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Breeding of Bivoltine Breeds of *Bombyx mori* L Suitable for Variable Climatic Conditions of the Tropics

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The success of rearing with presently available conventional bivoltine is unpredictable in some seasons of the tropical regions due to highly fluctuating adverse climatic conditions. Thus, in order to popularize bivoltine breeds in tropical parts of India, it is very much essential to have a bivoltine breed(s), which can give stable cocoon crop under variable environments. With this objective a breeding programme was undertaken to improve the survival trait in bivoltine silkworm by introducing multivoltine genes into bivoltine through back crossing. Resultant bivoltine lines showed significantly higher survival in compared to the receptor (Bivoltine) parent and control bivoltine breed. Esterase isozyme analysis revealed similar banding pattern in the developed bivoltine and in the donor multivoltine, which predicts the introgression of multivoltine character into evolved bivoltine.

Key words: *Bombyx mori*, Bivoltine, Backcross, Introgression, Esterase isozyme

Introduction

India is a vast country with varying climatic conditions in different agro-climate zones. The precipitation rate, temperature and humidity vary from season to season and zone to zone. Among different zones, the tropical regions experience the highest temperature as well as humidity (Sengupta *et al.*, 1997). In traditional sericulture practicing countries like Japan and China, considerable achievement have been made from time to time in respect of qualitative and quantitative improvement of silkworm breeds in accordance with the demand of the industry. In India considerable work have also been done on breed improvement (Datta, 1984; Basavaraja *et al.*, 1995; Krishana Rao, 1998; Suresh Kumar *et al.*, 1999; Das *et al.*, 2000; Ghosh *et al.*, 2001; Basavaraja and Dandin, 2002; Suresh Kumar *et al.*, 2004). Yet, popularization of bivoltine in tropical parts especially in Eastern India is difficult owing to the prevalence of adverse environmental conditions and socio economic conditions of the farmers as well. In Eastern India, the multi x bi hybrids are very much popular in the autumn (Nov-Dec) and spring (Feb-Mar) crop seasons. However during autumn (Nov-Dec), the major commercial crop season of this region especially in the major silk producing state West Bengal, even the required quantity of multi x bi hybrid layings production is not possible, mainly due to unavailability of bivoltine male seed cocoon. Since, the preceding seed crop (Sept-Oct) experiences high temperature, high humidity and unpredictable rainfall, which threat rearing of bivoltine parent silkworm.

The breeding of silkworms since long has been aimed towards evolving superior and hardy breeds, either by means of selection alone or by out crossing and back crossing followed by selection in subsequent generations (Raju and Krishnamoorthy, 1993; Das *et al.*, 1998; Das, 2001). Introduction of polyvoltine blood into bivoltine is more useful in developing hardy bivoltine breeds and it is also widely used in China (Das, 2001; Das *et al.*, 2005a, b; Chandrasekaraiah and Ramesh Babu, 2003). Isozymes have been widely used as an important tool to screen variability present in the population and to select desired genotypes in the population. Isozyme and total protein-banding patterns are also used to identify species (Ladizinsky, 1975) cultivars (Bringhurst *et al.*, 1981) and inbred lines (Stuber and Goodman, 1981).

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Keeping this in view, a breeding programme was undertaken to develop bivoltine breeds, which can give cocoon crop stability under variable environments.

Materials and Methods

For this programme initially twelve bivoltine parents were selected on the basis of multivariate analysis from sixty genetic stocks maintained at Central Sericulture Research and Training Institute, Berhampore, West Bengal, India. Finally, out of those twelve, four parents i.e., CSR2, SK3 (Oval shaped cocoon) and D6 (P), SK4 (Dumbbell shaped cocoon), were selected for the present programme based on isozyme variability and ranking through multivariate analysis (Moorthy *et al.*, 2007). Like wise polyvoltine breeds viz., Nistari (Indigenous) and Cambodge (South East Asia origin) were selected as donor parents. The breeding plan was presented in Fig. 1. Since improvement was required in bivoltine, the crosses were made between female component of bivoltine and male component of multivoltine. Accordingly, eight combinations viz., D6 (P) × Nistari, SK4 × Nistari, SK3 × Nistari, CSR2 × Nistari, D6 (P) × Cambodge, SK4 × Cambodge, SK3 × Cambodge, and

