

The extent of variability in economic traits viz., larval, silk gland, cocoon and cocoon shell weights was studied in seven pure breeds (two multivoltines and five bivoltines) and their 42 direct and reciprocal Fls. The extent of heterosis exhibited by the F1 hybrids is also reported (Govindan *et al.*, 1987).

Effect of exposure to high temperature (38 °C) in two phases of fifth instar, i.e., spinning period and pupal stages of eight silkworm breeds were analyzed. It was proven that exposure to high temperature during the later developmental stages considerably reduce the survival rate, pupation rate, cocoon quality and fecundity of the breeds and result in increased pupal mortality and unfertilized eggs. Among the breeds, Hosa Mysore, Kalimpong 'A' and the hybrid HM X KA revealed increased tolerance to high temperature conditions and overall better crop results (Venugopala Pillai and Krishnaswami, 1987).

The rearing and reeling tests were conducted with crosses, bivoltine female x multivoltine male, and the results were compared with conventional crosses, multivoltine female x bivoltine male. Three way crosses of (bivoltine x bivoltine) hybrid female x multivoltine male and its reciprocal were also tested to study the possibility of using bivoltine x bivoltine hybrid as one of the parents, instead of pure breeds. Results indicated crosses using bivoltine as female parent are at disadvantage with significant reduction in hatching (by 5.7 to 9.3 %), survival rate (by 4.6 to 10.1 %), cocoon yield (by 8.3 to 17.9 %), cocoon weight (by 4.9 to 7.3 %), shell weight (by 6.4 to 9.2%) and high floss content (by 10.0 to 22.0 %) and renditta (by 4.5 to 6.4 %). The clear advantages in such crosses were shorter larval duration (by 8 to 22 h) and higher fecundity (by 34 to 74 eggs/laying). Results also

indicated that bivoltine x bivoltine hybrid can be used as male parent with multivoltine as female parent, at par with crosses PM x NB18 or PM x NB7 (Benchamin *et al.*, 1988).

Combining ability for cocoon yield and other five basic independent characters among hybrids of five multivoltine and three bivoltine silkworm (*B. mori* L.) breeds were studied in an 8 x 8 partial diallel crosses programme including reciprocals, to select the best combiners for utilization in replacing the existing hybrids as well as in the heterosis breeding programme. Parental performance as judged by GCA quantities indicated that Hosa Mysore and Mysore Princess multivoltines with NB7 bivoltine were good general combiners. The crosses of Nistari and Daizo multivoltines with NB7 and Hosa Mysore with NB4D2 bivoltine proved better specific combiners for cocoon yield and pupation rate (Datta and Pershad, 1988).

A spontaneous oily mutant larval form was detected in a polyvoltine strain of the silkworm. It was identified as the og mutant, which is situated on chromosome 9 at locus 7.4. Although the mutation has a deleterious effect, the cocoon characters of survivors were better than those of the normal form (Rama Mohana Rao *et al.*, 1989).

A 7 x 7 diallel cross analysis involving 7 breeds of *B. mori* and their 42 F₁s, including reciprocals, was conducted for their combining ability for 6 quantitative traits. The comparison of parents indicated that none of them was good for all the traits. Predominance of additive genetic variance existed for larval weight, cocoon weight and cocoon shell weight, and non-additive genetic variance for larval duration, cocooning and pupation (Satenahalli *et al.*, 1989).

In India, ninety per cent of the silk production is of multivoltine variety. In South India, indigenous multivoltine race - Pure Mysore is the only female parent used for the bulk of the silk production. Pure Mysore, though resistant to various stress conditions, is very poor with regard to yield attributes and larval span which is around thirty days. The CSRTI, Mysore evolved a new

multivoltine breed namely, MY1, through cross breeding and selection. Sengupta *et al.* (1989) assessed the performance of MY1 x Bivoltine hybrids based on 19 trials data and in comparison with PM x bivoltine hybrids.

Sericulture is practiced both under irrigated and rain-fed conditions in Karnataka. The cocoon productivity and quality are of lower order in the rain - fed tracts. The farmers prefer to rear the traditional Pure Mysore x C. Nichi crosses. During 1983-89, a new hybrid combination viz., PCN x bivoltine was introduced successfully in the rain-fed tracts of Karnataka (Sengupta *et al.*, 1989).

Development of polyvoltine breeds in the 90s

Six new multivoltine breeds evolved in 1990s through hybridization and selection were found superior to Pure Mysore (an indigenous race of Karnataka) in all economic characters viz., cocoon weight, shell weight and cocoon shell percentage. However, no significant difference was observed in survival rate (Noamani *et al.*, 1990).

In another attempt, a local polyvoltine race of Karnataka, Pure Mysore was introduced with sex-limited larval markings from an inbred polyvoltine strain maintained in germplasm. F₂ generation was backcrossed and subsequently inbred up to F₉ generation. The strain was also tested for its hybrid forming ability with bivoltine males of KA, NB7 and NB18. Various economic characters such as differential viability of sexes, larval duration and other cocoon characters of both the inbred line and hybrids were studied and proposed for economic utilization (Nagaraju *et al.*, 1991). The genomes of two available bivoltine strains KA and NB4D2 and two exotic races J111 of Japanese origin and E112 of European origin were used in breeding in order to extract the desired genotypes. By following selection pressure at every generation, two sub-lines, MV 281 and MV 287 exhibiting desired combination of genes for productivity and survival were isolated at the end of 12th generation. After 26 generations, the sub-lines displayed significant improvement in survival over the parents. The importance of inbreeding the hybrids and back crossing are discussed in the study with respect to the evolution of superior races

for regional climates (Subramanya *et al.*, 1991).

New breeding approaches to improve productivity through recombination breeding have been described by Chatterjee *et al.* (1992). Breeding programmes were implemented to evolve improved multivoltine breeds of silkworm, *B. mori* L. with better pre and post cocoon parameters to replace Nistari. It was possible to isolate two promising multivoltine breeds, A-23 and A-25 exhibiting desired combination of genes for optimum productivity and survivability at the end of 12th generation. These two breeds bred through over 19 generations revealed a significant ($p < 0.01$) improvement over Nistari race with regard to various economic parameters *viz.*, cocoon and shell weight, shell percentage, absolute silk content, filament length, reelability and calculated yield/100 dfls both under favourable and unfavourable rearing seasons. Negative correlation between survival and cocoon character was not observed in A-25 during both the rearing seasons (Jayaswal *et al.*, 1992).

Nagaraju *et al.* (1992) bred and evaluated a new polyvoltine breed of the silkworm, *B. mori* L. Later, a few more superior breeds suitable for tropical conditions were developed by Kalpana (1993). In the very next year, a new strain of *B. mori* L., A-23, was produced out of 3 multivoltine breeds (M2, Nistari and G). The new strain spun golden yellow elongated oval cocoons with good processing qualities (Singh and Rao, 1994).

A large portion of the sericulture track in India is characterized by rain-fed condition without any assured irrigation facilities. Such conditions restrict the use of high yielding breeds. For such zones, efforts should be made to marginally improve upon the yield accompanied with improvement of fibre quality. In order to breed improved polyvoltine strains, a few indigenous and exotic silkworms were hybridized and desired characters were selected in the inbred populations. Two breeds namely, BL23 and BL25 were evolved after inbreeding and selection. These breeds are characterized by higher cocoon weight (>1.2 g) shell weight (>20 cg) and shell

percentage (15 %). Besides, the new breeds recorded higher heterotic values for the yield attributes when hybridized with bivoltine male parent NB4D2, than the control hybrid, PM x *C. nichi*. New hybrids have recorded an yield of > 17 kg/10,000 larvae, >1.7 g cocoon weight and > 18 % shell percentage as against control hybrid, PM x *C. nichi*, with 11 kg yield, 1.2 g cocoon weight and 13 % shell percentage. In India, more than 90 % of the silk production accounts for the hybrids of polyvoltine and bivoltine strains. Pure Mysore females are generally crossed with bivoltine males in the preparation of cross breed in South India. Pure Mysore race is characterized by longer larval duration and poor quantitative traits. To improve upon the productivity in polyvoltines and cross-breeds, indigenous and exotic parents were selected based on the GCA values and hybridized. Selections for desired traits were carried out at different stages of breeding. After a few generations of inbreeding coupled with selection, two new productive polyvoltine breeds, BL24 and BL26 were evolved which have higher cocoon weight (>1.2 g) shell weight (>20 cg) and shell percentage (>16 %) compared to Pure Mysore performance of 1 g, 14 cg and 14 %, respectively. The new breeds when crossed with bivoltine male parent NB4D2, the resultant F1 hybrids have shown higher yield/10,000 larvae (>18 kg), shell weight (>35 cg) and shell percentage (>18 %) with less renditta (8.9). The performance of new breeds and its hybrids are compared with control hybrid and the prospects of the new hybrids in raising productivity in irrigated areas are discussed by the authors (Ramam Mohana Rao *et al.*, 1995). Another new, sex limited polyvoltine strain, SL-KSPM of the silkworm, *B. mori* L. was developed towards the end of the decade (Mundukar *et al.*, 1999).

Development of polyvoltine breeds in the 2000s

The bulk of the silk produced in India is from multi x bi hybrids such as PM x NB4D2 and Nistari x NB4D2. These hybrids have some inherent defects like poor silk quality, low silk content and less yield. Keeping the above constraints in view, attempts were made to evolve productive multivoltine silkworm breeds / hybrids with

high silk content, better yield with quality silk (Raghavendra Rao *et al.*, 2000). In southern parts of India, sericulture is sustained on the firm foundation of polyvoltine Pure Mysore race. This race is being used as female parent in the preparation of hybrids with *C. nichii* race as male parent in rain-fed area though this combination is very inferior in terms of productivity. However, this hybrid combination is very popular among farmers due to its adaptability to local conditions. Keeping this in view, Raju *et al.* (2000) have evolved two new polyvoltine breeds C104 and C110 with better productivity and adaptability as possible replacement for *C. nichii*.

Studies on variability estimates, heritability, correlation and path analysis in 56 multivoltine silkworm breeds (*B. mori* L.) consisting of 46 indigenous breeds and 10 exotics were carried out for cocoon shape, size and weight variables. Wide range of variability for all the characters was observed and the variability was normally distributed except for cocoon volume where it was positively skewed. High heritability coupled with high genetic advance as percentage of mean was observed for cocoon volume and the rest of the traits exhibited high heritability associated with moderate genetic advance. All the correlations were in positive direction. Cocoon volume and area were significantly associated with other characters. Cocoon width was significantly correlated with cocoon weight and shell weight. Path analysis studies revealed the importance of selection pressure on cocoon width, cocoon volume and shell weight in breeding programme aimed at improving the cocoon weight (Mukherjee *et al.*, 2000).

Heterosis, general and specific combining abilities (GCA, SCA) were estimated for seven economically important traits *viz.*, fecundity, total larval duration, yield per 10,000 larvae by number and weight, cocoon weight, shell weight and shell percentage for 46 polyvoltine silkworm strains by line x tester analysis. ANOVA estimates showed highly significant GCA effects for many characters indicating the importance of additive gene action for the expression of traits studied

(Premalatha *et al.*, 2000).

Genetic variability and selection indices for seven economically important quantitative traits of fifty-six multivoltine mulberry silkworm races were estimated. ANOVA showed a highly significant variability for all the six commercial characters under study. High value of GCV and PCV percentages along with heritability and genetic advancement for filament length, shell weight and cocoon weight showed that the above characters would be highly effective for improvement. The correlation between cocoon weight and shell weight as well as shell percentage were found highly significant. The filament length has shown a highly significant and positive correlation with four economic characters (Kumaresan *et al.*, 2000).

The efforts made till date in silkworm breeding for the development of silkworm breeds/hybrids suitable for the tropical conditions of India are based on conventional breeding strategies. These efforts usually lay emphasis on single, two or three characters which may be quantitative or a mix of both (such as higher survival rate, pupation rate, yield, shell percentage and silk quality). These approaches are not holistic to suit field conditions as most of the productivity-linked characters in silkworm are controlled by polygenes. Silk output in India is entirely dependent on productivity achieved through rearing of cross-breed (PM x NB4D2) variety that produces inferior quality of silk. The bivoltine breeds/hybrids synonymous with higher productivity and superior quality silk are reared on a limited scale (Chandrashekaraiah, 2001).

In an experiment involving 45 polyvoltine strains as lines and 3 bivoltine strains as testers, in a line x tester programme, heterosis, general and specific combining abilities (gca and sca) were estimated for seven quantitative traits *viz.*, fecundity, larval period, cocoon yield per 10,000 larvae by number and weight, cocoon weight, shell weight and shell percentage. ANOVA estimates revealed significant effects for all the characters studied for all the breeds indicating the significance of both additive and non-additive gene action in the

expression of the traits. The breeds, 95B and 95G were judged as good general combiners among the 45 breeds, as these breeds showed significant gca effects for as many as six characters indicating the importance of additive gene action for the expression of traits studied. Out of 135 hybrids studied, no single hybrid indicated positive heterobeltiosis and /or sca effects for all the quantitative traits, however, the hybrids, BL25 x NB4D2 and 95L x CSR5 expressed desirable sca effects and heterobeltiosis for cocoon yield by weight, cocoon weight, shell weight and cocoon yield/10000 larvae by number and cocoon weight, respectively, and hence deserve commercial exploitation (Datta *et al.*, 2001).

Exploitation of heterosis for the improvement of both quantitative and qualitative traits in sericulture is a matter of practice using multivoltine x bivoltine silkworm (*B. mori* L.) strains. The highly heterogenic gene pool in tropical silkworm strains produce hybrids with variable performances. Therefore, an experiment was designed with an entirely different breeding approach for the first time in sericulture by developing successively syngenic line, recurrent backcross line (RBL) and congenic line (Con. L) or near isogenic line (NIL) causing transgression of cocoon shell weight (SCSW), the target character in all the lines in comparison to the original population *i.e.*, CB5 (GP). The target character in congenic or near isogenic line is very close to bivoltine - the donor parent (JPN9LmP). Single cocoon filament length being the most important linked character has also increased substantially keeping larval marking, cocoon colour, cocoon shape and voltinism as that of multivoltine - the receptor parent (CB5Lm-5). Such breeds having higher yield and better silk quality may directly be released for commercial exploitation bypassing conventional hybridization (Chattopadhyay *et al.*, 2001).

In order to promote the utilization of silkworm genetic resources, 56 multivoltine silkworm accessions maintained at CSGRC, Hosur, were evaluated for 10 post cocoon parameters, which include reeling and quality of raw silk. The results revealed highly significant variability among all the races ($P > 0.01$) for all parameters

studied. Based on the evaluation, ranking was also done and observed that the breed CB5. (BMI-0021) performed better for 09 parameters followed by LMO (BMI-0055) and MW13 (BMI-0043) for 08 parameters each. Further, 10 best accessions have also been identified for all the post cocoon parameters, which could be utilized in breeding programme and also for commercial exploitation (Mahadevamurthy *et al.*, 2001).

Heterotic effects for seven characters *viz.*, fecundity, total larval period, cocoon yield/10,000 larvae both by number and weight, cocoon weight, cocoon shell weight and shell percentage were studied among F1 hybrids of 45 polyvoltine and 3 bivoltine breeds. Among 135 hybrids, five hybrids *viz.*, Tamil Nadu white x CSR2, PMS2 x NB4D2, Nistari x NB4D2, 95B x CSR5 and 95L x CSR5 showed significant positive effects for all the seven characters studied whereas, 35 hybrids exhibited significant heterosis for six characters and 52 hybrids for five important quantitative characters. Majority of the hybrids exhibited significant positive heterotic effects for three important characters such as cocoon yield per 10,000 larvae by weight, cocoon weight and shell weight. These results point towards the need for exploitation of hybrid vigour among polyvoltine x bivoltine hybrids to increase silk production in India (Raghavendra Rao *et al.*, 2001).

Increasing demand for quality silk in the domestic markets of India has necessitated evolution of suitable breeds. Accordingly, a few multivoltine breeds were developed for quality improvement (Datta *et al.*, 2001). Fourteen improved polyvoltine breeds were developed through hybridization and selection, which proved much superior to the traditional breeds and withstand high temperature and high humidity conditions (Ghosh *et al.*, 2001).

Concerted efforts of silkworm breeding over the years have resulted in the development of a few multivoltine x bivoltine hybrids *viz.*, BL 23 x NB4D2, BL24 x NB4D2 and BL67 x CSR101 (cocoon shell percentage: 19-20 %) which are superior in most of the productivity traits over the existing local hybrids, besides providing higher recovery of A grade silk. Besides, the progress achieved

in improved productivity and quality under four special programmes namely, breeding for high temperature tolerance, breeding for disease tolerance especially, BmNPV, BmDNV-1 and BmIFV, breeding for thin denier races and breeding for sex limited races to facilitate sustainable seed production programme, have also yielded quite encouraging results (Basavaraja and Dandin, 2002).

Search for suitable hybrid combinations of silkworm (*B. mori*) for superior rearing traits was made among 28 F1 hybrids synthesized from seven multivoltine and four bivoltine silkworm breeds in line x tester fashion through heterosis breeding. The cross HM x NB18 ranked top for extent of progression to fourth instar and effective rate of rearing by yielding significantly positive heterobeltiosis and standard heterosis for full grown larval weight. The hybrids, P2D1 x NB18 recorded the highest negative standard heterosis for fifth instar and total larval duration. The rearing traits *viz.*, extent of progression to fourth instar, larval duration up to fourth instar, fifth instar duration, total larval duration, full grown larval weight and effective rate of rearing revealed that the hybrids, P2D1 x NB18 and KJ x KA produced the highest standard heterosis for rearing traits in general (Narayanaswamy *et al.*, 2002).

Under a breeding programme initiated during November, 1995, utilizing two multivoltine breeds *viz.*, BL-24 and BL-27 as breeding resource materials from the silkworm germplasm maintained by CSRTI, Mysore, a new multivoltine breed namely, BL-67 was evolved. The evolved breed was crossed with five tropical bivoltine breeds *viz.*, NB4D2, CSR2, CSR5, CSR18 and CSR101 to study the combining ability, and identified a superior hybrid, named as CAUVERY. The hybrid is characterized by high pupation rate (>95 %), high shell weight (>35 cg), high cocoon shell percentage (>20 %), longer filament (>900 m) and high neatness (93) with a renditta of 6.5 producing 2A-3A grade silk (Raghavendra Rao *et al.*, 2002).

In an another attempt, ten promising polyvoltine mulberry silkworm strains (SDMG1, SDMG2, SDMG3,

SDMG4, SDMW1, SDMW2, RMW1, RMW2, RMW3 and RMW4) that are superior in quantitative and qualitative traits were synthesized in the polyvoltine breeding laboratory of Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur through systematic hybridization and appropriate selection methods. After the genotypes were found homozygous for the desired traits, they were crossed with 3 bivoltine testers (APS8, APS4 and NB4D2). Evaluation Index and Subordinate Function methods were employed for the assessment of hybrid performance since they are widely used in silkworm hybrid evaluation. Total of seven poly x bivoltine combinations, which ranked high in both the methods, were selected as potential combinations for further field test. These combinations also ranked far above the control hybrid (APM1 x APS8) (Rao *et al.*, 2004 a).

In the process of synthesizing more potential polyvoltine hybrids (polyvoltine x bivoltine) of superior quantity and quality, APSSRDI, Hindupur has developed 8 promising polyvoltine breeding lines (SDMG2, APM16, APM15, APM14, APM5, RM2, APM3 and APM13). After crossing with 4 potential bivoltine testers (SDD1, SDD2, SDD3 and APS8), three superior polyvoltine hybrid combinations namely, RM2 x APS8, APM3 x APS8 and APM15 x SDD2 were adjudicated as potential heterotic hybrid combinations of superior silk yield and hence recommended for further large scale field trials and commercial exploitation (Rao *et al.*, 2004 b).

Breeding programmes were initiated during 2001 for the development of polyvoltine breeds /hybrids tolerant to high temperature and humidity. Initially, forty-four polyvoltine breeds maintained at CSRTI, Mysore were evaluated under high temperature and humidity ($36 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH) for one year. Eight top ranked polyvoltine breeds for temperature tolerance and seven polyvoltine breeds resistant to BmNPV were utilized and ten breeding plans were initiated. Utilizing two better performing polyvoltine breeds *viz.*, BL67 and MAR, the breed ND5 was developed. At F12, ND5 was subjected to hybrid evaluation with six bivoltine breeds *viz.*, NB4D2, CSR2,

CSR4, CSR17, CSRR18 and CSR19. The hybrid 'ND5 x CSR17 exhibited its superiority by recording 96.2 % survival, 1.965 g cocoon weight, 0.413 g cocoon shell weight, 21.0 cocoon shell percentage, 16.08 raw silk percentage and 928 m filament length whereas the control (PM x CSR2) recorded 96.00 % survival, 1.921 g cocoon weight, 0.352 g cocoon shell weight, 18.3 cocoon shell percentage, 14.00 raw silk percentage and 805 m filament length (Raghavendra Rao *et al.*, 2005).

A number of Congenic silkworm breeds consisting of three multivoltines (M Con.1, M Con.2 and M Con. 3) with high shell weight and high survival for adverse climatic condition in Eastern India have been developed by adopting congenic breed developing approach through transferring the genes by repeated back crossing. In multivoltines, more than 50 % improvement in shell weight (0.265 g) over the receptor (0.175 g) was achieved. Bivoltine congenic breeds recorded more than 60 per cent survival in adverse seasons when the receptor (JPN) failed to survive at all. Biochemical studies revealed that two apparent native proteins of 224 kDa and 180 kDa-possessors of 6 Esterase are the marker for high survival and high cocoon shell weight, respectively in *B. mori* L. Development of these Congenic breeds opens up the possibility to rear productive multi x bi silkworm hybrids in Eastern India throughout the year (Das *et al.*, 2005).

Some multivoltine and bivoltine breeds have been developed with high silk content and with high survival through the application of new breeding technique. Three congenic multi x bi hybrids (MB1, MB2 and MB3) were tested at Mothabari (malda) and Berhampore (Murshidabad) along with ruling hybrids N x YB and N x NB4D2 during Nov. - Dec. (Agrahayani), Jan. - Feb., (Chaitra), and March-April (Baisakhi) commercial crop seasons. The average yield/10000 larvae was observed to be the highest in the case of MB2 *i.e.*, 14.57, 13.95 and 12.19 kg, respectively during the three seasons against 11.20, 10.26 and 10.34 kg, respectively in N x YB and 12.05, 11.24 and 9.45 kg in N x NB4D2. The filament length and renditta in respect of MB2 during three crop

seasons were 804 m, 7.00; 775 m, 7.4 and 757 m, 7.61, respectively. The corresponding data of N x YB were 668 m, 8.83; 594 m, 9.53 and 674 m, 8.34 and in the case of N x NB4D2, it was 723 m, 8.12; 701 m, 8.43 and 712 m, 8.25, respectively (Verma *et al.*, 2006).

A breeding programme was initiated during August-September, 2003 utilizing one polyvoltine silkworm breed, BL67. During the maintenance of BL67, some larvae with prominent eye spots on second thoracic segment were observed and separated. By following directional selection for subsequent generations, the line, ND7 was isolated and stabilized. The F1 hybrid performance of ND7 was evaluated at 7th generation by crossing with eleven bivoltine silkworm breeds. One hybrid, ND7 x CSR2 showed its superiority for several economic characters such as fecundity, pupation rate, cocoon yield/10,000 larvae by weight, filament length and raw silk per cent. The new hybrid exhibited the highest average evaluation index value and recorded evaluation index value 50 for nine out of ten characters. Ten hybrids revealed higher magnitude of hybrid vigour for cocoon yield /10,000 larvae by weight, cocoon weight, cocoon shell weight and reelability (Dandin *et al.*, 2006).

Rama Mohana Rao *et al.* (2007) evaluated 22 polyvoltine breeds maintained at CSRTI, Mysore, based on quantitative and qualitative traits by utilizing multiple trait evaluation index method. Ten top ranked breeds were identified as potential parents and among that, NDV6 ranked the top most both for the reeling and rearing parameters. The other top ranked breeds were BL67, BL68, 2000H, 2000K, NP1, ND7, 96A, BL62 and BL61. The identified polyvoltine breeds can be utilized in the future breeding programmes as breeding resource materials for the development of superior polyvoltine breeds/hybrids.

In another such attempt, twenty-six polyvoltine breeds of the mulberry silkworm, *B. mori* maintained at CSRTI, Mysore were evaluated for seven characters utilizing multiple trait evaluation index method. Eight breeds *viz.*, DNP5, DNP3, NP1, ND5, BL24, ND7, 96E and BL68 exhibiting high average evaluation index values were

found promising. Five breeds among this viz., DNP5, DNP3, BL68, NP1 and ND7 were utilized as breeding resource materials (Nirupama and Singh, 2007).

A promising multivoltine x bivoltine hybrid, "ND7 x CSR2" (Jayalakshmi) was evaluated in fifteen locations covering the three southern states, Karnataka, Tamil Nadu and Andhra Pradesh during three different seasons. The results showed significant genotype x environment interactions. Higher cocoon yield was recorded during winter (67.42 kg/100 dfls) followed by summer (65.99 kg/100 dfls). Samayanallur and Salem in Tamil Nadu recorded the highest average yield of 80.60 and 79.80 kg/100dfls, respectively. Hence, it was resolved that the hybrid can be popularized in these areas in large numbers for improving the cocoon yield of the farmers (Sudha *et al.*, 2007).

Haemolymph and ionic isozyme pattern based (at pH 7.0 and 8.5) silkworm lines viz., V3CB5-Lm5 and V3M6DPC-Lm were used as receptors having potentiality to withstand adverse climatic situation, and JPN-Lm and D6p as donors for high cocoon shell weight for developing multivoltine (V3) congenic breeds viz., V3CB5Con.Ow and V3M6DPC Con.C with high cocoon shell weight. Simultaneously, D6p was used as a receptor and V3M6DPC-Lm as a donor to develop V2D6p Con.Ow characterized with high survival. Both multivoltine and bivoltine congenic breeds were developed by adoption of method developed by Chattopadhyay *et al.* (2001) for introgression of a desired trait controlled by multiple genes. Another bivoltine congenic breed V2CB5Con. Ow was isolated from the developed multivoltine congenic breeds V3CB5Con.Ow. Rearing performance of isozyme based lines was at par with their parents (GP) whereas, the cocoon shell weight of congenic breeds V3CB5Con. Ow and V3M6DPC Con.C were 0.265 and 0.246 g in comparison to their parents *i.e.*, 0.172 and 0.202 g, respectively. Pupation rate of bivoltine Con. B (s) was also equivalent to that of ruling bivoltine breed NB4D2 (control) during favourable season and was significantly higher than control during unfavourable seasons. Performance of NB4D2 and D6 as

bivoltine and V3CB5 and V3M6 DPC as multivoltine breeds developed through general / conventional breeding approach (RIL) showed that all the characters considered for assessment were inferior to that of breeds developed through the adoption of congenic breeding approach (Chattopadhyay *et al.*, 2008).

Silk productivity is expressed in cg of cocoon shell weight/day of 5th instar larval duration and is an important breeding index in silkworm *B. mori* L. Rama Mohana Rao *et al.* (2008 a) crossed five polyvoltine breeds, Pure Mysore (PM), RD1, ND1, NP1 and ND7 with a popular bivoltine breed CSR2 (used in the preparation of commercial silkworm seed) to calculate silk productivity in pure races and their F1 hybrids. Results indicated significant ($P < 0.01$) differences in the silk productivity and also other cocoon traits. For silk productivity, the breeds studied were in the order: ND7 (4.6 cg/day) > NP1 (3.91 cg/day) > ND1 (3.83 cg/day) > RD1 (2.73 cg/day) > PM (2.2 cg/day). The same trend was also observed in the corresponding F1 hybrids. The highest silk productivity of 7.24 cg/day was recorded in ND7 x CSR2, followed by NP1 x CSR2 (6.28 cg), ND1 x CSR2 (6.13 cg). The least productivity was recorded in PM x CSR2 (5.65 cg/day). Heterosis for silk productivity was also estimated in the hybrids studied. Data on heterosis indicate that for silk productivity, only ND7 x CSR2 has recorded positive standard heterosis which is 13.53 %. All the other crosses recorded negative standard heterosis. Overall results indicate that to improve productivity traits like silk productivity, use of improved polyvoltine as a female component is a must in the cross-breed seed preparation for commercial exploitation.

