



Synthesis of improved crossbreeds (ICB) of mulberry silkworm (Poly x Bivoltine) of *Bombyx mori* L. for commercial exploitation in tropical regions

SV Seshagiri^{1*}, PJ Raju²

^{1,2} Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Kirikera, Hindupur, Andhra Pradesh, India

Abstract

Indian Sericulture Industry is crossbreed oriented which amounts to be more than 80% of mulberry raw silk production. Due to certain field constraints like economic, social compulsions and agro-climatic conditions; still there is a prominence for crossbreeds which would continue to hold a place of significance. Hence, to meet the local requirement i.e. the tropical stress of Andhra Pradesh and marginal farmers, twenty new hybrid combinations were tested in Line x Tester method involving five promising polyvoltine oval lines viz., MF4, APMV2, APMV3, APMV4, and APMV5 and four bivoltine silkworm breeds viz., BV105, BV115, BVFC1 and BVS8 as testers. These hybrids were reared under standard rearing conditions to evaluate their performance. Data for eight economically important economic traits viz., fecundity, hatching %, cocoon yield per 10,000 larvae by number, cocoon yield per 10,000 larvae by weight, survival rate, cocoon weight, cocoon shell weight and cocoon shell ratio was recorded. The relative merit of the hybrids under study was assessed based on Evaluation Index and Subordinate Function methods. Based on the both evaluation index methods, two hybrid combinations such as MF4 x BVFC1 and APMV2 x BVFC1 were selected for further laboratory trials.

Keywords: silkworm, hybrid combinations, performance data, evaluation index, subordinate function

Introduction

Breeding as an important tool has been utilized by many breeders which has played a vital role in increasing the productivity in sericulture. The silkworm, *Bombyx mori* L. offers one of the very important insects of choice with large number of strains which is best exemplified for utilization of heterosis by crossing them in different combinations (Datta and Nagaraju, 1987) ^[20]. In fact, silkworm crop is the only exception where hybrids are invariably used (Yokoyama, 1979) ^[29]. Heterosis is being constantly exploited to improve the productivity of plants and animals. It is one of the phenomenon where there is increase in size and vigour resulting from crossing. In silkworm, *Bombyx mori*, this phenomenon is documented as early as in 1905 in Japan (Toyama, 1905) ^[28]. As far as Indian sericulture is concerned, a quantum jump in the cocoon productivity was realized only after the introduction of four new bivoltine races during 1970's and crossing of males of bivoltine races with multivoltine pure races was adopted. With the result there is a significant increase in the silk productivity. On the other hand the quality of silk produced is inferior and the inherent defects of multivoltine races frequently appeared in the hybrids.

Most of the breeding programmes aim to improve the yield potential and qualitative parameters of the breeds/ hybrids over the existing ones. A new era came into existence with the exploitation of 'heterosis' which has increased the productivity of silk contributing to the development and expansion of sericulture industry. However, there is always a scope of improvement of genetic traits and identification of superior hybrids through estimation of heterosis by combining ability studies (Rao *et al.*, 1968). Very limited number of polyvoltine cross breeds are available in the field with all the desired traits. The polyvoltine race (Pure

Mysore) is ruling the Indian Sericulture Industry since decades in the production of crossbreeds. At this juncture, there is a need for the development of quantitatively and qualitatively superior hybrids to cater to the vagaries of various climatic conditions of tropical regions.

The recent trend of global silk production centered mainly in tropical countries and perhaps India is the second largest producer of silk in the world next to China. More than eighty percent of the silk produced is mainly from cross breeds (polyvoltine x bivoltine). In spite of continuous efforts for the development of sericulture through various breeding programs many hybrids have been developed by the breeders (Rao *et al.*, 2004; Seshagiri *et al.*, 2011; Seshagiri and Raju, 2016), still there exists a wide gap between domestic requirement and production in India. To fulfill the gap and to face global competitiveness in silk production there is a need to develop more productive breeds or hybrids with quantitative and qualitative merit. In the present study, an attempt is made to evaluate new hybrids through Line x Tester analysis to adjudicate the best hybrid combinations for commercial exploitation at farmer's level.

