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Development of cocoon colour sex-limited breeds/foundation crosses of silkworm *Bombyx mori* L. in the production of commercial hybrid

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Abstract
Indian sericulture industry depends on Poly x Bivoltine hybrids and are being reared over decades. The crossbreeds are prepared through the mating of polyvoltine female with bivoltine male parent. Which involves sex separation of pupae in bivoltine parent and only males are retained and females are rejected to avoid selfing. In this context, introduction of viable breeds with sex-limited traits *i.e*., the character found on only one sex at different stages of life cycle assume significance in silkworm seed production. This study was designed to develop sex limited breeds and foundation crosses for cocoon colour in the production of commercial hybrid. The advantages of sex limited foundation cross is easy at farmer's level with improved fecundity and reduced labour cost since the yellow cocoons can be reeled and males can be utilized in the preparation of cross breed. Keeping this as strategy, 10 productive bovoltine breeds along with the sex-limited donor (SLO1) were reared and based on the pupation rate and cocoon uniformity, the females of donor were crossed with the recipient males. The replication with consistency in yellow colour was selected at F1 wherein all the yellow cocoons are females and white cocoons are males. Cellular rearing was followed at early generation and back cross with recipient males was carried out from F1 - F6 generation to establish breed characters in addition to sex limited trait followed by selection and inbreeding was done from F7 to F12. After F12 generation, the consistency in the breed characters were assessed in the 10 productive sex-limited breeds. Further studies are in progress to establish sex-limited foundation crosses. The paper discusses on all the above aspects.

Keywords: Backcross breeding, Cocoon colour, Donor, Poly x Bivoltine hybrid, Recipient, Sex-limited breeds and foundation Cross

Introduction
The silk production in India depends mainly on Poly x Bivoltine hybrids. To meet the demanding agro-climatic conditions of tropical India and socio-economic status of farmer, development of new economically viable Poly x bivoltine hybrid is quite essential and the popularity of these hybrids is very prominent and 90% of the raw silk produced in our country is from Poly x bivoltine hybrid (Datta, 1994) [4]. The Andhra Pradesh State Sericulture and Research and Development Institute (APSSRDI) introduced 'Swarnandhra' in the state of Andhra Pradesh and it was very popular for a significant period and thereafter, Kolar gold (PM x CSR2) shown popularity and first hybrid was widely accepted by the reelers.

The crossbreeds in general practice are prepared in commercial silkworm seed production center through mating of polyvoltine female parent with bivoltine male parent. Due to this discarding of the respective other sex in both the parents could be avoided which otherwise result in selfing. Separation of sex at larval stage requires expertise which is difficult, hence, this practice is not being used for large scale sexing of larvae (Hirobe, 1968) [6]. Thus, sex separation is usually carried out at pupal stage is laborious too and is expensive and time consuming in addition to damage caused while cutting open the cocoons. Efforts were also made to sex separate at cocoon stage based on weight differences as average weight of female cocoon is usually higher than the male cocoon and this mechanical method was practically difficult and failed due to negligible variation or overlapping cocoon weights of opposite sexes either as a result of rearing lapse or racial characteristics. In this context, introduction of viable breeds with sex-limited traits *i.e*. the character found on only one

sex at different stages of life cycle assume great significance of practical utility to the sericulture industry, especially during silkworm seed production.

Tanaka (1916) [17] first discovered sex-limited inheritance in the silkworm and Hashimoto (1933) [5] showed that, the W chromosome is the determinant of femaleness through the finding that ZZW and ZZZW individuals of triploids and the tetraploids are normal females. Since the discovery of the sex-determining mechanism by Tanaka (1916) [17], many genes responsible for morphological traits have been mapped on the Z chromosome but no genes of morphological significance has been found on the W chromosome. Tazima (1951) [18], in his experiment showed that Z and W chromosomes are always segregated and has demonstrated that, a special translocation from the 2nd chromosome to one end of the W chromosome, which became marked by two noticeable genes ($+^{p}$ and $+^{sa}$) both for larval marking. Tazima (1951) [18] provided strong evidence to Hashimoto's theory. When the translocated dominant gene was present, the larvae carrying the marking were invariably females, while all the non-marked larvae were males. This discovery not only confirmed the females determining nature of the W chromosome but also led to the discovery of so-called sex-linked strain of practical utility to screen males and females at the larval stage. After this initial discovery, strain with sex-limited cocoon colour was developed by translocating the yellow cocoon colour gene from 2nd chromosome and the yellow colour gene from 10th chromosome to the W chromosome (Kimura *et al* 1971) [9]. Sex-limited strains became a reality and heralded a new era in the application of fundamental genetic principles to the commercial use in Sericulture. In all the sex-limited strains, whether the discrimination is at larval, egg or cocoon stage, females and males can be easily separated and little labour is required for such a task.

