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Research Article

PHYLOGENETIC ANALYSIS OF TASAR ECORACES AND HYBRID POPULATIONS AS REVEALED THROUGH SSR MARKERS

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ABSTRACT

The tropical tasar silkworm, *Antheraea mylitta* D. is a semi-domesticated, trivoltine, wild, sericigenous insect (Lepidoptera: Saturniidae), existing in the form of more than forty ecoraces /ecotypes based on geographical and basically feeding on *Terminalia sp.* The improved varieties of these silkworms can be evolved by hybridization between Andhra local and Daba TV ecoraces by backcross method, to improve the traits in the upcoming generation by selective parental selection. The present studies of genetic relations is based on phylogeny of Tasar ecoraces using SSR markers, which further provides molecular evidence of the fact that climatic factors, the changes at DNA level and its wide range of distribution in varied geographic conditions would lead to genetic divergence, ultimately leading to the formation of new ecorace.

Keywords: *Antheraea mylitta*, Ecoraces, Breeding, Backcross, SSR markers, Phylogeny.

INTRODUCTION

The Tasar silkworm, *A. mylitta* has rich genetic resource as 44 ecoraces, however, the Tasar culture, an important co-discipline of applied forest biology, needs special understanding and addressing towards breeding perspective to promote the sustainable utilization of this precious natural resource (Reddy *et al*., 2009). 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. The backcross breeding of silkworm using parents with preferred traits and selection in subsequent generations offer superior varieties (Reddy *et al*., 2009).

Assessment of genetic diversity is essential for efficient management and conservation of any animal genetic resources in gene banks. Since, SSRs are co - dominant markers and can reveal multiple alleles at a single locus and been extensively used in the diversity analysis of animal and plant system. This present work undertaken characterizes the ecoraces of Tasar silkworm, *A. mylitta*, from different parts of tropical forest zones, as basis for identification and genetic diversity among the tasar populations. Based on these reports, a comprehensive breeding programme could be evolved to conserve the dwindling population of Tasar silkworm, *A.mylitta*, Andhra local ecorace.

The present investigation is an attempt to study the genetic proximity of the ecoraces of *A. mylitta*, bring about an idea of breeding of Andhra local ecorace with other ecoraces, without losing its beneficial commercial characters and suggest methods to overcome its weaknesses and as a strategy to conserve the dwindling population, which is on the brink of extinction. Its conservation focused in the lines of carrying out hybridization with more than viable variety. The selection of parental strains for a breeding program is a based on economically desirable quantitative traits of the parental ecotypes.

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MATERIAL AND METHODS

The parental stocks of ecoraces *viz.,* Andhra Local and Daba TV of Tasar Silkworm *A. mylitta* raised during the seed crop rearing season, July-August. The Andhra local and Daba TV seed cocoons were collected from RTRS (Warangal district) and Telangana State Silk Board (Chennur Mandal, Adilabad district) respectively. They arranged in the form of garlands in grange chambers. The disinfection of room is using with 2% formaldehyde, prevented by arranging suitable nylon net, ventilated grainage chambers in the laboratory. The date of emergence of each of the ecorace (male/female) noted. The male and female moths emerged out of Non - diapause cocoon stocks of the above divergent geographic ecoraces used for the study.

Isolation of Genomic DNA

Genomic DNA was extracted by the use of the phenol-chloroform method modified by Nagaraja & Nagaraju, (1995).

SSR based molecular analysis of polymorphic study on the different ecoraces of *Antheraea mylitta* D.

Simple sequence repeats (SSR) otherwise known, as microsatellites are randomly repeated DNA sequence motifs present in eukaryotic genomes. SSR markers became very popular because they are highly reproducible, multi-allelic and Co dominant markers

The reaction mixture is giving a momentary spin for thorough mixing of the cocktail components. Then 0.2ml PCR tubes are loaded in a thermal cycler (Research Master Cycler PTC 200, Eppendorf) Table 3.