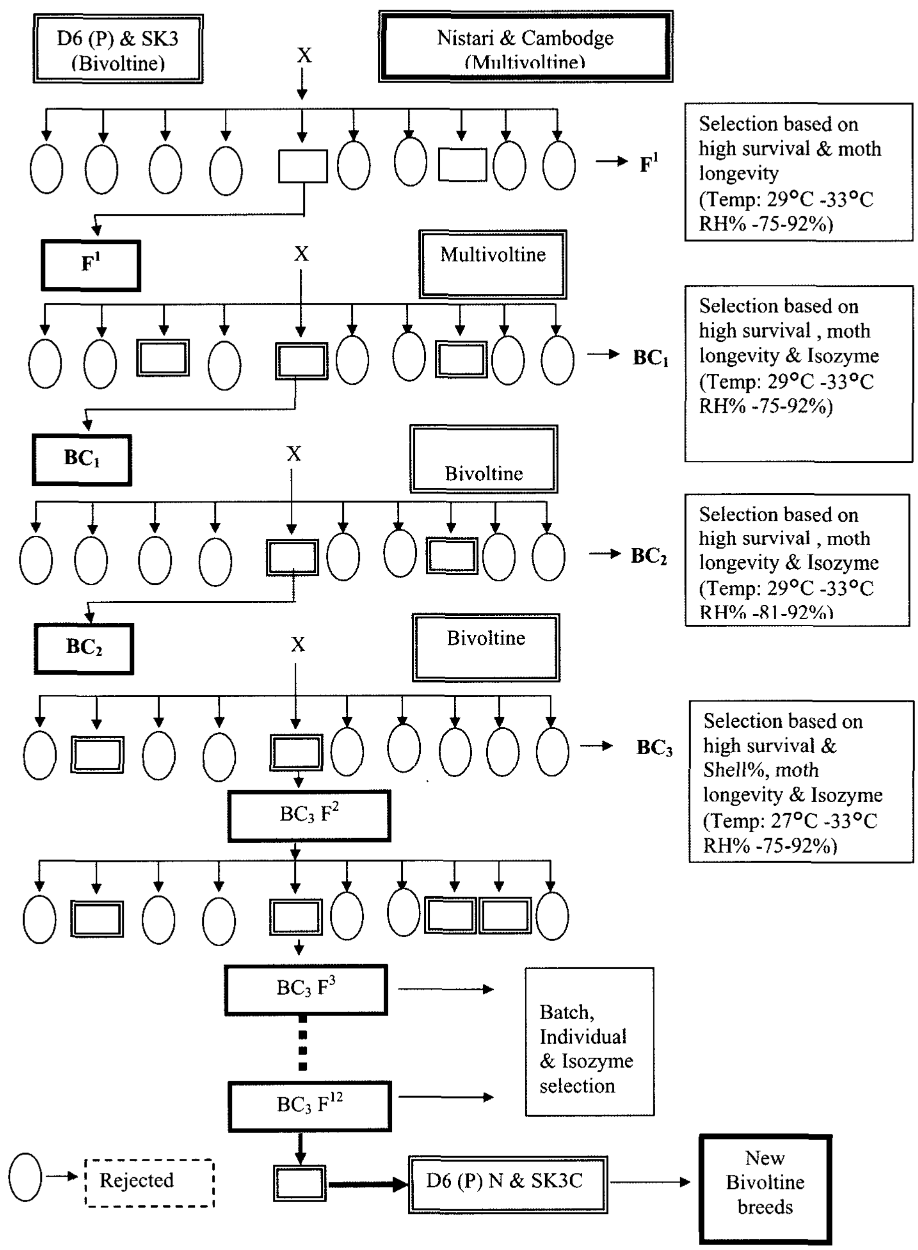

Fig. 1. Breeding Scheme.

