ISSN 2250-3153

Influence of cross breeding of wild and semi-domestic populations of tropical tasar silkworm *Antheraea mylitta* D on Grainage and silkworm rearing traits

G. Lokesh, A. K Srivastava, P. K. Kar and M. K. Sinha

Silkworm Breeding & Genetics, Central Tasar Research & Training Institute, Central Silk Board, Ranchi, 835303, India

Abstract- The immense variability in the population of tropical tasar silkworm *Antheraea mylitta* D is an intriguing aspect to exploit heterosis effect in important quantitative and qualitative traits. In the present study, two reciprocal crosses with four combinations were prepared such as semi-domestic Daba (S-d) x Laria, Laria x Daba (S-d), Daba (wild) x Daba (S-d), Daba (S-d) x Daba (wild) and Parallely Daba (S-d) is maintained as control. The important grainage parameters such as coupling behavior, fecundity and emergence pattern of parent in captive condition was observed. The larval hatching and rearing (growth) performance of F1 larva was recorded, followed by assessment of cocoon traits. Erratic moth emergence was observed in the wild populations, while, in Daba (S-d) almost synchronized emergence and 100% self coupling observed although there was low coupling rate in the different crosses. Significantly higher fecundity was recorded in the F_1 crosses Daba (S-d) x Daba (wild) [245 ± 08] over Daba (Control) [225 ± 15]. Higher hatching recorded in the Daba (S-d) [82% ± 04] and in Laria x Daba [82% ± 05], lower values observed in Daba x Laria cross [68 ± 04]. Linear growth pattern was observed during development of larva. No significant difference in larval growth was noticed. Variability was observed in growth rate between the different batches. Cocoon characters indicated higher in the F_1 of different crosses over the Daba. Increased cocoon weight (> 7.41%), shell weight (>12.07%) and corresponding shell ratio recorded with Daba x Daba (wild) compared to the Daba (S-d). The study demonstrates the possibilities to harness potentials of heterosis effects in developing breeds with higher productive traits

Index Terms- *Antheraea mylitta*, Reciprocal cross, Grainage, F_1 Population, Daba, Laria

I. Introduction

Silkworm breeding has prevailed in most of Sericulture countries with an overwhelming improvement in the silk production and produced prominent commercially important silkworm breeds. Yet tropical tasar silkworm *Antheraea mylitta* D is one of the important sericigenous insect species is commercially exploited for the production of tasar silk in India. Tasarculture being practicing in Central, Northern and Eastern regions mostly on *Terminalia arjuna* (Arjun) and *T. tomemtosa* (Asan) forest plants as well as economic plantation. Besides, large number of tasar cocoons is also collected from *Shorea robusta* (Sal) and other secondary food plants. Tropical tasar silkworm has a wide range of distribution in varied geo-climatic condition (Jolly *et al.*, 1968; Sengupta *et al.*, 1993; Singh and Srivastava, 1997; Srivastava *et al.*, 2003 and Suryanarana and Srivastava, 2005). Foraging of silkworm on variety of food plants, annual precipitation, day length, plant succession *inter alia* factors like latitude, longitude, the altitude *etc.*, of different areas lead to marked life cycle differences expressing wide variations in phenotypic, physiological and behavioural traits resulted in formation of ecoraces (Srivastava *et al.*, 2004). Though, the *A. mylitta* has wide genetic as well as phenotypic variability in its population, also a good material for the exploitation of heterosis, besides it has many constraints in silkworm hybridizations Siddiqui (1997). Since, variability being the genetic basis of any crops, this is the basic requirement for genetic improvement. Therefore, in order to create new reservoirs of genetic variability, the use of crossing method the most genetically diverse parents has become essential as it helps in recombination of genes from diverse sources (Reddy *et al.*, 2008). Genetic variants in the natural population and diverse gene in the individual of the population are the prime need for evolving improved breeds and cross breeds with better tolerance and quantitative traits. Since, tropical tasar silkworm *A. mylitta* is under commercial exploitation in the country, improved breeds/races are need of the hour (Reddy *et al.*, 2010).

