



NEW BREEDING RESOURCE MATERIAL FOR THE DEVELOPMENT OF POLYVOLTINE BREEDS OF SILKWORM, *BOMBYX MORI* L. TOLERANT TO HIGH TEMPERATURE

Dayananda^{a*}, Premalatha Varadaraj^a, Murikinati Balavenkatasubbaiah^a, Kuniyil Chandrashekarana^a, Sundaramurthy Nirmal Kumar^b and Bharat Bushan Bindroo^a

^aCentral Sericultural Research and Training Institute, Central Silk Board, Mysore-570008, Karnataka, India

^bCentral Sericultural Research and Training Institute, Central Silk Board, Berhampore- 742101, West Bengal, India

* Corresponding author e-mail: dayananda1966@yahoo.co.in

ABSTRACT: Though many productive polyvoltine breeds have been evolved earlier, their full potentiality has not been exploited in the field due to various reasons. It was primarily due to their non-tolerance to wide fluctuating environmental conditions especially high temperature. Hence, development of polyvoltine silkworm breeds with genetic plasticity to buffer against high temperature is of primary importance to keep sericulture sustain. The selection of initial parents to be used as breeding resource material is of prime importance in the success of any breeding programme. The selection of suitable parents determines the degree of success of the breeding programme to a large extent. Therefore, proper and appropriate evaluation and identification of suitable parents among the large population is crucial for the breeders. Accordingly, thirty polyvoltine breeds were evaluated by employing multiple trait evaluation index and index scoring method. Based on the evaluation index values and index score, fifteen breeds viz., NDV6, L13, L14, L15, ND5, NP1, AGL3, 96A, PV2, NDV3, ND2, NP4, BL65, BL68 and BL69 were identified as relatively tolerant to high temperature. These identified polyvoltine breeds can be used as breeding resource material for further development of polyvoltine breeds tolerant to high temperature and subsequent identification of cross breeds.

Key words: Breeding resource material, Evaluation Index, Temperature tolerant, Index scoring, Selection

INTRODUCTION

Recent efforts in silkworm breeding have led to the development of many productive polyvoltine breeds, which have potential to produce gradable silk. Even though they are known for their productive merit, absence of genetic plasticity to buffer against the tropical environmental stress act as a constraint to exploit the full economic potential of these hybrids under large scale in the field. The newly evolved breeds continue to suffer badly in adverse conditions of high temperature, due to which there is a wide gap between the results of laboratory and field [1]. Since silkworm is a poikilothermic insect, temperature plays a major role on its growth and development. The major aspect is that many quantitative characters decline sharply at high temperature (>28°C) preferably during late age [2]. However, polyvoltine races / breeds of indigenous origin in tropical countries are known to tolerate slightly higher temperature [3], which is not true with evolved polyvoltine breeds that have been developed specially for their productivity. The effect of temperature higher than 30°C on the growth and the development of the silkworm larvae were reported earlier [4, 5]. Survival rate of silkworm is a main parameter for evaluating thermo tolerance [6,7]. Resistance to high temperature is a heritable character and it may be possible to breed silkworm races tolerant to high temperature [8]. The environmental effect on the performance of an insect is improved by selection in the environment in which it is subsequently exploited [9]. The hot climatic conditions of the tropics and universal rise in temperature due to the global warming are contributing to the poor performance of the evolved polyvoltine breeds. Since, more than 90% of the Indian raw silk production is from the cross between polyvoltine and bivoltine, it is very much essential to develop polyvoltine breeds which can withstand high temperature.

In the above context and also with the expansion of sericulture, need for development of silkworm breeds tolerant to various stress factors has assumed greater significance. The success of the breeding to develop silkworm breeds tolerant to various stress factors depends on the initial selection of parents, their judicious utilization and choice of mating to generate ample genetic variability for facilitating enough scope for selection.

Therefore, it is of paramount importance that utmost care has to be taken by the breeder in verifying and analyzing the genetic worth of the initial parents to be utilized as potential resource material by employing various selection methods. Therefore, it is imperative for a breeder to identify distinct and different gene pools existing in a group of resource materials. The lines which have been identified through selection adopting high temperature as stress factor could be effectively utilized in further breeding programmes to develop polyvoltine breeds tolerant to high temperature.

