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Evaluation of indigenous bivoltine silkworm (*Bombyx mori* L.) Hybrids under sub-tropical conditions

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Abstract
The investigation was carried out to evaluate ten silkworm bivoltine hybrids raised by involving half (5 × 5) diallel set of bivoltine breeds including five hypersericigenous (NSO, SPO, SPDL, Z and ZZ) breeds along with ruling bivoltine hybrid, $SH_6 \times NB_4D_2$ in order to identify the adaptable, high yielding hybrids suited for sub-tropical conditions. Observations were made on twelve economically important traits namely; larval weight, larval survival, cocoon yield per 10,000 larvae (by weight and by number), good cocoon, pupation, single cocoon weight, single shell weight, shell ratio, average filament length, non-breakable filament length and denier. The data was analyzed using Evaluation index (E.I.) method. Six hybrid combinations recorded mean evaluation index (E.I.) values of >50 ranging from 51.63 to 57.51, whereas, control scored E.I. value of 31.38 only. Three hybrid combinations, Z × SPO (57.51), SPDL × NSP (56.89) and SPO × NSP (56.84) recorded Average E.I. value >56 for all the characters under sub-tropical conditions.

Keywords: bivoltine, adaptable, hypersericigenous, heterosis, evaluation index (E.I.)

1. Introduction
Silkworm, *Bombyx mori* L. spins valuable silk fiber, making it the most beneficial insect to mankind. Sericulture being an agro based and welfare oriented industry is capable of providing employment to a larger section of people as a subsidiary occupation in rural areas (Trivedi and Sarkar, 2015) [28]. The trend of sericulture development in India has shown a quantum jump in mulberry silk production with an annual production of 20,000 MT during the last three decades (Lakshmanan and Kumar, 2012) [10] and it enjoys the comfortable second position in the production of silk in the world next only to China, in which silkworm breeding and development of bivoltine hybrids have played a vital role. India is predominantly a tropical country with marginal sub-tropical and temperate sericulture zones. Mostly multi × bivoltine hybrids are reared in tropical areas of the country which do not meet the international standards, thus, there is great need and scope for improving the bivoltine sericulture of the country (Moorthy *et al*., 2007) [15]. As a result, more emphasis needs to be given to bivoltine sericulture, which is the need of the hour if India has to produce international silk grade. Realizing the importance of bivoltine sericulture, efforts are being made by silkworm breeders of the country to evolve high yielding bivoltine silkworm breeds for commercial purpose Rao *et al*. (2006) [20]. Most of the silkworm breeding programmes now-a-days are oriented towards boosting the bivoltine silk yield and fiber quality.

The northern states of India, such as Jammu & Kashmir, Himachal Pradesh, Uttrakhand and some pockets of Punjab assumes special significance due to its salubrious climate congenial for rearing of bivoltine silkworms and these states has observed an increasing trend in cocoon production and has a high potential for bivoltine sericulture activities (Datta *et al*., 2001) [2]. The state produces quality bivoltine cocoons for the production of silk yarn and has the potential to increase the raw silk provided locally developed region and season specific hybrids are commercially exploited. In order to meet the diverse requirements of the silk industry, different silkworm hybrids, tolerant to high temperature and high humidity, needs to be developed so that they get well adapted under local climatic conditions for stable cocoon production. Thus, there is a need to evaluate highly adaptive indigenous productive hybrids suitable for this region. Considering the economic importance of silkworm rearing as an employment and income generating activity, therefore, the present investigation was aimed to develop and evaluate indigenous silkworm hybrids for spring season that can results in better yield, adaptability and quality for successful commercial exploitation of bivoltine crop.

2. Materials and Methods

The present investigation was conducted during spring 2015 at Division of Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu with an intention to develop, evaluate and identify new bivoltine hybrid combinations suitable for rearing during spring season in sub-tropical conditions of northern India. For this purpose, five hypersericigenous bivoltine silkworm breeds (NSO, SPO, SPDL, Z and ZZ) evolved at Division of Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu were taken to prepare bivoltine hybrids by employing half diallel method (Table 1) and studied along with ruling bivoltine hybrid, $SH_6 \times NB_4D_2$ in order to identify the adaptable, high yielding bi × bivoltine silkworm hybrids. These hybrids along with the control were reared in the completely randomized block design (CRBD) with three replications each. Standard rearing techniques as suggested by Dandin *et al*. (2003) [1] were followed. Data was recorded replication- wise for all the treatments viz., larval weight, larval survival percentage, cocoon yield per 10,000 larvae (by weight and by number), good cocoon percentage, pupation percentage, single cocoon weight, single shell weight, shell ratio, average filament length, non-breakable filament length and denier and the data was analyzed using multiple trait index (Evaluation index) method.