In the present study, the genetic capabilities of tasar silkworm have been explored through conventional breeding methods to demonstrate the implication of introgression of wild and semi-domestic genotypes and its influence on different characters of tasar silkworm. The reciprocal crosses of two wild populations such as Daba (wild) and Laria were prepared by combining with semi-domestic Daba ecorace. The important characters of each ecoraces are mentioned in Table-1. The possible influence of cross breeding on heterosis effect due to introgression of genes in the different crosses prepared was examined through grainage and silkworm rearing traits.

Table. 1: Characteristic features of the three ecoraces of *A.mylitta*

Ecorace	Native	Food Plant	Fecundity	Cocoon traits	Voltinism
Daba (Semi-domestic)	West Singhbum, Jharkhand Reared in 9 States in India	*Terminalia arjuna, T. tomentosa*	200 -220	Large size shell with short peduncle, weighs 10-12g, shell wt.1.5-2.0 g. medium denier (10) filament	Bivoltine trivoltine
Daba (wild)	West Singhbum forest area, Jharkhand	*Terminalia arjuna, T. tomentosa*	250-350	Large and tough shell weighs 11-12.5 g, shell wt.1.8-2.3 g. filament length >1200 mts.	Bivoltine trivoltine
Laria (wild)	Peterbar forest area, Jharkhand	*Shorea robusta*	160- 200	medium size shell, long peduncle and robust cocoons with low denier silk filament	Uni, bi & trivoltine

II. MATERIALS & METHODS

The present study was undertaken at the Breeding & Genetics Grainage house for the preparation of reciprocal crosses and rearing of tasar silkworm was conducted at well maintained host plant field laboratory of Central Tasar Research & Training Institute, Ranchi, during second crop (September-October) season.

Collection of cocoons for breeding: Daba semi-domesticated cocoons were collected after first crop harvesting of Silkworm breeding & Genetics section's germplasm bank, CTR&TI, Laria and Daba wild cocoons were collected from its natural eco-niches *ie.*, Sal forest of Peterbar area of Jharkhand and forest area near Hatgamaria, Jharkhand respectively. All the cocoons were preserved separately in separate out-door cages specified for preservation of cocoons at the Grainage area till the moth emergence.

Moth Emergence pattern: Moth emergence was studied during second grainage season (September), the number of moth emerged and male - female synchronization was recorded in Daba (wild), Daba (S-d) and Laria ecoraces.

Preparation of reciprocal crosses: Immediately after emergence of moth, male-female moths selected from different batches as per the requirement to prepare reciprocal crosses among Daba, Laria and Daba wild ecoraces. Following crosses were prepared.

1. Laria x Daba (S-d), 2. Daba (S-d) x Laria, 3. Daba (S-d) x Daba (wild), 4. Daba (wild) x Daba (S-d). Along with these crosses a control/ reference of Daba (S-d) also used for comparison.

Coupling Behaviour: Coupling behaviour of different crosses prepared was recorded based on the self (natural) and hand coupling (induced) of 50 each individual crosses in all the batches. The coupling of moths was represented in the percentage of coupling.

Fecundity Assessment: Male and female mated moths of different combinations were allowed for copulation for about 6-7 hours in standard grainage conditions. Female moths were decoupled and kept in paper box (Ovi-position/egg laying device) for ovi-position up to 72 hrs. The number of eggs laid by individual mother moth in three days (72 hrs.) was counted and recorded as fecundity (Sinha, 1998). Methodical mother moth examination was conducted to detect any pebrine disease. The eggs were washed to remove muconium layer and surface sterilized to prepare disease free layings.

Hatching performance: Larval hatching was recorded on the 9th day after coupling date. Comparative hatching performance of different crosses and control as recorded. Hatching performance was considered for three day hatching and calculated in percentage (Sinha, 1998).

Silkworm rearing: Silkworms of different reciprocal crosses on the day of hatching were brushed on different well maintained *Terminalia arjuna* (Arjun) plants. Rearing was conducted and different batches were maintained on separate, labeled Arjun (*Terminalia arjuna)* plants following standard tasar silkworm rearing practices.

