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Research article

BREEDING OF NEW POTENTIAL CROSSBREED *(Bombyx mori L.)*, 'MSO3 x APS45' For TROPICAL REGIONS

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ABSTRACT: Keeping the various objectives of the industry in view, silkworm breeders are contributed significantly for the development of new strains through inbreeding coupled with appropriate selection procedures. Continuous renewal and change of existing breeds/hybrids with superior varieties and their commercialization is the need of the hour to increase silk quality and quantity. In this direction an attempt has been made at Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur, India and contributed for the development of potential cross breed, MSO3 x APS45. Twenty four hybrid combinations were prepared in Line x Tester method involving eight newly developed polyvoltine lines with the three testers. The relative merit of the hybrids over multiple traits was assessed adopting Evaluation Index method. Based on the evaluation methods and performance, the hybrid MSO3 x APS45 were adjudicated as promising and chosen for further laboratory evaluation during different seasons. The performance data obtained was further subjected for multiple trait evaluation index method and the ranks were adjudicated based on the index values. The hybrid MSO3 x APS45 performed well under diversified environmental conditions indicating their overall stability and superiority which could be exploited at field level for the benefit of the sericulture farmers.
Key words: Silkworm, breeding, crossbreed, heterosis, percent improvement, conventional method.

INTRODUCTION

The recent trend of global silk production centered mainly in tropical countries. India is the second largest producer of silk in the world next to China and more than ninety percent of the silk produced mainly by cross breeds (polyvoltine x bivoltine). To face the global competitiveness in silk production there is a need to develop more productive breeds or hybrids with quantitative and qualitative merit. The silkworm, *Bombyx mori* L. is a lepidopteran economic insect known for the production of mulberry silk which is aptly named as "*the Queen of Natural Fibers*" and has been successfully retaining its esteemed position even in the face of growing competition from other man-made fibers. Enrichment of silkworm breeds has always been one of the important factors contributing to increase in productivity in sericulture. Continuous renewal and change of existing breeds/hybrids with new superior varieties and their commercialization is the need of the hour to increase silk quality and quantity.

During the early and later part of 1960, several attempts made by Indian breeders to replace the indigenous polyvoltine races resulted in the isolation of improved races. However, these races were not exploited due to their non-acceptance in the field due to certain negative parameters. Of late, the approach of breeding new productive breeds by genetic manipulation through conventional breeding has emerged as one of the powerful tools in exploiting the commercial qualities. The systematic hybridization coupled with appropriate selection procedures have contributed to amalgamate the major economic traits of choice from selected breeds and to synthesize genotypes of desirable genetic constitution [24]. The attempts to evolve highly productive bivoltine races have met with little success [16]. Hence, prominent breeders and geneticists of Japan [14,26] stressed the importance of polyvoltine breeding in the tropical regions of India.

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 [4]. In fact, silkworm is the only exception crop where hybrids are invariably used [32].

The systematic rearing of F1 hybrids was undertaken for the first time in Japan after establishing the superiority of hybrids [27]. The final results in silkworm breeding are judged by the superiority on the commercial traits of the parental races that appear in F1. The advantages of rearing F1 hybrids are that they exhibit shorter larval duration, lower mortality, higher cocoon and shell weight and longer filament length [10]. 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.

The combining ability analysis is a tool, widely used to determine the magnitude of heterosis and to identify perspective parents or hybrids for commercial exploitation of the hybrid vigor in agriculture, animal husbandry and poultry [23, 11]. An extensive study on the combining ability of silkworm was made to identify the superior and promising hybrid combinations for commercial exploitation in polyvoltine x bivoltine hybrids [2, 29, 18, 19, 22]. However, there is further scope in the improvement of genetic traits and identification of superior hybrids through estimation of heterosis by combining ability studies. Very limited number of polyvoltine cross breeds are available with all desired traits, which are not sufficient to meet the demand. At this juncture, there is a need for the development of quantitatively and qualitatively superior polyvoltine breeds and hybrids with high genetic plasticity to cater various climatic conditions.

