AN OVERVIEW ON THE DEVELOPMENT AND EVALUATION OF BREEDS/ HYBRIDS OF THE MULBERRY SILKWORM, *BOMBYX MORI* L.

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ABSTRACT: Response of different silkworm breeds towards parthenogenetic development has been studied in newly developed bivoltine and polyvoltine silkworm breeds. Japanese type bivoltine silkworm breeds showed pronounced parthenogenetic developments as compared to Chinese ones. An increased tendency towards parthenogenetic development was observed in hybrids obtained from a mother moth having a high tendency of parthenogenesis. Three bivoltine breeds *viz.,* DNB_1, DNB_6 and DNB_7 were developed and evaluated. Evaluation was carried out through various statistical tools like analysis of combining ability, hybrid vigour and cocoon size uniformity. Androgenetic development was also induced in different silkworm breeds/hybrids. Polyvoltine hybrids exhibited higher androgenic development as compared to bivoltine hybrids. A breed with dominant cocoon colour gene was utilized as genetic marker to identify androgenic male individuals. Induction of androgenesis was performed by exposing the oviposited eggs to hot air (38 °C) for 200 min. Repeated backcrosses were adopted utilizing androgenic males to introgress homozygosity in the breeding lines. Among six polyvoltine silkworm breeds developed through androgenesis, two breeds AGL_3 and AGL_5 were found promising. Two bivoltine hybrids *viz.,* $DNB_1 \times CSR_4$ and $DNB_7 \times CSR_2$ and 2 polyvoltine × bivoltine hybrids *viz.,* $AGL_3 \times CSR_2$ and $AGL_5 \times CSR_2$ were found promising.

KEY WORDS: Androgenesis, *Bombyx mori,* evaluation, hybrid vigour, combining ability, parthenogenesis, performance, silkworm breed and hybrids.

Continuous domestication and selection of the mulberry silkworm, *Bombyx mori* L has made a diversified genotype (Tazima, 1964). Though, conventional breeding approaches have remarkably increased the silk production, continuous selection showed a decline of targeted characters (Seidel & Brackett, 1981). Selection of desirable individuals based on phenotypic observation is not always accurate. Breeders often opt to preserve the exact genotypic copy of the parent in the descendants (Strunnikov, 1983). Parthenogenesis and androgenesis would be useful in the development of superior silkworm breeds and hybrids with more viability, hybrid vigour, combining ability and less phenotypic variability (Dznealaidze & Tabliashvili, 1990; Takei et al., 1990; Plugaru et al., 1993; Strunnikov, 1995). Androgenic development in silkworm has been induced through several activating agents like X-ray and gamma irradiation of mated female moths, hot air, hot water, CO_2 and super cooling of oviposited eggs at low temperature (Whiting, 1955; Tazima & Onuma, 1967; Ye et al., 1989; Nagoya et al., 1996). Attempts have been made to isolate bisexual lines of the mulberry silkworm through application of dispermic androgenesis (Xu et al., 1997; Nacheva et al., 1999). Information on the role of artificial parthenogenesis and androgenesis in the development of silkworm breeds / hybrids is lacking. In the present study, an attempt was made to explore the possibility of using breeding strategies like artificial parthenogenesis and androgenesis in the development and evaluation of outstanding hybrids of high viability, more hybrid vigour, combining ability and phenotypically uniform population.

Astaurov (1940) method was followed to induce ameiotic parthenogenesis in the unfertilized eggs. Eggs were extracted by rubbing through a muslin sieve, washed in running tap water, dried and kept in a cotton bag for 12 h at room temperature. Then they were dipped in a hot water bath at 46 °C for 18 min and abruptly cooled at 20 °C water bath for 10 min. After drying, eggs batches were put in a Petri dish and incubated at 15 °C and 80 % RH for 3 - 5 days. Egg were soaked in hot HCl (Sp. Gr. 1.075) for 5 min to terminate the egg diapause and rinsed in tap water (20 °C) to eliminate acid traces before being dried. Care was taken to incubate the eggs at normal temperature (25 ± 0.5 °C) and relatively high humidity of 90-95% till larval hatching. Appearance of reddish-brown to dark-brown pigmentation in the serosa was considered as parthenogenetic development. The rate of parthenogenesis was estimated by counting pigmented eggs about 7 - 8 days after transfer from 15 to 25 °C. The ratio of reddish-brown / dark pigmented eggs and total number of eggs treated was expressed as percentage of parthenogenesis whereas the ratio of hatched larvae and pigmented eggs was considered as percentage of hatching. Data were recorded for number of pigmented eggs, number of non-pigmented eggs, number of larvae hatched, percentage parthenogenesis and hatching.

