DEVELOPMENT OF A POLYVOLTINE BREED OF THE MULBERRY SILKWORM, *BOMBYX MORI* L. BY MEANS OF DISPERMIC ANDROGENESIS

Ravindra Singh*, R. Nirupama and Yumnam Debaraj***

*Regional Sericultural Research Station, Jorhat - 785 005, INDIA. E-mail: ravin_sin@yahoo.com
**Central Sericultural Research and Training Institute, Mysore - 570 008, INDIA.

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ABSTRACT: A polyvoltine silkworm breed "AGL_3" was evolved by using dispermic androgenesis. The breed was developed by crossing F_2 males derived from a polyvoltine hybrid $BL_{68} \times BL_{69}$ with another polyvoltine race "Nistari" and exposing the eggs at 38 °C for 200 minutes. Androgenetic larvae expressed paternal characters. Repeated backcrosses were adopted with androgenetic males for several generations. The line was evaluated utilizing five productive bivoltine silkworm breeds. Laboratory evaluation showed superiority of the hybrid $AGL_3 \times CSR_2$ in terms of higher fecundity, pupation rate, yield/10,000 larvae by weight, cocoon shell weight, cocoon shell percentage, filament length and neatness. The hybrid exhibited maximum average evaluation index value and manifested high hybrid vigour for several characters. The possibility of application of androgenesis as a breeding tool in silkworm breeding for the evolution of superior silkworm breeds and hybrids has been discussed.

KEY WORDS: Androgenesis, *Bombyx mori* L., Development, Polyvoltine breed.

Induction of androgenetic development in the silkworm, *Bombyx mori* L. has been carried out by means of several agents such as ultraviolet light (Strunnikov, 1983), gamma rays (Xu et al., 1988; 1997), CO_2 and dry ice (Tazima & Onuma, 1967; Li et al., 1988), low temperature (Sugai et al., 1987) and high temperature (Ravindra Singh et al., 1991). Ravindra Singh et al. (2009a) have devised an improved method of androgenetic development in the eggs of the silkworm. Attempts have been made to explore the possibility of developing silkworm breeds through application of androgenesis (Strunnikov et al., 1982; Malinova et al., 1996, Xu et al., 1997; Nacheva et al., 1999). Recently, Ravindra Singh et al. (2009b; 2010) have developed some polyvoltine breeds of the mulberry silkworm, *Bombyx mori* L. utilizing androgenesis as a breeding tool. Hybrid vigour has been reported to a greater extent due to the homozygous nature of the breed involved (Strunnikov, 1986; Nacheva et al., 1999). In the present study, an attempt was made to explore the possibility of application of androgenesis in the evolution of superior silkworm breed to raise hybrids of high viability, vigour, cocoon yield and better silk quality.

MATERIALS AND METHODS

Androgenetic development in the eggs of the silkworm was induced as per Strunnikov (1983). Nistari, an indigenous polyvoltine race of West Bengal which gives marked larvae and golden yellow spindle shaped cocoons and two evolved polyvoltine silkworm breeds BL_{68} and BL_{69} which give plain larvae and greenish yellow cocoons, were utilized in this study. F_2 males obtained from the polyvoltine hybrid BL_{68} x BL_{69} were crossed with Nistari females. Moths were allowed to mate for 3 h at 25 °C and after depairing, females were kept at 5 °C for 48 h to synchronize the age of the egg. Oviposition was carried out in dark condition for 50 – 60 minutes. Then the eggs were exposed to hot air at 38 °C for 200 minutes and transferred to 15 °C for 72 h. Eggs were incubated at 25 °C and 75 % relative humidity till hatching. Androgenetic development of eggs was identified with the appearance of reddish-brown dark pigmentation in the serosa cells. The polyvoltine silkworm line "AGL_3" was developed by a series of backcrosses with androgenetic males. The breeding plan of AGL_3 has been depicted in Fig. I. The breed was evaluated utilizing five productive bivoltine silkworm breeds *viz.*, CSR_2, CSR_3, CSR_4, CSR_{12} and NB_4D_2. The promising polyvoltine × bivoltine hybrid AGL_3 × CSR_2 was evaluated along with the control PM × CSR_2 in the laboratory as well as in Technology Validation and Demonstration Centre (TVDC) of CSRTI, Mysore. Evaluation of the developed hybrids has been carried out through multiple traits evaluation index method of Mano *et al.* (1993). Evaluation Index (E. I.) for different characters was calculated using the following formula

