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ORIGINAL ARTICLE

Genetic diversity of *Macrophomina phaseolina* isolates from certain agro-climatic regions of India by using RAPD markers

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Abstract Genetic diversity analysis of *Macrophomina phaseolina* isolates obtained from different host range and diverse geographical locations in India was carried out using RAPD fingerprinting. Of the thirteen 10-mer random primers used, primer OPB-08 gave the maximum polymorphism and the UPGMA clustering could separate 50 isolates in to ten groups at more than 65% similarity level. The ten clusters correlated well with the geographical locations with exceptions for isolates obtained from Eastern and Western Ghats. There was a segregation of isolates from these two geographical locations in to two clusters thus, distributing 10 genotypes in to eight geographical locations. All the isolates *M. phaseolina* irrespective of their host and geographical origin, exhibited two representative monomorphic bands at 250 bp and 1 kb, presence of these bands suggests that isolates might have evolved from a common ancestor but due to geographical isolation fallowed by natural selection and genetic drift might have segregated in to subpopulations. Genetic similarity in the pathogenic population reflects the dispersal of single lineage in all locations in India.

Keywords *Macrophomina phaseolina* · Random amplified polymorphic DNA · Genetic diversity

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Introduction

Macrophomina phaseolina (Tassi) Goid is primarily a soil borne pathogen which causes disease in more than 500 plant species spread over 100 families [1]. *M. phaseolina* persists in soil as sclerotia formed in infected host tissue and later released in the soil during decaying process [2]. Though the genus *Macrophomina* contains only one species *M. phaseolina* [3], its host specificity and geographic distribution over which it is found suggests that the pathogen is quite heterogeneous. This variability has been confirmed by reports demonstrating differences in pathogenicity of isolates obtained from both a single plant and a single host species [4]. Attempts to study the genetic diversity in relation to geographical distribution and pathogenic variation will facilitate developing effective/resistant cultivars. Little is known about the genetic complexity of *M. phaseolina* population in India or about the origin and spread of the pathogen i.e. whether it arose as a single lineage in one location or arose independently in several locations. Significant advances in molecular detection and differentiation of *M. phaseolina* has been achieved using polymerase chain reaction (PCR) and hybridization based techniques [4–8]. The lack of strong correlation between genotype and geographical origin suggests a high level of diversity within and among the population of *M. phaseolina* [9, 10]. Random Amplified Polymorphic DNA (RAPD) analysis has many advantages as a means of characterizing genetic variability such as speed, low cost and minimal requirement for DNA. Polymorphism among major bands in RAPD pattern indicates genetic distinctness and can be used to distinguish unrelated groups.

The current study was undertaken to elucidate the genetic diversity of *M. phaseolina* isolates obtained from different

hosts and geographical locations in India using arbitrarily primed PCR fingerprinting technique.

Materials and methods

Fungal samples

A total of 50 *M. phaseolina* isolates were obtained from 8 major agro-climatic regions in India. Details regarding the geographical and biological origin of all the isolates are given in Table 1.

Extraction of genomic DNA

Cultures were retrieved on MEYE (malt extract 1%, yeast extract 0.5%) broth and mycelium developed from single microsclerotia was transferred on to potato dextrose agar (PDA) plates and incubated at 30°C. A 5 mm disc of 2-day-old culture growing on PDA medium was transferred in to 150 ml of MEYE broth, in a 250 ml Erlenmeyer flask and gyrated at 125 rpm for 4–6 days at 28 ± 2°C. The mycelia were filtered through a double layer of cheese cloth, frozen at –80°C overnight and dried under vacuum freezing. Total genomic DNA was extracted as described by Babu et al. [8]. The pure quantified DNA samples were stored at 4°C for further use.

RAPD fingerprinting

DNA from 50 *M. phaseolina* isolates was amplified by the RAPD method using 13 randomly selected 10-mer random oligonucleotide primers from Operon kit (OPA-09, 10, 11, 15, OPB-06, 07, 08, 17, OPC-10, OPD-11, OPE-01, OPV-17 and Oligo-09). The PCR were carried out in a total volume of 25 µl reaction mixture containing 0.2 mM of each dNTPs, 50 pmol primer 2.5 µl of 10 × PCR buffer (100 mM, Tris-HCl, pH 8.3, 250 mM KCl, 15 mM $MgCl_2$), 1 U of Taq DNA polymerase (Bangalore Genei; India), and 50 ng genomic DNA in H_2O. The PCR was carried out for 5 min of initial denaturation at 94°C, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 35°C for 2 min and extension at 72°C for 2 min, with a final extension at 72°C for 5 min.