In addition, Mano *et al*., (1991) [11] has developed sex limited larval marking silkworm breeds viz., N147 and C145 by utilizing N123, N115 and Daizo as initial breeding resource material. The physical properties of silk filament of sex limited breed 'Ouhaku' was studied in relation to size and compared with male and female cocoons (Iizuka and Yang. 1993) [8].

At present, for the preparation of existing cross breed, the bivoltine cocoons procured by the grainages are to be sex separated and only males will be retained and females will be rejected. The bivoltine breed is a highly productive race and has to be reared only in the favourable season of the year *i.e*., between August and February. But to utilize bivoltine for the preparation of Cross Breed (PM x CSR2), the bivoltine breed has to be reared in summer also which causes stress to the parental breed and to avoid stress and increased survivability of the bivoltine pure breed in the field, the sex limited foundation crosses with cocoon colour has developed and released to the field. The advantages of sex limited foundation crosses is highly beneficial as the yellow cocoons (female) can be sent for reeling while the white cocoon (Male) can be utilized for hybrid preparation.

The males of foundation crosses are more active and highly vigorous when compared to the normal males. Realizing the difficulties of P1 seed farmer's, instead of releasing pure breeds, the foundation crosses were popularized at P1 seed farmer's level. The foundation crosses are highly advantageous as rearing is easier when compared to pure races. Assured crop of P1 at seed farmer's is highly beneficial to graineurs as pupation rate is higher than pure races and males are more active in foundation cross (Sohn *et al.,* 1987; Mal Reddy *et al.,* 2003) [14, 2]. Keeping this in view, the present work has been designed to develop sex-limited cocoon colour breeds and foundation crosses to use as male parent in the preparation of poly x bivoltine hybrid.

Materials and methods

'SLO1' is a sex-limited breed for cocoon colour (All yellow colour cocoons are female & white cocoons are males) which is being maintained in the germplasm repository of APSSRDI, was used as a donor parent for transferring the sex-limited cocoon colour character into 10 productive bivoltine breeds *i.e.* APS5, APS67, APS45, AP71, APSHTO5, APSHTO2, APS9, APDR105, APDR115 and AC9, of which some of them are authorized parents. 5 dfls each of the selected breeds were reared by following the standard rearing techniques (Basavaraja *et al*., 2002) [1]. Out of five replications, the replication with highest pupation rate and cocoon weight, shell weight and cocoon shell percentage as per the breed character were selected. The females of SLO1- donor were crossed with the recipient breed males and cellular rearing was followed. The replication with consistency in yellow colour was selected at F1. At every generation, the single cocoon assessment of selected cocoons was undertaken and at F2 colour segregation was observed and yellow (female) and white (male) coloured cocoons were selected.

An Index for cocoon shell weight was made as one of the important selection criteria (Basavaraja *et al.,* 2003) [2] as follows.

$$\text{Cocoon shell weight index} = \frac{\text{Shell weight of females}}{\text{Shell weight of males}} \text{ X } 100$$

By using this index, normally 3 categories of batches were selected having individuals with (i). Index value less than 100, (ii). Index value equal to 100 and (iii). Index value more than 100. In this particular breeding programme, the batches in the 3rd category were selected and continued.

The females with high shell weight when compared to the male cocoon was selected at every generation and cellular rearing was followed at early generation and back cross with recipient males was carried out from F1 - F6 generation to fix the breed characters in addition to sex limited trait and fixation of the metric traits from F7 to F12 (Fig. 1). After F12 generation, the consistency in the breed characters was assessed. The sex-limited cocoon colour breeds thus developed *i.e.* APS5(*SL*), APS67(*SL*), APS45(*SL*), AP71(*SL*), APSHTO5(*SL*), APSHTO2(*SL*), APS9(*SL*), APDR105(*SL*), APDR115(*SL*) and AC9(*SL*) (Fig. 2) will be further studied involving 45 sex-limited foundation crosses (SLFCs) will be prepared by following Partial Diallele Method and the performance of SLFCs as a male component with PM will be evaluated and identified the best combiner. Finally, the performance of PM x SLFC and PM x CSR2 will be evaluated and compared at farmer's level.

