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Genetic diversity of *Persea bombycina* from goalpara district of Assam, India

Azizur Rahman[1], Bhaben Tanti[2*], Gajen Chandra Sarma[2], Jatin Kalita[3]

[1]Department of Sericulture, Government of Assam, Guwahati, India
[2]Department of Botany, Gauhati University, Assam, India
[3]Department of Zoology, Gauhati University, Assam, India
Email: [*]bt53@rediffmail.com

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ABSTRACT

Assam of the northeastern region of India is unique in terms of its rich biodiversity and multiple ethnicity of its people. The impact of the resultant socio-religio-cultural diversity is also reflected in the diverse traditional ways of silkworm farming. We report the genetic diversity of *Persea bombycina* "*Som*" from different locations of Goalpara district of Assam, India, where random amplified polymorphic DNA (RAPD) marker was used in this study. RAPD analyses of ten genotypes of "*Som*" from Goalpara district of Assam, India with B19 RAPD primer generated 86 bands, showing an average of 8.6 bands per sample and 30.2% (26 bands) of these were polymorphic. The number of bands per accession ranged from 5 to 10 with a mean of 8.6 and the size range of the amplified bands was 250 - 6000 bp. In a UPGMA phenetic dendrogram based on Jaccard's coefficient, the *P. bombycina* accessions showed a high level of genetic variation, as indicated by genetic similarity and revealed 10 "*Som*" genotypes in to three major clusters. This study may be useful in identifying diverse genetic stocks of *P. bombycina*, which may then be conserved on a priority basis. The RAPD primer used in this study was able to distinguish all the 10 genotypes of "*Som*" plants, which can be used to assess genetic diversity.

Keywords: *Persea bombycina*; Genetic Diversity; Molecular Markers; RAPD Analyses

1. INTRODUCTION

Persea bombycina (King ex Hook. f.) Kost, locally known as "*Som*" is one of the primary food plants of *Antheraea assama* Westwood, the silkworm that produces muga, or golden colour natural silk, no where in the world [1-3]. "*Som*" belongs to the family Lauraceae, a medium size evergreen tree with spreading branches, bark and foliage usually aromatic, alternate leaves grows abundantly in its natural habitat in Assam particularly Brahmaputra Valley up to an elevation of about 500 meters, apart from its distribution extend to Khasi and Jayantia Hillls in India, along the Lower Himalaya and as far as to the west of Nepal [4,5]. The commercial use of *Persea bombycina* (King ex Hook. f.). Kost is restricted to Northeast India, although the plant grows abundantly in almost all parts of India, Nepal, Myanmar, Cambodia, Malaysia and Indonesia [6].

Different researchers have reported variations among "*Som*" [7,8]. The macro and micro morphological variation may differentiate according to their growth, leaf-yield, shape and size of the leaf, colour, its different taste and odour. Different authorities have approached the classification of "*Som*" from different perspectives. The reason for these contradictory estimates is due the presence of some elements of instability in the classification of "*Som*". The family is notorious for the paucity of taxonomically useful morphological characters, and generic limits are often vogue and in dispute [9].

A more recently employed approach in plant systematic and population biology is random amplified polymorphic DNA (RAPD) markers, a PCR-based (polymerase chain reaction) technique. The simplicity, efficiency, and relative ease of performing RAPD techniques, without sequence information, have led to their expanded use for taxonomic and systemic analysis and phylogenetic studies of plants, species differentiation, and phylogeographic variation, as well as for studying breeding and genetic relationships [10-20]. RAPD techniques have recently been used in genetic diversity studies of a wide range of tree plants [21,22].

Characterizing the types and extent of genetic variation is essential to identifying genotypes so that they can be effectively used by breeders, geneticists, and conservationists [23]. Earlier classifications and evaluations of

[*]Corresponding author.

P. bombycina were based solely on morphological and physiological characteristics, which are easily influenced by the environment. Although reliable and consistent classification can be obtained through genetic information, a literature survey failed to uncover any published work in this area. Moreover, given the lack of knowledge of genetic differentiation among the "*Som*" genotypes and the wild nature of the muga silkworm, it is difficult for farmers to retain a uniform quality of silk [24]. Therefore, the present study was emphasized to discriminate the 10 "*Som*" genotypes on the basis of RAPD analyses from Goalpara district of Assam.