The Evaluation Index method developed by Mano *et al.* (1993) [12] was found to be very useful in selecting potential hybrids. Data on the economically important traits was collected, pooled and analyzed. The Evaluation Index (E.I.) was calculated as per the procedure:

$$\text{E.I.} = \frac{\text{A - B}}{\text{C}} \times 10 + 50$$

Where, A = Value obtained for a particular trait of a particular hybrid combination

B = Mean values of a particular trait of all the hybrid combinations

C = Stand deviation of a particular trait of all the hybrid combinations

10 = Standard Unit

50 = Fixed Value

The E.I. value fixed for the selection of hybrid is 50 or >50 for the traits. The hybrid which scored above the limit is considered to possess greater economic value.

3. Results and Discussion

Mulberry silkworm, *Bombyx mori* L. is an insect of economic importance, which is commercially exploited for silk. Ultimate objective of silkworm breeding is not only to synthesize new genotypes but also to identify sustainable silkworm hybrids for commercial purpose. In order to improve the yield and quality of silk, it is pertinent to characterize the potential of available genetic resources for transferring the desirable components in hybrids. Selection of parents on the basis of performance does not always lead to fruitful results. More diverse the parents more are the chances of heterosis (Talebi and Subramanya, 2009) [26]. The earnest efforts of silkworm breeders have resulted in the evolution of a large number of silkworm strains expressing well-defined qualitative and quantitative traits (Singh *et al*., 2006) [25]. The introduction of hybrid concept for greater productivity and evaluation of hybrids derived from the selected pure lines is undoubtedly the most widely tested method for identifying the superiority of the hybrid. Realizing the need, many productive hybrids has been developed continuously and made available to the industry by the breeders (Singh and Gangopadhyay, 2013) [24].

Silkworms are voracious eaters of mulberry during its larval stages and around 80 percent leaf is consumed in the last two instars (Fukuda, 1960) [4]. Highlighting the importance of food intake, Horie *et al.* (1978) [6] reported that for the production of 1 g larval dry weight, requirement of ingestion and digestion of food is 4.2 mg and 1.8 mg respectively. The intake of food during total larval life is also reflected by the larval weight and is a cocoon and shell contributing parameter. For hybrids, maximum E.I. value of 73.84 was recorded in hybrid Z × SPO followed by SPDL × SPO (65.04) and control hybrid, ($SH_6 \times NB_4\ D_2$) recorded E.I of 47.07. Minimum E.I. of 30.99 was found in Z × SPDL followed by SPDL × NSP (35.95) (Table 2). This may be attributed to higher digestibility of food leading to higher weight gain during V instar. Yamamoto and Gamo (1976) [29] also reported positive correlation between food ingestion and weight of mature larvae. This reflects higher consumption of mulberry leaves. The present study reveals that the bivoltine worms which consumed more mulberry leaves and attained robust growth at larval stage resulted in the higher larval weight and ultimately cocoon weight, shell weight and raw silk production. Present finding is supported by Kumar *et al.,* (2013) [8] who found similar results. Commercially larval survival constitutes an important character for rearer's point of view that contributes to produce a more number of cocoons for better crop production. Hybrid, Z × NSP displayed maximum E.I. value of 69.23 followed by SPO × NSP (65.72), whereas, minimum E.I. of 34.14 was displayed by hybrid ZZ × Z. Control hybrid $SH_6 \times NB_4D_2$ scored E.I of 23.61 only (Table 2). The significant variation in larval survival can be attributed to rearing conditions and the occurrence of disease or may be due to the non-adaptability. Ohi *et al*. (1970) [16] also worked out multiple co-relations between yield components and found that larval survival is directly correlated to number of cocoons harvested. This is in accordance with the findings observed in the present study.