Materials and Methods

In the present study, involving five promising polyvoltine oval lines viz., MF4, APMV2, APMV3, APMV4, and APMV5 and three bivoltine silkworm breeds viz., BV105, BV115, BVS8 and one foundation cross i.e. BVFC1 as testers, twenty hybrid combinations were prepared in Line x Tester mating design. All the hybrid combinations along with the ruling hybrid (PM x CSR2) as control were reared in three replications following standard rearing techniques (Krishnaswami, 1973) ^[9]. Three hundred larvae were retained after 3rd moult in each replication. During silkworm

rearing, the data pertaining to eight economic traits viz., fecundity, hatching %, cocoon yield per 10,000 larvae by number, cocoon yield per 10,000 larvae by weight (kg), survival rate (%), cocoon weight (g), cocoon shell weight (g) and cocoon shell ratio (%) were recorded. The silkworm hybrid rearing performance data was analyzed by adopting two evaluation methods widely used in sericulture viz., Multiple Trait Evaluation Index (Mano *et al.*, 1993)^[13] and Subordinate function method (Gower, 1971). Based on the average Evaluation Index values and Subordinate Function values, ranks were assigned to each of the hybrid combinations. The top ranked three hybrid combinations in both the evaluation methods were adjudicated as the potential and would be subjected for large scale laboratory trials and subsequently for commercial exploitation.

Multiple Trait Evaluation Index Method

Evaluation index over multiple traits was calculated for each of the hybrid combinations as per the formula suggested by Mano *et al.* (1993)^[13] which is as follows.

A - B

Evaluation Index = ----- x 10 + 50

C

Where,

A = Value obtained for a trait by the specific hybrid

B = Overall mean value of particular trait over all the hybrids

C = Standard deviation of the trait over all the hybrids

10 = Constant used for change of scale

50 = Constant used for change of origin

Subordinate function method

The subordinate function values for each of the trait were calculated for all the hybrid combinations by using the following formula.

$$X_u = (X_i - X_{\min}) / (X_{\max} - X_{\min})$$

Where,

X_u = Subordinate Function

X_i = Measurement of character of a tested genotype

X_{\max} = The maximum value of the character among all the tested genotypes

X_{\min} = The minimum value of the character among all the tested genotypes

Based on average Evaluation Index and Subordinate function method values ranks were assigned to all the hybrid combinations.

Results

The mean rearing performance data pertaining to eight economic traits of the twenty hybrid combinations along with the control hybrid are presented in Table 1. Perusal of the data revealed that, the fecundity ranged from 430 eggs/laying (MSO5 x APS45) to 497 eggs/laying (MF4 x BVFC1), hatching % to a maximum of 98.41 (MF4 x BV115) and to a minimum of 94.20 (APMV3 x BV105), yield per 10,000 larvae by number varied from a minimum of 9331 (APMV5 x BVS8) to a maximum 9903 (APMV4 x BV115), minimum yield per 10,000 larvae by weight of 15.270 kg was recorded in MF4 x BV115 and with a maximum of 19.245 kg in MF4 x BVFC1, survival rate ranged from 90.41% (APMV4 x BV115) to 98.75% (MF4 x BVFC1), cocoon weight varied from 1.533 g (MSO4 x

APS8) to 1.985 g (MSO3 x APS45), Shell weight ranged from 0.254 g (APMV4 x BV115) to 0.362 g (MF4 x BVFC1) and shell ratio was recorded to be minimum in APMV4 x BV115 (16.56%) with a maximum of 19.25% in MF4 x BVFC1.

Evaluation of the New Combinations: The new hybrid combinations were evaluated by the statistical methods viz., multiple trait evaluation index (EI) and subordinate function method. The evaluation of data reveals that the average EI values ranged to a maximum of 67.62 (MF4 x BVFC1) and to a minimum of 41.09 (APMV4 x BV115). The hybrid combinations such as MF4 x BVFC1, APMV2 x BVFC1 and APMV5 x BVFC1 were stood top three under evaluation index method (Table 2). Further, under the subordinate function method also the hybrid combinations such as MF4 x BVFC1 (7.38), APMV2 x BVFC1 (6.29) and APMV5 x BVFC1 (4.85) were shown higher values and stood top among all the hybrid combinations (Table 3). In both the evaluation methods, the top ranked three hybrid combinations were selected for further trials and subsequently for commercial exploitation (Table 4).

