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Evaluation and development of bivoltine double hybrids of silkworm *Bombyx mori l.* for commercial exploitation

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Abstract In India, of late much emphasis is being given for bivoltine silkworm rearing to boost up the quality silk production matching international standards. Indian sericulture is dominated by the usage of only one or two bivoltine hybrids, either double/single, that are developed for usage under optimal conditions particular for favourable seasons only. There is a requirement for the development of silkworm double hybrids for commercial exploitation and therefore, attempts are being made to develop bivoltine silkworm hybrids. Earlier on these lines had resulted in the development of bivoltine hybrids and these hybrids have shown minimum impact on the Indian sericulture industry. Keeping this in view, an attempt has been made to develop bivoltine double hybrids tolerant to high temperature with disease resistance. After the screening for high temperature and disease tolerance, 10 promising stocks, five each of oval (APS67, APDR105, APS27, APSHTO5 & AP71) and peanut (APS12C, APS50, APS20, CTIPP & AP72), were selected as breeding resource material and a total of 20 oval foundation crosses and 20 peanut foundation crosses were prepared and reared. Out of which, 7 oval and 8 peanut FC's were short listed and a total of 56 double hybrid combinations (Oval x Peanut type) were prepared and evaluated. During rearing, the important parameters viz., fecundity, yield / 10000 larvae by weight, pupation %, single cocoon weight, shell weight and shell ratio (SR %) measured. Two single hybrids, such as APS45 x APS12 and CSR2 x CSR4 and one double hybrid (CSR6 x CSR26) x (CSR2 x CSR27) were kept as control. The superiority of the hybrids was assessed through evaluation index method and heterosis is also calculated. The study identified two promising bivoltine double hybrids of (FC 25 x 90) and (FC 42 x 96) for large scale laboratory trials.

Keywords Bivoltine, Bombyx mori, Double hybrid, Disease resistance, High temperature

Introduction

Bivoltine silk production in India had not reached most of the farmers despite best efforts made by Scientists and extension officials. The major constraints for the popularization of bivoltine silkworm hybrids in India are their inconsistency in cocoon yield and not being suitable for adverse climatic conditions. In India, of late much emphasis is being given for bivoltine silkworm rearing to boost up the quality silk production matching international standards. Exploitation of heterosis through single hybrids in silkworms for economic traits triggered a revolutionary change in overall qualitative and quantitative silk output [1-3]. Maintenance of pure breeds at P1 seed farmers is very important and the failure of one breed affects the production of single hybrid seed. It is well known that survival and fecundity are affected greatly with increase in quantitative traits beyond the threshold level. Although survival could be maintained in single hybrids, they are handicapped by less number of eggs laid by parental races. The works of various scientists [2, 4] demonstrated the superiority of single, three way and double hybrids over their parental races.

The double hybrids have clear advantages like easy rearing, better growth and vigour, and yield on par or better than single hybrids [5] and hence the double hybrids could be popularized in India. The polygenic expression in double hybrids is more stable than single crossed hybrids in unfavourable environment due to their flexibility in gene constitution within the population. The increase in egg number is possible only with foundation crosses and in addition, the foundation crosses are easy for rearing at P1 seed farmers and produces quality seed cocoons with high pupation rate than single parents. By utilizing this advantage, all the sericulturally advanced countries like China and Japan have successfully exploited the double hybrid concept commercially and more than 50% of the silk production in China is attributed to double hybrids. For popularization of bivoltine sericulture as in China and Japan, it is imperative to utilize silkworm parental breeds that are tolerant to silkworm diseases and high temperature for synthesizing silkworm double hybrids. The utilization of highly productive bivoltine breeds for the development of single/double hybrids hampered the popularization of bivoltine sericulture in India. Several silkworm hybrids developed for optimal conditions in favourable seasons did not perform up to the level of expectation and failed under adverse conditions. In a single hybrid, hybrid vigour between two genetically distant parents has the potential for better productivity, but the double hybrids involving four parental breeds of various qualitative characteristics (high temperature tolerance, disease tolerance, high productivity) ensure the sustainability and in turn better financial returns to the farmer. The flexibility in genetic constitution within the population and polygenic expression of several economic characteristics in silkworm double hybrids is more stable than the single hybrids under sub-optimal conditions. The development of sustainable silkworm double hybrids characterized for disease tolerance and high temperature tolerance would provide better opportunities for the popularization of bivoltine sericulture in India.