Fig 1. DEVELOPMENT OF SEX-LIMITED BREEDS

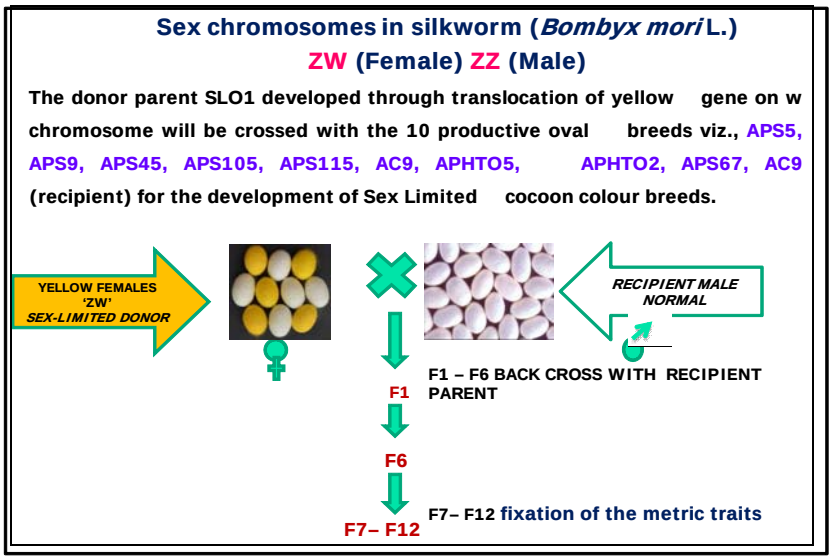

Fig 1: Development of sex-limted breeds

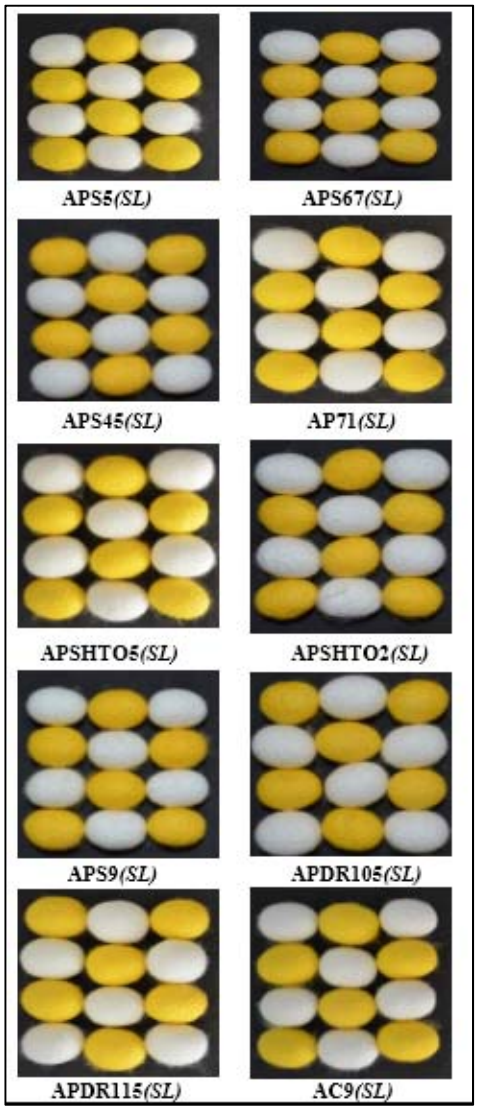

Fig 2: Sex-limited breeding lines

Results and discussion

Sex-limited character for cocoon colour was introduced in to productive bivoltine breeds and developed 10 sex-limited (SL) breeds for cocoon colour *viz.*, APS5(*SL*), APS67(*SL*), APS45(*SL*), AP71(*SL*), APSHTO5(*SL*), APSHTO2(*SL*), APS9(*SL*), APDR105(*SL*), APDR115(*SL*) and AC9(*SL*). During the development of sex-limited breeds the index for cocoon shell weight was made as one of the important selection criteria especially for improving the yellow cocoon shell. In the present study the batch with more than 100 index value was selected. The perusal of data (Table 1) for pupation rate ranges from 90.2 (APS5SL) to 96.9 % (APS45SL). The shell ratio ranges from 21.40 to 23.12%. The performance of these SL breeds is on par with their parents along with the sex-limited character for cocoon colour.

Generally, when the donor female is used for transferring the yellow gene into the productive breeds there is a possibility of transferring deleterious effects along with yellow colour sex-limited trait into the productive recipient parents but these undesirable characters were eliminated through repeated back cross breeding with the recipient parent after F1 generation followed by selection and inbreeding for desired traits as envisaged in the study. More over the 'W' chromosome bearing the yellow gene (y) which is responsible for the yellow colour cocoon trait hence, these yellow cocoons (females) under perform because of hyperploidy as compared to white (male) cocoons. In normal cases also, where 'W' chromosome does not carry any translocated part of the autosomes, females are less viable when compared to males (Nagaraju *et al.*, 1989)[12].