Discussion

Heterosis is being exploited to improve the productivity in plants and animals and more so in silkworm, *Bombyx mor.* L. with its larger number of strains is best exemplified for utilization of heterosis by crossing those strains which differ in their silk yield attributes both quantitatively and qualitatively (Tazima, 1964; Bhargava, 1995)^[1]. In general, silkworm races of Indian origin are poor in silk yield as compared to Chinese and / or Japanese ones (Thiagarajan *et al.*, 1993). It has been noticed that silk productivity of crosses is better than that of parents (Hirobe, 1961, Kobayashi *et al.*, 1968)^[4, 8]. While selecting the parental inbred races for hybridization, attention is given to their genetic diversity, geographical origin as well as voltinism in developing commercially viable high yielding hybrids. The commercial exploitation of hybrid vigour created a new era in sericulture which contributed substantially to the increased silk production. The hybrid combination possesses favourable characteristics in comparison with pure breeds such as greater survival rate, shorter larval duration and higher yields of cocoons and raw silk (Kovalov, 1970)^[6]. Tazima (1958) reported that the indigenous Pure Mysore race though hardy can withstand varied agro-climatic conditions, spins inferior quality of silk with neatness defect and emphasized the necessity for replacing it for the improvement of silk industry. Yet, the hybrids of Pure Mysore have become very popular among farmers (Nanavathy, 1965).

Rearing of hybrid varieties of silkworm in India began as early as 1922 but could achieve only limited success in producing quality cocoons as the parental breeds used were both multivoltines viz., Pure Mysore and C. Nichi (Raju and Krishnamurthy, 1995)^[17]. Cross breeding of silkworm races using multivoltine and bivoltine breeds of different quantitative characters was initiated during 1960's and later, the hybrids like PM x C108 and PM x NN6D were introduced (Jolly, 1983)^[5]. During 1970's, promising bivoltine breeds of NB series such as NB4D2, NB18, NB7 were evolved (Datta, 1984) and utilized for the development of improved multivoltine x bivoltine hybrids namely PM x NB4D2 and PM x NB18, which ruled the silk industry of

India for more than two decades covering major group of sericulture farmers.

Selection of potential hybrid combinations for commercial exploitation is one of the prime importance under tropical regions. In the present study, five promising polyvoltine breeds were tested with three bivoltines pure breeds and one bivoltine foundation cross to identify promising crossbreeds. To achieve desired goals, cross breeding is widely used in commercial animal production as a means of exploiting heterosis (Sang, 1956 and Bowman, 1959). Development of productive silkworm strains suitable to local conditions play a pivotal role in the overall development of sericulture industry. The polygenic nature of the quantitative traits and role of different intensities of selection in changing the mean expression have been demonstrated in plants and animals. Selection cannot create new gene combinations and however, it can increase the frequency of desirable genes existing in the population. Silkworm breeding which has been in practice since many decades in Japan, wherein desirable goals were achieved with certain specific objectives. The poor adaptability of the bivoltines to the fluctuating environmental conditions of the tropical climates makes them unsuitable for their commercial exploitation throughout the year. Under such circumstance the need was felt for synthesis of new breeds and accordingly in the present study twenty hybrid combinations were evaluated keeping the ruling hybrid as control.

The ultimate result in silkworm breeding is judged by the excellency of characters of the parental strains that appear in F₁. The superiority of the hybrids over parental strains is undoubtedly due to variable magnitude of heterosis for the quantitative characters in silkworm and the results of present study are corroborating the findings of Gamo (1976). Several attempts were made in India by eminent silkworm breeders to identify suitable hybrid combinations (Dandin *et al.*, 2007). For this, it is necessary to test large number of hybrid combinations to identify the pure races which can be utilized for identification of the superior hybrid combinations. The wider variability observed for important economic traits in the hybrids can be attributed to the genetic diversity present with the resource material which is utilized in hybrids.