Induction of androgenesis: Diagramatic representation of androgenetic development in silkworm has been depicted in Fig.1 (Singh et al., 2009a).Twenty six polyvoltine silkworm breeds *viz.*, BL_{23}, BL_{24}, BL_{61}, BL_{62}, BL_{65}, BL_{67}, BL_{68}, BL_{69}, 96A, 96C, 96B, ND_5, ND_7, NP_1, PM, P_2D_1, MY_1, D_1, GNP, Sarupat, Moria, Nistari, Kollegal Jawan, Kolar Gold, DNP_3 and DNP_5 were screened to shortlist superior breeds based on average evaluation indices as per Mano et al. (1993). Five polyvoltine silkworm breeds *viz.*, NP_1, ND_7, BL_{68}, DNP_3 and DNP_5 exhibiting higher evaluation index values were utilized as breeding resource materials (Nirupama & Singh, 2007).

Induction of androgenesis in the oviposited eggs was carried out in three different hot air treatments I) at 38˚C for 200 min, II) 40˚C for 135 min and III) 42˚C for 210 min to standardize the procedure(Nirupama & Singh, 2007). Soon after treatment, the eggs were transferred to 15˚C till the appearance of pigmentation in the serosa. Bivoltine and bivoltine × polyvoltine hybrid eggs were treated with hot hydrochloric acid to terminate the egg diapause. Incubation of eggs was done at 25˚C till hatching. The ratio of dark bluish pigmented eggs and total number of eggs treated was expressed as percentage of androgenetic eggs. Nistari, a polyvoltine race possessing dominant gene for golden yellow cocoon colour with marked larvae was utilized as genetic marker to identify androgenetic male individuals. Repeated backcrosses were adopted utilizing the androgenic males. Astaurov (1957) method was followed for the induction of androgenetic development. In order to increase the rate of androgenetic development in the eggs of the polyvoltine hybrid [Nistari × (BL68 × BL69)], the method of Astaurov was modified (Singh et al., 2009b).

Artificial parthenogenesis and silkworm breeding: Five breeding plans were initiated during the course of breeding for the development of homozygous breeds of the silkworm utilizing Chinese and Japanese type bivoltine silkworm breeds as breeding resource materials. Breeds with sex-limited characteristics were kept as genetic marker to identify the parthenogenetic female individuals. Six parthenogenetic lines namely, DNB_1 and DNB_2 of Chinese type (plain larvae;

oval cocoons) and DNB_3, DNB_4, DNB_6 and DNB_7 having Japanese racial characteristics (marked larvae; dumbbell cocoons) were evolved.

Evaluation of silkworm hybrids: Six newly developed bivoltine breeding lines *viz.,* DNB_1, DNB_2, DNB_3, DNB_4, DNB_6 and DNB_7 were evaluated with 4 bivoltine breeds namely, CSR_2, CSR_4, CSR_{17} and NB_4D_2. Selection of the silkworm breeds / hybrids was carried out through multiple traits evaluation index method of Mano et al. (1993). The breeds / hybrids showing greater average evaluation index value and evaluation index value for a particular character higher than 50 for more characters were identified as promising. Newly developed 6 breeding lines and 4 popular bivoltine breeds were utilized as lines and testers, respectively. General combining ability of lines and testers and specific combining of bivoltine and polyvoltine × bivoltine hybrids were determined as per Kempthorne (1957).

