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Molecular variability in *Pythium aphanidermatum* causing rhizome rot of ginger

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Molecular variability among 12 isolates of *Pythium aphanidermatum* was studied by using PCR based RAPD method. RAPD analysis of using 40 random primers, resulted in total of 313 amplicon levels. Out of these, total of 245 polymorphic bands levels were observed. The cumulative analysis of similarity values placed 12 isolates in 2 clusters. In the present investigations the results obtained showed the possiblity of using RAPD technique to distinguish variability among the isolates of *P. aphanidermatum.* The information could then be used to determine specific primers that would allow the identification of the fungus directly from plant material or in the soil.

Key words: Ginger, *Pythium aphanidermatum,* RAPD, rhizome rot.

INTRODUCTION

India is considered as *'the land of spices'* and enjoys from time immemorial a unique position in the production and export of ginger. These crops are cultivated for their underground rhizomes, which are used in many ways. Ginger is used as flavoring agent, a preservative, used in pickling and ginger oil in soft drinks. Among the major constraints for growing ginger is the rhizome rot. Even though important foliar diseases do exist in these crops, rhizome rot is very important in view of severe crop losses. It occurs in several parts of India wherever these crops are grown. The term rhizome rot is loosely used for all the diseases affecting the rhizome irrespective of pathogens involved, since the ultimate result is the partial or total loss of rhizome. The pathogens involved decide the nature of damage and also symptoms expression. The major disease identified is the soft rot resulting in wet rot caused by *Pythium aphanidermatum.* Studies on physiological specialization of predominant pathogen like *Pythium aphanidermatum* is important in order to screen the germplasm for resistance against the virulent race. PCR based technique like Random Amplification Polymorphic DNA helps to distinguish variability among the isolates of the organism. This information helps to determine specific primers that would allow identification of the fungus directly from plant material or in the soil. Hence the present investigation on the molecular variability of *Pythium aphanidermatum* was undertaken.

MATERIALS AND METHODS

Collection of isolates

During survey, rhizome rot samples were collected from different localities and isolated. *Pythium aphanidermatum* was isolated from all the locations collected and proved to be the most predominant pathogen among rhizome rot pathogens. These isolates were designated as *viz., Pa1, Pa2, Pa3, Pa4, Pa5, Pa6, Pa7, Pa8, Pa9, Pa10, Pa11,* and *Pa12,* as per the locations of collections *viz.,* Basavakalyan, Humnabad, Chickmagalur, Hassan, Hanagal, Hirekerur, Kodagu, Mysore, Sorab, Sagar, Sirsi and Siddapur.

Total genomic DNA extraction and purification

2-3g of fungal mat grown V-8 juice broth was taken and homogenized using pestle and mortar in 4 ml of 2 per cent SDS for 5 minutes. To the solution 6 ml of lysis buffer was added. The suspension in pestle

and mortar was extracted with equal volume of phenol : Chloroform : isoamylalcohol (1 : 1 W/V) in centrifugation tube and centrifuged at 10,000 rpm for 10 minutes. Supernatant was taken in fresh centrifuge tube. To this 1/10th volume of 3M Sodium acetate and 0.54 volume of isopropanal were added. Mixed by gentle inversion and kept for 30 minutes at –20°C. Centrifuged @ 10,00rpm for 10 minutes at 4°C for the necessary of DNA pellet and was washed with 70% ethanol, air dried and resuspended in 500 μl of $T_{10}E_1$. This DNA obtained was further quantified by agarose gel electrophoresis.