MATERIALS AND METHODS

During evaluation, emphasis was laid on to select silkworm breeds with relatively tolerance to high temperature. The study involved 30 polyvoltine breeds available at Germplasm stock of Central Sericultural Research and Training Institute (CSR&TI), Mysore, Karnataka, India. The morphological features of the breeds utilized for the study are given in Table.1. All the 30 breeds were brushed enmass in three replications and reared up to V instar as per standard rearing method [10]. On 3rd day of 5th instar, 300 larvae from each replication were subjected to high temperature ($36\pm 1^{\circ}\text{C}$) and high humidity ($85\pm 5\%$) in the Sericatron for a period of six hours per day till spinning. Observations on survival percent (number of live cocoons harvested per 100 larvae subjected for stress), cocoon weight, cocoon shell weight and cocoon shell percent were recorded.

Table 1. Morphological features of polyvoltine silkworm breeds utilized for the study

S.No.	Breed	Origin	Larval markings	Cocoon colour	Cocoon shape
1	ND7	India	Plain	Greenish yellow	Oval
2	NDV6	India	Plain	Greenish yellow	Oval
3	L13	India	Plain	Greenish yellow	Oval
4	L14	India	Plain	Greenish yellow	Oval
5	L15	India	Plain	Greenish yellow	Oval
6	ND5	India	Plain	Greenish yellow	Oval
7	NP1	India	Plain	Greenish yellow	Oval
8	AGL3	India	Plain	Greenish yellow	Oval
9	AGL5	India	Plain	Greenish yellow	Oval
10	96A	India	Plain	Greenish yellow	Oval
11	96E	India	Plain	Greenish yellow	Oval
12	2000H	India	Plain	Greenish yellow	Oval
13	PV1	India	Plain	Greenish yellow	Short Oval
14	PV2	India	Plain	Greenish yellow	Dumb-bell
15	MAD	India	Plain	Greenish yellow	Short Oval
16	NDV1	India	Plain	Greenish yellow	Oval
17	NDV3	India	Plain	Greenish yellow	Oval
18	NDV7	India	Plain	Greenish yellow	Dumb-bell
19	ND2	India	Plain	Greenish yellow	Oval
20	ND10	India	Plain	Greenish yellow	Oval
21	NP3	India	Plain	Greenish yellow	Oval
22	NP4	India	Plain	Greenish yellow	Oval
23	BL24	India	Plain	Dark Greenish yellow	Oval
24	BL62	India	Plain	Greenish yellow	Oval
25	BL65	India	Plain	Greenish yellow	Oval
26	BL67	India	Plain	Greenish yellow	Oval
27	BL68	India	Plain	Greenish yellow	Oval
28	BL69	India	Plain	Greenish yellow	Oval
29	MR1	India	Marked	Off white	Spindle
30	MO6	India	Plain	Greenish yellow	Oval

Observations on the above economic characters recorded from replicates under high temperature rearing were pooled together and analyzed statistically by employing Evaluation Index and Index scoring method. Evaluation indices (E.I) were determined by using the formula [11]:

Evaluation Index =	$\frac{A - B}{C}$	$\times 10 + 50$

Where,

A= Value of a particular breed for a character.

B= Mean value of particular trait of all the breeds.

C= Standard deviation of particular trait of all the breeds.

10= Standard Unit.

50= Fixed value

The cumulative score index of all the characters of a breed were also determined by using the formula [12]:

Score Index (%) =	Total score obtained	× 100
	Maximum possible score	

The average evaluation index and index score, ranks the breed based on the higher score. The breeds scoring average evaluation index of above 50 and index score percent of above the average under the stress was identified as breeds relatively tolerant to high temperature.

RESULTS

The main objective of the study was to identify the potential polyvoltine breeds, which are relatively tolerant to high temperature to use them as breeding resource material for the development of productive polyvoltine breeds tolerant to high temperature. The mean performance under high temperature rearing of the thirty polyvoltine breeds considered for the study is given in Table.2.