Identification of silkworm breeds with high parthenogenetic ability: Initially, response towards artificial parthenogenesis in bivoltine silkworm breeds was studied (Gangopadhyay & Singh, 2004, 2006a). Among Japanese type bivoltine breeds, CSR_{19} expressed maximum parthenogenetic development (58.55 %) followed by NB_4D_2 (51.09 %) and CSR_4 (50.85 %). Among Chinese type bivoltine breeds, CSR_{12} exhibited maximum parthenogenetic development (39.85%) followed by CSR_2 (39.25 %) and CSR_{18} (37.17 %). In order to establish parthnogenetic characters, crosses were made between the breeds possessing higher parthenogenetic ability. Among Japanese type bivoltine hybrid, maximum parthenogenetic development was observed in $CSR_{19} \times CSR_6$ (91.20 %) followed by $CSR_{19} \times CSR_4$ (88.56 %). Hatching % was recorded maximum in $CSR_{19} \times CSR_4$ (78.13 %) followed by $CSR_{19} \times CSR_6$ (51.05 %). Among Chinese type hybrids, maximum parthenogenetic development was observed in $CSR_{18} \times CSR_{12}$ (81.65 %) followed by $CSR_3 \times CSR_{17}$ (60.34 %) Hatching % was maximum in $CSR_{18} \times CSR_{12}$ (10.57 %) followed by $CSR_2 \times CSR_{27}$ (6.78 %). Though, the rate of parthenogenetic development was higher in F_1 hybrids; there was no further improvement in the subsequent generations. A new improved method (Gangopadhyay and Singh, 2006b;c) was devised to improve the rate of parthenogenetic development and results were compared with the routine one.

Development of homozygous silkworm breeds with parthenogenetic origin: Five breeding plans were initiated for the development of homozygous breeds utilizing Chinese and Japanese type bivoltine breeds as breeding resource materials. Astaurov (1940) method was followed for the induction of artificial parthenogenesis. Breeds with sex-limited characteristics were used as genetic marker to identify the parthenogenetic individuals. Eggs extracted from 30 virgin female moths were individually tested and the egg batch of each female was kept separately. The egg batch showing maximum parthenogenetic development and hatchability was continued further. To achieve a balance between viability and productivity characters in the parthenoclones, two backcrosses were adopted in the earlier generations. Six parthenogenetic lines namely, DNB_1 and DNB_2 of Chinese type (plain larvae; oval cocoons) and DNB_3, DNB_4, DNB_6 and DNB_7 having Japanese racial characteristics (marked larvae; dumbbell cocoons) were developed. Improvement in parthenogeneic development in parthenogenetic lines were observed (Gangopadhyay and Singh, 2006b).Promising lines were evaluated in limited scale (Gangopadhyay & Singh, 2007) Mean performance of two promising lines DNB_1 and DNB_7 has been given in Table 1.

Assessment of advantages of parthenogenesis: Two bivoltine breeds *viz.,* DNB_1 and DNB_7 and two bivoltine hybrids *viz.*, DNB_1 × CSR_4 and DNB_7 × CSR_2 were found promising based on average evaluation indices. Estimation of GCA revealed superiority of DNB_1 for 8 characters. Less GCA effects in DNB_7 may be due to existence of only females. Two hybrids DNB_1 × CSR_4 and DNB_7 × CSR_2 showed significant SCA effects for 5 - 6 characters. Significant hybrid vigour for pupation rate, yield / 10,000 larvae by weight, cocoon weight, cocoon shell weight, filament length and filament size was observed. The hybrids have shown standard deviation less than 8 and their CV % ranged from 4.04 to 4.96 % (Table 2). DNB_7 × CSR_2 was identified as promising and evaluated along with control CSR_2 × CSR_4 in the Technology Validation and Demonstration Centre, CSRTI, Mysore (Table 3). The hybrid was characterized with high viability, quantitative characters and each kg of cocoons fetched 15 - 20 rupees more than the control.

Genotypic variability of the silkworm breeds developed through DNA fingerprinting: For the assessment of homozygosity, 8 random decamer primers from Operon Technologies Inc., Alameda, USA (OPAA2, OPAA5, OPAH1, OPA5, OPA11, OPA20, OPC4, and OPD2) were tested on 8 individuals each taken from the bisexual line DNB_1, female parthenoclonal lines DNB_6 and DNB_7. No polymorphism in DNA was detected among the individuals randomly selected indicating the attainment of homozygosity in their genetic make-up (Singh et al., 2009c). Further, shared (common) RAPD fragments found in all individuals of DNB lines with fixed frequencies (monomorphic) observed in all investigated primers, imply their close genetic relationships. The RAPD DNA pattern of all the randomly selected individuals belonging to parthenogenetic bisexual line DNB_1 (both male and female) and entirely female parthenoclonal line DNB_7 have shown identical banding pattern clearly suggesting the attainment of homozygosity (Fig. 2). The amplification product with decamer OPA20 revealed 1 band of 1700 base pairs (bp) specific to DNB_7. Thousand two fifty bp bands specific to DNB_6 and DNB_7 appeared in the DNA profile indicate their close genetic relationship. DNB_1 males and females shared 2 bands (1100 and 1000 bp).