$$E. I. = A – B / C × 10 + 50$$

Where,
A = value obtained for a particular hybrid combination.
B = mean value of particular trait of all the hybrid combinations.
C = standard deviation of particular trait of all the hybrid combinations.
10 = standard unit.
50 = fixed value.

Heterosis over mid parent (MPH) and better parent (BPH) was calculated by using the following formulae

$$MPH = 100 \times (F_1 – MPV) / MPV$$

$$BPH = 100 \times (F_1 – BPV) / BPV$$

Where, MPV = mid parent value
BPV = better parent value

RESULTS AND DISCUSSION

Performance of AGL_3 during breeding process: Generation-wise performance of AGL_3 for thirteen characters namely, fecundity, hatching, total larval span, pupation rate, yield / 10,000 larvae by weight, cocoon weight, cocoon shell weight, cocoon shell percentage, filament length, filament size, reelability, raw silk percentage and neatness has been presented in Table I. Fecundity ranged from 474 (F_9) to F_2 (521). Maximum pupation rate was observed at F_4 (95.20 %). Cocoon yield / 10,000 larvae by weight ranged from 1.511 (F_8) to 12.634 kg (F_2). Higher cocoon weight was observed at F_4 (1.438 g) whereas it was minimum at F_8 (1.142 g). Cocoon shell weight ranged from 0.193 (F_8) to 0.262 g (F_4).) Cocoon shell percentage (18.46 %) was recorded maximum at F_1. Filament length ranged from 525 (F_8) to 785 m (F_4). Maximum reelability was observed at F_2 (83.0 %). Raw silk percentage ranged from 11.6 (F_2) to 13.9 % (F_9). Maximum neatness of 90 p was observed from F_8 onwards. To know stability of the breeding line, data pertaining to different economic characters at later generations from F_{10} – F_{12}

were statistically analysed and CV % was calculated. The line was stabilized as the CV % ranged below the level of 10 % for all the characters.

Performance of polyvoltine breed “AGL_3” with bivoltine breeds in the laboratory: The performance of AGL_3 × bivoltine hybrids along with evaluation index values is given in Table II. Two hybrids *viz*., AGL_3 × CSR_4 and AGL_3 × CSR_{12} have recorded a fecundity of 505 and 513, respectively. The survival rate was observed maximum in PM × CSR_2 (97.60%) followed by AGL_3 × CSR_3 and AGL_3 × CSR_{12} (97.47 %). The cocoon yield/ 10,000 larvae by weight was recorded maximum (18.322 kg) in AGL_3 × CSR_2 followed by PM × CSR_2 (16.917 kg). Maximum cocoon weight (1.928 g), cocoon shell weight (0.412 g), cocoon shell percentage (21.38 %) filament length (870 m), reelabilty percentage (82 %), raw silk percentage (16.2 %) and neatness (91 p) were recorded in AGL_3 × CSR_2.

The hybrid AGL_3 × CSR_2 exhibited higher evaluation index values of above 50 for nine characters namely, hatching (75.00), cocoon yield / 10,000 larvae by weight (68.48), cocoon weight (60.50), cocoon shell weight (69.33), cocoon shell percentage (61.27), filament length (69.13), reelability (61.62), raw silk percentage (64.57) and neatness (66.90). The hybrid ranked first on the basis of highest average evaluation index value of 63.85 followed by AGL_3 × CSR_3 (52.90) which recorded higher index value for ten characters.