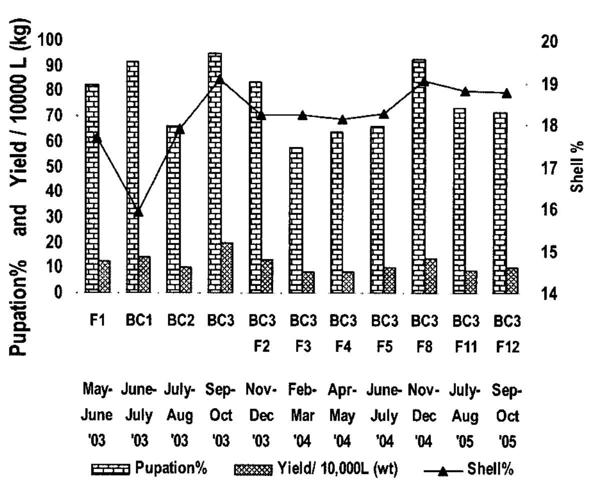

Fig. 2. Generation wise performance of the newly developed bivoltine breed, D6 (P)N.

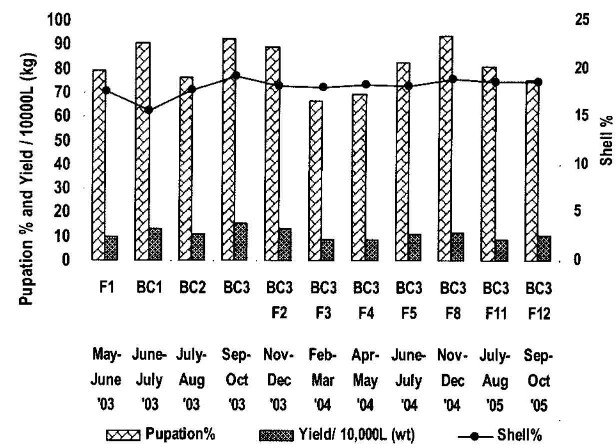

Fig. 2a. Generation wise performance of the newly developed bivoltine breed, SK3C.

CSR2 × Cambodge were made. Rearing was conducted under prevailing environmental conditions. The F_1 progeny was backcrossed with polyvoltine (BC_1) followed by back crossing with bivoltine (BC_2 & BC_3) to regain productive traits. After back crossing (BC_3), inbreeding was continued. From F_1 to BC_3 F^{12}, batch rearing was conducted and directional selection was imposed during F_1 to BC_3. Emphasis was given to select batches having higher survival in each generation. During inbreeding, individual & batch selection are applied. During course of breeding the following selection parameters were considered at different stages of development in addition to larval marking, cocoon colour, shape and other quantitative characters. (1). Moth longevity, (2). Hibernation character, (3). High fertility, (4). Uniform hatching, (5). Uniform moulting. Besides, each breeding generation, esterase isozyme analysis was done on both the parents and developing lines following the methodology suggested by Harris and Hopkinson (1977). From each batch, randomly selected larvae were subjected to esterase isozyme analysis and subsequently the batches having high survival with similar banding pattern to multivoltine parent were selected.

Results

Out of eight combinations made initially, two promising breeds viz., D6(P)N and SK3C were developed from combination of D6 (P) × Nistari and SK3 × Cambodge respectively. The generation wise performances of these lines are presented in the fig. 2 & 2a. The pupation% was recorded higher during F_1 & BC_1 generation and declination was observed in BC_3F^3 to BC_3F^5 generation in both the lines followed by an approximate stabilization in subsequence. Contrary to this, shell% was observed low during F_1 to BC_2 and subsequently increased.