Preliminary field trail of promising bivoltine hybrids: Two selected bivoltine hybrids *viz.,* DNB_1 × CSR_4 and DNB_7 × CSR_2 along with control CSR_2 × CSR_4 were further evaluated both in the laboratory and with a few farmers located in Karnataka. Rearing results of 400 dfls each of the selected bivoltine hybrids tested with the farmers recorded an average cocoon yield of 65.450, 69.770 and 60.350 kg / 100 dfls, cocoon weight of 1.789, 1.827 and 1.741 g, cocoon shell weight of 0.371, 0.399 and 0.354 g and cocoon shell percentage of 20.74, 21.85 and 20.35 % in DNB_1 × CSR_4, DNB_7 × CSR_2 and CSR_2 × CSR_4 respectively Gangopadyay et al., 2009; Singh et al., 2009d). Results showed superiority of DNB_7 × CSR_2 over other hybrids both in the laboratory as well as in the field.

Development of homozygous silkworm breeds with androgenetic origin: Twenty six polyvoltine silkworm breeds were screened based on higher average evaluation indices and 5 breeds *viz.*, DNP_5 (59.95), DNP_3 (58.25), NP_1 (55.52), ND_7 (54.42) and BL_{68} (53.68) possessing higher average evaluation indices were selected for breeding resource materials (Nirupama and Singh, 2007). Treatment of eggs at 38 ˚C for 200 min, maximum hatching percentage (12.78 %) was observed in the modified method as compared to the (6.36 %) in the routine method. Nistari, a polyvoltine silkworm race possessing marked larvae and golden yellow spindle shaped cocoons was kept as a genetic marker to identify the androgenetic individuals. Androgenetic individuals were utilized as donors to transmit homozygosity into bisexual lines by a series of backcrosses. Five breeding plans were initiated and five lines *viz.,* AGL_1, AGL_2, AGL_3, AGL_4 and AGL_5 were developed. Mean performance of selected polyvoltine androgenetic lines namely, AGL_3 and AGL_5 has been given in Table 4.

Assessment of advantages of androgenesis : Five polyvoltine androgenetic lines were evaluated following different statistical analyses like multiple traits evaluation index method (Mano et al., 1993), combining ability and hybrid vigour analysis (Kempthorne, 1957) and cocoon size uniformity test etc. Polyvoltine androgenetic lines *viz.,* AGL_1, AGL_2, AGL_3, AGL_4 and AGL_5 were evaluated utilizing popular bivoltine breeds namely, CSR_2, CSR_3, CSR_4, CSR_{12} and NB_4D_2. Two polyvoltine × bivoltine hybrids $AGL_3 \times CSR_2$ and $AGL_5 \times CSR_2$ were found promising based on subordinate function index method, average evaluation indices and cocoon size variability (Nirupama et al., 2008a;b). AGL_3 and AGL_5 exhibiting significant GCA effects for majority of the characters were good general combiners. Among 30 polyvoltine × bivoltine hybrids, $AGL_3 \times CSR_2$ and $AGL_5 \times CSR_2$ expressed highly significant (SCA) effects for fecundity, cocoon yield/10,000 larvae by weight, cocoon weight, cocoon shell weight and cocoon shell percentage (Singh et al., 2010). $AGL_3 \times CSR_2$ and $AGL_5 \times CSR_2$ were found promising and manifested highly significant hybrid vigour over MPV and BPV for cocoon yield/ 10,000 larvae by weight, cocoon weight, cocoon shell weight, cocoon shell percentage, filament length and raw silk percentage over the control PM × CSR_2. Cocoons of $AGL_3 \times CSR_2$ and $AGL_5 \times CSR_2$ were found relatively uniform with their SD < 8 and CV % of 4.23 and 4.09 %, respectively (Table 5). $AGL_3 \times CSR_2$ and $AGL_5 \times CSR_2$ were evaluated in the Technology Validation and Demonstration Centre, CSRTI, Mysore along with control PM × CSR_2. Data showed that the new hybrids performed better in terms of cocoon yield, cocoon weight, cocoon shell weight, cocoon shell percentage, filament length and raw silk percentage over the control (Table 6).