Sericulture plays a unique role in the socio-economic development of Eastern region of India. Eastern India experiences extreme variations in topography, soil characteristics, temperature, humidity and rainfall. This region is characterized by luxuriant growth of mulberry due to high temperature, humidity, rainfall and rich soil, but during the period when the leaf production is at the peak, it is not congenial for silkworm rearing. The successful rearing of presently available conventional

bivoltine and multivoltine breeds is unpredictable in such seasons due to highly fluctuating adverse climatic condition. Seed crop failure is frequently experienced. In order to overcome this problem, CSRTI, Berhampore has developed some improved multivoltine breeds viz., M12 (W), M6M81, M6DP(C), M.Con.1 and M.Con.4 with high productivity and quality and bivoltine breeds viz., YB, B.Con.1, B.Con.4, D6(P)N and SK4C and Foundation cross (SK6 x SK7) with high survival and productivity. Utilizing these breeds, season specific productive multi x multi hybrids namely, N x M12 (W), M. Con.1 x M Con. 4 and M12 (W) x M6M81 for unfavourable seasons (June-September) and multi x bi hybrids viz., N x YB, M. Con. 1 x B. Con 4, M.Con.4 x B.Con.4, M6DP (C) x (SK6 x SK7), M6DP (C) x SK4C and M6DP(C) x D6 (P)N for favourable seasons (October-April) were developed with encouraging performance (Das *et al.*, 2008).

Under an elaborate breeding programme, 22 polyvoltine breeds maintained at CSRTI, Mysore, were evaluated based on quantitative and qualitative traits by utilizing multiple trait evaluation index method (Rama Mohana Rao *et al.*, 2008 b). Data indicated that the size of the filament decreased from the outer to inner layer, the rate of decrease being sharp in some breeds and gradual in others. Data indicate that the slope of the denier is gradual in BL68 (-0.00268) and MY1 (-0.00295). In the case of BL43 (-0.00725) and BL25 (-0.00653), the slope was sharp in nature. In silkworm breeding programmes, breeds with gradual slope has to be selected in order to improve the fibre quality. Boil off loss ratio with reference to cocoon shell was determined. Highly significant correlation was observed between cocoon weight and cocoon shell weight, cocoon shell weight and cocoon shell percentage, cocoon shell weight and filament length and cocoon shell percentage and filament length. Among multivoltine silkworm breeds, some breeds such as PV1, BL61, NDV6 and 96A with low boil off loss ratio may be utilized as breeding resource materials in future breeding programme for the development of superior silkworm breeds with quality silk. Filament denier is mainly dependent on the race/hybrid used. Since raw silk is

composed of many filaments, variation in thickness of the individual filament influences the size and thickness of the thread. Under these circumstances, reeling characters have to be given priority in silkworm breeding. It was observed from the results that significant variation existed among the polyvoltine races and MAD can be effectively utilized in the fibre quality improvement breeding programmes. Index values were calculated for rearing and reeling parameters separately. Ten top ranked breeds were identified as potential parents and NDV6 ranked the top most both in the reeling and rearing parameters. These breeds were utilized in the breeding programmes as breeding resource materials for the development of superior polyvoltine breeds/hybrids with improved fibre characteristics. Role of sex in improving the cocoon traits in cross-breeding of the silkworm, *B. mori* L. has also been described (Rama Mohana Rao *et al.*, 2008 c).

Through a conventional breeding approach, a few quantitatively and qualitatively superior polyvoltine crossbreeds were synthesized. For the development of superior hybrids, the existing polyvoltine resource material of APSSRDI was screened for the desired qualitative and quantitative traits and four inbred lines were selected. Using the selected parents, twelve foundation crosses were prepared in diallel mode and based on their performance, five promising heterotic lines viz., APMG4, APMG6, APMG8, APMG10 and APM12 were chosen for selection and inbreeding. The breeding lines were also subjected for their thermal tolerance. After fixation of the desired traits, the breeds were subjected for hybrid testing using the five promising bivoltine testers. Based on two popular evaluation methods such as multiple traits evaluation index and Subordinate function methods, one hybrid combination, APM12 x APMG10 that showed superiority among all the hybrids tested was selected for further field trials (Ramesha *et al.*, 2008).

A study was carried out to develop bisexual polyvoltine breeds of the silkworm, *B. mori* L. with androgenic origin. The breeds were developed by crossing F2 males derived from a polyvoltine hybrid BL68 x BL69 with a polyvoltine race 'Nistari' and the eggs were exposed at 38 °C for 200

minutes. The larvae hatched out from these eggs expressed paternal characters. Five polyvoltine breeding lines were developed by repeated backcrosses with androgenic males for several generations. Two polyvoltine x bivoltine hybrids, AGL3 x CSR2z and AGL5 x CSR2 were found promising. This method will be useful to shorten the breeding cycle and to produce homozygous breeds which are of utmost importance in silkworm breeding (Ravindra Singh *et al.*, 2009).

Breeding methods coupled with the application of basic principles of genetics have enabled the silkworm breeders to develop an array of genotypes with desirable characters. Sex-limited strains assume a special significance in Indian sericulture as the bulk of the silk production is derived from polyvoltine x bivoltine crossbreeds at reduced cost of production. Hence, an attempt has been made to introgress sex-limited trait for larval marking into a commercially exploited crossbreed of silkworm (*B. mori* L.), Swarnandhra, APM1 x APS8 developed by APSSRDI, Hindupur. The recipient parent, APM1 (polyvoltine) and APS8 (bivoltine) are devoid of larval marking and characterized by spinning greenish yellow, oval cocoons and white dumbbell cocoons, respectively. The donor breeds, PMSL (polyvoltine) and 318 SLD (bivoltine) are characterized by the male larvae which are plain bodied without any larval marking and the females with an eye spot marking on the 2nd thoracic segment and a pair of crescent markings on the dorsal side of the 2nd abdominal segment and a pair of star marks on the dorsal side of 5th abdominal segment in addition to the sooty grey random mosaic colour all over the body. For the introgression of sex-limited trait, PMSL spinning greenish yellow, oval cocoons is used as donor to APM1 and the bivoltine sex-limited breed, 318 SLD spinning white dumbbell cocoons is used as donor parent to APS8. Introgressive hybridization, recurrent back crossing for 4 to 6 generations, selection of families with nearer to 50:50 sex ratio and cocoon shell index above 100 followed by sib-mating (6-8 generations) along with selection pressure was employed for development of auto-sexing silkworm strains with desired characteristics of recipient parents. The newly developed strains, APM1SL

(polyvoltine) and APS8SL (bivoltine) were tested for hybrid performance and found on par when compared with a commercial crossbreed, Swarnandhra (APM1 x APS8) (Ibrahim Basha *et al.*, 2011).

Genetic resources contribute as raw materials for crop improvement. The success of any breeding depends on the initial selection of parents and their effective utilization in desirable combinations. It also depends on the ability of breeds to assemble and recombine the genetic variability, to get the potential gene combinations from the gene pool based on phenotypic expression leading to genetic fixation of the traits over generations. It is important that the genetic difference between parents should be wide to get heterosis. Varied silkworm germplasm stocks contribute immensely to the development of viable and hardy silkworm breeds for commercial exploitation. The very purpose of the existence of a conservation centre is to collect, characterize, evaluate and conserve the germplasm material and also to prevent these valuable genetic material evolved over a very long period from extinction. An attempt has been made in this direction to identify potential and promising multivoltine silkworm genetic resources conserved at CSGRC, Hosur from among a collection of 73 multivoltine silkworm genetic resources (exotic-10 and indigenous-63) for 28 important economic parameters including rearing as well as post cocoon traits. The fact that these silkworm genetic resources have completed more than 70 generations and stabilized to the tropical conditions is an added advantage (Balachandran *et al.*, 2011).

Of late, in silkworm breeding programmes, more emphasis is being given to the fibre quality improvement. The main quality parameters tested for high grade silk are size deviation, evenness, cleanness, neatness, tenacity, elongation and cohesion and winding breaks. Among these, some are breed / hybrid specific. Size deviation of the yarn mainly depends on the individual silk filament size variation from outer to the inner layer and filament size. Some of the new improved polyvoltine breeds and polyvoltine x bivoltine hybrids have been studied and assessed for their fibre characteristics. Special emphasis

was also given to study the impact of early generation selection in respect of cocoon size variation, neatness and filament length in fibre quality improvement programmes (Rama Mohana Rao *et al.*, 2011 a, c).

In Eastern India, particularly in West Bengal (the major silk producer in Eastern states), five commercial crops are practiced due to distinct variation in climatic conditions. They are spring (February – March/Falguni), early summer (April - May/ Baisakhi) dry summer (June-July/Shravani), wet summer (August-September/Bhaduri & Aswina) and autumn (November-December/Agrahayani). During favourable seasons (spring and autumn), multi x bi hybrid and in unfavourable seasons (dry & wet summer), multi x multi hybrids are reared. During early summer, multi x bi as well as multi x multi hybrids are reared. Though 2 -3 commercial crops are congenial for multi x bi rearing, the seed crop rearing during the respective preceding seasons is not congenial with respect to the bivoltine parent. Nowadays, the bivoltine (male) seed cocoons are brought from other parts of the country for preparing multi x bi hybrids. In view of the above, it is essential to develop a multi x bi hybrid which gives crop stability with its bivoltine parent able to sustain the fluctuating climate during seed crops. Accordingly, a three way cross (multi x bi) hybrid namely, N x (SK6 x SK7) was developed (Moorthy *et al.*, 2011).

Concerned efforts of research and development contributed to development of improved breeds and increased raw silk production but the quality of raw silk is yet to match the international standard (Figure 1). This has necessitated the development of new silkworm hybrids with improved fibre characteristics. Twenty-two polyvoltine breeds maintained at CSRTI, Mysore were evaluated based on quantitative and qualitative traits by utilizing multiple trait evaluation index method. Ten top ranked breeds were identified as breeding resource material. By following the directional selection for subsequent generations, based on the fibre characteristics, ten breeding lines were isolated. The breeds namely, 113, 114 and 115, found as promising were stabilized. The new breeds were evaluated by crossing

with nine bivoltine breeds. Three new hybrids namely, 113 x CSR2, 114 x CSR2 and 115 x CSR2 exhibited their superiority over other hybrids for several yarn characteristics and recorded the highest average evaluation index values. The improvement recorded in the new hybrids ranged from 2 to 2.3 % in pupation, 14.3 to 14.8 % in cocoon yield, 23.1 to 28.9 % in cocoon shell weight, 10.8 to 15.1 % in cocoon shell percentage, 25.5 to 27.1 % in filament length and 18.8 to 22.5 % in raw silk per cent. Authors have discussed the feasibility of their usage for commercial exploitation in the field (Rama Mohana Rao *et al.*, 2011 b).

In order to meet the domestic and international demand for thin denier silk, experiments were carried out for the development of breeds with special characters such as longer filament and thin denier. Ten polyvoltine lines were selected from the polyvoltine germplasm and the selected parents were further multiplied and based on their reeling performance, a few polyvoltine lines in the category of having 650 m of filament length and 2.5 denier were short listed as initial parents for further breeding programme. Directional selection was employed in each generation and three polyvoltine breeds *i.e.*, APMG2, APMG4 and APMG16, found as breeding true for the desired traits were subjected to line x tester analysis by utilizing two bivoltine breeds, APS67 and APS20 having longer filament and thin denier as testers. Evaluation of selected hybrid combinations was carried out under both laboratory and field conditions and the hybrid combination, APMG16 x APS20 was selected as the most promising hybrid with special traits (Ramesh *et al.*, 2011).

Breeding programmes were initiated during 2004 for the development of multivoltine breeds/hybrids tolerant to high temperature and humidity. Initially, forty-four multivoltine breeds maintained at CSRTI, Mysore were evaluated at high temperature and humidity ($36\pm1^{\circ}\text{C}$ and $85\pm5\%$ RH) for one year. Eight top ranked multivoltine breeds for temperature tolerance and six multivoltine breeds resistant to BmNPV were utilized and ten breeding plans were initiated. The new Line4 developed and subsequently subjected to hybrid evaluation revealed Line4 x CSR2 as the best combination and also superior to PM x CSR2 (Umadevi *et al.*, 2011).



Pure Mysore - Indigenous race quite popular with the farmers of South India



Nistari x M.Con.4 - Productive polyvoltine x polyvoltine hybrid developed for Eastern India



Nistari - Indigenous race quite popular with the farmers of Eastern India



M.Con.1 x M.Con.4 - Productive polyvoltine x polyvoltine hybrid developed for Eastern India



PM x NB4D2 - Traditional polyvoltine x bivoltine hybrid popular in irrigated areas of South India



M.Con.1 x B.Con.4 - Productive polyvoltine x bivoltine hybrid developed for Eastern India



PM x C. Nichi - Traditional polyvoltine x bivoltine hybrid popular in rain-fed areas of South India



M.Con.4 x B.Con.4 - Productive polyvoltine x bivoltine hybrid developed for Eastern India



CAUVERY (BL67 X CSR101) - Productive polyvoltine x bivoltine hybrid developed for South India



Nistari x (SK6 x SK7) - Most popular polyvoltine x bivoltine hybrid in Eastern India



PM X CSR2 (KOLAR GOLD) - Most popular polyvoltine x bivoltine hybrid in South India

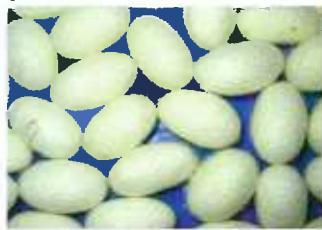


Figure 1: Indigenous races and poly x bivoltine and poly x polyvoltine hybrids developed in India

Future strategies

Of late, In India, many polyvoltine breeds/hybrids have been developed. However, the quality of silk realized from these hybrids are of inferior quality. Therefore, it is highly pertinent to develop qualitatively and quantitatively superior polyvoltine breeds/hybrids. Development of silkworm breeds through conventional breeding has many limitations. In order to accelerate the breeding process, there is an urgent need to incorporate molecular biology techniques into breeding process.

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DEVELOPPEMENT DE LIGNEES / HYBRIDES POLYVOLTINS DU VER A SOIE *BOMBYX MORI* L. POUR DES CONDITIONS INDIENNES - REVUE

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RESUME

L'Inde est un acteur-clé dans le scénario global de la production de soie. Etant un pays tropical, la majeure partie de sa soie est d'origine polyvoltine. Comme elle est d'une qualité comparativement inférieure, des efforts concertés ont été entrepris par de nombreux chercheurs pour développer des lignées polyvoltines qualitativement et quantitativement supérieures à celles existantes. Cette revue fournit un compte-rendu de la littérature relative aux études réalisées dans cette direction sur une période de nombreuses décennies.

Mots-clés: *Bombyx mori*, lignées polyvoltines.



DEVELOPMENT OF OPTICAL IMAGING TECHNIQUE FOR THE IDENTIFICATION OF MATURE 5TH INSTAR MUGA LARVA (*ANTHRAEA ASSAMENSIS* HELFER)

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ABSTRACT

The cycle of muga culture consists of four distinct phases of activities such as (i) collection and hatching of eggs (ii) feeding and growth of larvae from 1st to 5th instar (iii) cocoon spinning and (iv) emergence of moth. In addition, reeling of muga cocoon is practiced by some muga farmers as an additional means of income. In muga farming, the 5th larval instar is a vital decision making stage, where the mature muga larva has to be identified and separated from immature ones in the collected lots to ensure recycling of the immature muga larvae back to their feeding zone. In this backdrop, an optical imaging technique has been developed for determining the maturity level of muga larvae (*Antheraea assamensis* Helfer) at its 5th instar. Since this is otherwise a laborious and time consuming task, the new technique would ensure quick and proper selection and collection of mature muga larvae during a cultivation period. Moreover, it also paves way for the improvement in quality of the thread because of the least interruption from immature larvae during spinning.

Key words: *Antheraea assamensis*, muga, optical characteristic, sericigenous insect, silk.

INTRODUCTION

Muga (*Antheraea assamensis* Helfer) is a wild silkworm prevalent in Assam and the North East India. It is a unique sericigenous insect of the world, since its habitat exists nowhere else other than these areas. Out of several sericigenous insects producing silk, muga silkworm (*A. assamensis* Helfer, syn. *A. assama* Westwood) secretes the unique, golden yellow lustrous silk (Neog *et al.*, 2008). Its habitat is considered as one of the twenty five biodiversity hotspots of the world (Mayers *et al.*, 2000). Under the rich diversity in forestry, the sericigenous insects form a separate habitat called "Seri-biodiversity" in the region (Barah, 2010). Silkworms are characterized to feed on species specific host plants. The primary host plants of muga silkworm are som (*Persea bombycina*) and soalu (*Litsaea monopetala*). Like other lepidopterans, muga silkworm is a holometabolous insect, passing through a complete metamorphosis from egg to adult stage through two intermediate stages of larva and pupa (Thangavelu *et al.*, 1988).

Larva feeds on the host plant and passes through five instars intercepted by four moults, spanned over a period of 20 days in summer and 60 days in winter season. The mature larva weighs about ten to fifteen grams and measures eight to ten cm in length (Figure 1a).

At the end of fifth instar, mature larvae descend from the host plant in preparation to spin its cocoon. During this stage, some immature larvae also come down along with the mature ones. They descend usually in groups at the hour of dusk. The separation of immature larvae from the mature larvae is a slow and laborious task and any delay in the process causes partial crop loss. There is hardly any information about any device or system used for the identification, separation and collection process of muga larvae at this stage. Hence, we undertook a study to develop a sensor based system to select mature larvae from a mixed collection by exploring the optical characteristics of mature and immature larvae.



Figure 1: a - Mature muga larva; b - Excretion of muga larvae.

Before descending from the host plant, at the end of the 5th instar, muga larva excretes a viscous fluid from its food canal. The first part of this fluid is sometimes colored red (Figure 1b) and the remaining is colorless. At this stage, the body of muga larva is light green or light bluish green. This fluid while inside the food canal, is observed as an opaque thick line when the larva is exposed to visible light in dark ambient condition. After excretion, the food canal appears semi transparent, again under the exposure of visible light. Photograph of muga larvae taken by placing the light source on one side of the object and the camera on the other side, reveals this characteristic. Therefore, images of different muga larvae with varying maturity level captured by a digital camera can be used to determine the maturity by quantifying the optical characteristics of the image using image processing techniques.

Extraction of optical characteristics needs processing of image directed towards a specific goal. The basic objective of this work is to quantify the optical characteristic of the image of a muga larva. A Maturity Index (MI) has been proposed to quantify the degree of maturity using the energy level of the pixels of an image of the larva. Image of a muga larva contains many gray levels that have to be minimized to remove confusion in the detection process. Traditional image processing procedure utilizes image segmentation techniques to improve quality of the targeted or selected portion of an image by eliminating the unnecessary gray levels. Segmentation of image deals with operations of image enhancement and separation of different components of an image. Thresholding is a major processing involved with image segmentation. Thresholding is achieved by a number of approaches. Application specific image thresholding has been addressed in many works (Kapur *et*

al., 1985; Sahoo *et al.*, 1988; Abutaleb, 1989; Chang *et al.*, 1995). Through this, an image is converted from multilevel intensity to binary level. Based on intensity, single, double or adaptive thresholding are used where different values of thresholds are employed for the purpose. Multi stage thresholding techniques have been proposed by Chi and Yan (1993) which are extended for automatic thresholding of gray level image (Wu and Amin, 2003). In this work, single thresholding method has been adopted for segmentation of the image of a muga larva, as it requires less computation time and is easy to implement. At first, the image of a muga larva of any maturity level is captured and stored in matrix format in a computer. The energy level of the stored matrix (which represents the energy level of the pixels) is computed using image processing technique which involves the stages such as i) image segmentation by single thresholding and ii) computation of maturity index of the larva using energy level of the pixels of the segmented image. It has been observed that the MI thus computed shows significant difference between that of a mature and an immature larva enabling an easy differentiation between them.

MATERIALS AND METHODS

At the end of the 5th instar, a mature muga larva discharges all its feed (*i.e.*, the feeding canal becomes completely empty) and descend from the feeding trees to find a suitable place for spinning cocoon using its fibroin. Thus, a mature muga larva is optically more transparent compared to an immature larva.

To capture image of a larva, an LED light source was placed on the opposite side of a digital camera facing the larva descending from its host plant at the end of its 5th instar. Several colored images were captured and downloaded to a computer in jpeg format. Image processing technique was used to convert a colored image into a grey scale image which is stored as a two dimensional matrix. After this, the size of the matrix is determined followed by image segmentation by single thresholding technique to discard the unnecessary part of the image. The colored images of muga larvae before conversion are shown in Figure 2.

The immature larva has many dark regions in its food canal, thus scoring grey scale value near to 0 for most of the elements in the image matrix. The mature one has grey scale value near to 255 for almost whole of the food canal, except at the tail end. Image segmentation by single thresholding technique is used to convert the image into black and white mode by using a chosen threshold, X , where every pixel of the original image is tested for its own grey level in comparison with the chosen threshold, X . The pixel under consideration is turned into white if its grey level is greater than X , or otherwise, to black *i.e.*,

$$\begin{aligned} x_{rc} &\text{ is white if its grey level } > X, \\ x_{rc} &\text{ is black if its grey level } < X \end{aligned}$$

Here x is the image matrix, r and c are indices for row and column, x_{rc} is a grey scale value for the element stored in the r^{th} row and c^{th} column.

In image processing, grey scale value of the darkest element is 0 and that for the brightest element is 255. The image of a mature muga larva provides sufficiently large number of matrix element having grey value around 255. However, the beginning and the end of the food canal is characterized by matrix element of grey value below 150. On the other hand, image of an immature larva provides large number of matrix elements having grey scale value below 150. Therefore the value of X (threshold) is considered as 150. After image segmentation by single thresholding with threshold value 150, the MI is computed as follows:

$$MI = \sum x_{rc} \quad r = 1, 2, \dots, n, \quad c = 1, 2, \dots, m \quad (1)$$

Figure 3 represents two grey scale images of mature and immature larvae after converting them from color to grey images. Figure 4 depicts the images of the mature and immature larvae after trimming the head and tail portion of the images and enhancing contrast by single thresholding technique with threshold value 150.

RESULTS AND DISCUSSION

Several images were captured for different larvae of different seasons and of different places at its 5th instar with varying maturity levels for determination of MI. It

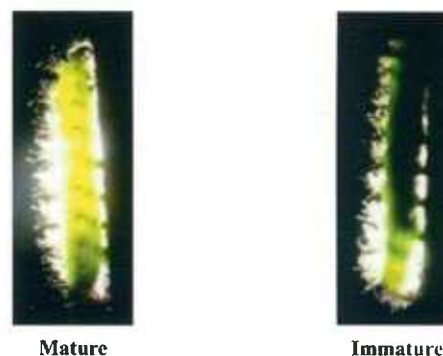


Figure 2: Colour image of mature and immature muga larva under the exposure of visible light.

has been found that MI provides good measure of maturity level of muga larvae. Table 1 provides MI values for a few mature and immature larvae. The MI is significantly high for mature larvae and their mean is 299013 and standard deviation is 22109. At the same time, the mean of MI for immature muga larvae is 68646 and standard deviation is 28973. It shows that the standard deviation of MI for immature larvae is high as compared to its mean value. It is due to the fact that the immature larvae used for the investigation have different levels of immaturity. As a result, MI values also vary significantly (*i.e.*, 24735 to 101745).



Muga 1(mature)

Muga 2 (immature)

Figure 3: Grey scale image of mature and immature larva



Muga 1cut piece

Muga 2 cut piece

Figure 4: Matlab generated image after trimming head and tail of original image

Table 1: Maturity indices of muga larvae of different maturity levels

Sample	Mature			Immature		
	Σr_{α}	Mean	Standard Deviation	Σr_{α}	Mean	Standard Deviation
1	325890	299013	22109.83	68085	68646	28973.88
2	306000			85935		
3	275910			62730		
4	310845			24735		
5	276420			101745*		

* The value is that of a semi-matured larva that might require another couple of days to become matured.

It is evident that the mean MI value of mature larvae sample is 299013, being a high value in comparison to that of immature muga larvae for which even the highest MI value is 101745 (Table 1). In addition to this, the minimum value of MI of mature larvae also vary with a standard deviation of 22109. The minimum value of MI of mature larva is found to be 275910. Therefore, to qualify a muga larva as a mature one, it should have a threshold MI value (MI_{Th}) less than 275910. The value of MI_{Th} is represented as

$$MI_{Th} = MI_{mean} - (MF)(MI_{stdev}) \quad (2)$$

Where,

MI_{mean} is the mean value of MI of mature samples,

MI_{stdev} is the standard deviation of MI of mature larvae and

MF is the multiplication factor.

MF is introduced to set a proper threshold value of MI for a muga larva. For the sample under investigation, the MI_{Th} value should be less than 275910. It may be expected that there may be more variations of MI of mature larvae around this value. Therefore, the MI_{Th} must be lower than 275910 and must be higher than 101745. Putting $MF = 1.5$ in equation (2), the MI_{Th} becomes 265849. Therefore, the threshold value of MI for a mature larva can be taken as 265800 (by rounding 265849 to the nearest hundred).

This investigation proves that the optical characteristic *i.e.*, optical transmissibility of muga larva can be utilized to determine the maturity level as it significantly differs between a mature and immature larva.

CONCLUSION

Muga cultivation is an intangible cultural heritage of Assam and the adjoining geographical areas of North East India. Due to the rapid changes in socio-economic scenario, the number of muga cultivators opting for modernization has been increased. This paper reveals the concept of an optical imaging technique that can be used to select mature muga larvae in its 5th instar in a mechanized process. A maturity index (MI) has been derived using the image segmentation by single threshold technique with threshold value 150 from an image of a muga larva. The experimental result shows that this MI value offers a very good measure for identifying a mature larva. In fact, the measure of MI provides the optical characteristics *i.e.*, the optical transmissibility of a muga larva. Therefore, it would be possible to implement this concept using simplified optical intensity based sensor with simplified electronic circuits.

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APPENDIX

Matlab code for the extraction of optical characteristic of muga larvae

```
w1=rgb2gray(imread('muga1.jpg')); % muga1.jpg is the name of the mature image
figure,imshow(w1),axis on
[r c]=size(w1);
%Trimming is done here
midy = r/2;
midx = c/2;
upy = midy-30;
downy = midy+30;
leftx=midx-10;
rightx=midx+10;
p=1;
q=1;
for l=upy:downy
    for m=leftx:rightx
        x = w1(l,m);
        A(p,q) = x;
        q = q+1;
    end
    p=1;
    q = p+1;
end
%Contrast enhancement is done here
threshold = 150;
[r c]=size(A);
for p = 1: r
    for q = 1: c
        if(A(p, q)<150)
            A(p, q)=0;
        else
            A(p, q)=255;
        end
    end
end
end
figure,imshow(A),axis on
%The sum of all the values of the elements of the image matrix is computed here
%This summation is termed as Maturity Index (MI)
x = sum(A);
MI = sum(x);
disp(MI)
% This code can be used to find MI for muga2.jpg ( immature) and other images also.
```

DEVELOPPEMENT D'UNE TECHNIQUE D'IMAGERIE POUR IDENTIFIER DES LARVES MURES MUGA (*ANTHERAEA ASSAMENSIS* HELFER) DU 5^{ème} AGE

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RESUME

Le cycle d'élevage du muga comprend quatre phases distinctes telles que (i) la collecte et l'éclosion des oeufs (ii) le nourrissage et la croissance des larves du 1^{er} au 5^{ème} âge (iii) le filage du cocon et (iv) l'émergence du papillon. De plus, certains fermiers dévident les cocons pour obtenir un supplément de revenus. Pour les fermiers, le 5^{ème} âge larvaire nécessite une décision importante, puisque les larves mûres doivent être distinguées et séparées des larves immatures afin de remettre les larves immatures vers leur zone de nourrissage. A cet effet, une technique d'imagerie a été développée pour déterminer le niveau de maturité des larves muga (*Antheraea assamensis* Helfer) au 5^{ème} âge. Alors que ceci est laborieux et long, la nouvelle technique devrait permettre une sélection rapide et fiable des larves muga mûres pendant la période d'élevage. De plus, c'est aussi une voie pour améliorer la qualité du fil en diminuant le temps d'interruption des larves immatures pendant le filage.

Mots-clés: *Antheraea assamensis*, muga, caractéristique optique, insecte séricigène, soie.



