

Molecular Phycogenetic Aspects in Tasar Culture

Vineeta Ateesh¹, Sunita Thakur², P.K. Tewary³, P.K. Kar⁴, O.P. Dubey⁵

^{1,2}Department of Botany, Doranda College, Ranchi University, Ranchi, Jharkhand, India

^{3,4,5}Central Tasar Research & Training Institute, Nagri, Ranchi-835303, Jharkhand, India

Abstract: *Tasar culture is an agro forest based industry of the indigenous tribes, who are in dire need of high yielding, season specific and resistant breed(s) of tasar silkworm (Antheraea mylitta Drury) and of food plants (Terminalia spp.). The biotechnology arsenals operated through genomics, proteomics, bioinformatics and transgenesis hold the key to facilitate the bringing up of desired products in tasar culture. The paper portrays about the latest biotechnological tools and their applicability. A comprehensive review on the molecular genetic works attempted so far in tasar silk worm and host plant have been reviewed. followed by adoption of suitable strategies on use of the biotechnological tools so solve the constraints of tasar culture.*

Keywords: Biotechnology, tasar culture, markers, tissue culture, Antheraea, Terminalia, Shorea

1. Introduction

Tasar silkworm (*Antheraea mylitta* Drury) is commercially utilized for production of vanya or wild silk. The species is polyphagous in nature and distributed in a wide range of geo-climatic conditions of Indian subcontinent expressing wide variation in phenotypic, physiological, behavioral and commercial traits. Various geographically isolated population of *A. mylitta* inhabiting small territories favors the adaptation to local conditions and thus resulted in the evolution of ecoraces. The primary food plants of tasar silkworm include *Terminalia arjuna* Bedd, *T. tomentosa* W. & A., *Shorea robusta* G. whereas there is a dozen of secondary host plants also, such as *T. chebula*, *T. bellirica*, *Lagerstroemia* spp., *Anogeissus* spp. Etc. These potential genetic resources are the ideal material to be used for crop improvement, evolution of disease resistant breeds and introgression of useful traits in commercially utilized breeds. In recent years the biotechnological tools have been applied in various crops on a global scale. The same can be applied in tropical tasar also. Hence, the paper deals with a general account of the work done so far in tasar silkworm and its host plant so as to facilitate the researchers to acquaint themselves and work on the future aspects.

2. Status on Molecular Studies in Tasar Culture

2.1 Tasar Silkworm

Molecular Phylogenetic aspects

Under molecular phylogeny aspects a number of efforts have been made for establishing phylogenetic relationship among the evolutionary offshoots of the phylogenetic tree. Prasad et al., (2002) have reported the phylogeny and pattern of divergence of mariner like elements (MLEs) in silkmths in relation to the phylogeny of the host species. Almost all the silkmths MLEs contain conserved features that are characteristic to MLEs such as the D₁D₂(34)D motif. Out of 33 partial MLEs sequenced 31 were defective due to stop codons or frame shifts in the transposase ORF. So also was the case with the three copies of the full-length mariner elements isolated from *A. mylitta*. Their results indicated that, in general, phylogenetic relationships between MLEs

obtained from diverse silkmths are similar to the phylogeny of the host species consistent with the vertical inactivation stage of the MLE life cycle. It looks probable that most of these elements were present in the ancestral lineage prior to the divergence of these species and neutral evolution has occurred independently in each copy with respect to coding of amino acids in the transposase gene. For example, the MLEs from *Antheraea* species, *A. roylei*, *A. pernyi*, *A. proylei*, *A. mylitta*, and *A. yamamai* clearly belong to a subgroup of closely related elements within the *Cecropia* subfamily. *A. assama* is considered different from the other *Antheraea* species, and more close to the common ancestor of *Antheraea* and *Philosamia*. Accordingly, the MLEs from *A. assama* comprise a separate subgroup within the *Cecropia* subfamily, along with the MLEs from probably related species, *A. polyphemus*. These results imply that the MLEs from *Cecropia* subfamily existed in the genome of the common ancestor of the *Antheraea* and *Philosamia* species.

Prasad and Nagraju (2003) reported that Mariner like elements (MLEs) are widely distributed type II transposons with an open reading frame (ORF). Comparative phylogenetic evolution and inverted terminal (ITR) conservation of MLEs from Indian saturniid silk moth *A. mylitta* were studied with other full length MLEs. They have described that full length MLEs were inactive with multiple mutations. Many conserved amino acid blocks were identified after aligning transposase sequences. Mariner signature sequences were almost invariable although a few new classes of elements had different signatures. *A. mylitta* MLEs (Anmmar) get phylogenetically classified under *Cecropia* subfamily and *Bombycoidea* superfamily.

