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Evaluation of the Reproductive Potential of Bivoltine Silkworm Hybrids of *Bombyx Mori* L under High Temperature and High Humidity and High Temperature and Low Humidity Conditions of the Tropics

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Abstract:
The challenging task of breeders is to develop silkworm breeds which can produce eggs under prevailing conditions of temperature and humidity. The present study was undertaken to evaluate the reproductive potential of single and double hybrids under high temperature and high humidity as well as high temperature and low humidity conditions of the tropics. The moth emergence percentage, laying recovery percentage, egg recovery/kg cocoon, number of eggs/gram and percentage of unfertilized eggs were calculated and compared among single and double hybrids.

Keywords: *Bombyx mori*, reproductive potential, single hybrid, double hybrid, foundation cross

1.0 Introduction:
Silkworm breeding aims to achieve superior performances in respect of egg yield, cocoon raw silk yield, cocoon stability and production followed by expansion to new areas besides others. Silkworm breeders continue to strive for an inherent gain in resistance by incorporating resistant genes into the genetic backgrounds of high yielding temperate bivoltines. Besides this, the cocoon crop stability also relies more on improving other production technologies which have to be explored. It is interesting to note that in breeding experiments, besides choice of parents , selection and inbreeding the hybrids is very important which has to be carefully executed since both inbreeding and hybridization are forms of non-random mating or selective mating, but operate in opposite ways. Inbreeding is a kind of genetic assortative mating as compared with phenotypic assortative mating in hybridization. The major effect of inbreeding which is most apparent in the reduction of mean performance of the population in question. While gene frequencies do not change on the whole, genotypic frequencies do change towards the production of more homozygotes and fewer hetrozygotes. Thus, any change in the population mean as a result of inbreeding must be related to difference in genotype value between homozygote and heterozygote (Suresh Kumar, 2005).

The success of sericulture industry depends upon several factors of which the impact of the environmental factors such as biotic and abiotic factors is of vital importance. Among the abiotic factors, temperature plays a major role on growth and productivity of silkworm, as it is a poikilothermic (cold blooded) insect (Benchamin and Jolly, 1986). It is also known that the late age silkworms prefer relatively lower temperature than young age and fluctuation of temperature during different stages of larval development was found to be more favourable for growth and development of larvae than constant temperature. There are ample literature stating that good quality cocoons are produced within a temperature range of 22-27°C and above these levels makes the cocoon quality poorer (Krishnaswami *et al.,* 1973). However, polyvoltine races reared in tropical countries are known to tolerate slightly higher temperature (Hesieh *et al.,* 1995), which is also true with cross breeds, that have been evolved specially for tropical climate.

Fecundity and fertility are the two main factors of seed cocoon production. Several factors affect the fecundity and fertility of silkworm races including aberrations in sex organ, faulty handling of moth during mating and egg laying, defective preservation of cocoons and environmental stress during larval rearing and cocoon storage (Biram et al., 2009). Temperature and humidity are key environmental factors that influence the physiology of insects. The range of adaptations to changing environments and maintenance of homeostasis is a complex and dynamic display of species inherent potential to keep internal changes within tolerable limits under wide fluctuations in their surroundings. The silkworm is sensitive to environmental fluctuations and unable to survive naturally due to continuous domestication since the dawn of sericulture. This has resulted in varied response to the environmental conditions (Temperature, humidity and photoperiod etc.) as compared to wild insect species. It means a significant interaction of environmental conditions and developmental stages governs the physiology of silkworm which affect the growth, development, productivity and quality of silk.

The challenging task of developing silkworm breeds which can produce eggs under prevailing conditions of temperature and humidity. Perusal of literature reveals that no work has been carried out on the effect of temperature and relative humidity fluctuations on the fecundity and fertility of silkworm lines. The present study, therefore, was conducted to find out the effect of variations in temperature and humidity during rearing on silk worm egg production and egg-fertility of the silkworm, *B. mori.*

2.0 Materials and Methods:

The main objective of the study was to identify silkworm hybrids tolerant to high temperature and high and low humidity conditions. Considerable variation exists among the bivoltine silkworm breeds at different temperature and humidity conditions, which opens an avenue for selection of temperature tolerant breeds suitable for tropical conditions.