2. MATRIALS AND METHODS

2.1. Sample Collection

Young leaves were selectively collected from ten phenotypically distinct *Persea bombycina* plants from the "*Som*" plantation at Goalpara District, Assam, India (**Figure 1**). After collection, these specimens were lyophilized, placed in sealed plastic bags, and chilled (–80˚C) until genomic DNA was extracted.

2.2. DNA Extraction

Genomic DNA was isolated from 10 plant leaves of *Persia bombycina* by following the protocol as mentioned in GeneiPure™ plant Genomic DNA Purification Kit (Catalogue#117298). The quantity of genomic DNA was determined by electrophoresis on a 0.8% agarose gel against a known quantity of unrestricted lambda DNA.

2.3. RAPD Fingerprinting

To optimize PCR amplification, experiments were carried out with varying concentrations of template DNA, random primer, *Taq* polymerase, and $MgCl_2$, as well as dNTPs. RAPD reactions were performed using 10-mer RAPD primer [B19; 5'-ACCCCCGAAG-3' (Bangalore Genei)] under PCR conditions as mentioned bellow.

All PCR reactions were carried out in 25 µl volumes containing 2.5 µl of 10× PCR buffer with $MgCl_2$, 2 µl of a mixture containing each of the dNTP's at a concentration of 2.5 mM, primer at a final concentration of 5.0 pM and 2 U of *Taq* DNA polymerase (Bangalore Genei). At first, the amplification was run for 8 cycles and each cycle comprised of 4 minutes of denaturation at 94˚C, again 45 seconds of denaturation at 94˚C, 60 seconds of annealing at 35˚C, and a 90 seconds of extension step at 72˚C. This was followed by 35 cycles, where each cycle comprised of 45 seconds of denaturation at 94˚C, 60 seconds of annealing at 38˚C and 60 seconds of extension at 72˚C. After 35 cycles, there was a final 7 minutes extension at 72˚C. The PCR amplifications were performed using a Perkin Elmer Thermal Cycler (GeneAmp PCR2400, Perkin Elmer, USA). The PCR products were purified by following manufacturer's protocol with QIAquick PCR purification kit obtained from Qiagen.

Amplified PCR products (15 µl) were separated on 1.5% agarose gel (w/v) in TBE buffer at 70 V for 150 min. The gel was visualized by ethidium bromide staining and documented under UV light by a Gel Doc-2000 system (BioRed).

2.4. Statistical Analysis and Construction of Dendrogram

The banding patterns generated by RAPD analyses were converted to binary matrices assigning 1 for present and

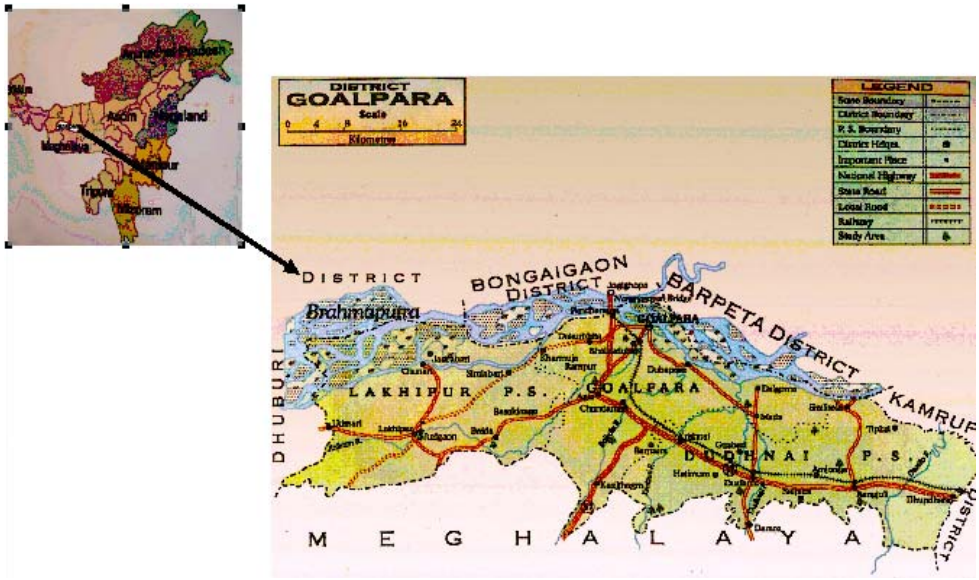

Figure 1. Map of Goalpara district showing study areas.

