ITS-5.8S-rDNA REGION AND DISEASE SEVERITY COMPARISON OF *Rhizoctonia solani* ANASTOMOSIS GROUPS ISOLATED FROM COMMON BEAN (*Phaseolus vulgaris* L.) AT ISABELA, PUERTO RICO

By

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ABSTRACT

Rhizoctonia solani is a widespread soilborne pathogen of common bean (*Phaseolus vulgaris* L.). This major pathogen is a species complex classified in 14 anastomosis groups (AG). Some AGs can cause web blight (WB), one of the most important diseases of bean plants planted in the Caribbean, while others are responsible for root rots (RR). Knowledge of these subgroups and their interactions with plant hosts represents an essential contribution to the mycological and plant breeding communities. For this reason the variability of web blight and root rot isolates were compared by detecting differential reactions among bean lines using a detached leaf method in leaf evaluations and a mycelia suspension technique in root evaluations. Disease severity evaluations demonstrated that line PR0401-259 was resistant to WB and line PR0650-27 expressed moderate levels of RR resistance. The most aggressive WB isolate was AG 4WB2 and the most severe RR isolate was AG 4RR1.

RESUMEN

Rhizoctonia solani es un patógeno, con distribución mundial, de habichuela común (*Phaseolus vulgaris* L.). Es un complejo de especies clasificado en 14 grupos de anastomosis (GA). Algunos GA causan mustia hilachosa (MH), una de las enfermedades más importantes de habichuelas sembradas en el caribe, mientras que otros causan pudrición de raíz (PR). El conocimiento de estos subgrupos y sus interacciones con plantas hospederas representa una contribución esencial para comunidades micológicas y de fitomejoración. Por tal razón se comparó la variabilidad de aislados de MH y de PR utilizando un método de hoja desprendida para evaluar hojas y una técnica de suspensión micelial para evaluar raíces. Evaluaciones de severidad de enfermedad demostraron que la línea PR0401-259 fue resistente a MH y la línea PR0650-27 tuvo niveles moderados de resistencia a PR. El aislado más agresivo de MH fue GA 4WB2 y el aislado más severo de PR fue AG 4RR1.

DEDICATION

To God, to my parents and sister, and
to my grandmother who is in heaven,
for supporting and believing in me
all these years.

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INTRODUCTION

Rhizoctonia solani Kühn is known to cause diseases in common beans (*Phaseolus vulgaris* L.) such as foliar blight, stem and root rots, and damping-off (Harikrishnan and Yang, 2004). These diseases limit seed yield due to root cortex lesions and stunted growth (Smith et al., 2003). They also cause seed quality losses due to blemishes and discoloration and over 50 percent of marketable seed loss, resulting in economic losses that can surpass millions of US dollars (Coyne et al., 2003; Godoy-Lutz et al., 2003; Harikrishnan and Yang, 2004; Smith et al., 2003). In the USA over 24% of acres planted with sugar beet suffer an economic damage from *Rhizoctonia* (Stojsin et al., 2007). Web blight (WB) is one of the most important diseases of bean plants planted in the humid lowland tropics of Central America and the Caribbean and is also increasing in importance in eastern Africa (Galvez et al., 1989; Godoy-Lutz et al., 2008; Montoya et al., 1997). It can be easily spread by airborne basidiospores produced by the pathogen's teleomorph *Thanatephorus cucumeris* (Frank) Donk, or by rain-splashed mycelium, sclerotia or infested soil which fall onto other plants (Godoy-Lutz et al., 2003). Because *R. solani* has a wide host range, chemical, cultural and biological control is difficult (Zhao et al., 2005). Systemic fungicide application is used in Panama, but this practice is expensive since fungicide applications must be carried out repeatedly in order to be effective (Coyne et al., 2003; Godoy-Lutz et al., 2003; Takegami et al., 2004).

WB infection is most severe under high temperature and relative humidity conditions. These are typical climatic conditions of humid tropics during wet season (Polanco et al., 1996). When the climate favors disease development, web blight causes

rapid defoliation resulting in total loss of the bean crop, especially when it occurs during the pod filling stage (Montoya et al., 1997). In addition to bean seed yield reduction, it also discolors the seed and diminishes market value (Godoy-Lutz et al., 2008). Widespread crop infection by *Rhizoctonia* AG-4 in Georgia coastal plain in USA causes root rot of snap bean or unripe fruit of *P. vulgaris* and pod rot in peanut (*Arachis hypogaea* L.) (Summer and Bell, 1994). *R. solani* AG-4 causes damping-off during the early growth stages of common bean and produces stem and root rot during the later stages of development (Balali and Kowsari, 2004). Testing for *Rhizoctonia* root rot resistance is difficult because *R. solani*'s growth in the field is unpredictable and patchwise (Büttner et al., 2004). Root infections depend on the fungal hyphae's ability to spread many millimeters or centimeters through the soil (Otten et al., 2004). *R. solani* can translocate nutrients through the hyphal network. This allows it to spread substantially at distances away from the nutrient source (Otten et al., 2004).

This major pathogen is a species complex currently classified in 14 anastomosis groups (AGs) based on hyphal fusion, cultural morphology, pathogenicity or virulence, and DNA homology (Godoy-Lutz et al., 2003; Harikrishnan and Yang, 2004). Some of these AGs are known to cause aerial and web blight while others are responsible for root, stem or seed rots (Harikrishnan and Yang, 2004). Anastomosis group variability is the result of adaptations to different ecological areas. Little is known about the regional predominance of certain AG types (Dorrance et al., 2003). Studies reported WB of common bean is caused by *R. solani* subgroups AG 1-IA, AG 1-IB, AG 1-IE, AG 1-IF and AG 2-2 (Godoy-Lutz et al., 2008). There is variability in pathogen's isolates virulence (Polanco et al., 1996). Different AG 1-IB isolates recovered from diverse

lettuce crop fields in Germany showed a pathogen population which had both highly and less aggressive isolates, which had either high or low hyphal growth rate. This study suggests there might not be a relationship between isolate aggressiveness and hyphal growth rate (Grosch et al., 2004). They suggest that isolate aggressiveness may be directly related to cell-wall digesting enzymes (Grosch et al., 2004). Balali and Konsari (2004) suggest some isolates seemed to attack specific plant parts, while the more aggressive isolates were able to invade different plant parts. Disease expression varies annually and from field to field because it is influenced by environmental conditions and disease pressure (Büttner et al., 2004). Certain common bean lines have an intermediate level of expressed resistance to web blight in the field (Montoya et al., 1997). There are no commercial bean varieties with high levels of resistance (Godoy-Lutz et al., 2008). The lack of information, concerning on *R. solani* genetics and disease resistance mechanisms, has restrained WB resistance breeding of dry beans with greater levels of WB resistance (Godoy-Lutz et al., 2008).

Cultural and chemical control methods have not been successful in reducing disease severity or its spread (Godoy-Lutz et al., 2008). Some pathogens co-evolved with the common bean forming distinct Middle American and Andean populations. These pathogens can survive over a prolonged period of time in crop debris and can be disseminated by vectors, seed, rain, irrigation water, wind, or mechanically from contaminated machinery (Godoy-Lutz et al., 2008; Monteagudo et al., 2006). This makes chemical control difficult and expensive for bean growers (Monteagudo et al., 2006). Some fungicides provide partial chemical control of *R. solani* (Montoya et al., 1997). *In vitro* fungicide assays using amended agar plates demonstrated that the efficacy of

specific (PCNB, carboxin, triadimfon and liprodione) fungicides against *R. solani* were variable and dependent on the AG (Dorrance et al., 2003). Because different *Rhizoctonia* AGs differ in sensitivity to the same fungicide chemical disease control is difficult (Tewoldenmedhin et al., 2006). Environmental concerns, increased costs, and the potential development of fungicide resistant pathogen populations might limit fungicide use in the future (Sayler and Yang, 2007). When fungicide is used in excess the input costs increase. The inappropriate use of fungicides can threaten human health, damage the environment and contaminate water supplies (Coyne et al., 2003). Small scale bean farms have limited economic resources thus reducing the possibility of using expensive fungicides (Montoya et al., 1997). Reduction in pesticide usage is an important benefit of the availability of an adapted and disease resistant cultivar (Monteagudo et al., 2006).

Before a standard greenhouse or laboratory screening methods are adapted, the techniques should be tested in diverse controlled environments which include multiple pathogen isolates that vary in aggressiveness on the host and also represent the range in aggressiveness found in a field environment (Kull et al., 2003). Classical and molecular bean breeding programs both benefit from reliable and accurate screening methods in the identification of different levels of disease resistance (Kull et al., 2003). An important objective in the development of an adequate screening technique is the need to identify partial resistance of evaluated plants and to achieve repeatable high correlations between screening laboratory method results and field disease ratings (Kull et al., 2003).