All the amplifications were repeated three times to check the reproducibility of the banding pattern. The PCR amplified products along with 1 kb (Fermentas, USA) were run on 1.4% agarose gel in 1 × TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) containing 0.5 mg/ml ethidium bromide, at constant voltage (5 V/cm) and visualized under UV transilluminator.

Data analysis

The fingerprints generated by different primers were compared for their relatedness among isolates. The presence (scored 1) or absence (scored 0) of a band of a particular molecular weight was scored as two alleles at single locus to compile binary matrices. The dendrogram were constructed by Un-weighted Pair Group Method with Arithmetic mean (UPGMA) cluster analysis with Jaccard [11] coefficient (J) by using NTSYSpc (V 2.0) [12].

Results

In order to study the genetic relationship between 50 isolates and to obtain a robust dendrogram, 13 10-mer arbitrary primers were used. Of the 13 random primers used, 5 (OPA-15, OPB-06, OPC-10, OPD-11 and OPE-01) showed amplification of few fragments, ranging from 6 to 10, and the polymorphism was <60%. Rest of the 8 primers showed more than 80% polymorphism among the different isolates. However, primer OPB-08 (5′-GTGAGCTAGG-3′) gave the maximum polymorphism and could reveal significant correlation between genotype and geographical locations. Thus, the results obtained with the OPB-08 was only presented and discussed.

Essentially the banding pattern produced by the OPB-8 ranged between 100 bp and 3 kb (Fig. 1), and the UPGMA clustering produced a dendrogram that separated 50 isolates into 10 groups at 65% similarity level (Fig. 2). The 10 clusters correlated well with the geographical locations with exceptions for isolates obtained from Eastern ghats (GC-IV and X) and Western ghats (GC-VIII and IX). There

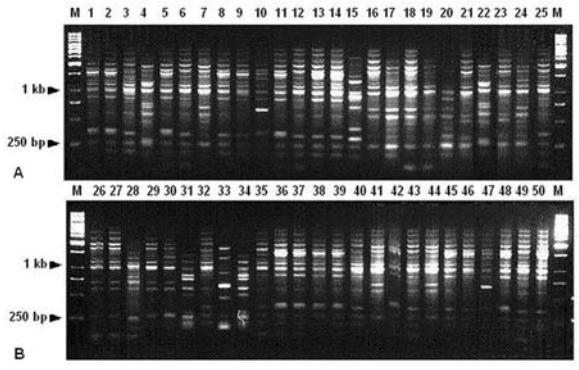

Fig. 1 RAPD-PCR fingerprinting: 50 isolates of *M. phaseolina* collected from different geographical regions of India were subjected to PCR amplification by using 10-mer RAPD primer OPB-8. (A) Lane 1 to 25 and (B) Lane 26 to 50 indicating isolates of *M. phaseolina* listed in the Table 1. M- Represents 1 kb ladder. ◄- Indicates monomorphic bands present in all isolates.

Table 1 List of *M. phaseolina* isolates used in this study

Isolate No.	Geographical origin	Biological origin	G.C by RAPD (OPB-08)
mpk1	Himachal Pradesh	Potato	I
mpk2	Himachal Pradesh	Potato	I
mpk3	Ballia, Uttar Pradesh	Chickpea	II
mpk4	Maharashtra	Sorghum	VI
mpk5	Varanasi, Uttar Pradesh	Chickpea	II
mpk6	Varanasi, Uttar Pradesh	Sorghum	II
mpk7	Jhansi, Uttar Pradesh	Soybean	II
mpk8	Mau, Uttar Pradesh	Corn	II
mpk9	Ballia, Uttar Pradesh	Soybean	II
mpk10	Kerala	Soil	VIII
mpk11	Varanasi, Uttar Pradesh	Chickpea	II
mpk12	Mau, Uttar Pradesh	Soil	II
mpk13	Uttar Pradesh	N.A	II
mpk14	Varanasi, Uttar Pradesh	Pea	II
mpk15	Karnataka	Corn	IX
mpk16	Gujarat	Sorghum	III
mpk17	Karnataka	Chickpea	VI
mpk18	Delhi	Mung bean	III
mpk19	Delhi	Soybean	III
mpk20	Jorhat, Assam	Sorghum	VII
mpk21	Delhi	Sorghum	III
mpk22	Chennai	Corn	X
mpk23	Delhi	Chickpea	III
mpk24	Andhra Pradesh	Soybean	VI
mpk25	Delhi	Pea	III
mpk26	Madhya Pradesh	Soil	V
mpk27	Madhya Pradesh	Soybean	V
mpk28	Tamil Nadu	N.A	X
mpk29	NA	N.A	V
mpk30	Madhya Pradesh	Ground nut	V
mpk31	Solapur, Maharashtra	Sorghum	IX
mpk32	Varanasi, Uttar Pradesh	French bean	II
mpk33	Jorhat, Assam	Chickpea	VII
mpk34	Karnataka	Soybean	IX
mpk35	Varanasi, Uttar Pradesh	Soybean	II
mpk36	Jhansi, Uttar Pradesh	Soil	II
mpk37	Uttar Pradesh	Soybean	II
mpk38	Varanasi, Uttar Pradesh	Chickpea	II
mpk39	Ballia, Uttar Pradesh	Sorghum	II
mpk40	Mau, Uttar Pradesh	Chickpea	II
mpk41	Gulberga, Karnataka	Sorghum	VI
mpk42	Andhra Pradesh	Sorghum	IV