PERFORMANCE OF FIVE BIVOLTINE *BOMBYX MORI* L. (LEPIDOPTERA: BOMBYCIDAE) STRAINS ON THREE MULBERRY VARIETIES IN THE COASTAL SAVANNA AGRO- ECOLOGICAL ZONE OF GHANA

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ABSTRACT

The silkworm, *Bombyx mori* L. feeds on a variety of mulberry plants and the impact of the host plant is reflected on the quantity and quality of silk produced. The nutritional effect of three mulberry (*Morus alba* L.) varieties (Mysore local, Kanva 2 and S36) on the quality of cocoons spun and silk fibre produced by five bivoltine *B. mori* strains (G2xV2xH1xKK, M2xN2xSN1xI1, Z/Y, ICIPE 1, and ICIPE 2) was evaluated. The quality of cocoon and silk fibre was assessed on the basis of cocoon size, cocoon weight, shell weight, shell percentage, silk fibre tenacity, elongation, filament length, cleanliness and neatness. In terms of raw silk production, this study has revealed that the three silkworm strains from Bulgaria (Z/Y, G2- and M2-) yielded more than the two Kenyan strains (ICIPE 1 and ICIPE 2). However, ICIPE 1 silkworm strain had longer filament, good tenacity and elongation which contribute to fibre quality. No significant differences were noticed in the cleanliness and neatness of the fibre of different silkworm strains irrespective of the food plants they were fed upon. All the mulberry varieties were generally good for feeding the silkworms for cocoon production.

Key words: *Bombyx mori*, cocoon quality, mulberry variety, silk fibre quality.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. (Lepidoptera, Bombycidae) is an important economic insect that produces about 95-99 % of the valuable silk fibres for industrial and commercial purposes making it one of the most beneficial insects to mankind (Tsukada *et al.*, 2005). This insect is monophagous and depends solely on mulberry leaves for its source of food and nutrition (Sabhat *et al.*, 2011). The quality of mulberry leaves has direct bearing on the growth and development of the larvae and the quality of cocoons produced (Adolka *et al.*, 2007; Nguku *et al.*, 2009; Gangwar, 2010). Differences in mulberry varieties used for rearing may lead to varying effects on growth and development, cocoon and silk production among the silkworm strains (Mahmood *et al.*, 1987, Giridhar and Reddy, 1991). Cocoon quality in turn

has considerable impact on the quality and quantity of raw silk that is produced (Srinivasa, 2012).

Globally, about 40 countries produce silk amounting to an annual raw silk yield of around 1,50,000 tonnes which is still far below the world production of cotton and other synthetic fibres. The demand for silk has been increasing annually by 5 %, and is even bound to increase further as the world population continues to grow along with the demand for fashionable clothing and items (Anonymous, 2003). In Ghana, mulberry sericulture depends mainly on introduced silkworm strains and mulberry varieties. It has therefore become imperative to ascertain the quality of cocoons and raw silk fibre obtained from these strains. Highly productive silkworm strains and mulberry varieties that are adapted to the local conditions and are also tolerant to adverse climatic conditions and diseases are the present

day requirements (Zhao *et al.*, 2007; Nguku *et al.*, 2009). This will improve the yield and quality of cocoons produced in order for growers in Ghana to access international market. According to statistics from Institute of Industrial Research (IIR) of Centre for Scientific and Industry Research (CSIR) which is in charge of the silk industry in Ghana, the quantity of cocoons produced is very low and of inferior quality (Anonymous, 2012). The number of farmers who were actively involved in silkworm rearing has also reduced drastically (Ntaanu, 2007). The information on the silkworm strains and mulberry cultivars that yield superior quality cocoon and are suitable to the local climate being scarce, the present study was conducted to assess the performance of five *B. mori* strains fed on three mulberry varieties namely, Mysore local, Kanva 2 and S36 in the Coastal Savanna agro-ecological zone of Ghana.

MATERIALS AND METHODS

Experimental site

The experiments were conducted at University of Ghana (UG), Legon, Accra in the Coastal Savanna agro-ecological zone of Ghana. Mulberry plantation was raised at the University of Ghana farm located at latitude 5°39'37.264N and longitude 0°11'40.687W with an altitude of 97.24 m ASL. The rearing of the silkworm and data collection were undertaken under the African Regional Postgraduate Program in Insect Science (ARPPIS) Entomology Laboratory at the University of Ghana. The quality assessment of silk fibre was undertaken at the Silk Quality Control Laboratory at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.

Establishment of mulberry farm

Stem cuttings of healthy mulberry varieties namely, Mysore local, Kanva 2 and S36 with 4-5 buds were cultivated in June, 2012 with a planting distance of 0.9 m x 0.9 m. Chemical fertilizers (Urea, Sulphate of Ammonia

and N.P.K. 15: 15: 15) were applied to two months old mulberry plants at equal rate of 5 g/plant by ring method at 4 weeks interval for two consecutive times. The experimental plot was irrigated immediately after the application of fertilizer (Adolka *et al.*, 2007) and on daily basis during the dry season.

Silkworm rearing

Two sets of silkworm rearing were done during the major rainy season of 2013, and the average values were tabulated in tables. Healthy eggs of three F1 bivoltine silkworm hybrids (G2xV2xH1xKK, M2xN2xSN1xI1 and Z/Y) procured from Sericulture Experimental Station, Vratza, Bulgaria and of two strains, ICIPE 1 and ICIPE 2 from ICIPE, Kenya were used for the study. The eggs were incubated at 28 ± 2 °C and 70 – 80 % relative humidity with photoperiod of 12: 12 h (L: D). The silkworms were reared following the method suggested by Nguku *et al.* (2009). The 15 treatments (5 silkworm strains x 3 mulberry varieties) were laid in a Completely Randomised Design with three replications per treatment comprising of 150 larvae per replication. Matured silkworms (21-23 day old) were handpicked and mounted until they spun cocoons. Freshly spun cocoons were harvested on the seventh day.

Measurement of cocoon characteristics

Representative samples of cocoons (*i.e.*, cocoons with live pupae inside) from each treatment were selected at random to record cocoon size (length and width), cocoon weight and shell weight. Cocoon size was measured with digital vernier calliper (150 mm) and an electronic weighing balance (Kern 870) was used for recording the weight. Shell weight was determined by cutting open the cocoons using a razor blade to release the pupa and the moulted skin to obtain the weight of the empty shell. Shell percentage was estimated from the values of average shell weight and cocoon weight. The remaining cocoons were oven dried at declining temperatures of 115-100-85-70-55 °C for 5 hours (Gowda and Reddy, 2007) to prevent the emergence of adult moths.

Cocoon processing and quality analysis of the silk fibre

Dried cocoons obtained from each treatment were sorted and defective ones (double cocoons, outer stained, malformed and thin end cocoons) were discarded. Cocoons were boiled for about 5 minutes in boiling water to soften the sericin which binds the protein fibroin strands from which the silk fibre was reeled. Cooked cocoons were brushed manually using a paddy husk brush and transferred to reeling basin for picking. A multi-end reeling machine was used to reel cocoons into raw silk.

Tenacity and elongation percentage test

A serigraph was used to test the degree of elongation and tenacity of the raw silk. A sizing reel of 1.125 m in circumference (100 revolutions equal to 1.125 m) with a constant speed of 100 revolutions per minute was used to prepare the test sample. The sample skeins were weighed and conditioned in a room where standard humidity and temperature was maintained. The clamp distance was 10 cm and the extension speed, 15 cm per min. The sample skeins were mounted on the serigraph and elongation and breaking force (N) recorded. The breaking force in Newton was converted to grams and the tenacity was expressed as grams/denier using the formula:

$$\text{Tenacity (g/d)} = \frac{Z}{n \times d}$$

Where, Z= breaking force in grams,

d= denier and

n= number of strands tensioned.

Cleanliness and neatness

These traits were estimated to ascertain the imperfections in the silk filament. Cleanliness defects were classified as super major defect, major defect and minor defect (Lee, 1999). The cleanliness inspection was done from a distance of 0.5 meters directly in front of the Seriboard inspection panels on a Seriplane. The actual number of defects of each kind was counted on the yarns on both sides of the inspection panel. Each defect

determined was categorized by comparing it with standard photographs for cleanliness defects. Each defect carried a penalty point and the difference of the total penalty point from 100 gave the test result (Lee, 1999). Neatness defects are imperfections in silk yarn which are smaller than minor cleanliness defects. Each panel on any one side of the inspection board was carefully compared with the standard photographs for neatness and its neatness value estimated in percentages (Lee, 1999).

Filament length

The cocoons randomly selected from each treatment were boiled and reeled to determine the length of the cocoon using eprouvette. The length of the filament was determined by the following formula.

$$L = R \times 1.125$$

Where, L is the filament length,

R is the number of revolutions recorded by the eprouvette and 1.125 is the circumference of eprouvette reel in meters.

Data analysis

Data on cocoon measurements and raw silk quality test were subjected to ANOVA statistical procedure of SAS (Anonymous, 2011). Student-Newman-Keuls (SNK) test was used to separate the significant differences between treatment means. Differences were considered significant at $P < 0.05$. Data in percentages were arcsine square root transformed prior to analysis. However, back transformed means are presented in the tables.

RESULTS

Cocoon size (length and width)

The mean cocoon length of silkworm strains G2- and M2- differed significantly among the treatments of host plants they fed upon (Table 1). Cocoons of silkworm strain G2- were longer when fed on Mysore local (ML) than on S36. Cocoons from M2- silkworm strain attained a greater length when fed on K2 host plant than on S36. Cocoons from M2- and Z/Y reared on K2 host plant were the

Table 1: Mean cocoon size ($\pm$ SE) of five silkworm strains reared on three mulberry varieties

Variety/ Strain	G2	ICIPE 1	ICIPE 2	M2	Z/Y	F, P values
Mean cocoon length (cm) $\pm$ SE						
K2	3.06 $\pm$ 0.07bAB	2.80 $\pm$ 0.03cA	2.79 $\pm$ 0.05cA	3.24 $\pm$ 0.04aA	3.19 $\pm$ 0.05abA	18.76, 0.0001*
ML	3.24 $\pm$ 0.05aA	2.74 $\pm$ 0.04bA	2.85 $\pm$ 0.03bA	3.16 $\pm$ 0.04aAB	3.25 $\pm$ 0.06aA	26.33, 0.0001*
S36	3.02 $\pm$ 0.07bB	2.66 $\pm$ 0.09cA	2.82 $\pm$ 0.04cA	3.05 $\pm$ 0.04bB	3.25 $\pm$ 0.06aA	12.10, 0.0001*
F, P values	3.40, 0.0430*	1.23, 0.3018	0.51, 0.6033	5.15, 0.0100*	0.38, 0.6846	
Mean cocoon width (cm) $\pm$ SE						
K2	1.65 $\pm$ 0.03aA	1.54 $\pm$ 0.02bA	1.47 $\pm$ 0.02bB	1.73 $\pm$ 0.02aA	1.71 $\pm$ 0.03aA	21.32, 0.0001*
ML	1.71 $\pm$ 0.04aA	1.49 $\pm$ 0.03cA	1.59 $\pm$ 0.03bA	1.69 $\pm$ 0.02aA	1.73 $\pm$ 0.02aA	12.66, 0.0001*
S36	1.58 $\pm$ 0.05aA	1.59 $\pm$ 0.11aA	1.49 $\pm$ 0.02aB	1.59 $\pm$ 0.03aB	1.71 $\pm$ 0.04aA	1.79, 0.1399
F, P values	2.81, 0.0713	0.54, 0.5883	6.43, 0.0037*	11.44, 0.0001*	0.23, 0.7921	

Mulberry varieties; ML = Mysore local, K2 = Kanva 2. Silkworm Strains; G2 = G2xV2xH1xKK, M2 = M2xN2xSN1xII, Means within a column followed by the same capital letter and within a row followed by the same small letter(s) are not significantly different ($P < 0.05$, Student-Newman-Keuls SAS, 2011).

Table 2: Mean cocoon weight, shell weight and shell percentage ($\pm$ SE) of five silkworm strains reared on three mulberry varieties

Variety/ Strain	G2	ICIPE 1	ICIPE 2	M2	Z/Y	F, P values
Mean cocoon weight (g) $\pm$ SE						
K2	1.33 $\pm$ 0.07aA	1.11 $\pm$ 0.05bA	1.01 $\pm$ 0.03bA	1.46 $\pm$ 0.05aA	1.39 $\pm$ 0.02aB	16.21, 0.0001*
ML	1.46 $\pm$ 0.06bA	1.02 $\pm$ 0.03cA	1.04 $\pm$ 0.05cA	1.64 $\pm$ 0.07aA	1.44 $\pm$ 0.06bB	26.15, 0.0001*
S36	1.41 $\pm$ 0.08bA	0.90 $\pm$ 0.03cB	0.95 $\pm$ 0.02cA	1.44 $\pm$ 0.08bA	1.83 $\pm$ 0.06aA	43.72, 0.0001*
F, P values	0.89, 0.4175	6.32, 0.0040*	1.85, 0.1692	2.76, 0.0745	21.91, 0.0001*	
Mean shell weight (g) $\pm$ SE						
K2	0.28 $\pm$ 0.01aA	0.21 $\pm$ 0.01bA	0.20 $\pm$ 0.01bA	0.30 $\pm$ 0.01aA	0.28 $\pm$ 0.00aB	23.77, 0.0001*
ML	0.28 $\pm$ 0.01bA	0.20 $\pm$ 0.01cA	0.21 $\pm$ 0.01cA	0.32 $\pm$ 0.01aA	0.28 $\pm$ 0.01bB	32.61, 0.0001*
S36	0.28 $\pm$ 0.01bA	0.17 $\pm$ 0.01cB	0.18 $\pm$ 0.00cA	0.29 $\pm$ 0.01bA	0.34 $\pm$ 0.01aA	64.23, 0.0001*
F, P values	0.05, 0.9481	8.43, 0.0008*	2.53, 0.0917	2.67, 0.0808	19.43, 0.0001*	
Mean shell % $\pm$ SE						
K2	20.76 $\pm$ 0.43aA	19.24 $\pm$ 0.32bA	19.99 $\pm$ 0.26abA	20.54 $\pm$ 0.38aA	19.74 $\pm$ 0.16abA	3.52, 0.0113*
ML	19.32 $\pm$ 0.33aA	19.78 $\pm$ 0.24aA	19.81 $\pm$ 0.26aA	19.54 $\pm$ 0.44aA	19.73 $\pm$ 0.28aA	0.40, 0.8096
S36	19.97 $\pm$ 0.47aA	18.91 $\pm$ 0.23abA	19.34 $\pm$ 0.18abA	20.04 $\pm$ 0.51aA	18.56 $\pm$ 0.31bB	3.12, 0.0201*
F, P values	2.95, 0.0631	2.68, 0.0804	1.99, 0.1491	1.25, 0.2972	6.59, 0.0032*	

Mulberry varieties; ML = Mysore local, K2 = Kanva 2. Silkworm Strains; G2 = G2xV2xH1xKK, M2 = M2xN2xSN1xII, Means within a column followed by the same capital letter and within a row followed by the same small letter(s) are not significantly different ($P < 0.05$, Student-Newman-Keuls SAS, 2011).

Table 3: Mean tenacity and elongation of raw silk ($\pm$ SE) from five silkworm strains reared on three mulberry varieties

Variety/ Strain	G2	ICIPE 1	ICIPE 2	M2	Z/Y	F, P values
Mean tenacity (g/d) $\pm$ SE						
K2	3.81 $\pm$ 0.22bA	4.81 $\pm$ 0.04aA	4.17 $\pm$ 0.31abA	4.36 $\pm$ 0.09abA	4.19 $\pm$ 0.05abA	4.12, 0.0316*
ML	3.57 $\pm$ 0.24cA	4.98 $\pm$ 0.09aA	4.61 $\pm$ 0.36abA	4.04 $\pm$ 0.17bcA	3.89 $\pm$ 0.11bcAB	6.98, 0.0060*
S36	3.09 $\pm$ 0.07bA	4.46 $\pm$ 0.23aA	3.88 $\pm$ 0.24abA	3.80 $\pm$ 0.33abA	3.65 $\pm$ 0.17abB	4.83, 0.0198*
F, P values	3.59, 0.0942	3.52, 0.0972	1.45, 0.3056	1.64, 0.2707	4.94, 0.0539	
Mean elongation (%) $\pm$ SE						
K2	17.67 $\pm$ 0.33aA	21.00 $\pm$ 0.00aA	19.67 $\pm$ 0.67aA	20.67 $\pm$ 1.67aA	19.00 $\pm$ 0.00aA	2.70, 0.0924
ML	19.33 $\pm$ 0.88aA	21.33 $\pm$ 0.33aA	20.00 $\pm$ 1.00aA	19.33 $\pm$ 1.33aA	18.33 $\pm$ 0.66aA	1.49, 0.2778
S36	16.67 $\pm$ 0.67bA	21.67 $\pm$ 0.88aA	20.67 $\pm$ 1.33aA	18.00 $\pm$ 1.00abA	18.33 $\pm$ 0.33abA	5.09, 0.0168*
F, P values	4.08, 0.0760	0.38, 0.7023	0.24, 0.7928	0.96, 0.4348	0.80, 0.4921	

Mulberry varieties; ML = Mysore local, K2 = Kanva 2. Silkworm Strains; G2 = G2xV2xH1xKK, M2 = M2xN2xSN1xI1. Means within a column followed by the same capital letter and within a row followed by the same small letter(s) are not significantly different ($P < 0.05$, Student-Newman-Keuls SAS, 2011).

longest, followed by G2-, with ICIPE 1 and 2 being the shortest. Cocoons from Z/Y, G2- and M2- reared on ML were longer than those from ICIPE 1 and 2 silkworm strains. Z/Y cocoons reared on S36 were the longest, followed by M2- and G2- with ICIPE 1 and 2 being the shortest.

Similarly, the mean cocoon width of strains ICIPE 2 and M2- differed significantly among the host plants they fed upon (Table 1). Cocoons from M2- silkworm strain were wider when reared on K2 and ML than when reared on S36. Cocoons from ICIPE 2 silkworm strain were wider when reared on ML than on S36 and K2. Among the silkworm strains, cocoons produced by M2-, Z/Y and G2- reared on K2 were wider than those of ICIPE 1 and 2. The cocoons of Z/Y, G2- and M2- when reared on ML were the widest, followed by ICIPE 2 with ICIPE 1 being the least wide. Interaction effect on cocoon width between silkworm strains and host plants was also significant ($P < 0.0477$).

Cocoon weight

The mean cocoon weight of the silkworm strains Z/Y and ICIPE 1 differed significantly among the host plants they fed upon (Table 2). Z/Y silkworm strain had heavier cocoons when fed on S-36 host plant than on K2 and ML.

ICIPE 1 produced heavier cocoons when fed on K2 and ML than on S36. Cocoons from M2-, Z/Y and G2- reared on K2 were heavier than those from ICIPE 1 and 2. M2-cocoons were the heaviest in this case followed by G2- and Z/Y, with ICIPE 1 and 2 cocoons possessing the least weight. When fed on S36, Z/Y cocoons were the heaviest, followed by M2- and G2-, with ICIPE 1 and 2 being the lightest. There was significant interaction effect on cocoon weight due to silkworm strains and host plants ($P < 0.0001$).

Shell weight and shell percentage

The mean shell weight of Z/Y and ICIPE 1 silkworm strains differed significantly among the mulberry food plants they were fed on (Table 2). Cocoons obtained from Z/Y strain had heavier shell when fed on S36 food plant than on ML and K2. Cocoons of ICIPE 1 strain reared on K2 and ML had heavier shell than that when reared on S36. The silkworm strains M2-, Z/Y and G2- when reared on K2 had heavier shell than ICIPE 1 and ICIPE 2 strains. M2- cocoons produced on ML had the heaviest shell followed by G2- and Z/Y strains, with ICIPE 1 and ICIPE 2 having the least. Z/Y strain reared on S36 had the heaviest shell followed by M2- and G2- with ICIPE 1 and ICIPE 2 having the least.

Table 4: Mean filament length, cleanliness and neatness of raw silk ($\pm$ SE) from five silkworm strains reared on three mulberry varieties

Variety/ Strain	G2	ICIPE 1	ICIPE 2	M2	Z/Y	F, P values
Mean filament length (m) $\pm$ SE						
K2	551.25 $\pm$ 115.53aA	495.15 $\pm$ 58.31aB	549.00 $\pm$ 115.47aA	697.50 $\pm$ 173.84aA	1013.64 $\pm$ 57.80aA	3.49, 0.0495
ML	1033.88 $\pm$ 173.27aA	664.88 $\pm$ 173.85aAB	708.46 $\pm$ 57.55aA	821.25 $\pm$ 115.53aA	732.38 $\pm$ 116.11aA	1.19, 0.3729
S36	894.38 $\pm$ 57.09abA	982.13 $\pm$ 57.75aA	590.38 $\pm$ 57.58cA	658.13 $\pm$ 58.38cA	740.25 $\pm$ 57.79bcA	7.96, 0.0037*
F, P values	3.97, 0.0798	4.96, 0.0535*	1.03, 0.4128	0.46, 0.6503	3.82, 0.0852	
Mean Cleanliness (%) $\pm$ SE						
K2	99.80 $\pm$ 11.60aA	98.90 $\pm$ 8.66aA	98.00 $\pm$ 10.91aA	97.60 $\pm$ 11.43aA	99.80 $\pm$ 9.29aA	0.01, 0.9998
ML	99.80 $\pm$ 5.83aA	98.00 $\pm$ 9.75aA	99.80 $\pm$ 12.76aA	99.50 $\pm$ 9.93aA	99.00 $\pm$ 10.91aA	0.01, 0.9999
S36	99.80 $\pm$ 12.12aA	99.50 $\pm$ 6.24aA	99.70 $\pm$ 11.66aA	99.73 $\pm$ 13.94aA	99.40 $\pm$ 12.82aA	0.00, 1.0000
F, P values	0.00, 1.0000	0.01, 0.9919	0.01, 0.9927	0.01, 0.9904	0.00, 0.9987	
Mean Neatness (%) $\pm$ SE						
K2	98.00 $\pm$ 5.77aA	95.00 $\pm$ 14.43aA	93.00 $\pm$ 13.27aA	94.00 $\pm$ 15.01aA	98.00 $\pm$ 5.77aA	0.04, 0.9966
ML	98.00 $\pm$ 11.55aA	96.00 $\pm$ 10.96aA	98.00 $\pm$ 12.70aA	97.00 $\pm$ 7.51aA	95.00 $\pm$ 16.16aA	0.01, 0.9997
S36	98.00 $\pm$ 8.66aA	95.00 $\pm$ 10.18aA	97.00 $\pm$ 12.12aA	97.00 $\pm$ 8.66aA	97.00 $\pm$ 13.8aA	0.01, 0.9998
F, P values	0.00, 1.0000	0.00, 0.9977	0.04, 0.9579	0.03, 0.9752	0.01, 0.9858	

Mulberry varieties; ML = Mysore local, K2 = Kanva 2. Silkworm Strains; G2 – G2xV2xH1xKK, M2 = M2xN2xSN1xI1, Means within a column followed by the same capital letter and within a row followed by the same small letter(s) are not significantly different ($P < 0.05$, Student- Newman-Keuls SAS, 2011).

The mean shell percentage of Z/Y silkworm strain differed significantly among the mulberry food plants (Table 2). Z/Y strain obtained higher shell percentage when fed with K2 and ML food plants than with S36. Cocoons of G2- and M2- strains had more shell percentage when reared on K2 than ICIPE 1 strain reared on the same host plant. Cocoons of M2- and G2- strains had higher shell percentage when reared on S36 than Z/Y strain reared on the same host plant. Interaction effect between silkworm strains and mulberry food plants was significant ($P < 0.0454$) for this trait.

Raw silk tenacity and elongation

The mean raw silk tenacity of silkworm strain Z/Y differed significantly among the different host plants it fed upon (Table 3). Z/Y silkworm strain obtained the highest raw silk tenacity when fed on K2 mulberry food plant. Among the silkworm strains, ICIPE 1 attained the highest tenacity irrespective of food plants (Table 3). There were no significant differences in raw silk

elongation when the different silkworm strains were fed on K2 and ML food plants. However, ICIPE 1 and ICIPE 2 strains obtained higher raw silk elongation than G2- when fed on S36 food plant (Table 3).

Filament length

The mean raw silk filament length of ICIPE 1 silkworm strain differed significantly among the host plants (Table 4). ICIPE 1 silkworm strain had the longest filament when fed on S36. Among the silkworm strains, there were no significant differences in the raw silk filament length when they were fed on K2 and ML varieties. However, ICIPE 1 and G2- silkworm strains produced the longest filament when fed on S36 food plant. Interaction effect between silkworm strains and mulberry food plants on the raw silk filament length was also significant ($P < 0.0130$).

Cleanliness and neatness

There were no significant differences in the cleanliness and neatness of silk filament of the different silkworm

strains irrespective of the food plants they were fed upon (Table 4). Interaction effect between silkworm strains and mulberry food plants on the cleanliness and neatness of the raw silk was also not significant ($P = 1.0000$).

DISCUSSION

Cocoon quality contributes to about 30 % of the raw silk quality (Vishuprasad, 2004). Several factors contribute to the quality and yield, of which, the weight of cocoons, the characteristics of the cocoon (unattacked or attacked by natural enemies, or with exit holes created by moth), degumming loss and the age of the cocoon being important (Sonwalker, 1993; Fening *et al.*, 2009). Furthermore, cocoon size is a critical characteristic for evaluating the raw material (Lee, 1999), and the cocoon and shell weight are the most important characters evaluated for productivity (Gaviria *et al.*, 2006). The significant interaction obtained for cocoon and shell weight revealed variation among the silkworm strains and mulberry varieties they were fed on. Cocoons obtained from Z/Y, M2- and G2- strains were larger and heavier than those from ICIPE 1 and ICIPE 2 strains.

The results of the present study underline the significant effect imposed by the mulberry varieties on the cocoon characters. Qader *et al.* (1992) investigated the nutritive effects of three mulberry varieties on larval growth and cocoon characters of three *B. mori* races, and the results revealed that mature larval weight, single cocoon weight, shell percentage and the length of filament were greatly influenced by the nutritive value of different mulberry varieties. Our study revealed that the highest cocoon and shell weight were obtained by feeding Z/Y silkworm strain with S36 variety. Our finding concurs with the results obtained by other researchers that S36 variety was superior in silkworm rearing in terms of cocoon yield (Saratchandra *et al.*, 1992; Ogunleye and Johnson, 2012).

Shell percentage is an indicator of the amount of raw silk that can be reeled from a given fresh cocoon which varies according to the breed of the silkworm (Lalfelpui

et al., 2014). G2- and M2- obtained the greatest shell percentage among strains. Shell percentage is one of the important and deciding factors in selecting silkworms for higher cocoon production. Since this trait offers an estimation of the amount of raw silk present in each cocoon, the higher the value, the better the cocoon quality (Fening *et al.*, 2009).

Testing of silk yarn is essential for the production of quality yarn and fabric as it ensures efficiency in the production process and high quality end product (Anonymous, 1995). The results on tenacity of raw silk registered ICIPE 1 as the strain with the highest tenacity and elongation. The present results obtained in respect of these two traits confirm the findings of Lee (1999), which indicated that the tenacity of a typical bave (filament) was 3.6 to 4.8 g per denier and has an elongation of 18-23 % of its original length. Datta and Nanavaty (2007) also noted that silk has a tenacity of about 4g/d and an elongation of 20 %. Hariraj and Somashekar (2006) had shown that cocoon strains have significant influence on their silk quality characteristics which include tenacity and elongation.

Neatness defects denote imperfections in silk yarn, which are smaller than minor cleanliness defects. Aruga (1994) attributed cleanliness and neatness defects to the technique adopted for cooking and reeling of the cocoon. On the other hand, Lee (1999) reported that the characteristics of the silkworm strain may give rise to cleanliness and neatness defect. In the present study, silk filament of cocoons recovered from ICIPE 1 and G2- silkworm strains reared on S36 were lengthier. According to FAO (Anonymous, 1999), silk filament length ranges from 600 to 1500 m out of which 80 % is reelable. The present results fall well within FAO range.

In terms of raw silk production, this study has revealed that the three silkworm strains from Bulgaria (Z/Y, G2- and M2-) yielded higher than the two silkworm strains (ICIPE 1 and ICIPE 2) from Kenya. However, ICIPE 1 silkworm strain possessed longer filament, better tenacity and elongation. Furthermore, the three mulberry varieties (K2, ML and S36) could be used for feeding silkworms for

cocoon production, as generally not many differences were observed among the silkworm performance on them. Thus, the three silkworm strains from Bulgaria, especially Z/Y, and the ICIPE 1 strain from Kenya could be promoted in the Coastal Savanna agro-ecological zone of Ghana for cocoon and silk production. The results on quality testing of raw silk obtained were within International Silk Association (ISA) standards. It is recommended that the performance of these promising silkworm strains on the different mulberry plants should be tested under the different agro-ecological zones and seasons of Ghana so as to select the best for each zone based on the seasons.

