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RAPD-PCR ANALYSIS OF *Aulacobolus newtoni* IN PALANI HILLS

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ABSTRACT

In this present investigation, to study the genetic polymorphism in the field populations of *A. newtoni*, collected from eight different localities of Palani Hills by using RAPD-PCR. The two primers amplified a total of 75 scorable bands in the molecular weight range of approximately 266bp to 1352bp. The number of amplification products obtained was specific to each primer and ranged from thirty forty five (Kit A15) to thirty (Kit A3). The UPGMA based dendrogram of all the primers grouped the populations of *A. newtoni* in two clusters. The similarity coefficients values were ranged from 0.03 to 0.86, indicating that no two populations were 100% similar. The similarity matrix of all the primers indicated that most of the populations exhibited <50% similarity coefficient. Thus RAPD banding patters suggested that all the populations of *A. newtoni* exhibit a great degree of intraspecies variation, and this could be attributed to the varied agronomical practices, stress on the insect, selection pressure etc.

KEYWORDS : RAPD-PCR, *Aulacobolus newtoni* , Diplopoda, Phylogeny

Millipedes, otherwise known as Thousand Leggers, are striking in appearance and they occupy a unique position among organisms that live on soil substrates. These arthropods are generally long in relation to its width, cylindrical animals with two pairs of legs in each body segment and they have fewer than a hundred pairs of legs. Usually they are seen on damp floors and they feed on rotten plant materials or detritus.

The presence 12,000 described species in 145 families and 16 orders and an estimated global fauna of more than 80,000 species, the Diplopoda (Adis, 2002; Sierwald et al., 2007and Shelley, 2007) is the third largest class of terrestrial Arthropoda following Insecta and Arachnida. A major part of terrestrial ecosystems right through the temperate, subtropical and tropical zones of the world, they are ecologically essential as detritivores (Hopkin and Read, 1992).

Within the last decade, technological advancement has increasingly supported the use of genetics in determining population diversity. Many molecular techniques are now available which allow ecologists and evolutionary biologists to determine the genetic architecture of a wide variety of closely related individuals. RAPDs are viewed as having several advantages over other molecular markers and DNA fingerprints as the technique randomly samples the genome and hence multiple amplifiable fragments are present for each primer (Lynch and Milligan, 1994).

In this present investigation, to study the genetic polymorphism in the field populations of *A. newtoni*, collected from eight different localities of Palani Hills by using RAPD-PCR.

MATERIALS AND METHODS

Aulacobolus newtoni collected from eight different locations of Palani Hills (Figure, I) and DNA isolation was done by Phenol: Chloroform extraction method. RAPD analysis were done by following procedure. 20ng of DNA was dissolved in 20µl PCR reaction buffer containing 10mM Tris-HCl (pH 9.0), 1.5mM $MgCl_2$, 50mM KCl, 0.01% gelatin, 0.2mM dNTPs, 21 pM of primer and 0.5 U of DNA polymerase. Twenty primers (RAPD Kit A1 to RAPD Kit A20) obtained from IDT were used for RAPD-PCR studies. PCR was conducted as initial heat step (94ºC for 5min.), 40 cycles of denaturation (94ºC for 1min.), annealing (36ºC for 1min.) and extension (72ºC for 2min.) and a finial extension step (72ºC for 7min.). Amplification was performed using a programmable thermal Cycler PTC-150 (MJ Research, USA). The products of PCR and DNA size markers [λ DNA digested with EcoRI and HindIII (Bangalore Genei)] were loaded onto a 1.6% tris-borate-EDTA agarose gel and run for 4hrs at 50V. The gels were stained with ethidium bromide and photographed. Each lane of RAPD profiles was subjected to gel documentation system (Vilbert-Lourmat, France). The dendrogram analysis was carried out using Bioprofile 1D software.