Statistical analysis: One-way *ANOVA* using computer application SPSS 10.0 statistical package to test the significance of differences, between the means of individuals and between the groups studied. Comparisons were performed with Duncan's Multiple Range Test (DMRT, $P< 0.05$) (Duncan, 1955).

III. RESULTS

The grainage and rearing performance of different reciprocal crosses was observed for different parameters and recorded as follows;

Moth emergence and Coupling behavior: highly erratic emergence was observed in the wild tasar silkworm ecoraces compared to semi-domesticated Daba, which recorded almost synchronized moth emergence. The coupling behavior was recorded that most of the crosses, there was no self (natural) coupling but 100% natural coupling was found in the Daba. In the cross Daba x Daba (wild) 10% natural coupling was recorded followed by 8% in Daba (wild) x Daba, the remaining crosses did not found self (natural) coupling they were resorted to induced mating to prepare crosses (Table. 1).

Fecundity and vigourity of egg : Fecundity was recorded and found highly variable among the different reciprocal crosses and showed higher fecundity in the crosses over the control batch, Daba. Higher fecundity (252±15) was recorded when Daba crossed with its wild relative [Daba (wild)] followed by Laria x Daba 245±08, Daba (wild) x Daba 235±18, Daba (control) 230±15 and least recorded with Daba x Laria 203±15 (Table. 2).

Vigourity of the eggs was recorded based on the weight in different batches of reciprocal crosses and compared with the

Daba. Variability was observed in the egg weight in different batches and also higher weight was recorded Daba x Daba (wild) which weighs 98 eggs per one gram (Table. 2).

Larval hatching performance: Hatching performance was recorded on ninth day after oviposition. Higher hatching was recorded in Daba and in the cross of Laria x Daba (82%) followed by Daba x Daba (wild) (78%), Daba (wild) x Daba (73%) and least was recorded in the Daba x Laria (68%). There was no significant difference in the hatching performance of different crosses over control Daba.

Larval Growth performance: Linear growth pattern was observed in the larvae during different stages of development. There was marginal variation was observed among different batches of reciprocal crosses and the control Daba. The data was recorded gravimetrically of individual larva from different batches separately from 1st Instar up to the spinning. The growth rate was recorded Instar wise for 1st, 2nd & 3rd instars. During 4th and 5th instars day-day growth rate was recorded. Growth rate was observed increased rapidly during initial days of the instar ie., immediately after the moulting period. There was no uniformity in the growth rate during middle and end of the instars were recorded (Table. , Fig.).

Cocoon characters: Cocoon weight, shell weight and shell ratio was recorded in the cocoons harvested from different batches. Higher cocoon weight was recorded with F1 of Daba x Daba (wild), followed by its reciprocal ie., Daba (wild) x Daba and the least was recorded with Daba x Laria crossing. Corresponding shell weight was also recorded. No significant improvement observed over Daba.

IV. Discussion

Cross-breeding signifies an efficient method for animal population amelioration. For silkworms, the cross-breeding is intended on one hand for the amelioration of the breeds and on the other hand for immediate economical purposes consisting in producing commercial hybrids, for which the superiority against pure breeds. The introgression of traits present in both domesticated and wild populations of tasar silkworm for heterosis effect in F1 was reported by Talebi and Subramanya (2009), Reddy *et al* (2009b) and Moorthy *et al* (2007) to avail a viable breed for sustainable tasar cocoon production.

The fecundity and egg fertility are considered as one of the most desired quantitative characters of commercial importance in silkworms (Reddy *et al.,* 2010). The genotype-environment interaction associated with host plant quality has highly significant influence on the fecundity of the silkworms (Venugopal *et al*., 1987). The healthy and robustness of the mating male-female moths are very important for the subsequent quality and quantity of egg laying. Also the males of different breeds can influence the egg laying performance in silkworms (Kumar and Paul, 1993; Krishnaprasad *et al*., 2002). The fecundity of different reciprocal crosses recorded in the present study indicates the variability in the number of eggs laid by mother moths. Higher fecundity recorded in some of the crosses compared to the Daba (control). The effect of hybridization is very much apparent and the due to the inter-ecorace cross. Also the robustness and vigority of the wild relatives are influenced the higher fecundity and quality of the eggs in the progeny. In contrast, the hatching performance was low in the F_1 of different crosses compared to the hatching in Daba. This may be due to the compatibility barriers and fertility complications between mating individuals of different ecoraces.