The analysis of the performance of the newly developed bivoltine vis-à-vis their parents are shown in the Table 1 & 1a. Results revealed highly significant ($P<0.01$) increase of survival in the developed lines as compared to

Table 1. Comparative performances of the newly developed bivoltine breed, D6 (P) N (BC_3F^{12}) their donor & recipient parents and control

Season: Sep-Oct (2005). Temperature range: 28-32°C; Humidity range: 78-93%

Lines / Parents	Fecundity	Pupation (%)	Yield/ 10,000L (wt-kg)	SCW (g)	SSW (g)	Shell (%)	FL (mt)	Denier
D6(P)N	501	71.73	9.972	1.411	0.265	18.78	784	2.18
Nistari (Donor)	360	90.30	8.831	1.008	0.133	13.22	361	2.05
D6(P) (Recipient)	554	11.04	1.696	1.418	0.272	19.18	850	2.22
NB4D2 (Control)	525	8.28	1.055	1.414	0.267	18.89	866	2.25
Mean	485	45.34	5.389	1.313	0.233	17.451	715.25	2.17
SD	86.41	41.91	4.66	0.20	0.07	2.83	238.8	0.089
CD at 5%	37.920	7.507	1.220	0.038	0.012	1.086	150.24	0.012
CV%	4.150	8.790	12.050	1.540	2.856	3.890	24.25	1.20

Table 1a. Comparative performances of the newly developed bivoltine breed SK3C (BC_3F^{12}) their donor & recipient parents and control

Season: Sep-Oct (2005) ; Temperature range: 28-32°C; Humidity range: 78-93%.

Lines / Parents	Fecundity	Pupation (%)	Yield/ 10,000L (wt-kg)	SCW (g)	SSW (g)	Shell (%)	FL (mt)	Denier
SK3C	430.67	75.20	10.628	1.307	0.243	18.59	744	2.20
Cambodge (Donor)	422	86.37	7.061	1.020	0.143	13.98	374	2.09
SK3 (Recipient)	520	17.55	2.485	1.363	0.258	18.97	845	2.20
NB4D2 (Control)	525	8.28	1.055	1.414	0.267	18.89	866	2.25
Mean	474	46.85	5.307	1.276	0.226	17.51	707.25	2.185
SD	55.79	39.63	4.38	0.18	0.06	2.38	228.46	0.0675
CD at 5%	39.48	7.98	1.52	0.11	0.02	1.39	142.51	0.014
CV%	4.21	8.98	15.24	4.64	6.30	4.21	26.44	1.85

their respective receptor parent. About five fold increase of survival in D6 (P) N and three fold in SK3C have been noticed comparing to the receptor parents D6 (P) and SK3 respectively. Similarly, as compared to NB4D2 (control) both the lines registered around eight fold more survival.

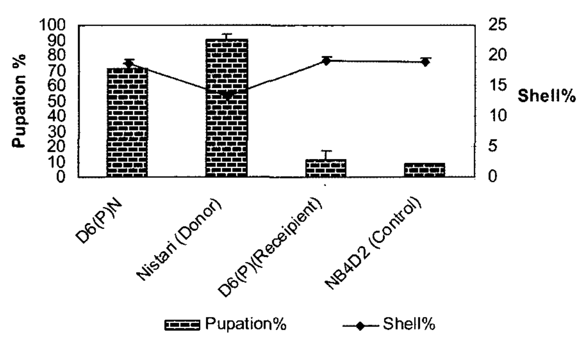

Fig. 3. Comparative performance of pupation (%) and shell% in the developed bivoltine breed D6 (P) N with donor, recipient parents and control.

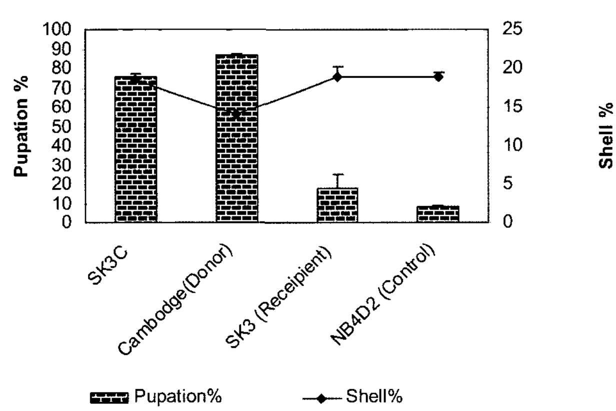

Fig. 3a. Comparative performance of pupation (%) and shell% in the developed bivoltine breed SK3C with donor, recipient parents and control.