The exploitation of heterosis is an important step towards achieving desirable economic effects from the hybrids. The multiple trait evaluation of the twenty hybrid combinations revealed that, seven combinations which recorded average cumulative index values above 50 possess economic merit. Among them, the combination of MF4 x BVFC1 and ranked first followed by APMV2 x BVFC1. These observations are

in conformity with the observation of Vidyunmala *et al.*, (1998) and Ramesh Babu *et al.*, (2002)^[18] that superiority of one or a couple of characters may not reflect in the overall merit of the hybrid. Since the comprehensive merit of the hybrid over a range of traits depends on relative superiority of many individual traits, selection needs to be based on multiple traits contributing to overall silk output. The fecundity, determined on the genotype of maternal parent and environmental conditions prevailing at the time of oviposition and it is one of the fitness components reflecting on the productivity. In the present study with regard to this trait, the newly evolved strains revealed that they differ from one another in the total number of eggs laid by a single female moth. The trait, yield per 10,000 larvae by number which showed variations among the hybrid combinations can be attributed to the heterosis, influence of environmental factors and interaction of alleles. As the cocoon yield is related to pupation rate and cocoon weight, it could be increased by emphasizing on these traits. Pupation rate is an important parameter which reflects the viability of the breed and the fluctuations observed among various hybrid combinations could be partially attributed to the influence of environmental conditions and the interaction of alleles responsible for expression of the trait (Sudhakar Rao, *et al.*, 2006)^[27]. The cocoon weight is considered to reflect the vigor of the silkworm breed and similarly the cocoon shell weight showed wider variations in different combinations. Thus, the results of the present study are in agreement with the earlier works (Darlington, 1939) who pointed out the selection of resource material is a basic tool for generating desired hereditary changes in the improvement of commercial qualities. As the ultimate goal being the development of productive and qualitatively superior silkworm hybrids, it is envisaged for conducting a comprehensive hybrid test involving all the newly evolved polyvoltine strains. It has been pointed out by Toyama (1906)^[28] that the F₁ hybrids in silkworm, *Bombyx mori* L. in several aspects are superior to their pure line parents and the present results are in support with the findings of Harada, 1961; Yokoyama, 1979^[29]. It is noteworthy to point out that, most of the hybrid combinations excelled for the economic traits analyzed over their parents.

Conclusion

Based on the silkworm rearing performance and as evidenced by statistical tools, two hybrid combinations such as MF4 x BVFC1 and APMV2 x BVFC1 were shortlisted for further trials prior to their commercial exploitation.

Table 1: Mean Performance of Poly x Bivoltine hybrids of silkworm, *Bombyx mori* L.

Sl. No.	Breed	Fecundity (No.)	Hatching %	Yields/10000 Larvae		Pupation rate (%)	Cocoon assessment		
				No.	Wt. (Kg)		Cocoon Wt. (g)	Shell Wt. (g)	SR %
1	APMV2 x BV115	463	94.85	9371	16.507	96.75	1.800	0.320	17.80
2	APMV2 x BVS8	458	97.44	9794	15.972	97.33	1.765	0.315	17.83
3	APMV2 x BVFC1	483	97.87	9854	17.983	98.17	1.825	0.336	18.43
4	APMV2 x BV105	445	98.07	9652	16.361	92.74	1.694	0.306	18.04
5	MF4 x BVFC1	495	98.40	9785	19.245	98.75	1.985	0.362	18.23
6	MF4 x BV105	458	96.82	9524	16.245	95.07	1.707	0.300	17.60
7	MF4 x BVS8	461	94.78	9505	15.956	94.00	1.680	0.298	17.73
8	MF4 x BV115	446	98.41	9407	15.270	93.46	1.613	0.310	19.25
9	APMV3 x BV115	430	97.83	9627	15.325	95.49	1.602	0.285	17.77
10	APMV3 x BV105	439	94.20	9818	15.315	96.17	1.559	0.279	17.90

