J. Phytopathology **154**, 542–544 (2006)

College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India

Genetic Variability in *Phytophthora palmivora* (Butl.) Using RAPD Markers

K. G. Sudheesh[1] and V. B. Sreekumar[2]

Authors' address: [1]College of Forestry; [2]College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India (correspondence to K. G. Sudheesh. E-mail: korthalsia@rediffmail.com)

Received December 21, 2005; accepted May 12, 2006

Keywords: *Phytophthora palmivora*, genetic variability, unweighted pair-group method with arithmatic averaging dendrogram, AMOVA

Abstract

Phytophthora palmivora causes bud rot, fruit and immature nut fall in India, and causes significant coconut yield losses. Genetic variability was estimated in 70 isolates representing seven populations from the Kerala and Karnataka regions using random amplified polymorphic DNA (RAPD) markers. RAPDs generated a total of 163 markers with 15 decamer primers, of which 92% were found to be polymorphic. The number of polymorphic loci within a single population varied from 45 to 92 with estimated hetrozygosities ranging from 0.08 to 0.23. The majority of the genetic diversity was distributed within populations (68.25%) and only 31.75% were among populations. Genetic relationships estimated by the unweighted pair-group method with arithmatic averaging (UPGMA) revealed clear separation of Kerala and Karnataka populations.

Introduction

Bud rot, an apical meristem decay and fruit rot or immature nutfall (Teodoro, 1925) are two destructive diseases known to be caused by *Phytophthora palmivora* in coconuts. This pathogen produces a 'dry' rot before the development of rotting symptoms that are associated with other organisms such as *Fusarium* and *Erwinia* species (Joseph and Radha, 1975). Bud rot was first reported in India in 1906 (Butler, 1906), its heavy incidence from Kerala in 1997 (Veena et al., 1997) that continued to affect coconut production in Kerala. Despite the huge economic costs of *Phytophthora* diseases, relatively little is known about the population genetics of the causal organisms. RAPD markers have been successfully used to detect intraspecific polymorphisms among different species of *Phytopththora* such as *Phytopththora sojae* (Meng et al., 1999) and *Phytopththora infestance* (Backonyls et al., 2001). The purpose of the present study was to characterize isolates based on virulence and to examine the pattern of genetic variability within and among populations of *Phytopththora palmivora* using random amplified polymorphic DNA (RAPD) markers. RAPD technique gained importance due to its simplicity, efficiency, relative ease to perform and non-requirement of sequence information (Williams et al., 1990).

Materials and Methods

Pathogenicity studies

The pathogenicity of five isolates from each population was tested by artificially inoculating on Chowghat Orange Dwarf (COD) nuts by a wound inoculation method. For every isolate three nuts were inoculated and three wounded uninoculated nuts were served as control. The length and breadth of the lesions were measured 10 days after inoculation and lesion area was calculated by average of length and width of the lesion at 90° angles in cm^2. To study the varietal reaction to artificial inoculation and variabilities in pathogenicity, 6-month old tender nuts from West Coast Tall (WCT), Andaman Giant, Kappadam Tall (KP), Fiji Ringed nut and Strait Settlement Green were inoculated using the three isolates (Pala14, Kasar17 and Kann09) and lesion area were calculated after 10 days of incubation by taking the average of length and width of lesion at 90° angles in cm^2.

DNA isolation and polymerase chain reaction (PCR) amplification Seventy isolates representing seven different populations of *P. palmivora* were examined for RAPD patterns obtained from young petiole, spear leaf, axil and crown tissue of the coconut palm. DNA from each isolate were extracted using a modified CTAB protocol (Doyle and Doyle, 1990). PCR-RAPD amplifications were carried out according to Williams et al. (1990) protocol using fifteen primers viz. OPA03, OPA11, OPA18, OPF01, OPF03, OPG15, OPD01, OPD03, OPD04, OPF08, OPD02, OPD08, OPF02, OPF04, OPA05 (Operon Technologies, Alameda, CA, USA) selected out of the 100 primers, based on the repeatability of DNA band profile. The amplification products were electrophoresed on 1.5% horizontal

agarose gel (Sigma, St. Louis, MO, USA) in TBE buffer and stained with ethidium bromide. The bands were compared with DNA markers and the gels were documented using Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (Kodak, Rochester, NY, USA).