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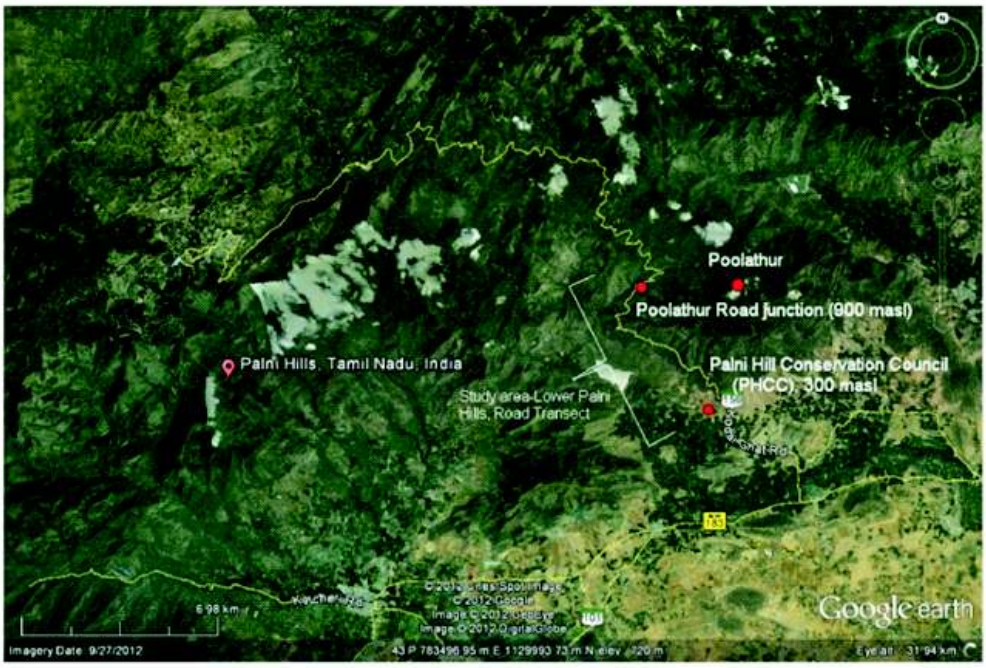


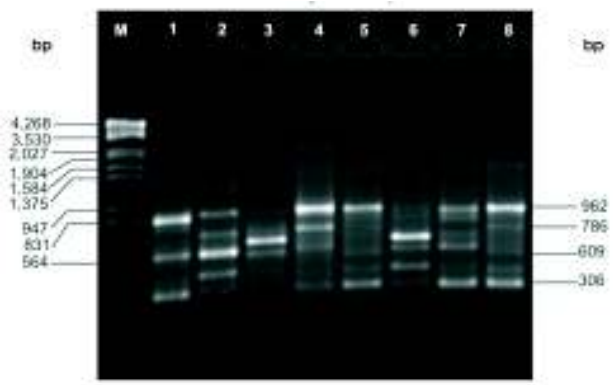
Figiure I: Location OIntensive Study Area in Palani Hills, Western Ghats

RESULTS

The populations of *A. newtoni* obtained from eight different locations in Palani hills were analyzed by RAPD-PCR. A total of twenty decameric oligonucleiotide primers (RAPD Kit A1 to Kit A20) were tested for their ability to provide suitable banding pattern for eight different populations. Of the primer set tested, RAPD KitA3 and A15 yielded clear, consistent and discrete banding patterns for the populations of A. newtoni. Dendrogram and similarity index were constructed based on the RAPD profiles generated by A3 and A15 primer using UPGMA (Bioprofile 1D software).

A total of 30 scorable fragments were produced by KitA3 primer with the molecular weight range of 306bp to 962bp for the population of *A. newtoni* (Figure, II). The RAPD profile showed a maximum of five fragments in S-2 population and a minimum of two fragments in S-3 population. The UPGMA dendrogram showed two major clusters, one comprised populations of S-1, S-5, S-2 and S-8, and the other included S-3, S-4, S-7 and S-6 populations. The similarity index revealed a maximum of 86% similarity among S-2 and S-5 populaions (Figure, III).

The Kit15 primer produced 45 scorable fragments of the range 266bp to 1,352bp (Figure IV). Maximum of



Figiure II: RAPD of different populations of *Aulacobolus newtoni*
Generated by the Primer RAPD Kit A3

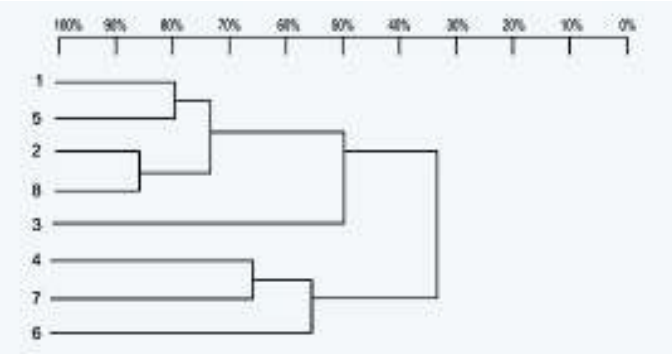


Figure III: Dendrogram with Homology
Coefficient %: 0.0 (UPGMA)