The thermal cycler programmed as follows

Profile 1: 94°C for 10 min - Initial denaturation

Profile 2: 94°C for 30 sec - Denaturation

Profile 3: 48-60°C for 35sec - Annealing

Profile 4: 72°C for 45 sec - Extension

Profile 5: 72°C for 10 min - Final extension

Profile 6: 4°C for infinity to hold the sample.

Profile 2, 3 and 4 programmed to run for 35 cycles.

Phylogenetic Study of different ecoraces of Tasar Silkworm, *Antheraea mylitta* D.

The Phylogenetic relationship among tasar ecoraces was analyzed by generating the Phylogenetic tree by (Nei, 1972) genetic distance using UPGMA analysis through POPGENE software 1.32 version (Yeh & Boyle, 1999).

The PCR amplified bands scored visually by different ecoraces of *A. mylitta* because of their presence (1) or absence (0). The scores obtained were then pooled for constructing a single data matrix, which was used for estimating the proportion of polymorphic loci, (Yeh *et al.*, 1999) gene diversity (h), gene flow (Nm), coefficient of gene differentiation GST, (Nei, 1978) unbiased genetic distance (D). Significant test and construction of a UPGMA (Un weighted Pair Group Method of Arithmetic Means) dendrogram among populations were carried out by using POPGENE version 1.32 (Yeh *et al.*, 1999) computer program. Band sharing based intra- population similarity indices (S1) were calculated for all possible comparisons according to the following formula: Similarity index (S1) = 2NAB / (NA + NB).

Scoring for Co-dominant markers (SSR)

With co-dominant markers, such as allozymes, RFLP and SSR, each recognizable allele at a given locus is ordinarily associated with a single band at a unique position in the gel. Thus, in the case of diploid organisms for a given locus, a homozygote will have one band and a heterozygote will have two. Null alleles (no band) rarely seen. In addition, if there are multiple alleles per locus, as expected for SSRs, which are highly variable, the total number of bands expressed by all the individuals in a sample will likely be much greater than the number of loci involved.

In the profile of dendrograms for SSR using popgene 1.32., the level of polymorphism expressed as the percentage of all loci that are polymorphic. It also gives details about no. of alleles, gene flow, genetic distance, gene diversity, *etc*. Genetic Distance (D) Genetic distances designed to express the genetic differences between two populations as a single number. If there are no differences, the distance sent to Zero, whereas if the population has no allele in common at any locus the distance may be set equal to its maximum value, 1. The genetic distance (D) was calculated by POPGENE software (Yeh *et al.*, 1999) using (Nei, 1972) standard genetic distance equation.

RESULTS

The Instar wise average Temperature (°C) and its standard deviation of Tasar silkworm *Antheraea mylitta* (F1and F2 Hybrid) were as follows. F1 Hybrid: 32.21 ± 0.966 (S.D), 32.22 ± 2.108 (S.D), 30.14 ± 1.463 (S.D), 31.16 ± 1.834 (S.D), 29.20 ± 2.592 (S.D), and F2 Hybrid: 28.50 ± 1.322 (S.D), 28.91 ± 0.948 (S.D), 28.35 ± 0.748 (S.D), 28.78 ± 0.9063 (S.D), 29.37 ± 0.882 (S.D), for I, II, III, IV and V instars respectively. The instar wise average Relative Humidity (%) and its standard deviation for F1 Hybrid were 86.78 ± 6.619 (S.D), 86.11 ± 6.050 (S.D), 88.42 ± 7.390 (S.D), 90.16 ± 3.544 (S.D) and 90.50 ± 4.377 (S.D)

and F2. Hybrid were 93.14 ± 2.672 (S.D), 91.50 ± 4.764 (S.D), 94.00 ± 2.516 (S.D), 92.28 ± 4.644 (S.D) and 87.58 ± 3.648 (S.D) in I, II, III, IV and V instars respectively (Table 1). The no of dfls of Tasar silkworm *Antheraea mylitta* (F1 and F2 Hybrid) were 02 and 01; the mortality was 109 and 54 *i.e.,* 33.5% and 41% respectively, and cocoon yield was 80 and 2 Andhra local parental, Daba-TV parental, F1 Hybrid, were found to have a genetic distance of 0.2126, 0.1629, and 0.1920 with that of F2 Hybrid. The dendrogram produced by UPGMA of Nei's genetic distance for all populations (4 ×13 = 52) is presented on figure 3 (Phylogenetic tree of SSR), in (Table 4).