Manifestation of hybrid vigour over mid and better parent values was estimated in six polyvoltine × bivoltine hybrids along with the control PM × CSR_2 (Table III). Positive and significant heterosis over mid parent value for survival was observed in all the six hybrids except AGL_3 × CSR_4. All the six hybrids have manifested positive and significant hybrid vigour over mid parent values for six characters namely, yield/10,000 larvae by weight, cocoon weight, cocoon shell weight cocoon shell percentage, filament length and raw silk percentage. AGL_3 × CSR_2 exhibited maximum hybrid vigour over better parent value for four characters *viz*., yield/10,000 larvae by weight (26.53), cocoon weight (16.19), cocoon shell weight (15.72) and filament length (7.94).

The hybrid AGL_3 × CSR_2 was evaluated on large scale in TVDC of CSRTI, Mysore along with the control PM × CSR_2. The average performance of 3 trials is given in Table IV. The new hybrid recorded maximum per cent gain over for cocoon rate/kg (20.81 %) followed by filament length (16.70 %) and cocoon shell weight (14.46 %). The hybrid was also evaluated with few farmers in a limited scale. The performance of the hybrid along with the control PM × CSR_2 is given in Table V. The new hybrid recorded maximum per cent gain over for cocoon rate/kg (25.68) followed by cocoon shell weight (15.53) and cocoon yield/ 100 dfls (10.34).

Androgenesis has been used for the development of homozygous silkworm breeds (Strunnikov et al., 1982; Malinova et al., 1996). Recently, Ravindra Singh et al. (2009b) have developed some bisexual polyvoltine silkworm breeds through application of androgenesis. Self bred silkworm lines have been isolated through irradiation of female pupae with 80 KR gamma rays, crossing the female moths with normal males and exposing the eggs at 38 °C for 200 minutes (Xu et al., 1997). Nacheva et al. (1999) have developed bisexual silkworm lines by crossing F_2 hybrid males to Ps race used as marker and exposing the eggs at 42 °C for 200 minutes.

In the present study, an attempt has been made to develop promising polyvoltine silkworm breed with better silk quality. The hybrid AGL_3 × CSR_2 is characterized by high survival (>95 %), high cocoon weight (1.9 g), high cocoon shell weight (>0.35 g), high cocoon shell percentage (>20.0 %), longer filament length (> 800 m) and high neatness (90 p). Dandin et al. (2007) have isolated one promising polyvoltine breed "ND_7" with better silk quality without sacrificing other quantitative characters.

Manifestation of hybrid vigour was observed relatively high for some characters *viz.*, yield/10,000 larvae by weight (46.63), cocoon weight (37.67) and cocoon shell weight (50.12) in $AGL_3 \times CSR_2$. Efforts have been made on manifestation of hybrid vigour in hybrids involving polyvoltine and bivoltine silkworm breeds (Ravindra Singh et al., 1998; 2001; Datta et al., 2001; Gangopadhyay & Ravindra Singh, 2006, Dandin et al., 2007).

Laboratory evaluation of AGL3 × CSR_2 has demonstrated the superiority of the new hybrid over the control PM × CSR_2. The cocoons fetch more price which is more than 20 rupees as compared to the control. Presently, the hybrid is being tested on farm trials through the nested units of CSRTI, Mysore. Large scale trials at TVDC have recorded an average cocoon yield of 71.945 kg/100 dfls as against 68.643 kg in the control. The hybrid recorded cocoon yield of 70.139 kg/100dfls as against 63.561 kg in the control when evaluated with few farmers in a limited scale (Table VI).The striking features of the new hybrid are that it produces cocoons with high cocoon shell weight, cocoon shell percentage, filament length and neatness.

The findings of this study can be useful to the silkworm breeders to assess the practical significance of androgenesis in the development of outstanding homozygous genotypes with uniform populations, high viability and hybrid vigour. Androgenesis can be successfully utilized in the transmission of homozygosity into existing pure lines to develop promising silkworm breeds. Recently, level of homozygosity using random amplified polymorphic DNA (RAPD) has been assessed in some silkworm breeds developed through application of androgenesis and parthenogenesis (Ravindra Singh et al., 2009 c). The main advantages of utilization of androgenesis in silkworm are that it provides an opportunity to shorten the breeding cycle and to develop bisexual homozygous silkworm lines which are of utmost importance in silkworm breeding in order to obtain high hybrid vigour.