Reduction of survival in D6 (P) N (26%) and in SK3 C (15%) was observed, when compared to their donor parent, Nistari and Cambodge respectively. On the other hand, 42% and 32% increased shell% were observed in D6 (P) N and SK3 C, when compared to multivoltine donor parents Nistari and Cambodge respectively (Fig.3 & 3a). However 2% reduction in shell% was observed in both the lines viz., D6 (P) N and SK3C, when compared to their original bivoltine parent D6 (P) and SK3 respectively.

The α-esterase isozyme pattern in the two developed bivoltine at BC_3F^{12} generation and their parents (Donor and receptor) revealed a total of four bands (Fig. 4) viz., Est-1, Est-2, Est-3 , Est-4 with Relative mobility (Rm) values of 0.425, 0.346, 0.247 and 0.217 respectively. . Of which, the characteristic band Est-3 with Rm value of 0.247 present only in the multivoltine (donor) parents are also predominantly available in the developed bivoltine lines. This shows the introgression of multivoltine genes/ characters in the developed bivoltine lines.

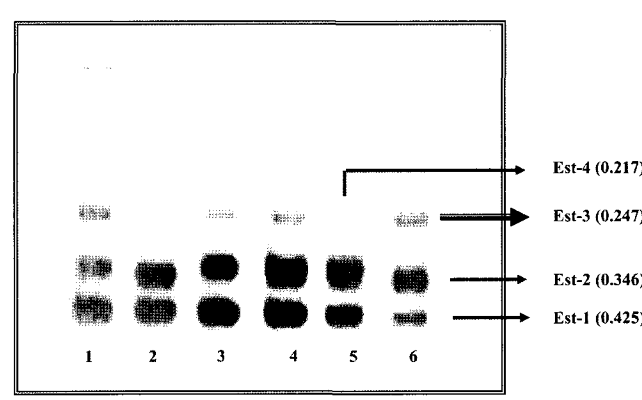

Fig. 4. Zymogram of α-esterase in the two developed bivoltine (at BC_3F^{12} generation), their donor and recipient parents. Lane 1. D6 (P) N; 2. D6 (P); 3. Nistari; 4. SK3C; 5. SK3; 6. Cambodge. Where D6 (P) N and SK3C are developed breeds, D6 (P) and SK3 are recipient bivoltine parents, Nistari and Cambodge are donor multivoltine parents.