Hwang et al., (2004) have worked on comparative molecular study between *Bombycoidea* and *Saturniidea* based on mtDNA RELP and cytochrome oxidase I gene sequences. Implication for molecular evolution, reported comparative sequence analysis have shared a 9%, 85% and 8% sequence identity with *B. mandarina*, *A. yamamai*, and *A. pernyi* respectively. *A. yamamai* shared 92% sequence identity with *A. yamamai* and *A. pernyi* respectively. This exhibits monophyle and confidence limits of more than 99% in all for both *Bombycoidea* and *Saturniidea*.

Molecular Characterization aspects

Under characterization for establishing relationship among the ecoraces or breeds or evolved lines, various efforts have been made, using mostly the dominant molecular markers. Chatterjee et al., (2004) attempted molecular characterization and worked on ISSR profiling of genetic variability in the Raily ecotypes of *A. mylitta* and presented the DNA polymorphism unraveled by 12 ISSR primers for 11 population of *A. mylitta* belonging to 6 ecotypes and 41 individuals of Raily ecotypes collected from five zones. This gives molecular evidence on genetic differences between eleven ecotype populations and highlights the genotypic diversifications of a single ecotype into further separate discrete gene pools. The analysis revealed grouping of the five population of Raily ecotype into two clumps. The analysis also identified nine markers which can be utilized to characterize specific population. In one set 34 accessions of tasar silkworm were PCR amplified using 13 ISSR primers (UBC) a total of 274 bands were obtained of which 259 bands were polymorphic. Variation was observed within and between ecotypes. The analysis showed that significant variation exist within a single population.

Kar et al., (2005) studies the genetic diversity in the wild and semi-domestic populations of Daba ecorace of *A. mylitta* to ascertain the distribution of variability within and among populations with ISSR markers. A total of 138 markers were produced among 56 individuals of the three populations, of which 98% were polymorphic. The population genetic aspects were also worked out. Considerable intra- and inter-population variability is found in all three populations. The population structure analysis further suggests that the semi-domestic populations of Daba ecorace are at the threshold of differentiating themselves. The high genetic variability present within wild Daba population of *A. mylitta* is of much importance for conservation as well as utilization in systematic breeding programme.

Ghosh et al., (2005) worked on molecular characterization of Pao like long terminal repeat retrotransposons, Tamy in saturniid silkworm *Antheraea mylitta* and presented that a long terminal (LTR) retrotransposon Tamy was obtained by screening *Antheraea mylitta* sub genomic DNA library with PCR amplified partial fibron gene sequences as probe. Tamy has 1305 nucleotide long LTRs at its 5' – and 3' – ends with characteristic features of a functional LTR retrotransposon. Starting from its N terminus nucleic acid binding motif (cys) protease, reverse transcriptase, RNase and integrase domains were present in sequential order. RT domain in Tamy showed high homology with Pao like retrotransposable elements.

Datta et al., (2000) have reported purification and characterization of fibroin gene from *A. mylitta* and further on differential expression of this gene in development stage of silkworm *A. mylitta*. Qanungo et al., (2000) reported characterization of cypoviral isolates from tropical and temperate Indian saturniid silkworm. Qanungo et al., (2002) made studies on molecular cloning and characterization of *A. mylitta* cytoplasm polyhedrons virus (CPV) genome segment. Datta et al., (2005) studies the molecular

characterization of *A. mylitta* CPV polyhedron gene and its variant forms.

Scientists at Indian Institute of Technology, Kharagpur have reported that a total of 24 sets of RAPD primers produced 415 reproducible bands and were used to generate the distance matrix and subsequent clustering. Out of seven sets of SCAR primers, a single primer pair produced polymorphic SCAR bands which are used to diagnose five of the ten ecoraces. The southern hybridization and sequence analysis show the repetitive nature of the Taq DNA fragment designated as *A. mylitta* Taq repeat family, AmTRF (Ghosh et al., 2005)

2.2 Tasar Food Plants

Molecular Genetic Studies

Tewary and Suryanarayana (2007) developed the protocol for isolation and purification of genomic DNA from the tasar silkworm host plants viz. *Terminalia tomentosa*, *T. arjuna*, *T. belerica* and *T. chebula*. They have also used these DNA for PCR amplification using random decamer primers. Not much work has been done on primary food plants species, therefore works on allied species are reviewed. Pither et al., (2003) have studied *Terminalia Amazonia* using RAPD analysis. In total 30 RAPD bands were generated by five decamer primer which were used to compare the genetic diversity of six populations in two groups. Genetic variation within the population as estimated by Shannon diversity index ranged from 0.32 to 0.38 with an overall diversity of 0.38. Analysis of molecular variance revealed that most of the variation was attributable to differences among individual within population. Population differentiation was significantly lower among the fragmented population than among the continuous forest population. On an average the fragmented population also had slightly but statistically significant lower level of genetic diversity. One gallery forest site had higher level of genetic diversity than two of the continuous forest sites. It is suggested that long-term fragmentation of genetic diversity of tropical trees will depend upon the amount of local forest cover in proximity to the fragmented population.