Silkworm cocoon is important commercial and economic product of rearing. Cocoon characters are commercially most important and do have close relation with mulberry leaves as food. Minagava and Otsuka (1975) [14] has reported interrelationship between multiple characters in silkworm. It therefore becomes essential to evaluate the breeds and their hybrids to understand the magnitude of potential and heterosis towards improvement in a cocoon and silk productivity as suggested by Gowda *et al*. (2013) [5]. Malik *et al.* (2006) [11] suggested that cocoon yield/10,000 larvae by weight and by number, good cocoon percentage, pupation percentage, single cocoon weight, shell weight and shell ratio percentage are important parameter for quality cocoon crop as well as potential hybrid. The cocoon yield per 10,000 larvae (by weight) depicted maximum E.I. value of 65.71 in hybrid SPDL × NSP followed by hybrid SPO × NSP 61.93), while as the lowest E.I. value for this trait was observed in hybrid ZZ × NSP (32.77). Control hybrid $SH_6 \times NB_4D_2$ scored least E.I. of 28.82 only (Table 3). Cocoon yield by number is an important parameter for contributing viability. Maximum E.I. value for cocoon yield per 10,000 larvae (by number) was recorded in hybrid Z × NSP (65.49) followed by hybrid SPO × NSP (64.15), while as the lowest E.I. value for this trait was observed in hybrid ZZ × Z (33.36). Control hybrid SH_6 × NB_4D_2 scored least E.I. of 18.63 only (Table 3). The higher yield (by weight and by number) in these hybrids may be due to higher larval survival rate. Positive correlation for cocoon yield, single cocoon weight and the hatching percentage has been reported by Kumar *et al*. (2012) [9] which is in conformity with the present findings. Saratchandra *et al.* (2002) [22] also reported mulberry varietal effect on cocoon yield by weight and number. Good cocoon and pupation percentage is a positive sign for cocoon reeling performance as well as seed production. These are generally influenced by rearing environment and other abiotic factors. The genetic and environment gets more reflected in this character. Maximum E.I. value of 65.00 for good cocoon percentage was recorded by hybrid Z × NSP followed by SPO × NSP (63.95), while as hybrid Z × ZZ scored a minimum E.I. value of 38.84 (Table 3). For pupation rate, maximum E.I. value of 63.58 was scored by SPO × NSP and Z × NSP worms followed by SPDL × NSP (56.01) and lowest E.I. value of 28.23 in Z × ZZ. Control hybrid SH_6 × NB_4D_2 scored E.I. of 20.65 only (Table 3). Uniformity in these characters can be attributed to the rearing practices rather than food type. Major factors among these include spacing, hygiene, seriposition material and appropriate time for picking of mature larvae for seriposition. The observations are in accordance with the findings of Gowda *et al*. (2013) [5]. The cocoon weight, shell weight and shell ratio are important commercial parameters of cocoon for yield and silk reeling performance. The cocoon weight has a negative correlation with shell ratio but positive correlation with shell weight whereas, shell weight has positive correlation with shell ratio. Maximum E.I. value of 83.64 was recorded in hybrid SPDL × NSP for single cocoon weight followed by Z × SPO (62.73), while as hybrid ZZ × NSP obtained minimum E.I. value of 34.54 only. Control hybrid SH_6 × NB_4D_2 recorded E.I. of 37.27 (Table 3). This character depicts the superiority of hybrids and indicates that the phenomenon of heterosis could be either due to additive gene action or due to dominance as reported by Petkov (1989) [17]. Single shell weight contributes to the silk content. In the present study, maximum E.I. value of 75.00 was achieved by Z × SPO followed by SPDL × NSP (65.00). The control hybrid could not qualify the index value of 50 for single shell weight (Table 3). Similar trend with respect to shell weight was observed by Maqbool *et al.* (2008) [13]. Shell ratio is an important parameter of quality depicting actual silk content of a cocoon. For shell ratio, maximum E.I value of 55.72 was depicted by hybrid Z × SPO followed by ZZ × SPO (55.56) and a minimum value of 33.14 was recorded in hybrid SPDL × NSP. The control hybrid scored E.I of 18.47 for said trait (Table 3). Saratchandra *et al.* (2002) [22] has reported that superior mulberry varieties, particularly triploids responsible for higher cocooning characters. Similar findings were also recorded by Rao *et al*. (2006) [20].