Discussion

The cocoon crop stability and other yield attributes characteristics in silkworm, *Bombyx mori* are largely dependent on a wide range of environmental conditions and attaining this objective is a challenge to a breeder particularly in tropical countries (Ren *et al.,* 1988). Basically, a silkworm breed has to satisfy the egg producer, cocoon producer and reeler. But it is well known that in silkworm *Bombyx mori,* most of the economic traits are under the control of polygenes and especially, silk productivity and viability are negatively correlated. So, there is an obligation to develop bivoltine breed with high survival and all other economic characters by balancing them at moderate level for rearing during unfavourable season (Chandrasekaraiah and Ramesh babu, 2003). Success of any breeding programme depends on the selection of parents, their effective utilization and proper adoption of selection procedure. In this programme, Nistari and Cambodge (Polyvoltine) are taken as a donor parents. Nistari is an indigenous and highly adapted breed to local environmental conditions of the Eastern India (Sengupta *et al.*, 1997) while Cambodge is a resistance strain to high temperature and has the dominant gene for robustness (Suresh Kumar *et al.,* 2004). Tazima and Ohnuma (1995) also used Pure Mysore an indigenous polyvoltine breed of southern part of India as parent to develop silkworm strain tolerant to high temperature. According to Wu and Hou (1993), environment prevailed in the rearing localities is the base temperature for developing/understanding the thermo tolerance of the silkworm. Keeping this in mind, breeding programme was conducted in the prevailing environmental conditions of the region and especially F_1 - BC_3 , BC_3F^4 - BC_3F^7 , BC_3F^{10} - BC_3F^{12} generations rearing was conducted during selective summer months viz., May – September (2003), April – September (2004) and April – September (2005) respectively in order to get exposure in high temperature & high humidity conditions. Rajanna and Sreeramareddy (1998) opined that, initiating breeding experiments in native environment would facilitate 'direct response' of the suitable genotype to the prevailing environment and yield consistent cocoon crops. According to Falconer (1960), the improvement obtained by selection under favourable conditions will not help in realizing the full potential when the selected strains are transferred to unfavourable conditions. Since improvement of survival was required in productive bivoltine, back crossing was adopted (Das, 2001) and bi x multi crosses were considered as base material for breeding. Besides, He and Oshiki (1984), who developed bivoltine breeds suitable for autumn rearing in China, reported that resistance to adverse environment was greater in bi x multi form as compared to its reciprocal. The present study clearly indicates that there is improvement of survival in developed bivoltine breeds comparing to their original parents (Bivoltine) and control. It also confirms the introgression of survival character from multivoltine in to bivoltine. The variability like high productivity and higher viability present in the genetic materials (Bivoltine & Multivoltine respectively) and, the correct selection of the character which expressed in specific environment, pave the way to amalgamate more desirable characters into a single breed that may have acquired buffering capacity against the prevailing environmental impediments. Shao *et al.* (1987) and He *et al.* (1989) also adopted hybridization and back crossing followed by rearing at high temperature and humidity to develop robust silkworm breeds. Morrison and Milkman (1978) succeeded in increasing heat resistance and heat sensitivity within the isofemale lines of Drosophila melanogaster using an indirect selection method mainly due to the availability of potential genetic variability in the population. So creation of variability is one of the important criteria for the target oriented breeding programme.

Back crossing and introgression are useful for genetic improvement in breeding programme. In backcross, progeny are selected on the basis of character of interest and then back cross to recurrent parent (Hospital, 2005). Tankley and Rick (1980) suggested exploiting differences in isozymic allele between the donor and recurrent parent and it would be possible to screen the back cross progeny for recurrent parent in genotype two or more backcross generation. Esterase isozyme pattern revealed the presence of new band (Est-3) in the developed lines, which was available only in the polyvoltine parents. This may be the reason for increased survival in the developed lines, since we have sleeted the batches having the esterase banding pattern similar to donor parent in order to retain the higher survival in the new lines. However, it is yet to establish that the particular band is related to survival character. It requires further study for a full-proof ad vocation. Bouchez *et al.* (2002) performed the introgression of favourable allele at three QTL for two traits (earliness & yield) between maize elite lines with marker-assisted breeding. They found that use of markers would improve the background selection efficient. Ashwath and Morrison (2001) used digestive amylase, as a surrogate marker to develop high survival bivoltine using multivoltine as a donor. When the survival character was improved to some extent, there was reduction in the shell ratio by about 10% in the developed lines compared to the original parent. Kato *et al.* (1998) and Suresh Kumar *et al.* (2001) also found that reduction of cocoon weight and cocoon shell weight, when silkworms are exposed to high temperature and reported that it is possible to develop high temperature tolerant breeds as because resistance to high temperature is a heritable character. Though the developed parental strains are superior in respect of survival than the control and their parents, the genetic worth of the any line can be determined by evaluating their combining ability. So for determining their ability hybridization with other bivoltine and multivoltine is under progress.

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References

Ashwath, S. K. and M. N. Morrison (2001) Isozyme marker assisted silkworm breeding; in *Concept papers in Silkworm Breeder's Summit.* 18th & 19th October, 2001 held at Dehradun, India, pp. 91-97.