MATERIALS AND METHODS

Two polyvoltine silkworm breeds viz., APMG4 and APMG16 maintained at Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur constitute the parental material for the development of the breed MSO3. The breeding plan was initiated with the crossing of APMG4 and APMG16. The resultant line MSO3 was developed with qualitatively and quantitatively superior traits and subjected for hybrid testing by employing the Line x Tester analysis method along with other 7 newly evolved breeds with productively superior bivoltine breeds such as APS12, APS45 and APS8. The data pertaining to eight economic traits viz., fecundity, 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), cocoon shell ratio (%) and filament length (m) were analyzed for various hybrid combinations and the superiority of new polyvoltine hybrid MSO3 x APS45 was established when compared with other newly hybrid combinations. The merit of the each of the hybrid combinations was analyzed through multiple trait evaluation index method [13] as detailed below.

Multiple Trait Evaluation Index Method

Evaluation index over multiple traits was calculated as per the following formula which is suggested by Mano *et al*., 1993.

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

Where,

A = Value obtained for a trait in a race

B = Overall mean value of a particular trait of all the races

C = Standard deviation of a trait of all the breeds

10 = Standard unit

50 = Fixed value

Breeding and Selection Methods

Emphasis was laid to select the cocoons of intermediate size and no selection pressure was applied during F1 – F3 to retain gene pool responsible for different economic traits. Cellular rearing was carried out from F4 generation with minimum five replications. At the time of brushing, emphasis was given to high fecundity and good hatching percentage at every generation. The selected cocoons were subjected for sex separation to assess the cocoon, shell weight and cocoon shell ratio. This method of individual selection was followed to facilitate the fixation of desired economic traits at a faster rate. To select the parents at every generation, selection pressure was applied at every stage of development viz., egg, larva, pupa / cocoon and moth.

Development of Breed 'MSO3'

The breeds APMG4 (females) and APMG16 (males) were used as initial parents for this plan. The F_1 progenies were raised by brushing composite layings and mass rearing was carried up to F_3 followed by cellular rearings. The cocoons were categorized into two types *i.e.*, medium and elongated oval based on cocoon shape and breeding was continued by selecting medium oval type of cocoon. The population which showed uniformity in cocoon shape and consistent in the economic characters by F_{12} generation was considered as fixed and it was named as MSO3.

Fig. 1. Silkworm Larvae and cocoons of MSO3

Characteristics and Generation wise rearing performance of MSO3

The newly evolved polyvoltine silkworm line 'MSO3' which is characterized by bluish white larvae, plain and spinning green medium oval cocoons with medium grains. The generation wise rearing performance of the breed is as follows.

Table 1. Generation wise rearing performance of MSO3

Generation	Fecundity (No.)	Yield /10,000 Larvae		Survival Rate (%)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)
		No.	Wt.(kg)					
F_1	536	9367	14.781	92.17	1.589	0.260	16.36	701
F_2	540	9400	11.860	91.00	1.596	0.250	15.66	687
F_3	494	9333	12.325	91.42	1.495	0.241	16.12	680
F_4	505	9500	12.958	92.33	1.460	0.245	16.78	699
F_5	488	9267	13.020	93.00	1.418	0.251	17.70	729
F_6	496	9400	13.124	94.00	1.407	0.232	16.49	717
F_7	487	9300	12.379	91.03	1.397	0.237	16.96	712
F_8	489	9283	12.901	91.67	1.401	0.224	15.99	700
F_9	497	9300	12.879	92.00	1.390	0.228	16.40	695
F_{10}	480	9217	12.768	91.89	1.386	0.224	16.16	690
F_{11}	477	9167	12.623	91.10	1.379	0.227	16.46	699
F_{12}	486	9200	12.598	91.68	1.380	0.230	16.67	704
Average	498	9311	12.851	91.94	1.442	0.237	16.48	701
S.D.	20	95	0.704	0.87	0.078	0.012	0.52	13
CV (%)	4.06	1.02	5.48	0.95	5.44	5.02	3.17	1.91

Characteristics of APS_{45}

The potential bivoltine breed 'APS45' was utilized for the development of the hybrid and the larvae of the breed is characterized by plain and bluish white in colour, cocoons are white and oval in shape with medium grains. The total larval period is 22 - 23 days (Fig.3).