Post cocoon/reeling characters may not be of much importance to rearer but have greater significance not only from reeler's point of view but also from industrial view. Three post-cocoon parameter viz. total filament length, non-breakable filament length and denier (filament size) mainly contribute towards the end product i.e. silk. In the present study, maximum E.I. value for total filament length was achieved in ZZ × SPO (78.01) followed by SPDL × NSP (67.18) and minimum value of 38.96 was recorded in hybrid ZZ × NSP. Control hybrid SH_6 × NB_4 D_2 recorded E.I. value of 47.70 (Table 4). This finding can be attributed to longer larval duration and higher larval weight (Satenahalli *et al.*, 1999) [23]. Increase or decrease in filament length is dependent on the increase or decrease in the thickness of silk filament and cocoon shell weight of breeds and hybrids. Rajalakshmi *et al.* (2000) [19] opines that the quality of a good hybrid is to have minimum or no breaks during the process of reeling. Hybrid ZZ × SPO scored maximum E.I value to the tune of 83.47 for non-breakable filament length followed by SPDL × NSP (64.15) and hybrid ZZ × Z scored minimum E.I value of 39.73 for the said trait. Control hybrid, SH_6 × NB_4D_2 recorded the E.I. of 48.28 for non-breakable filament length (Table 4). Similar were the findings of Kumar *et al*. (2013) [8]. Denier represents the thinness or thickness of the filament and maximum E.I value was recorded in ZZ × SPDL (63.46) closely followed by ZZ × NSP (63.08). Hybrid ZZ × SPO scored least E.I. value of 40.38 and control hybrid SH_6 × NB_4D_2 recorded E.I. of 38.08 only (Table 4). Thiagarajan *et al*. (1993) [27] reported that silk yield is the main contributing factor among major twenty characters usually considered by the breeders. Premlatha *et al*. (2000) [18] suggested that low magnitude of heterosis in hybrids for particular traits indicates the presence of partial dominance. The results are in accordance with the findings of Dayananda *et al*. (2011) [3].

In the silkworm (*Bombyx mori* L.) even though the parental strains are superior they do not have much value if the same is not refuted in the hybrids. The ultimate results in silk worm breeding are judged by the excellency of commercial traits the appear in the hybrids (Reddy *et al*., 2012) [21]. Therefore large numbers of hybrids are tested and promising ones are selected based on the economic traits (Kumar and Naik, 2011) [7]. Evaluation of different breed/hybrids is undoubtedly the most important method to identify their superiority. This could be achieved precisely by adopting a common index giving adequate weight age to all the commercially important traits (Reddy *et al*., 2012) [21]. Based on the superior index values for twelve qualitative and quantitative traits six hybrid combinations were short listed with average E.I. value >50. The hybrids were: Z × SPO (E.I. 57.51) followed by SPDL × NSP (E.I. 56.89), SPO × NSP (E.I. 56.84), ZZ × SPO (E.I. 55.43), Z × NSP (E.I. 54.80) and ZZ × SPDL (E.I. 51.63). Control hybrid, SH_6 × NB_4D_2 stood at cumulative E.I. value of 31.38 only (Table 5). Thus, based on the Evaluation Index values for qualitative and quantitative traits three hybrid combination viz., Z × SPO, SPDL × NSP and SPO × NSP (Table 5) were identified as potential combinations both by evaluation and ranking through E.I. values respectively.

Table 1: Half diallel crosses of five parental breeds

Breeds	NSP	SPO	SPDL	Z	ZZ
NSP	NSP × NSP				
SPO	SPO × NSP	SPO × SPO			
SPDL	SPDL × NSP	SPDL × SPO	SPDL × SPDL		
Z	Z × NSP	Z × SPO	Z × SPDL	Z × Z	
ZZ	ZZ × NSP	ZZ × SPO	ZZ × SPDL	ZZ × Z	ZZ × ZZ

Control – SH_6 × NB_4D_2
No. of Parents – 5 (n)
Half diallel formula = n (n-1)/2

= 5(5-1)/2
= 5(4)/2
= 20/2
= 10

Table 2: Evaluation Index values of bivoltine silkworm hybrids for larval characters

Traits → Hybrids ↓	Larval weight (g)	Larval survival (%)
SH6 × NB_4D_2 (control)	47.07	23.61
SPO × NSP	52.89	65.72
SPDL × NSP	35.95	55.19
SPDL × SPO	65.04	48.18
Z × NSP	56.16	69.23
Z × SPO	73.84	49.93
Z × SPDL	30.99	46.45
ZZ × NSP	39.01	46.45
ZZ × SPO	57.73	48.18
ZZ × SPDL	54.09	49.93
ZZ × Z	37.64	34.14

Table 3: Evaluation Index values of bivoltine silkworm hybrids for cocoon characters