Table 2. Mean performance of polyvoltine silkworm breeds under high temperature

S.No.	Breed	Survival (%)	Cocoon weight (g)	Shell weight (g)	Shell percent	Average E.I
1	ND7	23.50 (31.67)	1.214 (57.79)	0.195 (51.02)	16.08 (37.85)	44.58
2	NDV6	46.00 (47.17)	1.244 (60.50)	0.210 (57.48)	16.86 (47.21)	53.09
3	L13	54.00 (52.67)	1.117 (49.09)	0.206 (55.87)	18.46 (66.40)	56.01
4	L14	78.00 (69.20)	1.139 (51.06)	0.197 (52.02)	17.34 (52.97)	56.31
5	L15	52.00 (51.30)	1.144 (51.52)	0.201 (53.54)	17.56 (55.61)	52.99
6	ND5	49.00 (49.23)	1.285 (64.14)	0.213 (59.00)	16.59 (43.97)	54.09
7	NP1	43.50 (45.44)	1.169 (53.80)	0.215 (59.82)	18.39 (65.56)	56.16
8	AGL3	51.00 (50.61)	1.214 (57.81)	0.220 (61.81)	18.08 (61.84)	58.02
9	AGL5	43.50 (45.44)	1.161 (53.03)	0.189 (48.44)	16.31 (40.61)	46.88
10	96A	57.00 (54.74)	1.145 (51.64)	0.199 (52.87)	17.41 (53.81)	53.26
11	96E	67.00 (61.63)	0.960 (35.08)	0.170 (39.96)	17.72 (57.52)	48.55
12	2000H	29.50 (35.80)	1.184 (55.11)	0.201 (53.79)	17.01 (49.01)	48.43
13	PV1	74.00 (66.45)	1.046 (42.73)	0.177 (42.82)	16.89 (47.57)	49.89
14	PV2	71.00 (64.38)	1.115 (48.91)	0.185 (46.70)	16.63 (44.45)	51.11
15	MAD	38.00 (41.66)	0.970 (35.96)	0.160 (35.46)	16.49 (42.77)	38.96
16	NDV1	37.00 (40.97)	1.163 (53.26)	0.194 (50.55)	16.68 (45.05)	47.46
17	NDV3	35.50 (39.94)	1.188 (55.48)	0.223 (63.31)	18.76 (70.00)	57.18
18	NDV7	47.50 (48.20)	1.091 (46.81)	0.187 (47.45)	17.15 (50.69)	48.29
19	ND2	39.50 (42.69)	1.227 (58.99)	0.218 (61.26)	17.79 (58.36)	55.32
20	ND10	41.00 (43.72)	1.036 (41.87)	0.172 (40.55)	16.55 (43.49)	42.41
21	NP3	50.50 (50.26)	1.184 (55.10)	0.186 (46.82)	15.68 (33.06)	46.31
22	NP4	53.50 (52.33)	1.270 (62.79)	0.211 (58.11)	16.63 (44.45)	54.42
23	BL24	50.00 (49.92)	0.700 (11.79)	0.109 (12.89)	15.57 (31.74)	26.58
24	BL62	48.00 (48.54)	1.150 (52.07)	0.191 (49.12)	16.60 (44.09)	48.46
25	BL65	48.00 (48.54)	1.106 (48.11)	0.197 (51.96)	17.84 (58.96)	51.89
26	BL67	38.00 (41.66)	1.091 (46.79)	0.189 (48.35)	17.34 (52.97)	47.44
27	BL68	58.50 (55.77)	1.150 (52.08)	0.211 (58.20)	18.38 (65.44)	57.87
28	BL69	78.00 (69.20)	1.181 (54.89)	0.206 (55.79)	17.43 (54.05)	58.48
29	MR1	73.00 (65.76)	1.055 (43.59)	0.174 (41.57)	16.47 (42.53)	48.36
30	MO6	28.50 (35.12)	1.107 (48.21)	0.178 (43.46)	16.09 (37.97)	41.19
	Min.	23.50	0.700	0.109	15.57	26.58
	Max.	78.00	1.285	0.223	18.46	58.48
	Mean	50.12	1.127	0.193	17.09	50.00
	SD	14.52	0.112	0.0226	0.83	6.70
	CV %	28.98	9.915	11.715	4.88	13.40

Values in the parenthesis are the multiple trait evaluation index (EI) values on the mean performance

