

Pythium root rot of common bean: biology and control methods. A review

John Rusagara Nzungize ⁽¹⁾, François Lyumugabe ⁽²⁾, Jean-Pierre Busogoro ⁽³⁾,
Jean-Pierre Baudoin ⁽¹⁾

⁽¹⁾ Univ. Liege - Gembloux Agro-Bio Tech. Tropical Crop Husbandry and Horticulture. Passage des Déportés, 2. B-5030 Gembloux (Belgium). E-mail: nzungizej@gmail.com

⁽²⁾ National University of Rwanda. Biotechnology Unit. P.O. Box 117. Butare (Rwanda).

⁽³⁾ Belgium Technical Cooperation. IPM project. Kigali (Rwanda).

Received on September 2, 2011; accepted on March 20, 2012.

Pythium root rot constitutes a highly damaging constraint on the common bean, *Phaseolus vulgaris* L., grown in several areas of Eastern and Central Africa. Here, this food legume is cultivated intensively under poor conditions of crop rotation due to the exiguity of the land in the region. Yield losses of up to 70% in traditional local bean cultivars have been reported in Kenya and Rwanda. In this study, a detailed analysis of the biology and diversity of the *Pythium* genus was carried out in order to understand the mechanisms leading to the development of the disease. Various control methods for reducing the damage provoked by this disease were analyzed.

Keywords. *Phaseolus vulgaris*, *Pythium*, root rots, plant disease control, disease resistance, Africa.

Pourriture racinaire du haricot commun causée par *Pythium* : biologie et méthodes de lutte. La pourriture racinaire causée par *Pythium* constitue une importante contrainte pour la production du haricot commun (*Phaseolus vulgaris* L.) dans plusieurs régions de l'Afrique centrale et orientale où la production du haricot est intense dans des conditions de non-respect des schémas de rotation à cause de l'exiguïté des terres. Des pertes de rendement allant jusqu'à 70 % au sein des cultivars locaux traditionnels de haricot ont été rapportées au Kenya et au Rwanda. Dans ce travail, une analyse détaillée de la biologie du genre *Pythium* et de sa diversité a été conduite pour comprendre les mécanismes qui mènent au développement de la maladie. Pour réduire les dégâts provoqués par ce pathogène, diverses méthodes de contrôle ont été analysées.

Mots-clés. *Phaseolus vulgaris*, *Pythium*, pourritures des racines, lutte antimaladie des plantes, résistance aux maladies, Afrique.

1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most widely cultivated food legume species in the world (Baudoin et al., 2001). The crop is exploited in a wide range of areas extending from around 52° North latitude to 32° South latitude and from sea level to 3,000 m above sea level, and it displays great variation in growth habits and length of vegetation (FAO, 2005). On a practical level, common bean is a major source of low cost calories, protein, dietary fiber, minerals and vitamins for poor populations (Hillocks et al., 2006). In Rwanda, this food source provides up to 25% of the total calories and 45% of the total dietary protein; this level of contribution of common bean to the population's diet is considered to be the highest in the world (Pachico, 1993). *Pythium* species are spread worldwide (Paul, 2004). Over the last 20 years, there has been an

increase in the importance of *Pythium* bean root rots in several countries of Eastern and Central Africa, such as Burundi, the Democratic Republic of Congo, Kenya and Uganda (Otsyula et al., 2003). For example, in Western Kenya and in Rwanda, many farmers stopped growing beans between 1991 and 1993 due to a severe outbreak of root rots, which caused serious food shortages and price increases beyond the reach of many resource-poor households (Nekesa et al., 1998). In tropical regions, common bean is characterized by low and unstable grain yields due to various ecological and agronomic parameters. Among these parameters, bean root rot and a decline in soil fertility have been cited as being among the major causes leading to bean yield losses (Miklas et al., 2006). In Rwanda, Western Kenya and South Western Uganda, *Pythium* spp. are the fungal pathogens most frequently associated with severe root rot epidemics (Rusuku et al., 1997). Yield

losses of up to 70% in commercial bean cultivars have been reported in Rwanda and Kenya (Otsyula et al., 2003). In other studies carried out in these countries the following species have been isolated from bean samples affected by root rot symptoms. In view of understanding the biology of root rot diseases, a study was conducted in Kenya, Rwanda and Uganda (Mukalazi, 2004) to identify the causal agents. In these countries the following species were isolated from bean samples affected by root rot symptoms: *Pythium nodosum* Bhatn, *Pythium echinulatum* Matthews, *Pythium pachycaule* Shtayeh, *Pythium oligandrum* Drechsler, *Pythium acanthicum* Drechsler, *Pythium chamaehyphon* Sideris, *Pythium folliculosum* Paul, *Pythium indigoferae* Butler, *Pythium irregulare* Buisman, *Pythium lutarium* Shtayeh, *Pythium macrosporum* Vaartaja, *Pythium myriotylum* Drechsler, *Pythium paroecandrum* Drechsler, *Pythium torulosum* Coker, *Pythium vexans* de Bary, *Pythium zingiberis* Takah, *Pythium graminicola* Subraman, *Pythium spinosum* Sawada, *Pythium ultimum* Trow, *Pythium arrhenomanes* Drechsler, *Pythium catenulatum* Matthews, *Pythium diclinum* Tokun, *Pythium dissotocum* Drechsler, *Pythium rostratum* Butler, *Pythium salpingophorum* Drechsler and *Pythium deliense* Meurs (Nzungize et al., 2011; Buruchara et al., 2007). Root rot diseases are widespread in the world and are often considered as a major constraint to bean production, reducing both yield and quality (Abawi et al., 2000). Depending on the pathogen(s) involved in the development of the disease, general root rot symptoms might include any combination of various traits such as poor seedling establishment, damping-off, uneven growth, leaf chlorosis, premature defoliation, death of severely infected plants, and lower yield (Abawi et al., 2006; Schwartz et al., 2007). *Pythium* is a complex genus containing over 200 described species with a broad host range and occupying a variety of terrestrial and aquatic ecological habitats (Dick, 2001). The presence of the pathogens responsible for producing root rot and the severity of the disease are associated with intensification of land use, inappropriate crop rotations and/or reduced fallow periods, leading to a decline in soil fertility and a build-up of soil pathogen inoculum (Abawi et al., 2006).

