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Breeding of Two Bivoltines, A3×935E and A3×916B of Silkworm *Bombyx Mori* L. for Higher Survival and Moderate Silk Productivity

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ABSTRACTS

Twelve pure bivoltine silkworm lines identified based on higher G.C.A values from a line×Tester crossing programme, were crossed with twelve breeds showing cocoon shell ratio>22% and hybrids prepared, evaluated in a Multiple index method. Seven hybrids showing average index value>50 in all the 10 economic characters were selected and evaluated in the laboratory. However, two hybrids viz., A3× 935E and A3×916 B were finally selected based on the superiority of the breeds in one day shorter larval duration and with higher reeling characters compared to control KA×NB4D2. The breeding procedure involved in the evolution hybrids are discussed.

Key words : Line×Tester analysis, hybrid evaluation, Silkworm, *Bombyx mori* L.

INTRODUCTION

India being in the tropical zone of Asian Continent, is characterized by fluctuating climatic condition, high temperature and scanty rainfall which are not condusive to high quality mulberry and which in turn affect the bivoltine cocoon production. Bivoltine silkworms are temperate in origin which produce quality silk, whereas most of the Indian farmers traditionally rear crossed-breed to meet the internal silk requirement (Jolly, 1983).

Such silk produced by cross-breed cocoons neither quantitatively meet the indigenous silk demand nor does it qualitatively and competitively conform to international silk grade (Thiagarajan *et al.*, 1993 b). In order to overcome these dual constraints, mass production of bivoltine cocoons is the need of the hour.

Even though rearing of bivoltine silkworms was introduced during late seventies (Krishnaswami and Tikoo, 1971), even now the bivoltine rearing is limited to a meagre 10% only, (Sengupta *et al.*, 1971; Nagaraju *et al.*, 1996). Therefore, there is an urgent need for evolving farmer-friendly, silkworm breeds which could be reared in the farmers' dwellings and which do not require stringent laboratory conditions.

Keeping in view of the farmers' constraints and country's qualitative and quantitative silk demand, an attempt was made to evolve bivoltine silkworm breeds with survival rate of more than 90%., in line with the cross-breeds but with better cocoon characters. Silkworms showing distinct characters were crossed to combine genes and isolate the genotypes of desirable gene combination for the optimum expression in economic traits. (Harada, 1949, 1961; Hirobe, 1961; Yokoyama, 1976).

MATERIALS AND METHODS

Data (last three years 1987-1990) on 10 rearing characters of 150 bivoltine breeds of silkworm, *Bombyx mori* L maintained in the germplasm collection of this institute were subjected to ranking analysis. The ranking analysis has resulted in the identification of six breeds as parents for the initiation of hybridization breeding programme. Those were : CPP1 and Hu204 (for high survival rate>90 %) TC1., JJ1 and EM for high shell weight (0.34 to 0.36 g) and EM and HUA1×A2 for high shell ratio 22-23 %).

Approach

Selection of breeds with higher adoptability and low yielding hibernating stock (Chinese, Japanese and European) as breeding resource material for improving the survival.

A total of 12 hybrid combinations prepared by crossing high survival breeds with high shell weight breeds and high survival breeds with high shell ratio breeds. Breeding initiated during April, 1991. Targets are as follows: 1) To evolve bivoltine silkworm breeds with the high survival rate of>90 % and moderate cocoon characters viz., single cocoon weight 1.4 to 1.6 g., singel shell weight 0.30 to 0.32 g and shell ratio 20 to 21% and 2) To identify hybrids with survival>90 %., single cocoon weight 1.6 to 1.8 g., single shell weight 0.35 to 0.40 g and shell ratio 21-22%.

Breeding Procedure

Eggs from 25 dfls were pooled and all the newly hatched larvae (Approximately 10,000) were reared till III ecdysis/moult. After III moult, 3 replications of 500 Larvae in each replication was retained. A replication was represented by a tray (60×90 cm). After the harvesting of cocoons, they were distributed into 3 parts (i.e) : i) one part was utilized for general assessment (n=100), ii) second part for reeling (n=200), iii) third part of the cocoons for seed preparation (n=200) Mass rearing was followed for infusion of desirable genes from F1 to F4 generation (Re-combination of genes).