A summary of genetic variation statistics for all loci depicted in the (Table 7) indicated. The average number of alleles observed was for 1.7143 ± 0.4688, 1.6429 ± 0.4972, 1.7857 ± 0.4258, and 1.5714 ± 0.5136, for ALP, DTVP, F1 Hybrid, and F2 Hybrid respectively, the average number of effective alleles were 1.3828 ± 0.3748, 1.3417 ± 0.3819, 1.4205 ± 0.3394, and 1.2599 ± 0.2728 respectively, while average for the total populations was 1.5555 ± 0.4047 when all populations were taken together, Genetic diversity (h) 0.2290 ± 0.1930, 0.2031 ± 0.1979, 0.2574 ±0.1774 and 0.1724 ± 0.1701. An alternative approach for calculating the within population variation is Shanon's diversity index which does not assume Hardy-Weinberg equilibrium. Average Shannon's diversity index was 0.3499 ± 0.2701, 0.3106 ± 0.2786, 0.3934 ± 0.2491, and 0.2707±0.2578 ALP, DTVP, F1 Hybrid, and F2 Hybrid respectively (Table 7). The genetic diversity in the four populations presented in (Table 7). The total genetic diversity (Ht) was 0.2290 ± 0.1930, 0.2031 ± 0.1979, 0.2574 ± 0.1774 and 0.1724 ± 0.1701. Within-population sample genetic diversity (Hs) of ALP, DTVP, F1 and F2 Hybrids were as 0.229 ± 0.1930, 0.2031±0.1979, and 0.2574 ± 0.1774 and 0.1724 ± 0.1701. While overall Gene differentiation (Gst) was 0.3013 (Table 7).

Table 1. Instar-wise Temperature (°C), Relative humidity (%) of F1 and F2 hybrids of Tasar Silkworm *Antheraea mylitta* D.

Instar	Temperature (°C)		Relative Humidity (%)	
	F1	F2	F1	F2
I	32.21 ± 0.966	28.50 ± 1.322	86.78 ± 6.619	93.14 ± 2.672
II	32.22 ± 2.108	28.91 ± 0.948	86.11 ± 6.050	91.50 ± 4.764
III	30.14 ± 1.463	28.35 ± 0.748	88.42 ± 7.390	94.00 ± 2.516
IV	31.16 ± 1.834	28.78 ± 0.9063	90.16 ± 3.544	92.28 ± 4.644
V	29.20 ± 2.592	29.37 ± 0.882	90.50 ± 4.377	87.58 ± 3.648

*The values expressed in terms of Standard Error of the mean.

Table 2. Rearing performance of F1 and F2 hybrids of Tasar silkworm, *Antheraea mylitta* D in 2015 (for 1 DFL = 150 eggs).

S.No.	Hybrid	No. of dfls	Mortality	Percent Mortality	Cocoon yield	% Cocoon yield
1	F1	2	109	33.5**%**	80	26
2	F2	1	54	41**%**	26	17.3

Table 3. PCR amplification of DNA with SSR primers (The cocktail for PCR amplification for respective SSR fragments is prepared. The reaction mixture (10µl) contains).

PCR products	Amysat 005	Amysat 007	Amysat 024	Amysat026	Amysat033	Amysat034	Amysat036
Genomic DNA(10ng/µl)	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl
10xPCR buffer	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl
25mM $MgCl_2$	0.6 µl	0.6 µl	0.6 µl	0.6 µl	0.6 µl	0.6 µl	0.6 µl
1Mm dNTPs	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl
Forward primer	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl
Reverse primer	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl
Taq DNA polymerase	0.1µl	0.1µl	0.1µl	0.1µl	0.1µl	0.1µl	0.1µl
MQ	4.3 µl	4.3 µl	4.3 µl	4.3 µl	4.3 µl	4.3 µl	4.3 µl

Table 4. Nei's original measures of genetic identity and genetic distance.