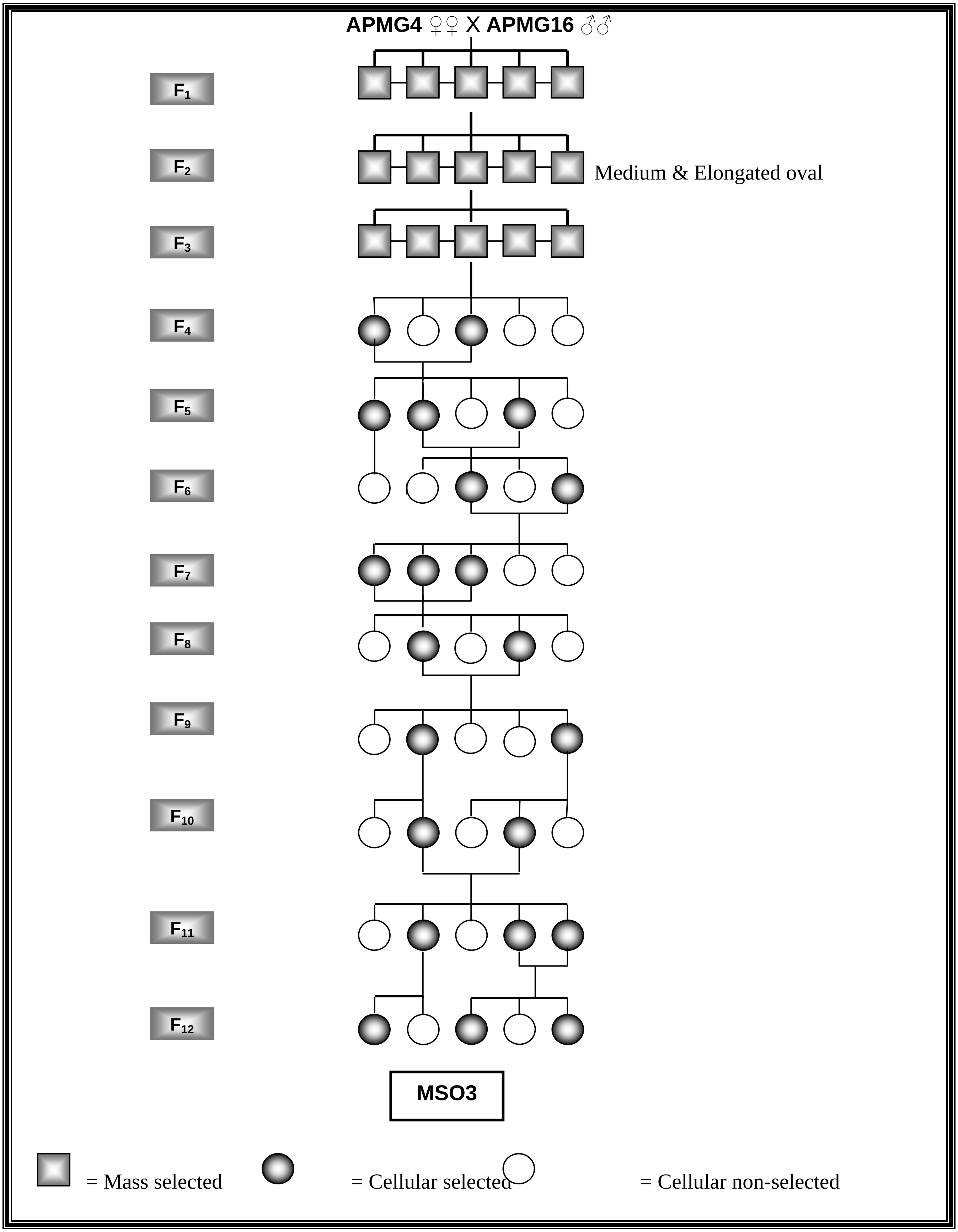

Fig.2. Breeding plan of MSO3

Fig. 3. Larvae and cocoons of APS45

Hybrid evaluation

The newly developed line 'MSO3' along with other seven evolved polyvoltine breeds *viz*., MSO1, MSO2, MSO4, MSO5, MSO6, MSO7 and MSO8 as lines and productively superior bivoltine breeds such as APS12, APS45 and APS8 as testers were subjected for hybrid evaluation study following the Line x Tester analysis method. A total of twenty four new hybrid combinations were prepared and reared in replications by following the standard rearing techniques as per the procedure suggested by [10]**.** The data pertaining the major silk contributing traits. *viz.*, fecundity, 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), cocoon shell ratio (%) and filament length (m) were recorded and the superiority of the hybrid MSO3 x APS45 was established with the average evaluation index value

Characteristics of MSO3 x APS45

The larvae are bluish white in colour and cocoons are greenish yellow with medium grains. The larval period varied between 21 – 22 days. The hybrid (Fig. 4) showed overall merit for quantitative and qualitative traits compared to the control hybrids *viz*., APM1 x APS8 and PM x CSR2.

Fig.4. Silkworm larvae and cocoons of MSO3 x APS45

$$\text{Percent improvement} = \frac{\text{(Evolved hybrid value – Control hybrid value)}}{\text{Control hybrid value}} \times 100$$

$$\text{Heterosis \% (MPH)} = \frac{\text{F1 – Mid Parent Value (MPV)}}{\text{Mid Parent Value}} \times 100$$

RESULTS