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PERFORMANCE DE CINQ BIVOLTIN *BOMBYX MORI* L. (LEPIDOPTERA: BOMBYCIDAE) SOUCHES SUR TROIS VARIÉTÉS DE MÛRIERS DANS LA ZONE AGRO-ÉCOLOGIQUE SAVANE CÔTIÈRE DU GHANA

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RESUME

La quantité et la qualité de la soie produite par différentes lignées de vers à soie *Bombyx mori* dépendent aussi des variétés de mûrier utilisées pour les nourrir. Nous avons évalué l'effet de trois variétés de mûrier (*Morus alba* L.) c'est-à-dire Mysore local, Kanva 2 et S-36, sur la quantité et la qualité des cocons et de la fibre de soie produits par cinq lignées bivoltines de *B. mori* (G2xV2xH1xKK, M2xN2xSN1xI1, Z/Y, ICIPE 1 et ICIPE 2). La qualité des cocons et de la fibre de soie a été évaluée selon la taille du cocon, le poids du cocon, le poids de la coque, la richesse soyeuse, la ténacité, l'élongation, la longueur du filament, la propreté et la netteté. En terme de production de soie grège, les trois lignées de vers à soie d'origine bulgare (Z/Y, G2- et M2-) donnent un meilleur rendement que les deux souches provenant du Kenya (ICIPE 1 et ICIPE 2). Cependant, ICIPE 1 a un filament plus long, une bonne ténacité et une élongation qui contribuent à une fibre de qualité. Nous n'avons constaté aucune différence significative quant à la propreté et la netteté de la fibre entre les différentes souches de vers à soie quelle que soit l'origine des feuilles de mûrier. Toutes les variétés de mûrier apparaissent bonnes pour alimenter les vers à soie et produire des cocons.

Mots-clés: *Bombyx mori*, qualité du cocon, variété de mûrier, qualité de la fibre de soie.



EVALUATION OF SELECTED BIVOLTINE SILKWORM GENETIC RESOURCES UNDER ABIOTIC STRESS CONDITIONS OF NORTHERN INDIA

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ABSTRACT

Despite the availability of mulberry leaves in plenty during autumn season, the cocoon crops are generally a failure in North India due to adverse climatic conditions. In order to identify suitable bivoltine silkworm races which can combat the abiotic stress conditions of high temperature and high humidity prevailing during autumn season in North India, a few selected bivoltine silkworm germplasm conserved at CSGRC, Hosur were evaluated. Ten out of 230 bivoltine accessions, selected based on superior performance in rearing and post cocoon parameters were evaluated under RSRS, Sahasapur conditions. The experimental accessions were BBE-0173, BBE-0174, BBE-0178, BBE-0186, BBE-0189, BBE-0192, BBE-0198, BBE-0226, BBI-0239 and BBE-0266 along with two control accessions *i.e.*, one local [BBI-0045(SH-6)] and one common national control [BBI-0290(CSR-2)]. Six trials conducted in both the favourable (spring) and unfavourable seasons (autumn) revealed accessions, BBE-0266, BBE-0178, BBE-0198 and BBI-0239 as better performers during both the seasons for rearing and post cocoon parameters. These accessions are recommended as possible potential resources to evolve silkworm breeds suitable for autumn season.

Key words: Abiotic stress, autumn, bivoltine accessions, germplasm, spring.

INTRODUCTION

In Northern India, autumn crop is unsuccessful, inspite of having substantial quantum of mulberry leaves available for rearing. Autumn crop is reared under the stress conditions of high temperature and high humidity and needs to have hardy bivoltine hybrids. Autumn rearing generally constitutes only < 15 % of total seed intake with only < 20 % of total cocoon production over the years (Pankaj Tewary *et al.*, 2003). Kashmir valley had witnessed a tremendous decline in cocoon production from 4.83 lakh kg in 1980-81 to just 1.49 lakh kg in 1995-96 (Kamili and Masoodi, 2000). Rapussas and Gabriel (1976) reported that environmental factors especially temperature and humidity play a very important role in the lifecycle of silkworm in determining the cocoon characters and its existence in a particular zone. Among other reasons, lack of productive silkworm

breeds/hybrids has been identified as an important constraint in boosting cocoon production of J & K state. According to Khan *et al.* (2003), due to monocropping system adopted by the farmers, sericulture has not been economically viable. Besides, due to non-availability of cocoons round the year, reeling sector is affected because in Jammu region, autumn crop is scarce inspite of having mulberry in abundance for rearing. Similarly, according to Chauhan *et al.* (2003), in Dehradun region, suitable thermo-tolerant breeds are required to withstand the adverse temperature conditions of autumn season. Likewise, silkworm genotypes suitable for rearing during spring, summer, autumn and late autumn should be developed to make sericulture more remunerative and economically viable.

The Central Sericultural Germplasm Resources Centre (CSGRC), Hosur is having a collection of 443 silkworm

genetic resources, comprising 350 bivoltine, 73 multivoltine, and 20 mutant stocks. The bivoltine collections are accumulated from 14 countries around the world and include Chinese, Japanese and European races. The purpose of germplasm centre is to conserve these valuable collections, at the same time, since mere conservation will not sustain the existence of a germplasm resources centre, the genetic resources need to be evaluated for their potential on adaptability to different agro climatic conditions with abiotic and biotic stresses.

Hence, CSGRC identified some promising bivoltine silkworm germplasm based on the important economic traits to suit the need of above regions and formulated a collaborative project with RSRS, Sahaspur to screen them against the abiotic stress conditions prevailing in Sahaspur region especially during autumn season. The accessions have been identified by standard statistical analysis through Mano Evaluation Index (Mano *et al.*, 1993) and also through multiple trait evaluation index.

MATERIALS AND METHODS

Ten elite bivoltine accessions were selected for evaluation along with the local and national checks *i.e.*, local control for Sahaspur SH-6(BBI-0045) and one national control CSR-2(BBI-0290). The test accessions (BBE-0173, BBE-0174, BBE-0178, BBE-0186, BBE-0189, BBE-0192, BBE-0198, BBE-0226, BBI-0239 and BBE-0266) were short listed based on five important economic parameters for rearing (fecundity, larval duration, pupation rate, shell percentage, cocoon yield /100dfls) and eight reeling traits (total filament length, denier, renditta, raw silk recovery %, neatness, boil-off loss, cleanness and evenness). Dfls of the selected bivoltine silkworm genotypes (10 test accessions) along with that of 2 control races were supplied to collaborating station (RSRS, Sahaspur) for conducting rearing in both the seasons (favourable-spring and unfavourable-autumn seasons). The rearings were conducted for three years and totally 6 crops were undertaken. Standard silkworm rearing techniques were adopted (Krishnaswami, 1978) and experiment was designed in completely randomized

block design (CRBD) with three replications of three hundred larvae each.

Data on important economic parameters viz., fecundity, hatching percentage, weight of 10 grownup larvae, total larval duration, fifth age larval duration, cocoon yield - (No.) /10000 larvae and (weight) /10000 larvae, pupation percentage, cocoon weight, shell weight and shell percentage were recorded during all six crops. The cocoons were stifled and sent back to CSGRC, Hosur for reeling tests considering four reeling parameters viz., total filament length, non-broken filament length, renditta, and denier. Data were subjected to statistical analysis for adjudicating the accessions which performed better under abiotic stress condition (autumn season) in comparison with local control and national control.

Further, the performance under favourable rearing season (spring season) was compared with that of unfavourable season (autumn season).

RESULTS AND DISCUSSION

Mean data on the rearing performance of races at RSRS, Sahaspur during spring season and the Mano Evaluation Indices worked out are presented in Tables 1 and 2, respectively. Based on Multiple Trait Evaluation Index, accessions performed better than national control (CSR2) and local control(SH-6) during spring season at Sahaspur are presented in Tables 3 and 4, respectively. Based on Mano's Evaluation Index, better accessions identified during spring season are BBE-0266 and BBE-0198 with 9 qualifying parameters and this was again confirmed through multiple trait evaluation index in comparison with national control BBI-0290 (CSR2) whereas, when compared with local control BBI-0045 (SH6), BBE-0266 ranked first followed by BBI-0239.

Mean data on the rearing performance during autumn season is presented in Table 5 and the corresponding Mano Evaluation Indices in Table 6. In autumn season, accession BBE-0266 performed well in terms of 11 parameters followed by BBE-0178 with 9 parameters. When the data were analysed by multiple trait evaluation index, the same

Table1: Performance of selected bivoltine germplasm during spring season at Sahaspur

Acc. No.	Fec	H%	10 Lwt	TLD	VLD	YLDNo.	YLDwt.	P%	CWT	SWT	S%	FL	NBFL	D
BBE-0173	525	93.0	41.0	154	614	8600	13.3	81.7	1.5	0.3	18.3	758.2	575.0	2.8
BBE-C174	504	95.0	38.4	158	602	8552	13.1	83.7	1.5	0.3	19.0	773.1	671.0	2.8
BBE-C178	511	95.4	37.7	165	618	8685	13.4	84.6	1.5	0.3	17.4	843.1	693.5	2.4
BBE-C186	525	97.7	42.8	172	635	8467	14.5	83.8	1.6	0.3	19.8	940.1	842.1	2.6
BBE-C189	526	96.9	38.1	172	643	8507	13.6	83.1	1.6	0.3	17.0	784.4	784.4	2.2
BBE-G192	473	86.4	37.6	179	650	8262	12.6	78.6	1.6	0.3	20.3	901.0	580.1	2.4
BBE-C198	520	91.5	42.9	162	627	8746	15.4	92.0	1.6	0.3	19.3	804.6	609.0	2.7
BBE-0226	501	96.4	39.6	177	628	9537	13.9	94.8	1.5	0.3	19.6	678.0	442.8	3.2
BBI-0239	506	93.7	37.2	156	632	9462	14.7	93.8	1.5	0.3	19.2	738.3	738.3	2.4
BBE-0266	539	97.3	43.3	172	634	9089	16.1	90.5	1.7	0.3	19.2	885.0	611.1	2.4
BBI-0045	534	98.9	44.7	159	651	9767	16.8	95.3	1.7	0.3	16.8	727.2	503.4	2.8
BBI-0290	502	98.1	39.1	165	636	8600	14.6	78.7	1.6	0.4	22.1	880.7	733.9	2.8
Mean	515	95.2	40.2	166	630	8905	14.5	87.1	1.6	0.3	18.9	792.1	636.2	2.6
Min	473	86.4	37.2	154	602	8262	12.6	78.6	1.5	0.3	16.8	678.0	442.8	2.2
Max	539	98.9	44.7	179	651	9767	16.8	95.3	1.7	0.4	22.1	940.1	842.1	3.2
SD	17	3.5	2.5	8	14	498	1.3	6.1	0.1	0.0	1.5	99.3	120.7	0.3
SE	5	1.0	0.7	2	4	144	0.4	1.8	0.0	0.0	0.4	28.7	34.8	0.1
CV%	3	3.6	6.3	5	2	5.6	9.0	7.0	5.1	9.0	7.9	12.5	19.0	9.8

Table 2: Mano Index values of mean data of RSRS-Sahaspur for spring season

Acc. No.	Fec	H%	10 Lwt	TLD	VLD	YLDNo.	YLDwt.	P%	CWT	SWT	S%	No.Par
BBE-0266	64.0	55.9	62.2	58.1	52.3	66.3	61.4	52.4	53.7	62.7	55.6	9
BBE-0198	53.2	39.1	60.8	45.4	47.6	54.6	55.1	52.5	46.8	57.0	58.1	9
BBI-0045*	61.4	60.7	67.8	41.2	64.7	60.7	43.1	36.0	67.3	68.2	63.6	8
BBE-0186	55.9	57.1	60.5	57.7	53.4	55.7	58.8	56.4	41.2	50.5	44.6	7
BBI-0290**	43.0	58.2	45.7	49.5	54.1	56.4	72.6	71.6	43.9	50.9	36.2	6
BBE-0226	42.1	53.4	47.8	63.6	48.3	34.2	45.4	54.9	62.7	45.7	62.7	5
BBI-0239	45.2	45.6	38.0	37.4	51.3	39.2	45.4	52.2	61.2	51.7	61.0	5
BBE-0173	55.9	43.4	53.2	36.0	38.5	43.5	42.7	45.7	43.9	41.3	41.1	4
BBE-0174	44.1	49.2	42.9	40.3	29.4	39.3	45.0	51.0	42.9	39.7	44.4	3
BBE-0178	48.2	50.4	40.2	48.6	41.3	41.6	37.5	40.0	45.6	41.6	45.8	3
BBE-0189	56.8	54.7	41.5	57.8	59.3	53.2	40.1	37.4	42.0	43.5	43.5	3
BBE-0192	26.3	24.4	39.5	66.5	64.2	45.3	55.4	59.3	37.1	35.6	36.1	3

*local control;** national control

Fec-Fecundity (No.), H%-Hatching%, 10Lwt- Weight of 10 larvae (g), TLD-Total Larval duration (hours), VLD-Fifth age larval duration (hours), YLDNo. - Cocoon yield/10000 larvae by no., YLDwt. - Cocoon yield/10,000 larvae by wt. (kg), P%-Pupation percentage, CWT - Cocoon weight (g), SWT-Shell weight (g), S%- Shell %, FL-Filament length (m), NBFL- Non - broken filament length (m), D-Denier (d), No.Par-Number of parameters qualified.

accessions (BBE-0266 and BBE-0178) performed better in comparison with National control, CSR2 and local control, SH6.

The success of sericulture industry depends upon several factors, of which the impact of the environment

through its biotic and abiotic components is of vital importance. The environment is dynamic and environmental differences bring about profound changes in the physical and biotic factors governing the expression of commercial characters in the silkworm (Kobayashi *et al.*, 1986).

Table 3: Superiority of accessions in comparison to national control (CSR2) during spring at Sahaspur based on Multiple Trait Evaluation Indices

Acc. No.	No. Traits	Trait number with actual values
BBE-0266	9	1(539), 3(43.2), 5(633), 6(9088), 7(16.1), 8(90.4), 9(1.72), 12(885), 14(2.43)
BBE-0198	8	1(520), 3(42.9), 4(162), 5(627), 6(8745), 7(15.3), 8(92), 14(2.66),
BBI-0045	8	1(534), 2(98.9), 3(44.6), 4(158), 6(9766), 7(16.8), 8(95.3), 9(1.67),
BBI-0239	8	1(506), 4(155), 5(632), 6(9461), 7(14.6), 8(93.7), 13(738), 14(2.36)
BBE-0173	7	1(524), 3(40.9), 4(154), 5(614), 6(8599), 8(81.6), 14(2.76)
BBE-0178	6	1(511), 4(164), 5(618), 6(8685), 8(84.5), 14(2.42)
BBE-0186	6	1(524), 3(42.8), 5(635), 8(83.8), 12(940), 13(842)
BBE-0226	4	3(39.6), 5(628), 6(9536), 8(94.8)
BBE-0189	4	1(526), 8(83.1), 13(784), 14(2.24)
BBE-0174	4	1(504), 4(157), 5(601), 8(83.7)
BBE-0192	2	12(900), 14(2.41)

1. Fecundity (No.), 2. Hatching %, 3. Weight of 10 larvae (g), 4. Total larval duration (hours), 5. Fifth age larval duration (hours) 6. Cocoon yield/10000 larvae by no., 7. Cocoon yield/10,000 larvae by wt. (kg), 8. Pupation percentage, 9. Cocoon weight (g), 10. Shell weight (g), 11. Shell %, 12. Filament length (m), 13. Non - broken filament length (m), 14. Denier (d).

Table 4: Superiority of accessions in comparison to local control (SH6) during spring at Sahaspur

Acc. No.	No. Traits	Trait number with actual values
BBE-0266	9	1(539), 3(43.2), 5(633), 9(1.72), 10(0.32), 11(19.2), 12(885), 13(611), 14(2.43)
BBI-0239	8	4(155), 5(632), 6(9461), 10(0.28), 11(19.2), 12(738), 13(738.3), 14(2.36),
BBE-0198	6	5(627), 10(0.31), 11(19.2), 12(804), 13(608), 14(2.66)
BBE-0186	6	5(635), 10(0.32), 11(19.8), 12(940), 13(842), 14(2.63)
BBE-0174	6	4(157), 5(601), 10(0.28), 11(19.0), 12(773), 13(671)
BBI-0290	6	5(636), 10(0.35), 11(22.1), 12(880), 13(733), 14(2.79),
BBE-0192	6	5(650), 10(0.31), 11(20.2), 12(900), 13(580), 14(2.41),
BBE-0178	6	5(618), 8(84.5), 11(17.3), 12(843), 13(693), 14(2.42)
BBE-0173	6	4(154), 5(614), 11(18.2), 12(758), 13(574), 14(2.76),
BBE-0189	5	5(643), 11(17.0), 12(784), 13(784), 14(2.24),
BBE-0226	3	5(628), 10(0.28), 11(19.6),

1. Fecundity (No.), 2. Hatching %, 3. Weight of 10 larvae (g), 4. Total larval duration (hours), 5. Fifth age larval duration (hours) 6. Cocoon yield/10000 larvae by no., 7. Cocoon yield/10,000 larvae by wt. (kg), 8. Pupation percentage, 9. Cocoon weight (g), 10. Shell weight (g), 11. Shell %, 12. Filament length (m), 13. Non - broken filament length (m), 14. Denier (d).

Among the abiotic factors, temperature and humidity play important role on growth and productivity in silkworm, as the silkworm is a poikilothermic insect (Benchamin and Jolly, 1986). It is known that the late age silkworm prefers relatively lower temperature than young age (Krishnaswami, 1994) 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 showing that good quality cocoons are produced within a temperature range of 22-27 °C and beyond this range, the crop suffers (Krishnaswami *et al.*, 1973). In a tropical country like India, it is very much essential to develop bivoltine breeds/hybrids that can withstand the high

Table 5: Performance of selected bivoltine germplasm during autumn season at Sahasapur

Acc. No.	Fec	H%	10 Lwt	TLD	VLD	YLDNo.	YLDwt.	P%	CWT	SWT	S%	FL	NBFL	D
BBE-0173	513	87.1	34.0	144	562	8733	12.9	84.1	1.52	0.27	17.68	714.5	295.6	2.44
BBE-0174	495	89.9	33.9	140	562	8470	11.7	81.4	1.43	0.26	17.93	738.4	489.6	2.81
BBE-0178	515	96.8	32.6	147	573	9207	13.3	90.6	1.70	0.33	19.56	704.2	408.1	2.54
BBE-0186	513	98.3	33.6	148	570	8055	12.0	76.3	1.48	0.29	19.96	888.7	526.1	2.28
BBE-0189	512	97.6	34.1	151	579	8996	13.1	87.3	1.47	0.24	16.10	753.0	349.1	2.10
BBE-0192	487	82.6	32.2	137	563	8296	11.1	77.3	1.42	0.27	18.69	678.6	444.0	2.46
BBE-0198	522	95.0	35.3	142	563	9331	13.8	91.6	1.50	0.27	18.35	777.8	414.4	2.51
BBE-0226	502	94.4	33.4	151	575	8922	12.9	86.5	1.45	0.27	18.99	684.3	372.6	3.00
BBE-0239	485	84.8	32.7	153	579	9229	12.8	90.6	1.42	0.26	18.66	558.7	316.5	3.08
BBE-0266	530	95.2	37.2	145	575	9337	15.4	92.1	1.67	0.32	19.24	710.8	326.7	2.86
BBE-0045	550	98.3	45.3	150	600	8941	14.4	83.4	1.64	0.29	17.97	876.4	629.5	2.84
BBE-0290	526	98.1	36.0	165	608	8989	14.7	83.9	1.66	0.35	21.50	928.8	631.6	2.84
Mean	514	93.6	35.4	148	576	8930	13.3	86.0	1.53	0.28	18.59	741.2	425.9	2.67
Min	485	82.6	32.2	137	562	8055	11.1	76.3	1.42	0.24	16.10	558.7	295.6	2.10
Max	550	98.3	45.3	165	608	9337	15.4	92.1	1.70	0.35	21.50	928.8	631.6	3.08
SD	18.8	5.6	3.6	7	14	440	1.3	5.5	0.10	0.03	1.36	105.9	113.7	0.30
SE	5.4	1.6	1.0	2	4	127	0.4	1.6	0.03	0.01	0.39	30.6	32.8	0.09
CV%	3.7	6.0	10.3	5	2	5	9.9	6.4	6.69	11.63	7.31	14.3	26.7	11.36

Table 6: Mano Index values of mean data of RSRS-Sahasapur for autumn season

Acc. No.	Fec	H%	10 Lwt	TLD	VLD	YLDNo.	YLDwt.	P%	CWT	SWT	S%	No.Par
BBE-0266	58.2	52.9	55.0	46.0	48.9	59.3	65.8	61.3	62.9	60.5	54.8	11
BBE-0178	50.5	55.8	42.3	47.9	47.5	56.3	49.9	58.4	65.9	64.1	57.1	9
BBE-0290	56.3	58.0	51.8	73.5	72.2	51.3	60.5	46.3	61.9	71.1	71.4	8
BBE-0198	54.3	52.5	49.9	41.9	40.5	60.3	53.5	60.3	46.3	46.2	48.2	7
BBE-0045	69.3	58.5	77.4	52.0	66.8	50.2	58.0	45.4	60.0	52.6	45.4	7
BBE-0297	60.4	58.8	61.5	58.0	54.8	63.6	63.6	61.7	56.4	45.9	38.7	7
BBE-0186	49.3	58.5	45.0	50.1	45.5	30.1	39.8	32.3	44.7	53.2	60.0	4
BBE-0226	43.6	51.5	44.6	55.6	48.9	49.8	47.2	51.0	41.7	46.5	53.0	4
BBE-0189	48.8	57.1	46.4	53.6	51.7	51.5	48.1	52.4	44.2	35.3	31.6	3
BBE-0192	35.4	30.4	41.4	34.3	40.9	35.6	33.1	34.1	38.9	44.4	50.7	3
BBE-0239	34.6	34.3	42.6	56.8	51.9	56.8	46.1	58.5	39.1	43.8	50.5	3
BBE-0173	49.3	38.4	46.2	44.1	40.1	45.5	47.0	46.6	48.4	45.0	43.3	2
BBE-0173	40.0	43.3	45.9	33.1	40.2	39.5	37.5	41.8	39.6	41.4	45.1	2

Fec-Fecundity (No.), H%-Hatching%, 10Lwt- Weight of 10 larvae (g), TLD-Total Larval duration (hours), VLD-Fifth age larval duration (hours), YLDNo. - Cocoon yield/10000 larvae by no., YLDwt. - Cocoon yield/10,000 larvae by wt. (kg), P%-Pupation percentage, CWT - Cocoon weight (g), SWT-Shell weight (g), S%- Shell %, FL.- Filament length (m), NBFL- Non - broken filament length (m), D -Denier (d), No.Par-Number of parameters qualified.

temperature stress conditions. In India, the adverse climatic conditions during summer is not the same throughout with some location having high temperature coupled with high humidity and in some, high temperature with low humidity besides, poor leaf quality

at times. The acquisition of desirable level in cocoon production depends on the successful selection of initial breeding source (Suresh Kumar *et al.*, 2004). In order to select efficient breeds with high temperature tolerance, it is very important to analyze critically the impact of high

Table 7: Superiority of accessions in comparison to national control (CSR2) during autumn season at Sahasapur

Acc. No.	No. Traits	Trait number with actual values
BBE-0266	8	1(529), 3(37.1), 4(145), 5(574), 6(9337), 7(15.4), 8(92.1), 9(1.66)
BBE-0178	6	4(146.667), 5(572.667), 6(9207.444), 8(90.554), 9(1.697), 14(2.539)
BBI-0045	6	1(550), 2(98.3), 3(45.3), 4(149), 5(600), 14(2.84)
BBE-0198	5	4(142.444), 5(562.667), 6(9331.333), 8(91.59), 14(2.513)
BBE-0189	5	4(150.667), 5(578.667), 6(8996.111), 8(87.256), 14(2.098),
BBI-0239	4	4(152.889), 5(578.889), 6(9229.444), 8(90.627)
BBE-0226	3	4(150.667), 5(574.667), 8(86.497)
BBE-0186	3	2(98.316), 4(148.222), 5(569.778)
BBE-0173	3	4(144), 5(562), 8(84.07)
BBE-0192	3	4(137.111), 5(563.111), 14(2.462)
BBE-0174	3	4(139.778), 5(562.222), 14(2.807)

Table 8: Superiority of accessions in comparison to local control (SH6) during autumn season at Sahasapur

Acc. No.	No. Traits	Trait number with actual values
BBE-0266	8	4(145), 5(574), 6(9337), 7(15.4), 8(92.1), 9(1.66), 10(0.32), 11(19.2)
BBE-0178	8	4(146), 5(572), 6(9207), 8(90.5), 9(1.69), 10(0.33), 11(19.5), 14(2.53)
BBI-0290	8	6(8988), 7(14.7), 8(83.9), 9(1.65), 10(0.35), 11(21.4), 12(928), 13(631)
BBE-0198	6	4(142), 5(562), 6(9331), 8(91.5), 11(18.3), 14(2.51)
BBE-0186	6	4(148), 5(569), 10(0.29), 11(19.9), 12(888), 14(2.27)
BBI-0239	4	5(578), 6(9229), 8(90.6), 11(18.6)
BBE-0189	4	5(578), 6(8996), 8(87.2), 14(2.09)
BBE-0173	4	4(144), 5(562), 8(84.07), 14(2.43)
BBE-0192	4	4(137), 5(563), 11(18.6), 14(2.46)
BBE-0226	3	5(574), 8(86.4), 11(18.9)
BBE-0174	3	4(139), 5(562.2), 14(2.80)

1. Fecundity (No.), 2. Hatching %, 3. Weight of 10 larvae (g), 4. Total larval duration (hours), 5. Fifth age larval duration (hours) 6. Cocoon yield/10000 larvae by no., 7. Cocoon yield/10,000 larvae by wt. (kg), 8. Pupation percentage, 9. Cocoon weight (g), 10. Shell weight (g), 11. Shell %, 12. Filament length (m), 13. Non - broken filament length (m), 14. Denier (d).

temperature on many silk yielding attributes of silkworm races. It was reported that the lines selected under high temperature and high humidity perform better than the lines selected under normal temperature and humidity (Nagaraju *et al.*, 1996). Kato *et al.* (1989) in a series of experiments observed that resistance to high temperature is a heritable character and it may be possible to breed silkworm races tolerant to high temperature.

Based on the results of the present study, the germplasm accessions BBE-0266, BBE-0178 and BBE-0198 have performed better than both the respective local ruling breeds and national control (CSR2) for rearing and reeling parameters at high temperature and high humidity which prevails during the autumn season of Dehradun region. In addition, accessions BBE-0198 and BBE-0266 have recorded less disease incidence also during the

experimental rearings. These results corroborate with the findings by Muthulakshmi *et al.* (2011) that the bivoltine germplasm accessions BBE-0266, BBE-0178 and BBE-0198 performed better than local ruling breed (Jam-25) and national control (CSR2) with respect to rearing and reeling parameters under high temperature and high humidity condition prevailing during the autumn season in Jammu region also.

Hence, these germplasm can be exploited by breeders to evolve suitable F1 hybrids for exploitation under high temperature and high humidity condition. Information on the general and specific combining abilities (GCA and SCA) of these races with ruling breed as against that of popular F1 hybrids would also be useful in drawing efficient breeding plans for the future. Naseema Begum *et al.* (2001) also had worked in the same line by screening 15 bivoltine silkworm breeds under high temperature and humidity conditions leading to the identification of 8 thermotolerant breeds. Suresh Kumar and Yamamoto (1995) and Suresh Kumar *et al.* (2001) indicated that hybrids are more tolerant than pure races and there was maternal effect regarding temperature tolerance as evidenced from the better performance of those hybrids where the female parents used were more tolerant as pure races. According to Maribashetty *et al.* (1998), the breeding experiments aimed at evolution of hardy bivoltine races for tropics indicated that genetic diversity, productivity and viability are complexly related, being influenced by the intrinsic and extrinsic factors. Considering the poor performance of bivoltines during summer season, Krishna Rao *et al.* (1996, 1997) and Krishna Rao (1998) also evolved three new bivoltine races namely, NP-2, SP2 and KSO-1, involving pure Mysore. These findings underline the need to evaluate genetic resources for their potential on adaptability to different agro climatic conditions characterized by abiotic stresses.

The present study revealed that accession BBE-0266 and BBE-0178 have performed better than both the respective local ruling breeds and CSR2 for rearing and reeling parameters even under high temperature and high

humidity conditions prevailing during the autumn season of Sahaspur. The performance of these races may further be studied as F1 hybrids with suitable male showing better combining ability and also, these germplasm may be exploited by breeders to evolve hardy races suitable for autumn crop in the northwestern region of India.

