BIOLOGIA (PAKISTAN)
June, 2016, 62 (1), 127-131

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Eco-genetics and Random Amplified Polymorphic DNA (RAPD)

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ABSTRACT

This paper describes the efficiency of RAPD technique to assess the genetic diversity of *Sordaria fimicola* in response to environmental exposure. Three strains were obtained from divergent microscale environments, harsh South facing slopes (SFS) and mild North facing slopes (NFS) of evolution canyon (EC). The dominant marker RAPD primed the whole genome of filamentous fungi. The fungus 1 primer show high level of polymorphism (69%). Total amplified fragments were 401 out of which 247 were polymorphic bands. Band size was between 20 to 2000 base pairs (bp). The 71 polymorphic bands scored in (strain S3 and its progeny), 66 (strain N5 and its progeny), 110 (strain S*w* 92.1 and its progeny). Sixty seven percent polymorphism was observed in strain S3. Genome of *Sordaria fimicola* in this study was dug out 37.06 kb with five random markers (RAPD). The individuals showed heritable differences (polymorphism) within strains on South Facing Slope (SFS) in response to environmental agents. The phylogenetic tree derived on the basis of the degree of similarity reflected the genetic relationship among strains and their progeny. The hierarchical analysis was used to compare inter and intra specific variants. This was performed by using the unweighted pair group method (UPGM) with arithmetic average.

Key Words: RAPD, *Sordaria fimicola*, Diversity, Environmental stress.

INTRODUCTION

Nature always favors genetic variability. All genetic markers are estimators of natural polymorphism which occur in DNA sequence motif. A positive correlation has been examined between diversity in quantitative traits and DNA markers (Merila & Crnokrak, 2001). Genetic variation in individuals is measured when they are examined in collection of individuals in a population or species. The severe bottle neck population degrades genetic diversity and cannot cope with environmental stresses which lead to extinction of species. Genetic diversity is essential for a population to evolve; species somehow must adapt to environmental changes and it enables an organism to survive in a more varied habitat (Duran *et al*., 2009).

A population responds to its surroundings in multiple ways; an organism runs away from harsh climatic conditions, it adjusts to the changed abiotic component of the ecosystem by means of phenotypic plasticity. Reshuffling, INDEL (insertion, deletion) inversion and translocation and polymorphism are ever-present in nature so most species are chosen by natural selection. The environmental stress induces genomic instability and mutation which are the raw materials in regulation of evolution (Galhardo *et al*., 2007).

Evolution Canyon (EC) which is more than a million years old, acts as a natural laboratory for research, polymorphism, evolution as well as molecular characterization and biodiversity in fungi, plants and other organisms. (Nevo, 2001) The sample for this study was obtained from EC which has two slopes, one slope is the African /south- facing AS/SFS which receives 200-800% solar radiation. Its condition is sunny and xeric, with sparse vegetation like the Savanna. The other European /North Facing Slope ES/NFS is cooler and wetter with lush green vegetation.

The genetic markers are very old. At the time of Mendelian they were called “classical markers”. The phenotypic genetic markers in the fruit fly led to the Theory of Linkage. With new advancement in molecular biology, DNA based marker techniques such as non-PCR-based (DNA-DNA hybridization) RFLP and PCR-based

Author’s Contribution: S.J., Performed experimental work, Wrote manuscript; **M.S**., Helped in experimental work; **M.S.**, Planned and supervised the project; **A.C**., Helped in manuscript. *Correspondence author: sjavaid.phd@gmail.com*

advanced techniques have emerged (SSR, ISSR, SNP) and routinely being used in detection of polymorphism, evolution and phylogenetic studies. Williams *et al*., (1990) used single, short random oligonucleotides of arbitrary sequence to prime the whole genome this is called RAPD (Random Amplified of Polymorphic DNA). This is most widely used for DNA polymorphism and segregation of genetic markers between and within strains of fungus (Sunnucks, 2000). The RAPD is used for detection of relatedness between and within species, and to study population genetics (Althoff *et al*., 2007). The PCR amplified products are separated on gel.