Contd. on next page

Table 1 List of *M. phaseolina* isolates used in this study (Contd.)

mpk43	Uttar Pradesh	Corn	II
mpk44	Rajasthan	Sorghum	III
mpk45	Varanasi, Uttar Pradesh	Sorghum	II
mpk46	Andhra Pradesh	Corn	IV
mpk47	Chenni	Soybean	IV
mpk48	Varanasi, Uttar Pradesh	Chickpea	II
mpk49	Varanasi, Uttar Pradesh	Sunflower	II
mpk50	Jhansi, Uttar Pradesh	Corn	II

N A- Data not available, G.C- Geographical cluster, Roman numeral- Genotypes.

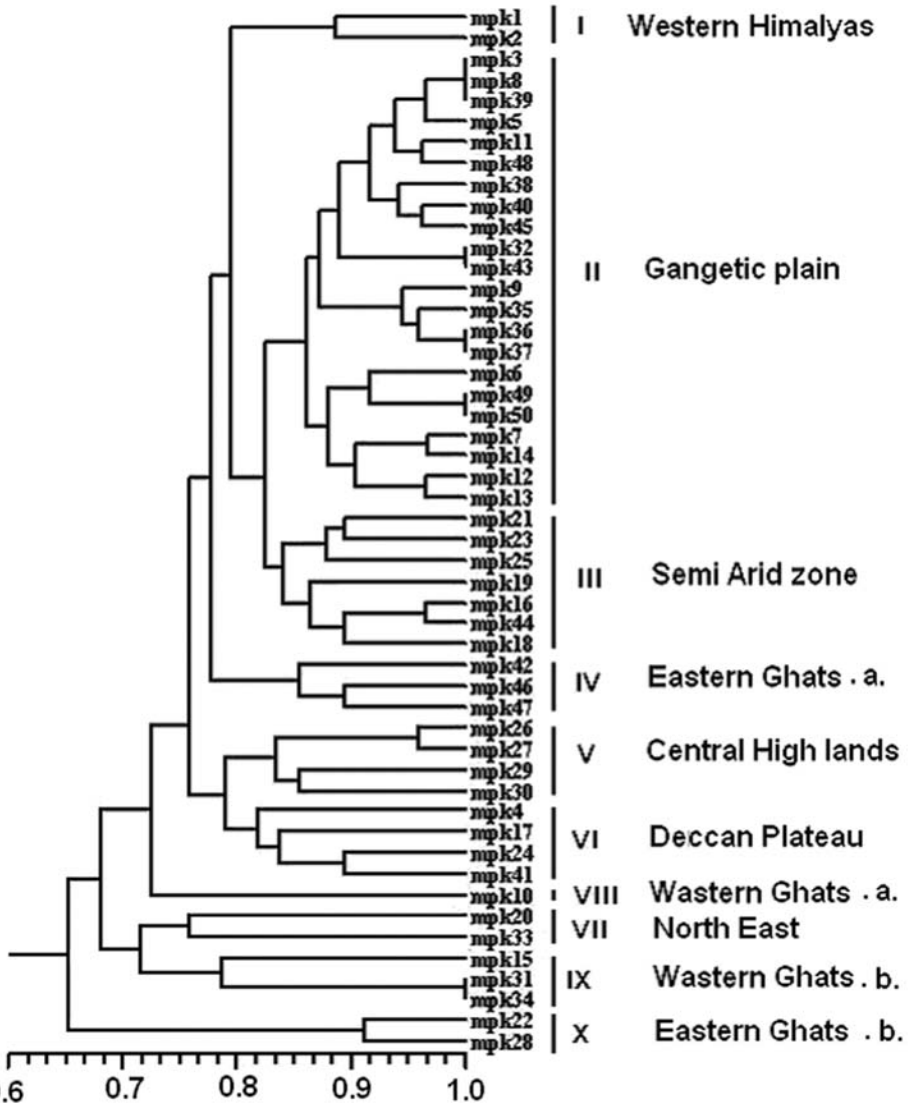

Fig. 2 UPGMA-SAHN clustering dendrogram constructed by the data obtained from the primer OPB-8 in RAPD assay of *M. phaseolina* isolates labeled as mpk 1 to mpk 50. Geographical clusters I to X. Scale in the dendrogram shows the genetic similarity coefficient calculated according to Jaccard.

was a segregation of isolates from these two geographical locations in to two clusters thus, distributing 10 genotypes in to eight geographical locations. In each group, isolates shared 75–100% similarity among themselves. With in each geographical cluster (GC), however no correlation was obtained with regards to biological origin, for example isolates obtained from sorghum (mpk39), chickpea (mpk3) and corn (mpk8) from Indo-Gangetic plains (GC-II) showed 100% similarity among themselves. Similarly, all isolates from Semi arid zone (GC-III) produced similar banding patterns even though they were from sorghum, chickpea, pea, soybean and mungbean. Conversely the isolates obtained from the same host-like chickpea from Indo-Gangetic plain did not show 100% similarity among themselves. All the isolates of *M. phaseolina* irrespective of their host and geographical origin, exhibited two representative monomorphic bands at 250 bp and 1 kb (Fig. 1).

Discussion

Macrophomina phaseolina is known to occur throughout India. Genetic characterization of this prevailing plant pathogen is of importance for the effective disease management. In the present study, examination of a large sample of *M. phaseolina* isolates for genetic diversity, host specificity and geographical origin by using molecular markers indicated significant genetic diversity among the isolates collected from eight major agro-climatic zones in India. Various studies devoted to the genetic, geo-diversity and pathogenic variability of *M. phaseolina* collected from Mexico and other countries have shown clear differentiation [4, 5, 13]. Jana et al. [14] developed taxonomic marker for population studies by using a single RAPD primer that distinguishes isolates of *M. phaseolina* from soybean, sesame, ground nut, chickpea, cotton, common bean and 13 other hosts. Among the Indian populations there are no reports available on correlation between the genotype and geographical or biological origin. Recently, sorghum isolates of Indian origin were distinguished based on chlorate sensitivity [15]. Significant variations among the pathotypes obtained from different continents [7] have also been reported.