In a tropical country like India, for the introduction of bivoltine silkworm breeds it is necessary that the hybrid to have stability in cocoon yield under high temperature and not-so-perfect hygienic environment. The hybrids are usually selected after the hybridization and evaluation for quantitative characteristics alone under optimal conditions. The prolonged and fluctuating hot climatic conditions are not conducive to rear high yielding bivoltine hybrids because of their sensitivity to high temperature and silkworm diseases. This situation warrants reorientation in the approach in identifying silkworm double hybrids which are suitable for favourable (August to February) conditions with emphasis on qualitative and quantitative characteristics and better survival rate. Keeping the facts in view, an attempt has been made at APSSRDI to develop bivoltine double hybrids their viability at field level.

Material and Methods

Thirty eight productive silkworm genetic stocks from the germplasm repository of APSSRDI were screened for their resistance/tolerance to high temperature (36 ºC) and silkworm viruses *viz.*, *Bm*NPV, *Bm*DNV1 and *Bm*IFV. Based on the screening results, 10 promising stocks, five each of oval (APS67, APDR105, APS27, APSHTO5 & AP71) and peanut (APS12C, APS50, APS20, CTIPP & AP72) were selected as breeding resource material and by utilizing these breeds, a total of 100 combinations were prepared in diallel pattern. In the present paper short listing of foundation crosses (FC) and their double hybrids is discussed.

A total of 20 oval foundation crosses and 20 peanut foundation crosses were prepared and reared. Out of these FC combinations, 7 oval (FC2, FC25, FC32, FC33, FC41, FC42 and FC44) and 8 peanut FC's (FC57, FC59, FC69, FC77, FC79, FC90, FC96 and FC98) were short listed and a total of 56 double hybrid combinations (Oval x Peanut type) were prepared.

During rearing, the important parameters like fecundity, yield / 10000 larvae by weight, pupation %, single cocoon weight, shell weight and shell ratio (SR%) were measured. The performance of the newly developed double hybrid combinations were evaluated by subjecting data for evaluation index [6] method. Two single hybrids such as APS45 x APS12 and CSR2 x CSR4 and one double hybrid (CSR6 x CSR26) x (CSR2 x CSR27) were kept as control.

Results and Discussion

The mean rearing performance pertaining to economic traits of the 56 double hybrid combinations are presented in Table 1**.** Perusal of the data revealed that maximum variation was observed among all the traits of the hybrids such as fecundity which ranged from 514 eggs/laying (FC 2 x 77) to 680 eggs/laying (FC 41 x 59), yield per 10000 larvae by weight ranged from 14.32 kg (FC 25 x 98) to 18.52 kg (FC 33 x 77), pupation rate ranged from 79.50% (FC 33 x 69) to 98.70% (FC 42 x 59), cocoon weight ranged from 1.518 g (FC 25 x 69) to the 2.018 g (FC 25 x 90), shell weight ranged from 0.307 g (FC 25 x 96) to the 0.438 g (FC 42 x 96) where as shell ratio ranged from 18.60% (FC 32 x 98) to 24.20% (FC 41 x 96). Based on the performance and evaluation index (EI) values a total of 15 hybrids were short listed and in the subsequent trials two double hybrids were short listed for further laboratory trials.