In a study conducted in South Western Uganda, seven *Pythium* species from various crops associated with beans were obtained: *Pythium macrosporum*, *Pythium oligandrum*, *Pythium spinosum* Sawada isolated from sorghum, *Pythium glomeratum* B. Paul isolated from potato, *Pythium arrhenomanes* isolated from maize, *P. ultimum* isolated from peas and *Pythium heterothallicum* W.A. Campb. & F.F. Hendrix isolated from sweet potato (Gichuru, 2008). In cases where these various *Pythium* species are identified on plant species intercropped with beans, it is likely that

controlling bean *Pythium* root rot with crop rotation practices will be of limited efficiency. In the present study, an investigation was undertaken into the various biological characteristics of the *Pythium* agents present in Rwanda. The aim was to attain a better understanding of the conditions leading to disease development and to establish appropriate control methods to reduce the yield losses caused by *Pythium* root rot.

2. TAXONOMY AND BIOLOGICAL CHARACTERISTICS OF *PYTHIUM* SPP.

The genus *Pythium* belongs to the family Pythiaceae, order Pythiales, class Oomycetes, Phylum Oomycota and kingdom Chromista (Kirk et al., 2008).

Pythium species are fungal microorganisms with a filamentous vegetative body called a mycelium. The mycelium of *Pythium* species is colorless, sometimes lustrous, and occasionally slightly yellowish or a grayish lilac (Owen-Going et al., 2008). The mycelia in *Pythium* species branch out apically at right angles to form structures known as hyphae. These hyphae are hyaline, with the main hyphae being mostly 5-7 μm wide, occasionally reaching a width of up to 10 μm . Cross septa are lacking except in old, mostly empty hyphae or where the cross septa delimit the hyphae's reproductive organs (Van der Plaats-Niterink, 1981). Protoplasmic streaming is often clearly visible in young hyphae. According to Postma et al. (2009), hyphal walls are composed of 80-90% polysaccharides, mainly β 1-6 linked glucans and β 1-3 and β 1-4 (cellulose). It should be noted that *Pythium* spp. do not contain chitin or chitosan in the hyphal walls, but that they do contain protein and lipid at levels varying from 3-8% and from 1-3%, respectively (Postma et al., 2009). Pathogenic *Pythium* spp. may produce hyphae with swollen digitate regions, called appressoria, which enable the fungus to attach and penetrate the host cells (Lévesque et al., 2004).

Pythium spp. can reproduce both asexually and sexually. Asexual reproduction takes place through the zoosporangia and zoospores. In *Pythium* the zoospores are not formed in the sporangium itself but in a vesicle outside it (Stanghellini et al., 1971). The sporangium is separated from the rest of the mycelium by a cross wall. The development of a tube can be observed on the sporangium. The undifferentiated content of the sporangium moves through this tube and forms a vesicle at its end. At this level of development, the zoospores are delimited and start moving (Stanghellini et al., 1971). After 10 to 20 minutes, the wall of the vesicle disappears and the zoospores swim away in divergent directions. Zoospores are only liberated under wet conditions. Production of sporangia or hyphal swellings can be stimulated by Mg, K, and

Ca ions (Postma et al., 2009). Exudates of roots and germinating seeds have a stimulatory effect on the germination of sporangia and on mycelial growth (Stanghellini et al., 1971). Sexual reproduction in *Pythium* spp. takes place through the oogonia and antheridia. The female organs, the oogonia, are spherical to limoniform and are intercalary or terminal. The oogonial wall can be smooth or ornamented with projections. The antheridia, the male organs, consist of an antheridial cell, which can be sessile on a hypha, intercalary, or formed terminally on an antheridial stalk (Postma et al., 2009). During sexual reproduction, the antheridial cell touches the oogonium and forms a fertilization tube, which penetrates the oogonium. (Van der Plaats-Niterink, 1981). The antheridia are termed monoclinal if they originate from the oogonial stalk and declinal if they originate from a different hypha not closely connected with the one subtending the oogonium (Hendrix et al., 1969). After fertilization, the oogonial content forms a zygote, which evolves into an oospore. Only in rare cases is more than one oospore produced inside an oogonium. The oospore wall is smooth, except in *Pythium dictyosporum* where oospores are reticulate (Van der Plaats-Niterink, 1981). Some stimulatory effects of Ca, Mg, K, Zn and Mn ions on growth and reproduction by oospores have been identified (Hsu et al., 1972). Sterols (cholesterol, β -sitosterol, etc.) represent important factors in the sexual propagation process. Sterols stimulate growth and reproduction by oospores and allow the survival of these structures at high temperatures. Such temperatures make the cell membranes of the oospores less permeable to antifungal constituents (Pystina, 1974). After maturation of the oospore, a dormant phase is usually necessary, before germination takes place. At germination, the oospore is converted into a thin-walled structure, which produces a germ tube (Lumsden et al., 1987). Oospore germination consists of two stages: first, the absorption of the endospore, depending on an exogenous calcium supply (Lévesque et al., 2004), and secondly germ tube formation, which depends on the presence of exogenous carbohydrate sources (Stanghellini et al., 1973).