RAPD analysis has been reported as a useful tool for detecting genetic variation in *M. phaseolina* population in different countries [4, 16, 17]. In the present study RAPD analysis with one random primer OPB-08 revealed considerable variation among *M. phaseolina* isolates obtained from different geographical locations and hosts. Purkayastha et al. [6] has found some degree of genetic diversity and pathogenic variability among the Indian isolates with the help of Internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) and RAPD analysis. However Indian isolates obtained from a single host, sorghum [15] have been reported to have limited genetic variation.

The UPGMA clustering could segregate 50 isolates in to 10 groups and revealed significant correlation between genotype and geographical location. However, the presence of two monomorphic bands among the isolates irrespective of geographical or biological origin indicates genetic similarity among the isolates. In absence of sexual reproduction in *M. phaseolina*, genetic variability could occur by slow recombinant process-like fusion of cells or parasexual recombination [18]. The presence of two monomorphic bands suggests that isolates might have evolved from a common ancestor but due to geographical isolation followed by natural selection and genetic drift might have segregated in to subpopulations. Thus the clustering of genotypes in to geographical clusters is an indication of adaptation of isolates over the years to particular location.

The little amount of genetic diversity observed among the tested isolates might be due to genetic diversity in the native population that has infected various crops through the years [17]. The RAPD-PCR with primer OPB-8 has produced reproducible results under optimized conditions; this primer can be used as for rapid and routine genetic diversity analysis from large numbers of isolates representing similar phenotypic characteristics. Genetic similarity in the pathogenic population also reflects the dispersal of single lineage in all locations in India. In summary, our results proved that OPB-8 primer can be used as a suitable marker to measure genetic relatedness of the isolates, within and between various geographic locations and also helps to understand the ecology and biology of this fungus.

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