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*******************************************************************************
**                                                                           **
**         Nei's Original Measures of Genetic Identity and Genetic distance  **
**                        [See Nei (1972) Am. Nat. 106:283-292)]             **
**                                                                           **
*******************************************************************************


================================================
 pop ID    1         2         3         4
================================================
 1          ****    0.9120    0.8301    0.8085
 2        0.0921      ****    0.8295    0.8497
 3        0.1862    0.1869      ****    0.8253
 4        0.2126    0.1629    0.1920      ****
================================================

 Nei's genetic identity (above diagonal) and genetic distance (below diagonal).
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Table 5. Calculation of mean genetic distance of parental and hybrid ecoraces of Tasar Silkworm *Antheraea mylitta* D (as calculated from the Table 4).

Sl. No.	Ecoraces	Mean values
1.	Andhra local parent	0.1473
2.	Daba-TV parent	0.1883
3.	F1 Hybrid	0.1891

Table 6. Polymorphism of Hybrid populations of *Antheraea mylitta* D, as revealed by phylogenetic analysis based on SSR primers**.**

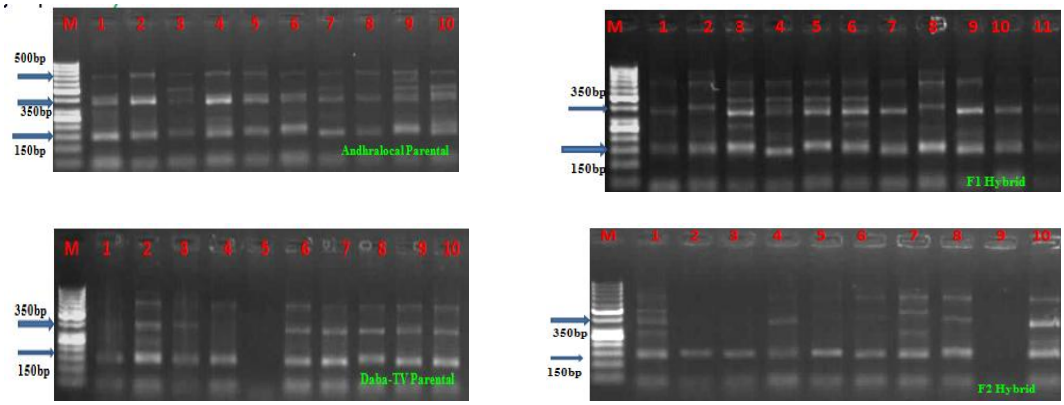
Ecorace	Place of collection	Number of polymorphic loci	Percentage of polymorphic loci%
Andhra local (Parental)	RTRS,Warangal, Telangana	10	71.43
Daba TV (Parental)	Adilabad, Telangana	9	64.29
F1 Hybrid	K.U.Warangal, Telangana	11	78.57
F2 Hybrid	K.U. Warangal, Telangana	8	57.14

Table.7. Nei's Analysis of gene diversity in subdivided populations.

S.No.	Ecoraces	Na	Ne	h	I	Ht	Hs
1	Andhra local Parent	1.7143± 0.4688	1.3828 ± 0.3748	0.2290 ± 0.1930	0.3499 ± 0.2701	0.2290± 0.1930	0.2290 ± 0.1930
2	Daba TV Parent	1.6429± 0.4972	1.3417± 0.3819	0.2031 ± 0.1979	0.3106 ± 0.2786	0.2031± 0.1979	0.2031± 0.1979
3	F1 Hybrid	1.7857± 0.4258	1.4205± 0.3394	0.2574 ± 0.1774	0.3934± 0.2491	0.2574± 0.1774	0.2574± 0.1774
4	F2 Hybrid	1.5714± 0.5136	1.2599± 0.2728	0.1724± 0.1701	0.2707± 0.2578	0.1724± 0.1701	0.1724± 0.1701

Population genetics parameters for the 11 populations of hybrid Populations of *A.mylitta* D. Population - wise data on the number of alleles (Na), number of effective alleles (Ne), h = genetic diversity; I = Shannon Index, Ht = Total genetic diversity, Hs = Sample genetic diversity, Gst = Gene differentiation, Nm= Gene flow.

