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Establishing Host Pathogen Relationship between *Arachis hypogea* and *Cercospora arachidicola* S. Hori by RAPD (Random Amplified Polymorphic DNA) Markers

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ABSTRACT: RAPD (Random amplified polymorphic DNA) analysis was performed to establish host-pathogen relationship between Arachis hypogea and *Cercospora arachidicola*. The relationship between the host and pathogen was analyzed based on the dendrogram of RAPD patterns using UPGMA (Unweighed Pair GroupMethod with Arithmetic Mean). RAPD analysis in our study showed that there is a clear host pathogen relationship between *Arachis hypogea* and *Cercospora arachidicola*.

Key words: RAPD, Host- pathogen interaction, *Arachis hypogea*, *Cercospora arachidicola*
Abbreviations: RFLP- Restriction fragment length polymorphism, PCR- polymerase chain Reaction, c-DNA- Complementary DNA

Introduction

Groundnut (*Arachis hypogaea* L.) is grown in many agro environments. It is cultivated in some 90 countries around the world[1]. In semi-arid tropical areas it is an important cash crop in subsistence and in commercial farming systems, as well as an important food source. Its successful production has been drastically affected by a number of problems; some of which is leaf spot disease, which is economically important[2]. Early leaf spot disease caused by the fungus *Cercospora arachidicola* S. Hori is one of the major destructive diseases of groundnuts worldwide[3]. Problems related to leaf spot diseases cause nearly complete defoliation and yield losses of up to 50% or more.

When there is infection or attack on a plant by pathogens, the plant would defend itself by various defence mechanisms. Producing resistant genes is one kind of defence mechanism present in plants which keeps them uninfected by new evolving pathogens. Since pathogens adopt new genes for different mode of infections and plants produce new genes for their defence, both develop new types of genes and mechanisms for their benefit.

In the present study, an attempt has been made to establish the host and pathogen relationship between *A.hypogea* and *C.arachidicola* using random amplified polymorphic DNA (RAPD) markers.

Materials and Methods

Isolation of the plant DNA

Dry leaf tissue (200 mg) was ground to a fine powder in liquid nitrogen and Isolation of plant genomic DNA was done by the method described by Chen and Dellaporta[4].

The nucleic acids dissolved in TE buffer were treated with ribonuclease (RNase, 10 mg/mL) and stored at –20°C until use. DNA was quantified via spectrophotometric measurement of UV absorption at 260 nm (Shimadzu UV-260).

Isolation of fungal DNA

DNA extraction was done by the method of Saghai-Maroof et al.[5] with modifications. The fungal cell wall was disrupted by grinding with pestle and mortar in liquid nitrogen. The powdered mycelium was then transferred to an extraction buffer that contained, cetyl tri-methyl ammonium bromide (CTAB) and 2-_mercaptoethanol, EDTA and polyvinyl pyrolidone (PVP). Quantification of DNA was done with spectrophotometer determination. Working concentration of DNA was adjusted to50 ng/ml and stored at 4°C.

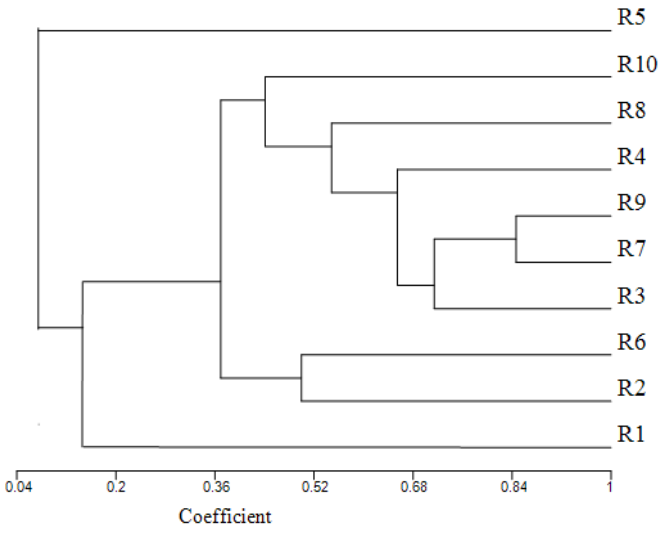
Random amplified polymorphic DNA (RAPD)

The procedure described by Williams et al.[6] with minor modification was done for carrying out PCR reaction to produce RAPD profiles. Amplification of DNA fragments was carried out by the PCR using 10-mer arbitrary primers. The reaction mixture consisted of 3 mM MgCl2, 100 µM each of dATP, dCTP, dGTP, dTTP, 0.2 µM primer, 15 ng of genomic DNA and 1 unit of Taq polymerase. to a total reaction volume of 25 µl performed in thermocycler with one cycle of initial denaturation 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, extension at 72°C for 2 min and with a final extension at 72°C for 5 min. Amplified products along with markers (Bangalore Genei) were resolved by gel electrophoresis on 1.2 % agarose gels in 1X TAE buffer containing 6 µl of 10 mg/mL ethidium bromide.

