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Silk Filament Progression with Backcross Breeding Generations in Tropical Tasar Silkworm, *Antheraea mylitta* D

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Silk filament progression study applying backcross breeding with recipient parent Jata and donor parent Daba ecoraces of tropical tasar silkworm, *Antheraea mylitta* Drury conducted during 2006 to 2008, revealed introgression of filament denier (10.2 d) superior to both parents at BC4 level. Also, the silk waste (0.35%) and filament breaks (2.6 nos) were reduced compared to both parents, while the filament length (973 m) and non break filament (296 m) improved over donor and could attain closer to recipient parent. The best performance of Jata x Daba at F1 level, with highest silk filament length (1646 m) of 12.5 d denier, denotes heterosis impact on silk trait with parental heterogenousity, an advantage to exploit silk filament yield. The progression of quality in terms of finer filament denier, less silk waste and least number of filament breaks over both parents and improvement in filament length and non break filament over donor parent except for a marginal shortage against recipient at BC4 level indicates the prospects of Jata and Daba ecoraces as source of breeding material for qualitative improvement of tasar silk filament. The study suggests that the commercially important finer denier of tasar silk filament can be attained with minor reduction in silk yield of wild Jata ecorace by adopting repeated backcrossing for four generations with semi domesticated Daba ecorace.

Key words: *Antheraea mylitta,* Denier, Silk filament, Backcross breeding, introgression.

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Introduction

Tasar silk is produced by a wild sericigenous polyphagous insect, *Antheraea mylitta* Drury; (Lepidoptera: Saturniidae) distributed all along central India (12-31°N latitude & 72-96°E longitude). Though insect has rich genetic resource as 44 ecoraces, only Daba and Sukinda were semi domesticated and applied for commercial rearings and the need is exploration and commercial exploitation of economic trait(s) variation of their wild relatives. Backcrossing is a well known and long established breeding plan where a character is introgressed from a domesticated or wild relative donor parent into the genomic background of a recurrent parent, which progress better with selection of genetically diverged parental breeds (Moorthy *et al*., 2007; Nakada, 1992; Tazima, 1984; Tzenov and Guzman, 2004; Zamir, 2001). The recipient will attain near-isogenic stage in preferred trait by 4^{th} to 6^{th} backcrossing generation (Babu *et al*., 2005; Nakada, 1992; Semaga *et al*., 2006). The proportion of donor genome is expected to be reduced by one-half (50%) at each generation on desired trait, except on chromosome holding the character (Hospital, 2005). The introgression of quantitative trait depends on its heritability, number of genes involved and their interaction and distribution of genes over the genome (Ooijen, 1992). The backcross breeding of silkworm using parents with preferred traits and selection in subsequent generations offer superior varieties (Raju and Krishnamurthy, 1993). The cocoon and shell weights shown higher co-heritability with silk yield (Siddiqui and Sengupta, 1994), the cocoon weight and filament length shown positive correlation, while, the filament thickness and length are negatively associated (Sekharappa *et al*., 1999). The better reeling performances were reported in hybrids over pure races (Takabayashi *et al*., 1994) and intensity of hybrid vigor on filament size deviation found varying with changing environments (Kumar *et al*., 2002). The out crossing or repeated back-crossing with exotic races can improve targeted character(s) and induction of polyvoltine traits into uni/ bivoltines enhances resistance in breeds (Moghaddam *et al*., 2005). The introgressive hybridization with parental genetic diversity has heterosis impact on silk quality with cumulative effect of genes (Verma *et al*., 2003). The dominant epistatic gene interactions followed by additive-additive epistatic gene exchanges will elevate filament length during hybridization (Petkov *et al*., 2003).

This inadequacy of amenable tasar silkworm genetic wealth, limited cycles (uni & bivoltine), pupal diapause, lack of adaptability and their outdoor rearings under fluctuating environment compels to utilize limited breeds and suitable methods to improve silk associated traits. The choosing of longer silk filament of univoltine wild Jata and finer filament denier of bivoltine semi domesticated Daba ecoraces and transformation of silk filament quality of Jata race through repeated backcrossings of Daba is the objective of present study.