Figure 1. Moths of Tasar Silkworm, *Antheraea mylitta* D., Andhra local and DabaTV ecoraces



Andhra local parental : Lanes1–10 represent different strains of Andhra local ecorace

Daba TV parental : Lanes 1–10 represent different strains of Daba TV ecorace

F1 Hybrid : Lanes 1–11 represent different strains of Daba BV ecorace

F2 Hybrid : Lanes 1–10 represent different strains of Modal ecorace

M: 50 bp DNA Ladder

Note: Primer *Amysat* 026*;* Fragment size is 142 bp

Figure **2.** SSR profiles generated from genomic DNA of 11 strains from different individuals of (Andhra local parental, Daba- TV parental, F1 Hybrid, and F2 Hybrid) Populations of Tasar Silkworm, *A.mylitta* D using the primer Amysat026.

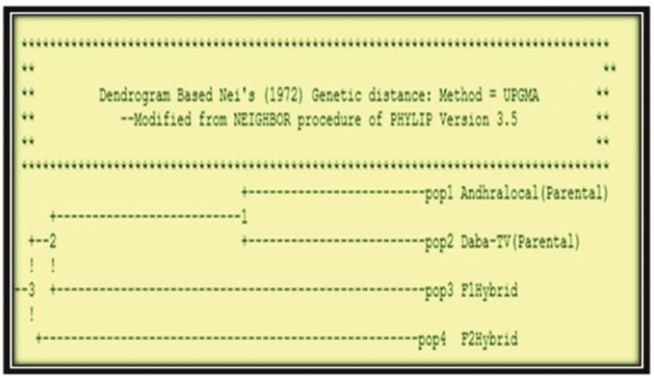


Figure 3. UPGMA dendrogram depicting genetic diversity of Tasar Silkworm, *A. mylitta* D genotypes were obtained by PCR-SSR marker data (POPGENE version 1.32).

DISCUSSION

In India, rearing of silkworm hybrids is in vogue to get better cocoon yield. Since bivoltine silkworm hybrids cannot survive the harsh climatic conditions in the tropics, especially during the summer and rainy months, hardy tropical silkworm hybrids need to be developed (Srivastava *et al*., 2009).

The DNA isolation and the quantification of the 1-11 samples of Tasar Silkworm, *A. mylitta* D Parental ecoraces (Andhra local, Daba TV, F1 and F2) revealed an ideal concentration of DNA ng / µl of the genomic sample *i.e*., between 1.8 – 1.9 in 260 / 280 ratio) checked against 1 kb standard DNA ladder was obtained. It been observed that DNA has been isolated without any protein or any other contamination and was used for further studies in PCR and phylogenic analysis. Genetic characterization of 4 populations *viz.,* parental ecoraces (Andhra local, Daba TV), F1 and F2 was done. Out of the 15 primer combinations tried 6 primer combinations have shown to have desired size fragments and three primers *viz.,* Amysat024, Amysat026 and Amysat034 (of allelic size 200 - 250, 130 - 500 and 150 - 400 respectively) have shown polymorphism, while three primers *viz.,* Amysat007, Amysat033 and Amysat036 (of allelic size 200, 200 and 250 respectively) have shown monomorphism.

The SSR amplification of 4 tasar populations (11 individuals in each, with six primers, which generated polymorphism) using 6 SSR primers yielded a total of 335 bands, out of which 166 bands were polymorphic (49.55%). During scoring, all the bands present in both polymorphic and monomorphic profiles selected. The average no. of amplicons produced per DNA sample was 2 - 6 per primers, most of the bands observed within the range of 150-500 bp, which is in accordance with the allelic size of the primers taken for studies (Figure 2).