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EVALUATION DE RESSOURCES GENETIQUES SELECTIONNEES DE VERS A SOIE BIVOLTINS SOUS DES CONDITIONS DE STRESS ABIOTIQUES DE L'INDE DU NORD

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R E S U M E

En dépit de la disponibilité totale de feuilles de mûrier pendant l'automne, les récoltes de cocons sont généralement perdues en Inde du nord en raison de conditions climatiques défavorables. Afin d'identifier des races de vers à soie bivoltins capables de combattre les conditions de stress abiotiques dues à la haute température et à la forte humidité qui règnent en automne en Inde du nord, quelques lignées de vers à soie bivoltins conservées au CSGRC à Hosur ont été évaluées. Dix lignées bivoltines sur 230, sélectionnées pour leurs performances supérieures quant aux paramètres d'élevage et post cocons ont été évaluées dans les conditions du RSRS à Sahaspur. Les lignées expérimentales sont BBE-0173, BBE-0174, BBE-0178, BBE-0186, BBE-0189, BBE-0192, BBE-198, BBE-0226, BBI-0239 et BBE-0266 ainsi que deux témoins, l'un local [BBI-0045(SH-6)] et l'autre national usuel [BBI-0290(CSR2)]. Six essais conduits à la fois dans des conditions favorable (printemps) et défavorable (automne) révèlent que les lignées BBE-0266, BBE-0178, BBE-0198 et BBI-0239 sont les meilleures pendant les deux saisons pour les paramètres d'élevage et post-cocon. Ces lignées sont recommandées comme ressources afin de développer des souches adéquates pour l'automne.

Mots-clés: Stress abiotique, automne, lignées bivoltines, collection, printemps.



DESTRUCTIVE STAGES OF *ANTHRENUS SCROPHULARIAE* LINN. (COLEOPTERA: DERMESTIDAE), THE COMMON CARPET BEETLE INFESTING STORED COCOONS

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ABSTRACT

The biological features of *Anthrenus scrophulariae* Linn., commonly known as carpet beetle as well as its nature of damage on stored cocoons were documented at DMMMSU-SRDI filature of Region 1, Philippines. It is a holometabolous insect pest which completes its development in 126 to 205 days. The adult male and female beetles were observed to be active from 7 to 14 and 9 to 17 days, respectively. The female lays 20 to 88 eggs with hatchability ranging from 68 to 100 %. The eggs are usually laid in small batches on the surface of the cocoons. The eggs hatch after 15 days, the larvae undergo five moults, the developmental period spanning from 123 - 126 days during which they feed on silkworm pupae inside the cocoon. After sixth moult *i.e.*, pupal to adult stage, the body appears reddish brown, covered with many dark hairs and measure 5.20 – 9.21 mm long, while adult carpet beetles are oval in shape and vary from 1.0 to 3.50 mm in length. Stored cocoons are rendered unreelable due to numerous holes made by the dermestid larvae. Thus, the larval stage of the beetle causes an economic loss of 3.42 % per kilogram of cocoon amounting to P-13.68 during a storage period of 15 to 60 days. The findings are indicative of the remarkably destructive role played by *A. scrophulariae* Linn. in the silk production of Region 1, Philippines.

Key words: *Anthrenus scrophulariae*, filature, holometabolous, metamorphosis, unreelable cocoon.

INTRODUCTION

Common carpet beetles of *Anthrenus* spp. pose a threat to stored silk cocoons. The members belonging to the genus *Dermestes*, *Attagenus* and *Anthrenus* are important, as they attack silk cocoons, raw silk or silk products (Ashraf, 1988). Extensive amount of damage was reported on silk cocoons stored at silk factory, Srinagar, India in 1983 and at Central Sericultural Research and Training Institute (CSRTI), Mysore, India (Ansari and Bajalingappa, 1991). Studies made on the beetle pests infesting stored silk cocoons at Department of Entomology, Agriculture College, Dharwad, Karnataka, revealed that the cocoons are attacked by three different species such as, *A. fasciatus* (Thumb), *D. maculatus* (DeGeer), and *D. leechi* Kalik. However, *Tribolium castaneum* (Hbst.), a flour beetle, belonging to Tenebrionidae is also reported to infest cocoons. Among these, the infestation due to *A. fasciatus* was

comparatively severe (Rajasekharan *et al.*, 1985).

In the Philippines, information on the bionomics of *Anthrenus* is meager especially on its relation to sericulture. Detailed studies on *A. scrophulariae* with reference to their feeding behavior, life history, nature of damage and causes of their prolonged infestation are much needed to help in devising suitable control measures to keep the infestation at sub-economic level. The cocoons stored at the Don Mariano Marcos Memorial State University Sericulture Research and Development Institute (DMMMSU - SRDI) are often infested by common carpet beetle, *A. scrophulariae*, which affect the reeling characters, thereby reducing the quantity and quality of raw silk. The presence of hide outs, animal or insect skins, or dried fish at farmers' rearing houses either attract or act as the primary source of infestation of these beetles which subsequently infest silkworm cocoons.

Since their feeding causes serious damage to leather, woolen goods, stored meat, silk cocoons and silk products, they are considered as household/cosmopolitan pests. The larvae, being voracious eaters feed on silkworm pupae, cocoons and occasionally eggs. They make small holes on the cocoons which render them unfit for reeling. Hence, this study was undertaken to generate information on its biology, behavior and extent of damage to stored cocoons.

METHODOLOGY

The experiment was conducted at the screen house of Pest Clinic, DMMMSU-SRDI, Bacnotan, La Union, Philippines.

Mass rearing of *A. scrophulariae*: Cocoons infested by Dermestid beetles were collected from the filature of the Institute for laboratory trials. The nucleus culture was multiplied and maintained to have an adequate number of beetles for the biological investigation. The male and female beetles in pairs were kept in petri dishes and provided with uninfested cocoons for egg deposition besides artificial diet composed of pulverized dog food (50 g), dry silkworm pupa (10 g), sugar (5 g), sodium benzoate (1.5 g), water (250 ml), brewer yeast (30 g) and HCl (pH 4.5). The dermestid eggs laid on cocoons were collected from time to time and replaced with cocoons previously fumigated with phostoxin (at 1.5 g a.i. per m³ of sack pile) to ensure no previous insect infestation. Cocoons with deposited eggs were placed in plastic jars containing moist soil up to 2-3 cm height, as pupation site. The jars were properly labeled to determine the emergence of the adult beetles which were then caged like the initial stock. This procedure was repeated throughout the study.

Identification: Adult beetles were collected from different sericulture sites and fresh specimens were identified using the original description of the species by Hinton and Corbet (1975) and available taxonomic information. The identity of specimens was confirmed by Dr. Victor P. Gapud, Professor, and insect taxonomist of Department of Entomology, UPLB- Los Banos, Laguna, Philippines.

Biology of *A. scrophulariae*: The duration of developmental stages namely, embryo, larva, pupa and adult, fecundity, pupation rate (%) and adult emergence were recorded. Different stages of the insect were also described by determining the body measurements (in 0.05mm caliper), while insect behavior was also observed and documented.

Assessment of damage to silk cocoons: Bivoltine silkworms were reared to produce fresh cocoons, free from *A. scrophulariae*. A kilogram each of newly harvested good bivoltine cocoons was placed in jute sacks of size 8" x 24". These were inoculated with 10 carpet beetle larvae per bag and were placed in a rack to protect them from ants and other destructive house insects. The experiments were arranged following the Complete Randomized Design (CRD) with three replications. The relative humidity (%) and room temperature (°C) were recorded. The number of damaged cocoons per treatment after 15, 30, 45 and 60 days of storage was noted by counting either the partially or completely infested cocoons. The per cent infestation was computed using the following formula:

$$\% \text{ infested cocoons} = \frac{\text{Total number of infested cocoons}}{\text{Total number of cocoons provided}} \times 100$$

RESULTS AND DISCUSSION

Life Cycle

Table 1 provides information on the developmental period of *A. scrophulariae* under laboratory conditions. The incubation period of eggs ranged from 9 to 22 days, with an average of 15.80 days. The eggs are usually laid in small batches (2-4) on the surface of the cocoons. The freshly laid eggs are small, white, soft, elliptical in shape with projections.

Adult females produced an average of 41.06 eggs which hatched in 15 days. The hatchability of the eggs varied from 68 to 100 %, with an average of 85.48 %. The total larval period from hatching to pupation ranged from 98-148 days, with an average of 123 days and from 98-154 days with an average of 126.50 days, for the females and males, respectively. The mean fecundity of common

Table 1: Growth and development of *A. scrophulariae* on artificial diet under laboratory condition

Parameter	Developmental status of <i>A. scrophulariae</i> (Mean $\pm$ SD)
Incubation Period of Eggs (days)	15.80 $\pm$ 5.26
Total larval Period (days)	126.50 $\pm$ 9.57
First Instar	28.75
Second Instar	30.25
Third Instar	34.00
Fourth Instar	33.00
Pupation rate (%)	84.4 $\pm$ 8.52
Pupal period (days)	13.50 $\pm$ 4.35
Adult emergence (%)	75.6 $\pm$ 12.65
Oviposition period (days)	15 $\pm$ 5.55
Adult fecundity ¹ (eggs laid/female)	41.06 $\pm$ 18.18
Hatchability (%)	85.48 $\pm$ 14.7
Adult longevity (days)	10.6 $\pm$ 3.18
Male adult	9.0 $\pm$ 3.8
Female Adult	11.7 $\pm$ 5.16
Total life cycle ² : egg to adult (days)	169.30 $\pm$ 10.26

¹Average of 30 female adults

²Average of 15 cultures

carpet beetle was 40 with hatchability of 87.50 under laboratory condition. The pupal period ranged from 9-16 days, with an average of 12.5 days for the females; 12-15 days with an average of 13.5 days for the males. While, the adult life span ranged from 9 to 17 days, with a mean of 13 days for the females, it was 7-14 days, with a mean of 10.5 days for the males.

The findings corroborate with the observation of Sengupta *et al.* (1990) that the adults emerge, copulate and the females soon deposit eggs in small batches. The grubs and adults are attracted by the smell of stifled and stored cocoons and the dried pupae inside. They bore into the cocoons and eat the dried pupae. They also damage pierced and melted cocoons which are stored within the grainage building for longer duration. Occasionally, they also attack adult silk moths, eggs, and rarely the young silkworm larvae. Presence of multi pierced cocoons in the storage rooms and also the presence of the egg laying silkworm moths in the grainages damaged mostly on the abdominal parts are indication of infestation by dermestid beetles.

The study is also in consonance with claims of Griswold and Greenwald (1941) that adult common carpet beetles live outdoors, feeding on pollen and nectar. It is thought that the pollen and nectar encourage mating

and oviposition outside; the female may lay her eggs in animal nests, bee hives, or on dead animals. Indoors, she may lay her eggs on textiles, carpets, clothing, preserved specimens, or other materials of animal/plant origin. The larvae feed on these materials and require about 66 days to complete their development at room temperature (Griswold and Greenwald 1941, Hasan *et al.*, 2007).

However, the above study is in contrast with the claims of Black (2004) and Smith and Whitman (1996), that say that the larvae progress through six larval instars and at the end of the sixth, they pupate in the last larval skin. Pupation lasts between 7 and 15 days and after pupation, the adults emerge and rest in the larval skin for about 18 days before becoming active adults (Black 2004, Smith and Whitman 1996, Hasan *et al.*, 2007). The adults then make their way outdoors to feed and mate.

Description of different life stages of *A. Scrophulariae* Linn.

The different life stages of *A. scrophulariae* Linn. are depicted in Figure 1. Eggs of the carpet beetle are soft, white and elliptical in shape with 0.5 mm length and 0.34 mm diameter. It varies in color from a translucent to creamy white. The eggs hatch in two weeks' time and the grubs immediately attack silk cocoons. Black (2004) and Smith and Whitman (1996) have also reported that the eggs of genus *Anthrenus* are small, white, and have projections on them to enable adherence to surfaces. They observed that the female lays 30 to 60 eggs that hatch within 10 to 20 days after being oviposited.



Figure 1: Different stages of *A. scrophulariae* Linn. under laboratory condition: (a) first instar (1.99-4.34 mm); (b) second instar (6.03-6.05 mm); (c) third instar (6.71-7.26 mm); (d) fourth instar (7.37-7.48 mm); (e) pupal stage; (f) male adult (1.0-2.05 mm) and (g) female adult (2.0-3.5 mm).

The larvae of *Anthrenus* are elongated, spindle-shaped, possessing numerous short to fairly long, reddish to deep brown and dim black sensory hairs or bristles. Three pairs of five segmented legs on the thorax, bearing single claw

makes it possible for the larvae to crawl rapidly, especially when exposed to light as they are negatively phototropic. There are 10 abdominal segments which tapers towards the rear end. The dorsal side of the ninth segment bears a pair of prominent spines. The color of both male and female beetles are similar. Generally, the females are longer than the males. The antenna of carpet beetle is club-shaped and is buried in the front of the prothorax. Body is convex, round, 1-3.5 mm long and dorsal surface conspicuously covered with alternate patches of white, blackish or brownish scales. Legs are short and when retracted, exhibited grooves on the ventral surface. The above taxonomic description was confirmed true and correct by Dr. Victor Gapud, Insect Taxonomist and Professor, Entomology Department-UPLB, College, Laguna, Philippines. However, Smith and Whitman (1996), Black (2004) and Hasan *et al.* (2007) claimed that in the sixth instar, the larva is 2.5–5.5 mm long and reddish brown, covered with many dark hairs.

Behavioral studies

The first and second instar larvae bore into the cocoons and eat the dried pupae (Figure 2). The larvae spend the whole of their developmental period inside the cocoon *i.e.*, until it is about to pupate. They feed also on pierced and melted cocoons, produce the frasses and complete the duration of about 126.50 days. These conditions rendered the cocoons unfit for reeling, thereby reducing the quantity and quality of raw silk. Signs of damage was seen externally when the larva had already made its way inside the cocoons and had caused considerable damage. This stage is characterized by the presence of frasses accumulated on the surface of the cocoons where initial burrows had been made. The increase in size of the larva inside the cocoons caused some of the accumulated frasses or waste material to move outward the tunnel. The larvae were observed to cease feeding for as long as 8-15 hours prior to each ecdysis. This may be attributed to the duration of cuticle hardening.

Copulation occurs immediately after adult emergence. It lasts for one to four hours and 45 minutes. Mating usually occurs at five o'clock in the early morning. A female and male adult mate usually only once but seldom

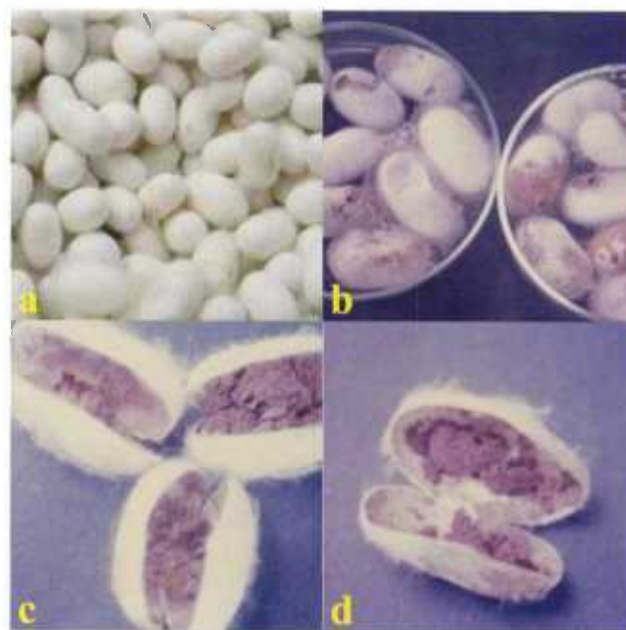


Figure 2: (a) Good quality cocoons; (b) Cocoons infested by *A. scrophulariae*; (c-d) Silkworm pupae damaged & powdered due to Dermestid attack.

3 to 4 times. The female and male adults live for 10.5 – 13 days (Table 1). Oviposition usually occurs at night. A female oviposits 3 or 4 times. The young larvae hatched from eggs laid on the surface of cocoons bore easily into the cocoons than from those laid on other animal and plant products.

Damage to silk cocoons

Table 2 shows the yield loss estimated due to damage of stored cocoons by common carpet beetle. The cocoons stored for different durations *i.e.*, 15, 30, 45 and 60 days showed differences in the degree of damage caused. Cocoons stored for 60 days had the highest mean damage of 3.42 %.

The results are in supportive of the findings of Ansari and Bajalingappa (1991) who observed the occurrence of two species of dermestid beetles, *Dermestes ater* and *Attagenus fasciatus* attacking silkworm cocoons during their survey at different cocoon markets, filature centers, grainages and sericulture museums in Karnataka, India. Different varieties of silkworm cocoons of *B. mori* and non-mulberry silkworm cocoons at CSRTI, Mysore, were

Table 2: Cocoon yield loss due to *A. scrophulariae* infestation under laboratory condition

Storage (Days)	Cocoon yield loss	
	Percentage infestation (%)	Economic value (P/kg)
15	2.58 b	10.32 b
30	2.43 b	9.72 b
45	2.33 b	9.32 b
60	3.42 a	17.28 a

Means in a column with the same letter are not significantly different at 5 % level, DMRT; Price of dried cocoons is P 400.00 per kilogram.

infested and damaged by the larvae. At the government Mini Filature Center, Hubli, percentage of deterioration and weight loss caused to Hosa Mysore and Hosa Mysore x WB18 cocoons of *B. mori*, by the larvae of *D. ater*, was studied after 15, 20 and 25 days storage. The larvae of *A. fasciatus* were not found boring into the cocoons up to 25 days. After 15, 20 and 25 days storage, the deterioration of Hosa Mysore through infestation was 4.1, 5.5 and 7.5 %, respectively. Weight loss of Hosa Mysore /100 cocoons after 15, 20 and 25 days was 2.73, 3.64, and 4.35g, respectively. Similarly, the infestation of cocoons of Hosa Mysore x WB18 was 2.2, 3.8 and 5.6 % and weight loss per 100 cocoons was 3.17, 5.41 and 7.61 g, respectively, percentage deterioration was more in Hosa Mysore cocoons than in Hosa Mysore x WB18 cocoons.

The biological features and behavioural characteristics of the carpet beetle and the extent of damage caused to stored cocoons were worked out in view of the severe outbreaks of *A. scrophulariae* Linn., in the cocoon storage premises in Philippines. The information generated may prove vital in formulation of environmentally sustainable control strategies for effective management of the pest.

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**STADES DESTRUCTIFS DE *ANTHRENUS SCROPHULARIAE* LINN.
(COLEOPTERA: DERMESTIDAE), LE SCARABEE COMMUN
DES TAPIS INFESTANT LES COCONS STOCKES**

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R E S U M E

Les données biologiques de *Anthrenus scrophulariae* Linn., communément nommé le scarabée des tapis, ainsi que la nature des dommages causés sur les cocons stockés ont été établis à la filature DMMMSU-SRDI de la Région I aux Philippines. C'est un insecte holométabole qui accomplit son développement en 126 à 205 jours. Les adultes mâles et femelles sont actifs de 7 à 14 jours et de 9 à 17 jours respectivement. Les femelles pondent de 20 à 88 œufs dont le taux d'éclosion est de 68 à 100 %. Les œufs sont habituellement pondus par petits paquets à la surface des cocons. Les œufs éclosent après 15 jours, les larves subissant cinq mues, la période de développement s'étalant de 123,0 à 126,0 jours au cours desquels elles se nourrissent des chrysalides à l'intérieur des cocons. Après la sixième mue, entre le stade puppe et le stade adulte, le corps devient brun rouge, couvert de nombreux poils sombres et mesure de 5,20 à 9,21 mm de long, alors que le scarabée des tapis adulte est ovale et mesure de 1,0 à 3,50 mm de long. Les cocons stockés deviennent indévidables en raison des nombreux trous formés par les larves du dermeste. Ainsi, le stade larvaire du scarabée cause une perte économique de 3,42 % par kilogramme de cocon allant jusqu'à 13,68 pour un stockage de 15 à 60 jours. Ces données montrent le rôle remarquablement destructif joué par *A. scrophulariae* Linn. Pour la production dans la Région I des Philippines.

Mots-clés: *Anthrenus scrophulariae*, filature, holométabole, métamorphose, cocon indévidable.



Research Paper

IMPROVEMENT OF SOIL CHARACTERS IN ORGANIC CULTIVATION OF MULBERRY *VIS-À-VIS* CHEMICAL BASED FARMING

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ABSTRACT

The sustained production of silk is adversely affected by the poor quality of mulberry leaf. In many a case, it is due to the inadequate application of chemical fertilizers in mulberry cultivation by small and marginal sericultural farmers, because of its escalating costs and non-availability in time. Since organic farming is gaining popularity of late, with its proven advantages, the present study was undertaken to assess the effect of application of different organic manures and biological inputs in mulberry cultivation and its effect on soil fertility. The required quantity of nutrients (NPK) for mulberry were supplemented to the soil through easily available organic sources viz., farm yard manure (FYM), sericompst, poultry manure, neem oil cake, green manure, bio-inoculants [*Azotobacter* biofertilizer, vesicular arbuscular mycorrhiza (VAM), phosphate solubilizing bacterial biofertilizer (PSB)] and rock phosphate. There was significant improvement in the physical, chemical and biological properties of the soil after three years of organic supplementation leading to the enhancement in soil fertility. Thus, the present study confirms the possibility of adoption of absolute organic farming as an alternative method of mulberry cultivation *in lieu* of chemical based farming for sustained quality leaf production.

Key words: Mulberry cultivation, organic farming, soil fertility.

INTRODUCTION

Soil is defined as the unconsolidated mineral material on the immediate surface of the earth that serves as a natural medium for the growth of land plants. Degradation of soil health and fertility has emerged as a major factor responsible for stagnation in agricultural production. Indiscriminate use of chemical fertilizers in farming has deleterious effect on soil health and fertility. It is well known that intensive cultivation leads to a rapid decline in organic matter content and nutrient level of soil besides affecting its properties. Conversely, crop residue management practices influence agricultural sustainability by improving physical, chemical and biological properties of soil. The maintenance of good soil health needs balanced fertilization, which includes application of all the required plant nutrients in proper amount and form (Yadav and Kumar, 2009). The concept of soil health has gained importance because of the prominent role it plays in sustaining crop productivity

(Brar and Preethi, 2007). The imbalanced use of chemical fertilizers continuously for many years can lead to deterioration of soil health rendering it unproductive for further cultivation (Subehia *et al.*, 2005). Organic farming is an eco-friendly system of farming which can maintain the soil health in terms of its biological fertility. Mulberry is a perennial plant mainly cultivated for its foliage, used as feed for *Bombyx mori* L., a monophagous insect which produce 'silk', the queen of textiles. The present study envisaged supplementation of required nutrients through different means of available organic and biological inputs in mulberry cultivation to maintain soil fertility and health for sustainable leaf production.

MATERIALS AND METHODS

The present study was conducted at Central Sericultural Research and Training Institute (CSRTI), Mysore. The experiment was laid with V1 mulberry variety under four treatments with ten replications each in

a randomized block design (RBD), adopting paired row system of cultivation spacing *i.e.*, (90 cm + 150 cm) x 60 cm. The treatments are detailed below:

T-0 (Control): Chemical fertilizers (NPK @ 350:140:140 kg/ha/yr) + FYM 20 MT/ha/yr as recommended for the V1 mulberry variety.

T-1: Chemical fertilizers (50 % of recommended dose) *i.e.*, NPK @ 175:70:70 kg + FYM 20 MT + Sericompst 2 MT + Poultry manure 1 MT/ha/yr + Green manuring (Sunhemp 45 kg seed/ha one time only in monsoon) + *Azotobacter* biofertilizer 10 kg + Rock phosphate 200 kg + Phosphate Solubilizing Bacterial biofertilizer 5 kg/ha/yr.

T-2: FYM 10 MT + Sericompst 5 MT + Poultry manure 10 MT + Neem oil cake 1 MT/ha/yr + Green manuring (Sunhemp 45 kg seed/ha one time only in monsoon) + *Azotobacter* biofertilizer 5 kg/ha/yr + Vesicular Arbuscular Micorrhyza (VAM @ 1000 kg soil based inoculum/ha one time only) + Phosphate Solubilizing Bacterial fertilizer 5 kg/ha/yr.

T-3: FYM 10 MT + Sericompst 5 MT + Poultry manure 5 MT + Neem oil cake 1 MT/ha/yr + Green manuring (Sunhemp 45 kg seed/ha/yr two times during monsoon) + *Azotobacter* biofertilizer 10 kg + Rock phosphate 400 kg + Phosphate Solubilizing Bacterial biofertilizer 5 kg/ha/yr.

A total of 40 plots, with 52 Sq m gross area and 72 plants each were maintained for the study. The soil was red loam with pH 8.34, electrical conductivity, 0.23 mmhos/cm, organic carbon, 0.43 % and available P and K, 14 and 242 kg/ha, respectively. Chemical fertilizers were applied in 5 equal split doses in all crops while organic manures were applied in 3 splits with alternate crops. Sunhemp seeds @ 45 kg/ha were sowed in the wider space of the mulberry rows during monsoon season for the corresponding treatments and mulched with soil prior to flowering but with root nodules. The plots for the treatments receiving VAM were inoculated following Maize intercropping method (Katiyar *et al.*, 2000).

The soil bulk density (g/cc) and water holding capacity (%) were calculated by 'Keens cup' method as described

by Gupta (1999). The soil pH and EC were estimated by following the standard method of Jackson (1973). The organic carbon content (OC) was estimated by modified Walkley and Black (1934) rapid titration method. Available phosphorus was estimated by the method of Olsen *et al.* (1954). Available Potassium was extracted from the soil with the help of saturated ammonium acetate solution. The potash content was estimated using Elico flame photometer method as described by Tandon (1993).

Total bacteria, fungi, *Azotobacter*, P-solubilizing bacteria and actinomycetes were estimated by determining the number of colony forming units (cfu / g soil) from soil samples on recommended culture media in order to monitor the build-up of microbial population in the rhizosphere of different treated and control plots. The different culture media, *viz.*, Nutrient Agar, Rose Bengal Agar, Actinomycetes Agar, Waksman No. 77 medium and Pikovskaya's medium were prepared for the isolation of bacteria, fungi, actinomycetes, *Azotobacter* and phosphate solubilizing bacteria, respectively. The mycorrhizal colonization and spore formation in mulberry rhizosphere were assessed following the method of Gerdmann and Nicolson (1963).

RESULTS AND DISCUSSION

The initial level of soil physical, chemical and biological properties of the experimental site is presented in Table 1 in order to assess the changes in the parameters after the treatment. The data on soil pH, bulk density, water holding capacity and EC after three years are presented in Table 2. Initial level of bulk density of soil before imposition of the treatments was recorded as 1.35 g /cc. The bulk density of the soil did not show any significant difference among treatments for three years. However, it was observed that bulk density decreased from the initial level of 1.35 to 1.25 g/cc in T-2. Further, it has been clearly observed that in both the cases of organic treatments T-2 and T-3, the bulk density consistently decreased through the years from the initial value, contrary to the control where there was no such reduction. This is an indication of improvement in soil physical properties, especially texture, due to application of organics over a period of

Table 1: Initial status of physical, chemical and biological properties of soil

Parameter	Value
Bulk density (g/cc)	1.35
Water holding capacity (%)	14
pH	8.34
EC (mmhos/cm)	0.23
Organic Carbon (%)	0.43
Phosphorus (kg/ha)	14
Potassium (kg/ha)	242
Total bacteria (cfu/g)	3.5×10^4
Total fungi (cfu/g)	2.33×10^3
Actinomycetes (cfu/g)	0.6×10^3
<i>Azotobacter</i> (cfu/g)	0.29×10^3
PSB (cfu/g)	0.22×10^3
VAM propagules (No. / 20 g soil)	52

Table 2: Effect of different organic and biological sources of nutrients on pH, bulk density, water holding capacity and EC after 3rd year

Treatment	pH	Bulk density (g/cc)	Water holding capacity (%)	EC (mmhos/cm)
T0	8.12	1.3	14.0	0.27
T1	8.01	1.28	15.0	0.21
T2	8.18	1.25	15.8	0.17
T3	8.20	1.25	15.6	0.21
CV (%)	0.54	2.26	2.17	5.4
F test	**	NS	**	**
CD @ 5 %	0.056	—	0.541	0.015

NS – Non significant, ** - significant at 1 % level.

time. The long term studies conducted by Kahle *et al.* (1992) on the influence of soil organic matter on physical properties of soils, revealed that supplementation of nutrients through organic sources lowered the bulk density and improved the porosity of soil. It was further supported by the findings of Bairathi *et al.* (1974), Shanmugam and Ravikumar (1980), Bellakki and Bahadur (1994), Srikanth *et al.* (2000) and Patil *et al.* (2003). Incorporation of organic sources is reported to considerably improve the soil properties such as decrease in bulk density and increase in infiltration rate and

available NPK status of the soil (Bajpai *et al.*, 2006).