0 absent of the bands. Similarity matrices were constructed by using SPSS version 11.0 software (Information Technology Service Center, Lingnan University, 2002) followed by Jaccard similarity coefficient analysis. To construct the dendrogram, levels of similarity among the banding profiles were calculated by using the band matching Dice coefficient (S_D) and the cluster analysis of similarity matrices was calculated with the unweighted pair group method with arithmetic average (UPGMA).

3. RESULTS

Although earlier studies have reported some results of research on chemical, cytological, pollen morphological, ecological, geographical, and karyotypic characters of the species; neither the genetic diversity nor the divergence were clear. The purpose of the present study is to assess genetic diversity and divergence within and among populations of the "*Som*" species from Goalpara district of Assam, India.

In the RAPD analyses, a total 86 bands were scored for the B19 RAPD primer corresponding to an average of 8.6 bands per sample, and 30.2% (26 bands) of these were polymorphic. The primers used for the study are known to provide reproducible RAPD profiles. We screened B19 RAPD primer for its ability to generate a consistently amplified band pattern and to assess polymorphism in the tested genotypes. We discarded poorly stained, unique, and very low-frequency bands from the data set used for further analysis. The level of polymorphism revealed by this study was high. Morphologically, the 10 selected genotypes also showed variations which is identical with the study of some earlier workers, reported that the use of a small number of primers is sufficient when morphological variation is high [24,25]. The number of bands per accession ranged from 5 to 10 with a mean of 8.6, and the size range of the amplified bands was 250 - 6000 bp. The B19 primer used in this study was able to distinguish all the 10 genotypes of "*Som*" plants, which can be used to assess genetic diversity (**Figure 2**).

According to the genetic distances obtained and the relative position of each genotype in the UPGMA tree, using a combined data set for the B19 primer revealed three major clusters. The cluster I consisted of 5 genotypes obtained from three different blocks *viz.*, *Balijana*, *Lakhipur* and *Kuchdhowa*, whereas the second and third group comprised germplasm collected from only *Balijana* block under Goalpara district of Assam, India.

Bands were scored for their presence or absence in 10 genotypes of *P. bombycina.* The pairwise similarity values between the genotypes, based on both shared and unique amplification products, were calculated using Jaccard's coefficient. There is great genetic variation among the genotypes belonging to cluster I. Out of five genotypes grouped in cluster I, genotype S1 and S8 showing 80% similarity whereas S6 and S7 showing only 55% similarity on the basis of RAPD analysis. Again, S9 under same cluster is only 40% similar with S6 and S7, whereas these three genotype revealed only 20% similarity with S1 and S8. Genotypes S3 and S4 belonging to cluster II showing 70% similarity and these two genotypes showing 50% similarity with S10 under same cluster. But, cluster II revealed only 15% similarity with cluster I. Again, genotypes S2 and S5 under cluster III showed only 30% similarity with each other whereas, this cluster did not show any similarity with cluster I and cluster II on the basis of the RAPD results (**Figure 3**).