3. MORPHOLOGICAL CHARACTERISTICS USED TO IDENTIFY THE *PYTHIUM* GENUS

In the past, the morphological characteristics and size of each of the structures of *Pythium* have been used as the criteria to identify species within this genus. The most important of these criteria include:

- the presence of sexual reproductive structures (homothallic or heterothallic);
- the type of sporangial morphology (spherical, filamentous or lobulated);

- the character of the oogonial wall (smooth or ornamental);
- the character of the oospore (plerotic or aplerotic);
- the characteristics of the antheridia (Matsumoto et al., 1999).

The major morphological criteria for *Pythium* species identification are based on qualitative characteristics that may vary depending on the culture conditions and the isolate tested (Dick, 2001). Differences in the value attributed to each characteristic have resulted in a confusing taxonomic system for the *Pythium* species (Uzuhashi et al., 2010). As a consequence, a more relevant approach to identifying *Pythium* species would be to combine traditional morphological characterization with molecular analyses (Kageyama et al., 2005).

4. MOLECULAR CHARACTERIZATION AND THE PHYLOGENY OF *PYTHIUM* GENUS

The ITS (Internal Transcribed Spacer) region of the rDNA has become a useful tool in fungal taxonomy and can be used to identify or detect different *Pythium* species (Matsumoto et al., 1999; Belbahri et al., 2008). In a study conducted on 116 *Pythium* species by Lévesque et al. (2004), the ITS region containing the 5.8S gene was shown to have a size varying between 750 and 1,050 bp. The results from Lévesque et al. (2004) but also from Kageyama et al. (2005) revealed that sequences of the rDNA- ITS region were very different between *Pythium* species. Thus, sequence data of this region have been frequently used to identify and classify *Pythium* species (Matsumoto et al., 1999; Vasseur et al., 2005). Molecular data have also been used for phylogenetic analyses of *Pythium* and related genera based on the rDNA large subunit (LSU) D1/D2 on the ITS region, and on either the β -tubulin gene, or the mitochondrial cytochrome oxidase II (coxII) gene (Belbahri et al., 2008). The phylogeny of *Pythium*, as based on ITS sequences, reveals a divergence between *Pythium* species. *Pythium aphanidermatum* Edson and *P. deliense* represent one of the best demonstrations of speciation between two very closely related species. The spacers differ by only 3% and yet the species exhibit differences in several morphological characteristics, which are slightly but consistently different between the two species (Herrero et al., 1998). *Pythium attrantheridium* has been described on isolates from cavity spot lesions on carrots as well as on apple and cherry seedlings from various widely distributed locations in Canada and the USA (Allain-Boulé et al., 2004). In their study, Allain-Boulé et al. (2004) showed that *P. attrantheridium* presented only 5% ITS divergence in comparison with *Pythium*

intermedium, but that this species was morphologically distinct and could not be mated with *P. intermedium*. The ITS region of the nuclear ribosomal DNA of *Pythium longisporangium* is comprised of 890 bases and a BLAST search reveals the closest resemblance of this oomycete with the following species: *Pythium bifurcatum* (AY083935), *Pythium longandrum* (AY039713), *Pythium terrestris* (AY039714) and *Pythium hypogynum* (AY455804), with the following respective similarity percentages: 99.6, 96.2, 84.6 and 84.6. Other species are also relatively close to *P. longisporangium*, on the basis of a morphological trait (hypogynous type of antheridia) and also of some resemblances between their ITS sequences: *Pythium segnitium* (AY149173) with 74.8% similarity, *Pythium canariense* (AY06561) with 60.7% similarity and *P. rostratum* (AJ233456) with only 57.6% similarity with *P. longisporangium* ITS sequence (Paul et al., 2005).

5. ECOLOGY OF *PYTHIUM* SPP.

Pythium species can be found in various ecological areas such as soils in arable land, pastures, forests, nurseries, and marshes, and in water (Van der Plaats-Niterink, 1981). In general, soil temperature can affect spore germination, germ tube growth and zoospore discharge (Tedla et al., 1992). However, each *Pythium* species has its specific optimal development conditions. For example, *P. ultimum* and *P. dissotocum* inhabit cool (10-15 °C) and wet soil as saprophytes on crop residues. Other *Pythium* root rots, such as those caused by *P. aphanidermatum*, *P. irregulare*, *Pythium sylvaticum* Campbell and *P. myriotylum* occur in warm (25-36 °C) and wet soil (Owen-Going et al., 2008).

Pythium species have been recovered in soils with a pH ranging from 3.6 to 7.2 (Martin et al., 1999). However, in the same study, *Pythium* spp. populations were found to be higher in soils with a pH ranging from 6.8 to 7.2 and lower in soils with a pH ranging from 3.6 to 5.5 (Martin et al., 1999). The pH of soil influences some phases of the *Pythium* species life cycle, such as the formation of oospores and sporangia. Alkaline soils (above a pH of 7) favor the growth of *Pythium* species (Lumsden et al., 1987). *Pythium* species are more abundant in cultivated than in uncultivated soils: cultivation and incorporation of plant residues into the soil tend to create favorable conditions for the faster decomposition of organic matter, and thus of the availability of fungal food in that environment (Hendrix et al., 1971). However, some *Pythium* species are mycoparasites. For example, *P. oligandrum* is nonpathogenic on 12 species of crops from six families, including sugar beet, cucumber, wheat, peas, nephrolepis and common beans (Dušková,

1995; Wulff et al., 1998). *Pythium oligandrum* does not attack the tissues of these crops but occurs on the root surface, predominantly in the regions of the hypocotyl (taproot), together with plant pathogenic fungi. *Pythium oligandrum* utilizes the root exudates and fungus hyphae on the root surface, including those of the plant pathogens, for its own nutrition (Brožová, 2002).