The water holding capacity of soil increased significantly in the treatment T-2 and attained the highest level of 15.8 % in the third year against the control having 14 % (Table 2). This can be attributed to the addition of only organic manures in the treatment, which significantly improved the water holding capacity of soil. This is in conformation with the results of the studies by Biswas *et al.* (1971) who reported that application of different organic manures combined with *in-situ* green manuring enhanced the organic matter of the soil, which in turn helped in soil conservation. This is also supported by the findings of Das *et al.* (1990), Shivaprakash and Narayangowda (2006) and Selvi and Kalpana (2009). The overall data on EC revealed that application of different organic sources of nutrients resulted in a significant decrease in the EC in treatment T-2 from initial level of 0.23 to 0.17 mmhos / cm after three years (Table 2). This suggests a good amount of improvement in soil health through application of organics over a period of three years. Unlike inorganic fertilizers used in conventional farming, organic manures do not contain salts and hence there was no influence of organic farming on the accumulation of salts in soil.

The soil pH gradually decreased in all the treatments compared to the initial value and was the lowest in the third year (Table 2). However, in the treatments T-0 and T-1, which included application of chemical fertilizers, the decrease was more marked in comparison to the organic treatments. This is perhaps the result of the acidifying activity of the chemical fertilizer, ammonium sulphate used for Nitrogen supplementation. This finding is in agreement with that of Subbuswamy *et al.* (1999).

The initial level of organic carbon (OC) content in the soil of the experimental plot was recorded as 0.43 %. Significant increase in the OC content was recorded in the treatments T-1, T-2 and T-3 in comparison to T-0 (control) in all the three years. The highest OC content in the first year was recorded in the treatment T-1, while it was the highest in T-1 and T-2 during second and third year, respectively (Table 3). This might be due to gradual

Table 3: Effect of different organic and biological sources of nutrients on OC, phosphorus and potassium level after 3rd year

Treatment	OC (%)	Phosphorus (kg/ha)	Potassium (kg/ha)
T0	0.45	74.6	312
T1	0.65	55.5	224
T2	0.67	70.1	224
T3	0.59	68.6	269
CV (%)	2.87	3.73	5.02
F test	**	**	**
CD @ 5 %	0.021	2.435	5.73

** - Significant at 1 % level

increase in the organic carbon content of soil by virtue of decomposition and mineralization of the organic inputs. Various studies undertaken in different agricultural crops revealed that the application of organic manures increased the organic carbon content significantly (Gupta *et al.*, 1988; Pattar *et al.*, 1998). In the present study, the organic carbon content improved significantly in the treatments where different organic manures combined with green manuring and bio-inoculants were applied. Similar findings were reported in mulberry cultivation by Mesilamani *et al.* (2007) and Kumar *et al.* (2005).

In the present study, it was observed that phosphorus content increased in treatments where phosphate solubilizing/ mobilizing organisms were applied together with organic manures as they could convert fixed phosphorus into available form. In the third year, the soil potassium content increased significantly in the control

which may be due to regular application of potassium through chemical fertilizers. This is in conformity with the findings of Reddy *et al.* (2006) who observed increase in potassium content of soil due to application of potassium through chemical fertilizers.

The data on various microorganisms viz., total bacteria, total fungi, actinomycetes, *Azotobacter*, PSB and VAM (Tables 4 and 5) revealed a significant improvement in their population throughout the period due to application of various organic inputs. This is attributed to the continuous application of organic materials which favored the growth of all these microorganisms. Organic manures release large quantity of enzymes which stimulate the growth of microbes already present in the soil and also enhance the population of rhizosphere micro flora, which in turn improves the plant growth (Balasubramanian *et al.*, 1972; Gaur *et al.*, 1992). The *in-situ* green manuring also promoted the saprophytic fungal growth as the nutrient availability was high due to large quantity of biomass. Krishnakumar *et al.* (2005) observed that application of FYM and Neem oil cake in combination with other organic manure increased the actinomycetes population. Continuous use of organic manures improved the microbial load of the soil rather than single organic manure application. Briggs and Spedding (1963) and Kiss *et al.* (1986) reported that the application of organic manures favours more enzyme activity in the soil ecosystem, which enhances the growth of more microorganisms and satisfies the nutritional need of plants by humification of the added organic materials.

Table 4: Effect of different organic and biological sources of nutrients on population of microorganisms after 3rd year

Treatment	Total bacteria (cfu/g x 10 ³)	Total fungi (cfu/g x 10 ³)	Actinomycetes (cfu/g x 10 ⁵)	<i>Azotobacter</i> (cfu/g x 10 ⁴)	PSB (cfu/g x 10 ⁴)
T0	0.55	8.79	0.72	4.34	2.94
T1	1.08	11.46	1.94	12.2	9.56
T2	2.35	13.65	2.65	10.83	5.28
T3	2.28	14.49	2.51	13.73	13.03
CV (%)	3.27	2.12	1.2	0.37	0.47
F test	**	**	**	**	**
CD @ 5 %	0.063	0.335	0.025	0.029	0.057

** - Significant at 1 % level



Figure 1: Well - grown mulberry under organic farming

Table 5: Effect of different organic and biological sources of nutrients on VA-mycorrhizal colonization and propagules in mulberry roots (%)

Treatment	VA-mycorrhizal colonization	VA-mycorrhizal propagules
T0	62.15	68
T1	70.33	78
T2	85.40	88
T3	82.86	85
CV (%)	1.82	1.62
F test	**	**
CD @ 5 %	2.938	2.513

** - Significant at 1 % level

It is thus established that the soil fertility status was improved through organic farming which favours the growth and yield of mulberry on par with that of chemical based farming (Figure 1).

CONCLUSION

The present study proves that the supplementation of plant nutrients in the form of different organic and biological sources has improved the soil physical, chemical and biological properties, which in turn determined the soil fertility. The year wise improvement in the biological properties of soil due to the continuous

application of the organic inputs can be a promising approach towards soil improvement. Thus, organic farming may be adopted as an alternative practice to chemical fertilizer based farming in mulberry cultivation for sustainable crop production.

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AMELIORATION DES CARACTERES DU SOL DANS LA CULTURE BIOLOGIQUE DU MURIER PAR RAPPORT A LA L'AGRICULTURE BASEE SUR L'EMPLOI DE PRODUITS CHIMIQUES

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R E S U M E

La production durable de soie est contrariée par la faible qualité de la feuille de mûrier. Dans de nombreux cas, ceci est dû à l'application inappropriée de fertilisants chimiques dans la culture du mûrier par les petits et marginaux sériculteurs, en raison de l'accroissement des coûts et de la non-disponibilité au bon moment. Bien que l'agriculture biologique gagne en popularité avec ses avantages démontrés, l'étude présente a été entreprise pour évaluer l'effet de l'application de différents engrais biologiques et les apports dans la culture du mûrier et leurs effets sur la fertilité du sol. La quantité requise de nutriments (NPK) pour le mûrier a été ajoutée au sol à travers des sources biologiques variées telles que le fumier (FYM), le compost séricicole, le fumier de volailles, l'engrais vert, des bio-inoculant (*Azotobacter*, les mycorhizes arbusculaires vésiculaires (VAM), un bio-fertilisant bactérien solubilisant le phosphate (PSB) et du phosphate de roche. On constate une amélioration significative des propriétés physiques, chimiques et biologiques du sol après trois ans d'ajouts biologiques entraînant une augmentation de la fertilité du sol. Ainsi, la présente étude confirme la possibilité d'adopter une agriculture entièrement biologique comme alternative à une agriculture basée sur la chimie dans la culture du mûrier pour une production durable de feuilles.

Mots-clés: Culture du mûrier, agriculture biologique, fertilité du sol.



IDENTIFICATION OF NEWLY ISOLATED MICROSPORIDIAN NIK-1Pr CAUSING PEBRINE DISEASE IN SILKWORM *BOMBYX MORI* L., USING CONVENTIONAL PCR TECHNIQUE

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ABSTRACT

The bio-chemical and PCR analyses for identification of NIK-1Pr, a newly isolated microsporidian strain from *Pieris rapae* were conducted and results compared with the type species, *Nosema bombycis*. The NIK-1Pr was found to infect the commercial silkworms. The protein content in NIK-1Pr was significantly higher when compared with that of *N. bombycis*. The lowest OD recorded was 2.95×10^{-2} and 1.75×10^{-2} in NIK-1Pr and *N. bombycis*, respectively on a concentration of 10 μ l / ml. The highest OD was observed at a concentration of 1000 μ l / ml (13.15×10^{-2} and 10.80×10^{-2} , respectively). To confirm the identity of pathogen as a true microsporidian, the DNA was isolated and tested in a PCR using the universal rRNA primers. The primers generated an expected PCR fragment (1250 bp) and these results clearly suggest that the identified pathogen is in fact a microsporidian isolate and possibly belonging to the genus, *Nosema*. It is concluded that the insect pests associated with mulberry may harbor a number of infective microsporidia thus posing a threat of pebrine infection to commercial silkworms.

Key words: *Bombyx mori* L., NIK-1Pr, *Nosema bombycis*, PCR analysis, pebrine.

INTRODUCTION

In India, the productivity of raw silk is lower when compared to China. The reasons attributed to low per hectare production of raw silk in India are frequent crop failures due to silkworm diseases and inclement weather conditions. The loss due to diseases in India is about 15-20% as against 3-4 % in China. The silkworm, *Bombyx mori* L. is a delicate and sensitive insect, which has been domesticated for silk production since time immemorial as a captive. Microsporidiosis is considered to be one of the serious diseases in the sericultural world including India. The disease is caused by different parasitic microsporidians. Among them, *Nosema bombycis* (Naegeli) is commonly prevalent during all the seasons and is a potential threat to the sericulture industry if not managed effectively. Of late, there are reports on different strains of microsporidians isolated from the insect pests causing pebrine disease (Bhat and Nataraju, 2004). Hence, the present study is made on NIK-1Pr, a newly

isolated microsporidian strain from *Pieris rapae* which causes pebrine disease in the silkworm (Bashir, 2008). Normally, the *per oral* infection of silkworm larvae with *N. bombycis*, *Nosema* sp. NIS-M11, *Vairimorpha* sp. NIS-M12, NIS-M14 and NIS-M25 is systemic and severe. They form environmental spores in all tissues and organs *viz.*, mid-gut epithelium, muscles, fat body, silk gland and malpighian tubules. They are eukaryotes with distinct nucleus and nuclear envelope but lacking centrioles or mitochondria and are considered unique among the eukaryotes because of their small sub-unit ribosomal RNA (SSU-rRNA) which is smaller than those of typical eukaryotes (Vossbrinck *et al.*, 1993). All microsporidia are intracellular parasites of great reproductive capacity and are characterized by the possession of thick walled spores containing a polar filament and a sporoplasm. But these characterizations have become traditional and generate lots of redundant data. Thus, in the present study, identification of newly isolated strain NIK-1Pr is carried

out using conventional PCR technique and compared with type species *N. bombycis* to confirm its identity.

MATERIALS AND METHODS

Isolation and purification of microsporidian spores: The silk moths infected with NIK1Pr were homogenized individually in 1 ml of 0.6 % K_2CO_3 solution and the smear was observed for the presence of microsporidian spores at 600 x magnification under Nikon (Type-104) phase contrast microscope. The microsporidian spores were purified as per density gradient method (Sato and Watanabe, 1980). The final sediment of the microsporidia was suspended in physiological saline (0.85 % NaCl) and stored at 5 °C for further experimentation.

Biochemical characterization of microsporidian spore, NIK-1Pr: Estimation of spore protein levels: The spore proteins of both microsporidians (NIK-1Pr and *N. bombycis*) were isolated by following the method described by Kawarabata and Hayasaka (1987). The purified spores of NIK-1Pr and *N. bombycis* were suspended in 15 % TCA (1×10^8 spores / ml) for 30 min. at room temperature. The suspension was gently stirred at interval of 5 min. and the pH of the spore suspension was adjusted to 7.0 using 0.1N HCl. The spore suspension was centrifuged at 8000 rpm for 15min. and the residue containing spore protein was collected. An equal volume of Sodium hydroxide (1N NaOH) was added to the residue and kept at 4 °C over night.

Protein content of spores was determined by the method of Lowry *et al.* (1951). Aliquot was taken in a test tube and made to 1ml with distilled water. Five ml of alkaline copper reagent was added, mixed and kept standing for 15 minutes at room temperature. To this mixture, 0.5 ml of diluted folin reagent was added, mixed well and left for 30 minutes in dark at room temperature for colour development. For reagent blank, distilled water was used instead of protein solution. The standard strain, *N. bombycis* and the reagent blank were run simultaneously with test samples each time. All the samples were read at 660 nm using the quantitative mode of a Shimadzu 160Å spectrophotometer.

Identification of newly isolated microsporidian spores: DNA extraction: Genomic DNA was extracted from the sporoplasms discharged from spores using the glass bead method described by Undeen and Cockburn (1989). The microsporidians isolated were suspended in an aqueous solution, containing 100 mM NaCl, 200 mM sucrose, 10 mM EDTA and 30 mM Tris-HCl buffer (pH 8.0), to osmotically protect the nuclei from disruption. To this, an equal volume of spore suspension (5×10^{10}) and glass beads (0.425 to 0.600 microns, 30-40 U.S. Sieve size; Sigma, St. Louis, MO) were added in a 1.5 ml eppendorf tube. The tubes were vigorously shaken for 5 to 10 min. at maximum speed on the vortex. When > 85 % of the spores had ruptured, as indicated by their dark and empty appearance under a phase contrast microscope, the homogenate was centrifuged at 3000 rpm for about 2 min. and the supernatant solution was transferred to a fresh eppendorf tube using a Pasteur pipette. Proteinase K (100µg/ml; USB, Cleveland, USA), 1/4th volume of 2.5 % Sodium dodecyl sulphate (SDS), 250 mM EDTA and 500mM Tris-HCl (pH 9.2) were added and the mixture was incubated for 1 h at 55°C to release DNA from the nuclei. The SDS and proteins were precipitated by adding 1/5th volume of 1M potassium acetate, incubated for 1 h at 4°C and centrifuged at 5000 rpm for 20 min. The DNA was precipitated by adding double the volume of cold absolute ethanol. RNA was degraded by digestion with 100 µg / ml RNase A (USB, Cleveland, USA) for 1 h at 37°C. DNA concentration and quality was determined both by spectrophotometry at 260 and 280 nm and on 0.8 % agarose gel, using a known quantity of λDNA (10 ng / µl) as a standard before use in subsequent PCRs. A working solution of DNA (10 ng / µl) was prepared in sterile autoclaved double distilled water.

PCR amplification of SSU-rRNA gene: The genomic DNA of the microsporidian isolate, NIK-1Pr and other three standard type species were amplified (Figure 2) for the SSU-rRNA gene. The SSU-rRNA genes were amplified from NIK-1Pr and *N. bombycis*, using the universal primers VN001F 5' - CTGCAGGTACCACCAGGTTGATTCTGCCTGAC-3' and VN001R5'GAGCTCGCATGCGGTTTACCTTGTTACGACTT-3' (PAGE purified), designed by Hatakeyama *et al.* (1997) and procured from Genei pvt. ltd., Bangalore,

India. In detail, the PCR amplification was carried out in a Peltier Thermal Cycler PTC-200 (MJ Research Inc. Watertown, Mass.), in 20 µl of reaction mixture containing approximately 50 ng of template DNA obtained from each of the microsporidia, 10 pmol of each primer, 200 µM each dNTP's (Fermentas Life Sciences, Vilnius, Lithuania) and 1 U of *Taq* DNA polymerase in 1 x PCR buffer containing 75 mM Tris-HCl pH 8.8, 20 mM (NH₄)₂SO₄, 0.01 % Tween 20, and 2 mM MgCl₂ both provided by the manufacturer of the enzyme (Fermentas Life Sciences, Vilnius, Lithuania). Amplification reactions were carried out for 40 cycles after an initial denaturation for 3 min. at 94 °C. Each PCR cycle comprised three steps: denaturation at 94 °C for 2 min. annealing at 55 °C for 2 min. and extension at 72 °C for 30 sec. with a final extension of 30 min. at 72 °C. The amplified PCR products were size fractionated by electrophoresis on 1.5 % agarose gel (Gibco BRL, Grand Island, NY) in 1x Tris borate EDTA buffer (89 mM Tris, 89 mM Boric Acid, 2 mM EDTA, pH 8.0) and gels were stained with ethidium bromide (0.5 µg/ml) for 30 minutes. A standard molecular weight marker (Mass ruler DNA ladder, Fermentas Life Sciences, Vilnius, Lithuania) was used and the UV trans-illuminated gels were photographed by using Gel Documentation System (Syngene Corporation, Cambridge, U.K).

RESULTS

Biochemical characterization of microsporidian spore, NIK-1Pr: Protein profiles: The microsporidian NIK1Pr and *N. bombycis* spore protein were successfully isolated, purified and quantified. The spore protein were subjected to spectrophotometric study and the OD values obtained for microsporidian spore, NIK-1Pr and *N. bombycis* are shown in Figure 1. The profile of the solubilised spore protein showed higher content of protein in NIK-1Pr compared to *N. bombycis*. There was a gradual increase recorded from the concentration of 10 to 1000 µl / ml in both NIK-1Pr and *N. bombycis*. The lowest OD was recorded as 2.95×10^{-2} and 1.75×10^{-2} in NIK-1Pr and *N. bombycis*, respectively for concentration of 10 µl / ml. The highest OD was observed for a concentration of 1000 µl / ml in NIK-1Pr (13.15×10^{-2}) and *N. bombycis* (10.80×10^{-2}).

Total Spore Protein Estimation

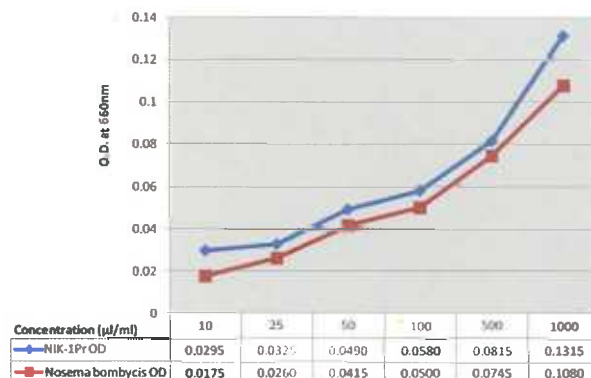


Figure 1: Comparison of spore protein between NIK-1Pr and *Nosema bombycis* at 660 nm

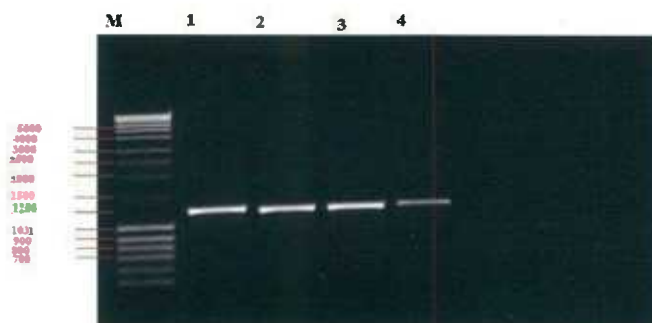


Figure 2: PCR amplification of genomic DNA of 4 microsporidian isolates with primers designed based on small subunit rRNA (SSU-rRNA) sequences on 1.5 % agarose gel: 1-NIK-1Pr; 2-NIS-001(Standard); 3-NIK-1S (Standard); 4-*Vairimorpha* sp. NIS-M12; The lane marked M shows the molecular-size marker.

Molecular identification of the microsporidian isolate: SSU-rRNA-PCR analysis: The extracted DNA of NIK-1Pr and *N. bombycis* was quantified using 0.8 % agarose gel and accordingly run in the PCR with the universal primers specially designed to amplify the unique SSU-rRNA of the microsporidia. The primers generated expected size fragment of 1250 bp for the microsporidian species, NIK-1Pr, standard strain of *N. bombycis* showed 1170 bp, NIK-1S showed 1200 bp and *Vairimorpha* sp. has shown 1031 bp (Figure 2).

DISCUSSION

The present investigation consisted of collection of the new microsporidian spore NIK-1Pr infecting silkworm, its purification, isolation, biochemical and molecular identification using conventional PCR technique, to

confirm its identity and comparison with *N. bombycis*. In the recent past, many microsporidia have been isolated from silkworms which are morphologically different from the standard strains of *Nosema* and exhibiting varying degree of pathogenicity in silkworm (Ananthalakshmi *et al.*, 1994). Microsporidian spores meet the environment with a resilient spore wall that consists of a protein coat and chitinous endospore. Protein is the most complex and most characteristic living matter in the viable cells. Due to the unusual functional and solubility properties of the polar tube, the proteins present in it are likely to be members of a protein family with a highly conserved amino acid composition among the various microsporidia. Thus, estimating the soluble protein in the spore is a necessary study which appropriately gives a clear insight into the protein content of the spore which differs with different microsporidian species (Undeen and Cockburn, 1989). The present study revealed that NIK-1Pr is having higher content of spore protein than the standard strain of *N. bombycis* (NIS-001). There was a gradual increase recorded from the concentration of 10 to 1000 $\mu\text{l/ml}$ in both NIK-1Pr and *N. bombycis* batches. The highest OD was observed (Figure 1) at a concentration of 1000 $\mu\text{l/ml}$ in NIK-1Pr (13.15×10^{-2}) and *N. bombycis* (10.80×10^{-2}).

Development of molecular biological techniques has resulted in the availability of new tools for taxonomic and phylogenetic studies. In the present study, the extracted DNA of NIK-1Pr and *N. bombycis* was quantified using universal primers with expected size fragment of 1250 bp for the microsporidian species, NIK-1Pr (Figure 2) indicated its variability from *Nosema*. Hatakeyama *et al.* (1997) used SSU-rRNA sequences to draw taxonomic inferences about 5 microsporidians infecting *B. mori*. The studies based entirely on ultra-structural characters and life-cycle features sometimes mislead the identity. In the related studies on microsporidia classification (Hazard and Oldacre, 1975; Sprague, 1982), *Vairimorpha* species have been described twice, each time in a different genus, *Nosema* (diplokaryotic and disporoblastic) and *Thelohania* (uninucleate and octosporoblastic) on the basis of ultra-structural features. Studies involving large

subunit rRNA sequences from different *Vairimorpha* and *Nosema* sp (Baker *et al.*, 1994) showed that the *Nosema* sp. was a paraphyletic group and this was later confirmed by Malone and Mclover (1996) using the SSU-rRNA gene. The results indicate that the rRNA sequence-based phylogeny is useful in resolving problems related to species identification in microsporidia. Thus, the SSU-rRNA amplification analysis using PCR technique in the present study indicates that the NIK-1Pr is infective microsporidia probably belonging to genus *Nosema* and differs from *Vairimorpha*. This needs further confirmation through studies on cloning of the obtained PCR product, sequencing and constructing the genetic dendrogram.

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IDENTIFICATION PAR LA TECHNIQUE CONVENTIONNELLE DE PCR D'UN NOUVEAU MICROSPORIDIEN, NIK-1Pr, CAUSANT LA PEBRINE CHEZ LE VER A SOIE *BOMBYX MORI* L.

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R E S U M E

L'analyse biochimique et PCR pour l'identification de NIK-1Pr, une souche de microsporidien nouvellement isolée à partir de *Pieris rapae* a été comparée avec celle de l'espèce type *Nosema bombycis*. NIK-1Pr infecte les vers à soie commerciaux. Le contenu en protéines de NIK-1Pr est significativement plus élevé que celui de *N. bombycis*. La plus faible densité optique enregistrée est respectivement de 2.95×10^{-2} et de 1.75×10^{-2} chez NIK-1Pr et chez *N. bombycis* à une concentration de 10 µl/ml. La densité optique la plus élevée est respectivement de 13.15×10^{-2} et de 10.80×10^{-2} à une concentration de 1000 µl/ml. Pour confirmer que le pathogène est un vrai microsporidien, l'ADN a été extrait et soumis à PCR en utilisant des amorces ARNr universelles. Les amorces ont généré un fragment PCR attendu (1250pb) et ce résultat suggère clairement que le pathogène est un microsporidien et possiblement appartenant au genre *Nosema*. Nous concluons que les insectes associés au mûrier peuvent présenter un nombre de microsporidiens infectieux et constituer ainsi une menace pour les vers à soie commerciaux.

Mots-Clés: *Bombyx mori* L., NIK-1Pr, *Nosema bombycis*, analyse PCR, pébrine.



EVALUATION OF DOUBLE HYBRIDS AND PARENTS OF SILKWORM, *BOMBYX MORI* L. BASED ON HYBRID VIGOUR

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ABSTRACT

Twelve bivoltine parental breeds (6 each of oval and dumb-bell) developed at CSRTI, Mysore were utilized to prepare fifteen each of oval and dumb-bell foundation crosses (FC) in a selective method. All possible (oval x oval) x (dumb-bell x dumb-bell) four-way crosses prepared out of these FCs were reared in three replications during different seasons of the year to evaluate their performance for both pre and post-cocoon traits and also to estimate the magnitude of heterosis. The study reveals that, out of 15 each of bivoltine oval and dumb-bell FCs and 21 four-way crosses studied, 6 each of oval and dumb-bell FCs and 5 four-way crosses exhibited better performance with desirable positive heterosis for most of the economic traits, which may be attributed to better inherent advancement obtained in the study. The results of the present study also show prophecy of identifying promising bivoltine double hybrids for field testing based on overall performance and heterosis for each of the quantitative trait.

Key words: Bivoltine foundation crosses, field testing, four-way crosses, heterosis.

INTRODUCTION

The silkworm *Bombyx mori* L. is an insect of great importance not only to the sericultural economy but also to biological studies and has become one of the most studied model systems in lepidopteron genetic research. Silkworms have been reared for over 5000 years in China (Nagaraju and Goldsmith, 2002 a, b) for silk production purpose and about 4,310 silkworm germplasm stocks comprising of geographical races, inbred lines and mutants are thought to be available worldwide (Goldsmith *et al.*, 2005). The genetic diversity of silkworm is derived from hybridization of races of different geographical origins, mainly Japanese, Chinese, European and also Indian having distinct genetic traits. Among these four geographical races, silkworm of temperate origin produces superior quality silk fibre, whereas the tropical races are hardy, tolerant to pathogen load and resistant to diseases. However, the tropical races produce less quantity of silk, which is coarser and weaker.

Hence, these geographical races are very valuable genetic stocks for further improvement of silkworm strains (Goldsmith *et al.*, 2005). An extensive study is needed to improve existing races / strains for commercial purposes and to develop new strains through breeding programs aimed at improving silk productivity, adaptability to local environments and disease resistance / tolerance capabilities (Sen *et al.*, 1999). Keeping these in mind, productive and robust bivoltine breeds were developed at Central Sericultural Research and Training Institute (CSRTI), Mysore by utilizing the exotic bivoltine hybrids of Japan. The breeds developed with great caution have been utilized in preparation of single and double hybrids.

Four-way crosses of bivoltine silkworm are essentially preferred to overcome adverse environmental conditions. India, being a tropical country possesses such challenges to any agricultural avocation in abundance because of the fluctuations in ambient environmental conditions during different seasons of the year and the silkworm rearer often requires a silkworm breed / hybrid owning inherent

potential to ease such challenges. Exploitation of heterosis through hybrids for economic traits triggered a revolutionary change in overall qualitative and quantitative silk output (Harada, 1961; Gamo, 1976; Subba Rao and Sahai, 1990; Nagaraju *et al.*, 1996). The F1 hybrids are more productive and robust, which can easily be reared by the farmers by adopting appropriate rearing technology (Ramesha *et al.*, 2009; Seshagiri *et al.*, 2009). Generally, the breeds with high cocoon shell percentage show low viability and produce less number of eggs. Moreover, owing to the existence of negative correlation between cocoon shell percentage and pupation rate in pure races, their handling needs more care and attention. To overcome this, the four-way crosses with suitable foundation crosses (FCs) were developed. There were clear advantages such as easy rearing, better growth and vigour of FCs and economic characters and yield of double cross on par or better than single hybrids (Nirmal Kumar *et al.*, 1998). This helps not only in generation of quality seed cocoons but also in improved egg recovery. Hence, the bivoltine four-way crosses evolved are being commercially exploited to the maximum potential (Mal Reddy *et al.*, 2005).

Though there were distinct merits observed in four-way crosses exploitation, certain disadvantages were also noticed. The maintenance and multiplication of bivoltine parental breeds, which involve in preparation of two way crosses, at different downstream multiplication centers is obviously a difficult task. If the performance of one of the breeds is not up to optimum, the whole four-way cross preparation program will be hampered. To overcome these demerits during multiplication of four-way crosses, a novel idea was implemented to converge the selected six each of oval and dumb-bell parental breeds in the preparation of fifteen FCs by crossing (O x O) and (D x D) breeds and by utilizing them, 21 four-way crosses were prepared to know their performance in different seasons. Present study was undertaken to assess the overall rearing performance and heterosis estimates of bivoltine silkworm foundation crosses and four-way crosses involving selected oval and dumb-bell parents during summer, rainy and winter seasons of the year.