Basavaraja, H. K., S. Nirmal Kumar, S. Sureshkumar, N. Mal reddy, Kshama Giridhar, M. M. Ahsan and R. K. Datta (1995) New productive bivoltine hybrids . *Indian Silk* **34**, 5-9.

Basavaraja, H. K. and S. B. Dandin (2002) Recent breakthrough in breeding of silkworm, *Bombyx mori* L. in tropics; in *International Sericulture Congress*, Bangkok, Thailand.

Bouchez, A., F. Hospital, M. Causse, A. Gallais and A. Charcosset (2002) Marker assisted introgression of favourable allele at quantitative trait loci between maize elite lines. *Genetics* **162**, 1945-1959.

Bringhurst, R. S., S. Arulsekar, J. F. Hancock and V. Voth (1981) Electrophoretic characterization of strawberry cultivars. *J.Ameri.Soci.Hort. Sci.* **106**, 684-687.

Chandrasekariah and M. Ramesh Babu (2003). Silkworm breeding in India During the last five decades and what next? ; in *Concept papers in Silkworm Breeder's submit.* 18th & 19th July, 2003 held at APSSRDI, Hindupur, A.P., India. pp. 6-13.

Das, S. K., S. K. Sen and B. Saratchandra (1998) Improvement of commercial traits in mulberry silkworm *Bombyx mori* L by hybridization; in *Perspective in cytology and genetics* **9**, 101-108.

Das, S. K., S. M. Moorthy, P. R. T. Rao, S. K. Sen and B. Saratchandra (2000) Breeding bivoltine silkworm varieties of *Bombyx mori* L. for rearing under adverse climatic condition. Nat. Conf. Strategies for Seric. Res. & Dev. CSR&TI, Mysore, India. pp. 19.

Das, S. K. (2001) Techniques of breeding for evolving improved breeds of bivoltine mulberry silkworm *Bombyx mori* L; in *Perspective in cytology and genetics* **10**, 129-134.

Das, S. K., G. K. Chattopadhyay, A. K. Verma, A. K. Sengupta and A. Sarkar (2005a). Development of High yielding Silkworm Breeds of *Bombyx mori* L. for Eastern India through Congenic line breeding approach; in "*20th Congress of the international Sericultural commission*", Bangalore. Vol .I, pp. 268-272.

Das, S. K., S. M. Moorthy, G. K. Chattopadhyay, A. K. Verma, B. Ghosh, P. R. T. Rao, A. K. Sengupta and A. Sarkar (2005b) Silkworm breeding for tropical conditions of Eastern India; in "*Proceedings of 12th AICCG*", Bombay, India. pp. 4-5.

Datta, R. K. (1984) Improvement of silkworm races (*Bombyx mori* L) in India. *Sericologia* **24**, 393-415.

Falconer, D. S. (1960) Selection in mice for growth on high and low planes of nutrition . Genet. Res. **1**, 91-113.

Ghosh, B., A. K. Sengupta, P. R. T. Rao, G. C. Roy, S. M. Moorthy and B. Saratchandra (2001) Evolution of improved polyvoltine silkworm breeds for Eastern India; in *Perspectives in cytology and Genetics* **10**, 709-716.

Harris, H. and D. A. Hopkinson (1977) Hand book of Enzyme Electrophoresis in Human Genetics. North Holland publishing Co, Amsterdem. pp. 297.

He, S. M., X. Yan, Y. Mi and L. Xia (1989) Breeding of new silkworm variety "Feng I × 54A' for summer-autumn rearing. *Sci.Seri.* **15**, 79-87.

He, Y. and T. Oshiki (1984) Study on cross breeding of a robust silkworm race in low latitude are in China. *J.Seri.Sci.Jpn.* **54**, 320-324.

Hospital, F. (2005) Selection in backcross programme. *Phil. Trans.R.Soci.* **360**, 1503-1511.