Table 1: Rearing performance of bivoltine double hybrid combinations

S. No.	Combination	Fecundity (No.)	Yield/10000 L by wt.	Pupation (%)	SC Wt. (g)	SS Wt. (g)	SR (%)	EI
1	FC42 x 96	597	16.120	93.60	2.011	0.438	21.78	**59.20**
2	FC 25 x 90	624	16.190	94.40	2.018	0.413	20.44	**57.86**
3	FC 32 x 69	577	18.100	95.90	1.910	0.394	20.60	**57.39**
4	FC 2 x 79	622	17.840	93.20	1.786	0.405	22.70	**57.39**
5	FC44 x 90	522	18.220	93.60	1.906	0.417	21.90	**57.34**
6	FC41 x 96	590	16.420	90.20	1.700	0.412	24.20	**57.14**
7	FC42 x 57	568	16.740	94.40	1.769	0.411	23.30	**56.93**
8	FC44 x 96	639	17.860	92.70	1.839	0.407	22.10	**56.88**
9	FC42 x 59	624	17.200	98.70	1.818	0.409	22.50	**56.87**
10	FC32 x 77	654	17.100	93.40	1.746	0.392	22.40	**56.45**
11	FC41 x 90	630	16.330	89.40	1.750	0.398	22.70	**56.32**
12	FC41 x 59	680	16.980	91.50	1.759	0.373	21.20	**55.98**
13	FC 33 x 77	608	18.520	94.70	1.782	0.380	21.30	**55.90**
14	FC44 x 79	582	17.480	94.70	1.884	0.405	21.50	**55.83**
15	FC 33 x 57	588	17.950	92.10	1.824	0.387	21.20	**55.70**
16	FC 42 x 77	555	17.560	95.90	1.877	0.402	21.42	**54.93**
17	FC41 x 57	610	17.560	93.80	1.830	0.394	21.50	**54.43**
18	FC 2 x 90	589	17.320	90.50	1.768	0.393	22.20	**54.25**
19	FC42 x 79	598	16.570	94.70	1.758	0.370	21.10	**54.16**
20	FC44 x 69	568	16.820	88.70	1.748	0.412	23.60	**53.59**
21	FC 2 x 57	582	16.700	89.40	1.888	0.395	20.90	**52.88**
22	FC 2 x 69	624	17.000	90.40	1.741	0.359	20.60	**52.81**
23	FC41 x 98	572	16.810	94.10	1.744	0.362	20.80	**52.53**
24	FC41 x 79	565	17.230	89.60	1.875	0.402	21.40	**51.78**
25	FC42 x 98	536	16.210	90.90	1.720	0.384	22.30	**51.69**
26	FC44 x 57	517	15.810	88.50	1.744	0.412	23.60	**51.65**
27	FC 2 x 59	621	16.500	84.70	1.718	0.364	21.20	**50.44**
28	FC42 x 90	557	16.080	95.90	1.743	0.372	21.30	**50.41**
29	FC44 x 98	567	16.480	90.40	1.794	0.371	20.70	**49.69**
30	FC 2 x 96	542	17.200	90.00	1.833	0.362	19.70	**49.68**
31	FC44 x 77	527	17.140	86.40	1.871	0.379	20.30	**49.31**
32	FC32 x 98	581	17.100	91.50	1.838	0.342	18.60	**49.00**
33	FC33 x 90	598	16.100	89.50	1.737	0.352	20.30	**48.32**
34	FC32 x 59	598	15.460	90.40	1.679	0.348	20.70	**48.19**
35	FC32 x 96	591	16.520	84.70	1.753	0.345	19.70	**47.45**

36	FC42 x 69	547	16.240	95.20	1.677	0.361	21.60	**47.19**
37	FC41 x 77	578	17.100	84.40	1.748	0.370	21.20	**47.11**
38	FC 25 x 57	568	15.240	89.70	1.646	0.355	21.60	**47.08**
39	FC 32 x 57	574	16.890	91.20	1.864	0.364	19.50	**47.05**
40	FC33 x 79	571	15.740	85.70	1.747	0.359	20.60	**46.86**
41	FC 2 x 98	578	15.940	86.70	1.783	0.367	20.60	**46.71**
42	FC41 x 69	547	16.100	81.60	1.773	0.373	21.00	**46.28**
43	FC44 x 59	534	15.990	86.40	1.794	0.387	21.60	**46.10**
44	FC33 x 98	615	15.240	86.10	1.659	0.345	20.80	**46.06**
S. No.	**Combination**	**Fecundity (No.)**	**Yield/10000 L by wt.**	**Pupation (%)**	**SC Wt. (g)**	**SS Wt. (g)**	**SR (%)**	**EI**
48	FC 2 x 77	514	15.900	85.70	1.700	0.364	21.40	**43.73**
49	FC 32 x 79	547	14.560	92.60	1.568	0.326	20.80	**43.00**
50	FC 25 x 79	564	15.110	91.60	1.602	0.322	20.10	**42.20**
51	FC33 x 59	567	14.510	80.50	1.552	0.345	22.30	**40.47**
52	FC 33 x 69	597	14.680	79.50	1.652	0.332	20.10	**40.06**
53	FC 25 x 69	587	14.920	88.70	1.518	0.314	20.70	**39.89**
54	FC 25 x 98	534	14.320	87.90	1.552	0.323	20.80	**39.71**
55	FC 25 x 96	574	14.520	86.70	1.558	0.307	19.70	**38.70**
56	FC 25 x 77	555	15.450	88.90	1.637	0.317	19.40	**36.71**
	Min	**514**	**14.32**	**79.50**	**1.518**	**0.307**	**18.60**	
	Max	**680**	**18.52**	**98.70**	**2.018**	**0.438**	**24.20**	
	Mean	**580**	**16.40**	**90.01**	**1.753**	**0.372**	**21.20**	
	SD	**34.80**	**1.03**	**4.31**	**0.109**	**0.031**	**1.13**	
	CV	**6.00**	**6.26**	**4.79**	**6.218**	**8.323**	**5.35**	