Materials and Methods

Maintenance of parental ecoraces

The disease free layings (Dfls) of parents were prepared following package of tasar egg production using seed cocoons of Daba ecorace obtained from stabilized stocks of the Institute and Jata ecorace from Tasar Rearers Cooperative Society (TRCS) areas of Orissa, India during June/ July, 2006. The two races were reared following package of tasar silkworm rearing in a randomized block design with three replications each during seed and commercial crop seasons of 2006, 2007 and 2008 on economic plantation of *Terminalia tomentosa* at field laboratory of Research Institute, Ranchi, India. The cocoons generated in each crop were assessed for shell weight with twenty random samples, silk yield based on average shell weight (of twenty randomly selected cocoons/Dfl) and cocoon harvested per Dfl. The silk filament technological characters like filament length, non break filament, filament denier, silk waste and filament breaks were studied with ten randomly collected cocoons at post cocoon technology division of the Institute. The required Dfls were also prepared using cocoons after every crop season for rearings to continue parental progenies parallel to F1 and BC generations for comparison studies.

Maintenance of backcross stocks

The female moths of Jata ecorace obtained from the cocoon stocks of TRCS areas of Orissa were crossed with males of Daba race of stabilized stocks of Institute to get F1 [Jata × Daba] and the females of F1, BC1, BC2 and BC3 progenies were back crossed with Daba males to obtain BC1, BC2, BC3 and BC4 hybrids. After every crop rearing, the hybrid cocoons (F1 to BC3) were assessed sex wise and the selected female cocoons were used for preparing BC hybrid Dfls using males of Daba with simultaneous rearings during seed and commercial crop seasons of 2006, 2007 and 2008 on the economic plantation of *Terminalia tomentosa* at field laboratory of Research Institute, Ranchi India.

Evaluation of parents, F1 and BC (BC1 to BC4) generations

The parental ecoraces Daba and Jata along with F1 and BC hybrids (BC1 to BC4) raised using wild Jata and semi domesticated Daba ecoraces of *Antheraea mylitta* D were reared simultaneously in a randomized block design with three replications each during seed and commercial crops of 2006, 2007 and 2008 on the economic plantation of *Terminalia tomentosa* at field laboratory of Research Institute, Ranchi, India. During the course of breeding the parental cocoons were considered based on period and duration of spinning, uniform cocoon shape and color, better shell weight and silk ratio percentage, higher total silk yield for grainage and better fecundity and egg hatching for rearings. Larvae of one Dfl in each parent as well as hybrid were considered as one replication in all seed and commercial crop rearings and recorded observations. The silk related technological characters like filament length, non break filament, filament denier, silk waste and filament breaks were considered on single cocoon basis (with ten randomly selected cocoons) in respect of parents, F1 and BC hybrids (BC1 to BC4) at post cocoon technology division of the Institute. The data recorded on different parameters during experiment were analyzed statistically.

Results

Performance of parental ecoraces under *in-situ* and *ex-situ* habitats

The variability in origin, food plants, voltinism, adaptability, cocoon and silk related commercial traits of parental ecoraces (Daba & Jata) under *in-situ* conditions (Table 1), indicates the superiority of wild race, Jata in all traits except for its semi adaptability and higher filament denier, where the semi domesticated Daba scored superior, but has shown lower performances in cocoon traits and filament lengths. The average performances of donor (Daba) and recipient (Jata) parents at Institute field laboratory

Table 1. Origin and character variations of parental ecoraces under *in-situ* conditions

Parameter	Daba		Jata	
	Range	Ave.	Range	Ave.
Origin of race	Singhbhum (Jharkhand)		Thakurmunda (Orissa)	
Altitude (AMSL)	500'		1000'	
Voltinism (*in-situ*)	Bivoltine		Univoltine	
Level of adaptability	Wider adaptability		Semi adaptability	
Cocoon weight (g)	9.20~12.83	10.63	12.24~14.80	13.52
Shell weight (g)	1.25~2.36	1.80	1.60~2.34	2.05
Silk ratio (%)	14.13~16.28	15.07	15.94~18.95	17.45
Filament length (m)	475~1240	962	840~1550	1184
Non break filament (m)	79~475	230	52~635	325
Filament denier (d)	9~11	10	11~13	12