Genotypic variability of the silkworm breeds developed through DNA fingerprinting: For the assessment of homozygosity, 8 random decamer primers from Operon Technologies Inc., Alameda, USA (OPAA2, OPAA5, OPAH1, OPA5, OPA11, OPA20, OPC4, and OPD2) were tested on 8 individuals each taken from androgenetic lines AGL_1, AGL_2, AGL_3, AGL_4 and AGL_5. No polymorphism in DNA was detected among the individuals randomly selected indicating the attainment of homozygosity in their genetic make-up (Singh et al., 2009c). A total of 28 scorable, discrete amplicons were generated when the template DNA of AGL series (AGL_1 to AGL_5) were amplified with 7 random primers at an average of 4 bands per primer. The analysis has shown identical RAPD profiles within the inbred lines of AGL series when amplified with OPA11, and OPD2 (Fig. 3) indicating the accomplishment of homozygosity of the lines at the molecular level. The two bands with 1870 and 1650 bp are seen common in all the AGL series analyzed.

Development of silkworm breeds by conventional breeding has played a vital role in upgrading both quality and quantity of silk produced (Datta, 1984). Since most of the quantitative characters in silkworm are governed by polygenes, their inheritance shows variation and therefore, more emphasis is being paid for selection of silkworm breeds based on their phenotypic expression (Nagaraju, 1998). Sometimes, a suitable phenotype may not exhibit a suitable genotype and due to low heritability, the subsequent progeny may lose its unique genotype.

Artificial parthenogenesis enables one to produce from one outstanding individual hundreds of parthenoclones each of which is an exact genotypical copy of its parent (Astaurov, 1957; Strunnikov, 1975). In sexual reproduction, the offsprings receive only a random half of alleles from each parent and the results are not predictable accurately (Seidel and Brackett, 1981). Application of new breeding strategies like parthenogenesis and androgenesis would be beneficial to the silk industry in the development and cloning of homozygous silkworm breeds with either entirely females (completely heterozygous) via ameiotic parthenogenesis or predominantly males (homozygous) via androgenesis to improve the selection efficiency (Strunnikov, 1983, 1986; Retnakaran & Percy, 1985; Takei et al., 1990). Though, the practical significance of artificial parthenogenesis and androgenesis has been realized, less attention has been given to explore the possibility of utilizing these strategies for the development of silkworm breeds / hybrids found in India. Attempts have been made to develop superior breeds / hybrids of the silkworm through the application of artificial parthenogenesis and androgenesis (Singh et al., 2004; Gangopadhyay & Singh, 2007, 2008, 2009; Singh et al., 2009a, 2011).

Response of different silkworm breeds towards parthenogenetic development has been studied. Among the different bivoltine and polyvoltine breeds, Japanese type bivoltine silkworm breeds showed pronounced parthenogenetic development (Gangopadhyay & Singh, 2006a). An increased tendency towards parthenogenesis was observed in hybrids obtained from a mother moth having a high tendency of parthenogenesis. Breeds with higher parthenogenetic ability were crossed to establish parthenogenetic character in the lines. Three bivoltine breeds *viz.,* DNB_1, DNB_6 and DNB_7 were developed. The bisexual line DNB_1 was characterized by sex-limited characteristics with white oval cocoons while DNB_6 and DNB_7 were characterized by entirely female parthenoclones possessing white dumbbell cocoons. Hybrids were prepared by crossing the developed silkworm breeds and productive bivoltine breeds. Evaluation of the developed breeds / hybrids was carried out through various statistical measures like analysis of combining ability, hybrid vigour and cocoon size uniformity. Studies showed more combining ability, hybrid vigour and cocoon size uniformity in the new hybrids.