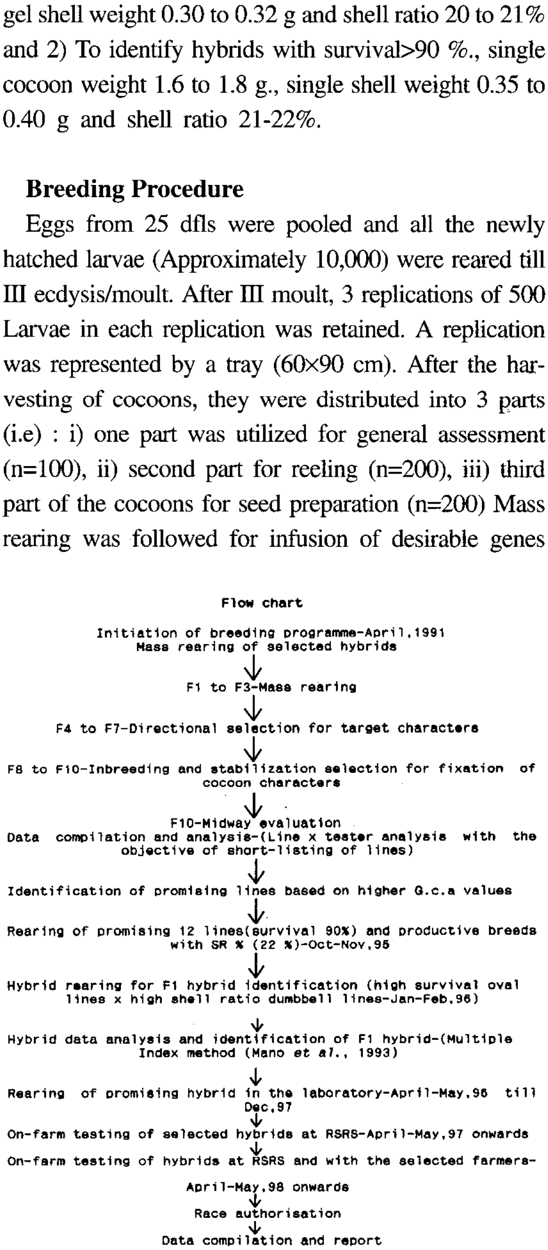

Fig. 1. *Flow chart for selection of hybrids.*

Cellular rearing was followed from F4 to F7 generation. From F5 onwards until F7, the family selection was taken up. Inbreeding coupled with stabilization. Selection for fixation of cocoon characters was followed from F8 till F10 generation. Mid-way evaluation of the evolved line using KA and NB4D2 as testers was taken at F10 generation. The hybrids were analysed in a Line×tester crossing programme. Based on higher G.c.a values, 12 lines were identified.

RESULTS

All the 24 lines were crossed with two widely adapted testers namely KA and NB4D2 as male parents. The combining ability effects of 24 new hardly lines and their F1 hybrids made in a Line×tester (Kempthorne, 1957) crossing programme was analysed for five quantitative traits of economic importance viz., cocoon yield (by number and weight) per 10,000 larvae brushed, single cocoon weight, single shell weight and shell ratio.

Based on G.c.a effects, the lines found to be superior for different traits are as follows: 931D, 935E, 934D1, 930G and 934B for cocoon yield by weight: 931D, 935E, 934D1, 930G, 934B, 916A, 916B, 912B2, 915A for cocoon yield by number: 931D, 931C, 935C, 930E, 916B, 915A for single cocoon weight: 932B, 934B, 916B for single shell weight ; 932B, 933A, 935A, 934B, 916D for shell ratio.

Similarly, the crosses identified to be superior based on specific combining ability (S.c.a) effects and their per se performance for cocoon yield, single cocoon weight, single shell weight and shell ratio are as follows: 932D×KA and 931C×NB4D2, 915B×KA, 912A×NB4D2 for cocoon yield by number; 932A×NB4D2 and 935B×NB4D2 for cocoon yield by weight; 931C×KA, 931B×KA, 916B×NB4D2, 915A×NB4D2 for single cocoon weight; 934B×NB4D2, 933A×NB4D2, 916B×KA, 916A×NB4D2 for single shell weight; 934B×NB4D2, 930A1×KA, 912D×NB4D2, 915B×NB4D2, 915D×NB4D2 for shell ratio.