Variations of limited term inoculation methods have been employed by researchers to screen for disease resistance. These methods consist of using a mycelial-infested medium that is then inoculated onto the plant and after a specified time, removed

from the evaluated plant. An example of limited time term inoculation technique is the use of mycelial plug to inoculate a specific plant part (Kull et al., 2003). The detached-leaf technique is a fast disease screening protocol that produces repeatable results. It is nondestructive and can be used to test plants grown in field or controlled environments (Kull et al., 2003). There has insufficient emphasis placed on the development of screening methods that utilize statistical approaches to detect cultivar differences, or to reveal cultivar-by-isolate interactions, or the comparison of the effectiveness of different inoculation techniques to separate resistant and susceptible hosts (Kull et al., 2003).

Due to diverse disease reactions on bean lines, plant breeders must take into consideration such subgroups when developing resistant cultivars (Coyne et al., 2003). Knowledge of these subgroups and their interactions with their plant hosts represents an essential contribution to the mycological and plant breeding communities. Previous researchers have studied AGs and their relation to a specific disease. More knowledge is needed to determine if a specific AG can cause more than one disease in the same host plant. This research will focus on root rot and web blight AG isolates of *R. solani*. We plan to determine if root rot AGs have the capacity to induce web blight if exposed to bean leaf surfaces under conditions that are favorable for the development of disease and the potential of web blight AGs inducing root rot in host bean plant roots.

OBJECTIVES

1. Compare the variability of previously characterized and sequenced web blight isolates of *Rhizoctonia solani* with isolates obtained from field samples in common bean plants that had root rot and web blight symptoms.
2. Determination of anastomosis groups of root rot pathogenic isolates of *R. solani* using PCR amplification of the ITS-5.8S-rDNA region.
3. Use a detached-leaf screening technique to detect differential reactions among bean lines when inoculated with root rot and web blight *R. solani* pathogenic isolates.
4. Test for differential root rot reactions among bean lines when inoculated with web blight and root rot isolates of *R. solani*.
5. Identify dry bean lines that have at least moderate levels of resistance to web blight and root rot caused by diverse *R. solani* isolates.

LITERATURE REVIEW

The genus *Phaseolus* originated in the Americas and consists of over thirty species. Only five of these species are domesticated, of which *Phaseolus vulgaris* L. (the common bean) is the most widely grown (Singh, 2001). This species comprises over 90 percent of production area sown for this specific genus world wide (González et al., 2006). Common bean is the third most important grain legume crop in the world and is considered a primary source of protein in human diets (González et al., 2006; Infantino et al., 2006). In Latin America and the Caribbean it is the major legume crop grown, with over 7 million hectares planted annually, and consumed by over 500 million people (Godoy-Lutz et al., 2008). Worldwide, the area of common bean production is estimated to be over 14 million hectares (Singh, 2001). In recognition of health benefits the consumption of dry and green beans in the United States as well as other developed countries has increased in recent years (Monteagudo et al., 2006). Common bean is not a centric crop, having multiple sites of domestication throughout Mexico, Central America and Andean South America (Singh, 2001). Large-seeded dry beans of Andean origin are grown in North and South America, Asia, Europe and Africa. It is due to such a wide geographic distribution that new combinations of genes favoring adaptation to diverse environments have generated (Santalla et al., 2005). Crosses between the Andean and Middle American gene pools help in the development of populations with important traits sought by breeders (Santalla et al., 2005). Domestication produced bush plant types from a climbing growth habit. Humans also selected common beans that are insensitive to long photoperiods. Its seeds have changed through domestication from smaller to larger forms

that lack dormancy and have a water permeable seed coat. This great variability permits common beans to be produced in diverse cropping systems and environments such as Europe, China, the Middle East, Africa and the Americas (González et al., 2006; Singh, 2001). Improving breeding lines for seed quality through selection within landraces has often left them susceptible to diseases (Monteagudo et al., 2006).

The environment plays a major role in disease development and severity (Román-Avilés and Kelly, 2005). Disease expression varies annually and from field to field because it's influenced by environmental conditions and disease pressure (Büttner et al., 2004). In addition to bean seed yield reduction, web blight caused by *Rhizoctonia solani* also discolors the seed, negatively affecting its market value (Godoy-Lutz et al., 2008). A specific plant species can be simultaneously infected by different AGs (Guillemaut et al., 2003). Some of the control methods proposed against legume diseases range from cultural practices or traditional breeding and adoption of marker assisted selection techniques, to the use of biological and chemical control (Sillero et al., 2006). Breeding plants for disease resistance is an effective and widely used method to control soil-borne diseases (Li et al., 2004). Breeding for resistant cultivars has proven to be an effective strategy used to control this disease (Jung et al., 1996). Common bean lines with moderate levels of resistance to web blight resistant germplasms have been identified (Godoy-Lutz et al., 2008; Montoya et al., 1997).

Progress in breeding for root rot resistance is limited because this trait is genetically complex and difficult to evaluate, lowering the efficiency of phenotypic selection (Román-Avilés and Kelly, 2005). Much of the effort to select for disease resistance in bean has focused on monogenetic resistance which targets only one disease.

This can produce varieties which are vulnerable to other diseases or to new races of the pathogen to which they were originally bred against (García-E. et al., 2003).

Crop rotation is an effective method used to minimize yield reductions caused by root rot (Li et al., 2004). Induced systemic resistance is the proposed plausible mechanism by which the non-pathogenic *Rhizoctonia* spp. protects *P. vulgaris* from pathogenic *Rhizoctonia* AG-4 (Wen et al., 2005). In greenhouse assays conducted in Iran, researchers reported that young common bean plants inoculated with AG-4 were more susceptible than older plants. They proposed that a lack of calcium pectate in young plant cell structure could cause susceptibility to fungal diseases and suggested that resistance to *R. solani* in older plants could be related to the development of calcium pectate in cell walls (Balali and Kowsari, 2004). Balali and Kowsari (2004) observed how greenhouse isolates seemed to attack specific plant parts, while the more aggressive isolates were able to invade different plant parts.

There are over 1.6 million species of fungi in the world (Pewitt et al., 2008). Fungal diseases are one of the major constraints for bean production world wide by reducing crop yield and seed quality. These diseases are considered to be one of the most limiting factors that bean producers encounter in tropical agro-ecosystems (García et al., 2003; Infantino et al., 2006). An important soil-borne pathogen in the warm humid tropics, which causes various diseases in bean crops, is the basidiomycete *Thanatephorus cucumeris* (A.B. Frank) Donk. *T. cucumeris* is the teleomorph of the anamorphic (asexual stage) fungal plant pathogen, *Rhizoctonia solani* (Carling et al., 2002). It is a widespread soilborne pathogen able to adversely affect over 142 plant species causing severe economic damage (Guillemaut et al., 2003; Mazzola, 1997). It causes yield losses

of approximately 20% yearly in over 200 crops around the world (Salazar et al., 2000). *R. solani* affects growth of annual and perennial crops, in both agricultural and forest ecosystems (Tewoldenmedhin et al., 2006). In tropical regions the soilborne pathogen can destroy an entire harvest (García-E. et al., 2003). *Thanatephorus cucumeris* belongs to the subdivision Basidiomycotina, class Hymenomycetes, and subclass Holobasidiomycetidae (Sneh et al., 1991). *R. solani* AG 2-2 has been proven to have a bipolar heterothallic mating system (Toda and Kyakumachi, 2006).

The genus *Rhizoctonia* is given to the anamorph state of a heterogenous group of fungal species which do not produce conidia (Mordue et al., 1989). *R. solani* belongs to the cantherelloid clade of the phylum Basidiomycete (Ceresini et al., 2007). Julius Kühn first observed *R. solani* growing on diseased potato tubers in 1858 (Polanco et al., 1996). It is considered a soilborne pathogen with a worldwide distribution. The economic significance attributed to the genus arises from the number of important pathogens of crop plants causing diseases on a large variety of field crops, ornamentals, as well as on fruit and forest trees (Mordue et al., 1989; Salazar et al., 2000). *R. solani* is considered mainly as a soilborne plant pathogen with a wide host range. Because of its facultative parasitic properties it can also subsist as a worldwide saprotroph in soil (Otten et al., 2001; Zhao et al., 2005). Only multinucleate hyphae (Ceratobasidiales) of *Rhizoctonia* have *T. cucumeris* as their perfect stage (Tupac-Otero et al., 2002). This pathogen has evolved as an aerial, soil surface and subterranean parasite, causing web blight, hypocotyl and root rots (Allen, 1997; Galvez et al., 1989). *R. solani* is distributed world wide and has a large number of host species. Multinucleate *R. solani* have a host range of over 250 species. They are able to incite diseases under varying environmental conditions in

almost any stage of plant development (Tewoldenmedhin et al., 2006). *R. solani* can spread by airborne basidiospores, rain-splashed sclerotia, infested soil-debris, mycelial bridges between plants and by infected seeds (Godoy-Lutz et al., 2003). Both, the sclerotia, the asexual stage that can remain viable in soil for several years, and the basidia, the sexual stage, can initiate the disease. Nonetheless, they differ in symptomology (Allen, 1997; Galvez et al., 1989).

