

## Genetic Variation Between *Colletotrichum lindemuthianum* Isolates

K. I. ANSARI<sup>1\*</sup>, N. PALACIOS<sup>2</sup>, C. ARAYA<sup>3</sup>, T. LANGIN<sup>2</sup>, D. EGAN<sup>1</sup> and F. M. DOOHAN<sup>1</sup>

<sup>1</sup>Department of Environmental Resource Management, UCD, Dublin-4, Ireland; <sup>2</sup>Institute de Biotechnologie des Paris, Universite Paris XI-Orsay, 91405 Orsay Cedex, France;

<sup>3</sup>Laboratorio de Fitopatologia, Escuela Ciencias Agrarias, Universidad Nacional, Heredia-863000, Costa Rica

E-mail: ansari750@rediffmail.com

### Abstract

We characterized the genetic diversity of seventy-three *C. lindemuthianum* isolates collected from 10 different countries by Amplified Fragment Length Polymorphism (AFLP) analysis. The results of this research highlighted the fact that there is huge variation in the genetic diversity between isolates from different countries. The molecular profile of the isolates showed correlation with geographic origin of the isolates.

**Keywords:** anthracnose; *Phaseolus vulgaris*; phylogenetics

### INTRODUCTION

Anthrachnose disease is a major constraint of common bean (*Phaseolus vulgaris*) production in both tropical and temperate regions of the world. The causal organism *Colletotrichum lindemuthianum* (Sacc. et Magnus) (Figure 1) has worldwide distribution and high pathogenic variation. The highest pathogenic variation of the fungus was observed in what are thought to be the centres of origin of its host; the Mesoamerican the Andean region. Recent studies of *C. lindemuthianum* from Latin America distinguished two gene pools of the pathogen, corresponding with the host gene pools (GEPTS & DEBUOCK 1991; SICARD *et al.* 1997). In this current study the genetic diversity of the isolates was compared with their origin.

### MATERIAL AND METHODS

The *C. lindemuthianum* isolates used in this study originated from Honduras, Dominican Republic, Costa Rica, Guatemala, Bolivia, Peru, Argentina, Colombia, France and Tanzania (Figure 2). Most of the isolates

were recovered from cultivated common bean varieties (*Phaseolus vulgaris*) maintained on potato dextrose agar (PDA) (Difco, UK) at 22°C.

Genetic variation was assessed by Amplified Fragment Length Polymorphism (AFLP) analysis using

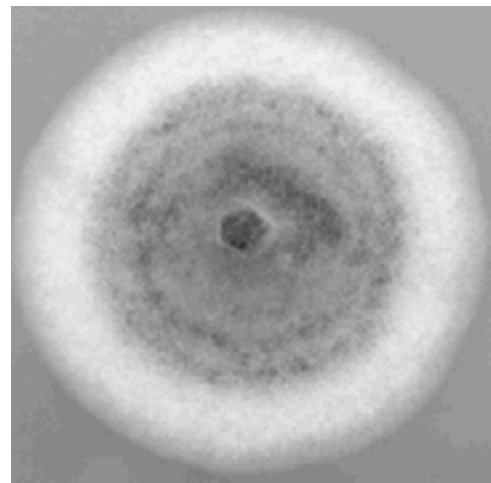


Figure 1. Growth of *Colletotrichum lindemuthianum* on potato dextrose agar medium

Supported by EU INCO-DC Project IC18-CT98-0317.