Based on higher G.c.a values, 12 lines isolated from a line x tester programme (Kempthorne, 1957) showing survival>90 % were crossed with lines having high shell ratio>22 %. Oval type female parents were crossed with dumbbell type male parents and 143 F1 hybrids prepared. Hybrids along with 24 parents were reared using 3 replications in each group. Standard rearing practices were always followed (Krishnaswami, 1978). Two popular hybrids viz., Pure Mysore×NB4 D2 and KA×NB4D2 were reared along with the hybrids. The maximum-minimum rearing room temperature and the average relative humidity recorded during the experiment were 29°C-24°C and 72% respectively. The observations were recorded on the rearing and reeling characters. Those were ; 1) total larval period (hrs.), 2) V instar period (hrs.), 3) cocoon yield by number (Nos.), 4) cocoon yield by weight (kg), 5) single cocoon weight (g), 6) single shell weight (g), 7) shell ratio (%), 8) filament length (m), 9) raw silk % and 10) reelability (%).

From each hybrid combination 500 g cocoons were reeled in a Japanese multiend reeling machine at the Reeling and Fibre Technology Division of Central Sericultural Research and Training Institute, Mysore, India.

An evaluation index (e.i) was calculated for each character by the formula mentioned below :

$$e.\text{i} = \frac{A-B}{C} \times 10 + 50$$

Where A=Value of a particular hybrid
B=Mean value of all the hybrids
C=Standard deviation of all the hybrids
10=Standard unit
50=Fixed value

An Evaluation Index (E.I) is defined as the sum of all the characters in a population. The concept of 'Evaluation Index' is very popular in 'Japanese educational system' to test students merit. It was developed from variation index value method. In silkworm breeding experiments this 'Evaluation Index' was first applied by mano *et al.* (1993) and later on widely put in to practice by others (Singh *et al.*, 1993 and Thiagarajan *et al.*, 1993a).

Evaluation index values were calculated for each hybrid in all the 14 characters by following the above method. The indices obtained from all the characters in each hybrid was combined and the average E.I value was calculated (E.I). The average E.I value fixed for the selection of hybrid was 50 or>50. The hybrids which scored above the limit were considered to possess greater economic value.

Mean values and standard deviation for each trait in all the 143 F1 hybrids were calculated. For each trait the evaluation index (e.i) was calculated. The e.i. calculated for 10 traits were used in calculation of 'Evaluation Index' (E.I) for each hybrid. Different hybrids indicated higher values for different economic traits. Those were : 934A1×916B for cocoon yield by number (9483), 9147A3×CSR4 for cocoon yield by weight (18.33 kg), A3×916B for single cocoon weight (1. 815 g), 9147A3 ×NK25 for single shell weight (0.410 g), CSR13 x 916B for shell ratio (24.61 %), CSR13×935E for average filament length (1147. 90 m), 934A1×935A for denier (2.00), 933A×NK25 for reelability (95.93 %), CSR13×916B for raw silk % (19.00 %), 934B×B25 for total larval duration (535.00 hrs), CSR13×935A for V age duration (138.00 hrs.).

Although there are about 21 characters contributing to silk yield (Thiagarajan *et al.*, 1993a) but it is necessary to select hybrids with the combination of 10 economic characters contributing to silk yield studied. Therefore, evaluation index method was employed giving due importance to all the economic traits contributing to silk yield in selection of hybrids. The results of the present study showed that only 72 hybrids had E.I value>50. However, 15 hybrids indicated E.I value>50 for most of the characters studied. Those were : 1)A3×935A (scored>50 for all the characters), 2) A3× 935E (scored>50 of the all the characters), 3) A3× 916B2 (except for average filament length 48.65), 4) A3×916B (except for reelability 33.77), 5) A3×J10 (scored>50 for all the characters), 6) A25×935A (except for average filament length 46.14), 7) A25×935E (except for average filament length-36.59), 8) A25× 916A2 (except for shell Ratio % 48.45), 9) A25×B25 (except single cocoon weight and reelability 48.48 and 49.59), 10) A25×CSR4 (scored>50 for all the characters), 11) A25×NK 25 (except for single cocoon weight and average filament length 48.12 and 49.04), 12) CSR13× 916B2(scored>50 for all the characters), 13) CSR13× 916A2 (scored<50 for all the characters), 14) CSR13×