Data analysis

RAPD products were scored for presence (1) and absence (0) of bands and genetic diversity parameters were determined using POPGENE Version 1.31 (Yeh et al., 1999). The AMOVA (Excoffier et al., 1992) was used to estimate – among population and within population – variance components, and 1000 random permutation were used to test the significance of variance components. Nei's (Nei, 1978) unbiased genetic distances were measured between all pairs of populations and relationships between populations were investigated by constructing a phylogenetic with the phenetic approach of the UPGMA using TFPGA software. A phylogenetic tree of all the haplotypes were generated by the Dollo parsimony algorithm method for discrete character data with two states (0 and 1) using PHYLIP Version 3.5 (Felsenstein, 1993) assuming no ancestral states. A mantel test were also performed study the correlation between genetic distance and geographic distance.

Results

In the pathogenecity studies, the lesion induced by the different isolates exhibited a wide variation compared with lesion formation on COD nuts. The lesion area ranged 102.75 cm^2 in Pala14 isolate and 272.43 cm^2 in Kasar17 after 10 days of incubation. *Phytophthora palmivora* isolates were categorized into three groups, according to the variation of lesion area produced on COD nuts. Group 1 consists of *Phytophthora* isolates, which produced lesion area between 102–160 cm^2, includes the isolates from Dakshina Kannada, Calicut and Palakkad. Group II consists of *Phytophthora* isolates which lesion area between 161–216 cm^2. The isolates from Thrissur, Kannur and Wayanad included in this group. The third group consists of the *Phytophthora*, isolates which produced lesion area higher than 216 cm^2 as found in Kasarkode. Regarding, the varietal reaction to isolates of *Phytophthora*, WCT was subject to the most rapid increments in lesion size, while KP had the slowest. The isolates showing maximum, medium and minimum lesion area, respectively, on COD nuts were used in the varietal nut reaction test.

Genetic variation

Out of the 100 RAPD primers screened from Kits OPA, OPD, OPF, OPG, OPAU and OPAW (Operon Technologies, Alámeda, CA, USA), 15 produced repeatable amplification products that yielded a total of 163 scorable markers and the number of scorable bands amplified by each primer varied from 6 (OPG 15) to 16 (OPA 11). The number of polymorphic loci within a single population varied from 45 to 92 with estimated hetrozygosities ranging from 0.08 to 0.23. Nei's (1978) gene diversity varied from 0.08 (Palakkad) to 0.16 (Kasarkode) and averaged to 0.39. The estimated average genetic distances between populations indicated that the genetic distances between population pairs were ranged from 0.29 (between Calicut and Kasarkode) to 0.57 (between Wayanad and Palakkad). The result of AMOVA indicated that 68.25% of the total variation was attributed to the differences among populations whereas 31.75% was due to the variation among the isolates of within populations and the random permutations test indicated that two variance components were both highly significant ($P < 0.001$). A mantel test using 1000 permutations indicated that the correlation between genetic and geographic distances were significant ($r = 0.4751$; $P = 0.0320$). In the UPGMA dendrogram, (Fig. 1) clear geographical separations were observed between the Kerala and Karnataka populations. The phylogenetic tree based on dollo-parsimony algorithm (Fig. 2) also revealed tendency of all the isolates to form distinct groups according to geographical localities.

Discussion

The characterization of virulence in *P. pamivora* is a primary step to understand strainal variation with regard to pathogenicity. Testing virulence of *Phytophthora* isolates on different coconut varieties showed that isolates exhibited significant variation with regard to lesion area on tender nuts. Similar results were obtained in the pathogeneic studies conducted on *Theobroma cacao* (Blaha and Lotode, 1982) and Coconut (Bennet et al., 1986). The initial results of the varietal nut reaction could be used in evaluation studies to determine the performance of promising hybrids in terms of disease reaction. Among the varieties tested, WCT showed maximum lesion area and KP showed minimum lesion area with all the three isolates tested. The estimates of average hetrozygosity and polymorphism indicate that surprisingly high levels of gene and genotypic diversity in all populations of

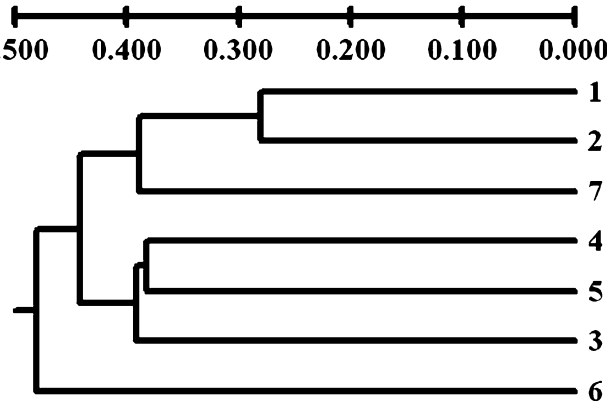

Fig. 1 Unweighted pair-group method with arithmatic averaging dendrogram of the seven populations of *Phythophthora palmivora* based on Nei genetic distance, 1978. 1, Calicut; 2, Thrissur; 3, Kannur; 4, Kasarkode; 5, D.kannada; 6, Wayanad; 7, Palakkad