Table 2. Average performance of daba and Jata parents for both crop rearing seasons of three years (2006 to 2008) under *ex-situ* conditions (values are mean and ± SE)

Parental ecoraces	Shell weight (g)	Total silk yield (g)	Filament length (m)	Non break filament (m)	Filament denier (d)	Silk waste (%)	Filament breaks (no.)
Daba (Donor)	1.53±0.09	98.95±9.63	835.2±65.04	231.3±9.99	10.04±0.13	0.89±0.15	6.21±1.36
Jata (Recipient)	1.79±0.06	75.63±17.12	1054.6±71.58	317.6±18.8	12.68±0.84	1.86±0.31	9.19±0.81

Table 3. ANOVA for silk filament traits among parents, F1 & BC hybrids (BC1 to BC4)

particulars	DF	Filament length (m)	Non break filament (m)	Filament denier (d)	Silk waste (%)	Filament breaks (no.)
SOURCE		MEAN SUM OF SQUARES				
Replicates	2	20083.0	185.3	0.032	0.07	4.9
Parents	1	193321.2***	6337.5**	9.35***	0.37***	10.7NS
Hybrids	4	353321.9***	3145.7**	0.53*	0.25***	23.2***
Parents VS Hybrids	1	119858.5**	676.8NS	2.65***	0.24**	12.9*
Error	12	7803.9	429.6	0.096	0.016	2.3
Total	14	119068.6	1341.8	0.882	0.114	10.2

*: Significant at 5%, **: Significant at 1%, ***: Significant at 0.1%, NS: Non significant

(*ex-situ*), Ranchi, over seed and commercial crop seasons of three successive years (Table 2) indicates better performance of recipient parent, Jata in productivity traits like shell weight (1.79 g), filament length (1045.6 m) and non break filament (317.6 m), while the donor, Daba in quality traits like filament denier (10.04 d), low silk waste (0.89%) and lesser filament breaks (6.21 no) besides better productivity in terms of total silk yield (98.95 g).

Analysis of variance (ANOVA)

The ANOVA (Table 3) among parents, Daba (donor) and Jata (recipient)), F1 & BC hybrids (BC1 to BC4) for silk filament commercial traits indicate significant variance (5 to 0.1% levels) except for filament breaks among parents and non break filament among parents versus hybrids. The filament length, filament denier and silk waste among parents; filament length, filament breaks and silk waste among hybrids and filament denier among parents versus hybrids were highly significant (0.1% level).

Performance of backcross hybrids

The Performance levels of Jata x Daba F1 and BC (BC1 to BC4) hybrids (Table 4) indicates considerable impact of backcrossing on filament denier which was consistently decreasing from F1 to BC4 (12.5 to 10.2 d) in spite of fluctuating trends in other silk filament related traits. The F1 hybrid has recorded highest filament length (1646 m) among all hybrids with better length of non break filament (288m) over donor parent, Daba and marginally lesser filament denier (12.5 d) over recipient parent, Jata. The BC1 hybrid shown 1043 m of filament length, 282 m of non break filament with 11.7 d filament denier, the BC2

Table 4. Performance levels of Jata × Daba backcross hybrids at F1, BC1, BC2, BC3 & BC4 levels (values are mean, ± SE and % change over donor & recipient parents respectively)

