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Molecular Phycogenetic Aspects in Tasar Culture

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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 n 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 ecorarces. 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 silkmoths in relation to the phylogeny of the host species. Almost all the silkmoths MLEs contain conserved features that are characteristic to MLEs such as the D,D(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 silkmoths 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 comsodered different from the other Antheraea species, and more close to the common ancestor of Anthereaea 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 transposans 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 clases of elements had different signatures. A. mylitta MLEs (Anmmar) get phylogenetically classified under Cecropia subfamily and Bombycidea superfamily.

Hwang et al., (2004) have worked on comparative molecular study between Bombycidea and Satumiidea 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 Bombycidate and Satumiidea.

Molcular Characterization aspects

Under characterization for establishing relationship among the ecoraces or breeds or evolved lines, various efforts have been made, usijng mostly the dominat molecular markers. Chatterjee et al., (2004) attempeted mmolecular characterization and worked on ISSr profiling of genetic variability in the Raily e\ecotypes of A. mylitta and presented the DNA polymorphism unravelted 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 diffences 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 indentified 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 retro transposable 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 characterizastion 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.

Seientists at Indian Institute of Technology, Kharagpur have reported that a total of 24 sets of RAPD primers produced 415 reporducible 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 diagonose five of the ten ecoraces. The sothern 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 studies 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 oppulations in two groups. Genetic variation within the population as estimated by Shanon 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, Terimnalia 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 studies 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 Neobalanocarpous. Ujino et al., (1998) have reported developmental and polymorphism of simple sequence repeats DNA markers for Shorea curtissi and other Diopterocarpaceae. Four SSR markers She01, She04, She07, She09 were4 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/I) and Kinetin (0.01/mg/I). 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 oxideants and absorbent on tissue browing metabolism in Terimnalia arjuna. Phenolic exudation in the explants/cultures was controlled by adding citric acid (10.0mg/I), PVP (50.0 – 150.0 mg/I) and ascorbic acid (50.0 – 100.0 mg/I) to the media. Genetic differences in response of in vitro shoot induction of Terminalia arjuna varieties were also studies 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/I) , Kn (2.0 mg/I) and NAA (0.1 mg/I).

Ramesh et. Al., (2002) reported shoot induction and proliferation from nodal explants of mature tree of Terminalia arjuna on TDZ (0.05 mg/I) fortified MS medium. The performance of TDZ as compared to BAP and Kinetin was found much better. Rhizogensis was obtained on ½ MS or B5 media supplemented with IBA (1.0 – 4.0 mg/I). 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. Subseqnelty, 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. Shyamkumae et al. (2007) have also studies 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 supplanted 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 condtions 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.

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