The rearing performance during breeding of the line 'MSO3' is presented in Table 1. Perusal of the data revealed that the fecundity recorded an overall mean of 498 and among the generations it was varied between 540 (F_2) and 477 (F_{11}). Further, the trait that showed variations in early (F_2 – F_5) and middle generations (F_6 – F_{10}) followed by consistency in the later generations (F_{11} to F_{12}) which indicates the fixation of the line for the trait. With regard to cocoon yield by number, it was varied between 9167 (F_{11}) to 9500 (F_4) with an average of 9311, SD of 95 and CV of 1.02 %.

The line 'MSO3' at F_1 was found with 14.781 kg and 12.623 kg at F_{12} with an overall mean of 12.851 kg for the trait cocoon yield per 10,000 larvae by weight and the maximum variability of the trait is evidenced from higher CV of 5.48 %. Over the twelve generations, the trait was ranged to the maximum of 94.00 % (F_6) and to the minimum of 91.00 % (F_2) and minimum variability of the trait is evidenced from lesser CV of 0.95 % for the trait survival rate. However for the various traits studied showed consistency in the later generations that suggests the fixation of the trait.

Hybrid Testing

Mean rearing performance data of the 24 new hybrid combinations and Evaluation index values (EI) is presented in Table 2 and the hybrid 'MSO3 x APS45' showed economic merit for the traits studied. The Percent improvement in economic characters in comparison to control hybrids and Hybrid vigour of MSO3 x APS45 is presented in the Table 3.