The amount of consumption and utilization of food are very important factors for determining the nutritional aspects of the phytophagous larvae. For instance, poor growth may not be due to the nutritional adequacy of the diet but, a low rate of intake due to the absence of a non nutrient with phyto-stimulatory activity which might lead to an increased growth, although the nutrient is neither required nor utilized (Dadd, 1960). The consumption of leaf by silkworms during final instar accounts for more than 80% of the total consumption during its larval life. Food consumed in this stage is effectively utilized for the production of silk proteins as well as to support its metabolism (Lokesh *et al*., 2006). Thus the energy acquired by the larvae as a consequence of feeding is utilized in the subsequent non-feeding stages. The growth rate represents the quantity of increase in the dry matter in the body of the animal for a given period. It influences the speed of development of the insect, which directly depends on the food to a maximum extent. Waldbauer (1968) and Delvi & Pandian (1971) reported that the higher feeding rates, better food assimilation, conversion efficiency and growth rate for lepidopterans can be taken as adaptive features to meet the energy requirement. These values will influence the non feeding life stages and further on the cocoon production. Silkworm larval growth is related to the difference in the intake and utilization of the food, although the diets are similar in their ability to support the growth. For instance, low intake might be offset by high digestibility or a high utilization of digested food for growth or vice-versa. Higher larval growth rate in the reciprocal crosses compared to the Daba in the present may be due to the acquirement of the vigourity in consumption of leaves and in turn better conversion/ digestibility by the hybrid silkworms. Tasar silkworm growth and subsequent cocoon production are normally influenced by the breed, feed and the rearing environment. The tasar silkworm rearing being an outdoor practice mostly on nature grown food-plants, the success of the crop go with breed engaged for commercial rearing (Reddy *et al*., 2010; Hansda *et al*., 2008).

The genotype-environment interaction has significant role in expressing the commercial cocoon traits in tropical silkworms (Petkov *et al*., 2000). Season specific performance of silkworm on commercial cocoon traits was studied (Kumar *et al.*, 2003; Malik and Reddy, 2007; Zhao *et al*, 2007) and particularly in tasar silkworm (Reddy et al, 2010; Ojha *et al*., 2009). The cocoon characters are important components, which determine the overall performance of the silkworm and also important for subsequent silk fiber quality. Various factors such as genetic background of silkworm, food quality, environment and etc., influence the economic traits of the silkworm (Lokesh *et al*., 2011). The variations observed in the cocoon weight, shell wt and respective shell ratio among F1 of different combinations features the compatibility and genetic capability of these crosses in expression of higher cocoon traits compared to the Daba (Control). It is also understood that crossing the commercially exploited domesticated silkworms with its wild counterparts would always yields better as in the case of Daba (S-d) x Daba (wild), and Daba (wild) x Daba (S-d). While, deterioration in some characters may also be occurred when crossing distantly related silkworms due to incompatibility as observed presently between Daba and Laria for commercial cocoon traits.

The positive improvement of some of the quantitative characters observed in the study indicates the possible heterosis effect due to inter-ecorace crossing. Also, variability in the tasar silkworm populations could be of immense application to exploit or to demonstrate heterosis effect in acquiring important quantitative and qualitative traits. Hence, the development of F1 hybrids from crossing different ecoraces of tropical tasar silkworm would contribute for the enhanced tasar cocoon production and total productivity. Whereas, imperative application of selection of parents to obtain desired commercial traits such as fecundity, better cocoon characters in developing F1 hybrids would always yields better.

REFERENCES

[1] Dadd, R. H. (1970). Digestion in insects. In Chemical Zoology. Edited by M.Florkin and B.T. Scheer. Academic Press. New York and London. 5 ; 117-145.

[2] Delvi, M. R. and Pandian, T. J. (1971). Eco-physiological studies on the utilization of food in the paddy field grasshopper Oxya velvox. Oecologia, 18 : 267-275.

[3] Duncan DB (1955). Multiple range and multiple F- tests. Biometrics 11, 1-42.