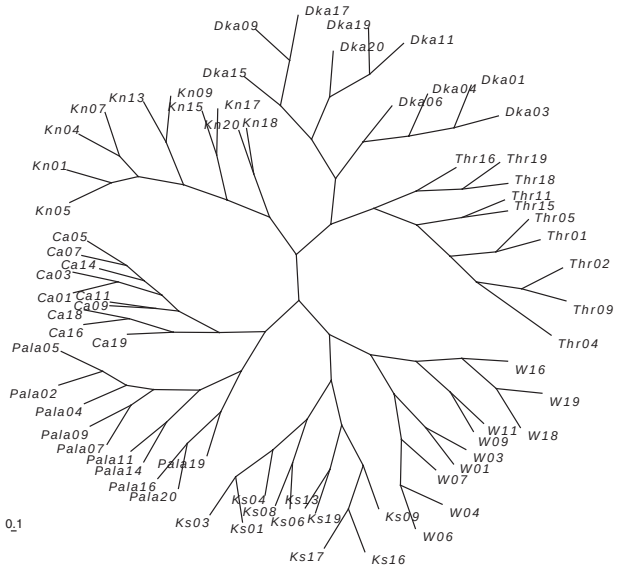

Fig. 2 Genetic relationships of 70 isolates of *Phythophthora palmivora* generated by the Dollo parsimony method (PHYLIP)

P. palmivora analysed. The estimated overall fixation index and AMOVA suggest that most of the total genetic variability is found among population, but relatively large component of genetic variability is found within populations. The significant correlation observed from the mantel test could simply reflect the genetic differentiation between Kerala and Karnataka populations as shown by UPGMA cluster analysis. The resulting dendrogram clearly separated Kerala and Karnataka populations indicating barrier to gene flow between Kerala and Karnataka. The dollo parsimony tree also revealed unambiguous groups corresponds to geographical locations. The proportion of polymorphic loci amplified in *P. palmivora* was 92%, which indicates that high level of genetic variation in this fungus.

References

Backonyls J, Dula T, Erse KT. (2001) Molecular characterization of Phytophthora infestans isolates from potato using RAPD. *Phytopathology* **123**:104–110.

Bennet CPA, Roboth O, Sitepu G, Lolong A. (1986) Pathogenicity of Phytophthora palmivora (Butler) causing premature nut fall disease of coconut. *Indones J Crop Sci* **2**:59–70.

Blaha G, Lotode R. (1982) Comparative study of the pathogenicity on seedlings of Theobroma cacao L. of Phytophthora species that are parasitic on cacao. *Cate–cacao* **26**:237–272.

Butler EJ. (1906) Some diseases of palms. *Agric J India* **1**:299–310.

Doyle JJ, Doyle JL. (1990) Isolation of DNA from fresh plant tissues. *Focus* **12**:13–15.

Excoffier L, Smouse PE, Quattro JM. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**:479–491.

Felsenstein J. *PHYLIP (Phylogeny Inference Package) Version 3.5*. Distributed by the author. Seattle, WA, Department of Genetics, University of Washington, 1993.

Joseph T, Radha K. (1975) Role of Phytophthora in bud rot of coconut. *Plant Dis Rep* **59**:1014–1017.

Meng XO, Shoemaker RC, Yang XB. (1999) Analysis of pathogenicity and genetic variation among Phytophthora sojae isolates using RAPDs. *Mycol Res* **103**:173–178.

Nei M. (1978) Estimation of average hetrozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583–590.

Teodoro NG. (1925) Coconut diseases and their control. *Philippine Agric Rev* **18**:585–592.

Veena SS, Peethambaran CK, Sharma YR. *Phytophthora katsurae as the causal agents of coconut bud rot in some parts of Kerala. International Conference on Integrated Plant Diseases Management For Sustainable Agriculture*. India, Indian Phytopathological Society, 1997, p. 272.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingley SV. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* **18**:6531–6535.

Yeh FC, Yang RC, Boyle T. (1999) POPGENE, Version 1.31. Microsoft Windows-Based Software for Population Genetics and Analysis. University of Alberta and Centre for International Forestry Research, Alberta, Canada.