Abstract

Ten ecoraces of *Antheraea mylitta* were screened with twenty-four random decamer primers in random amplification of polymorphic DNA PCR (RAPD-PCR), twenty simple sequence repeat primers anchored at 5′ or 3′ ends in inter-simple sequence repeat PCR (ISSR-PCR) and seven primers in long primer RAPD PCR (LP-RAPD PCR). The RAPD primers amplified a total of 415 bands of which 340 were polymorphic; ISSR primers amplified a total of 414 bands of which 304 were polymorphic and LP-RAPD primers amplified 188 bands of which 144 were polymorphic. The bands generated by each marker systems were used to estimate the genetic distances between the ecoraces. The overall genetic distance values calculated between the ecoraces by all the three systems were low. Cluster analysis was performed to graphically represent the relatedness between the ecoraces. Three separate phenograms were generated, one each with RAPD, ISSR and LP-RAPD data. The clustering of the ecoraces in RAPD and ISSR phenograms were similar and corresponded well with their geographical distribution. In LP-RAPD phenogram, the clustering of some of the ecoraces was different from the other two phenograms. Therefore, an integrated phenogram was also erected with the combined data of RAPD, ISSR and LP-RAPD and it showed closer proximity to the RAPD and ISSR phenograms. Despite low values of genetic distances, the clustering of the ecoraces was clear and unambiguous.

Average heterozygosity and marker index values were calculated for the three markers systems to estimate their performance and utility. The highest level of polymorphism was detected by RAPD markers and maximum utility was shown by LP-RAPD markers. However, standard two-sample't' test showed that the difference in values of average heterozygosity and marker index of the three marker systems were not significantly different.

Mantel's test of matrix correlation between genetic and geographical distance matrices gave positive 'r' value indicating lack of gene flow between the ecorace populations.

Six morphological traits which, at present, are widely used to identify ecoraces and estimate the closeness between them were subjected to principal component analysis (PCA). A 3D scatterplot and a dendogram were drawn to graphically represent the closeness between the ecoraces. The clustering revealed by these morpho-economic traits were not identical to those revealed by molecular DNA markers thereby indicating that morphological traits cannot be used to determine the genetic relatedness between ecoraces for cross-breeding experiments.

RAPD and SCAR PCRs were used to identify the same ecoraces of *A. mylitta* at the molecular level with specific markers. Ecorace specific fingerprint maps were generated with RAPD markers, SCAR markers and RAPD band based hybridized profiles. Despite the close genetic proximity between the ecoraces, all the ten ecoraces were successfully identified with 5 different fingerprint maps.

Keywords:

Saturniid silkworms, *Antheraea mylitta*, Ecoraces, RAPD PCR, ISSR PCR, LP-RAPD PCR, SCAR markers, Phenograms, Mantel's test, Average heterozygosity, Marker Index, PCA, RAPD fingerprint map, RAPD based hybridized maps.