[4] Hansda G., R. Manohar Reddy, M. K. Sinha, N. G. Ojha and N. B. V. Prakash, (2008). Ex situ stabilization and utility prospects of Jata ecorace of tropical tasar silkworm Antheraea mylitta Drury. Int. J. Indus. Entomol., 17: 1679-172.

[5] Jolly MS, Chaturvedi SN, Prasad S (1968) A survey of tasar crops in India. Indian. J. Seric.1, 50-58.

[6] Krishnaprasad, N K, B Sannappa and R Varalakshmi (2002). Effect of multiple mating on grainage performance of newly evolved bivoltine breeds of Bombyx mori L. Bulletin of Indian Academy of Sericulture 6(2); 50-55.

[7] Kumar , N. S., H. K. Basavaraja, B. Nanjegowda, P. G. Joge and G. V. Kalpana, (2003). Effect of high temperature and high humidity on the post cocoon parameters of parents, foundation crosses, single and double hybrids of bivoltine silkworm, Bombyx mori L. Indian. J. Sericulture, 42: 162-168.

[8] Kumar Kishore C M. And D C Paul (1993). Effect of age of males on ejaculation and behaviour of spermatozoa in the internal reproductive organs of the female moth, Bombyx mori L (Lepidoptera: Bombycidae). Sericologia, 33 (2): 219 – 222.

[9] Lokesh. G. and S. R. AnanthaNarayana (2011). Changes in the Protein profile of silkworm Bombyx mori. L (Lepidoptera: Bombycidae) in response to the chemical mutagen. Int. J.Sci. Nat. Vol 2(3): 559 -563.

[10] Lokesh. G., M. Narayanaswamy and S. R. Ananthanarayana (2006). The effect of chemical mutagen on hemolymph proteins of silkworm Bombyx mori L. J. Appl. Sci. Environ. Mgt. 10 (3) 21 – 25.

[11] Malik, F. A and Y. S. Reddy, (2007). Role of mulberry nutrition on manifestation of post cocoon characters of selected races of the silkworm Bombyx mori L. Serocologia, 47: 63-76.

[12] Moorthy, S. M., S. K. Das, N. B. Kar and S. R. Urs, (2007). Breeding of bivoltine breeds of Bombyx mori L. suitable for variable climatic conditions of tropics. Int. J. Ind. Entomol., 14: 99-105.

[13] Ojha N G., R M Reddy, G Hansda, M K Sinha, N Suryanarayana and N B V Prakash (2009), Status and potential of Jata, a new race of Indian tropical tasar silkworm (Antheraea mylitta Drury). Acad. J. Entomol. 2; 80-84.

[14] Petkov, N., I. Nacheva, P. Tsenov, D. Grekov, V. Golovko and M. Braslavskii, (2000). Hybridization schemes for developing new silkworm (Bombyx mori L.) races by using populations from different eco-geographical zones. Acta Agric. Serbica, 5: 3-9.

[15] Reddy R M, N Suryanarayana, M K Sinha, N S Gahlot, G Hansda, N G Ojha and N B Vijaya Prakash (2009a). Silk filament progression with backcross breeding generations in tropical tasar silkworm, Antheraea mylitta D. Intl. J. Indust. Entomol. 19(1); 187-192.

[16] Reddy R M, Suresh Rai, Srivastava A K, Kar P K, Sinha M K, Prasad B C (2010b) Heterosis pattern and commercial prospective of Assorted F1 hybrids of Indian tropical tasar silkworm, Antheraea mylitta Drury. Journal of Entomol., 7(3), 160-167.

[17] Reddy R M, Suryanarayana N, Vijaya Prakash NB (2008). Heterosis potential in selective parental F1 hybrids of divergent geographic ecoraces of tropical tasar silkworm, Antheraea mylitta D (Lepidoptera Saturniidae). Aca. J. Entomol., 1, 32-35.

[18] Reddy, R.M., M.K. Sinha and B.C. Prasad (2010a). Application of Parental Selection for Productivity Improvement in Tropical Tasar Silkworm Antheraea mylitta Drury-A Review. Journal of Entomology, 7: 129-140.