The heterosis (%) in double hybrids is presented in Table 2. The data reveals that heterosis percentage in double hybrid-1 (FC 25 x 90) ranged between 2.51 for shell ratio and 20.14 for yield per 10000 larvae by weight against their FC parents. Where as in the double hybrid - 2 (FC 42 x 96), the heterosis percentage ranged from a minimum of 4.30 for yield per 10000 larvae by number to a maximum of 27.10 in yield per 10000 larvae by weight against their FC parents.

Table 2: Heterosis in Double Hybrids

Heterosis in Double Hybrid-1 (FC 25 x FC 90)							
Breed / FC / DH	**Fecun-dity (No.)**	**Yield/10000 L (No.)**	**Yield/10000 L (wt.)**	**Pupation (%)**	**SC Wt. (g)**	**SS Wt. (g)**	**SR (%)**
HTO2	490	9283	14.500	88.00	1.632	0.328	20.10
APDR105	487	8867	14.000	90.50	1.640	0.333	20.30
Avg	**489**	**9075**	**14.250**	**89.25**	**1.636**	**0.331**	**20.20**
CT1PP	512	9250	14.400	97.00	1.508	0.311	20.62
APS12C	504	9083	12.956	89.00	1.530	0.315	20.59
Avg	**508**	**9167**	**13.678**	**93.00**	**1.519**	**0.313**	**20.61**
FC25	499	9480	15.900	92.80	1.856	0.378	20.37
FC90	592	9365	16.230	93.90	1.795	0.376	20.95
Avg	545.5	9423	16.065	93.35	1.826	0.377	20.66
DH (25x90)	627	9863	19.300	96.60	2.078	0.44	21.17

Heterosis % over oval parents in FC	**2.15**	**4.46**	**11.58**	**3.98**	**13.45**	**14.37**	**0.82**
Heterosis % over peanut parents in FC	**16.54**	**2.17**	**18.66**	**0.97**	**18.17**	**20.13**	**1.66**
Heterosis % over FC in DH	**14.94**	**4.67**	**20.14**	**3.48**	**13.83**	**16.71**	**2.51**
Heterosis in Double Hybrid-2 (FC 42 x FC 96)							
APDR105	487	8867	14.000	90.50	1.562	0.321	20.55
871PO	494	8322	13.700	83.60	1.523	0.319	20.95
Avg	491	8595	13.850	87.05	1.543	0.320	20.75
APS12C	521	9283	13.560	89.00	1.530	0.321	20.98
4KINSHU	512	8967	14.100	88.00	1.517	0.304	20.04
Avg	517	9125	13.830	88.50	1.524	0.313	20.51
FC 42	553	9450	15.200	92.80	1.758	0.366	20.82
FC 96	612	9434	15.800	90.10	1.785	0.381	21.34
Avg	583	9442	15.500	91.45	1.772	0.374	21.08
DH (42x96)	630	9848	19.700	98.20	2.010	0.452	22.49
Heterosis % over oval parents in FC	**12.74**	**9.95**	**9.75**	**6.61**	**13.97**	**14.38**	**0.34**
Heterosis % over peanut parents in FC	**18.49**	**3.39**	**14.24**	**1.81**	**17.16**	**21.92**	**4.07**
Heterosis % over FC in DH	**8.15**	**4.30**	**27.10**	**7.38**	**13.46**	**21.02**	**6.67**

Improvement of short listed double hybrid - 1 ((FC 25 x 90), double hybrids - 2 (FC 42 x 96)) against single and double hybrids is presented in Table 3. The data reveals that the improvement in double hybrid -1 against single hybrid (APS45 x APS12), is up to a maximum of 22.46 % for the trait fecundity where as against CSR2 x CSR4, 16.98 % improvement was noticed for this trait fecundity followed by 14.29 % for pupation rate. Against the double hybrid (CSR (6x26) x (2x27)) for the trait single cocoon weight, 3.64 % improvement was recorded followed by 2.55% in pupation rate. With regard to double hybrids - 2, the single hybrid (APS45 x APS12) reveals that maximum of 23.05 % was recorded for the trait fecundity where as against CSR2 x CSR4, 17.54 % improvement was noticed for the trait fecundity followed by 16.19 % for pupation rate. Against the double hybrid (CSR (6x26) x (2x27)), 4.25% improvement was recorded for the trait Pupation followed by 3.56% in yield per 10000 larvae by weight.