Out of a total of 335 bands produced in the 4 tasar populations, 94 bands in Amysat026, followed by 67 bands in Amysat034, 52 bands in Amysat024, 44 bands in Amysa033, 42 bands in Amysat007 and 36 bands in Amysat036 primers were generated. Among the 14 alleles, 11 alleles have shown polymorphism with a minimum of 2 and a maximum 4 bands in each lane.Amysat026 primer generated a total of 94 bands, out of which 30 bands belong to Andhra local. The no. of alleles produced 5 in Amysat026 followed by3 in Amysat024 and 1 each in Amysat007 and Amysat033 primers. From the above observations, it can to see that Amysat026 can consider as an effective SSR marker to identify the tasar ecotypes. From Table 4 and 5 on genetic distance, it can be seen that F1 Hybrid (mean value = 0.1891) shows higher genetic distance among the other three populations (*i.e*., Andhra local parent and Daba -TV parent), which implies that F1 Hybrid is genetically distant from other ecoraces. It observed that the lowest genetic distance found in ecorace Andhra local parent (0.1473).

In the present investigation, screening of genomic DNA from 11 individuals of 4 populations using 6 SSR primers yielded several reproducible amplicons. The average no. of amplicons produced per DNA sample were 2 - 6 per primers, with sizes ranging from 130-500 bp. The percent polymorphism was 86.16% in Amysat 026, whi1e it was 76.11 Amysat034 and 65.38% Amysat024 SSR primers (Figure 2). In the present studies, the germplasm collected from Andhra local, Daba -TV and their hybrids F1 and F2 displayed variable genetic polymorphism and was found to be highest in F1Hybrid population of Telangana (78.57%), followed by Andhra local (71.43%) of Telangana and Daba TV (64.29%) and F2 (57.14%) in Table 6.

The order of genetic closeness may summarize as follows: Daba TV parent is genetically less close than F1 Hybrid. Andhra local parent is genetically less close than Daba TV parent is. Andhra local parent and Daba TV parent found to be close within the populations according to phylogenetic tree. Earlier, studies using ISSR markers to analyze the intra-race diversity in Daba (Kar *et al*., 2005) and Raily (Srivastava *et al*., 2009) ecoraces of *Antheraea mylitta* D revealed considerable genetic differentiation across and within the populations of both the ecoraces. In another work, using RAPD markers, genetic variation among the different ecoraces of this moth were assessed (Saha *et al*., 2008). A study of the genetic structure of the different *A. mylitta* D ecoraces using polymorphic microsatellite can loci successfully done recently Chakraborty *et al*. (2015) and Renuka & Shamitha, (2016). The present work, based on genetic analysis of 7 tasar ecoraces using SSR and ISSR generated polymorphism, emphasized not only on the genetic closeness of Andhra local ecorace in relation to other ecoraces, but it went further probing its compatibility to mate with them. The resultant F1 and F2 population assessed genetically based on the SSR primer, which reported for the first time. The molecular characterisation and phylogenetic analysis revealed parental ecoraces (Andhra local and Daba TV) formed one cluster, while F1 and F2 formed individual clusters. It may note that F1 population is genetically closer to the parental cluster than that of F2 (Figure 3).

The present studies, which reveals a considerable improvement of commercial aspects (Sreenivas & Shamitha, 2017) is directed towards the conservation of Tasar silkworm, *A. mylitta* D, Andhra local ecorace needs further improvement of breeding practices, to enable the viability of F1 and F2 generations. PCR - SSR based phylogenetic analysis using popgene 1.32 in 4 tasar populations *viz.,* parental ecoraces (Andhra local, Daba TV), F1 and F2), revealed that F1 Hybrid shows higher genetic distance among the other 3 populations (*i.e*., Andhra local parent and Daba -TV parent), which implies that F1 Hybrid is genetically distant from other ecoraces. It observed that the lowest genetic distance found in ecorace Andhra local parent. Populations of Andhra local and Daba TV (Parental ecoraces) found to be close within the populations according to phylogenetic tree. The percent polymorphism was 86.16% in Amysat 026, whi1e it was 76.11 Amysat034 and 65.38% Amysat024 SSR primers.