[19] Sengupta AK, Sinha AK, Sengupta K. (1993). Genetic reserves of Antheraea mylitta Drury. Indian silk, 32, 39-46.

[20] Siddiqui AA (1997) Studies on heterosis and heterobeltosis in the tasar silkworm Antheraea mylitta D. Sericologia, 37, 59-65.

[21] Singh BMK, Srivastava A K (1997) Ecoraces of Antheraea mylitta Drury and exploitation strategy through hybridization pp 1-39, Base paper 6: Current Technology Seminar on Non-Mulberry Sericulture, CTR&TI, Ranchi, India.

[22] Sinha BRRP (1998) Survey of genetic resources and study of the genetic divergence in Antheraea mylitta Drury. Ph.D. Thesis, Ranchi University, Ranchi, India.

[23] Srivastava A. K., A.K.Sinha and B.R.R.P. Sinha (2003), Descriptor of tropical tasar silkworm, Antheraea mylitta Drury.(Lepidoptera Saturniidae), CTR&TI, Ranchi:1-14.

[24] Srivastava, A.K., A.H. Naqvi, A.K. Sinha, S.R. Vishwakarma and G.C. Roy, (2004). Genotype and environment interaction in Antheraea mylitta Drury and its implications. Perspectives in Cytology and Genetics, 11:219-224.

[25] Suryanarayana N, Srivastava AK (2005). Monograph on Tropical Tasar Silkworm. Central Tasar Research and Training Institute, CSB, India.

[26] Talebi, E and G. Subramanya, (2009). Genetic distance and heterosis through evaluation index in the silkworm, Bombyx mori L. World Applied Sci. J., 7: 1131-1137.

[27] Venugopal Pillai S, Krishnaswami S, Kashivishwanathan K (1987) Growth studies in silkworm, Bombyx mori. L. under tropical conditions. II. Influence of agronomical methods of mulberry on growth, cocoon crop and fecundity of silkworm. Ind. J. Seric. 26(1) , 38-45.

[28] Waldbauer G. (1968). The consumption and utilization of food by insect. Adv. Insect Physiol., 5 : 229-288.

[29] Zhao, Y., K. Chen and S. He, (2007). Key principles for breeding spring and autumn silkworm varieties; From our experience of breeding 873 x 874. J. Environ. Sci., 5: 57-61.

AUTHORS

First Author – Author name, qualifications, associated institute (if any) and email address.
Second Author – Author name, qualifications, associated institute (if any) and email address.
Third Author – Author name, qualifications, associated institute (if any) and email address.

Correspondence Author – Author name, email address, alternate email address (if any), contact number.

Table. 2: Grainage parameters studied in different reciprocal crosses of tasar silkworm *A.mylitta*

Reciprocal crosses	Coupling (%)	Fecundity (nos.)	Eggs per gram	Hatching performance (%)
Laria x Daba	00	245 ± 08	99 ± 01	82 ± 05
Daba x Laria	00	203 ± 15	102 ± 02	68 ± 04
Daba (wild) x Daba	08	235 ± 18	101 ± 02	73 ± 06
Daba x Daba (wild)	10	252 ± 13	98 ± 01	78 ± 03
Daba	100	230 ± 15	100 ± 01	82 ± 04

Fig. 1: Larval growth pattern of different reciprocal crosses during different larval stages (weight in grams)