2.1 Materials

The following breeds were screened under high temperature conditions to select suitable breeding resource materials to develop suitable single and double hybrids.

1) CSR2, 2) CSR3, 3) CSR4 , 4) CSR5, 5) CSR6, 6) CSR12, 7) CSR16, 8) CSR17, 9) CSR18, 10) CSR19, 11) CSR26, 12) CSR27, 13) CSR46, 14) CSR47) 15) CSR48, 16) CSR50, 17) CSR51, 18) S2, 19) S8, 20) D7, 21) D20 and 22) NB4D2

2.2 Methods

Silkworm rearing was conducted following the standard method under recommended temperature and RH till 2nd day of 5th instar. Three trials with three replicates were conducted for this study. On the third day of 5th instar, 200 larvae each for two thermal treatments (40 ± 1°C and 50 ± 5 % RH and 40 ± 1°C and 85 ± 5 % RH) were separated from each bed for thermal treatment. The remaining larvae in the tray were treated as control at normal temperature and humidity (25 ± 1°C and 65 ± 5 % RH). For thermal exposure, the larvae of 3rd day of the 5th instar were kept in plastic trays and reared in SERICATRON (Environment chamber with temperature and humidity control) at two different temperatures and humidity. Three replicates were kept in 40 ± 1°C and 50 ± 5 % RH and 40 ± 1°C and 85 ± 5 % RH and were fed with fresh mulberry leaves twice a day. The thermal exposure was given everyday for 6 hours duration. When the larvae started spinning, they were shifted to 25 ± 1°C and 65 ± 5 % RH. Plastic collapsible mountages were used for mounting the ripened larvae. After 48 hours of mounting, when the larvae formed hammock, the mountages were turned upside down. Cocoon harvesting was carried out on the 7th day of spinning. The cocoons were deflossed and the defective ones were sorted out. Assessment was carried out on the subsequent day. The survival rate was calculated as the number of live pupae to the number of larvae treated.

When the larvae ripened they were picked up by hand and were applied in mountages for cocooning at 25 ± 1°c and 65 ± 5% relative humidity which is suitable temperature for spinning. Plastic collapsible mountages were utilized for mounting, the mountage were turned upside downward and the dead and diseased worms were rejected. The cocoons from he room temperature and both he high temperature batches were harvested separately from the mountages on the sixth/seventh day of spinning. The harvested cocoons were deflossed and defective ones such as stained, flimsy, melted, thin shelled, Uzi infested and double cocoons were sorted out form each tray. The good harvested cocoons from each replicates were counted, mass weighed and ten male and ten female cocoons were cut from each replicates and kept separately to carried out assessment on the subsequent day. The pupation rate was calculated as the number of live pupae to the number of larvae reared after counting of third moult, doubled cocoons, stained cocoons and Uzi infested cocoons were also considered for pupation rate. Further directional selection was employed to obtain stability based on pupation rate, cocoon shape, size, compactness of shell and wrinkles as important selection criteria.

To continue breeding programme and to obtain new progeny for each breed the selected cocoons were subjected for pupal sex separation. Race wise sex separation was carried out and kept for moth emergence. On the day of emergence female and male moths were applied for mating (inbreeding) for 3½ to 4 hours. Each pair individually picked and depaired and the gravid female moths were allowed for oviposition in semi dark room. The egg laid moths were collected and subjected to individual mother moth examination to ensure disease free layings. Twenty five – thirty disease free laying were prepared from each breed and treated with HCL acid within 24 hrs oviposition to break the diapause following the hot acid treatment methods of Yokoyama (1963). Layings were incubated at 25 ± 1°c temperature and 70-80% relative humidity in incubators with 16 hrs light and 8hrs darkness. By utilized 15-20 rich egg laying showing good hatching percentage layings were prepared from individual with good fecundity as well as good hatching % layings were prepared, wrapped in tissue paper hen blank boxed to synchronize the development of embryo once after eggs turned to blue colour on seventh/eighth day of incubation to ensure healthy and uniform hatching on expected day.

