Pythium species associated with Pythium root rot of beans (*Phaseolus vulgaris* L.) in Eastern Africa

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L) is a popular grain legume crop mainly grown by women farmers throughout Africa. Over 4 million hectares of beans are grown annually, providing dietary protein for over 100 million people in rural and urban communities across the continent and is commonly regarded as the meat of the poor (PABRA Outlook). Beans also represent a significant and growing source of income for rural households. However, production is limited by a number of constraints that include diseases, insect pests and edaphic condition. Pythium root rot is one of the most important and destructive soil borne disease of beans (*Phaseolus vulgaris* L.) in the highlands of East and Central Africa, resulting in crop failures in some areas and seasons (Plates 1). The disease is associated with increased intensity of bean production, lack of crop rotation and declining soil fertility.

OBJETIVES

Characterization of the nature, variation and distribution of species causing Pythium root rot was analogous to understanding the nature and characteristics of your enemies so as to develop durable strategies to successfully counter their effects. In our case the objective was to use this information to identify and develop host resistance and other approaches to mange this disease. Besides, the information is useful as a basis for developing rapid, efficient and accurate detection and characterization techniques for *Pythium* spp pathogenic to beans.

MATERIALS AND METHODS

Isolate Collection and Isolations: About 340 samples of beans with typical Pythium root rot symptoms and soil from infected bean fields were collected from western and central Kenya, Rwanda, and southwest Uganda. Fungal isolation was done on a selective medium of cornmeal agar (CMA) amended with 30 mg/litre antibacterial rifamycine and 100 mg/litre antifungal pimaricin (White, 1988). After 24-48 hours, growing Pythium cultures were sub-cultured onto Corn Meal Agar without antibiotics and 24 hours, later cultures were transferred onto potato dextrose agar slants (PDA) and incubated at 25 C for 48 hours and stored in at 16 C

DNA Extraction: Mycelia from 10 day-old cultures grown in V-8 liquid medium were used to extract DNA using the isopropanol method.

DNA Amplification: Most of the isolates were characterized by sequencing using the protocol developed by Levesque (1998). Sequencing templates were prepared by amplifying the internal transcribed spacer (ITS) regions and the 5.8S gene of nuclear ribosomal DNA using the universal eukaryotic primers UN-UP18S42 with UN-LO28S576B (Levesque 2000). This first step also allowed the differentiation of *Pythium* and non-*Pythium* species (Figure 1).

Sequencing: Purified and quantified PCR products of several isolates were sequenced by targeting the ITS-1, ITS-2 and 5.8S rRNA gene regions using universal eukaryote primers (UNUP18S42, OOM-UP5.8S01 and PY-LO28S22) (Mazzola et al. 2002). The sequencing products were run on an ABI prism automated sequencer. After editing the sequences using Edit Seq (DNASTAR, Madison, WI; version 4. 05), multiple alignments were performed using SeqMan and compared to Pythium species database sequences managed by Dr A. Levesque.

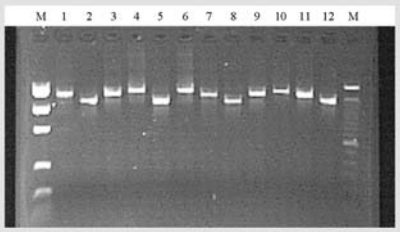

Figure 1. Banding patterns from amplifying ribosomal DNA spacer region using Oomycete specific primers and used to distinguish between *Mortierella* spp and *Pythium* spp. Lanes 1, 3,4, 6,7and 9 –11 represents DNA from Pythium spp, while DNA in lanes 2, 5, 8 and 12 contains DNA from *Mortierella* spp. Lane M a DNA molecular ladder

Pathogenicity: Pathogenicity of some of the identified species was determined using susceptible and resistant cultivars, CAL 96 and RWR 719, respectively, as described by Mukalazi et al. (2001). Seed was grown in soil (in wooden trays) inoculated with isolates representing different species (Figure 2). After germination seedlings were provided with soil environment conducive for disease development (e.g. high soil moisture). Assessment was done by uprooting seedlings washing in tap water and scoring them using the CIAT 1-9 scale (van Schoonhoven and Pastor-Corrales, 1987).

Figure 2: Evaluation of pathogenicity of Pythium species using a resistant (RWR 719) and susceptible (CAL 96) bean genotypes. Adding inoculum on pasteurised soil (A); Planting (B); and Evaluation for disease expression (C).

RESULTS

Characterization of Pythium species: Characterization done on the basis of cultural and sequencing techniques grouped 345 isolates into a total of 38 species which included known and previously unknown bean pathogenic species, putative new species and potential biological control agents. Species distribution maps developed for Uganda, Kenya and Rwanda showed that *P. ultimum* var *ultimum* is the most prevalent species, followed closely by *P. salpingophorum*, *P. torulosum* and *P. vexans* (Figure 3). In addition, some identified *Pythium* species (e.g. *P. oligandrum*) are known to have antagonistic effects against pathogenic species and are potential biocontrol agents. Five species were unique to and only found in each of the three countries. The results demonstrate a fair degree of diversity of the Pythium species occurring in the region: 19 species were identified in Rwanda 16 in Uganda and nine in Kenya. Five pathogens (*P. ultimum*, *P. salpingophorum*, *P. nodosum*, *P. spinosum*, *P. tulorosum*) were common to all the three countries

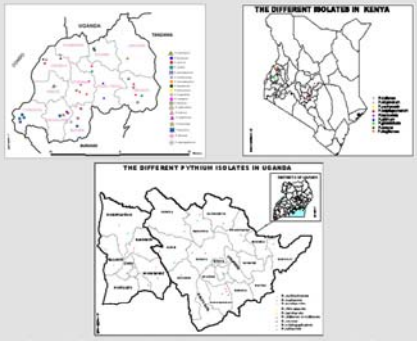

Figure 3. Pythium species associated with areas affected with Pythium root rots in Rwanda, Kenya and Uganda

Pathogenicity: Of significance were the observat-ions that isolates belonging to the species; *P. salpingophorum*, *P. nodosum*, *P. spinosum*, *P. tulorosum*, *P. graminicola*, *P. paroecandrum* and *P. pachycaule*, were shown to be pathogenic to the susceptible bean cultivar CAL 96 and possibly contributing to the root rot problem of beans in the region. RWR 719 was however, resistant to all pathogenic species (Table 1). The importance of these species depends on their distribution and levels of inoculum in the soil. The fact that some of the species pathogenic to beans such as *P. graminicola* and *P. paroecandrum* are known pathogens to other crops (maize or sorghum) in the predominant and existing cropping systems in the region indicate the probable role of intercrops or rotation with beans in perpetuating the root rot problem. We are screening germplasm using the pathogenic species identified and several potential sources of resistance including MLB-49-89A, AND 1062, AND 1055 and SCAM-80-CM/15 have been shown to be resistant.

Table 1. Pythium species pathogenic to beans and their relative occurrence in eastern Africa

	Isolates			
Pathogenic Species	Uganda	Kenya	Rwanda	Total
P. ultimum	17	9	23	50
P. aphanidermatum	1		1	2
P. irregulare	1	9	1	11
P. myriotylum		1	1	2
P. torulosum	8	11	10	29
P. salpingophorum	12	1	4	17
P. spinosum	7	1	7	15
P. pachycaule	3			3
P. graminicola		?		?
P. nodosum	2			2
P. echinulantum	1			
P. paroecandrum		3	3	6

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