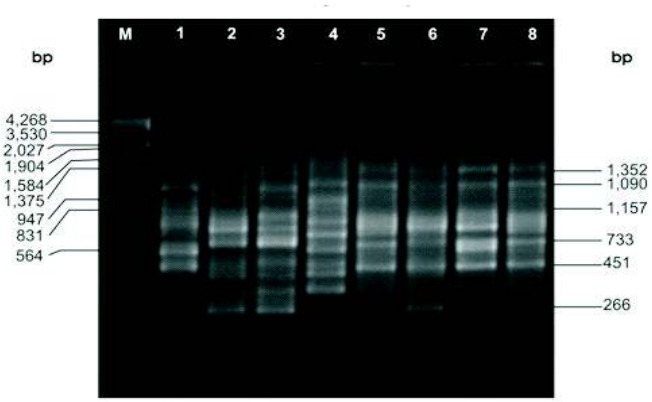


Figure IV: RAPD of Different Populaitons of *Aulacobolus newtoni* Generated By The Primer RAPD Kit A15

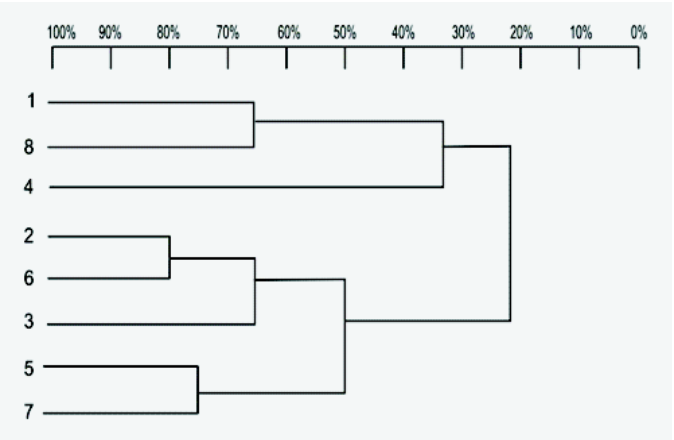


Figure V: Dendrogram With Homology Coefficient %: 0.0 (UPGMA)

eight fragments were exhibited by S-3 and S-5 populations and minimum of four in S-1 and S-2 populations. The dendrogram analysis grouped the populations of *A. newtoni* in two clusters, comprised of S-1, S-8 and S-4 in one cluster, and S-2, S-6, S-3, S-5 and S-7 in another cluster (Figure, V). The similarity coefficients were ranged from 0.21 to 0.80.

The two primers amplified a total of 75 scorable bands in the molecular weight range of approximately 266bp to 1352bp. The number of amplification products obtained was specific to each primer and ranged from thirty forty five (Kit A15) to thirty (Kit A3). The UPGMA based dendrogram of all the primers grouped the populations of *A. newtoni* in two clusters. The similarity coefficients values were ranged from 0.03 to 0.86, indicating that no two populations were 100% similar. The similarity matrix of all the primers indicated that most of the populations exhibited <50% similarity coefficient.

DISCUSSION

Molecular characterization of insects has been frequently conducted on the basis of existence of polymorphic DNA fragments amplified by PCR (Caterino et al., 2000). Haymer and McInnis (1994) have also pointed out a distinctive variation in PCR-amplified DNA patterns between laboratory-cultured and wild populations of Mediterranean fruit flies, Ceratitis capitata (Weidemann). RAPD as a tool to identify genetic variation in ecotypes of the population structure of Helicoverpa armigera using RAPD analysis (Zhou et al., 2000); RAPD markers linked to genetic diversity of Iberian populations of Bemisia tabaci based on RAPD-PCR has promise for displaying intra specific genetic variations in insect species. Skoda et al. (2002) have attempted to clarify PCR-based DNA patterns in *Cochliomyia* sp. collected from various ecotypes.

CONCLUSION

Thus RAPD banding patters suggested that all the populations of A. newtoni exhibit a great degree of intraspecies variation, and this could be attributed to the varied agronomical practices, stress on the insect, selection pressure etc.

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