Table 1. *Rearing and reeling performance of 15 hybrids*

Hybrid	Total larval period(h)	V Instar period(h)	Y1d/ 10000 no.	larvae wt.(kg)	Single cocoon weight(g)	Single shell weight(g)	Shell ratio(%)	Average filament length(m)	Raw silk(%)	Reel ability(%)
A3×935A	600	168	9350	16.73	1.652	0.399	24.18	985.30	18.00	91.45
A3×935E	612	177	9383	17.92	1.620	0.361	22.31	1103.40	17.00	91.16
A3×916B2	624	186	9217	16.03	1.660	0.358	21.61	901.20	17.30	88.88
A3×916B	600	168	9217	16.97	1.815	0.398	21.94	1058.80	16.80	83.41
A3×J10	603	159	9067	16.90	1.635	0.345	21.08	1017.90	16.50	89.92
A25×935A	621	168	9100	15.25	1.670	0.362	21.70	878.40	16.90	89.00
A25×935E	591	156	9050	16.83	1.595	0.348	21.83	791.70	16.80	90.28
A25×916A2	624	168	9133	16.50	1.607	0.330	20.54	993.90	16.60	89.98
A25×B25	573	147	9315	16.70	1.572	0.358	22.75	1038.20	17.60	87.83
A25×CSR4	639	159	9248	16.92	1.743	0.369	21.17	995.50	16.60	89.12
A25×NK25	603	165	9266	17.00	1.570	0.332	21.11	904.70	16.20	88.85
CSR13×916B2	603	168	9050	16.47	1.692	0.375	22.14	931.30	16.40	90.16
CSR13×916A2	618	168	9200	17.40	1.642	0.381	23.16	950.00	17.20	89.15
CSR13×916A1	630	180	9067	16.57	1.635	0.382	23.35	1112.20	18.10	88.52
9147A×916B	630	180	9047	15.75	1.650	0.400	24.20	975.50	18.10	89.79
Mean	611	168	9180	16.66	1.650	0.366	22.20	975.87	17.07	89.17
SD	±16.87	9.83	112.32	0.62	0.06	0.02	1.08	83.62	0.60	1.80

Table 2. *Evaluation index values of the 15 hybrids(Rearing and reeling)*

Hybrid	V1d/10000 no.	larvae wt.	Single cocoon weight	Single shell weight	Shell ratio	Average filament length	Raw silk	Reel ability	Average E.I	Total Larval period	V Instar period
A3×935A	61.47	61.42	59.91	72.66	72.17	57.92	67.44	62.55	64.44	46.11	52.33
A3×935E	62.53	70.23	55.38	60.19	60.01	70.93	58.69	61.52	62.43	51.91	60.89
A3×916B2	57.29	56.24	61.06	59.38	55.44	48.65	61.31	53.35	56.59	57.71	69.45
A3×916B	57.29	63.16	83.41	72.33	57.59	66.02	56.94	33.77	61.31	46.11	52.33
A3×J10	52.56	62.68	57.54	55.18	52.00	61.51	54.31	57.08	56.61	47.56	43.78
A25×935A	53.62	50.43	62.57	60.68	56.03	46.14	57.81	53.78	55.13	56.26	52.33
A25×935E	52.03	62.16	51.72	56.15	56.91	36.59	56.94	58.36	53.86	41.76	43.78
A25×916A2	54.66	59.72	53.51	50.32	48.45	58.87	55.18	57.29	54.75	57.71	52.33
A25×B25	60.39	61.16	48.48	59.22	62.90	63.75	63.94	49.59	58.68	33.06	32.37
A25×CSR4	58.28	62.79	72.99	62.95	52.61	59.05	55.18	54.21	59.76	64.96	43.78
A25×NK25	58.84	63.38	48.12	50.81	52.18	49.04	51.68	53.24	53.41	47.56	49.48
CSR13×916B2	52.04	59.46	65.73	64.73	58.88	51.97	53.43	57.94	58.02	47.56	52.33
CSR13×916A2	56.77	66.38	58.47	66.67	65.55	54.03	60.44	54.32	6033	54.81	52.33
CSR13×916A1	52.56	60.20	57.47	67.15	66.80	71.90	68.31	52.06	62.06	60.61	63.74
9147A×916B	552.03	54.17	58.98	72.33	72.35	56.84	68.31	56.61	61.45	60.61	63.74

916A1 (scored>50 for all the characters), and 15) 9147 A3×916B (scored > 50 for all the characters Table 1 and 2).