The survival ranged from 23.50 to 78.00% with the lowest of 23.50% recorded for ND7 and highest of 78.00% recorded for L14 and BL69. The lowest cocoon weight of 0.700g, cocoon shell weight of 0.109g and cocoon shell percent of 15.57 recorded for BL24. The highest cocoon weight of 1.285g was recorded for ND5. The highest cocoon shell weight (0.223g) and cocoon shell percent (18.76) were recorded for NDV3. The multiple trait evaluation index was computed and its values are depicted in parenthesis (Table. 2). The average evaluation index ranged from 26.58 to 58.48. The lowest evaluation index of 26.58 was recorded for BL24 and highest recorded for BL69 (58.48). The data were subjected for index scoring and the details of scoring allotted for the different class intervals of the traits considered for the study are presented in Table. 3. The total score ranged from 5 to 11 with the lowest of 5 recorded for BL24 and the highest score of 11 recorded for L13, L14, L15, NP1, AGL3, PV2, BL65, BL68 and BL69 (Table. 4).

Table 3. Scoring of traits considered for the study on the mean performance of polyvoltine silkworm breeds under high temperature

	Survival (%)	Cocoon weight (g)	Shell weight (g)	Shell percent
Range of means				
Min	23.50	0.700	0.109	15.57
Max	78.00	1.285	0.223	18.46
Index score				
1	23.50 – 41.66	0.700 – 0.894	0.109 – 0.146	15.57 – 16.53
2	41.67 – 59.83	0.895 – 1.089	0.147 – 0.184	16.54 – 17.49
3	59.84 – 78.00	1.090 – 1.285	0.185 – 0.223	17.50 – 18.46

Table 4. Index scoring on the mean performance of polyvoltine silkworm breeds under high temperature

S.No.	Breed	Survival	Cocoon weight	Shell weight	Shell percent	Maximum Possible score	Total score obtained	Score Index (%)	Average E.I
1	ND7	1	3	3	1	12	8	66.67	44.58
2	NDV6	2	3	3	2	12	10	83.00	53.09
3	L13	2	3	3	3	12	11	91.67	56.01
4	L14	3	3	3	2	12	11	91.67	56.31
5	L15	2	3	3	3	12	11	91.67	52.99
6	ND5	2	3	3	2	12	10	83.00	54.09
7	NP1	2	3	3	3	12	11	91.67	56.16
8	AGL3	2	3	3	3	12	11	91.67	58.02
9	AGL5	2	3	3	1	12	9	75.00	46.88
10	96A	2	3	3	2	12	10	83.00	53.26
11	96E	3	2	2	3	12	10	83.00	48.55
12	2000H	1	3	3	2	12	9	75.00	48.43
13	PV1	3	2	2	2	12	9	75.00	49.89
14	PV2	3	3	3	2	12	11	91.67	51.11
15	MAD	1	2	2	1	12	6	50.00	38.96
16	NDV1	1	3	3	2	12	9	75.00	47.46
17	NDV3	1	3	3	3	12	10	83.00	57.18
18	NDV7	2	3	3	2	12	10	83.00	48.29
19	ND2	1	3	3	3	12	10	83.00	55.32
20	ND10	1	2	2	2	12	7	58.33	42.41
21	NP3	2	3	3	1	12	9	75.00	46.31
22	NP4	2	3	3	2	12	10	83.00	54.42
23	BL24	2	1	1	1	12	5	41.67	26.58
24	BL62	2	3	3	2	12	10	83.00	48.46
25	BL65	2	3	3	3	12	11	91.67	51.89
26	BL67	1	3	3	2	12	9	75.00	47.44
27	BL68	2	3	3	3	12	11	91.67	57.87
28	BL69	3	3	3	2	12	11	91.67	58.48
29	MR1	3	2	2	1	12	8	66.67	48.36
30	MO6	1	3	2	1	12	7	58.33	41.19
Average								78.79	

After screening the breeds under high temperature, based on the evaluation index values and index score, fifteen breeds viz., NDV6, L13, L14, L15, ND5, NP1, AGL3, 96A, PV2, NDV3, ND2, NP4, BL65, BL68 and BL69 were identified as polyvoltine breeds comparatively tolerant to high temperature. The identified polyvoltines recorded an average evaluation index value of above 50 and index score percent of above the average under high temperature. These breeds can be used as breeding resource material for the development of productive polyvoltine breeds and cross breeds tolerant to high temperature