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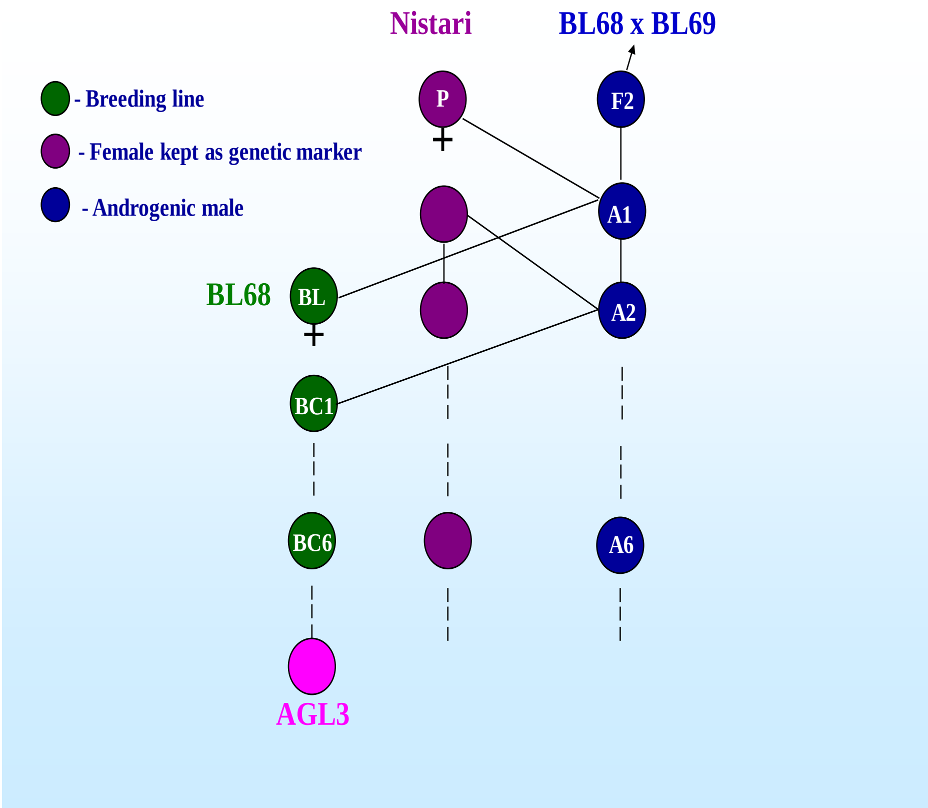

Figure 1. Breeding plan for AGL_3.

Table 1. Performance of selected polyvoltine line AGL_3 during the process of breeding.