Traits → Hybrids ↓	Cocoon yield/10,000 larvae		Good cocoon (%)	Pupation (%)	Single cocoon weight (g)	Single shell weight (g)	Shell Ratio (%)
	By weight (kg)	By number					
SH_6 × NB_4D_2 (control)	28.82	18.63	18.92	20.65	37.27	30.00	18.47
SPO × NSP	61.93	64.15	63.95	63.58	55.45	60.00	48.39
SPDL × NSP	65.71	56.12	54.53	56.01	83.64	65.00	33.14
SPDL × SPO	50.34	50.76	53.49	50.95	49.09	50.00	51.93
Z × NSP	59.49	65.49	65.00	63.58	49.09	50.00	52.66
Z × SPO	62.94	52.10	51.40	53.48	62.73	75.00	55.72
Z × SPDL	37.56	45.41	44.08	45.90	40.00	40.00	51.21
ZZ × NSP	32.77	45.41	43.03	43.38	34.54	30.00	46.53
ZZ × SPO	51.68	49.23	49.30	48.43	48.18	55.00	55.56
ZZ × SPDL	49.24	50.76	52.44	50.95	48.18	50.00	53.63
ZZ × Z	36.47	33.36	38.84	28.23	41.82	40.00	50.24

Table 4: Evaluation Index values of bivoltine silkworm hybrids for post-cocoon characters

Traits → Hybrids ↓	Total filament length (m)	Non-breakable filament length (m)	Denier (d)
SH_6×NB_4D_2 (control)	47.70	48.28	38.08
SPO × NSP	44.38	45.50	56.15
SPDL × NSP	67.18	64.15	46.15
SPDL × SPO	40.06	41.95	44.61
Z × NSP	40.43	42.28	44.23
Z × SPO	45.86	46.72	60.38
Z × SPDL	47.95	48.49	61.92
ZZ × NSP	38.96	48.28	63.08
ZZ × SPO	78.01	83.47	40.38
ZZ × SPDL	52.01	44.83	63.46
ZZ × Z	45.12	39.73	60.38

Table 5: Cumulative Evaluation Index values of bivoltine hybrids for twelve commercial traits

Hybrids → Traits ↓		SH6 × NB_4D_2 (control)	SPO × NSP	SPDL × NSP	SPDL × SPO	Z × NSP	Z × SPO	Z × SPDL	ZZ × NSP	ZZ × SPO	ZZ × SPDL	ZZ × Z
Larval weight		47.07	52.89	35.95	65.04	56.16	73.84	30.99	39.01	57.73	54.09	37.64
Larval Survival		23.61	65.72	55.19	48.18	69.23	49.93	46.45	46.45	48.18	49.93	34.14
Cocoon yield /10,000 larvae	By weight	28.82	61.93	65.71	50.34	59.49	62.94	37.56	32.77	51.68	49.24	36.47
	By number	18.63	64.15	56.12	50.76	65.49	52.10	45.41	45.41	49.23	50.76	33.36
Good cocoon		18.92	63.95	54.53	53.49	65.00	51.40	44.08	43.03	49.30	52.44	38.84
Pupation		20.65	63.58	56.01	50.95	63.58	53.48	45.90	43.38	48.43	50.95	28.23
Single cocoon weight		37.27	55.45	83.64	49.09	49.09	62.73	40.00	34.54	48.18	48.18	41.82
Single shell weight		30.00	60.00	65.00	50.00	50.00	75.00	40.00	30.00	55.00	50.00	40.00
Shell ratio		18.47	48.39	33.14	51.93	52.66	55.72	51.21	46.53	55.56	53.63	50.24
Total filament length		47.70	44.38	67.18	40.06	40.43	45.86	47.95	38.96	78.01	52.01	45.12
Non-breakable filament length		48.28	45.50	64.15	41.95	42.28	46.72	48.49	48.28	83.47	44.83	39.73
Denier		38.08	56.15	46.15	44.61	44.23	60.38	61.92	63.08	40.38	63.46	60.38
Cumulative E.I.		31.38	56.84	56.89	49.69	54.80	57.51	44.99	42.62	55.43	51.63	40.49

4. Conclusion

Thus based on the investigation, it can be concluded that hybrid, Z × SPO (57.51) followed by SPDL × NSP (56.89), SPO × NSP (56.84), ZZ × SPO (55.43), Z × NSP (54.80) and ZZ × SPDL (51.63) surpassed the E.I. bench mark value >50. Thus, based on the Evaluation Index values for qualitative and quantitative three hybrids viz. Z × SPO (57.51), SPDL × NSP (56.89) and SPO × NSP (56.84) were identified as potential hybrids both by evaluation and ranking through E.I. values respectively. These hybrids can be commercially exploited for sub-tropics for spring season after multi locational trials.

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