6. *PYTHIUM* ROOT ROT CONTROL METHODS

6.1. Chemical control of *Pythium* spp.

Once introduced into the soil, *Pythium* spp. may persist for many years through resistance structures such as oospores, zoospores and sporangia (Onokpise et al., 1999). In these conditions, applying chemical treatments to kill the pathogen may be an efficient method. There are many specific pesticides such as benomyl, captafol, captan, carboxin, metalaxyl, propamocarb hydrochloride and etridiazole, which have already proven to be efficient in controlling *Pythium* root rot diseases on beans. However, some pesticides, such as benomyl, are only active on growing mycelium, but not during the resting stage of the mycelium. In the same context, soil fumigants such as methyl bromide, chloropicrin and Vorlex are highly effective biocides that kill *Pythium* agents (Abawi et al., 2006). In Latin America and Africa, one of the safest and most economical uses of chemicals to control *Pythium* pathogens consists of coating the seeds of crops. This usually results in effective protection of seeds and young seedlings for about 2 to 3 weeks after sowing (Abawi et al., 2006; Schwartz et al., 2007). However, given the conditions prevailing in diverse developing countries such as those in Eastern and Central Africa, poor farmers cannot easily afford to apply a chemical control. Moreover, the large scale use of chemical treatment could constitute a source of soil and water contamination, while at the same time exposing poorly educated farmers to health risks related to handling chemical pesticides. Therefore, the one use of chemical control that could be applied in the context of developing countries – *i.e.* chemically coating the seeds of crops in order to protect them against *Pythium* pathogens – cannot be considered as sustainable in bean production by poor farmers in most of those countries.

6.2. Biological control of *Pythium* spp.

Biological control of soil-borne diseases is particularly complex because the pathogens occur in a dynamic environment at the rhizosphere interface. The

rhizosphere is typified by intense microbial activity involving firstly, a high population of microorganisms, and secondly, a rapid change in pH, in salt concentrations, and in osmotic and water potential (Handelsman et al., 1996). Microorganisms indigenous to the rhizosphere are ideal for biological control, since the rhizosphere provides a first-line defense for roots against attacks by plant pathogens (Weller, 1988). Microorganisms can protect the plant from fungal attacks through the production of antifungal metabolites, competition with the pathogen for nutrients, niche exclusion, parasitism or lysis of the pathogen, or through induction of plant resistance mechanisms (Whipps, 2001). Beneficial microorganisms of interest for biological control of plant pathogenic *Pythium* spp. have been identified among fungi and bacteria. Isolates of *Trichoderma* spp. and *Gliocladium* spp. are antagonists of *Pythium*-induced soil-borne diseases and several strains are already commercially available for the biological control of *Pythium* root rots (Howell et al., 1993; Fravel, 2005). Various actinomycete species including *Streptomyces*, *Actinoplanes*, and *Micromonospora* have considerable potential to inhibit *Pythium coloratum* cavity-spot on carrots (El-Tarabily et al., 1997). Other bacteria effective against *Pythium* are found in various genera including *Enterobacter*, *Erwinia*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, and *Rhizobium* but the most extensively studied group of bacterial biological control agents are *Pseudomonas* spp. (Chin-A-Woeng et al., 2003; Bardin et al., 2004). Competition for organic carbon and iron is one of the mechanisms through which some biocontrol agents suppress *Pythium* spp. (Hoitink et al., 1999). Sensitivity of *Pythium* spp. to competition and antagonism during its saprophytic phase of growth is one of the key factors in managing *Pythium* diseases through biological control (Martin et al., 1999). In contrast to this view, it is commonly known that *Pythium* spp. propagules germinate rapidly in response to seed or root exudates and quickly infect seeds or roots, and this complicates the application of biological control (Whipps et al., 1991). It is, therefore, of great importance that the activity of the biological control agent coincides with the period of host susceptibility and it should persist as long as the plant remains susceptible. Insufficient survival of the antagonists may lead to inadequate or partial control of the pathogen. From a field experiment conducted in Western Kenya, it was concluded that one approach to addressing this limitation is the introduction of a food base, such as compost, which supports the activity of antagonists but does not stimulate the activity of the pathogen (Otsyula et al., 1998; Hoitink et al., 1999). However, the compost must be free of *Pythium* root rot pathogens in order to increase the chance of effectively controlling *Pythium* root rot diseases (Martin et al., 1985).

6.3. Control through genetic resistance

The use of resistant common bean cultivars is the most efficient management strategy against root rot diseases. This approach is especially appropriate for small farmers with low inputs. However, the strategy requires the development of adapted common bean cultivars with resistance to all the major root rot pathogens that prevail in a given bean growing region (Abawi et al., 2006).