Warude et al., (2003) reported the method to obtain DNA in quality and quantity from plants such as "*Embolica officinal*", *Terminalia belerica*, *T. chebula* which have low pH and high amounts of secondary metabolites in tissue extracts. The modified DNA isolation method yields good quality high molecular weight DNA that is free of contaminants and colored pigments and is suitable for PCR amplification, also suitable for isolating DNA from dry powder.

The isozymes variation within and among population has been extensively studied in many wood plants including tropical species. Significant correlation between isozyme and quantitative traits is evident. Suoheimo et al., (1999) have reported isoenzyme based genetic diversity in natural population of *Shorea robusta* using 12 loci from 8 isozyme systems. The mean number of alleles per locus was 2.16 and 58.3% of the loci were polymorphic.

Kamiya et al., (2005) have reported phylogeny of PgiC gene in *Shorea* and its closely related general (Dipterocarpaceae),

the dominant trees in south east Asian tropical rain forest and informed that sequences generated in a gene tree with better resolution than previous cpDNA trees. The Pgi C tree is essentially consistent with cpDNA trees except for the placement of *Neobalanocarpus*. Ujino et al., (1998) have reported developmental and polymorphism of simple sequence repeats DNA markers for *Shorea curtissi* and other Dipterocarpaceae. Four SSR markers She01, She04, She07, She09 were found highly polymorphic.

2.3 Tissue Culture Studies

No many reports are available on tasar host plant tissue culture, except a few sporadic attempts. Nishi et al., (1998) induced callus from leaf explants of *Terminalia arjuna* on MS medium supplemented with 2,4-D (5.0mg/l) and Kinetin (0.01mg/l). Somatic embryogenesis was achieved from callus culture and in turn plant regeneration was reported on MS medium fortified with 2% sucrose and gelled with 0.8% agar. Tirkey et al., (2002) have studied the effect of anti oxidants and absorbent on tissue browning metabolism in *Terminalia arjuna*. Phenolic exudation in the explants/cultures was controlled by adding citric acid (10.0mg/l), PVP (50.0 – 150.0 mg/l) and ascorbic acid (50.0 – 100.0 mg/l) to the media. Genetic differences in response of in vitro shoot induction of *Terminalia arjuna* varieties were also studied by Tirkey et al., (2002). Out of five genotypes tested B2 responded maximum towards sprouting percentage and shoot growth on MS media supplemented with BAP (0.5 mg/l), Kn (2.0 mg/l) and NAA (0.1 mg/l).

Ramesh et al., (2002) reported shoot induction and proliferation from nodal explants of mature tree of *Terminalia arjuna* on TDZ (0.05 mg/l) fortified MS medium. The performance of TDZ as compared to BAP and Kinetin was found much better. Rhizogenesis was obtained on ½ MS or B5 media supplemented with IBA (1.0 – 4.0 mg/l). Regenerated plantlets were established in the greenhouse.

Micropropagation protocol from nodal explants of *Terminalia bellerica* seedlings has been developed by Ramesh et al., (2005) by culturing the explants on MS medium supplemented with 13.3 µM BAP. Subsequently, in vitro shoots were multiplied on MS medium with lower concentration of BAP i.e., 4.4 µM. Rooting was observed under in vitro condition on modified B5 medium or wood plant medium fortified with 4.9 µM IBA. Shyamkumar et al., (2003) developed in vitro propagation protocol for *T. chebula*. Multiple shoots from cotyledon node explants has been induced on ½ MS media supplemented with 0.3 mg dm⁻³ GA₃ + 1.0 mg dm⁻³ IBA + 10.0 mg dm⁻³ BAP. Somatic embryogenesis in callus cultures of *T. chebula* has been achieved by Anjaneyulu et al. (2004) exploiting the seedling explants (cotyledon, hypocotyls etc.). The cultures of somatic embryos open an avenue for genetic transformation in such important tree species. Shyamkumar et al. (2007) have also studied genetic transformation of *T. chebula* via *Agrobacterium tumefaciens* strain C-58. Cotyledon explants were found responsive to transformation and detection of nopaline in transformed callus was observed indicating presence of tannic acid in the tissue.