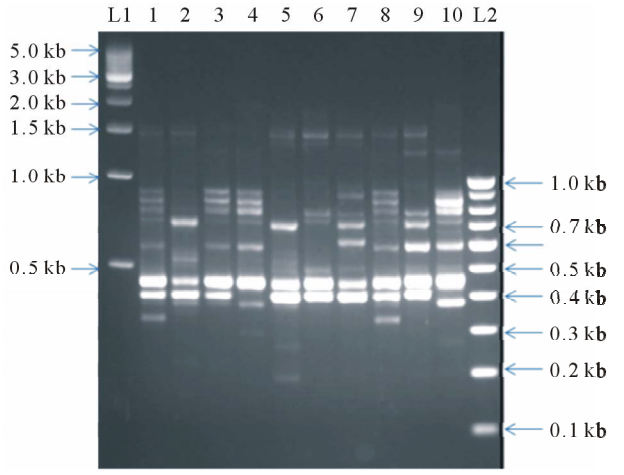

Figure 2. RAPD Profile of 10 plant leaves of *Persia bombycina* generated using RAPD primer B19. (L1: StepUpTM 500 bp Ladder, Banglore Genei, Cat#105670; Lane 1: Sample 1; Lane 2: Sample 2; Lane 3: Sample 3; Lane 4: Sample 4; Lane 5: Sample 5; Lane 6: Sample 6; Lane 7: Sample 7; Lane 8: Sample 8; Lane 9: Sample 9; Lane 10: Sample 10 and L2: StepUpTM 100 bp Ladder, Banglore Genei, Cat#107417).

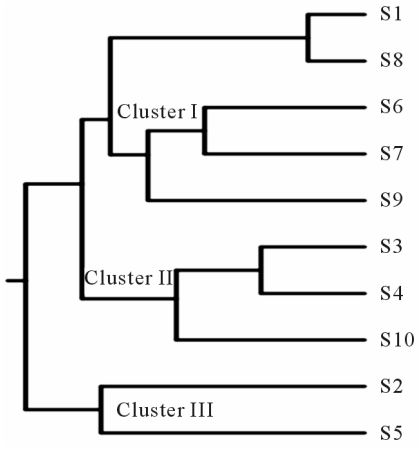

Figure 3. Dendrogram of the "*Som*" genotypes using cluster analysis method produced from Jaccards estimates.

4. DISCUSSION

The dendrogram generated from RAPD data revealed a highly consistent pattern of grouping, thus strengthening the validity of our data. RAPD analysis proved to be an effective technique for measuring the magnitude of diversity and discriminating between genotypes. In Assam, the scientific communities as well as general people believe that *Persea bombycina* (King ex Hook. f.) Kost, formerly named as *Michilus bombycina* King. is one of the most significant primary food plants of *Antherea assama* ww, locally known as "*Som*" belongs to single species. But, our study revealed that 10 genotypes of "*Som*" plants could be clustered into three major groups with a diverse range of variations among themselves. Out of 10 "*Som*" genotypes, four genotypes have already been identified at Central National Herbarium (CNH), Kolkata, India *viz.*, S1 as *P. bombycina* (King ex Hook. f.) Kost, CNH384752; (S5) as *P. gamblei* (King ex Hook. f.) Kost, CNH 384449; (S10) as *P. glucesences* (King ex Hook. f.) Kost, CNH384621 and (S7) identified as *P. odoratissima* (King ex Hook. f.) Kost, CNH13487 respectively. Although genotype S8 was showing ~80% similarity with S1 in RAPD results, but both were quite difference in terms of morphological and palenological parameters. Therefore, further RAPD analysis is required to establish their genetical identity in species level. As the genotypes S2, S3, S4, S6, and S9 are quite different in terms of morphological, palenological as well as RAPD results, therefore, these genotypes are considered as different species and yet to be identified through Quee Herbarium, Royal Botanical Garden, England.

"*Som*" is a highly cross-pollinated plant. The RAPD markers in this study may be attributed to more uniform clusters, which are expected for progenies of wild grown plants with predominant cross pollination [26]. Understanding patterns of genetic variation within tree species is of fundamental importance for successful management in tree conservation programs. Knowledge of possible adaptive variation among areas is essential to the evaluation of biodiversity within and among populations to reveal information on population evolution. The information obtained from this study could be of practical use for mapping the *P. bombycina* genome as well as for classical breeding. The putative species-specific bands can be used as genetic marker for proper identification of economically and commercially viable "*Som*" plants. Further, putative species-specific RAPD markers could be converted to sequence-characterized amplification regions after sequencing and designing primer pairs to develop robust species-specific markers.

The study also provides a basis for *P. bombycina* breeders to make informed selections of parental material based on genetic diversity to help to overcome some of the problems usually associated with a tree crop improvement program. The information can then be used in identifying and prioritizing areas with comparatively high genetic diversity for monitoring, management, and protection. Knowledge of population structure is important for *ex-situ* conservation and *in-situ* conservation of natural populations by maintaining the total evolutionary potential and minimizing consanguinity [27].

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