11	APMV3 x BVFC1	481	97.15	9835	16.082	95.29	1.618	0.282	17.44
12	APMV3 x BVS8	445	98.07	9714	15.805	96.70	1.628	0.287	17.65
13	APMV4 x BV115	432	97.21	9903	15.270	94.73	1.533	0.254	16.56
14	APMV4 x BVS8	431	95.84	9566	15.862	94.50	1.661	0.287	17.30
15	APMV4 x BVFC1	483	97.41	9863	15.996	96.44	1.619	0.299	18.48
16	APMV4 x BV105	491	94.53	9777	17.019	90.41	1.739	0.324	18.60
17	APMV5 x BV115	497	97.22	9705	16.451	94.14	1.692	0.306	18.08
18	APMV5 x BV105	486	98.24	9420	15.799	91.25	1.676	0.293	17.45
19	APMV5 x BVS8	442	97.21	9331	16.333	90.81	1.740	0.311	17.84
20	APMV5 x BVFC1	476	97.24	9691	16.861	95.23	1.740	0.323	18.56
21	PM x CSR2 (control)	464	95.37	9680	16.820	94.33	1.744	0.314	18.03
	Min	430	94.20	9331	15.270	90.41	1.533	0.254	16.56
	Maximum	497	98.41	9903	19.245	98.75	1.985	0.362	19.25
	SD	22	1.36	175	0.94	2.25	0.10	0.02	0.56
	Average	462	97	9658	16.31	94.85	1.696	0.304	17.93
	CV	5	1.40	1.81	5.78	2.38	5.95	7.521	3.13

Table 2: Evaluation index (EI) values of Poly x Bivoltine hybrids

Sl.No.	Breed	Fecundity	Hatching	Yield/10000 Larvae		Pupation rate	Cocoon assessment			Average EI
				No.	Wt.		Cocoon weight	Shell weight	SR %	
1	APMV2 x BV115	50.19	35.17	33.57	52.10	58.45	60.28	56.97	47.60	49.29
2	APMV2 x BVS8	48.10	54.19	57.77	46.44	61.04	56.78	54.49	48.19	53.37
3	APMV2 x BVFC1	59.33	57.42	61.18	67.77	64.74	62.74	63.99	58.94	62.01
4	APMV2 x BV105	42.26	58.85	49.66	50.56	40.64	49.82	50.76	51.87	49.30
5	MF4 x BVFC1	64.72	61.32	57.24	81.15	67.33	78.63	75.18	55.38	67.62
6	MF4 x BV105	48.10	49.67	42.35	49.33	50.98	51.04	48.24	44.02	47.97
7	MF4 x BVS8	49.59	34.63	41.26	46.27	46.25	48.44	47.22	46.47	45.02
8	MF4 x BV115	42.71	61.34	35.67	38.99	43.86	41.72	52.61	73.40	48.79
9	APMV3 x BV115	35.59	57.10	48.21	39.57	52.84	40.66	41.40	47.12	45.31
10	APMV3 x BV105	39.64	30.39	59.14	39.47	55.89	36.39	38.92	49.42	43.66
11	APMV3 x BVFC1	58.58	52.12	60.11	47.60	51.97	42.26	40.31	41.20	49.27
12	APMV3 x BVS8	42.11	58.87	53.20	44.66	58.25	43.25	42.56	45.02	48.49
13	APMV4 x BV115	36.42	52.54	63.96	38.98	49.50	33.80	27.93	25.58	41.09
14	APMV4 x BVS8	35.89	42.46	44.73	45.27	48.46	46.50	42.56	38.73	43.08
15	APMV4 x BVFC1	59.40	54.04	61.69	46.68	57.06	42.33	47.73	59.79	53.59
16	APMV4 x BV105	63.07	32.84	56.78	57.54	30.32	54.26	58.36	61.91	51.89
17	APMV5 x BV115	65.76	52.59	52.69	51.51	46.85	49.57	50.64	52.57	52.77
18	APMV5 x BV105	60.67	60.14	36.39	44.59	34.04	48.04	44.86	41.47	46.28
19	APMV5 x BVS8	40.91	52.53	31.29	50.26	32.07	54.34	52.68	48.43	45.32
20	APMV5 x BVFC1	56.03	52.76	51.89	55.86	51.72	54.35	58.14	61.22	55.25
21	PM x CSR2 (Control)	50.94	39.02	51.24	55.42	47.72	54.78	54.41	51.67	50.65