Table 2. Mean Performance of new hybrids

Sl. No.	Combination	Fecundity (No.)	Yield /10,000 larvae		Pupation (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio(%)	Filament length(m)	EI value
			No.	Wt.(kg)						
1	MSO1 x APS12	512	9539	17.798	94.02	1.888	0.353	18.70	937	65.50
2	MSO1 x APS45	502	9550	17.753	94.70	1.890	0.351	18.59	923	64.85
3	MSO1 x APS8	481	9122	16.195	89.56	1.788	0.323	18.08	834	50.04
4	MSO2 x APS12	469	9111	15.072	90.27	1.668	0.296	17.75	734	43.65
5	MSO2 x APS45	506	9533	17.784	94.45	1.875	0.351	18.72	953	65.28
6	MSO2 x APS8	482	9267	16.015	91.92	1.753	0.297	16.95	776	49.00
7	MSO3 x APS12	471	9383	16.855	92.73	1.833	0.337	18.38	825	55.57
8	MSO3 x APS45	516	9567	18.324	94.60	1.945	0.369	18.97	979	69.37
9	MSO3 x APS8	477	9250	16.243	90.32	1.765	0.321	18.17	805	50.54
10	MSO4 x APS12	457	9100	14.969	89.67	1.650	0.275	16.64	777	40.41
11	MSO4 x APS45	481	9222	15.354	90.88	1.676	0.277	16.51	763	44.61
12	MSO4 x APS8	487	9200	14.339	91.02	1.569	0.253	16.14	727	40.62
13	MSO5 x APS12	487	9422	15.798	93.20	1.748	0.277	15.84	701	47.95
14	MSO5 x APS45	454	9178	15.900	90.56	1.736	0.269	15.49	734	41.33
15	MSO5 x APS8	498	9211	15.983	91.12	1.742	0.284	16.32	812	48.50
16	MSO6 x APS12	494	9111	16.015	90.53	1.767	0.284	16.09	773	46.47
17	MSO6 x APS45	485	9322	15.351	92.23	1.654	0.274	16.58	759	46.33
18	MSO6 x APS8	484	9145	14.642	90.52	1.610	0.272	16.88	740	42.20
19	MSO7 x APS12	498	9189	15.676	90.73	1.714	0.279	16.30	715	45.55
20	MSO7 x APS45	474	9256	15.409	91.25	1.690	0.276	16.31	757	44.44
21	MSO7 x APS8	510	9495	17.794	94.19	1.887	0.360	19.08	926	65.42
22	MSO8 xA PS12	492	9322	15.527	92.57	1.675	0.283	16.90	761	48.32
23	MSO8 x APS45	487	9022	14.692	88.96	1.651	0.278	16.85	740	41.15
24	MSO8 xAPS8	473	9045	15.113	89.55	1.685	0.288	17.09	770	42.69
	Average	487	9273	16.025	91.65	1.744	0.301	17.22	801	
	SD	16	163	1.09	1.74	0.10	0.034	1.07	78	
	CV(%)	**3.24**	**1.75**	**6.81**	**1.90**	**5.56**	**11.12**	**6.23**	**9.79**	

Table 3. Percent improvement in economic characters of MSO3 x APS45

Combination	Fecundity (No.)	Yield /10,000 larvae		Pupation (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length(m)
		No.	Wt. (kg)					
MSO3 x APS45	516	9567	18.324	94.6	1.945	0.369	18.97	979
PM x CSR2 (Control)	491	9420	15.949	92.25	1.762	0.321	18.22	863
Percent improvement	5.09	1.56	14.89	2.55	10.39	14.95	4.12	13.44

Table 4. Hybrid vigour in MSO3 x APS45

Combination	Fecundity (No.)	Yield /10,000 larvae		Pupation (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio(%)	Filament length (m)
		No.	Wt. (kg)					
MSO3	471	9306	12.997	92.3	1.404	0.238	16.96	735
APS45	522	9247	15.989	91.1	1.712	0.378	22.10	1012
Average	**497**	**9277**	**14.493**	**91.7**	**1.558**	**0.308**	**19.53**	**874**
MSO3 x APS45	516	9567	18.324	94.60	1.945	0.369	18.97	979
MPH	**3.99**	**3.13**	**26.44**	**3.17**	**24.84**	**19.81**	**-2.86**	**12.08**