Pathogenicity comprises virulence and aggressiveness. Virulence is the qualitative ability of a given strain to infect a specific host genotype, and aggressiveness is its quantitative ability to infect a set of host genotypes (Tivoli et al., 2006). Colonization is a passive affair since plants have few means of avoiding airborne pathogen cell deposition on the host tissue (Sillero et al., 2006). *R. solani* can survive and disseminate by sclerotia, produced asexually, and by vegetative mycelium (Rosewich et al., 1999). Microsclerotia can cause secondary infections, when functioning as airborne propagules, thus facilitating pathogen spread and inoculum buildup (Godoy-Lutz et al., 2003). As an infectious inoculum source, sclerotia vary considerably in their germination rate based on their age and size. Smaller sclerotia have been reported to infect plants more rapidly than larger ones. Infection time might be intermediate with immature sclerotia inoculum (Park et al., 2008). Previous studies also showed that isolates producing macrosclerotia were more virulent than those which produced microsclerotia (Godoy-Lutz et al., 2003). *R. solani* thrives under variable conditions (García-E. et al., 2003). Temperature is one of the most important environmental factors affecting distribution, growth and survival of soil-borne fungi (Harikrishnan and Yang, 2004). Fungal pathogen invasion through soil depends on the hyphae's capacity to

colonize susceptible host structures such as roots and hypocotyls. Small differences in this ability may have a significant effect on epidemic dynamics to such an extent as to make the expansion of fungal colonies switch from an invasive to a noninvasive spread (Otten et al., 2001). Nonetheless, not much is understood about the ample geographic distribution and reasons for such variation of the different AGs which comprise the *R. solani* complex (Harikrishnan and Yang, 2004). Although the complex is diverse on the range of host plants they infect, both binucleate and multinucleate *Rhizoctonia* spp. found in agroecosystems appear to be crop-specific (Mazzola, 1997; Toda and Kyakumachi, 2006).

For *R. solani* the pathozone is dynamic, changing over time as its colonies expand towards an asymptotic limit. It is characterized by colony size, variability in the distribution of hyphae within the colony and colony expansion dynamics (Otten et al., 2001). Previous experiments have shown that the variability, extent and rate of *R. solani*'s mycelial spread—even in the absence of a host, is affected by soil physical conditions. These conditions might include soil water potential, aggregate size, bulk-density and air-filled pore volume (Otten et al., 2001; Otten et al, 2004). *Rhizoctonia solani* can spread more rapidly and further distances along surfaces than through soil. This is not a random process. When mycelium spreads through the soil and it encounters a large continuous pore volume. The spread is enhanced as the hyphae bypasses twisted pathways (Otten et al., 2004). Plant roots provide soil inhabiting microorganisms a primary source of nutrition, mainly carbon and energy, which can be obtained from detached cells from older roots, root exudates and roots from dead plants (Aziz et al., 1997). Unlike many soilborne pathogens, *R. solani* has the ability to grow quickly and

extensively from a host into natural soil (Paulitz and Schroeder, 2005). It can also transfer nutrients and other contents from its cytoplasm throughout the pathogen's extensive hyphal network, this allows it to spread substantially at distances away from the nutrient source (Otten et al., 2004; Paulitz and Schroeder, 2005). Root infections depend on the fungal hyphae's ability to spread many millimeters or centimeters through the soil, yet the geometry of the air-filled volume restrain fungal spread (Otten et al., 2004). The ability of fungi to invade a substrate may depend on the connectivity and tortuosity of air-filled pore space. The geometry of pore space also affects the dynamics of colonization (Otten, et al, 2001). Gaps in soil can either increase colonization efficiency or reduce it, depending on the width and orientation of the gaps (Otten et al., 2004). Nutrient status and competition with antagonists are other two reasons why pathogenic fungi cross gaps and colonize neighboring sites (Otten et al., 2004).

The teleomorph state of *R. solani* is often difficult to induce in culture (Mordue et al., 1989). Sexual stages seldomly occur in the field or laboratory. This is why the broad vegetative criteria for isolate identification have led to the creation of a paraphyletic taxonomy, with a large quantity of unrelated fungi being grouped together (Tupac-Otero et al., 2002). *R. solani* isolates from different bean production regions have different cultural characteristics, anastomosis group, classification and degree of virulence (Beaver et al., 2003; Galvez et al., 1989). *R. solani* is composed of related yet genetically isolated subspecific groups (Carling et al., 2002). Traditionally classification of the taxa and strains has drawn heavily on cultural morphology—pigmentation, hyphal dimensions and branching patterns, septal pore structures, number of nuclei, size and shape of monoloid cells and sclerotia appearance and rate of growth in culture (Mordue et al., 1989). *R.*

solani complex share similar characteristics, among which is the lack of conidia (Bounou et al., 1999). On potato dextrose agar (PDA) colonies first grow hyaline, but rapidly become brown as the hyphae grow older. The aerial mycelium can have a felted or mealy surface, some sparsely branched hyphae can also be observed (Galvez et al., 1989). Pathogen grows between temperature range of 12 to 35ºC, with optimum growth between 20-30ºC (Windels and Brantner, 2005). The quantification of nuclei per young cells is another morphological feature which helps in *Rhizoctonia*'s classification. There are multi-, bi-, and uninucleate cells (Tupac-Otero et al., 2002). *R. solani* have multinucleate cells, ranging from 2-25 nuclei per cell and have a dolipore septa. As the mycelium grows older its cells become shorter due to the formation of secondary septa (Galvez et al., 1989; Martin, 1988). Microscopically, *Rhizoctonia* isolates are characterized by hyphae with a right angle branching of nearly 90 degrees which develop at the distal end of the hyphae. They also bear a constriction at the branching point and a septum in the branched hypha near its point of origin. It is very common to observe chains of inflated hyphae, also known as monilioid cells (Galvez et al., 1989; Mordue et al., 1989; Tupac-Otero et al., 2002). The dolipore septae, is a septum close to the branch junction with the parent hyphae (Mordue et al., 1989). Many *Rhizoctonia* species form undifferentiated sclerotia or 'sporodochia' which are formed of aggregations of hyphae and/or monilioid cells. This occurs under natural conditions as well as in culture (Mordue et al., 1989). Sclerotia can develop as a crust radiating from the inoculum center or scattered throughout the colony surface (Galvez et al., 1989). It is common to observe monilioid cells, which are holoblastically proliferating, non-seceding, inflated segments (Mordue et al., 1989; Tupac-Otero et al., 2002). Because *R. solani* hyphae are

multinucleate and lack clamp connections, it is difficult to distinguish between homokaryons and heterokaryons (Rosewich et al., 1999). AG isolates produce meiotic basidiospores in nature (Rosewich et al., 1999).

Most basidiospores are monokaryotic and very fragile, while, field isolates are considered to be heterokaryotic with asexual reproduction occurring through heterokaryotization (Rosewich et al., 1999). For recombination to contribute to population structure—heterokaryotization, occurring through somatic mycelia fusion from two monokaryotic basidiospores—must occur before an infection takes place (Rosewich et al., 1999). It is hypothesized that homothallic strains of *T. cucumeris* conserve the genetic characters of their parents (Toda and Kyakumachi, 2006). Previous laboratory studies suggest that isolates within an AG with a heterothallic mating system might also undergo recombination through heterokaryon-homokaryon mating (Rosewich et al., 1999). Other AGs possess unknown mating systems yet they are presumed to be homothallic (self pairing) (Rosewich et al., 1999). *T. cucumeris* (AG 2-2) might have the ability to degrade the dolipore to allow nuclear migration (Toda and Kyakumachi, 2006). In observations done of AG 2-2, evidence demonstrated that genetic exchange occurs between *T. cucumeris* strains having homothallic and heterothallic mating systems. Other basidiomycetes have been reported to possess the ability of crossing between different mating systems (Toda and Kyakumachi, 2006). Sexual recombination, heterokariosis fusion and mutations are three possible causes for genetic variation in *R. solani* populations (Godoy-Lutz et al., 2003). Research done with AG 2-2 suggests that *T. cucumeris* crossings could occur under field conditions, recombining and producing genetic diversity (Toda and Kyakumachi, 2006).