Race/hybrid	Crop season	Filament length (m)	Non break filament (m)	Filament denier (d)	Silk waste (%)	Filament breaks (no.)
Daba (D) Donor	Jul/Aug '06	801±20	250±9.9	10.4±0.14	0.7±0.11	6.7±1.21
Jata (R) Recipient	Jul/Aug '06	1160±54	315±9.7	12.8±0.91	1.2±0.12	9.3±0.83
Jata×Daba (F1)	Sep/Nov '06	1646±86 +105.5+41.9	288±11.5 +15.2−08.6	12.5±0.30 +20.2−02.3	0.8±0.01 +14.3−33.3	8.3±1.21 +23.9−10.7
Jata×Daba (BC1)	Jul/Aug '07	1043±89 +30.2−10.1	282±9.2 +12.8−10.5	11.7±0.14 +12.5−08.6	0.8±0.03 +14.3−33.3	7.3±0.82 +08.9−21.5
Jata×Daba (BC2)	Sep/Nov '07	1317±21 +64.4+13.5	215±19.8 −14.0−31.7	11.3±0.18 +08.6−11.7	1.1±0.05 +57.1−08.3	9.0±1.13 +34.3−03.2
Jata×Daba (BC3)	Jul/Aug '08	757±41 −05.5−34.7	269±8.7 +07.6−14.6	10.5±0.10 +0.96−17.9	0.57±0.11 −18.6−52.5	4.0±0.54 −40.3−66.9
Jata×Daba (BC4)	Sep/Nov '08	973±35 +21.5−16.1	296±6.1 +18.4−06.0	10.2±0.12 −01.9−20.3	0.35±0.04 −50.0−70.8	2.6±0.34 −61.2−72.0

hybrid shown 1317 m of filament length, 215 m of non break filament with 11.3 d filament denier, while BC3 hybrid recorded least filament length (757 m) in spite of better non break filament (269 m) and filament denier (10.5 d). Although BC hybrid at BC4 level has recorded least filament denier (10.2 d), silk waste (0.35%) and filament breaks (2.6 no), the silk filament length (973 m) and non break filament (296 m) could surpass only donor parent, Daba, while they were nearer to recipient parent, Jata. The silk waste and filament breaks were least compared to both donor and recipient parents at BC4 level (0.35% & 2.6 no) followed by BC3 (0.57% & 4.0 no) respectively, while they were highest at BC2 level (1.1% & 9.0 no) followed by F1 and BC1 hybrids.

Discussion

The selection of breeding method in sericulture is to develop a breed with stability, productivity and quality of silk filament and for such aspire, the right technique is repeated backcrossing (Tazima, 1984). The selection of parents is always vital and hence Jata ecorace (wild), which proven its adaptability for rearings and seed production also under ex-*situ* conditions (Hansda *et al*., 2008) and Daba ecorace (semi domesticated), most commercially exploited and with wider adaptability have chosen for backcross breeding in the present experiment. These parental ecoraces exhibit variation both under *in-situ* and *ex-situ* habitats in filament length and filament denier apart from non break filament, silk waste, filament breaks, shell weight and total silk yields (Table 1 and 2).

The tasar ecoraces though undergo hybridization in nature; they live as ecoraces by acclimatizing to a particular ecological niche with specific phenotypic traits and based on amenability and commercial potential their application for repeated backcrossing found rational. The performance levels of ecoraces will come down, while acclimatizing to *ex-situ* condition, with retreat in superior traits seen in natural habitat and make them not suitable as donor to introgress trait of economic value. Hence, selecting a wild ecorace, Jata with superior traits as recipient to infuse qualitative trait from semi domesticated Daba with induction of strict selection for infused trait in following generations found coherent. Further, the retention of 50% of superior economic traits of *in-situ* grown wild ecorace in F1 generation and subsequent attainment of 75%, 87.5% and so on of domesticated blood in BC1, BC2 and so on progenies makes their stock maintenance easy, and to backcross with amenable Daba ecorace than choosing a wild ecorace (*in-situ* or *ex-situ*) as donor (recurring parent) for backcrossing.

The significant variance in silk associated traits among parents (donor and recipient), hybrids (F1 & BC1 to BC4) and parents versus hybrids indicate the genetic divergence among chosen parents of different ecozones like Jata from Orissa and Daba from Jharkhand of India. The other reasons might be the impact of repeated backcrossing and continuation experimental generations through seed and commercial crop rearing seasons during the years 2006 to 2008. The higher significant variance of filament denier (0.1%) in parents versus hybrids indicates the influence of chosen parents, introgression impact of selected trait and role of selection pressure at each generation in advancing the refinement of silk filament denier.