METHODOLOGY

In the present study, 12 bivoltine breeds *viz.*, BBE226, CSR27, CSR17, JPN8, JPN7 and S5 (oval) and BBE267, D13, S9, BBE247, CSR16 and CSR26 (dumb-bell) were utilized for preparation of 15 each of oval and dumb-bell foundation crosses. Utilizing 15 oval FCs as female parents with dumb-bell FCs, 21 (OxO)x(DxD) four-way crosses were prepared by employing partial diallel cross method and reared in three replications by following the standard rearing method (Datta, 1992). To ascertain the pre-cocoon, cocoon and post-cocoon performance, 13 important economic traits that govern quality and quantity of silk output, such as fecundity, larval duration (total and 5th age), yield/ 10,000 larvae by number, yield/10,000 larvae by weight, cocoon weight, shell weight, cocoon shell percentage, filament length, denier, raw silk percentage, neatness, renditta and boil-off loss were evaluated.

The heterosis for both foundation crosses (FCs) and four-way crosses were estimated for all the traits (except total and 5th age larval duration) in all the seasons by following the formula as detailed below.

$$\text{Heterosis (\%)} = \frac{\text{F1 value} - \text{Mid parent value (MPV)}}{\text{Mid parent value}} \times 100$$

F1 = Phenotypic mean value of hybrid (foundation cross / four - way cross)

MPV = Phenotypic mean value of parents

RESULTS

The mean performance of the bivoltine breeds, oval foundation crosses, dumb-bell foundation crosses and four-way crosses are presented in Tables 1, 2, 3 and 4, respectively. The hybrid vigour for the quantitative traits studied in oval FCs, dumb-bell FCs and four-way crosses are presented in Tables 5, 6 and 7, respectively.

1. Fecundity: Among the parental breeds, D13 expressed maximum fecundity of 562 (Table 1). Mean values of oval and dumb-bell FCs revealed maximum of 548 in CSR27 x CSR17 and 573 in (D13xS9), respectively (Tables 2 and 3). Among four-way crosses, (S5xCSR27) x (D13xS9)

Table 1: Performance of bivoltine parental breeds in different seasons(Mean values $\pm$ SD of 3 seasons)

Breed	Fecu- ndity	Larval duration		Yield/10000 larvae		Cocoon weight (g)	Shell weight (g)	Cocoon shell (%)	Filament		Raw silk (%)	Ren- ditta	Neat- ness (p)	Boil off loss (%)
		Total (h)	V age (h)	No.	Wt. (kg)				length (m)	size (d)				
Oval														
CSR17	537	537	149	9172	16.04	1.855	0.438	23.61	1053	2.81	18.37	6.76	92	23.00
	±31.88	±24.04	±8.31	±123.52	±0.86	±0.12	±0.02	±0.22	±80.28	±0.24	±0.87	±0.04	±0.51	±1.00
CSR17	536	537	149	9163	16.70	1.821	0.455	25.01	1030	2.80	18.40	6.70	92	23.00
	±40.30	±24.04	±8.31	±83.43	±0.73	±0.02	±0.02	±0.79	±44.76	±0.10	±0.30	±0.10	±0.19	±1.00
JPN8	530	533	149	9153	16.00	1.715	0.395	23.01	1203	2.11	18.26	6.68	92	21.50
	±23.06	±18.42	±8.31	±82.71	±0.34	±0.02	±0.01	±0.38	±35.50	±0.09	±1.41	±0.03	±0.69	±0.50
JPN7	537	534	149	9193	16.03	1.854	0.437	23.65	1000	2.26	18.15	6.77	92	21.50
	±37.17	±19.54	±8.31	±76.95	±0.60	±0.12	±0.03	±1.56	±20.41	±0.14	±0.79	±0.08	±0.38	±0.50
S5	524	534	149	9192	16.02	1.837	0.431	23.50	1108	2.67	18.30	6.85	92	23.00
	±18.83	±19.54	±8.31	±47.44	±0.56	±0.06	±0.03	±2.01	±30.50	±0.15	±0.26	±0.22	±0.69	±0.58
BBE226	521	539	149	8740	14.24	1.613	0.376	23.33	864	3.06	17.09	7.26	90	25.00
	±49.98	±22.74	±8.31	±109.55	±0.76	±0.06	±0.02	±0.93	±11.65	±0.26	±0.60	±0.08	±0.00	±0.58
Dumb-bell														
CSR16	529	533	149	9754	18.15	1.908	0.423	22.18	965	2.78	18.12	6.75	92	23.00
	±25.27	±31.48	±8.31	±86.33	±0.69	±0.14	±0.03	±0.37	±100.16	±0.35	±0.935	±0.05	±0.58	±1.00
CSR26	550	533	149	9723	17.97	1.910	0.419	21.95	966	2.84	18.32	6.72	92	23.00
	±35.22	±31.48	±8.31	±159.94	±1.50	±0.16	±0.03	±0.14	±66.12	±0.18	±1.33	±0.03	±0.51	±1.15
S9	555	533	149	9723	18.08	1.896	0.412	21.72	991	2.67	19.22	6.70	93	22.00
	±41.85	±31.48	±8.31	±234.26	±1.52	±0.10	±0.02	±0.42	±83.45	±0.17	±0.58	±0.05	±0.00	±0.58
D13	562	533	149	9653	18.43	1.902	0.405	21.35	950	2.69	17.80	6.77	92	23.00
	±31.29	±31.48	±8.31	±42.27	±0.56	±0.20	±0.02	±1.16	±121.11	±0.18	±1.31	±0.30	±0.58	±1.00
BBE247	516	533	149	9370	16.90	1.748	0.385	22.04	896	3.08	16.35	7.55	90	25.00
	±30.00	±31.48	±8.31	±451.57	±1.47	±0.13	±0.03	±0.37	±85.50	±0.23	±1.01	±0.06	±0.58	±0.58
BBE267	537	524	149	9382	16.83	1.781	0.321	18.15	915	3.16	16.30	7.45	90	25.00
	±25.92	±18.33	±8.31	±266.47	±1.51	±0.22	±0.01	±1.95	±30.83	±0.15	±0.42	±0.12	±0.58	±0.58

showed maximum fecundity of 644 (Table 4). The oval FCs expressed maximum heterosis of 5.98 in (S5xJPN8) and in dumb-bell FCs, the highest of 6.18 was recorded in (BBE267xD13) whereas in four-way crosses, (JPN8xCSR27) x (S9xCSR26) expressed maximum vigour of 18.42 % (Tables 5, 6 and 7).

2. Larval duration: Larval duration in oval breeds ranged from 533 (JPN8) to 539 h (BBE226) and in dumb-bell breeds, except in BBE267 (524 h), it was 533 h (Table 1). Oval FCs, dumb-bell FCs and four-way crosses showed mean larval duration of 545 h, 542 h and 553 h, respectively, with no variability during the different seasons and in the case of four way crosses, uniform mean total and 5th age larval duration was noticed (Tables 2, 3 and 4).

3. Yield / 10000 larvae (by number): Among pure breeds, CSR16 recorded the highest of 9754 cocoon yield per 10000 larvae (Table 1). Overall mean values of FCs showed the highest of 9775 in (CSR16xCSR26) (Tables 2

and 3). On the other hand, in four-way crosses, (JPN8xCSR27) x (S9xCSR26) recorded the highest of 9581 (Table 4). The highest magnitude of heterosis in oval FCs was recorded in BBE226xJPN7 (2.90 %) and in dumb-bell FCs, (D13xCSR16) expressed 0.61 % whereas, in four-way crosses, (JPN8xCSR27) x (S9xCSR26) expressed the highest heterosis of 1.49 % (Tables 5, 6 and 7).

4. Yield / 10000 larvae (by weight): Among the parental breeds, D13 showed the highest of 18.43 kg (Table 1). In oval FCs, maximum of 18.190 kg was recorded in JPN8xJPN7 and in dumb-bell FCs, 18.730 kg each was scored in CSR16xCSR26 and D13xS9. On the other hand, in four-way crosses, (CSR27xCSR17) x (D13xCSR26) recorded the highest of 21.140 kg (Tables 2, 3 and 4). The highest heterosis of 16.09 % was recorded in oval FCs (CSR27xCSR17) and in dumb-bell FCs, 3.71 % was recorded in (CSR16xCSR26). Among four-way crosses, (CSR27xCSR17)x(D13xCSR26) recorded maximum heterosis of 25.76 % (Tables 5, 6 and 7).

Table 2: Rearing performance of oval foundation crosses (FCs) during different seasons

(Mean values $\pm$ SD of 3 seasons)

Oval FC	Fecundity	Larval duration		Yield/10000 larvae		Cocoon weight (g)	Shell weight (g)	Cocoon shell (%)	Filament		Raw silk (%)	Re-n-ditta	Neat-ness (p)	Boil off loss (%)
		Total (h)	V age (h)	No.	Wt. (kg)				length (m)	size (d)				
BBE226xS5	518 ± 19.61	545 ± 23.64	144 ± 0.00	8645 ± 284.80	16.180 ± 0.78	1.89 ± 0.03	0.382 ± 0.01	20.25 ± 0.21	836 ± 65.68	2.76 ± 0.14	16.63 ± 0.52	6.94 ± 0.05	92 ± 0.00	24.50 ± 0.00
BBE226xJPN8	515 ± 12.04	545 ± 23.64	144 ± 0.00	8975 ± 249.51	17.034 ± 1.04	1.89 ± 0.02	0.378 ± 0.01	20.02 ± 0.50	844 ± 52.26	2.66 ± 0.19	16.45 ± 0.47	6.89 ± 0.09	92 ± 0.51	24.33 ± 1.26
BBE226xCSR27	532 ± 16.05	545 ± 23.64	144 ± 0.00	8745 ± 621.33	16.141 ± 0.96	1.79 ± 0.08	0.387 ± 0.01	21.65 ± 0.23	845 ± 84.14	2.66 ± 0.31	16.57 ± 0.67	6.62 ± 0.05	91 ± 0.58	25.17 ± 0.29
BBE226xCSR17	513 ± 18.28	545 ± 23.64	144 ± 0.00	8770 ± 112.40	16.770 ± 1.65	1.72 ± 0.18	0.361 ± 0.04	21.15 ± 2.55	841 ± 32.76	2.60 ± 0.08	16.30 ± 0.84	6.78 ± 0.55	91 ± 0.58	25.06 ± 0.41
BBE226xJPN7	511 ± 23.59	545 ± 23.64	144 ± 0.00	9227 ± 177.46	17.260 ± 1.26	1.79 ± 0.15	0.395 ± 0.02	22.13 ± 0.65	803 ± 61.94	2.84 ± 0.17	16.33 ± 0.81	6.31 ± 0.28	91 ± 0.29	24.97 ± 0.46
S5xJPN8	546 ± 15.87	545 ± 23.64	144 ± 0.00	9170 ± 112.86	17.640 ± 1.40	1.81 ± 0.08	0.421 ± 0.01	23.30 ± 0.83	1191 ± 94.89	2.89 ± 0.16	19.26 ± 0.69	6.55 ± 0.18	93 ± 0.25	24.50 ± 0.87
S5xCSR27	539 ± 15.48	545 ± 23.64	144 ± 0.00	9245 ± 104.00	17.660 ± 0.80	1.87 ± 0.09	0.439 ± 0.01	23.52 ± 0.77	1077 ± 45.85	2.91 ± 0.12	19.12 ± 0.27	6.53 ± 0.15	92 ± 0.25	24.50 ± 0.50
S5xCSR17	541 ± 16.37	545 ± 23.64	144 ± 0.00	9263 ± 116.06	17.400 ± 1.03	1.92 ± 0.03	0.440 ± 0.01	22.94 ± 0.64	1097 ± 44.26	2.80 ± 0.14	19.20 ± 0.54	6.65 ± 0.14	92 ± 0.25	24.17 ± 1.04
S5xJPN7	532 ± 12.26	545 ± 23.64	144 ± 0.00	9084 ± 48.81	17.440 ± 0.55	1.92 ± 0.07	0.432 ± 0.00	22.50 ± 0.57	1011 ± 44.39	2.76 ± 0.23	19.08 ± 0.66	6.57 ± 0.12	92 ± 0.21	24.08 ± 0.52
JPN8xCSR27	545 ± 8.19	545 ± 23.64	144 ± 0.00	9217 ± 224.04	17.410 ± 0.88	1.85 ± 0.04	0.426 ± 0.00	23.06 ± 0.34	1125 ± 60.55	2.73 ± 0.45	19.32 ± 1.56	6.54 ± 0.07	92 ± 0.44	24.33 ± 1.26
JPN8xCSR17	545 ± 7.88	545 ± 23.64	144 ± 0.00	9163 ± 117.55	17.740 ± 1.13	1.85 ± 0.08	0.423 ± 0.01	22.85 ± 0.54	1148 ± 44.22	2.62 ± 0.38	19.23 ± 0.92	6.62 ± 0.13	92 ± 0.25	24.56 ± 1.36
JPN8xJPN7	542 ± 12.33	545 ± 23.64	144 ± 0.00	9093 ± 88.38	18.190 ± 0.45	1.85 ± 0.06	0.407 ± 0.01	22.08 ± 0.43	1004 ± 8.19	2.41 ± 0.14	18.79 ± 0.95	6.63 ± 0.12	92 ± 0.51	23.50 ± 0.50
CSR27xCSR17	548 ± 18.99	545 ± 23.64	144 ± 0.00	9193 ± 69.12	17.910 ± 1.08	1.86 ± 0.07	0.450 ± 0.00	24.26 ± 0.66	1108 ± 99.12	2.73 ± 0.29	19.55 ± 1.19	6.61 ± 0.10	92 ± 0.58	23.83 ± 0.76
CSR27xJPN7	535 ± 9.52	545 ± 23.64	144 ± 0.00	9079 ± 163.94	17.300 ± 1.21	1.86 ± 0.10	0.407 ± 0.01	21.94 ± 0.68	1005 ± 22.05	2.68 ± 0.44	19.00 ± 0.68	6.58 ± 0.15	92 ± 0.21	23.86 ± 1.12
CSR17xJPN7	535 ± 8.70	545 ± 23.64	144 ± 0.00	8792 ± 585.27	16.960 ± 1.48	1.86 ± 0.09	0.402 ± 0.01	21.61 ± 0.33	978 ± 48.22	2.69 ± 0.36	18.92 ± 0.57	6.63 ± 0.07	92 ± 0.54	24.11 ± 0.35
CSR2xCSR27 (Control)	525 ± 13.33	545 ± 23.64	144 ± 0.00	8356 ± 120.77	17.150 ± 1.14	1.78 ± 0.16	0.391 ± 0.01	22.10 ± 0.61	1050 ± 40.19	2.65 ± 0.21	18.01 ± 0.57	6.75 ± 0.09	92 ± 0.25	25.00 ± 0.28

5. Cocoon weight: The mean values of cocoon weight in parental breeds revealed that CSR26 had the highest of 1.91 g (Table 1). Among FCs, the highest of 1.94 g cocoon weight was recorded in (D13xS9), (CSR16xCSR26) and (S9xCSR16). On the other hand, the four-way cross, (JPN8xCSR27) x (D13xCSR26) expressed maximum of 2.180 g (Tables 2, 3 and 4). Among oval FCs, (BBE226xJPN8) expressed maximum heterosis of 13.46% and in dumb-bell FCs, the highest of 4.85 % was recorded in (BBE267xBBE247) whereas, in four way crosses, (JPN8xCSR27) x (D13xCSR26) expressed maximum hybrid vigour of 18.48 % (Tables 5, 6 and 7).

6. Cocoon shell weight: Among the parental breeds, CSR27 expressed maximum shell weight of 0.455 g (Table 1). Mean values of oval and dumb-bell FCs revealed maximum shell weight of 0.450 g in (CSR27xCSR17) and 0.440 g in (S9xCSR26), respectively. The four-way cross, (JPN8xCSR27) x

(D13xS9) showed maximum shell weight of 0.496 g (Tables 2, 3 and 4). Among the oval FCs, (S5xJPN8) expressed maximum heterosis of 1.94 % and in dumb-bell FCs, the highest of 4.84 % was recorded in (BBE267xCSR16). On the other hand, the four-way cross, (JPN8xCSR17) x (D13xCSR26) expressed maximum heterosis of 14.63 % (Tables 5, 6 and 7).

7. Cocoon shell percentage: Comparison of oval and dumb-bell parental breeds revealed the highest mean value of 25.01 % in CSR27 (Table 1). Among FCs, CSR27xCSR17 scored maximum of 24.26 %. Similarly, four-way cross, (JPN8xJPN7) x (D13xS9) registered maximum of 23.60 % (Tables 2, 3 and 4). The oval FC, (JPN8xCSR27) expressed maximum heterosis of 3.96 % and in dumb-bell FCs, the highest of 6.84 % was recorded in (BBE267xCSR16). Similarly, among four-way crosses, (JPN8xJPN7)x(D13xS9) expressed maximum hybrid vigour of 5.22 % (Table 5, 6 and 7).

Table 3: Rearing performance of dumb-bell foundation crosses (FCs) during different seasons(Mean values $\pm$ SD of 3 seasons)

Dumb-bell FC	Fecundity	Larval duration		Yield/10000 larvae		Cocoon weight (g)	Shell weight (g)	Cocoon shell (%)	Filament		Raw silk (%)	Ren-ditta	Neat-ness (p)	Boil off loss (%)
		Total (h)	V age (h)	No.	Wt. (kg)				length (m)	size (d)				
BBE267xD13	516 ± 21.97	542 ± 17.09	144 ± 0.00	8910 ± 541.76	16.760 ± 0.97	1.84 ± 0.06	0.370 ± 0.01	20.29 ± 1.18	937 ± 28.68	2.58 ± 0.00	16.84 ± 0.04	6.84 ± 0.28	91 ± 0.58	25.11 ± 0.38
BBE267xS9	507 ± 19.86	542 ± 17.09	144 ± 0.00	8767 ± 402.90	16.770 ± 1.18	1.87 ± 0.06	0.380 ± 0.01	20.22 ± 0.88	911 ± 58.57	2.52 ± 0.00	17.08 ± 0.63	6.95 ± 0.22	91 ± 0.67	25.33 ± 0.29
BBE267xBBE247	499 ± 24.00	542 ± 17.09	144 ± 0.00	8806 ± 375.40	16.750 ± 0.90	1.85 ± 0.06	0.370 ± 0.00	20.02 ± 0.43	954 ± 18.50	2.68 ± 0.01	16.38 ± 0.17	7.10 ± 0.11	92 ± 0.00	25.28 ± 0.38
BBE267xCSR16	500 ± 21.32	542 ± 17.09	144 ± 0.00	9029 ± 188.72	17.420 ± 0.70	1.81 ± 0.05	0.390 ± 0.01	21.55 ± 0.75	928 ± 13.88	2.54 ± 0.03	17.27 ± 0.19	6.62 ± 0.21	92 ± 0.58	24.83 ± 0.29
BBE267xCSR26	502 ± 14.54	542 ± 17.09	144 ± 0.00	8918 ± 264.95	16.970 ± 0.70	1.82 ± 0.06	0.380 ± 0.01	20.93 ± 0.66	930 ± 17.17	2.86 ± 0.01	16.75 ± 0.85	6.78 ± 0.15	92 ± 0.48	24.83 ± 0.29
D13xS9	573 ± 2.89	542 ± 17.09	144 ± 0.00	9718 ± 90.54	18.730 ± 1.30	1.94 ± 0.06	0.430 ± 0.01	22.39 ± 0.33	1061 ± 44.01	2.56 ± 0.00	18.58 ± 0.94	6.62 ± 0.07	92 ± 0.67	24.17 ± 0.76
D13xBBE247	507 ± 19.63	542 ± 17.09	144 ± 0.00	9055 ± 167.38	16.380 ± 1.33	1.87 ± 0.12	0.410 ± 0.01	21.97 ± 0.80	893 ± 47.03	2.47 ± 0.01	18.03 ± 0.95	6.56 ± 0.17	92 ± 0.75	24.17 ± 0.76
D13xCSR16	548 ± 25.51	542 ± 17.09	144 ± 0.00	9763 ± 178.32	18.550 ± 1.03	1.93 ± 0.10	0.420 ± 0.02	21.61 ± 0.18	978 ± 88.55	2.65 ± 0.24	18.27 ± 0.92	6.66 ± 0.04	92 ± 0.93	24.14 ± 0.80
D13xCSR26	556 ± 21.59	542 ± 17.09	144 ± 0.00	9731 ± 214.73	18.600 ± 1.06	1.93 ± 0.12	0.430 ± 0.01	22.22 ± 0.68	999 ± 61.15	2.52 ± 0.02	18.35 ± 0.84	6.62 ± 0.14	92 ± 0.82	23.64 ± 0.48
S9xBBE247	531 ± 10.99	542 ± 17.09	144 ± 0.00	8930 ± 335.84	17.880 ± 1.31	1.89 ± 0.05	0.390 ± 0.01	20.82 ± 0.17	930 ± 34.64	2.22 ± 0.00	17.89 ± 0.69	6.80 ± 0.04	92 ± 1.06	24.33 ± 1.04
S9xCSR16	549 ± 31.86	542 ± 17.09	144 ± 0.00	9751 ± 516.96	18.360 ± 1.35	1.94 ± 0.09	0.439 ± 0.01	22.76 ± 0.47	1064 ± 89.08	2.76 ± 0.35	18.78 ± 0.52	6.58 ± 0.09	92 ± 0.67	23.50 ± 0.50
S9xCSR26	562 ± 16.05	542 ± 17.09	144 ± 0.00	9738 ± 521.89	18.320 ± 1.03	1.93 ± 0.05	0.440 ± 0.02	22.66 ± 0.25	1056 ± 105.52	2.32 ± 0.01	18.88 ± 0.36	6.57 ± 0.06	92 ± 0.67	23.50 ± 0.50
BBE247xCSR16	499 ± 10.12	542 ± 17.09	144 ± 0.00	9040 ± 570.93	16.780 ± 1.19	1.84 ± 0.02	0.390 ± 0.01	21.14 ± 0.45	899 ± 62.42	2.62 ± 0.09	17.09 ± 1.01	6.73 ± 0.10	91 ± 0.67	24.83 ± 0.76
BBE247xCSR26	498 ± 20.28	542 ± 17.09	144 ± 0.00	9114 ± 313.46	17.760 ± 1.04	1.82 ± 0.09	0.390 ± 0.02	21.53 ± 0.21	927 ± 59.27	2.81 ± 0.43	17.24 ± 0.92	6.67 ± 0.04	92 ± 0.43	24.83 ± 0.29
CSR16xCSR26	547 ± 15.84	542 ± 17.09	144 ± 0.00	9775 ± 148.75	18.730 ± 0.71	1.94 ± 0.12	0.430 ± 0.02	22.57 ± 0.00	1013 ± 84.17	2.55 ± 0.27	18.55 ± 1.02	6.42 ± 0.00	92 ± 0.29	23.50 ± 0.50
CSR6xCSR26 (Control)	534 ± 10.74	542 ± 17.09	144 ± 0.00	9257 ± 303.06	17.460 ± 0.47	1.74 ± 0.05	0.380 ± 0.01	21.62 ± 0.17	1015 ± 82.14	2.70 ± 0.29	17.80 ± 0.66	6.95 ± 0.12	91 ± 0.62	23.50 ± 0.51

8. Filament length: The mean values of filament length among pure breeds ranged from 864 (BBE226) to 1203 m (JPN8). On the other hand, among FCs, (S5xJPN8) registered the longest filament (1191 m). Among four-way crosses, (CSR27xCSR17) x (D13xS9) recorded the longest filament of 1121 m (Tables 2, 3 and 4). Among oval FCs, maximum hybrid vigour of 6.33 % (CSR27xCSR17) and in dumb-bell FCs, 5.36 % in (BBE267xBBE247) was recorded (Tables 5 and 6). Similarly, in four-way crosses, (CSR27xCSR17) x (D13xCSR26) recorded mean heterosis of 11.23 % (Table 7).

9. Filament size: The overall mean of filament size in oval FCs recorded thickness of 2.91 d in (S5xCSR27) and in dumb-bell FCs, (BBE267xCSR26) recorded 2.86 d. On the other hand, among four-way crosses, (JPN8xCSR27) x (S9xCSR26) expressed less size of 2.39 d and (CSR27xCSR17) x (S9xCSR16) recorded thicker size of

3.15d (Tables 2, 3 and 4). Magnitude of heterosis among oval FCs showed the highest of 20.92 % in S5xJPN8 and in dumb-bell FCs, it was 1.28 % in S9xCSR16. Similarly, in four-way crosses, (CSR27xCSR17) x (S9xCSR16) scored the highest mean heterosis of 14.13 % (Tables 5, 6 and 7).

10. Raw silk percentage: Among the oval and dumb-bell parental breeds, S9 expressed maximum raw silk percentage of 19.22 % (Table 1). Mean values of oval and dumb-bell FCs revealed maximum of 19.55 % in (CSR27xCSR17) and 19.32 % in (JPN8xCSR27) in oval FCs (Tables 2 and 3). The four-way cross (JPN8xCSR27) x (S9xCSR26) recorded higher quantity raw silk of 19.45 % (Table 4). The oval FC, (CSR27xCSR17) expressed the highest heterosis of 6.31 % and in dumb-bell FCs, 5.56 % was recorded in (D13xBBE247) whereas, the four-way cross, (CSR27xCSR17) x (D13xCSR26) expressed maximum mean heterosis of 4.48 % (Tables 5, 6 and 7).