Induction of androgenesis was performed in different silkworms to select potential breeds. Polyvoltine hybrids showed higher androgenic development as compared to bivoltine hybrids. Nistari, a polyvoltine breed possessing dominant gene for golden yellow cocoon colour with marked larvae was utilized as genetic marker to identify the androgenic male individual. Females of Nistari were crossed with the males of the hybrid $BL_{68} \times BL_{69}$ possessing plain larvae and oval shaped greenish yellow cocoons. Induction of androgenesis was performed by exposing the oviposited eggs at hot air of 38 ^{o}C for 200 min. Both marked and plain larvae were observed. Plain larvae were identified as androgenic individuals. Sex of the plain larvae at pupal stage was further determined. All the pupae derived from plain larvae were exclusively males. Backcrossing was adopted utilizing androgenic males to introgress homozygosity in the breeding lines (Singh et al., 2009c, 2011). By utilizing dispermic androgenesis, bisexual silkworm lines have been isolated (Xu et al., 1997). Some bisexual lines of the mulberry silkworm, *B. mori* with androgenetic origin have been developed (Nacheva et al., 1999). Level of homozygosity was assessed in the breeds developed via parthenogenesis and androgenesis through DNA fingerprinting (Singh et al., 2009c). Promising polyvoltine breeds and hybrids were short-listed utilizing different statistical tools (Singh & Nirupama, 2012).

Three bivoltine parthnogenetic lines *viz.,* DNB_1, DNB_6 and DNB_7 along with two polyvoltine androgenetic lines *viz.,* AGL_3 and AGL_5 were developed. One bivoltine hybrid DNB_7 × CSR_2 and two polyvoltine × bivoltine hybrids AGL_3 × CSR_2 and AGL_5 × CSR_2 were found promising exhibiting significant improvement for various quantitative characters like higher survivability, combining ability, hybrid vigour and more cocoon size uniformity and may be recommended for commercial exploitation to obtain stabilized cocoon crops and better silk quality. The developed bivoltine parthenogenetic and polyvoltine androgenetic breeds can be utilized as breeding resource materials for future breeding programmes. Besides, studies on combining ability, hybrid vigour and phenotypic uniformity would be of immense use to the silkworm breeders to assess the practical significance of artificial parthenogenesis and androgenesis in the development of superior silkworm breeds / hybrids.

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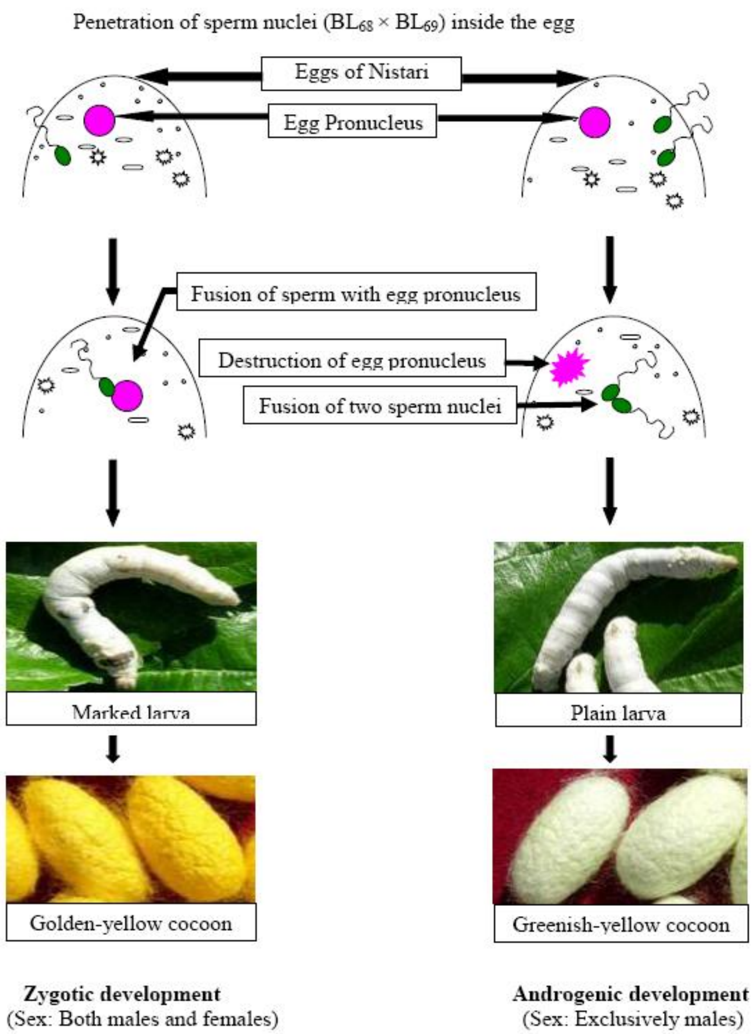

Figure 1. Diagramatic representation of normal zygotic and androgenic development in the silkworm, *B. mori* L. (Singh et al., 2009a).