3.0 Results and Discussion:

3.1 Emergence percentage of pure breeds and foundation crosses:

The emergence percentage of the four selected pure breeds along with control breeds is presented in Table 1. The pupation rate among the pure breeds ranged from 81.9 to 94.9 % with the lowest of 81.9 % recorded for HH3 and the highest of 94.9% recorded for HL1. The emergence percentage of male moths ranged from 88.5 to 91.2 % with the lowest of 88.5 % recorded for CSR2 and the highest of 91.2 % recorded for HH8. The emergence percentage of female moths ranged from 87.5 to 90.1 % with the lowest of 87.5 % recorded for CSR2 and CSR4 the highest of 90.1 % recorded for HH3.

The emergence percentage of four foundation crosses along control foundation crosses is presented in Table 3. The pupation rate among the foundation crosses ranged from 92.5 to 93.6 % with the lowest of 92.5 % recorded for HL1 x HL3 and the highest of 93.6 % recorded for Hl10 x Hl12. The emergence percentage of male moths ranged from 88.1 to 91.7 % with the lowest of 88.1 % recorded for CSR2 x CSR27 and the highest of 91.7 % recorded for HH8 x HH12. The emergence percentage of female moths ranged from 86.2 to 89.4 % with the lowest of 86.2 % recorded for CSR2 x CSR27 and the highest of 89.4 % recorded for HH1 x HH3.

Reproduction of identified single and double hybrids

Among the single hybrids, the reproductive efficiency of two identified hybrids viz., HL1 x HL7 for high temperature and low humidity and HH3 x HH8 for high temperature and high humidity along with its control CSR2 x CSR4 for productiveness were studied. Both sheet eggs and loose eggs were prepared. HH3x HH8 expressed high layings recovery of 83% followed by HL1 x HL7. The hybrid HH3 x HH8 expressed 3.75 improvement in laying recovery over the control CSR2 x CSR4. Similarly, HL1 x Hl7 showed 1.25 improvement over the control CSR2 x CSR4. During the course of preparation of loose eggs maximum recovery was recorded in HH3 x HH8 (61.36g/kg cocoon) followed by 59.65g/kg cocoon in HL1 x HL7 and control hybrid CSR2 x CSR4 with 57.5gm/kg. The new hybrids expressed improvement of 6.71% (HH3 x HH8) and 3.74% (HL1 x HL7) over the control hybrid CSR2 x CSR4 with regard to egg recovery/kg cocoon. Among two hybrids, 1813 grains/gm was recorded in HL1 x HL7 with 1.32 unfertilized egg percentage. HH3 x HH8 exhibited 1788 grains/gm with 2.13 unfertilized percentage.