Sordaria fimicola, a coprophilus filamentous fungus belongs to Ascomycota. It was selected, because we already know a great deal about their genetic characteristics. Ascus with eight ascospores have been studied for decades for mutation, recombination and gene conversion, it would be easier to interpret genetic linkage maps and DNA sequence motif. The short 7-12 day life cycle can be used to verify Mendelian laws directly not just statistically. In this study three strains have been used and random priming was done to cover the whole genome, RAPD tool is used due to its low cost, ease handling and to evaluate genetic variability and gene expression in plants sciences (Bauvet *et al*., 2004). Wild strain S3 was obtained from station 3 of EC, the mutant S*w* 92.1 collected from SFS was used for comparison between wild and mutant. In response to environmental stressor mutation (spontaneous and induced) accumulated in fungal hyphae, the molecular markers and genomic DNA were altered. Strain N5 is the original wild strain of NFS of station 5 of EC. Different response to the environmental stress on both slopes gives phenotypically and genotypically different organisms around EC and helps to determine genetic diversity and evolution in local fungi (Nevo, 1997; Nevo, 2006).

The aim of this study was to measure genetic variation in different strains of *Sordaria fimicola* and their F1 progeny, by applying molecular markers techniques to gauge environmental threats.

MATERIALS AND METHODS

The strains samples were obtained from Molecular Genetics Research Laboratory, University of Punjab, Lahore Pakistan. Culturing and sub culturing strains and their F1 generation were prepared in the research laboratory. Genomic DNA was extracted following the CTAB protocol (Gardes & Bruns, 1993). A total of three strains and 30 offsprings of fungus were used for PCR amplified using RAPD. The frozen- thawed mycelium was grinded in a chilled mortar pestle. The CTAB (cetyl-trimethy lammonium bromide) was used for DNA extraction. Five FUNGUS primers were used. 25µl amplification reaction mixture was prepared with 10x PCR buffer 2.5µl, primer 2.5µl, dNTP2.5µl, DNA 1.5µl, *Taq* DNA polymerase 1U, 1.2µl, 2mM $MgCl_2$. Sterile distilled water was added. PCR Amplification program was applied. Application of initial denaturation 94^0C for 7 min. and 94^0C for 30 sec, 36^0C for 45sec followed by 45 cycles. Final extension of 72^0C for 7min. was applied. Amplicons were electrophoretically separated on 1.5% agarose gels.

Scoring and Data Analysis

Fragments were amplified using different FUNGUS primers. Scorable bands were transferred to binary character matrices as discrete variable (1) for presence and (0) for absence. Data was analyzed by using PAST software version 2.17 to study population structure and molecular diversity. To construct dendrograms for RAPD simplest and common hierarchical cluster UPGMA (unweight pair group method with Arithmetic averaging algorithm) and by nearest NJ (neighbor joining) methods were used (Fig., 1). The dendrogram was constructed on the basis of Euclidean distance matrix between strains and their isolates (1-25). The isolates were located on branch tips of life tree. Euclidean distance matrix was used as similarity measure and pair group was taken as algorithm to generate triangular matrices.

The number of polymorphic bands and percentage of polymorphic bands (PPB) were calculated by profiles generated by all population/primers (Fig., 2).