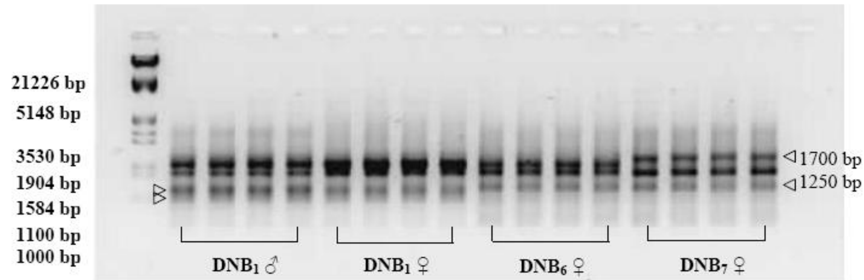

Figure 2. Identical RAPD profiles of DNB parthenogenetic lines amplified with OPA20 decamer. Arrows indicate 1700 bp product specific to DNB_7 line, 1250 bp band specific to DNB_6 and DNB_7, 1100 & 1000 bp amplified products found in DNB_1 males and females (Singh et al., 2009c).

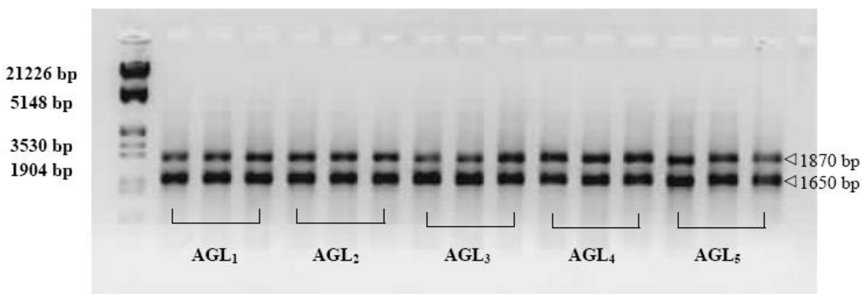

Figure 3. Identical RAPD profiles of AGL androgenetic lines amplified with OPD2 decamer. Arrows indicate 1870 and 1650 bp products common to all the AGL lines (Singh et al., 2009c).

Table 1. Performance of bivoltine parthenogenetic lines.

Breed	Fecundity (no)	Partheno-genesis (%)	Hatching (%)	Pupation rate (%)	Yield/10000 larvae by wt. (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell (%)	Filament length (m)	Reelability (%)	Raw silk (%)	Neatness (p)
DNB_1	551±21.70	-	97.42±0.6	94.00±2.43	14.760±1.00	1.577±0.07	0.331±0.02	20.96±0.60	885±51.32	82.4±0.84	16.45±0.62	92±0.50
DNB_7	-	94.37±1.60	73.46±2.1	93.64±2.45	15.080±0.83	1.600±0.07	0.301±0.02	18.82±0.51	777±28.67	81.2±1.82	14.49±0.36	92±1.26

Data are Mean ± SD of $F_9 - F_{12}$.

Table 2. GCA, SCA, Hybrid vigour, average evaluation indices and cocoon size uniformity in promising bivoltine silkworm breeds / hybrids.

Breed/ Hybrid	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Cocoon wt.	Cocoon shell wt.	Cocoon shell (%)	Filament length	Reelability	Raw silk (%)	Neatness	Average evaluation indices	Cocoon size uniformity
General Combining Ability Effects of Line													
DNB_1	-2.50	-0.04	2.60**	1.19***	0.03	0.024***	0.94***	44.54***	-2.27	1.14***	0.68**	58.16	169.33±8.29
DNB_7	5.92	-0.48	-2.05	-0.59	0.02	-0.001	-0.22	0.46	0.25	-0.27	-0.90	45.89	171.63±8.11
Specific Combining Ability Effects and hybrid vigour													
$DNB_1 \times CSR_4$	-15.17	0.02	2.02	0.76**	0.06	0.015*	0.21	23.96**	1.11	0.69**	0.76	58.92	167.98±7.66
	(0.53)	(-0.24)	(7.32**)	(18.63**)	(8.99*)	(13.96**)	(5.00*)	(12.30**)	(-1.97)	(12.61**)	(1.28*)		
$DNB_7 \times CSR_2$	28.69	2.65**	5.13**	0.55	-0.05	-0.011	-0.09	21.04**	3.64**	0.60*	0.51	56.99	163.36±7.96
	(2.28)	(36.62**)	(11.06**)	(2.59)	(1.54)	(5.64*)	(3.87)	(17.68**)	(5.97*)	(7.10**)	(-1.47)		

Data in parentheses are hybrid vigour over mid parent value; *, ** and *** denote significant difference at 5%, 1% and 0.1% level respectively.