Silkworm cocoon production depends on silkworm breed, rearing environment, mulberry quality and disease management during rearing. Widespread utilization of hybrids towards achieving sustainability and quality oriented increased production is well established in plants and animals. Silkworm is best exemplified insect where hybrids are invariably used for commercial silk production [7]. Realizing the significant impact of silkworm hybrids for increased quantitative and qualitative productivity of silk besides crop stability on commercial scale, many silkworm breeders in the sericultural countries succeeded in the development of bivoltine silkworm hybrids by exploiting the hybrid vigour that reflected in the improvement of several qualitative and economic traits [2, 8-10]. Successful silkworm breeding efforts also contributed in the evolution of many productive and qualitatively superior bivoltine hybrids for commercial exploitation in India during the last decade [11-19]. The double hybrids have been commercially exploited in advanced sericultural countries like China, Japan, Brazil and South Korea. Currently in China the double hybrids under use are Sfp.rb x 13a.137 and Sfp.137 x Rb.13a [17]. The advantages of silkworm double hybrids for silk productivity and their successful commercial exploitation in Japan and China enthused Indian R&D efforts to develop the double hybrids [12].

The results clearly indicate that major differences were observed between the pure breeds and foundation crosses with respect to fecundity and pupation. Further it is clear that foundation crosses are more robust than their parents as evidenced by improvement in fecundity, pupation rate, cocoon yield etc. Besides, the farmers can easily handle the foundation crosses for production of seed cocoons. In silkworms, it is well known that selection for one character is found to result in correlated changes in the other quantitative characters of economic importance [20]. With increase in quantitative traits beyond certain level, pupation rate and fecundity are affected greatly. Although pupation rate could be maintained in single cross hybrids, they are poor in egg number. Unless the mother is a hybrid, the fecundity cannot be increased [21]. Moreover, the cost of maintaining pure breeds especially in strains with low fecundity and pupation rate to supply parental stocks for preparation of single hybrids can be high. This necessitated the development of double hybrids possessing genetic material from four parental strains for obtaining sustainable cocoon yield commercially and in the process it is that the FCs are superior over their pure strains especially with reference to pupation rate and fecundity. The double hybrids have proved their merit in for easy rearing and in turn production of seed cocoons with high pupation rate paving way for high egg recovery and in quality seed production.

Table 3: Improvement of short listed double hybrids against control hybrids

Sl. No.	Hybrids / DH	Fecun-dity (No.)	Yield (No.)	Yield/10000 L by wt.	Pupation (%)	SC Wt. (g)	SS Wt. (g)	SR (%)
DH 1	FC 25 x FC 90	627	9863	19.30	96.60	2.078	0.440	21.17
DH 2	FC 42 x FC 96	630	9848	19.70	98.20	2.010	0.452	22.49
C1	APS45 x APS12	512	9240	17.89	89.25	1.889	0.415	21.97
C2	CSR2 x CSR4	536	8925	18.95	84.52	1.980	0.427	21.57
C3	CSR (6x26)x(2x27)	625	9625	19.02	94.20	2.005	0.445	22.20
Improvement in DH1 vs. Control								
1	DH1 vs. C1	22.46	6.74	7.88	8.24	10.01	6.02	-3.62
2	DH1 vs. C2	16.98	10.51	1.85	14.29	4.95	3.04	-1.82
3	DH1 vs. C3	0.32	2.47	1.46	2.55	3.64	-1.17	-4.64
Improvement in DH2 vs. Control								
1	DH2 vs. C1	23.05	6.58	10.12	10.03	6.41	8.92	2.36
2	DH2 vs. C2	17.54	10.34	3.96	16.19	1.52	5.85	4.27
3	DH2 vs. C3	0.80	2.32	3.56	4.25	0.25	1.53	1.27

Conclusion

In the present paper two double hybrids *viz*., FC (25 x 90) and FC (42 x 96) were identified based on their overall superiority and evaluation index values for large scale laboratory trials for further commercial exploitation.

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