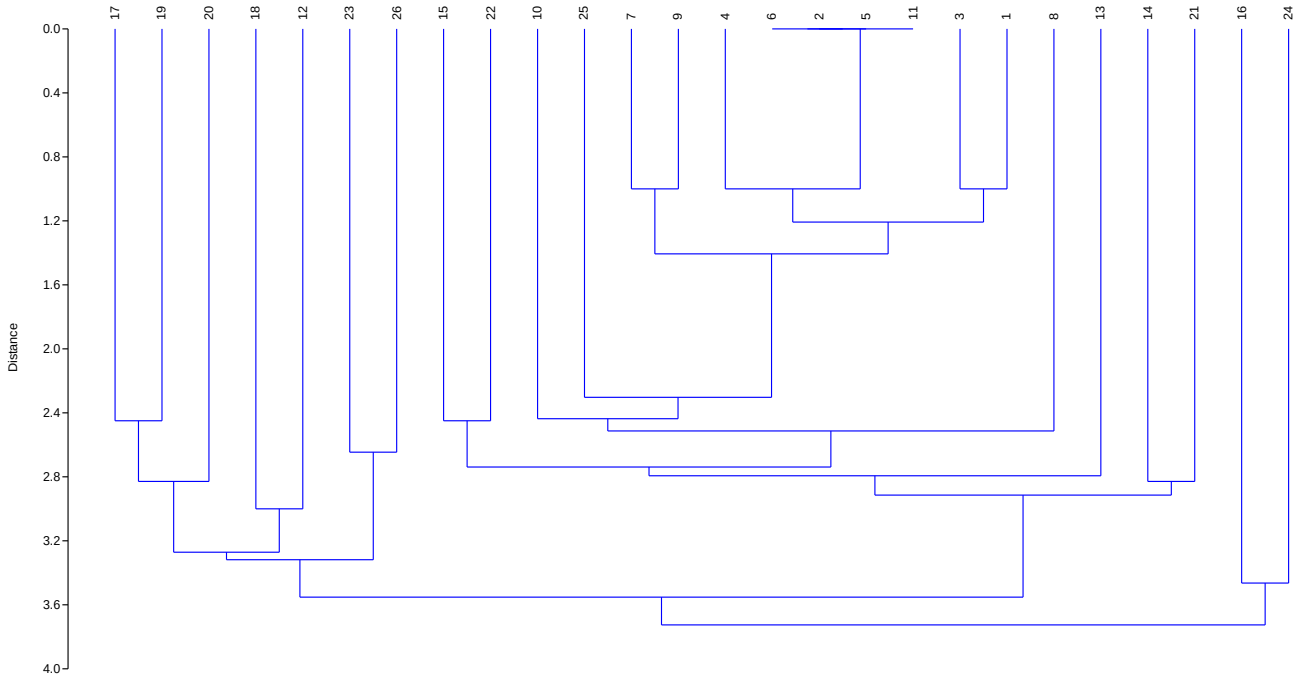

Fig., 1: Dendrogram of all the strains with all the primers generated by using PAST. See text for detail.

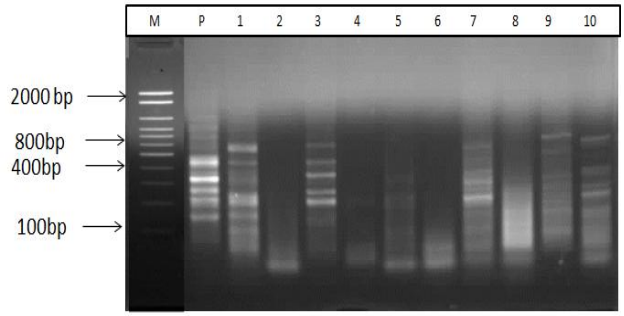

Fig., 2: RAPD-PCR band profile generated on agarose gel, fungus primer 2 used in strain S3 and its F1 progeny. Abbreviation: M: indicating weight marker, 1-10 lanes indicated isolates of strain S3, P: parental strain.

RESULTS AND DISCUSSION

Genetic diversity organism and environment form an eternal triangle of life. In contrasting hostile and no hostile environment using RAPD markers, genetic variability was studied in wild S3, N5 and mutant Sw 92.1 strains of *Sordaria fimicola*. Fresh fungal mycelium was used for DNA extraction.

In present studies RAPD tool was used to walk through nuclear DNA of different fungus strains to determine the genomic polymorphism among species and within species of fungal populations. Five oligonucleotide primers of 10-mer were selected out of seven. FUNGUS- I (CTCTGTCGG), FUNGUS- 2 (CGGCCANTGC), FUNGUS-3 (CGGCCACTNC), FUNGUS-4 (TCGGCTGAG) and FUNGUS- 7 (GTGAGCGTC) were used for RAPD analysis. The absence of polymorphic bands may have occurred due to changing sequence of primer sites or prevention of amplification target DNA due to INDEL. The annealing temperature (Ta) was set at 36^0C. Optimized conditions of PCR, genomic DNA concentration for reaction and uniform conditions of lab were maintained allowing the amplification of polymorphic DNA typing (Agarwal *et al*., 2008).