Unlike multivoltine mulberry silkworm (*Bombyx mori*), the tropical tasar silkworm (*Antheraea mylitta*), face seed crop (Jul-Aug) generating non diapause destined cocoons with simultaneous emergence, coupling and egg laying and commercial crop (Sep-Nov) with diapause destined cocoons undergoes long period of 6~7 months of hibernation. The seed crop cocoons are with thin shell and less silk, while, the commercial crop cocoons with thick shell and high silk content demonstrate specific commercial importance of seed and silk besides the wild insect's correlation and protection from foreseen environmental changes. As the present study on filament denier introgression (from F1 to BC4 generations) face seed and commercial crop rearings alternatively, the role of environment to be considered for better interpretation of results, as genotype and environment (G × E) interaction proven to play a key role on expression of silk associated phenotypic traits (Kumar *et al*., 2002; Reddy *et al*., 2009; Srivastava *et al*., 2004).

The induction of selection pressure on parents at every stage of breeding for desired traits and the repeated backcrossing resulted to introgress finer filament denier into Jata race at BC4 level and results are corroborating the earlier observations made by Moghaddam *et al*., 2005; Nakada, 1992; Raju and Krishnamurthy, 1993. The continuous refinement of silk filament denier in recipient parent over BC generations (BC1 to BC4) and its attainment at par with donor at BC4 level indicate the introgressive hybridization impact on silk quality, which might be a cumulative effect of genes and additive-additive epistatic gene exchanges due to higher genetic diversity among recipient (Jata) and donor (Daba) parental ecoraces (Babu *et al*., 2005; Ooijen, 1992; Petkov *et al*., 2003; Verma *et al*., 2003).

However, the decrease in filament length in BC1 and BC3 generations over its preceding generation clearly depicts the impact of changed environmental conditions during both crop seasons on larval duration and feed quality as nutrition besides the insects' diapause and non diapause predestined compatibility, (Kumar *et al*., 2002; Malik and Reddy, 2007; Mulder and Bijma, 2005). But, the descending trend in respect of filament denier over generations (from F1 to BC4) supports the progress of introgressive breeding on the selected trait of denier in the right direction. The trend in respect of non break filament was ascending, while it was descending in silk waste and filament breaks with advancement of generations, except at BC2 level, which recorded opposite. The trends in respect of filament denier, silk waste and filament breaks are commercially advantageous, as they enhance filament quality. The varying trends in filament length among alternative generations (between seed to seed & commercial to commercial crops), though, improves over donor parent, indicates correlation of silk quantity and quality and the polygenic control on quantitative trait i.e., silk yield (Nagaraju, 2002; Chatterjee and Mohandas, 2003). However, the least length of non break filament at BC2 (among hybrids of all generations), in spite of better filament length might be due to more number of breaks and higher silk waste or vice versa, besides the prevailed conditions during silkworm rearing and cocoon spinning (Kumar *et al*., 2002; Takabayashi *et al*., 1994).

Interestingly, the hybrid at F1 level had shown superior performance, which might be with the impact of heterobeltiosis on silk yield and higher heterogenousity among parental races with dominant epistatic gene interactions (Siddiqui and Sengupta, 1994), an additional advantage to exploit silk filament yield. The highest filament length (1646 m) with comparatively lower denier (12.5 d), silk waste (0.8%) and filament breaks (8.3 nos) over recipient parent, while the non break filament was little shorter than recipient (−8.6%), but improved over donor (+15.2%) indicates the influence of introgression hybridization, (Moghaddam *et al*., 2005; Sekharappa *et al*., 1999; Verma *et al*., 2003), besides better silk associated trait expressiveness of phenotype during commercial crop season. The optimal genotype environment relations with longer larval feeding period on better quality leaf under congenial climate (Malik and Reddy, 2007; Reddy *et al*., 2009; Srivastava *et al*., 2004) might be the other reason for elevated performance.

The study infers the prospects of Jata and Daba ecoraces as material source for backcross breeding to improve filament quality, besides the possibility of exploiting heterobeltiosis for filament yield at F1 level. The commercially important finer denier of tasar silk filament can be attained with slight reduction in silk yield of wild Jata (recipient) ecorace by adopting repeated backcrossing for four generations with semi domesticated Daba (donor) ecorace.

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