Plant resistance to diseases is defined as the ability of the host plant to hinder the growth and/or development of the pathogen (Parlevliet, 1979). Van der Plank (1963) classified plant resistance into two main categories: vertical and horizontal. Vertical resistance is race-specific and is characterized by specific interactions between the plant genotypes (or varieties) and the pathogen races (or strains). On the other hand, horizontal resistance is race-non-specific and is characterized by the absence of any specific interaction between host and pathogen genotypes (Robinson, 1987). The studies of Otsyula et al. (1998) and of Buruchara et al. (2001) aimed to identify resistance to *P. ultimum* root rot within *Phaseolus vulgaris*, through the evaluation of artificial inoculation under greenhouse conditions. In those studies, an isolate from *P. ultimum* was chosen for the artificial inoculation, due to its wide distribution and severity in East and Central Africa, as reported by Mukalazi (2004). The tested common bean materials belonged to the two major genepools of the food legume, *i.e.* the Mesoamerican and Andean genepool (as identified by Singh et al., 1991). The severity of lesions on the roots of the bean plants was scored using the CIAT 1-9 scale (as devised by Van Schoonhoven et al., 1987).

Following the work of Otsyula et al. (1998) and Buruchara et al. (2001), susceptible common bean varieties from Africa are now characterized by different seed sizes, such as the Kenyan varieties GLP 2 and GLP 585 with respectively large seeds (Andean genepool) and small seeds (Mesoamerican genepool), the Ugandan variety CAL 96 (Calima) with large seeds (Andean genepool) and the Rwandan variety Urugazi with an intermediate seed size (Mesoamerican genepool). All the resistant common bean varieties are advanced lines from an international breeding nursery run by CIAT (Cali, Colombia) and these varieties are also characterized by different seed sizes, such as the small-seeded variety RWR 719 from the Mesoamerican genepool, the intermediate-seeded varieties MLB 49-89A and SCAM 80-CM/15, both from the Mesoamerican genepool, and the large-seeded varieties AND 1055 and AND 1062, both from the Andean genepool (Otsyula et al., 2003). Identification of the genepool origin of the evaluated common bean genotypes has been helpful in predicting combining

ability and in the setting up of crossing programs likely to produce a wide segregation and ecological adaptation in common bean (Singh et al., 1991; Díaz et al., 2006). Due to its worldwide distribution, the Mesoamerican genepool is the most widely grown, with smaller seeds than those from the Andean genepool (Singh et al., 1991; Beebe et al., 2000). Cultivars of the Mesoamerican genepool are adapted to a range of hot, humid to moderate climates in the tropics and subtropics but these cultivars are also grown in cold high latitude climates in the United States and Argentina. As Mesoamerican genotypes are predominant in Rwanda, any breeding program needs to take into consideration the reality of climate suitability before choosing common bean varieties to be crossed (Buruchara et al., 2001; Díaz et al., 2006). In their experiments at the CIAT-Kawanda research station in Uganda, Otsyula et al. (2003) made crosses between susceptible varieties (GLP 2, GLP 585, CAL 96 and Urugezi) used as female parents and resistant varieties (RWR 719, MLB 49-89A, SCAM 80-CM/15, AND 1055 and AND 1062). The F1 hybrids were then backcrossed with the recurrent susceptible parents. Results showed that resistance to *P. ultimum* was expressed in all the F1 plants using the resistant genotypes as male parents. This shows that resistance to *P. ultimum* is inherited as a dominant characteristic by common bean (Otsyula et al., 2003; Mahuku et al., 2007). In order to determine the number of genes necessary for *Pythium* root rot resistance, the segregation of F2 and backcross plants was then analyzed. Chi square values revealed that goodness of fit was obtained for segregation ratios of 3:1 (resistant: susceptible) in the F2 plants, 1:1 when the F1 plants were backcrossed to GLP 2, GLP 585, CAL 96 and Urugezi, and 1:0 when the F1 plants were backcrossed to RWR 719, SCAM 80-CM/15, MLB 4889A, AND 1055 and AND 1062 (resistant varieties). From these results, it can be assumed that resistance to *P. ultimum* is controlled by one dominant gene, whatever the genepool origin and the parental genotypes used in the combinations (Buruchara et al., 2001; Otsyula et al., 2003). Molecular assisted selection could be applied in order to speed up the selection process in a breeding program. A SCAR marker named PYAA 19800 has been characterized as being associated with the *Pythium* root rot resistance gene in RWR 719 and AND 1062 and has been successfully used in selection for resistance to common bean *Pythium ultimum* root rot (Mahuku et al., 2007). This means that in a backcrossing program, all individuals devoid of the SCAR marker can be identified early and eliminated during the breeding process. Additional sources of resistance among the bean genepools need to be investigated with the aim of detecting new genes with resistance to different *Pythium* species and of developing varieties of bean adapted to the various

agro-ecological zones (Buruchara et al., 2001). This process would contribute to the sustainability of the varieties of bean that have been released and would thus ensure control of the disease for a longer period in common bean production systems (Otsyula et al., 2003).

6.4. Control by cultural practices

Certain cultural practices have been observed to reduce the severity and incidence of root rot diseases. In their experiments, Rosado et al. (1985) found that the incidence of *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* reduced when maize was included in rotation with beans.

Deep plowing and the use of raised ridges to grow beans has been found to reduce root rots favored by high moisture, such as *Rhizoctonia* root rot, southern blight, *Fusarium* root rot and *Pythium* root rot (CIAT, 1992). This is because ridging and deep tillage increase aeration and drainage, creating less favorable conditions for disease development. The application of organic soil amendments is also known to reduce root rot diseases (Volland et al., 1994). It has been shown that incorporating *Leucaena* spp. leaves and twigs of *Calliandra magrantha* and *Sesbania* as green manure two weeks before planting reduces plant mortality and increases bean grain yield (Buruchara, 1991; Buruchara et al., 1993).