R. solani isolates may lose aggressiveness after storing *in vitro* for long periods of time (Zhao et al., 2005). Pathogen aggressiveness is the relative ability to colonize a host and cause damage (Kull et al., 2003). Grosch et al. (2004) suggest that isolate aggressiveness may be directly related to cell-wall digesting enzymes (Grosch et al., 2004). Researchers believe that pectic enzyme (isosyme) secretion in *R. solani* could be related to pathogenicity and that the pathogen could, either with aid of other factors or alone, breakdown pectic components in plants (Balali and Kowsari, 2004). *R. solani* can secrete more than one type of pectic enzyme (Balali and Kowsari, 2004). These pectic enzymes are used in genetic variability and identity determination of *R. solani* isolates (Balali and Kowsari, 2004). Results from previous pectic enzyme secretion assays indicated variations in pathogenicity among AG 4 isolates (Balali and Kowsari, 2004).

Anastomosis, or hyphal fusion, of filamentous fungi is a worldwide phenomenon, which plays an important role in intrahyphal communication, nutrient and water translocation and colony homeostasis (Jakobsen, 2004). Anastomosis groups are a species complex composed of genetically isolated populations (Nicoletti et al., 1999). The genetic basis of the anastomosis phenomenon is not fully understood (Salazar et al., 2000). AG grouping of isolates is based on hyphal interactions (Ceresini et al., 2007). Hypahl fusion occurs if two isolates belong to the same AG (Tupac-Otero et al., 2002). Evolutionary units of the species (or AG types) are new group isolates with intermediate features (Nicoletti et al., 1999). *R. solani* subgroups are independent evolutionary units within the species (Ceresini et al., 2007).

R. solani is distinguished from other plant pathogenic fungi due to its taxonomy based on the concept of anastomosis groups. This grouping method classifies isolates

according to their ability to anastomose with established AG tester isolates (Kuninaga et al., 2000). Anastomosis grouping refers to the hyphal fusion which occurs only between isolates of the same AG. These groups are genetically unique, with the exception of isolates belonging to AG BI (Galvez et al., 1989). Subgroups differ in anastomosis frequency, biomolecular, biochemical, genetic, DNA homology, physiological and morphological features, and pathogenic characteristics (Harikrishnan and Yang, 2004; Kuninaga et al., 2000; Tewoldenmedhin et al., 2006). As a result of these variabilities within groups researchers have characterized subgroups which are regarded as evolutionary units of the species. Ecological types also exist for several AGs based on pathogenicity variations on different host plants (Kuninaga et al., 2000). AGs vary greatly in levels of host specificity (Tewoldenmedhin et al., 2006). Some AGs affect a broad range of plants while others show higher host specificity levels (Guillemaut et al., 2003). Isolates within an anastomosis group may produce similar symptoms on a host; and may also have similar host preferences (Dorrance et al., 2003).

In 1969, Parmeter initially reported four AGs (Nocoletti et al., 1999). *R. solani* is a taxonomic species complex composed of fungi which are morphologically similar. These fungi are isolated by hyphal incompatibility into 14 anastomosis groups (AGs), which comprise from AG 1 to AG 13 and the bridging isolate AG BI. AG-IB was proposed as a subset of AG 2-2 (Guillemaut, et al., 2003; Harikrishnan and Yang, 2004; Mazzola, 1997; Priyatmojo et al., 2001; Stojsin et al., 2007). Bridging groups, isolates that can fuse with more than one group, make it difficult to place an isolate in its proper AG. This makes classification based on hyphal anastomosis a more challenging process, augmenting the species taxonomic complexity (Nicoletti et al., 1999). Examples of

bridging groups are AG 8 and AG BI. AG BI can fuse with AG 8 as well as with isolates of AG 2. A higher number of subgroups may exist due to the capability of isolates of divergent populations to differentiate (Nicoletti et al., 1999). If anastomosis is incompatible (or vegetatively incompatible), cellular lysis occurs in the contact area (Salazar et al., 1999). If isolates belong to the same AG the fusion can either be classified as a C2 or C3 reaction. C2 reaction is when a genetically distinct isolates' cell wall fuse, cellular lysis occurs. This is known as somatic incompatibility. C3 reaction occurs between clones or closely related isolates. Complete somatic cell fusion occurs and there is a continuous flow of cytoplasm in the fusion point. This is known as somatic compatibility (Toda and Kyakumachi, 2006). It is considered positive anastomosis if the hyphae of two *Rhizoctonia* isolates observed fuse without discernable cell death. If hyphal fusion occurs with apparent cell death it is considered imperfect anastomosis. The anastomosis is negative if only simple hyphal contact occurs without cell fusion (Martin, 1988). When pairing with tester isolates the clonal anastomosis reaction, C3, resembles the appearance of self anastomosis (Carling et al., 2002). Sexual compatibility is only possible within an anastomosis group (Salazar et al., 1999). Isolates belonging to the same AG are considered vegetatively compatible, this is observed by complete anastomosis or perfect fusion of hyphae (Salazar et al., 1999).

AG 1 includes sheath blight pathogen of rice (*Oryzae sativa* L.). This isolate is adapted both morphologically and physiologically to an aquatic environment. It also includes an aerial web-blight pathogen of beans and other crops. This isolate, previously known as *R. microsclerotia* Matz, causes diseases such as rots and damping-off (Mordue et al., 1989). AG 1 has 6 intraspecific groups or subgroups (Priyatmojo et al., 2001). Its

isolates are mainly heterothallic, with a population structure possibly derived from outbreeding (Godoy-Lutz et al., 2008). The sexual stage of AG 1, which is the etiological agent of WB of common bean, has been reported to occur in regions of Latin America and the Caribbean (Godoy-Lutz et al., 2008). In AG 1, isolates have sexual compatibility independent from vegetative compatibility (Salazar et al., 1999). In previous studies, AG 1 isolates developed water soaked lesions and necrotic lesions after inoculation and were more virulent than AG 2 isolates (Godoy-Lutz et al., 2003). Subgroup AG 1-IA is a widespread fungus responsible for sheath blight disease of rice plant, and is known to form sclerotia on diseased leaf sheaths (Matsumoto et al., 1997). It is assumed that Japan is the center of origin of this subgroup being first described form the southwestern region of Japan, at the beginning of the 20th century, as the casual agent of sheath blight of rice (Rosewich et al., 1999). The isolate is considered to be endemic in the US, found present in most undomesticated plant species, and commercial crops (Rosewich et al., 1999). AG 1-IA is a successful pathogen because it takes advantage of its reproduction cycles. First, through sexual recombination favorable gene combinations take place. Then natural selection of successful individuals occurs. These are later increased to high frequency by asexual reproduction (Rosewich et al., 1999). Until 1999 it had been unclear if AG 1-IA was homothallic or heterothallic, but studies indicated that novel AG 1-IA genotypes are produced by sexual recombination and are heterothallic (outbreeding) (Rosewich et al., 1999). This subgroup also forms hymenia, the reproductive structure formed of basidia and basidiospores, from which fragile basidia form and discharge during night hours. Their survival is brief, lasting only a few hours, primarily if exposed to direct sunlight or dry conditions (Rosewich et al., 1999). AG 1-

IA is reported to cause aerial blight of soybean and AG 1-IB causes web blight; both subgroups cause seed, root and stem rot (Harikrishnan and Yang, 2004). These subunits are characterized by having broad hyphae (5.5-5.7µm approximately) (Mordue et al., 1989).

Different AG 1-IB isolates recovered from diverse lettuce (*Lactuca sativa* L.) crop fields in Germany showed a pathogen population which had both high and less aggressive isolates, which had either high or low hyphal growth rate. This study suggests there might not be a relationship between isolate aggressiveness and hyphal growth rate (Grosch et al., 2004). In previous research, AG 1-IB had the highest level of virulence on bean plants (Montoya et al., 1997; Polanco, 1993). Another subgroup, AG 1-IE, is also considered to occur in the USA (Godoy-Lutz et al., 2008). Although AG 1-IE and AG 1-IF isolates cause similar symptoms on common bean, they can be easily distinguished by their cultural traits. When grown on PDA AG 1-IE develop a light creme color or pigmentation and large sclerotia ranging from 5-20mm which may be single or aggregated (Godoy-Lutz et al., 2008). AG 1-IF develops dark brown pigmentation when grown on PDA and produces small or microsclerotia ranging from 1mm or less which are scattered on cultural surface (Godoy-Lutz et al., 2008). Microclerotia produced by AG 1-IF (WB etiological agent) function as airborne propagules. Rain splash can facilitate inoculum distribution. Rain, wind and running water also aid in microsclerotia local spread and pathogen survival (Godoy-Lutz et al., 2008). AG 1-IF causes web blight on common bean and had been previously characterized as AG 1-IB, which also produce microsclerotia (Grosch et al., 2007). Researchers believe that the characterization of subgroup AG 1-IF is related to the geographic origin and consider AG 1-IF a distinct

population of Central and South America, and the Caribbean (Grosch et al., 2007).