Seven hybrids (Table 3 and 4) viz., 1)A3×935A, 2) A3×935E, 3) A3×916B2, 4) A3×916B, 5) A25×935A, 6) A25×935E and 7) A25×916A2 were selected and tested 6 times in the laboratory to test the stability performance. However, after short-listing only two hy-

Table 3. *Rearing performance of the selected hybrids(Average of 6 trial)*

Hybrid	Eggs/laying(no.)	Larval period(h)	V instar period(h)	Yield/10000 no.	larvae wt.(kg)	Single cocoon weight(g)	Single shell weight(g)	Shell ratio(%)
A3×935A	520.83	549.00	146.00	9069.00	14.54	1.67	0.35	21.39
	±7.97	±24.91	±10.19	±287.18	±1.11	±0.08	±0.01	±1.4
	* 4.00	-2.37	-1.52	7.37	2.72	4.32	11.51	7.21
	**12.45	-4.92	-2.74	0.22	25.98	5.31	30.06	26.05
A3×935E	508.36	545.50	146.17	9252.00	15.32	1.66	0.35	21.12
	±9.00	±34.26	±14.01	±250.06	±2.28	±0.01	±0.02	±0.99
	* 1.64	-3.02	-1.41	9.20	7.69	3.36	9.34	5.99
	**10.3	-5.59	-2.62	2.20	29.76	4.36	28.35	25.00
A3×916B2	504.42	541.33	139.00	9135.00	14.68	1.60	0.32	20.08
	±13.33	±32.27	±5.38	±650.51	±1.46	±0.01	±0.02	±0.90
	* 0.88	-3.82	-6.63	8.04	3.65	0.07	1.20	1.15
	**9.60	-6.40	-7.91	0.94	26.69	1.10	21.91	21.20
A3×916B	511.28	544.33	145.33	9588.00	16.84	1.73	0.35	20.08
	±15.88	±30.43	±10.43	±268.91	±0.73	±0.05	±0.04	±1.04
	* 2.21	-3.25	-1.99	12.38	16.03	7.62	9.05	4.75
	**10.81	-5.82	-3.21	5.62	36.11	8.57	28.12	24.09
A25×935A	513.64	557.00	138.00	9132.00	15.22	1.68	0.35	20.84
	±12.24	±39.29	±9.41	±723.68	±1.88	±0.07	±0.03	±1.04
	* 2.66	-0.90	-7.41	8.01	7.05	4.78	9.32	4.75
	**11.22	-3.41	-8.70	0.91	29.28	5.76	28.33	24.09
A25×935E	509.00	543.00	144.50	9088.00	14.93	1.68	0.34	20.25
	±18.59	±24.60	±6.58	±319.25	±1.73	±0.09	±0.01	±0.82
	* 1.77	-3.50	-2.58	7.57	5.29	4.71	6.57	1.95
	**10.41	-6.08	-3.81	0.43	27.94	5.69	26.16	21.85
A25×916A2	510.00	554.00	138.00	9060.00	15.39	1.64	0.34	20.58
	±7.34	±25.02	±8.94	±703.63	±1.25	±0.04	±0.01	±0.99
	* 1.96	-1.44	-7.41	7.27	8.09	2.64	6.30	3.53
	**10.59	-3.97	-8.70	0.12	30.07	3.64	25.94	23.11
KA×NB4D2	500.00	562.00	148.22	8401.00	14.14	1.60	0.32	19.85
	±12.51	±46.56	±20.72	±1248.19	±2.51	±0.02	±0.01	±0.36
PM×NB42	456.00	576.00	150.00	9049.00	10.76	1.58	0.25	15.82
	±26.27	±8.94	±1.41	±612.61	±2.51	±0.02	±0.02	±1.40

*Indicates percentage increase/decrease over KA×NB4D2; over **PM×B4D2

brids viz., A3×935E and A3×916B were selected for multilocational test during 1997-1998 at Regional Sericultural Research Stations.

DISCUSSION

In sericulturally advanced countries like Japan and China silkworm breeds suitable for different seasons and regions are developed which are adoptable to both seasonal and regional environments. As survival is negatively correlated with silk content, in spring season, breeds with higher silk content and yield are reared. In summer season due to high temperature, humidity and poor leaf quality, breeds with lower silk content are reared. China being a tropical country has succeeded in evolving silkworm breeds suitable for spring, summer and autumn rearings (Sohn *et al.*, 1987; Shao, 1989; He *et al.*, 1989; He *et al.*, 1990 Shao *et al.*, 1990; Xu *et al.*, 1990; He *et al.*, 1991 Chen *et al.*, 1994; Zhang, 1994; yong *et al.*, 1996). In Japan also, silkworm breeds suitable for spring, summer and autumn seasons are evolved and are commercially exploited (Kamijyo *et*