It appears therefore that the approach most likely to be effective in the management of root rots of beans is one of integrated control. In Africa, the integration of organic amendments, raised beds and resistant varieties of beans has been shown to be advantageous over the use of single components in controlling the severity of root rots and crop yield (Buruchara et al., 1993; CIAT, 1993).

7. CONCLUSION

Root rot diseases are widespread and are considered as a major constraint to common bean production, as they reduce significantly the bean yield worldwide. Various disease control options are available, including different methods such as chemical control, biological control, genetic resistance methods and cropping practices. However, there is a crucial issue of the availability to users of resistant seed varieties, and this needs to be addressed in order that the majority of farmers may be reached. In these conditions, the use of improved bean varieties with disease resistance represents one of the most practical, economical, and easily adopted disease management strategies for the majority of bean producers in developing countries, within a context of small land area and limited economic resources

(Beebe et al., 1991; Otsyula et al., 1998). As previous studies have revealed that resistance to *Pythium* root rot is controlled by a single dominant gene (Otsyula et al., 2003; Mahuku et al., 2007), the varieties of bean that have been released and are deficient in resistance to *Pythium* root rot disease, can be improved by a backcrossing program. In order to speed up such a breeding program, the use of molecular markers associated with resistance to *Pythium* root rot is helpful in carrying out a rapid screening of large progeny populations. However, the fact that resistance to *Pythium* root rot is under the control of a single gene constitutes a risk factor that can lead to resistance breakdown. This risk is amplified by the fact that there is a high diversity in the pathogen populations. Thus, in order to improve the sustainability of this resistance-based control method, it is essential to use diversified sources of resistance and to integrate other control methods, mainly appropriate cropping practices. This will reduce the risk and encourage the rapid build-up of *Pythium* inoculums. For example, ridging and deep tillage increase aeration and drainage, creating less favorable conditions for disease development (Beebe et al., 1991; CIAT, 1992; Volland et al., 1994; Abawi et al., 2000).

Acknowledgements

The authors would like to express their warmest thanks to the Belgian Technical Cooperation, the Kirkhouse Trust and the Rwanda Agricultural Research Institute for their financial support.

Bibliography

- Abawi G.S. & Widmer T.L., 2000. Impact of soil health management practices on soil borne pathogens, nematodes and root diseases of vegetable crops. *Appl. Soil Ecol.*, **15**, 37-47.
- Abawi G.S., Ludwig J.W. & Gugino B.K., 2006. Bean root rot evaluation protocols currently used in New York. *Annu. Rep. Bean Improv. Cooperative*, **49**, 83-84.
- Allain-Boulé N. et al., 2004. *Pythium attrantheridium* sp. nov.: taxonomy and comparison with related species. *Br. Mycol. Soc. Mycol. Res.*, **108**(7), 795-805.
- Bardin S.D. et al., 2004. Biological control of *Pythium* damping-off of pea and sugar beet by *Rhizobium leguminosarum* bv. *Viceae*. *Can. J. Bot.*, **82**, 291-296.
- Baudoin J.P., Camarena F., Lobo M. & Mergeai G., 2001. Breeding *Phaseolus* for intercrop combinations in Andean highlands. In: Cooper H.D., Spillane C. & Hodgkin T., eds. *Broadening the genetic bases of crop*. Oxford, UK: CABI Publishing, 373-384.
- Beebe S. & Pastor-Corrales M.A., 1991. Breeding for disease resistance. In: van Schoonhoven A. & Voysest O., eds. *Common beans: research for crop improvement*. Wallingford, UK: CAB International; Cali, Colombia: CIAT, 561-617.
- Beebe S. et al., 2000. Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. *Crop Sci.*, **40**, 264-273.
- Belbahri L., Calmin G., Sanchez-Hernandez E. & Lefort F., 2008. *Pythium sterilum* spp. nov. isolated from Poland, Spain, and France: its morphology and molecular phylogenetic position. *FEMS Microbiol. Lett.*, **255**, 209-214.
- Brožová J., 2002. Exploitation of the mycoparasitic fungus *Pythium oligandrum* in plant protection. *Plant Prot. Sci.*, **38**, 29-35.
- Buruchara R.A., 1991. *Use of soil amendments in the management of root rots of beans*. CIAT African Workshop Series No. 17. Cali, Colombia: CIAT.
- Buruchara R.A. & Scheidegger U., 1993. *Development of cultural components in integrated management of root rots of beans. Paper presented at the 7th Regional seminar on the improvement of beans in the Great Lakes region, 2-6 November 1992, Goma, Zaire*. Cali, Colombia: CIAT.
- Buruchara R.A. et al., 2001. *A case study on developing and disseminating integrated pest management technologies for bean root rots in eastern and central Africa. Paper presented at the Global Forum on Agricultural Research, 21-23 May 2001, Dresden, Germany*, 423.
- Buruchara R.A., Mahuku G., Mukalazi J. & Levesque A., 2007. *Pythium species associated with Pythium root rot of beans (Phaseolus vulgaris L.) in Eastern Africa*. Cali, Colombia: CIAT, 42-53.
- Chin-A-Woeng T.F., Bloemberg G.V. & Lugtenberg B.J., 2003. Phenazines and their role in biocontrol by *Pseudomonas bacteria*. *New Phytol.*, **157**, 503-523.
- CIAT, 1992. *Pathology in Africa*. In: *CIAT Annual Report, 1992*. Cali, Colombia: CIAT Bean Program.
- CIAT, 1993. *Trends in CIAT commodities, 1993*. Working document No. 128. Cali, Colombia: CIAT.
- Díaz L.M. & Blair M.W., 2006. Race structure within the Mesoamerican genepool of common bean (*Phaseolus vulgaris* L.) as determined by microsatellite markers. *Theor. Appl. Genet.*, **114**, 143-154.
- Dick M.W., 2001. The peronosporomycetes. In: McLaughlin D.J., McLaughlin E.G. & Lemke P.A., eds. *The Mycota VII. Part A. Systematics and Evolution*. Berlin, Germany: Springer-Verlag, 39-72.
- Duřková E., 1995. New biological fungicides for plant protection registered in the Czech Republic. In: *Proceedings of the 3rd Conference of the European Foundation for Plant Pathology, Environmental Biotic Factors in Integrated Plant Disease Control, Poznan, 5-9 September 1994*. Poznan: The Polish Phytopathological Society, 211-217.
- El-Tarabily K.A. et al., 1997. The potential for the biological control of cavity-spot disease of carrots,