AG 2-1 isolates causes damping off and root rot in different hosts (Salazar et al., 1999). AG 2-2 and AG 4 are the most aggressive (Windels and Brantner, 2005). *R. solani* AG 2-2 causes root and crown rot of sugar beet (*Beta vulgaris* L.) worldwide (Büttner et al., 2004). An example of documented evidence of AG dominance in specific crop fields can be found in studies by Guillemaut et al. (2003) which report AG 2-2 isolate dominance in sugar beet fields exhibiting root rot. *R. solani* AG 2-IB contains multinucleate and heterokaryotic cells (Pannecoucque and Höfte, 2009). Two distinct ecological types are AG 2-2IIIB, known as rush type, and AG 2-2IV known as root rot type (Salazar et al., 1999). The particularity of AG 2-2IV is that its isolates have both homothallic and heterothallic mating systems which can pose as an evolutionary advantage (Toda and Kyakumachi, 2006). AG 2 isolates are also known to cause root canker diseases, while AG 3 is a more pathogenic strain causing stem and stolon cankers of potato (*Solanum tuberosum* L.). Substrate and enzyme studies indicate that they have a pathogenic dependence canker on their host for survival, although they have considerable competitive saprophytic abilities (Mordue et al., 1989). *R. solani* AG 3 is associated mainly with diseases of Solanaceous plants. Isolates from potato are mainly asexual surviving as mycelium and sclerotia on potato seed tubers and soil (Ceresini et al., 2007). The teleomorph, *T. cucumeris*, can also be observed growing during cool and moist weather periods, but its meiospores (basidiospores) do not contribute directly to disease epidemics on potato because their dispersal is very limited (Ceresini et al., 2007). It is considered that the sexual spores produced as a result of heterothallic mating might contribute to the genetic diversity and structure of field populations of this group on

potato (Ceresini et al., 2007). An example of this is a study that used isolates from Brazil and the U.S., between two sister populations of AG 3 from tobacco (*Nicotina tabacum* L.) and potato which represented two distinct and historically divergent lineages (Ceresini et al., 2007). Researchers considered that these populations had probably evolved within the range of their respective Solanacea host plants as sympatric species (Ceresini et al., 2007).

AG 4 is a single member cluster, and has reduced ability to develop sclerotia. It has slightly narrower hyphae than those of other AG isolates (approximately 5µm) (Mordue et al., 1989). Although Balali and Kowsari (2004) reported that AG 4 colonies recovered from different host parts varied in morphological traits, such as color and growth rate, these features do not seem to be associated with isolate-host specificity. Heterokaryon formation in AG 4 is controlled by the H-factor, a multi-allelle gene locus. Researchers suggest that heterokaryosis could directly influence AG 4 isolate pathogenicity (Balali and Kowsari, 2004). Researchers speculate that isolates with heterokaryons with genetically diverse nuclei could result in the more pathogenic AG 4 isolates (Balali and Kowsari, 2004). In previous studies with AG 4 and molecular markers based on RAPD (Random Amplified Polymorphic DNA) analysis, results supported that heterokaryotic mycelium occurred from two different homokaryons (Toda and Kyakumachi, 2006). AG 4 has a particularity, in that it has a heterothallic mating system, where single basidiospores progeny can fruit (Toda and Kyakumachi, 2006). AG 4 is the most common group, causing root and hypocotyls rot, isolated from soybean plants (Zhao et al., 2005). It also causes damping-off of common bean during the early growth stages of *P. vulgaris* and stem and root rot during the later stages (Balali and

Kowsari, 2004). In peanut, AG 4 is frequently isolated from shells of detached pods which remain in soil, rather than from the shells removed at harvest (Summer and Bell, 1994). Although many *R. solani* isolates are known to cause root and hypocotyls rot of cotton (*Gossypium hirsutum* L.), AG 4 seems to be the most common isolate associated with cotton root rot (Carling et al., 2002). AG 4 also infects turf grass, coffee (*Coffea arabiga*) seedlings, sugar beet, tomato, pea (*Pisum sativum* L.), spinach (*Spinacia oleracea* L.) and snap bean (Stojsin et al., 2007).

AG 1, 2-2, 3, 4, and 5 are commonly found in Minnesota and North Dakota, USA, and have been isolated from diseased sugar beet plants (Windels and Brantner, 2005). Laboratory crossings demonstrated that AG 1-IC, AG 4 and AG 8 possess heterothallic and bipolar mating systems (outcrossing) (Rosewich et al., 1999; Toda and Kyakumachi, 2006). *Rhizoctonia* isolates in South Africa have not been well characterized. Previous studies report that *R. solani* AG 6, AG 4, AG 2-t and AG 2-2 and *R. cerealis* have been characterized to species level (Tewoldenmedhin et al., 2006). AG 6 and AG 7 (a nonpathogenic or of minimal pathogenicity) are pigmented and produce sclerotia (Mordue et al., 1989). AG 8 causes root rot and bare patches. It is slow growing, making it difficult to isolate from soil and symptomatic roots (Paulitz and Schroeder, 2005). Weeds allowed to grow in the field between harvests can either maintain or increase inoculum potential of *R. solani* AG 8 increasing root rot severity (Smith et al., 2003). Multinucleate *R. solani* AG 6 and AG 12 differ from other AG because their isolates are known to be associated with orchids as mycorrhizae (Carling et al., 2002; Tupac-Otero et al., 2002). Previous studies showed that AG 13 affected cauliflower and produced tissue discoloration and small lesions on cotton seedling roots and hypocotyls, but showed low

virulence on greenhouse trials (Carling et al., 2002). Although traditional identification methods require experience and are time consuming, anastomosis grouping is still considered as a valuable, reliable and universal classification method of *R. solani* and its pathogenic isolates (Guillemaut et al., 2003; Salazar et al., 2000).

Fungal disease development begins with a contamination phase. During this phase contact occurs between the fungus propagule and a host plant. The fungus penetrates the host through the cuticle, stomates or wounded tissue (Tivoli et al., 2006). Then the infection phase occurs. The fungus settles and invades the host tissue, whether living or dead leading to the development of symptoms. Finally, secondary inoculum develops that will infect a nearby plant (Tivoli et al., 2006). After the fungus has formed a first haustorium and penetrated the stroma, it parasites the invaded plant cell by taking its nutrients, thus allowing further intracellular growth (Sillero et al., 2006). After penetration, by means of haustoria or intracellular hyphae, of a fungus into host cell the first type of resistance mechanism which is morphological occurs (Sillero et al., 2006). For a fungal soilborne infection to occur a propagule must disperse and/or sporulate within the pathozone, the soil region which surrounds roots, seeds or hypocotyls (Otten et al., 2001).

Partial resistance reduces pathogen multiplication; slowing disease progress because of an interference with one or more of the disease cycle steps (Tivoli et al., 2006). Complete resistance prevents the emergence of symptoms and pathogen multiplication interfering totally with disease cycle (Tivoli et al., 2006). Disease caused by *R. solani* include seed decay, damping-off, stem cankers, root rots, fruit decay, foliage disease, defoliation and pod infection may also occur (Aziz et al., 1997; Godoy-Lutz, et

al, 2003; Priyatmojo, et al, 2001). Although there is no definite relationship between AGs of pathogenic isolates and virulence, previous studies report that AGs 1 through 5 are casual agents of tobacco diseases (Nicoletti et al., 1999). Many tree species, including apple, are also susceptible to *R. solani* (Mazzola, 1997). *R. solani* causes diseases on cereals such as barley and wheat, vegetables, ornamentals and turf grasses (Paulitz and Schroeder, 2005; Toda and Kyakumachi, 2006). *Rhizoctonia* causes black scurf of potato. Its etiological agent is AG 3, but AG 1, AG 2-1, AG 2-2, AG 4, AG 5 and AG 9 have also been occasionally isolated from diseased potato (Bounou et al., 1999).