Jain et al., (2002) have reported in vitro propagation of shoot of *Shorea robusta* G on MS media supplemented with different growth regulators. Rhizogenesis has been achieved from cut end of in vitro shoots. Tewary et al. (2007) have reported callus induction and proliferation from leaf explants of *Shorea robusta* on MS media supplemented with 2,4-D + Kn and studied the growth rate of callus proliferation.

3. Future Biotechnological Applications

In the perspective of tasar culture constraints and potentials where the tasar silk producer are poor indigenous tribes who are neither monetarily nor literally equipped to translate the technologies should be provided with ready made high yielding and resistant food plant and silkworm breeds which can sustain adverse conditions with least input application. Biotechnology holds the key through which some significant achievement can be anticipated. The tools and techniques used for crop improvement in many economically important plant and animal genetic resources hold good for tasar silkworm as well as host plant. They include genome analysis, identification of QTLs, development of disease and stress resistant/tolerant races/breeds/varieties, attempting to manipulate the regulation and expression of desired gene or set of genes and host plant-insect interaction. Transgenic host plants and silkworm may also be one of the future propositions to have a new avenue in tropical tasar culture.

References

- [1] ANJANEYULU, C., SHYAMKUMAR, B. and GIRI, c.,c. (2004). Somatic embryogenesis from callus cultures of *Terminalia chebula* R. an important tree. *Tree Struct. Funct.*, 18: 547-552.
- [2] CHATTERJEE, S.N., VIJAYAN, K., ROY, G.C. and NAIR, C.V. (2004). ISSR profiling of genetic variability in the ecotypes of *Antheraea mylitta* Drury, The tropical tasar silkworm. *Russian J. Genet.*, 40, 8(2) : 152-159.
- [3] DATTA, A., GHOSH, A.K. AND KUNDU, S.C (2000). Purification and characterization of fibroin from the tropical saturniid silkworm *Antheraea mylitta*. *Insect Biochem. Mol. Biol.*, 31: 1013—1018.
- [4] GHOSH, A.K., ABHIK, DATTA, B. MAHAENDRAN AND JKUNDU, S.C. (2005). Molecular characterization of Pao like long terminal repeat retrotransposon Tamy in saturniid silkworm *Antheraea mylitta*. *Curr. Sci.*, 89(3):539-543.
- [5] Hwang, j.s., lee, j.s., gootw, k., sohn, h.r.kim, h.r. and kwon, o.y. (2004). The comparative molecular study between Bombycidae and Saturniidea based on mtDNA RFLP and cytochrome oxidase I gene sequences: implication for molecular evolution. National Sericultural and Entomological Research, RDA, Suwon Korea.
- [6] JAIN, M. AND CHATURVEDI, S.C. (2002). In vitro proliferation of shoot of *Shorea robusta* F. in (Eds. S.K. Nadi, L.M.S. Palvi and A. Kumar) *Role of plant Tissue culture in Biodiversity Conservation and Economic development*. Gyanodya Prakashan, Nainital. Pp 73-78.
- [7] KAMIYA, K. HARADA, K., TACHIDA, H. AND ASHTON P.S. (2005). Phylogeny of PgiC gene in *Shorea* and its closely related genera

- (Diptero0carpaceae) the dominant tree in south cast Asian tropical rain forests. *Am. J. Bot.*, 92:775-78.
- [8] KAR, P.K., VIJAYAN, K., MOHANDAS, T.P., NAIR, C.V, SARATCHANDRA, B. AND THANGAVELU, K. (2005). Genetic variability and genetic structure of wild and semi-domestic populations of tasar silkworm (*Antheraea mylitta*) ecorace Daba as revealed through ISSR markers. *Genetica*, 125:173-183.
- [9] NISHI, KUMARI, JAISWAL, UMA AND JAISWAL, V.S. (1998). Introduction of somatic embryo0genesis and plants regeneration from leaf callus at *Terminalia arjuna* Bedd. *Curr. Sci.*, 75 (10.25) : 1052-1085.
- [10] PITHER, R., SHORE, J.S. AND KELLMAN, M. (2003). Genetic diversity of the tropical tree *Terminalia Amazonia* (Combretaceae) in naturally fragmented population. *Heredity*, 91(3):307-13.
- [11] PRASAD, M. D. AND NAGRAJU, J. (2003). A comparative phylogenetic analysis of full length mariner elements isolated from the Indian tasar silk moth *Antheraea mylitta* (Lepidopteran : Saturniidae). *J. Biosci.*, 28: 443-453.
- [12] PRASAD, M.D., NUMINSKY, D.L. AND NAGRAJ, J. (2002). Characterization and Molecular Phylogenetic analysis of mariner elements from wild and domesticated species of wild silk moth. 25: 210-218.