- caused by *Pythium coloratum*, by streptomycete and non-streptomycete actinomycetes. *New Phytologist*, **137**, 495-507.
- FAO, 2005. *FAOSTAT database*, [http:// faostat.fao.org](http://faostat.fao.org), (1/7/2010).
- Fravel D.R., 2005. Commercialization and implementation of biocontrol. *Annu. Rev. Phytopathol.*, **43**, 337-359.
- Gichuru G.V., 2008. *Influence of farming systems and crop host varieties on Pythium root rots epidemics in a highland agroecology of South Western Uganda*. PhD thesis: Makerere University, Kampala (Uganda).
- Handelsman J. & Stabb E.V., 1996. Biocontrol of soilborne plant pathogens. *Plant Cell*, **8**, 1855-1869.
- Hendrix F.F.J.R. & Campbell W.A., 1969b. Heterothallism in *Pythium catenulatum*. *Mycologia*, **61**, 639-641.
- Hendrix F.F.J.R., Campbell W.A. & Chien C.Y., 1971. Some Phycomycetes indigenous to soils of oldgrowth forests. *Mycologia*, **63**, 283-289.
- Herrero M.L. & Klemsdal S.S., 1998. Identification of *Pythium aphanidermatum* using the RAPD technique. *Mycol. Res.*, **102**, 136-140.
- Hillocks R.J. et al., 2006. *Phaseolus* bean improvement in Tanzania, 1959-2005. *Euphytica*, **150**, 215-231.
- Hoitink H.A. & Boehm M.A., 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annu. Rev. Phytopathol.*, **37**, 427-446.
- Howell C.R., Stipanovic R.D. & Lumsden R.D., 1993. Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedlings diseases. *Biocontrol Sci. Technol.*, **3**, 435-441.
- Hsu D.S. & Hendrix F.F.J.R., 1972. Influence of temperature on oospore formation of four heterothallic *Pythium* spp. *Mycologia*, **64**, 447-451.
- Kageyama K. et al., 2005. Phylogenetic and morphological analyses of *Pythium graminicola* and related species. *J. Gen. Plant Pathol.*, **71**, 174-182.
- Kirk P.M., Cannon P.F., Minter D.W. & Stalpers J.A., 2008. *Ainsworth & Bisby's dictionary of the fungi*. 10th ed. Wallingford, UK: CAB International.
- Lévesque C.A. & de Cock A.W., 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycol. Res.*, **108**, 1363-1383.
- Lumsden L.D., Garcia R., Lewis J.A. & Frias G.A., 1987. Suppression of damping-off caused by *Pythium* spp. in soil from indigenous Mexican chinampa agricultural system. *Soil Biol. Biochem.*, **19**, 501-508.
- Mahuku G., Buruchara R., Navia M. & Otsyula R., 2007. Development of PCR markers tightly linked to *Pyult1*, a gene that confers *Pythium* root rots resistance in the common bean genotype AND 1062. *Phytopathology*, **97**, 69-79.
- Martin S.B., Abawi G.S. & Hoch H.C., 1985. Biological control of soilborne pathogens with antagonists. In: Hoy M.A. & Herzog D.C., eds. *Biological control in agricultural IPM systems*. Orlando, FL, USA: Academic Press, 433-454.
- Martin F.N. & Loper J.E., 1999. Soilborne diseases caused by *Pythium* spp: ecology, epidemiology, and prospects for biological control. *Crit. Rev. Plant Sci.*, **18**, 111-181.
- Matsumoto C., Kageyama K., Suga H. & Hyakumachi M., 1999. Phylogenetic relationships of *Pythium* species based on ITS and 5.8S sequences of the ribosomal DNA. *Mycoscience*, **40**, 321-331.
- Miklas P.N. et al., 2006. Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica*, **147**, 105-131.
- Mukalazi J., 2004. *Pathogen variation and quantification of Pythium spp. in bean fields in Uganda*. PhD thesis: Makerere University, Kampala (Uganda).
- Nekesa P., Ndiritu J.H. & Otsyula R.M., 1998. Bean research in Western Kenya: lessons and experiences. In: Farrell G. & Kibata G.N., eds. *Crop protection research in Kenya. Proceedings of the Second Biennial Crop Protection Conference, 16-17 September 1998*. Nairobi: Kenya Agricultural Research Institute (KARI)/ UK Department for International Development (DFID), 237-244.
- Nzunguze R.J. et al., 2011. Pathogenic and molecular characterization of *Pythium* species inducing root rot symptoms of common bean in Rwanda. *Afr. J. Microbiol. Res.*, **5**(10), 1169-1181.
- Onokpise O.U. et al., 1999. Evaluation of macabo cocoyam germplasm in Cameroon. In: Janick J., ed. *Perspectives on new crops and new uses*. Alexandria, VA, USA: ASHS Press, 394-396.
- Otsyula R.M., Ajanga S.I., Buruchara R.A. & Wortmann C.S., 1998. Development of an integrated bean root rots control strategy for Western Kenya. *Afr. Crop Sci. J.*, **6**, 61-67.
- Otsyula R.M., Buruchara R.A., Mahuku G. & Rubaihayo P., 2003. Inheritance and transfer of root rots (*Pythium*) resistance to bean genotypes. *Afr. Crop Sci. Soc.*, **6**, 295-298.
- Owen-Going T.N., Beninger C.W., Sutton J.C. & Hall J.C., 2008. Accumulation of phenolic compounds in plants and nutrient solution of hydroponically-grown preppers inoculated with *Pythium aphanidermatum*. *Can. J. Plant Pathol.*, **30**, 214-225.
- Pachico D., 1993. The demand for bean technology. In: Henry G., ed. *Trends in CIAT's Commodities*. CIAT working document No. 128. Cali, Colombia: CIAT, 60-74.
- Parlevliet J.E., 1979. Components of resistance that reduce the rate of epidemic development. *Annu. Rev. Phytopathol.*, **17**, 203-222.
- Paul B., 2004. A new species isolated from Burgundian vineyards and its antagonism towards *Botrytis cinerea*, the causative agent of the grey mould disease. *FEMS Microbiol. Lett.*, **234**, 269-274.