Generation	Fecundity (no.)	Hatching (%)	Total larval span (h)	Pupation rate (%)	Yield/10000 larvae by wt. (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell (%)	Filament length (m)	Filament size (d)	Reel ability (%)	Raw silk (%)	Neat-ness (p)
F_1	505	92.91	486	92.74	10.884	1.160	0.208	18.46	551	2.66	82	13.8	89
F_2	521	94.60	504	92.33	12.634	1.372	0.214	15.62	625	2.70	83	11.6	89
F_3	502	92.17	504	94.50	12.268	1.306	0.218	16.69	645	2.40	77	12.4	89
F_4	481	94.54	504	95.20	13.687	1.438	0.262	18.19	785	2.41	79	13.2	89
F_5	490	91.65	504	92.39	11.545	1.257	0.223	17.76	575	2.30	77	12.5	89
F_6	491	98.13	504	94.99	13.533	1.424	0.246	17.27	593	2.38	74	12.0	89
F_7	483	97.17	504	94.33	13.027	1.394	0.242	17.36	543	2.46	72	12.6	89
F_8	495	94.85	504	91.18	10.511	1.142	0.193	16.91	526	2.31	82	12.8	90
F_9	474	97.14	506	91.55	12.411	1.381	0.243	17.62	625	2.77	72	13.9	90
F_{10}	511	94.98	504	90.66	11.000	1.196	0.213	17.81	609	2.49	75	12.7	90
F_{11}	500	96.00	468	93.33	11.666	1.302	0.221	16.97	630	2.50	78	13.0	90
F_{12}	506	95.48	480	92.33	11.466	1.213	0.216	17.82	629	2.43	79	12.7	90
Mean	497	94.93	498	92.92	12.056	1.299	0.225	17.37	611	2.49	77	12.8	89
SD	13.59	2.01	12.50	1.57	1.04	0.10	0.02	0.76	67.00	0.15	3.68	0.68	0.55
CV %	2.74	2.12	2.51	1.69	8.59	8.04	8.60	4.40	10.96	6.10	4.75	5.14	0.62
(F10-F12)													
Mean	506	95.49	484	92.11	11.377	1.237	0.217	17.53	623	2.47	77	12.8	90
SD	5.51	0.51	18.33	1.35	0.34	0.06	0.00	0.49	11.85	0.04	2.08	0.17	0.00
CV %	1.09	0.53	3.79	1.46	3.00	4.60	1.87	2.78	1.90	1.53	2.69	1.35	0.00

Table 2. Performance of polyvoltine × bivoltine hybrids along with evaluation index values.

Hybrids	Fecundity (no)	Hatching (%)	Pupation rate (%)	Yield/10,000 wt. (kg)	Cocoon wt (g)	Cocoon shell wt (g)	Cocoon shell (%)	Filament length (m)	Reel-ability (%)	Raw silk (%)	Neat-ness (p)
$AGL_3 \times CSR_2$	493 (53.32)	96.21 (75.00)	96.60 (52.26)	18.322 68.48)	1.928 (60.50)	0.412 (96.33)	21.38 (61.27)	870 (69.13)	82 (61.62)	16.2 (64.57)	91 (66.90)
$AGL_3 \times CSR_3$	490 (52.41)	95.44 (53.61)	97.47 (57.44)	16.862 (51.11)	1.764 (50.25)	0.364 (53.33)	20.62 (55.93)	766 (47.73)	79 (51.66)	15.4 (56.06)	88 (52.41)
$AGL_3 \times CSR_4$	505 (56.95)	95.58 (57.50)	94.33 (38.75)	16.605 (48.41)	1.751 (49.43)	0.356 (50.66)	20.34 (52.67)	739 (42.18)	74 (35.04)	15.1 (52.87)	86 (42.75)
$AGL_3 \times CSR_{12}$	513 (59.37)	94.62 (30.83)	97.47 (57.44)	16.472 (47.01)	1.703 (46.43)	0.340 (45.33)	19.99 (48.60)	737 (41.76)	76 (41.69)	14.7 (48.61)	85 (37.92)
$AGL_3 \times NB_4D_2$	468 (45.75)	95.50 (55.27)	93.87 (36.01)	15.363 (35.33)	1.655 (43.43)	0.319 (38.33)	19.28 (40.34)	772 (48.97)	79 (51.66)	13.9 (45.57)	88 (52.41)
$PM \times CSR_2$	422 (31.85)	94.53 (28.33)	97.60 (58.21)	16.917 (51.69)	1.762 (50.12)	0.336 (44.00)	19.05 (37.67)	778 (50.20)	81 (58.30)	13.7 (37.97)	87 (47.58)
Mean	482	95.31	96.22	16.756	1.760	0.354	20.11	777	78.5	14.83	87.5
SD	33.07	0.36	1.68	0.95	0.16	0.03	0.86	48.66	3.01	0.94	2.00

Values in parentheses indicate evaluation index values

Table 3. Heterosis over mid and better parent values.