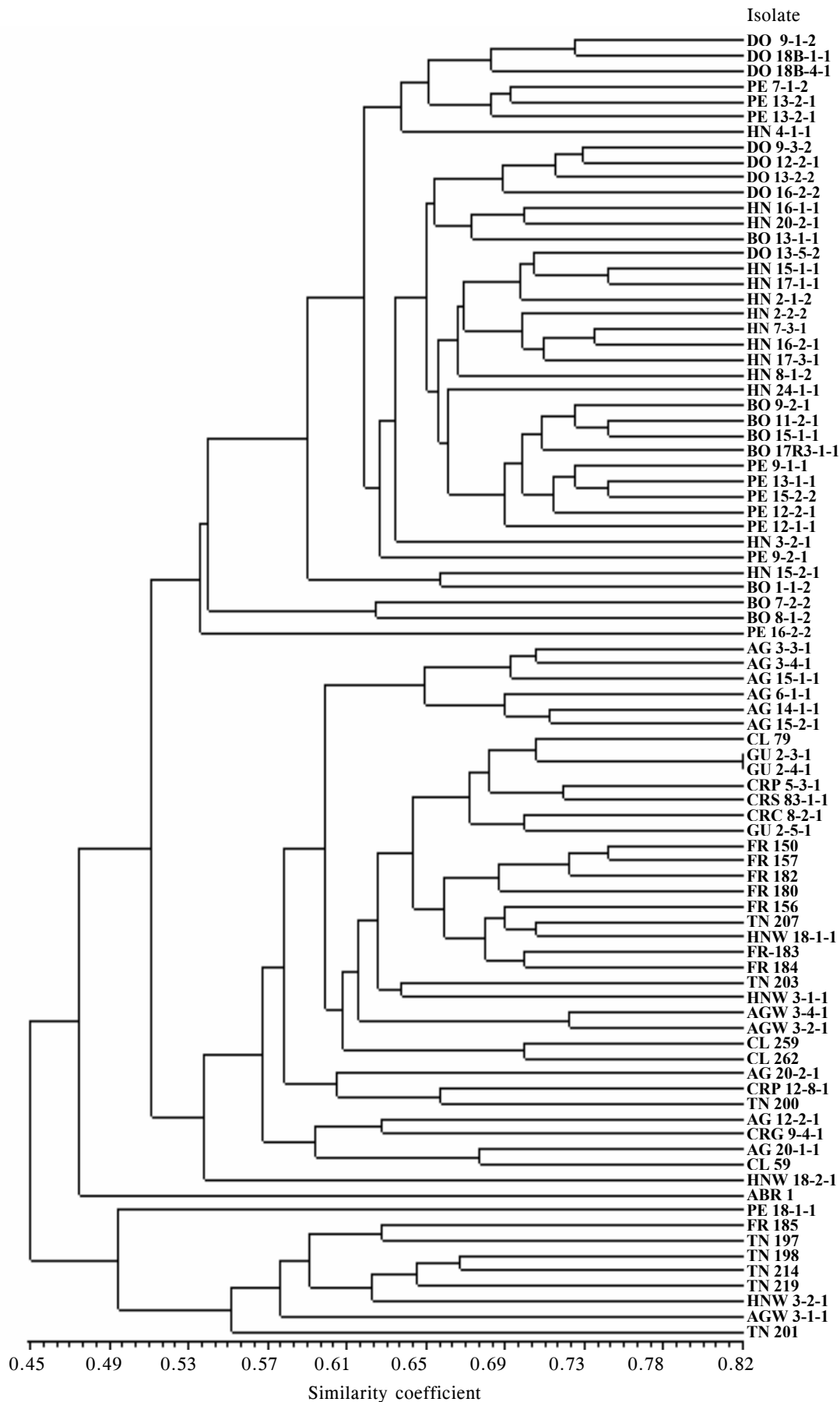


Figure 2. Dendrogram of *Colletotrichum lindemuthianum* isolates, based on UPGMA cluster analysis using the average Jaccard similarity coefficient of their *EcoR1* AT/Mse1 AG, *EcoR1* CA/Mse1 CA and *EcoR1*CT/Mse1 CG AFLP profiles. After: ANSARI *et al.* (2003)

three different selective primer pairs (5'-AGACT-GCGTACCAATTCAT-3'/5'-GACGATGAGTACCTGAGTAAAG-3', 5'-AGACTGCGTACCAAT-TCCA-3'/5'-GACGATGAGTCCTGATAACA-3' and 5'-AGACTGCGTACCAATTC/CG-3'+5'-GACGAT-GAGTCCTGATAACT-3') as previously described (VOS *et al.* 1995). AFLP products were visualized by polyacrylamide gel electrophoresis (PAGE) and silver staining (DOYLE 1996). Analysis of the AFLP profiles and construction of dendrogram based on Jaccard similarity coefficient were done using Image Master 1D Database software (Amersham Pharmacia Biotech AB, Sweden).

### RESULTS

Three selective AFLP primer combinations used in this study and the consensus dendrogram, constructed with average Jaccard similarity coefficient, showed a significant correlation between the genetic diversity of the isolates and their country of origin (Figure 2). This analysis showed that most of the isolates collected within a country clustered together. In general, the Latin American isolates showed more genetic similarity with other isolates collected in Latin America and the Central American isolates showed more genetic diversity. The European and Tanzanian isolates were genetically less diverse and showed greatest similarity to Central American isolates. However the Tanzania isolates were distinct in their genetic make up.

### DISCUSSION

The AFLP analysis showed that overall, there was a significant correlation between the genetic diversity and geographic origin. Higher diversity of the Central American isolates than Latin American isolates

were also reported earlier (SICARD *et al.* 1997). Our finding that European isolates were genetically most similar to Central American and Tanzanian isolates was in contrast to previous RAPD analysis. Also, this analysis re-affirmed that there are two possible centres of origin of *C. lindemuthianum* (Mesoamerica and Andes) and that the Latin American isolates are more homogenous than the Central American isolates. This study also revealed that, while the European isolates may have originated from Central and Latin America, those from Tanzania have a broader genetic base.

### References

- ANSARI K.I., PALACIOS N., CÁRDENAS C., ARAYA C., LANGIN T., EGAN D., DOOHAN F.M. (2003): Pathogenic and genetic variability among *Colletotrichum lindemuthianum* isolates from Europe, Africa, Central and Latin America. Mycol. Res. (submitted).
- DOYLE K. (1996): DNA sequencing. In: DOYLE K. (ed.): The sources for discovery, Protocol and application guide. Promega Corporation, USA: 147–162.
- GEPTS P., DEBUOCK D.G. (1991): Origin, domestication, and evolution of the common bean, *Phaseolus vulgaris*. In: VAN SCHOONHOVEN A., VOYSEEST O. (eds): Common Beans: Research for Crop Improvement. CAB Int., Oxford, England: 7–53.
- SICARD D., MICHALAKIS Y., DRON M., NEEMA C. (1997): Genetic diversity and pathogenic variation of *Colletotrichum lindemuthianum* in the three centres of diversity of its host *Phaseolus vulgaris*. Phytopathology, **87**: 807–813.
- VOS P., HOGERS R., BEEKER M., REIJANS M., VAN DE LEE T., HORNES M., FRIJTER A., POT J., PELEMAN J., KUIPER M., ZABEU M. (1995): AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res., **23**: 4407–4414.