The cocoon production in India is largely from the hybrids of indigenous polyvoltine females and males of bivoltine breeds. As a result, the separation of males and females to prevent the mating between the individuals of the same parental race is very important for the preparation of true hybrids. Due to practical difficulties of male and female separation at larval or cocoon stages, separation of sexes is generally carried out at pupal stage based on the sexual marking on the abdomen. This method involves cutting open of every cocoon rendering the cut cocoons unfit for reeling. Besides, this involves lot of labour to produce large number of hybrid layings. In bivoltines, weight differences between male and female cocoons for separation is not encouraging due to the overlapping of the cocoon weight of both males and females.

In this context, relevance of sex-limited breeds to Indian sericulture has been realized and breeding of viable sex-limited cocoon colour bivoltine silkworm breed NB4D2 (SL) has been developed (Mal Reddy *et al*., 2000) [1]. Such sex-limited breeds have been encouraged for commercial use in sericulture and proved to be highly useful tool in the preparation of polyvoltine x bivoltine hybrid. More recently, a new cocoon colour sex- limited breed CSR2 (SL) "Nandi" was developed for utilizing it as a male component with polyvoltines especially with Pure Mysore to meet the growing demand of cross breed layings at the commercial level (Basavaraja *et al*., 2004) [3]. To reduce the cost of production in the grainages, commercialization of sex limited races for the production of Poly x Bi hybrids is very much essential. The sex limited strains assume special significance for use as male component with polyvoltine females to produce F1 hybrids. The combining ability of Poly x bi hybrids by utilizing sex-limited cocoon colour breeds as male component revealed no difference in the hybrids of PM x Bi v/s PM x Bi (SL) (Sudhakara Rao *et al.*, 2002) [15]. In light of the above, the present work has been carried out on the evaluation of sex limited cocoon colour bivoltine breeds and foundation crosses as male component with polyvoltine breeds and their impact on seed production.

Table 1: Performance of sex-limited breeding lines

Sl. No	Combination	Fecundity (No.)	Hatch-ing (%)	Cocoon Yield/ 10000L by (wt)	Pupation Rate (%)	Single cocoon wt. (g)	Single shell wt. (g)	Shell %	Filament Length (m)	Reel-ability (%)	Neat-ness (P)
1	APS5SL	510±30	96.2±2.3	16.12±0.21	90.2±3.3	1.629±0.028	0.363±0.004	22.28±0.1	925±32	86.1±2.3	88±2
2	APS67SL	530±14	98.9±1.1	17.36±0.25	92.2±2.2	1.721±0.020	0.398±0.011	23.12±0.3	1050±12	88.0±1.2	89±1
3	APS45SL	512±21	96.5±0.5	16.10±0.15	96.9±0.4	1.643±0.026	0.361±0.003	21.97±1.0	1002±9	89.6±0.3	88±2
4	AP71SL	497±37	98.1±1.3	17.12±0.36	94.0±1.3	1.741±0.034	0.390±0.010	22.40±0.5	930±19	90.2±1.1	86±4
5	APSHTO5SL	505±11	97.1±1.2	16.01±0.24	92.9±3.4	1.615±0.043	0.363±0.004	22.47±0.2	1000±12	90.9±1.5	87±3
6	APSHTO2SL	509±13	93.6±2.2	16.26±0.38	91.8±1.4	1.729±0.027	0.375±0.005	21.68±2.1	975±21	88.1±1.4	86±4
7	APS9SL	496±40	95.3±3.4	16.24±0.47	92.0±2.3	1.728±0.023	0.367±0.003	21.23±1.0	950±46	89.3±2.4	86±4
8	APDR105SL	524±12	97.1±0.2	16.22±0.10	91.8±2.2	1.644±0.015	0.362±0.012	22.01±0.3	1010±21	86.5±3.3	87±3
9	APDR115SL	526±18	96.5±0.3	17.26±0.22	90.9±3.3	1.761±0.029	0.394±0.014	22.37±0.8	1025±24	85.9±4.1	88±2
10	AC9SL	514±34	96.2±1.4	16.20±0.32	92.5±2.2	1.727±0.014	0.372±0.006	21.54±1.2	1000±14	86.2±2.4	89±1
11	SLO1 (Control)	505±23	97.6±1.0	16.02±0.13	90.2±1.3	1.621±0.022	0.345±0.011	21.30±1.1	927±45	84.3±1.3	85±2

Values represent an average of 3 replications with 250 larvae/replication ± S.D

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