Web blight is a foliar disease of common bean and is caused by aerially dispersed isolates (airborne isolates) of *R. solani* Kühn (Coyne et al., 2003; Godoy-Lutz et al., 2003). In the Americas, AG 1, AG 2 and AG 4 have been identified as the infectious agents causing WB of common bean (Godoy-Lutz et al., 2003). Diseases, such as web blight, are considered an important bean production problem in the humid tropics, where there are high temperatures and abundant rainfall, with increased severity under conditions of high soil moisture and relative humidity (> 80 percent). This disease can occur at any stage of the bean-crop cycle causing defoliation, which in most cases can lead to complete crop failure (Allen, 1997; Galvez et al., 1989; Jung et al., 1996; Montoya et al., 1997; Singh, 2001). Web blight appears as small necrotic lesions, raging approximately from 5-10 mm across, on the primary leaves. The lesions have brown centers and pale green margins. These spots tend to expand rapidly becoming somewhat irregular and zonate. They coalesce until the entire leaf is affected. These leaves are covered with small brown sclerotia and light brown hyphae. Hyphae grow until the

mycelium eventually forms a web over the plant. The basidiospores of its teleomorph, *T. cucumeris* produce distinct small circular necrotic spots which rarely enlarge. Lesions may also be seen on pods and seeds (Allen, 1997).

Web blight isolates are fast growing, produce abundant sclerotia, and do not tolerate carbon dioxide (CO_2) (Galvez et al., 1989). The main inocula of web blight are sclerotia and mycelia found either free in soil or on colonized debris. Sclerotia have been reported to develop on a plant within 3-6 days of contact with infected soil. Under favorable environmental conditions the sclerotia germinate producing hyphae that branch out until they reach host tissue. This is followed by the development of an infection cushion which will penetrate through the stomata or directly through the tissue. Hyphae can develop both inter- as well as intracellularly (Allen, 1997; Galvez et al., 1989). Hyphae can grow over healthy leaves, flowers, petioles and pods; yet under dry environmental conditions web blight development can stop (Galvez et al., 1989). AG 2 isolates cause foliar blight of economic crops in Japan and the US (Godoy-Lutz et al., 2003). WB pathogen has variable disease reactions and has adapted to diverse ecological zones (Coyne et al., 2003). *R. solani* has a variable genetic composition which has aided its adaptation to diverse ecological niches and hosts (Godoy-Lutz et al., 2008). Previous studies reported that web blight isolates collected in Puerto Rico vary in levels of virulence to beans (Montoya et al., 1997; Polanco et al., 1993). Other *Rhizoctonia* spp. also cause similar symptoms on rice sheaths, making their identification by visual observation difficult (Matsumoto, 1997).

Root rot isolates are also fast growing, yet they produce less sclerotia and have a higher degree of CO_2 tolerance than web blight isolates (Galvez et al, 1989). In *P.*

vulgaris, root rot appears on the root cortex, lower stem and tap root of seedlings as lesions of a reddish brown color. These lesions later develop into sunken cankers of the same color, with discrete borders. The cankers may expand around the stem, encircling it, stunting the plant and eventually killing it (Allen, 1997). A serious infection can lead to severance of the root, also known as 'spear tip' symptom. Seed yield is limited when, under acute disease pressure, plant growth is stunted and bare patches are created in the field which can prevent a plant from researching a productive age (Garcia-E. et al., 2003; Smith et al., 2003). Pods can also become infected and develop sunken cankers, if they are in contact with infected soil. This increases the possibilities of seed invasion. Seed decay may also occur if it becomes infected from the soil before germinating (Allen, 1997). *R. solani* root rot infections also include root and hypocotyl rot of soybean as well as root rot and crown rot diseases of tomato which have been observed in Egyptian greenhouse experiments (Moataza, 2006; Zhao et al., 2005). *R. solani* is also considered a common pathogen of sugar beet (*Beta vulgaris* L.) (Windels and Brantner, 2005). *Rhizoctonia* spp. have been associated with roots of strawberries of which *R. solani* is one of the primary soil-borne pathogens causing root rot and crown rot in strawberries in Turkey, and the Northern Mediterranean region (Benlioğlu et al., 2004; Martin, 1988). *R. solani* has also proven to be pathogenic to coffee plant roots and stems (Rodríguez et al., 1996).

Other factors that affect pathogen development, growth and receptivity of host plant include host plant physiology and growth stage and environmental factors, such as temperature, humidity, light and wind (Tivoli et al., 2006). Successful managent of *Rhizoctonia* can be influenced by the variability among species and AGs to biological

control and chemical agents (Mazzola, 1997). Double-stranded RNA (dsRNA) elements in *R. solani* have been associated in up- or down- regulation of virulence and with cytoplasmic hypovirulence. These elements have been highly researched due to their potential adverse effects on fungal plant pathogens as well as plans to use them as biocontrol agents against the host fungus (Lakshman et al., 1998).

Binucleate *Rhizoctonia* spp. resemble anamorphic states of *R. solani* but most belong to the teleomorphic state *Ceratobasidium* (Martin, 1988). Binucleate *Rhizoctonia* possess the typical hyphal branching pattern, dolipore septa and binucleate hyphal cells regardless of culture color (Martin, 1988). These have also been grouped by anastomosis. Burpee, based on results obtained with isolates from the United States, separated them in seven groups ("CAG 1-7"), whereas, Ogoshi divided them into 15 anastomosis groups (AGs) from isolates obtained from Japan (Martin, 1988). There are 21 binucleate AGs and only one known uninucleate AG (Tupac-Otero et al., 2002). Their classification ranges from AG-A through –S (Tewoldenmedhin et al., 2006). Although some binucleate fungi can function as plant pathogens the majority are saprophytic. Only few binucleate isolates can develop as plant symbionts, mainly with orchids (Mazzola, 1997; Tewoldenmedhin et al., 2006). *R. solani* as well as other species of binucleate *Rhizoctonia* exhibit varying degrees of host specialization. On certain hosts binucleate *Rhizoctonia* spp. may induce distinct diseases from those induced by *R. solani* (Martin, 1988).

Various techniques have been developed for the qualitative and quantitative detection of *R. solani* in soil. Two of these techniques are baiting and culture plating. In spite of their effectiveness, these techniques require the researcher to have a considerable

level of taxonomic expertise, since the selective media alone cannot discriminate between *Rhizoctonia* spp. and *Rhizoctonia*-like fungi. For this reason, in recent years, nucleic acid-based diagnostic techniques, such as polymerase chain reaction (PCR) and DNA probes, have been employed in the detection and quantification of the pathogen from infested soil and within infected roots (Thornton et al., 2004). PCR procedure is preferred because it has high specificity, reproducibility and is easier to implement in diagnostics laboratories that aid in identifying pathogens that cause diseases (Bounou et al., 1999; Salazar et al., 2000). PCR amplification is also faster and more efficient than conventional isolation and identification methods of *R. solani* from infected plant tissue (Salazar et al., 2000). PCR and restriction fragment length polymorphism (RFLP) analyses are very useful in differenting and characterizing *Rhizoctonia* spp. rice sheath pathogens (Matsumoto et al., 1997). RFLPs and RAPD PCR have improved the understanding of *R. solani* population dynamics and has been useful in DNA typing its multi-, bi-, and uninucleate species (Bounou et al., 1999). Grosch et al, (2007) conducted studies using SCAR (Sequence-Characterized Amplification Region) primers for PCR, which is a more specific and sensitive diagnostic assay than conventional PCR. In this study they converted a RAPD amplicon to a SCAR marker of a specific gene, and reported that subgroup AG 1-IE was closely related to subgroup AG 1-IA (Grosch et al., 2007). Real-Time PCR (RT-PCR) is also used for AG pathogen detection of *R. solani* AG 1-IA. The pathogen is detected from infected plant tissue, even before fungal structures (i.e. mycelia or sclerotia) or symptoms appear. RT-PCR detection can also be used in the field with portable machines (Sayler and Yang, 2007). Molecular techniques based on rDNA sequence analysis add genetic support to traditional AG classification,

which rely on unknown isolate pairing with tester strains and identification of hyphal anastomosis reactions. They also aid in the investigation of the evolutionary relationship among the diverse *R. solani* isolates (Guillemaut et al., 2003).

Molecular analysis of ribosomal genes is another characterization technique used to differentiate between *R. solani* AGs and its subgroups by evaluating the intra- and inter-specific variation of the *R. solani* complex (Godoy-Lutz et al., 2003; Pannecoucque and Höfte, 2009). Molecular and biochemical approaches are identification alternatives to hyphal fusion frequency and pathogenicity tests (Salazar et al., 1999). Besides classification by anastomosis grouping, molecular techniques have been developed and proven to be efficient in analysis of evolutionary homology between isolates of this complex. Techniques which rely on molecular markers are more accurate, easier and faster than traditional techniques for determining anastomosis groups (Stojšin et al., 2007). DNA markers, thiamine requirements and fatty acids techniques are also employed to complement morphological, cultural and pathogenic tests in AG subdivision analysis (Godoy-Lutz et al., 2008).