Table 3: Subordinate function method values of Poly x Bivoltine hybrids

Sl.No.	Breed	Fecundity	Hatching	Yield/10000 Larvae		Pupation rate	Cocoon assessment			Cumulative values
				Number	Weight		Cocoon weight	Shell weight	SR %	
1	APMV2 x BV115	0.48	0.15	0.07	0.31	0.76	0.59	0.61	0.46	3.45
2	APMV2 x BVS8	0.41	0.77	0.81	0.18	0.83	0.51	0.56	0.47	4.55
3	APMV2 x BVFC1	0.79	0.87	0.91	0.68	0.93	0.65	0.76	0.70	6.29
4	APMV2 x BV105	0.22	0.92	0.56	0.27	0.28	0.36	0.48	0.55	3.65
5	MF4 x BVFC1	0.97	1.00	0.79	1.00	1.00	1.00	1.00	0.62	7.38
6	MF4 x BV105	0.41	0.62	0.34	0.25	0.56	0.38	0.43	0.39	3.38
7	MF4 x BVS8	0.46	0.14	0.31	0.17	0.43	0.33	0.41	0.44	2.68
8	MF4 x BV115	0.24	1.00	0.13	0.00	0.37	0.18	0.52	1.00	3.43
9	APMV3 x BV115	0.00	0.86	0.52	0.01	0.61	0.15	0.29	0.45	2.89
10	APMV3 x BV105	0.13	0.00	0.85	0.01	0.69	0.06	0.23	0.50	2.48
11	APMV3 x BVFC1	0.76	0.70	0.88	0.20	0.58	0.19	0.26	0.33	3.91
12	APMV3 x BVS8	0.22	0.92	0.67	0.13	0.75	0.21	0.31	0.41	3.62
13	APMV4 x BV115	0.03	0.72	1.00	0.00	0.52	0.00	0.00	0.00	2.26
14	APMV4 x BVS8	0.01	0.39	0.41	0.15	0.49	0.28	0.31	0.27	2.32
15	APMV4 x BVFC1	0.79	0.76	0.93	0.18	0.72	0.19	0.42	0.72	4.71
16	APMV4 x BV105	0.91	0.08	0.78	0.44	0.00	0.46	0.64	0.76	4.07

17	APMV5 x BV115	1.00	0.72	0.66	0.30	0.45	0.35	0.48	0.56	4.51
18	APMV5 x BV105	0.83	0.96	0.16	0.13	0.10	0.32	0.36	0.33	3.19
19	APMV5 x BVS8	0.18	0.72	0.00	0.27	0.05	0.46	0.52	0.48	2.67
20	APMV5 x BVFC1	0.68	0.72	0.63	0.40	0.58	0.46	0.64	0.75	4.85
21	PM x CSR2 (C)	0.51	0.28	0.61	0.39	0.47	0.47	0.56	0.55	3.83

Table 4: Comparative Ranking of Poly x Bi hybrids

Sl. No.	Breed	Ranking based on	
		Evaluation Index	Subordinate Function
1	MF4 x BVFC1	1	1
2	APMV2 x BVFC1	2	2
3	APMV5 x BVFC1	3	3
4	APMV4 x BVFC1	4	4
5	APMV2 x BVS8	5	5
6	APMV5 x BV115	6	6
7	APMV4 x BV105	7	7
8	PM x CSR2 (C)	8	9
9	APMV2 x BV105	9	10
10	APMV2 x BV115	10	12
11	APMV3 x BVFC1	11	8
12	MF4 x BV115	12	13
13	APMV3 x BVS8	13	11
14	MF4 x BV105	14	14
15	APMV5 x BV105	15	15
16	APMV5 x BVS8	16	18
17	APMV3 x BV115	17	16
18	MF4 x BVS8	18	17
19	APMV3 x BV105	19	19
20	APMV4 x BVS8	20	20
21	APMV4 x BV115	21	21

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