Table 3. Performance of promising bivoltine hybrids at TVDC (Mean of 3 trials).

Hybrids	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell (%)	Cocoon yield/100dfls (kg)	Cocoon price/kg (Rs.)
$DNB_7 \times CSR_2$	1.834 (0.49)	0.399 (2.31)	21.75 (2.31)	71.033 (10.34)	153.80 (7.63)
$CSR_2 \times CSR_4$ (Control)	1.825	0.390	21.36	63.690	142.06

Values in parentheses indicate per cent improvement over control.

Table 4. Performance of short listed multivoltine androgenetic lines Data are Mean ± SD of $F_9 - F_{12}$.

Breed	Fecundity (no)	Hatching (%)	Pupation rate (%)	Yield/10000 larvae by wt. (kg)	Cocoon wt. (g)	Cocoon shell wt. (g)	Cocoon shell (%)	Filament length (m)	Reelability (%)	Raw silk (%)	Neatness (p)
AGL_3	506±5.51	95.49±0.51	92.11±1.35	11.377±0.34	1.237±0.06	0.217±0.00	17.53±0.49	623±11.85	77±2.08	12.8±0.17	90±0.00
AGL_5	494±9.07	94.56±0.54	93.38±1.69	11.719±0.47	1.285±0.11	0.227±0.02	17.69±0.243	638±5.03	78±1.53	13.3±0.67	90±0.58

Table 5. GCA, SCA, Hybrid vigour, average evaluation indices and cocoon size uniformity in promising multivoltine silkworm breeds / hybrids.

Breed / Hybrid	Fecundity	Hatching	Pupation rate	Yield/10,000 larvae by wt.	Cocoon wt.	Cocoon shell wt.	Cocoon shell (%)	Filament length	Reel-ability	Raw silk	Neatness	Average evaluation indices	Cocoon size uniformity
General combining ability effects of lines													
AGL_3	46.43***	0.051	0.333	0.423***	0.031**	0.015***	0.505***	9.900	-0.111	0.696**	-0.544	53.90	185.97±8.09
AGL_5	-4.23	0.354	0.227	0.584***	0.046**	0.017***	0.419**	30.033*	1.356*	0.402*	1.189**	63.80	183.40±9.64
Specific combining ability effects and hybrid vigour													
$AGL_3 \times CSR_2$	-25.87 (-7.56)	0.664 (1.02)	-0.722 (3.22*)	0.170 (46.63**)	0.037* (37.67**)	0.016** (50.12**)	0.382 (11.43**)	38.267 (30.68**)	1.667 (-0.20)	0.593 (9.60**)	1.656* (-0.18)	65.25	167.42±7.08
$AGL_5 \times CSR_2$	31.12** (-8.21)	0.154 (0.38)	-0.016 (3.13*)	1.379*** (53.90**)	0.134*** (39.31**)	0.039*** (50.57**)	0.528* (9.46**)	32.467 (29.97**)	2.200 (1.00)	0.253 (4.84**)	-0.078 (-0.18)	68.52	161.72±6.62

Data in parentheses are hybrid vigour over mid parent value; *, ** and *** denote significant difference at 5%, 1% and 0.1% level respectively.

Table 6. Performance of promising multivoltine × bivoltine hybrids in TVDC (Mean of 3 trials).

Hybrid	Cocoon wt (g)	Cocoon shell wt (g)	Cocoon shell (%)	Yield/100 dfls (kg)	Cocoon rate / kg (Rs.)
$AGL_3 \times CSR_2$	1.806 (5.92)	0.364 (14.47)	20.15 (8.04)	71.945 (4.81)	137.76 (20.81)
$AGL_5 \times CSR_2$	1.879 (10.21)	0.383 (21.38)	20.38 (9.28)	76.359 (11.24)	137.03 (20.17)
$PM \times CSR_2$ (Control)	1.705	0.318	18.65	68.643	114.03

Values in parentheses indicate per cent improvement over control.