R. solani genetic variations have been studied by traditional methods and by molecular techniques which include nucleotide sequence of the rDNA-ITS region (ribosomal DNA internal transcribed spacers) (Coyne et al., 2003; Kuninaga et al., 2000). Internal transcribed spacer regions (ITS 1 and ITS 2) are found between the large (28S) rRNA sequence (subunit) and the small (18S) rRNA sequence (subunit). Between the ITS 1 and ITS 2 regions lies the 5.8S nuclear rDNA gene. ITS functional regions within rDNA genes, that produce ribosomes are important in taxonomic studies because they are highly conserved regions within many species, but the ITS 1 and ITS 2 regions are

variable between species evolving faster and differing between them (Pannecoucque and Höfte, 2009; Prewitt et al., 2008). Although they are transcribed, ITS regions do not play a structural role in the ribosome (Salazar et al., 1999). rDNA-ITS regions have been useful in studying fungal isolates at species level, as well as linking anamorphs to their respective teleomorphs (Sillero et al., 2006). Genes coding for ribosomal DNA are highly conservative, thus evaluating DNA sequence similarity of restricted portions of the genome allow a better understanding of the genetic affinity among taxonomic entities (Kuninaga et al., 2000). 18S rDNA, 28S rDNA and ITS sequences have revealed polymorphism between AGs (Guillemaut et al., 2003). ITS sequence aid in the classification of new isolates and differentiation of previously defined subsets within AGs based on their ITS sequence differences (Guillemaut et al., 2003). ITS region 5.8S rDNA sequence is completely conserved across all AGs. ITS 1 and ITS 2 rDNA sequences show greater differences between AGs (Stojsin et al., 2007). Isolates of the same subgroup have sequence homology in ITS region above 96%, whereas (homology between) isolates of different subgroups within AG range between 66-100% and isolates of different AG have a sequence homology between 55-96% (Stojsin et al., 2007).

Taking into consideration that various isolates have low anastomosis affinity with their representative tester isolates and that some AGs, such as AG-1, -2, -3 and -4 have a more heterogeneous ITS region, it would seem that biomolecular approaches tend to be more accurate for *R. solani* isolate AG classification (Kuninaga et al., 2000; Pannecoucque and Höfte, 2009). AGs closely relate with groups based on morphological and/or pathogenic traits. Resulting from investigations with electrophoresis of soluble proteins, non-specific esterase zymograms and pectic zymograms also support divisions

based on anastomosis data (Mordue et al., 1989). Subgroups are based on DNA homologies, ecological types and zymogram patterns (Nicoletti et al., 1999). PCR rDNA-ITS-5.8S region from Texas (AG 1-IA) isolates showed characteristics of a balanced dikaryon (Rosewich et al., 1999). Fatty acid analysis is used to differentiate closely related AG isolates. Previous studies demonstrated that with fatty acid analysis was useful when differentiating AG 1-IC from AG 1-IA and AG 1-IB, although AG 1-IA and AG1-IB could not be differentiated from each other. But many of the AG 2-2 could be differentiated from each other and AG 4 was differentiated from AG 7 as well (Priyatmojo et al., 2001). Although immunoclonal techniques based on monoclonal antibodies have been used to detect AG-4, its use is not very practical due to a lack of commercial kits available for *Rhizoctonia* spp. (Paulitz and Schroeder, 2005). Due to molecular techniques, AG 2-t was established as a new group after studies demonstrated that isolates, which were part of a homogeneous group, had lower anastomosis frequency with AG 2-1 as well as differences in pathogenicity. This is one of the reasons why anastomosis frequency may no longer have such an importance in AG classification when compared to more advance molecular techniques (Kuninaga et al., 2000).

MATERIALS AND METHODS

Fungal isolates

Previously characterized *R. solani* isolates AG 1-IA, AG 1-IF, AG 4 (WB 1), AG 4 (WB 2) and AG 4 (WB 3) used in this study were provided by Dr. Graciela Godoy-Lutz (University of Nebraska-Lincoln, Lincoln, NE, USA). These isolates are from common bean plant leaves that presented web blight symptoms at Isabela, Puerto Rico (Godoy-Lutz et al., 2008). Isolate AG 1-IE was provided by professor Myrna Alameda from the fungus collection maintained at the Alzamora Farm Laboratory on the University of Puerto Rico, Mayagüez campus. The uncharacterized field *R. solani* isolates AG 4 (RR 1), AG 4 (RR 2) and AG 4 (RR 3) were provided by Dr. Timothy Porch (USDA-ARS-TARS, Mayagüez, Puerto Rico) from common bean plant roots presenting typical root rot symptoms from the root rot field at the USDA-ARS Tropical Agricultural Research Station located at Isabela, Puerto Rico.

Molecular rDNA-ITS analysis of uncharacterized root rot field *R. solani* isolates

Fungal DNA extraction

R. solani isolates AG 4 (RR 1), AG 4 (RR 2) , AG 4 (RR 3) and AG 1-IA which was used as a positive characterized control, were grown on potato dextrose broth medium (MP Biomedicals, Ohio, USA) in 250 ml sterile Erlenmeyer flasks. Each flask was individually inoculated by one 6-mm diam. disk of the AG 4 and AG 1-IA isolates (Moataza et al., 2006). Flasks were incubated at 27ºC for 14 days in dark and maintained shaking at 117 rpm. After incubation, the mycelial pellets formed were harvested by

filtration, rinsed with distilled sterile water and ground to a fine powder with liquid nitrogen in cold (-80ºC freezer) sterile porcelain mortar and pestle. Genomic DNA extraction from approximately 40-50 mg of fungal tissue sample was conducted with a commercially available DNA extraction kit (UltraClean™ Microbial DNA Isolation Kit, MO BIO Laboratories, Inc., Solana Beach, CA, USA) following the manufacturer's instructions.

PCR amplification

R. solani root rot isolates RR 1, RR 2 and RR 3 were classified into anastomosis group (AG) 4 by polymerase chain reaction. Isolates AG 1-IA was used as a positive control. Nuclear rDNA and the 5.8S ribosomal-ITS region (ITS-5.8S-rDNA) were amplified using ITS specific primers for AG 4 and AG 1-IA. The primer pair sequence used for AG 4 specificity was 4-F (TGGGGGGGAAG-GAACTTTATTGGAC) and 4-R (CAGCTAATCCAAGAGGGCGG). The primer pair sequence used for AG 1-IA specificity was IA-F (CCTTATTTGGCAGGAGGGG) and IA-R (GACTATTAGAAG CGGTTCA) (Godoy-Lutz et al., 2008). PCR reactions consisted of a 25 µl volume which contained 2 µl genomic DNA (10ng μl^{-1}), 2.5 µl 10X PCR buffer (Promega, Madison, WI), 0.5 µl dNTPs (10.0 mM), 2.5 µl $MgCl_2$ (25mM), 0.25 µl of each primer pair (10 µM), 0.25 µl Go Taq® polymerase (5 units μl^{-1}) (Promega, Madison, WI) and 16.75 µl sterile highly purified water (HPLC H_2O). PCR was run in Touchgene-Gradient Techne Thermocycler (Barloworld Scientific Laboratory Group US, model TC-512, Burlington, NJ). Cycling parameters consisted of initial denaturation for 2 minutes at 94ºC, 30 cycles at these conditions: 94ºC for 40 seconds, 55ºC for 1 minute and 72ºC for

1 minute. Final extension at 72ºC for 5 minutes (Godoy-Lutz et al., 2008). Five microliters of the molecular marker GeneRuler™ 1 kb DNA Ladder Plus was used in PCR electrophoresis run (Fermentas Life Sciences, Glen Burnie, MD, USA). Fourteen microliters (12 µl PCR reaction and 2 µl loading dye) of resulting PCR products of 370 bp for AG 4 and 540 bp for AG 1-IA were analyzed by electrophoresis on 1% Agarose Low EEO/Multipurpose/Molecular Biology Grade (Fisher Scientific, Pittsburgh, PA, USA) gel in Tris-borate-EDTA buffer at 100V for 1.5h. The gel was stained with SYBR® Safe DNA gel stain (Invitrogen™ Molecular Probes™, Eugene, Oregon, USA). Fragments were viewed using a Fisher Biotech electrophoresis systems UV transilluminator (Fisher Scientific, Pittsburgh, PA, USA).