Random primers and PCR amplification

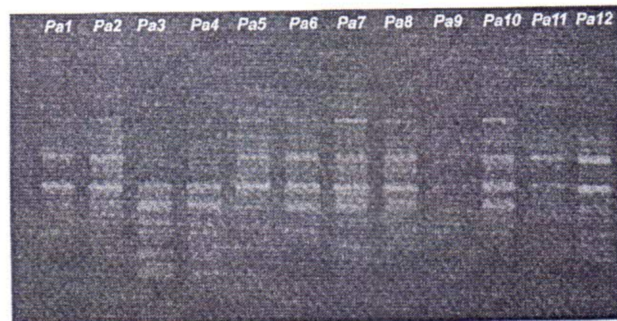
A total of 40 primers of 10 mer (Kits OPB and OPF) were used. The RAPD-PCR amplifications were carried out in 20 μl containing 15-25 ng of genomic DNA (Williams *et al* 1990). The reaction buffer consisted of 10 × assay buffer with 15 mM $Mgcl_2$ (2.00 μl), 0.17 μl of Taq DNA polymerase (6.0U μl^{-1}), 1.0 μl of dNTPs mix (2.5 mM each), 1.0 μl of RaPD primers (5MP/μl), 1.0 μl of template DNA (25 ng/μl) and sterile distilled water (14.83 μl). The PCR amplications were performed using Thermal Cycler programmed for initial denaturation of 94°C for 4 min followed by 40 cycles at 94°C for 1 min, 36°C for 1 min, 72°C for 2 min and a final cycle at 72°C for 5 min. All amplified DNA products were resolved by electrophoresis on agarose gel (1.2%) in TAE (IX) buffer, stained with ethidium bromide and photographed.

Data analysis

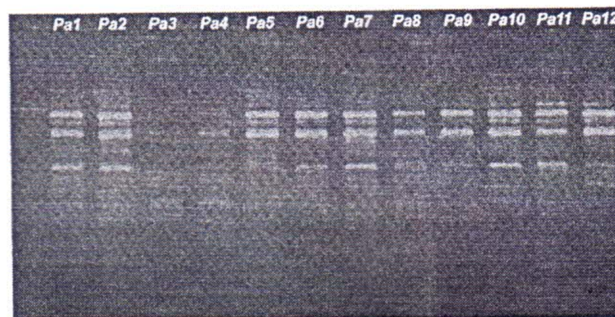
Gel photographs were scored for the presence (1) or absence (0) of bands of various molecular sizes. Binary matrices were analyzed to obtain Jaccard's coefficients among isolates using NTSYS-pc (Version 2.0) were clustered to generate dendrograms using the SAHN clustering program, selecting the unweighted pairgroup method with arithmetic average (UPGMA) algorithm in NTSYS-pc (Rohlf, 1993).

RESULTS AND DISCUSSION

Of the 40 primers used for amplification OPBI, OPB2, OPB14, OPB17, OPF6, OPF11 and OPF20 showed 100 per cent polymorphism (Fig. 1). Three primers showed monomorphism and two primers did not show any amplification. A total of 313 amplicon levels resulted from 38 primers and were available for analysis. Out of these, total of 245 polymorphic



OPB1



OPF 11

Fig.1 : Genetic variability in twelve ginger isolates of *Pythium aphanidermatum* by RAPD method.

bands levels were observed. On an average there were 7.82 amplicon levels per primer of which 6.12 were polymorphic, indicating there is a molecular variability among the isolates of *P. aphanidermatum.* Information on banding pattern for all the primers was used to determine genetic distance between isolates and to construct a dendrogram.

Based on simple matching coefficient a genetic similarity matrix was constructed to assess the genetic relatedness among the isolates of *P. aphanidermatum.* Further, the dendrogram constructed from teh pooled data clearly showed two major clusters A and B at similarity coefficient of 0.61 (Fig 2). Cluster A was classified upto sub-sub cluster A11 and Cluster B was classified upto two minor clusters comprising *Pa1* and *Pa2* isolates belonging to Bidar district. In the present investigation, the results revealed that, geographical locations of isolates were closely related. So the