Table 4. *Reeling performance of the selected hybrids(Average of 6 trials)*

Hybrid	Double cocoon(%)	Average filament length(m)	Denier	Raw silk(%)	Reel-ability	Neat-ness(pts)
A3×935A	2.00	964.28	2.15	13.90	87.30	90.00
	±1.80	±53.42	±0.08	±1.82	±4.28	±1.91
	*0.01	4.79	-13.74	7.91	1.05	1.45
	**50.00	16.41	-17.08	14.94	1.67	0.46
A3×935E	1.00	1014.52	2.40	14.76	89.30	90.00
	±0.97	±74.56	±0.00	±1.26	±2.95	±1.11
	*7.69	9.51	-1.88	13.21	3.29	1.45
	**53.85	20.55	-4.87	19.80	3.90	0.46
A3×916B2	1.00	950.56	2.30	14.37	89.42	90.50
	±2.43	±44.10	±0.05	±1.08	±4.53	±4.23
	*-100.00	3.42	-6.24	10.85	3.38	2.00
	**0.01	15.20	-9.35	17.60	3.99	1.01
A3×916B	1.00	960.97	2.32	14.31	88.89	91.00
	±0.97	±82.63	±0.13	±1.62	±3.49	±2.62
	*-100.00	4.47	-5.09	10.47	2.80	2.53
	**0.01	16.12	-8.18	17.30	3.41	1.56
A25×935A	2.00	919.47	2.39	13.43	89.71	91.00
	±2.54	±50.19	±0.16	±1.15	±2.44	±0.89
	*0.01	0.15	-2.02	4.63	3.69	2.53
	**50.00	12.33	-5.01	11.91	4.30	1.56
A25×935E	1.00	952.16	2.31	13.99	91.08	91.42
	±6.99	±59.82	±0.05	±0.73	±5.32	±2.24
	*-100.00	3.58	-5.85	8.42	5.14	2.98
	**0.01	15.34	-8.96	15.42	5.74	2.01
A25×916A2	1.00	947.88	2.33	13.53	88.58	89.58
	±4.32	±48.88	±0.13	±1.09	±1.23	±0.45
	*-100.00	3.15	-4.78	5.31	2.46	0.99
	**0.01	14.96	-7.85	12.54	3.08	0.01
KA×NB4D2	2.00	918.06	2.44	12.81	86.40	88.69
	±1.12	±52.41	±0.10	±1.22	±3.07	±1.33
PM×NB42	1.00	806.08	2.51	11.83	85.85	89.58
	±0.47	±61.22	±0.21	±0.47	±1.65	±2.74

*Indicates percentage increase/decrease over KA×NB4D2; over **PM×B4D2

al., 1985; Nakagama *et al.*, 1985; Midorikawa *et al.*, 1988; Kato *et al.*, 1989; Shirota, 1992; Eguchi *et al.*, 1995).

In India, rearing seasons are classified as spring, summer autumn and winter based on temperature rainfall, and humidity. Silkworm rearing is carried out only in some regions in few seasons. In southern state of Karnataka, Tamil Nadu and Andhra Pradesh, where seasonal differentiation is not very much marked, silkworm rearing is carried out in all the seasons, but in severe summer, rearing are skipped off during April-May and between middle of march to middle of July in Maharastra. In Utter Pradesh and Jammu, rearings are carried out only during spring and autumn (Iyengar, 1995).

In India due to varied agro-climatic conditions, different silkworm varieties are reared in different regions. In Karnataka, where more than 90% silk is produced, Pure Mysore×Bivoltine (NB4D2/NB18) is reared in irrigated areas in all the seasons, but KA×NB4D2 during September to February. In rainfed areas, Pure Mysore×Bivoltine during August to October and Pure× C.Nichi in all the other seasons. In Tamil Nadu and Andhra Pradesh, Pure Mysore×NB4D2 is reared in all the seasons. In the hilly areas of Utter Pradesh, SH6×NB4D2, C108.C110×J112.J122 are reared during spring and autumn whereas in plains, Nistari/Daizo is reared during summer and bivoltine hybrids during spring season. Besides these, silkworm breeds suitable for tropical climate have been evolved (Haqye *et al.*, 1990; Krishna *et al.*, 1996; Maribasetty *et al.*, 1996).

In the present study two hybrids viz., A3×935E (HS P1) and A3×916B (HSP2) have been identified. Based on the laboratory evaluation the survival rate of these hybrids is comparable with PM×NB4D2. The cocoon characters are better than the control KA×NB4D2 These hybrids will be further subjected to multilocational test and evaluation with the farmers during 1997-98. After successful evaluation, these hybrids may be recommended for summer and autumn rearing.

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