Kato, M., K. Nagayasu, O. Ninaki, W. Hara and A. Watanabe (1998) Study on resistance of the silkworm, *Bombyx mori* L to high temperature; in *Proc.Int. Congr. SABRAO* **2**, 953-956.

Krishna Rao, S. (1998) Bivoltine silkworm races evolved by KSSRDI; in *proceedings of the national workshop on Silkworm Breeding.* Sreeramareddy, G. (eds.), pp. 119-130. Oxford & IBH publishing Co, New Delhi and Calcutta.

Ladizinskey, G. (1975) Seed protein electrophoresis of the wild and cultivated species of selection of faba of vicia. *Euphytica* **24**, 785-788.

Moorthy, S. M., S. K. Das, P. R. T. Rao, S. Raje Urs and A. Sarkar (2007) Evaluation and selection of potential parents based on selection indices and isozyme variability in silkworm, *Bombyx mori* L. *Int. J. Entomology,* **14**(1), 1-7.

Morrison, W. W. and R. Milkman (1978) Modification of heat resistance in *Drosophila* by selection . *Nature* **273**, 49-50.

Rajanna, G. S. and G. Sreeramareddy (1998) New approach to silkworm improvement aimed at narrowing yield gaps; in *proceedings of the national workshop on Silkworm Breeding.* Sreeramareddy, G (eds.), pp. 272-277. Oxford & IBH publishing Co, New Delhi and Calcutta.

Raju, P. J. and N. B. Krishnamurthy (1993) Breeding of two bivoltines, MG511 and MG512 of silkworm, *Bombyx mori* L for higher viability and productivity. *Sericologia* **33**, 577-587.

Ren, L. L., L. Mini and C. Hell (1988) Stability of double cross hybrid combined with current silkworm varieties for spring and early autumn under normal rearing conditions. *Acta Serica Sinica* **14**, 42-44.

Sengupta, A. K., S. K. Das, P. R. T. Rao, B. Ghosh and B.

Saratchandra (1997) Silkworm breeds and their hybrids. *Indian silk* pp. 18-20.

Shao, Y-H., W. B. Li, J. Q. Xia, and J. R. Cao (1987) The breeding of the mulberry silkworm varities "Xinhang" and "Keming" for summer and autumn rearing. *Canye Kexue* **13**, 15-21.

Stuber, C. W. and M. M. Goodman (1981) Compilation of isozyme genotypes for 342 inbred lines. *Maize genetics cooperation of news letter* **55**, 126.

Suresh Kumar N., C. M. Kishore Kumar, H. K. Basavaraja, N. Mal Reddy, M. Ramesh Babu and R. K. Datta (1999). Comparative performance of robust and productive bivoltine hybrids of *Bombyx mori* L under high temperature conditions. *Sericologia* **39**, 567-571.

Suresh Kumar, N., T. Yamamota, H. K. Basavaraja and R. K. Datta (2001) Studies on the effect of high temperarture on F1 hybrids between polyvoltine and bivoltine silkworm races of *Bombyx mori* L. *Int.J.Indust.Entomology* **2**, 123-127.

Suresh Kumar, N., N. Mal Reddy, H. K. Basavaraja, B. K. Kariappa and S. B. Dandin (2004) Breeding strategies for the development of robust bivoltine hybrids of silkworm for the tropics; in *Recent trends in Applied biology*, Avinashilingam Institute of home science and higher education for women, January, 28-29, Coimbatore , India.

Tanksley, S. D. and C. M. Rick (1980) Isozyme gene linkage map of the tomato: application in genetics and breeding. *Theor.Appl.Genet.* **57**, 161-170.

Tazima, Y. and A. Ohnuma (1995) Preliiminary experiments on the breeding procedure for synthesizing high temperature resistant commercial strain of the silkworm, *Bombyx mori* L. *Silk.Sci. Res. Inst. Japan* **43**, 1-16.

Wu, Den-jen. and R. F. Hou (1993) Relation between Thermo tolerance and Heat-stable esterase in the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Appl.Entomol.Zool.* **28**, 371-377.