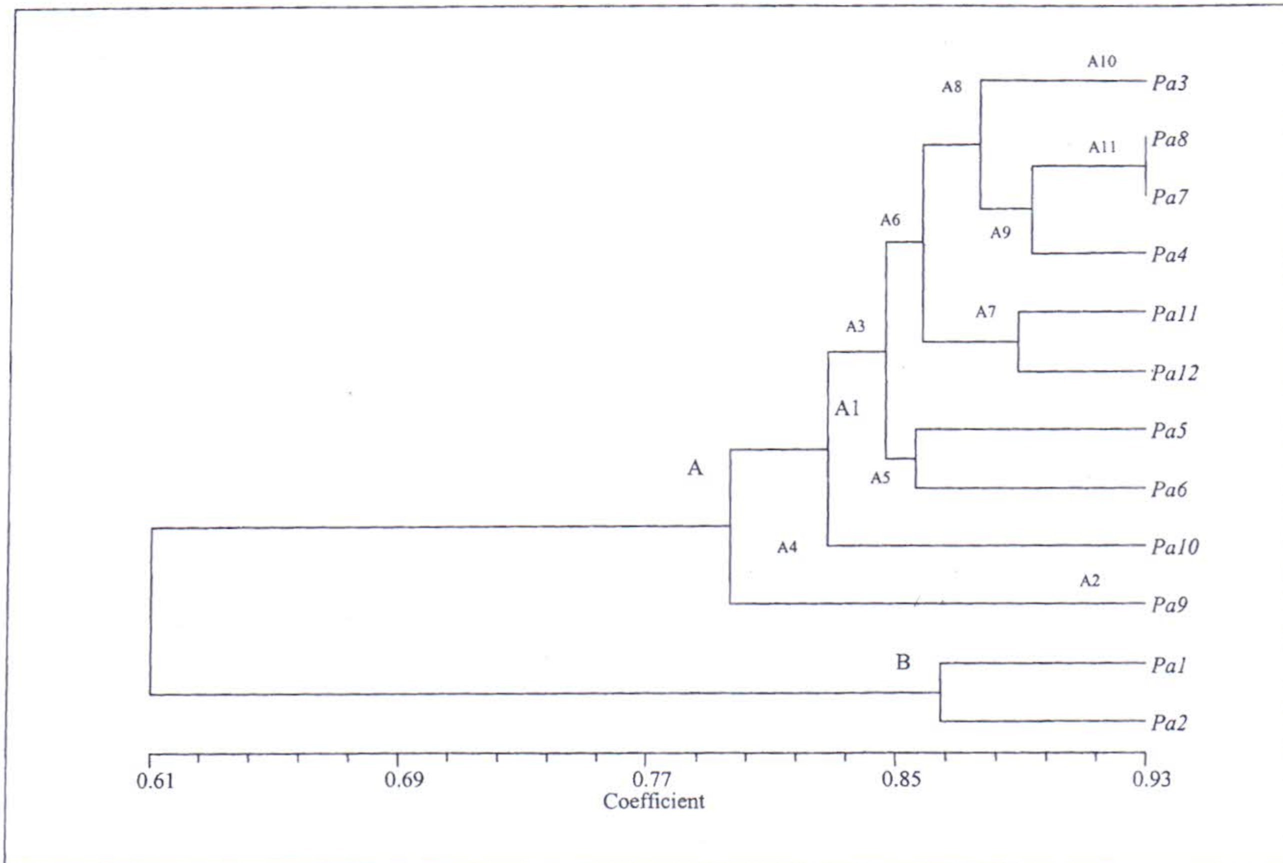


Fig. 2 : Dendrogram based on RAPD analysis of twelve isolates of *Pythium aphanidermatum.*

results obtained from the cluster analysis revealed that, sub cluster group composed of isolates belonging to same geographical locations with very less variability. The suitability of random amplified polymorphic DNA for identification of *Pythium apphanidermatum* was investigated by Herrero and Klemsdal, (1998). Two of the primers gave fingerprints that could be used to differentiate between isolates of the *Pythium* species studied. Matsumoto *et al.* (2000) collected forty-seven isolates of *Pythium irregular* from different hosts and geographic origins were compared from molecular, morphological and physiological viewpoints. They were divided into four groups (I-IV) based on RAPD analysis.

In the present study also, the results obtained here showed the possibility of using RAPD technique to distinguish variability among the isolates of *P. aphanidermatum.* The information could then be used to determine specific primers that would allow identification of the fungus directly from plant material or in the soil.

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