Data analysis

Comparison of each profile for each primer was done on the basis of the presence versus absence (1/0) of RAPD products of the same length. Bands of the same length were scored as identical. Analyses were based on the simple matching index [7], which measures the proportion of common data (either 0 or 1) between the isolates. A dendrogram (Fig.1) was derived from the distance matrix by UPGMA (Unweighted Pair Group Method of Arithmetic means)[8].

Fig: 1 Dendrogram obtained from RAPD analysis using UPGMA, showing host pathogen relationship



R- Rows in the matrix

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Results and discussion

We screened twenty RAPD primers (Table 1) to establish the host pathogen relationships between the invading pathogen and host plant. RAPD was used to establish the host pathogen interaction because there is movement of loci (small sequence or part of gene) from one genome to other. A study of the positions and numbers of loci aids in establishing the relationship easily.

Table 1: List of RAPD primers

Primer Code	Sequence
Arachis 1	5'-AGA AGA CCT C-3'
Arachis 2	5'-GGA TGC CCC A-3'
Arachis 3	5'-CTT TAC ACC T-3'
Arachis 4	5'- TTA GGC AAG A-3'
Arachis 5	5'- CCC TGG ATA T-3'
Arachis 6	5'-GGC TCT AAC C-3'
Arachis 7	5'-GTC AGG GGA C-3'
Arachis 8	5'-AGG CCA TCA A-3'
Arachis 9	5'- CCA TGG GGA G-3'
Arachis 10	5'- ATG CTG CGT G-3'
Arachis 11	5'- GGT GTG GCA T -3'
Arachis 12	5'-ATC CCC ACA T -3'
Arachis 13	5'- AGG CTG TCG T -3'
Arachis 14	5'- CTC GGC TTC A -3'
Arachis 15	5'- GGG TCC GTT A -3'
Arachis 16	5'- CCG ACA AAC C -3'
Arachis 17	5'- TGA ACC GGA G -3'
Arachis 18	5'- AGG GGA TCA G -3'
Arachis 19	5'- CTA TGC GGA C -3'
Arachis 20	5'- GAC GAA CGA T -3'

In the present study we had isolated the DNA from pathogen, non infected host and infected host and did RAPD analysis to establish the relationship. The limitations associated with pedigree data and morphological, physiological and cytological markers for assessing genetic diversity have largely been circumvented by the development of DNA markers such as restriction fragment length polymorphisms[9], random amplified polymorphic DNAs[6]. The RAPD technology is well suited to DNA fingerprinting[10] although it does suffer from a certain lack of reproducibility due to mismatch annealing[11].

The exploration of RAPD (random amplified polymorphic DNA) as genetic markers has improved the effectiveness of r-DNA techniques. This method does not require DNA probes or prior sequences information. This method utilizes a single, arbitrarily primer to amplify a number of fragments for a given template of DNA to generate a discrete "fingerprints"when resolved by gel electrophoresis[12].

The RAPD markers are very efficient in identification of flow of genes from host to pathogen or vice versa for assessment of host pathogen relationship. This method can be used for different pathogens affecting several hosts (plants). It has been seen that fragments of similar length amplified from different species are not always derived from corresponding loci, and that not all RAPD fragments within the same amplification pattern are independent but the band length can be used as a markers to establish the relationship[13].

Out of the twenty primers used, only five (Ara 6, Ara 9, Ara 14, Ara 15 and Ara 16) consistently generated reproducible RAPD patterns; therefore they were used for a comparative analysis . Based on the RAPD pattern obtained in the gel, we constructed a dendrogram. The dendrogram showed the relationship between host and pathogen, as the gel showed change in the position of the loci. The RAPD pattern of the infected host was different from the patterns of the uninfected host and the pathogen, thus indicating that infection created polymorphic regions in the host genome.

The main changes in the RAPD profiles of the present investigation were the appearance or disappearance of different bands with variation in their intensity. These effects might be due to the structural rearrangements in DNA caused by the interaction with the pathogen's genome. Thus, the estimate on the existence of structural alterations in plant DNA after infection by a pathogen on the bases of DNA patterns could be obtained after RAPD with the set of primers.

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