The genetic structure of parental (Andhra local and Daba TV) and Hybrid populations (F1 and F2) is presented

in Table 7. The study clearly demonstrates that the number of alleles (N_a), number of effective alleles (N_e), genetic diversity (h), Shannon Index (I) total genetic diversity (H_t) and sample genetic diversity (H_s) were highest in F1 hybrid population. This is in concurrence with the percent polymorphism, which is greater in F1 (78.57%) than that of the parental types which might be the reason for forming an individual cluster in the phylogenetic tree. Similarly, the above population genetics parameters were lesser in F2 hybrid populations than the parental as well as F1 types, which resulted in the formation of individual cluster. This is a significant observation from the breeding studies, which aimed at the improvement of Andhra local ecorace.

According to the recent reports, high intra population variability indicates optimum heterozygosity (Kar *et al*., 2010) which is clearly demonstrated by the F1 populations, on the other hand, the low genetic parameters of F2 populations appropriate attention towards conservation. As per the analysis of genetic diversity amongst parental (Andhra local and Daba TV) and hybrid populations (F1 and F2), the gene differentiation (G_{st} = 0.3013) and the gene flow of (N_m = 1.1597). Clearly indicate that populations are in the threshold of genetic differentiation, in section 4.6.1 and 4.6.2 Furthermore, the gene flow above 1.00 is the indication of mating between the adjacent populations (Slatkin, 1987).

Nei's analysis of gene diversity reveals the total gene diversity (H_t) was estimated to be (0.3084 ± 0.0412), which is a higher than Hs (0.2155 ± 0.0207), while average gene diversity ($D_{st} = H_t - H_s$) and gene differentiation were calculated to be 0.0929 ± 0.0205 and 0.3013 respectively. The values of G_{st}, N_m and D_{st} (D_{st} value was not zero) suggest that some genetic differentiation must have taken place between the populations, though at a very low pace, as revealed by gene diversity analysis (Su *et al*., 2009).

The study on the SSR based phylogenetic analysis in the parental and hybrid populations of tasar ecoraces is not so far reported. The present investigation of hybridization based on backcross method between two contrasting genetically variable ecoraces *viz.*, of Andhra local and Daba TV was successful for two successive commercial crops. It attributed to the fact that both these populations are inhabited in geographically almost same altitude and comparatively lesser, when compared to other ecoraces. It could be one of the reasons of their compatibility of mating and production of F1 and F2 generations with quality cocoons. This is also corroborated by the study on genetic differentiation as revealed by ISSR markers which indicated negligible possibility of genetic mixing in high altitude (Vijayan *et al.*, 2006).

On one hand, Daba TV is one of the sought after ecoraces, exploited commercially as it is more amenable to handling in the larval stages and better suited for cocoon production with its reproductive fitness (Sinha, 2011), while on the other, Andhra local ecorace, embodied with unique cocoon characteristics, is on the brink of extinction. It is the need of the hour to exploit the breeding techniques and develop hybrids with these ecoraces, which are co-existent in this geographical zone. It is indeed much favorable if it can be standardized in aspects like selection of parents, marker assisted breeding, optimizing temperature and relative humidity during rearing and investigate using more number of individuals in each ecorace, ultimately to yield cocoons without losing their distinctive characteristics.

CONCLUSION

The present study on genetic diversity of tasar ecoraces, selected parental ecoraces and F1 and F2 hybrid populations, as revealed by co-dominant markers helps the prospective breeders in the development of disease resistant, high yielding tasar populations and contribute towards their conservation. The study needs to be explored further using more number of primers and individuals, as genetic diversity and geographic distribution depend on several evolutionary factors, which need to study in detail.

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