DISCUSSION

More than 90% of the Indian silk is being produced from the hybrids of indigenous polyvoltine females crossed with males of bivoltine breeds [13]. Pure Mysore is an indigenous polyvoltine breed of southern India and reared hundreds of generations and is well adapted to the local environmental conditions. However, the race has got some inherent de-merits with regard to cocoon quality [14] which affected the quality production of Indian silk. Keeping this constraint, breeding programmes at CSR&TI, Mysore led into the development of many polyvoltine breeds. Though they are known for their productive merit, the absence of genetic plasticity to buffer against the tropical environmental stresses act as a constraint to exploit the full economic potential of these hybrids. The newly evolved breeds continued to suffer badly in adverse conditions of high temperature. Besides, understanding the genetic diversity of parental strains to be utilized in the breeding programme by their systematic evaluation, critical assessment of their quantitative nature that is greatly influenced by the high temperature environmental factor paves the way for the breeders for effective utilization [15,16]. Performance of the strain itself in a given environment indicates its superiority [17]. While evaluation, emphasis was given on the phenotypic expression of traits of economic importance under high temperature. However, as the objective of the study was for greater viability and high productivity merits, importance was given on those traits while selecting the parents. The significant variations observed for the traits analyzed can be attributed to the genetic constitution of the breeds and their degree of expression to which they are exposed during their rearing. The results are in line with the earlier findings [18,19,20,21]. It is important to measure the expression of the major contributing traits of economic importance in the silkworm strains under diversified environmental conditions to understand the genetic endowment pertaining to adaptability and productivity. The choice of potential material is critical and difficult to evaluate all the available silkworm breeds. However, a few strains of known genetic background, pedigree and specific traits desirable for the new breeding programme have to be taken into consideration while evaluating the breeding material. It is also equally important to understand the traits related to productivity and viability. All the 30 breeds selected for the evaluation are having one or more desirable traits as per the objectives of the present study. In the present study, the aim was to select the polyvoltine strains that can give rise to stable cocoon crop with better viability even though productivity is slightly low. The selection was primarily aimed at viability character such as yield by number without sacrificing much of the productivity traits like cocoon weight, cocoon shell weight and shell percent [22,23]. Assessment of multiple traits of the selected breed is an important task for predicting the potential resource material. Multiple trait evaluation index [11] method is widely used by the silkworm breeders. Though this index is more simple, easy and reliable to that of other index methods, the main disadvantage is that the index is based on the absolute values and accordingly, if the value of a particular character is very high on a particular breed than others, it may be scoring high in cumulative index, though the values of other characters are very low and there are chances that this particular breed may rank first among the others.

In addition to multiple trait evaluation index, a new index method using suitable class intervals was also considered for the selection of breeds with almost equal merits [12]. In this method, the range of variability with regard to the trait can be classified into three groups that gives index score value of 1, 2 and 3. The sum of the index scores with regard to all the characters indicates the breed's worth.

CONCLUSION AND RECOMMENDATION

After screening the breeds under high temperature, based on the evaluation index values and index score percent, fifteen breeds viz., NDV6, L13, L14, L15, ND5, NP1, AGL3, 96A, PV2, NDV3, ND2, NP4, BL65, BL68 and BL69 were identified as relatively tolerant to high temperature. These identified polyvoltine strains can be used as breeding resource material for the development of polyvoltine breeds tolerant to high temperature and subsequent identification of cross breeds.

REFERENCES

- [1] Dayananda. 2009. Studies on the performance and economic appraisal of new hybrids of the silkworm *Bombyx mori* L. through validation and demonstration. *Ph. D Thesis*, Mysore University, Mysore, India.
- [2] Shibukawa K. 1965. *Acta Sericologia*, 16:1.