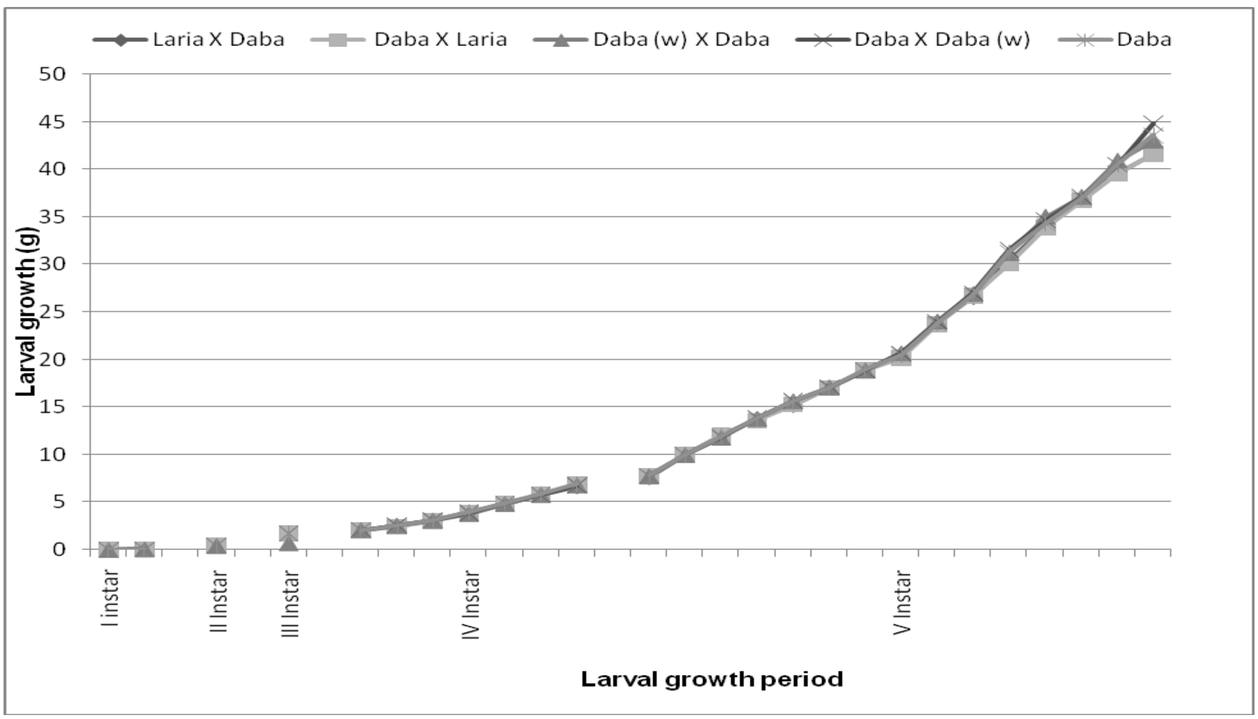

Fig. 2: Larval growth rate (%) of different reciprocal crosses during 4th Larval stage