Among the double hybrids, the reproductive efficiency of two identified hybrids viz., (HL1 x HL3) x (HL10 x HL12) for high temperature and low humidity and (HH1 x HH3) x (HH8 x HH12) for high temperature and high humidity along with its control (CSR2 x CSR27) x (CSR6 x CSR26) was studied. Both sheet eggs and loose eggs were prepared. (HH1 x HH3) x (HH8 x HH12) expressed high layings recovery of 83% followed by (HL1 x HL3) x (HL10 x HL12). The hybrid (HH1 x HH3) x (HH8xHH12) expressed 4.9% improvement in laying recovery over the control (CSR2 x CSR27) x (CSR6 x CSR26). Similarly, (HL1 x HL3) x (HL10 x HL12) showed 2.4 improvements in layings recovery over the control. During the course of preparation of loose eggs maximum recovery was recorded in (HH1 x HH3) x (HH8 x HH12) (84.95g/kg cocoon) followed by 82.48g/kg cocoon in (HL1 x HL3) x (HL10 x HL12) and control hybrid (CSR2 x CSR27) x (CSR6 x CSR26) with 80.60gm/kg. The new hybrids expressed improvement of 5.4% (HH1 x HH3) x (HH8 x HH12) and 2.3% (HL1 x HL3) x (HL10 x HL12) over the control hybrid (CSR2 x CSR27) x (CSR6 x CSR26) with regard to egg recovery/kg cocoon. Among two double hybrids, maximum of 1738 grains/gm was recorded in (HL1 x HL3) x (HL10 x HL12) with 3.68 unfertilized egg percentage. (HH1 x HH3) x (HH8 x HH12) exhibited 1680 grains/gm with 3.09 unfertilized percentage. The number of eggs/gm in (CSR2 x CSR27) x (CSR6 x CSR269 was lowest (1866/kg) indicates less numbers of grains/g.

Table1: Emergence percentage of pure breeds Mean of three trials)

Breed	Pupation rate (%)	Moth emergence %	
		Male	Female
HL1	94.9±0.78	90.7±0.42	89.9±1.13
HL7	94±0.49	90.3±0.57	90± 0.71
HH3	81.9±0.14	90.6±0.49	90.1±0.92
HH8	91.4±0.42	91.2±0.85	89.7±1.06
Control Breeds			
CSR2	88 ± 1.41	88.5±6.36	87±5.66
CSR4	88.5±4.95	90±4.24	87.5±4.95

Table 2: Grainage performance of the identified hybrids (Mean of three trials)

Hybrid	Laying recovery %			Egg rec. (g)/Kg cocoon	No. of eggs / grm.			UF%
	ULM	PL	GL		UF	Good	Total	
HL1xHL7	8±0.71	11±2.12	81±1.41 (1.25)	59.65±0.78 (3.74)	24±2.12	1789±2.83	1813±4.95	1.32±0.11
HH3xHH8	5±1.41	12±0.71	83±2.12 (3.75)	61.36±0.78 (6.71)	38±3.54	1750±2.12	1788±1.41	2.13±0.20
Control hybrid								
CSR2xCSR4	10+2.83	10+5.66	80+7.07	57.5+2.12	46.5+4.95	1676+8.49	1722.5+3.54	2.78+0.31

Values in parenthesis indicates percent improvement over control hybrid

Table 3: Emergence percentage of foundation crosses (Mean of three trials)

Foundation Crosses	Pupation rate(%)	Moth emergence %	
		Male	Female
HL1 x HL3	92.5±1.06	91.4±0.42	89.2±0.85
HL10 x HL12	93.6±1.13	90.3±1.41	88.9±0.57
HH1 x HH3	93.4±0.78	90±1.63	89.4±0.64
HH8 x HH12	93.1±0.14	91.7±1.20	89±0.35
Control foundation crosses			
CSR2 x CSR27	93.2±2.26	88.1±1.34	86.2±1.56
CSR6 x CSR26	92.8±1.27	88.4±0.07	87.5±0.92

Table 4: Grainage performance of the identified double hybrids(Mean of three trails)

Hybrid	Laying recovery %			Egg rec. (g)/Kg cocoon	No. of eggs / grm.			UF%
	ULM	PL	GL		UF	Good	Total	
(HL1xHL3) x (HL10xHL12)	12±2.83	4±3.54	84±0.71 (2.4)	82.48±0.72	64±1.41 (2.3)	1674±2.12	1738±0.71	3.68±0.08
(HH1xHH3) x (HH8xHH12)	10±2.12	4±0.71	86±1.41 (4.9)	84.95±0.18	52±2.83 (5.4)	1628±4.95	1680±7.78	3.09±0.16
Control hybrid								
(CSR2xCSR27) x (CSR6xCSR26)	8±3.54	10±2.83	82±6.36	80.6±0.39	75±4.24	1510±2.83	1585±1.41	4.73±0.26