- Paul B., Bala K., Gognies S. & Belarbi A., 2005. Morphological and molecular taxonomy of *Pythium longisporangium* sp. nov. isolated from the Burgundian region of France. *FEMS Microbiol. Lett.*, **246**, 207-212.
- Postma J. et al., 2009. Biological control of *Pythium aphanidermatum* in cucumber with a combined application of *Lysobacter enzymogenes* strain 3.1T8 and chitosan. *Biol. Control*, **48**, 301-309.
- Pystina K.A., 1974. Effect of sterols and vegetable oils on growth and sexual reproduction of fungi of the genus *Pythium*. *Mikol. Fitopatol.*, **7**, 493-498.
- Robinson R.A., 1987. *Host management in crop pathosystems*. New York, USA: MacMillan.
- Rosado May F., Garcia-Espinosa R. & Gliessmann S.R., 1985. Impact of soil borne plant pathogens on beans (*Phaseolus vulgaris*): cultivation in soil with different management practices in Chontalpa, Tabasco. *Rev. Mex. Fitopatol.*, **3**, 15-26.
- Rusuku G. et al., 1997. Effect of crop rotation on *Pythium ultimum* and other *Pythium* species in the soil. *Phytopathology*, **52**, 27.
- Schwartz H.F., Gent D.H., Gary D.F. & Harveson R.M., 2007. Dry bean, *Pythium* wilt and root rots. High plains IPM Guide, a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University.
- Singh S., Gepts P. & Debouck D., 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Econ. Bot.*, **45**, 379-396.
- Stanghellini M.E. & Hancock J.G., 1971. The sporangium of *Pythium ultimum* as a survival structure in soil. *Phytopathology*, **61**, 157-164.
- Stanghellini M.E. & Russell J.D., 1973. Germination *in vitro* of *Pythium aphanidermatum* oospores. *Phytopathology*, **63**, 133-137.
- Tedla T. & Stanghellini M.E., 1992. Bacterial population dynamics and interactions with *Pythium aphanidermatum* in intact rhizosphere soil. *Phytopathology*, **82**, 652-656.
- Uzuhashi S., Tojo M. & Kakishima M., 2010. Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience*, **51**, 337-365.
- Van der Plaats-Niterink J., 1981. *Monograph of the genus Pythium*. Studies in Mycology 21. Baarn, The Netherlands: Centraalbureau voor Schimmelcultures, 25-39.
- Van der Plank J.E., 1963. *Plant diseases: epidemics and control*. New York, USA: Academic Press.
- Van Schoonhoven A. & Pastor Corrales M.A., 1987. *Standard system for the evaluation of bean germoplasm*. Cali, Colombia: CIAT.
- Vasseur V. et al., 2005. Molecular characterization of *Pythium* group F isolates by ribosomal and intermicrosatellite-DNA regions analysis. *Eur. J. Plant Pathol.*, **112**, 301-310.
- Voland R.P. & Epstein A.H., 1994. Development of suppressiveness to diseases caused by *Rhizoctonia solani* in soils amended with composted and non-composted manure. *Plant Dis.*, **78**, 461-466.
- Weller D.M., 1988. Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.*, **26**, 379-407.
- Whipps J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.*, **52**, 487-511.
- Whipps J.M. & Lumsden R.D., 1991. Biological control of *Pythium* species. *Biocontrol Sci. Technol.*, **1**, 75-90.
- Wulff E.G. et al., 1998. Inoculation of cucumber roots with zoospores of mycoparasitic and plant pathogenic *Pythium* species: differential zoospore accumulation, colonization ability and plant growth response. *Eur. J. Plant Pathol.*, **104**, 69-76.
- (71 ref.)