- [3] Hesieh F K, Yu S, Su S Y and Peng S J. 1995. Studies on the thermo tolerance of the silkworm *Bombyx mori* L. Zsongriva.
- [4] Takeuchi Y, Kosaka T and Ueda S. 1964. The effects of rearing temperature upon the amount of food ingested and digested. Tech. Bull. Seric. Exp. Stn., 84:1-12.
- [5] Ohi H and Yamashita. 1977. On the breeding of the silkworm races J137 and C137. *Bull. Seric. Exp. Stn.*, 27: 97-139.
- [6] Huang P J, Chen J H, Hong D and Chen C N. 1979. Preliminary study on the inheritance of tolerance to high temperature in some silkworm strains. *J. Agric. Assoc. China*, 105: 23-39.
- [7] He Y and Oshiki T. 1984. Study on cross breeding of a robust silkworm race for summer and autumn rearing at low latitude area in China. *J. Seric. Sci. Jpn.*, 53: 320-324.
- [8] Kato M, Nagayasu K, Ninagi O, Hara W and Watanabe A. 1989. Studies on resistance of the silkworm *Bombyx mori* L. for high temperature. (in) Proc. 6th Internatl Congress of SABRAO (II), 953-956.
- [9] Falconer D S. 1990. Selection in different environments: effects on environmental sensitivity (reaction norm) and on mean performance. *Genet. Res.*, 56: 57-70.
- [10] Datta R K. 1992. Guidelines for bivoltine rearing. Bulletin of Central Silk Board, Bangalore.
- [11] Mano Y, Nirmal Kumar S, Basavaraja H K, Mal Reddy N and Datta R K. 1993. A new method to select promising silkworm breeds/combinations. *Indian Silk*, 31(10):53.
- [12] Suresh Kumar N, Palith AK, Kalpana G V and Joge P G. 2005. New index method for evaluation of silkworm hybrids of *Bombyx mori* L. (in) *Advances in tropical Sericulture*, Dandin SB et al., (Ed), *National Academy of Sericultural Sciences*, Bangalore, India, 74-78.
- [13] Datta R K, Raghavendra Rao D, Jayaswal K P, Premalatha V, Ravindra Singh and Kariappa B K. 2001. Heterosis in relation to combining ability in multivoltine and bivoltine strains of silkworm *Bombyx mori* L. *Indian J. Seric.*, 40 (1): 1-6.
- [14] Tazima Y. 1991. A view on the improvement of Mysore breeds. (in) *Proc. of International Congress on Tropical Sericulture Practices*, Central Silk Board, Bangalore. Feb. 18-23, 1988.
- [15] Naseema Begum A, Basavaraja H K, Rekha M, Ahsan M M and Datta R K. 2001. Identification of breeding resource material for the development of thermo – tolerant breeds in the silkworm *Bombyx mori* L. *Int. J. Indust. Entomol.*, 2(2):111-117.
- [16] Sudhakar Rao P, Datta R K, Ramesh Babu M and Vijaya Kumari K M. 2002. Breeding resource materials of silkworm *Bombyx mori* L. adaptive to tropical climates. *Int. J. Indust. Entomol.*, 5(1): 67-71.
- [17] Allard R B and Bradshaw A O. 1964. Implications of genotype environment interaction in applied plant breeding. *Crop Sci.*, 4: 503-506.
- [18] Kalpana G V. 1992. Breeding of superior races of silkworm *Bombyx mori* L. for tropical climates. *Ph.D thesis*, University of Mysore, Mysore, India, 288.
- [19] Nirmal Kumar S. 1995. Studies on the synthesis of appropriate silkworm (*Bombyx mori* L.) for tropics. *Ph.D thesis*, University of Mysore, Mysore, India.
- [20] Basavaraja H K. 1996. Studies on the synthesis of productive bivoltine strains of silkworm *Bombyx mori* L. through hybridization, selection and genetic evaluation. *Ph.D thesis*, University of Mysore, Mysore.
- [21] Sudhakar Rao P, Singh R, Kalpana G V, Nishitha Naik V, Basavaraja H K and Ramaswamy G N. 2001. Evaluation and identification of promising bivoltine hybrids of silkworm *Bombyx mori* L. for tropics. *Int. J. Indust. Entomol.*, 3(1): 31-35.
- [22] Lekuthai P and Butrachand S. 1974. Improvement of bivoltine silkworm races. *Bull. Thai Seric. Res. Trg. Cen.*, 2: 48-54.
- [23] Strunnikov V A and Strunnikov L V. 1986. The nature of genes controlling the silk productivity of silkworm cocoons. *Dokl Akad Nauk, USSR*. 290(1): 234-237.