Values in parenthesis indicates percent improvement over control hybrid

Thermal acclimatization and stress have been studied during different developmental stages of insects (larvae, pupae and adults) as well as different physiological conditions (Melanby,1954; Lee *et al.,* 1988). Surviving a thermal stress depends in part upon the individual organism's stress history. Brief exposure to a mild stress can protect an organism against a normally lethal stress. Brief exposure to a mild temperature than the optimum temperature induces tolerance (Thermo-tolerance) in insects. Once thermo-tolerance has been induced, it may persist for days (Carretero *et al.,* 1991; Yocum and Denlinger, 1992). Some stresses will even increase an organism's sensitivity to a future stress. Survival of high temperature stress appears to be energy dependent in insects and the loss should be compensated by nutrition (Haveman and Hahn,1981; James *et al*., 1986).

The variations in the environmental conditions during rearing influence the expression of various economic traits (Watanabe,1928: Hassanein and Sharawy, 1962: Kasivishwanathan *et al.,* 1970; Ueda *et al.,* 1969, 1971 and 1975). Moreover, the genotypic differences among breeds due to variable gene frequencies at many loci make the respective breeds to respond differently to changing environmental conditions (Watanabe, 1918,1919 and 1961; Kogure,1933; Nagatoma, 1942; Suzuki, 1954; Fukuda *et al.,* 1963; White and Richmond, 1963; Narayanan *et al.,* 1967; Morohoshi, 1969; Sengupta, 1969 and 1988; Subramanya and Sreerama Reddy, 1986; Ueda *et al.,* 1969; Ravindra Singh *et al.,* 1990,1992 and 1998: Sreerama Reddy *et al.,* 1992; Radhakrishna *et al*., 2001 and Sudhakar Rao, 2003).

The egg laying capability of *Bombyx mori* L. has been noticed to be influenced by the genotype of silkworm line and rearing temperature. Rearing under stress environments results consumption of energy in the metabolic activity (Gowda, 1988). Unfavorable environment during rearing results in poor performance of parental lines. Exposure of seed cocoons to high temperature of 35°C in combination with low relative humidity results in decreased egg recovery and increased incidence of unfertilized eggs (Ayuzawa *et al.,* 1972). The occurrence of unfertilized eggs was more common in summer as compared to other seasons (Biram *et al.,* 2009). Larvae and cocoon exposures to 35°C or above results in poor performance of silkworm moths in relation to egg number and egg fertility and high temperature during rearing, cocooning, mating and oviposition induced unfertilized egg layings (Gowda, 1988). Rate of development and physiology of larvae may be influenced by high temperature which results in alteration in metabolism causing reduced egg yield, increased mortality and enhanced disease incidence due to the production of apyrene sperm which results in the production of unfertilized eggs (Kovolov, 1970). The apyrene production reduces at 35°C by 50% (Osanai *et al*., 1989) resulting in increased egg infertility.

The number of investigations suggests that exposure of male silkworms at higher temperature from the time of spinning to pre-pupal period brings male sterility (Sugai and Takahashi, 1981; Das *et al*., 1996) and results in increasing of unfertilized eggs (Biram and Gowda, 1987; Ming *et al.,* 1994)). The results indicated that variations in temperature and humidity during the rearing of larvae have significant effect on the fecundity and fertility of silkworm moths. The number of eggs laid by a moth decreased significantly when the larvae were exposed to increased temperature and low humidity (T_1, T_4 and T_7). Fluctuations in temperature and humidity during rearing of larvae disturbed rhythm and efficiency of the larvae to harvest the energy during 5^{th} instar for the synthesis of cocoon before pupation and complete developmental process during the non-feeding stages. Thus, much of energy resources are utilized to restore internal homeostasis when external environment exerts stress. The fecundity and fertility are two major components of silkworm seed production. The study showed that fluctuations in rearing temperature and humidity have adverse effects on the egg production and egg fertility of the silkworm moths.

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