Table 5. Seasonal performance of MSO3 x APS45

Season	Fecundity	Yield / 10,000 Larvae		Survival Rate (%)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)
		No.	Wt. (Kg)				
Monsoon	495	9433	18.203	94.00	1.906	0.362	18.97
Post monsoon	516	9567	18.324	94.60	1.945	0.369	18.97
Pre monsoon	462	9300	17.004	92.33	1.847	0.344	18.63
Average	**482**	**9400**	**17.743**	**93.52**	**1.892**	**0.355**	**18.75**

The hybrid MSO3 x APS45 (69.37) with highest EI over multiple traits excelled over other combinations. Further the combination recorded higher percent improvement over its control for all the 8 traits studied with highest (14.95%) improvement was recorded for the trait shell weight. The hybrid also exhibited positive heterosis to a varying degree (Table 4) for different traits ranged to a maximum of 26.44% (Yield/10000 larvae by wt. (kg)). The performance of the hybrid in various seasons is presented in Table 5.

DISCUSSION

The silkworm breeding is the most important example where heterosis is being exploited commercially to the maximum extent. The conventional breeding methods adopted in silkworm breeding over the years have resulted in the larger number of inbred lines with distinct genotypic and phenotypic differences. Various systematic procedures have enabled the breeders to identify the best combiners and their combining ability in specific hybrid combinations. In view of this, the ultimate result in silkworm breeding is judged by the excellency of characters of the parental strains that appear in F_1. During the course of evaluation, it is essential to understand the nature of allelic differences between the pure lines as well as their phenotypic manifestation in heterozygous condition [31]. Moreover, the evaluation of general combining ability of the pure lines and the specific combining ability of the hybrids will be of utmost use in interpreting the genetic basis of phenotypic manifestation of most of the characters [5,8]. 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 [6].
It has been pointed out by [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 [9, 32]. To achieve desired goals, cross breeding is widely used in commercial animal production as a means of exploiting heterosis [1, 20]. Several attempts were made in India by eminent silkworm breeders to identify suitable hybrid combinations nevertheless there is an enormous requirement for development of productively and qualitatively superior hybrids [3]. For this, it is necessary to test large number of hybrid combinations to identify the pure races which can be utilized for ultimate identification of the superior hybrid combinations. In view of the above, the present investigation was made to identify the superior hybrid combinations by utilizing the eight newly evolved polyvoltine lines and three productively superior bivoltine breeds as testers by employing Line x Tester method.

The parental lines possessing high general combining ability (gca) are preferred for population development and for initiation of pedigree breeding as it is heritable which can be fixed. The wider variability observed for important economic traits in the hybrids can be attributed to the genetic diversity present among the newly developed lines utilized in hybrids. The magnitude of specific combining ability (sca) is an important aspect to be evaluated in the hybrids to estimate their superiority. In the present investigation the sca effects varied considerably among F_1 hybrids. The identification of superior hybrids based on sca effects are in agreement with the works of [7, 12, 25].

The exploitation of heterosis is an important step towards achieving desirable economic effects from the hybrids. The combination MSO3 x APS45 recorded highly significant desirable mid parent and better parent heterosis and the present findings are in agreement with [15]. The hybrids derived in the present study showed positive heterosis for most of the traits and overdominance for only some traits which corroborate the findings of [16,21] which is ascribed to the allelic interaction.

The multiple trait evaluation of the twenty four hybrid combinations revealed that, eight combinations which recorded average cumulative index values above 50 possess economic merit. Among them, the combination MSO3 x APS45 ranked first followed by MSO1 x APS12. These observations confirm the established fact as observed by [17, 30] that superiority of one or a couple of characters may not reflect 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. Based on the performance, heterosis, overdominance and evaluation index, the hybrid 'MSO3 x APS45' is adjudicated as most promising for commercial exploitation.

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