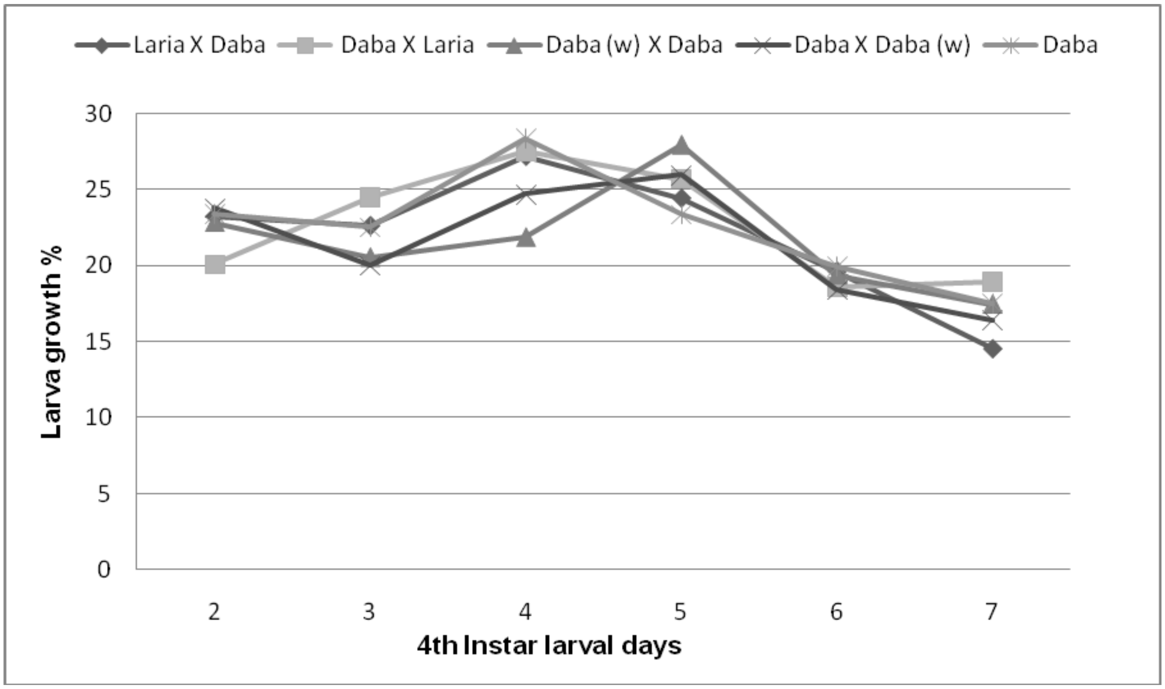

Fig. 3: Larval growth rate (%) of different reciprocal crosses during 5th Larval stage

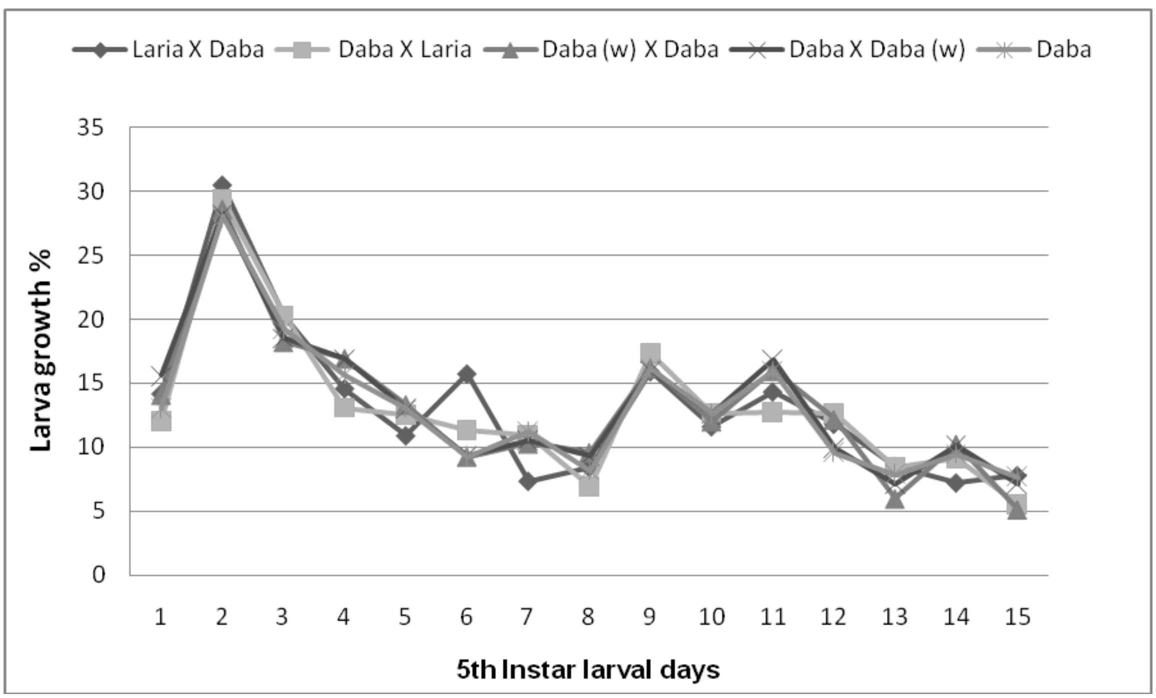

Table. 3: Analysis of Cocoon characters in different reciprocal crosses of tasar silkworm

	Cocoon wt. (g)	**Shell wt. (g)**	**Shell ratio (%)**
Laria x Daba	10.66 ± 2.10	1.70 ± 0.21	15.94
Daba x Laria	10.20 ± 1.98	1.61± 0.18	15.23
Daba (wild) x Daba	11.62 ± 2.40	1.91± 0.18	16.36
Daba x Daba (wild)	12.13 ± 2.61	2.07± 0.18	17.01
Daba	11.23 ± 2.40	1.82 ± 0.18	16.20