The genetic variability among different strains of *Sordaria fimicola* was examined by priming RAPD. The primers were used alone and in combinations. The total 15 RAPD fingerprints were observed in three parental strains and their F1 progeny. The primer that produced large number of amplified PCR products had high efficiency due to maximum pairing between primer and nuclear DNA. The primer efficiency of FUNGUS I, 2, 3, 4, and 7 were 14%, 25%, 18 %, 21% and 22% respectively. The primer discrimination powers were 15%, 26%, 17%, 22% and 20% which provided more information than the efficiencies of primers.

Primers generated total 401 bands, the number of polymorphic bands were 247. The percentage of polymorphic bands was 62% .The results showed that all strains and their F1 generation had different band patterns. The band mobile distance for each lane was determined and

molecular size of each amplified product was estimated by comparing its movement with standard size DNA marker or ladder. These results are in accordance with the results of Nwangburuka *et al*., (2011) when they used RAPD on Okra leaves. In this analysis strain S3 with five primers produced 106 markers out of which 71 were polymorphic and 35 were monomorphic. The size of amplicon was 20-1000 base pairs (bp). Wild strain N5 generated total 111 bands and 66 were polymorphic whereas 45 were monomorphic. The fragments bands range was 20-800 bp. The mutant strain S*w* 92.1 located on harsh and stressful conditions of SFS slope generated 184 markers 110 were polymorphic and 74 were common bands, ranging from 20-900 bp. The fungal strains differ in response to different environmental exposure, that lead to heritable differences and evolution as reported by Nevo *et al.* (2011).

The strain S3 and its F1 generation (HA isolates) with all primers produced 19 polymorphic bands, monomorphic bands were absent. Amplicon size was 80-500 bp. All primers dug out into 37.05 kb of total DNA of *Sordaria fimicola.* Strain N5 and its isolates (RA) had 12 markers while monomorphic bands were absent. Fragment length was 80-400 bp and primers probe was 6.05 kb. Mutant Sw 92.1 and F1 generation MS generated 23 bands and amplicon size was 100-900 bp. The genome harvested was 9.0kb. The comparison between *Sordaria fimicola* strains and their progeny located at SFS had more diversity and polymorphism than NFS. Saleem *et al*., 2001 mentioned high level of crossing over, gene conversion and recombination in *Sordaria fimicola* at SFS of EC. Arid environment conditions on SFS had induced high mutation frequency in ascospores pigmentation 2.2 -4.4% than in those strains from the NFS 0.9-1.3% (Lamb *et al*., 1998). RAPD is referred as an effective method for identification of polymorphism and showed level of genetic diversity similar to isoenzymes diversity as was examined higher on SFS (Nevo, 2001).

In this study, variation in banding patterns represented the allelic segregation of independent loci. The main dendrogram was at a Euclidean distance of 2.81 which was divided into two major groups at a distance of 3.21 and 3.62. The small cluster included 16 and 24 isolates (RA, MS) which indicated the early evolution from the main cluster. The major cluster was divided into two subgroups in which the isolates 17-26 (RA) were located in the first subgroup and 15-21 (HA) isolates were present in the second subgroup. There was a little mixing of some isolates which indicated the adaptations to the climatic conditions. The comparison of clusters between strains and F1 generation by using all RAPD primers indicated that more genetic diversity existed between strains than within parents and their isolates.

CONCLUSION

The result of this study has shown usefulness of RAPD as a reliable, easy and elegant tool to gauge environmental stress, evolution and polymorphism in genome of model organism *Sordaria fimicola*.

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Received: 09-11-2015 **Revised: 27-11-2015** **Accepted: 15-01-2016**