Hybrids	Fecundity	Hatching	Total Larval span	Pupation Rate	Yield/10,000 wt.	Cocoon wt	Cocoon shell wt	Cocoon shell (%)	Filament length	Filament size	Reel-ability	Raw silk	Neat-ness
Heterosis over mid parent values													
$AGL_3 \times CSR_2$	-7.56	1.02	0.00	3.22*	46.63**	37.67**	50.12**	11.43**	30.68**	8.42	-0.20	9.60**	-0.18
$AGL_3 \times CSR_3$	-0.57	0.44	-1.73	4.51**	28.56**	27.24**	36.29**	9.14**	21.56**	17.42	-2.46	6.08**	-2.21
$AGL_3 \times CSR_4$	7.41**	0.51	-4.55*	2.25	29.98**	25.62**	33.50**	8.20**	9.65*	14.34	-10.34	5.48**	-4.78
$AGL_3 \times CSR_{12}$	6.65**	-0.40	-3.41	4.89**	31.05**	29.10**	33.99**	5.43**	11.59**	1.49	-6.17	4.00**	-5.20
$AGL_3 \times NB_4D_2$	-0.92	1.01	-3.41	3.54*	25.63**	22.93**	29.41**	6.35**	18.49**	2.44	-1.24	1.46**	-1.68
$PM \times CSR_2$	-17.75	0.02	-4.35*	6.60**	44.92**	31.56**	32.41**	5.10**	31.38**	23.59	8.52**	0.61	-1.69
Heterosis over better parent values													
$AGL_3 \times CSR_2$	-13.76	0.62	0.00	0.62	26.53**	16.19**	15.72**	-0.42	7.94*	0.87	-1.20	-3.39	-1.45
$AGL_3 \times CSR_3$	-0.94	0.26	-4.49*	2.24	7.26**	8.13**	6.75**	-1.25	4.26	7.72	-2.86	-5.33	-2.93
$AGL_3 \times CSR_4$	2.02	0.25	-8.70**	1.07	10.41**	6.38**	4.60**	-1.68	-10.10	4.55	-10.89	-4.64	-5.82
$AGL_3 \times CSR_{12}$	3.70**	-0.55	-7.61**	2.96	12.61**	13.81**	8.04**	-5.05	-7.30	-0.42**	-6.94	-5.35	-5.20
$AGL_3 \times NB_4D_2$	-5.45	0.69	-7.61**	2.95	10.15**	6.73**	6.33**	-0.38	-0.73	-3.69**	-2.86	-4.81	-1.86
$PM \times CSR_2$	-26.12	-1.13	-12.00**	1.67	16.83**	637**	-5.80	-11.26	-3.51	7.84	-3.20	-17.93	-5.43

*and ** denote significantly different at 5 and 1% level, respectively

Table 4. Evaluation of promising polyvoltine × bivoltine hybrids in Technology Validation and Demonstration Centre of CSR&TI, Mysore (Average of 3 trials).

Hybrids	Yield/100 dfls (kg)	Cocoon wt (g)	Cocoon shell wt (g)	Cocoon shell (%)	Filament Length (m)	Raw silk (%)	Neatness (p)	Cocoon rate / kg (Rs.)
$AGL_3 \times CSR_2$	71.945 (4.81)	1.806 (5.92)	0.364 (14.46)	20.15 (8.04)	922 (16.70)	13.82 (9.07)	91.3 (2.23)	137=76
$PM \times CSR_2$ (Control)	68.643	1.705	0.318	18.65	790	12.67	89.3	114=03

Values in parentheses indicate per cent improvement over control.

Table 5. Field trial of promising polyvoltine × bivoltine hybrids of the silkworm, *Bombyx mori* L.

Hybrids	No. of Dfls.	No. of farmers	Yield/100 dfls (kg)	Cocoon wt (g)	Cocoon shell wt (g)	Cocoon shell (%)	Cocoon rate / kg (Rs.)
$AGL_3 \times CSR_2$	300	3	70.139 (10.34)	1.829 (7.27)	0.357 (15.53)	19.52 (7.72)	137=00 (25.68)
$PM \times CSR_2$ (Control)	300	3	63.561	1.705	0.309	18.12	109=00

Values in parentheses indicate per cent improvement over control.