Table 4: Rearing performance of four-way crosses (OxO)x(DxD) during different seasons

(Mean values $\pm$ SD of 3 seasons)

Four-way cross	Fecundity	Larval duration		Yield/10000 larvae		Cocoon wt. (g)	Shell wt. (g)	Cocoon shell (%)	Filament		Raw silk (%)	Renditta	Neatness (p)	Boil-off loss (%)
		Total (h)	V age (h)	No.	Wt. (kg)				length (m)	size (d)				
(CSR27xCSR17)x(CSR16xCSR26)	624 ± 12.52	553 ± 11.87	153 ± 7.57	9178 ± 31.18	18.830 ± 0.51	2.094 ± 32.32	0.489 ± 0.01	23.40 ± 0.65	993 ± 40.05	2.98 ± 0.08	18.62 ± 0.20	6.21 ± 0.17	91.89 ± 0.38	24.43 ± 0.12
(CSR27xCSR17)x(S9xCSR16)	621 ± 7.07	553 ± 11.87	153 ± 7.57	9274 ± 90.72	19.650 ± 0.18	2.120 ± 28.01	0.478 ± 0.01	22.50 ± 0.15	1033 ± 21.00	3.15 ± 0.16	18.70 ± 0.17	6.49 ± 0.04	91.67 ± 0.00	23.75 ± 0.00
(CSR27xCSR17)x(S9xCSR26)	636 ± 8.18	553 ± 11.87	153 ± 7.57	9341 ± 115.67	20.200 ± 0.62	2.162 ± 29.76	0.487 ± 0.01	22.51 ± 0.28	1108 ± 52.13	2.88 ± 0.23	19.10 ± 0.49	6.63 ± 0.06	92.33 ± 0.00	23.69 ± 0.10
(CSR27xCSR17)x(D13xCSR26)	636 ± 17.30	553 ± 11.87	153 ± 7.57	9324 ± 74.17	21.140 ± 0.33	2.143 ± 26.50	0.471 ± 0.02	22.01 ± 0.01	1112 ± 40.43	3.03 ± 0.29	19.34 ± 0.53	6.64 ± 0.00	92.44 ± 0.19	23.50 ± 0.00
(CSR27xCSR17)x(D13xS9)	637 ± 14.89	553 ± 11.87	153 ± 7.57	9356 ± 35.86	20.590 ± 0.80	2.170 ± 29.50	0.482 ± 0.02	22.20 ± 0.34	1121 ± 72.54	3.03 ± 0.19	19.44 ± 0.52	6.69 ± 0.08	92.44 ± 0.19	23.47 ± 0.05
(CSR27xCSR17)x(D13xCSR16)	630 ± 13.87	553 ± 11.87	153 ± 7.57	9460 ± 33.13	19.380 ± 0.40	2.072 ± 18.48	0.473 ± 0.02	22.80 ± 0.91	1022 ± 51.78	2.98 ± 0.13	19.05 ± 0.62	6.16 ± 0.21	91.67 ± 0.00	23.50 ± 0.00
(JPN8xCSR27)x(S9xCSR16)	624 ± 10.09	553 ± 11.87	153 ± 7.57	9203 ± 241.50	19.310 ± 0.14	2.176 ± 29.50	0.492 ± 0.03	22.60 ± 0.70	1049 ± 51.11	2.66 ± 0.02	19.06 ± 0.46	6.68 ± 0.15	92.22 ± 0.51	23.50 ± 0.00
(JPN8xCSR27)x(S9xCSR26)	634 ± 17.00	553 ± 11.87	153 ± 7.57	9581 ± 49.60	19.990 ± 0.31	2.143 ± 20.36	0.486 ± 0.01	22.70 ± 0.06	1092 ± 75.07	2.39 ± 0.05	19.45 ± 0.55	6.60 ± 0.01	92.33 ± 0.00	23.50 ± 0.00
(JPN8xCSR27)x(D13xCSR26)	632 ± 18.68	553 ± 11.87	153 ± 7.57	9331 ± 108.72	20.150 ± 0.58	2.180 ± 0.11	0.494 ± 0.03	22.62 ± 0.24	1118 ± 49.56	2.55 ± 0.08	19.18 ± 0.75	6.61 ± 0.03	92.33 ± 0.00	23.50 ± 0.00
(JPN8xCSR27)x(D13xS9)	635 ± 9.00	553 ± 11.87	153 ± 7.57	9336 ± 62.68	19.940 ± 0.23	2.156 ± 0.05	0.496 ± 0.12	23.01 ± 0.38	1066 ± 54.53	2.69 ± 0.15	19.40 ± 0.52	6.67 ± 0.10	92.33 ± 0.00	23.50 ± 0.00
(JPN8xCSR27)x(D13xCSR16)	606 ± 25.11	553 ± 11.87	153 ± 7.57	9157 ± 76.09	18.910 ± 0.37	2.034 ± 0.03	0.448 ± 0.01	22.03 ± 0.48	1039 ± 54.38	2.65 ± 0.05	19.12 ± 0.57	6.59 ± 0.10	91.67 ± 0.10	23.50 ± 0.00
(JPN8xCSR17)x(S9xCSR26)	639 ± 16.50	553 ± 11.87	153 ± 7.57	9381 ± 152.81	19.730 ± 0.26	2.158 ± 0.08	0.492 ± 0.02	22.79 ± 0.36	1100 ± 73.53	2.67 ± 0.16	19.29 ± 0.81	6.58 ± 0.18	92.67 ± 0.00	23.50 ± 0.00
(JPN8xCSR17)x(D13xCSR26)	635 ± 18.90	553 ± 11.87	153 ± 7.57	9447 ± 167.74	20.440 ± 0.36	2.144 ± 0.16	0.493 ± 0.02	22.99 ± 0.24	1108 ± 86.82	2.56 ± 0.09	19.28 ± 0.76	6.63 ± 0.05	92.44 ± 0.19	23.50 ± 0.00
(JPN8xCSR17)x(D13xS9)	636 ± 15.76	553 ± 11.87	153 ± 7.57	9365 ± 157.56	20.180 ± 0.29	2.147 ± 0.06	0.493 ± 0.01	22.96 ± 0.11	1105 ± 97.01	2.66 ± 0.09	19.22 ± 0.75	6.74 ± 0.06	92.33 ± 0.00	23.50 ± 0.00
(JPN8xCSR17)x(D13xCSR16)	623 ± 12.52	553 ± 11.87	153 ± 7.57	9195 ± 31.18	19.090 ± 0.51	2.072 ± 32.32	0.455 ± 0.02	21.96 ± 0.64	1021 ± 40.05	2.65 ± 0.08	18.92 ± 0.76	6.69 ± 0.17	91.67 ± 0.38	23.50 ± 0.00
(JPN8xJPN7)x(D13xCSR26)	627 ± 10.09	553 ± 11.87	153 ± 7.57	9159 ± 41.50	19.410 ± 0.14	2.144 ± 29.50	0.472 ± 0.03	22.01 ± 0.70	1026 ± 51.11	2.73 ± 0.02	18.93 ± 0.46	6.66 ± 0.15	91.44 ± 0.51	23.50 ± 0.00
(JPN8xJPN7)x(D13xS9)	630 ± 4.84	553 ± 11.87	153 ± 7.57	9136 ± 71.94	19.160 ± 0.32	2.086 ± 0.00	0.492 ± 0.01	23.60 ± 0.11	1033 ± 64.66	2.59 ± 0.05	19.02 ± 0.42	6.73 ± 0.03	91.67 ± 0.00	23.50 ± 0.00
(JPN8xJPN7)x(D13xCSR16)	627 ± 9.00	553 ± 11.87	153 ± 7.57	9204 ± 62.68	19.070 ± 0.23	2.121 ± 0.05	0.482 ± 0.02	22.70 ± 0.38	1012 ± 54.53	2.62 ± 0.12	18.85 ± 0.52	6.64 ± 0.10	91.67 ± 0.00	23.50 ± 0.00
(S5xCSR27)x(D13xS9)	644 ± 25.11	553 ± 11.87	153 ± 7.57	9326 ± 76.09	19.680 ± 0.27	2.172 ± 0.03	0.492 ± 0.02	22.70 ± 0.48	1099 ± 54.38	2.68 ± 0.04	19.11 ± 0.57	6.60 ± 0.10	92.28 ± 0.10	23.50 ± 0.00
(S5xCSR27)x(D13xCSR16)	621 ± 5.19	553 ± 11.87	153 ± 7.57	9197 ± 38.95	18.920 ± 0.31	2.123 ± 0.01	0.488 ± 0.01	23.00 ± 0.09	1040 ± 76.27	2.93 ± 0.28	18.95 ± 0.54	6.75 ± 0.02	91.78 ± 0.19	23.50 ± 0.00
(S5xJPN8)x(D13xCSR16)	610 ± 12.34	553 ± 11.87	153 ± 7.57	9256 ± 27.09	19.010 ± 0.34	2.179 ± 0.00	0.493 ± 0.02	22.60 ± 0.28	1022 ± 51.03	3.00 ± 0.16	18.87 ± 0.35	6.70 ± 0.07	91.67 ± 0.00	23.50 ± 0.00
(CSR2xCSR27)x(CSR6xCSR26) (Control)	595 ± 30.37	553 ± 11.87	153 ± 7.57	9155 ± 64.77	18.820 ± 0.30	2.043 ± 0.01	0.462 ± 0.01	22.60 ± 0.61	994 ± 36.72	2.63 ± 0.02	18.91 ± 0.56	6.66 ± 0.14	91.33 ± 0.33	24.00 ± 0.00

11. Renditta: The overall mean values of renditta in parental breeds ranged from 6.68 (JPN8) to 7.55 in BBE247 (Table 1). Among oval FCs, renditta was maximum (6.94) in BBE226xS5 and in dumb-bell FCs, BBE267xBBE247 recorded 7.10 (Tables 2 and 3). On the other hand, four-way cross (S5xCSR27) x (D13xCSR16) expressed mean renditta of 6.75 (Table 4). The heterosis estimation showed the range from -1.15 to -10.11 in oval FCs, -1.48 to -8.38 in dumb-bell FCs and -0.15 to -8.67 in four-way crosses (Tables 5, 6 and 7).

12. Neatness: The mean values of neatness among pure breeds showed maximum of 93 p in S9 (Table 1). Among oval and dumb-bell FCs, neatness was the highest in S5xJPN8 (93 p) (Tables 2 and 3). Among four-way crosses, (JPN8xCSR17) x (S9xCSR26) expressed the highest of 92.67 p (Table 4). Heterosis estimation in oval and dumb-bell FCs showed the highest of 2.22 % in BBE267xBBE247 (Tables 5 and 6). Among four-way crosses, (CSR27xCSR17) x (D13xCSR26) expressed maximum heterosis of 0.78 % (Table 7).

Table 5: Heterosis (%) in oval foundation crosses during different seasons

(Mean values of 3 seasons)

Oval FC	Fecundity	Yield/10000 larvae		Cocoon wt.	Shell wt.	Shell %	Filament		Raw silk	Renditta	Neatness	Boil off loss
		(No.)	(Wt.)				length	size				
BBE226xS5	0.86	-3.58	6.95	9.28	-11.98	-13.54	-15.21	-3.84	-6.05	-1.70	1.10	2.08
BBE226xJPN8	-2.00	0.32	12.67	13.46	-1.04	-13.60	-18.34	2.70	-6.96	-1.15	1.10	4.65
BBE226xCSR27	0.66	-2.31	11.14	4.07	-6.97	-10.50	-10.77	-9.22	-6.65	-5.16	1.10	4.88
BBE226xCSR17	-3.02	-2.08	10.77	-1.04	-9.99	-9.88	-12.30	-11.26	-8.07	-3.28	0.00	4.42
BBE226xJPN7	-3.40	2.90	14.05	3.41	-2.95	-5.79	-13.84	6.77	-7.32	-10.11	0.00	7.40
S5xJPN8	5.98	0.03	10.20	1.75	1.94	0.22	3.07	20.92	5.367	-3.25	1.09	10.11
S5xCSR27	1.70	0.73	14.57	2.24	0.90	3.05	0.75	6.40	4.21	-3.69	0.00	6.52
S5xCSR17	1.98	0.94	8.56	3.85	1.15	2.63	1.48	2.38	4.69	-2.35	0.00	5.09
S5xJPN7	0.28	-1.18	8.76	3.95	-3.00	-4.58	-4.08	11.97	4.66	-3.52	0.00	8.22
JPN8xCSR27	2.25	0.64	13.05	4.64	0.24	3.96	0.72	10.98	5.40	-2.24	0.00	9.35
JPN8xCSR17	2.16	0.03	10.72	3.64	1.68	1.97	1.77	6.50	4.68	-1.49	0.00	10.38
JPN8xJPN7	1.59	-0.87	13.53	3.42	-2.16	-5.36	-8.85	10.05	3.19	-1.48	0.00	9.30
CSR27xCSR17	2.14	0.28	16.09	0.92	0.67	0.21	6.33	-2.50	6.31	-1.78	0.00	3.61
CSR27xJPN7	-0.28	-1.08	12.18	1.14	-8.74	-9.82	-0.99	5.93	3.94	-2.37	0.00	7.24
CSR17xJPN7	-0.37	-4.25	5.76	0.27	-8.11	-8.55	-4.72	6.32	3.61	-2.07	0.00	8.36
CSR2xCSR27 (C)	1.02	-0.08	9.67	4.11	-2.19	-5.17	0.70	2.17	5.11	-2.97	1.08	4.44

Table 6: Heterosis (%) in dumb-bell foundation crosses during different seasons

(Mean values of 3 seasons)

Dumb-bell FC	Fecundity	Yield/10000 larvae		Cocoon wt.	Shell wt.	Shell %	Filament		Raw silk	Renditta	Neatness	Boil off loss
		(No.)	(Wt.)				length	size				
BBE267xD13	6.18	-6.39	-4.93	-0.08	1.93	2.73	0.48	-11.79	-3.77	-3.80	0.00	4.63
BBE267xS9	-7.14	-8.23	-3.91	1.69	-0.52	1.40	-10.11	-13.55	-3.83	-1.84	-0.55	7.78
BBE267xBBE247	-5.17	-6.08	-0.69	4.85	4.82	-0.35	5.36	-14.10	0.31	-5.33	2.22	1.12
BBE267xCSR16	-6.19	-5.63	-0.41	-1.87	4.84	6.84	-1.28	-14.48	0.35	-6.76	1.10	3.46
BBE267xCSR26	-7.64	-6.64	-2.47	-1.41	2.70	4.39	-1.12	-15.33	-3.24	-4.37	1.10	3.46
D13xS9	2.60	0.31	2.62	2.16	1.53	3.95	2.91	-4.48	0.44	-1.78	0.54	7.42
D13xBBE247	-5.23	-4.80	-7.25	2.47	3.80	1.27	-3.25	-14.38	5.56	-8.38	1.10	0.71
D13xCSR16	1.92	0.61	1.44	1.31	1.45	0.73	0.78	-3.11	1.73	-1.48	0.00	4.96
D13xCSR26	0.00	0.44	2.21	1.26	4.37	2.68	4.28	-8.86	1.61	-1.93	0.00	2.78
S9xBBE247	-0.84	-3.32	2.25	3.73	-5.80	-4.84	-7.37	-2.78	0.62	-3.95	0.55	3.53
S9xCSR16	1.29	0.13	1.38	2.01	1.73	3.69	2.46	1.28	0.60	-2.23	0.54	4.44
S9xCSR26	1.72	0.15	1.66	1.42	1.15	3.78	1.64	-15.79	0.59	-2.09	0.54	4.44
BBE247xCSR16	-4.50	-5.46	-4.24	0.66	-3.47	-4.39	-0.38	-4.10	-0.87	-5.87	0.00	3.46
BBE247xCSR26	-6.57	-4.53	1.88	-0.49	2.99	-2.11	-0.43	-5.07	-0.58	-6.58	1.10	2.17
CSR16xCSR26	1.39	0.37	3.71	1.62	2.14	2.27	4.92	-9.25	1.81	-4.01	0.00	2.17
CSR6xCSR26 (C)	-0.78	-5.19	-4.66	-0.46	-0.51	0.77	4.56	-13.97	0.61	-3.52	0.05	2.16

13. Boil-off loss (BOL): Parental breeds recorded maximum BOL of 25 % in BBE226, BBE247 and BBE267 (Table 1). Among oval and dumb-bell FCs, (BBE267xS9) expressed maximum BOL of 25.33 %. Among four-way crosses, (CSR27xCSR17) x (CSR16xCSR26) expressed the highest BOL of 24.43 %

(Tables 2, 3 and 4). Oval FCs recorded maximum heterosis of 10.38 % in (JPN8xCSR17) and in dumbbell FCs, (BBE267xS9) recorded 7.78 %. On the other hand, (JPN8xJPN7) x (D13xS9) four-way cross exhibited maximum heterosis of 6.82 % (Tables 5, 6 and 7).

Table 7: Heterosis (%) in four-way crosses during different seasons

Four-way cross	Fecundity	Yield/10000 larvae		Cocoon wt.	Shell wt.	Shell %	Filament		Raw Silk	Renditta	Neatness	Boil off loss
		No.	Wt.				length	size				
(CSR27xCSR17) x(CSR16xCSR26)	15.93	-5.60	14.79	11.76	-2.33	0.91	-1.04	6.43	-0.21	-7.73	0.31	6.22
(CSR27xCSR17) x(S9xCSR16)	15.08	-1.89	17.17	13.37	-2.27	-2.72	-0.66	14.13	-1.01	-3.57	0.36	4.40
(CSR27xCSR17) x(S9xCSR26)	16.74	-1.10	20.81	15.51	6.82	-2.43	6.53	3.60	0.84	-1.34	0.48	4.13
(CSR27xCSR17) x(D13xCSR26)	16.37	1.11	25.76	14.44	6.98	4.22	11.23	-8.25	4.48	-1.48	0.78	2.17
(CSR27xCSR17) x(D13XS9)	16.26	-0.76	22.45	16.04	6.97	-3.14	8.20	10.58	3.79	-0.59	0.48	3.16
(CSR27xCSR17) x(D13xCSR16)	16.38	0.26	14.95	10.70	-4.65	-1.04	2.26	7.58	3.20	-8.67	-0.17	2.17
(JPN8xCSR27) x(S9xCSR16)	17.89	1.41	19.27	15.05	9.30	-1.65	1.36	-7.72	0.90	-0.45	0.27	5.00
(JPN8xCSR27) x(S9xCSR26)	18.42	1.49	19.63	16.30	9.30	-0.96	1.34	-8.08	2.69	-1.49	0.39	5.36
(JPN8xCSR27) x(D13xCSR26)	16.39	1.20	19.94	18.48	11.90	-0.92	7.79	-1.92	3.62	-1.64	0.57	3.84
(JPN8xCSR27) x(D13XS9)	16.08	-0.92	18.48	18.03	9.52	1.05	-0.71	4.67	3.58	-0.60	0.27	5.00
(JPN8xCSR27) x(D13xCSR16)	12.32	-2.90	12.23	10.33	-2.38	-3.60	0.20	2.32	3.59	-2.08	-0.27	3.84
(JPN8xCSR17) x(S9xCSR26)	17.63	0.65	15.92	17.39	11.90	0.97	1.52	-1.15	1.69	-2.08	0.70	5.00
(JPN8xCSR17) x(D13xCSR26)	16.53	0.23	19.46	15.68	14.63	2.27	6.21	-1.92	3.99	-1.49	0.63	3.84
(JPN8xCSR17) x(D13XS9)	16.42	0.64	17.74	16.85	11.90	2.41	2.36	-0.39	2.45	-0.15	0.21	5.00
(JPN8xCSR17) x(D13xCSR16)	15.42	-2.52	9.40	11.89	0.00	-2.57	-2.10	0.03	2.33	-0.74	-0.33	3.84
(JPN8xJPN7) x(D13xCSR26)	15.11	-2.88	13.44	15.68	4.88	-2.13	-0.37	10.53	2.27	-1.19	-0.54	5.68
(JPN8xJPN7) x(D13XS9)	15.36	-3.12	11.85	13.59	0.00	5.22	-3.12	6.58	1.55	-0.15	-0.60	6.82
(JPN8xJPN7) x(D13xCSR16)	16.20	-2.48	11.19	15.22	2.44	0.67	-1.71	6.50	2.11	-1.48	-0.42	5.62
(S5xCSR27) x(D13XS9)	18.29	-1.13	16.93	16.67	9.30	-0.87	4.68	-1.11	2.08	-2.37	0.22	3.30
(S5xCSR27) x(D13xCSR16)	15.15	-2.58	12.30	13.37	0.00	-0.04	2.64	7.33	2.77	-0.30	-0.18	2.17
(S5xJPN8) x(D13xCSR16)	13.74	-1.93	10.85	18.40	4.88	0.40	-3.28	7.19	2.17	-0.91	-0.42	3.84
(CSR2xCSR27) x (CSR6xCSR26) (Control)	15.29	-1.14	15.78	14.20	4.70	-2.58	1.67	2.08	2.19	9.32	-2.39	4.75

DISCUSSION

The outcome of silkworm breeding is judged by the best desirable traits of the parental characters that appear in F1 hybrids. Several diallel cross techniques have been extensively used to evaluate the combining ability of different strains to adjudicate the potential hybrids for commercial purpose (Kalpana *et al.*, 1999). Based on the diallel analysis and heterosis, superiority of a breed or a hybrid can be determined by considering the positive vigour for all the metric traits except for filament size, renditta and boil-off ratio.

Though, the phenomenon of heterosis was exploited in plants much earlier to that of silkworm, the extent of exploitation in silkworm is unparallel to that in any of the commercial crops. The works of Harada (1961) and Pannegpet and Jaroonchai, (1975) demonstrated the superiority of single, three-way and double hybrids over

their parental races and confirms the present findings where maximum hybrid vigour was expressed in (CSR27 x CSR17) x (D13 x CSR26) for yield/weight (25.76 %), filament length (11.23 %), denier (-8.25 %), raw silk percentage (4.48 %), neatness (0.78 %) and boil-off ratio (lowest of 2.17 %) among the 21 four way crosses. However, heterosis with regard to each hybrid, derived from the parents is an important aspect, which enables to understand the manifestation of hybrid vigor in respect of each of the characters independently and in conjugation with other and in the present study, (JPN8 x CSR27) x (S9 x CSR26) expressed maximum hybrid vigour for fecundity (18.42 %). Similarly, the hybrids, (S5 x JPN8) x (D13 x CSR16) and (JPN8 x CSR27) x (D13 x CSR26) exhibited maximum vigour for single cocoon weight (18.48 %) and (JPN8xCSR17) x (D13xCSR26) showed maximum vigour of 14.63% for single shell weight. Moreover, skillful utilization of hybrid vigor is of utmost importance in sericulture or any other agricultural crops to

obtain maximum desirable economic benefits (Ramesha *et al.*, 2009; Seshagiri *et al.*, 2009). Realizing the essentiality of high cocoon shell percentage and raw silk percentage, as thrust areas, many viable single crosses were evolved and authorized for commercial exploitation (Basavaraja *et al.*, 1995; Datta *et al.*, 2002).

However, manifestation of heterosis may be differing on the extent of genetic advancement and the distance achieved in new genetic materials in a breeding study, as it is seen that many foundation crosses and four-way crosses have shown desirable manifestation of heterosis for many traits. This is in agreement with the earlier findings of Sathenahalli *et al.* (1989) who reported that manifestation of heterosis differ widely and such differences suggest that parental materials involved differ in their genetic makeup and the high degree of heterosis in any specific cross can be due to additive gene effects as reported by Rao *et al.* (1998).

In the present study, the parents are used to raise the foundation crosses and 15 each of oval and dumb-bell FCs were utilized for production of four-way crosses (OxO)x(DxD) which helps not only in high fecundity but also in exploiting the hybrids prepared between converged FCs for commercial purpose. These FCs and four-way crosses were evaluated by rearing performance and heterosis values for quantitative economic traits from the three different seasons. Based on the results of the present study, six combinations each of oval and dumb-bell FCs and five four-way crosses *viz.*, (CSR27xCSR17) x (D13xCSR26), (JPN8xCSR27) x (S9xCSR26), (S5xJPN8) x (D13xCSR16), (JPN8xCSR27) x (D13xCSR26) and (JPN8xCSR17) x (D13xCSR26) were identified which showed better potentiality as evidenced from the study by scoring higher heterosis for most of the pre-cocoon, cocoon and post-cocoon parameters. Considering the above facts, the information generated in the present study indicates that utilization of four-way cross hybrid *viz.*, (CSR27xCSR17) x (D13xCSR26) and its parents (both foundation crosses and parents) after field evaluation under tropical conditions in general and southern part of India in particular can contribute towards enhancement of total raw silk production.

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EVALUATION D'HYBRIDES DOUBLES ET DES LIGNEES PARENTALES DU VER A SOIE, *BOMBYX MORI* L. BASEE SUR LA VIGUEUR HYBRIDE

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RESUME

Douze lignées parentales bivoltines (6 à cocon ovale et 6 à cocon cintré) entretenues au CSRTI de Mysore, ont été utilisées pour préparer quinze croisements de fondation (FC) par sélection pour chaque catégorie, ovale et cintrée. Tous les croisements à quatre voies possibles (ovale x ovale) x (cintré x cintré) obtenus à partir de ces FCs ont été élevés en trois répétitions au cours de différentes saisons pour évaluer leurs performances pour les caractères pré- et post-cocon et également pour estimer le degré d'hétérosis. L'étude révèle que, sur chaque 15 FCs bivoltins ovales et cintrés et sur 21 croisements quatre voies, 6 FCs ovales et 6 FCs cintrés ainsi que 5 croisements quatre voies donnaient de meilleures performances avec une hétérosis positive pour la plupart des caractères économiques étudiés, ce qui peut être attribué à une meilleure avance inhérente. Ces résultats montrent également que l'on peut prévoir d'identifier des hybrides double bivoltins pour des essais de terrain en se basant sur la performance globale et l'hétérosis pour chaque caractère quantitatif.

Mots-clés: Croisements de fondation bivoltins, essais de terrain, croisements quatre voies, hétérosis.

NEWS CORNER

VISIT OF A SENIOR DELEGATION FROM DMMMS UNIVERSITY, PHILIPPINES



A three member high level delegation headed by Mr. Benjamin P. Sapitula President, Don Mariano Marcos Memorial State University (DMMMSU), Philippines and comprising Dr.(Ms.) Cristeta F. Gapuz, Director, Sericulture Research & Development Institute (SRDI), DMMMSU, Philippines and Mr. Engr. Roel D. Supsup, Planning Officer, DMMMSU-SRDI, Philippines visited the sericulture areas and facilities in Bangalore and Mysore from 5th to 8th May, 2014. The purpose of the visit was to initiate collaboration between Don Mariano Marcos Memorial State University (DMMMSU), Central Silk Board and International Sericultural Commission in the areas of research, future benchmarking / educational trip and short-term trainings in specialized areas of sericulture industry. The University also desired to gain some insights and knowledge from India that can improve the existing sericulture plans in Philippines.

A detailed interactive Meeting between the DMMMSU delegation and Secretary General, ISC, was held on 08.05.2014 at ISC HQs. The Philippine delegation expressed their desire to enroll their country as a Member Country of the International Sericultural Commission to avail the various facilities which are being extended under the Global Developmental Programme of ISC.

VISIT OF MR. JACQUES POULENARD, INTERSOIE, LYON, FRANCE



Mr. Jacques Poulenard, Technical Advisor, Lyon, France visited the sericulture areas and facilities in Bangalore and Mysore from 28th April to 2nd May, 2014. INTERSOIE FRANCE is an association of silk merchants, throwsters, weavers, dyers/printers/finishers, manufacturers, representatives of haute-couture and scientific and technical bodies. INTERSOIE FRANCE was created to meet the various requirements of the French silk sectors to deal with various National, European, and International bodies. After visiting the various facilities, a Meeting between Mr. Poulenard and the Officers of Central Silk Board and ISC was held on 30.04.2014. The meeting discussed the possibility of sourcing the support of Intersoie in improving the quality of silk in ISC Member countries, the need for instituting country specific silk mark labels for authenticating the quality and purity of silk products, source the support of Intersoie for linking with the Fashion Industry of France, the scope of promoting silk in European Markets, etc.

WELCOME TO DPR KOREA - THE 14TH MEMBER COUNTRY OF INTERNATIONAL SERICULTURAL COMMISSION



The Democratic People's Republic of Korea has become the 14th Member Country of the International Sericultural Commission (ISC). The Hon'ble Minister of Foreign Affairs, DPR Korea, Mr. Pak Ui Chun signed the Instrument of Accession signaling the entry of DPR Korea to ISC with effect from 19th February 2014. DPR Korea also nominated Mr. KIM Jong Bin, Director General, Sericultural and Silk Industry Guidance Bureau as the National Delegate to ISC. The enrollment of DPR Korea as a Member Country of ISC is expected to catalyze the development of sericulture and silk industry in the country. DPR Korea can now avail the various facilities of ISC such as, participate in the decision making process of ISC, depute candidates for training and scholarships, avail the services of volunteer experts, participate in the collaborative research programmes, source advanced innovations & technologies, and genetic materials from other member countries, source project related support from international agencies through the ISC platform and participate in scientific and technical meetings.

The International Sericultural Commission whole heartedly welcomes DPR Korea as its 14th Member Country.

EXECUTIVE COMMITTEE MEETING OF ISC HELD AT CAIRO, EGYPT DURING 24-25, JUNE 2014



The Executive Committee Meeting of the International Sericultural Commission was held at Baron Hotel Heliopolis, Cairo, Egypt, during 24th and 25th June 2014. The Meeting was hosted by the Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. Twelve delegates from various Member Countries attended the Meeting. The EC discussed on the various proposals submitted by the Secretary General, which are mandatory for the functioning of ISC. The SG presented a detailed report on the activities ISC for the last six months, which include many important developments *viz.*, the enrollment of DPR Korea as a Member Country, the initiation of membership enrollment by Bangladesh, enrollment of many Associate Members, finalization of ITEC training programme and the progress of the preparations of ISC Congress scheduled to be held in Bangalore during 24-27 November 2014. The EC also discussed and selected the winners of Louis Pasteur Award for 2014 based on the recommendations of a five Member Louis Pasteur Award Committee comprising of renowned scientists from different parts of the world.





INTERNATIONAL SERICULTURAL COMMISSION

An intergovernmental organization instituted in 1960 and registered with United Nations; Reg. No. 10418

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INFORMATION TO CONTRIBUTORS

SERICOLOGIA is a peer reviewed quarterly scientific journal dedicated to the science of sericulture, published by the International Sericultural Commission. Papers submitted to SERICOLOGIA should carry original contributions of scientific research (basic or applied) or reviews on any aspect of sericulture.

Submission of a paper to SERICOLOGIA implies that it has not previously been published, that it is not under consideration for publication elsewhere and that, if accepted in SERICOLOGIA, it will not be published elsewhere in the same form without the written consent of the Chief Editor.

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Russell E. W. (1961) Soil conditions and plant growth. (9th edition), John Wiley and Sons, New York.

Power J. F. (1990) Fertility management and nutrient cycling. In: *Dry land agriculture, strategies for sustainability. Advances in soil science*, Singh R. P., Parr J. F. and Stewart B. A. (Eds.), Springer Verlag, New York.

Fagoonee I., Schmuterer R. and Ascher K. R. S. (1987) Use of neem in vegetable crop protection in Mauritius. *Proceedings of the 3rd International Neem Conference*, Nairobi, Kenya, 10-15 July, 1986, pp. 419-429.

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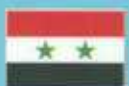
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