DNA sequencing

Basidiomycete specific primers ITS1-F (CTTGGTCATTTAGAGGAGTA) and ITS4-B (CAGGAGACTTGTACCGGTCCAG) were used to sequence USDA-TARS root rot field isolates *R. solani* AG 4 (RR1), AG 4 (RR2), AG 4 (RR3), AG 2-2 and a *Fusarium* species (Salazar et al., 2000). *R. solani* AG 1-IA and AG 1-IE previously sequenced by Godoy-Lutz et al. (2008) were included and used as comparative control isolates. PCR reactions consisted of 50 µl volume which contained previously described master mix reagents. Cycling parameters consisted of initial denaturation for 1.5 minutes at 94ºC, 40 cycles at these conditions: 95ºC for 55 seconds, 55ºC for 55 seconds and 72ºC for 1 minute. Final extension at 72ºC for 10 minutes (Prewitt et al., 2008). DNA material was sequenced by SeqWright DNA Technology Services (Houston, TX, USA). Forward and reverse sequences were aligned with ClustalW2 (Saitou and Nei, 1987). Sequence

analysis and construction of phylogram was performed using on-line program via http://www.ebi.ac.uk (European Bioinformatics Institute). A BLAST search in NCBI GenBank was conducted to match AG 4 root rot 1, 2 and 3 isolates with similar organisms**.** *Fusarium* spp. was used as an outgroup. *R. solani* AG 2-2 isolate was not used in the web blight and root rot virulence determination trials because it had not been isolated at the time.

Virulence determination for web blight and root rot

Web blight greenhouse evaluation

Web blight virulence of *R. solani* isolates was determined on common bean in a greenhouse experiment. Virulence determination was conducted following a detached leaf technique (Grosch et al., 2004; Kull el al., 2003; Priyatmojo el al., 2001 and Takegami et al., 2004). Twelve breeding lines and five cultivars of common bean were planted in a greenhouse at the Mayagüez Campus of the University of Puerto Rico (Table 1). Three evaluations were conducted during the months of July and October, 2007 and January, 2008. Plastic pots 12-cm diam. were disinfected with sodium hypochlorite and filled with sterilized mixture of peat moss and vermiculite. Planting medium was pasteurized in autoclave at Alzamora Field Laboratory, UPR Mayagüez Campus for two 1h cycles at 121ºC (Tewoldemedhin et al., 2006). Seeds were disinfected with 20% sodium hypochlorite for 10 minutes and 70% ETOH for 5 minutes followed by two rinses of $_{dd}H_2O$. Samples of the treated seeds were placed on potato dextrose agar (Difco, Detroit, MI, USA) and incubated for 4 days at 25 ± 2ºC to test the efficacy of the disinfection (Aziz et al., 1997; Balali and Kowsari, 2004). Plants were watered daily

with tap water to avoid stress.

Once the first and third trifoliolate leaves of the common bean plants were fully expanded (approximately 4 weeks after planting) they were detached at the stem. They were labeled, placed in a moistened paper towel, bagged, and immediately transported to the Biology laboratory to evaluate for a reaction to the web blight pathogen (Kull et al., 2003 and Takegami et al., 2004). The petioles were placed in orchid tubes filled with distilled-sterilized water to keep the leaflets turgid. The leaflets were placed in 26 x 38 x 7-cm aluminum roasting pans, which were previously disinfected with sodium hypochlorite and distilled water (Figure 1). Wet paper towels (moistened with sterilized distilled water) were placed inside the aluminum trays, simulating a humid chamber (Godoy-Lutz et al., 2003). To avoid the leaflets from coming in contact with water and/or the moistened paper towels they were placed atop inverted 95 X 15-mm Petri dishes. With the use of a cork borer agar disks were cut from the edges of 7 day old cultures. One 6-mm diameter disk of PDA colonized with an assigned *R. solani* isolate was placed fungus side down in the center of adaxial side of two leaflets (Kull et al., 2003). A non-inoculated leaflet was kept as a control. Two replicates were made for each isolate-host combination (Grosch et al., 2004).

Figure 1. Aluminum pan with inoculated and control trifoliolate leaves of *P. vulgaris*

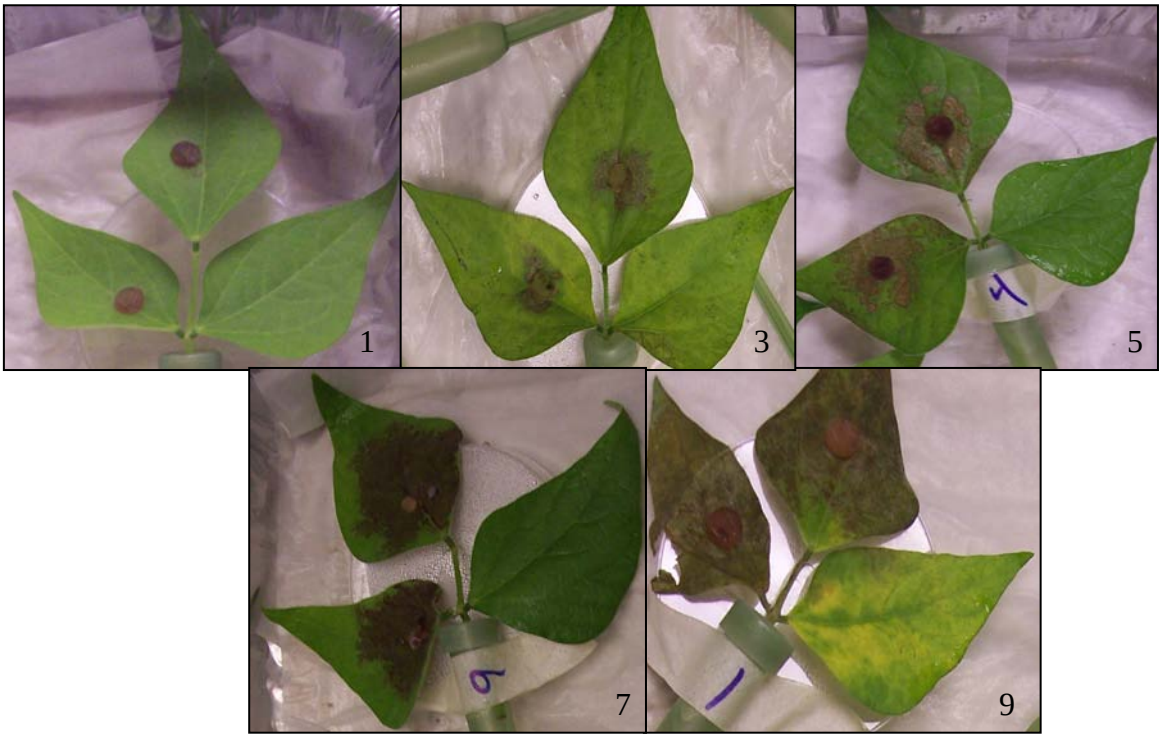
To create a high-humidity environment, the trays were placed inside a plastic bag after inoculation. The pans were placed on benches and incubated in the laboratory at 25 ± 2ºC. The trifoliolate leaves were evaluated at 24, 48, and 72 hours after inoculation in which degree of damage caused by the mycelium was rated using a subjective disease severity scale from the *Centro Internacional de Agricultura Tropical* (CIAT) which designates 1= no symptoms, 2= < 5% leaf area affected, 3= 6-10% leaf area with damage, 4= 10-20% leaf area with damage, 5= 20-30% leaf area with damage, 6= 30-40% leaf area with damage, 7= 40-60% leaf area with damage, 8= 60-85% leaf area with damage and 9= > 85% considered as severe leaf damage (Van Schoonhoven and Pastor-Corrales, 1991) (Figure 2). Leaflets with severity scores from 1 to 3 were considered resistant or partially resistant, severity scores from 4 to 6 were considered intermediate and scores >7 were considered susceptible (Schmidt and Baudoin, 1992). The experimental design consisted of a completely randomized design with three repetitions. The experimental

unit for WB was the trifoliolate leaf in which one bean line was evaluated. The two leaflets inoculated with *Rhizoctonia* disks were samples within the experimental unit.

Table 1. Common bean breeding lines and cultivars screened for web blight and root rot reactions in the greenhouse at the University of Puerto Rico, Mayagüez Campus

Entry	Identification	Seed Type	Pedigree
1	PR0401-257	Pink	VAX 6//MUS83/BelNeb///DOR483/BAT93
2	PR0401-259	"	"
3	PR0401-277	Small red	VAX 6/EAP9503-32A
4	PR0518-10	Black	Negro Veracruz/PR9607-29
5	PR0518-15	"	"
6	PR0518-16	"	"
7	PR0650-27	Purple	Amadeus 77PI417662/Bibri
8	PR0650-31	Black	BAT 93/PI417622//VAX 6
9	PR0650-32	"	"
10	PR0650-34	"	"
11	PR0650-41	"	"
12	Morales	White	Arroyo Loro/Don Silvio (DOR 482)
13	Verano	"	DOR 364/WBB-20-1//Don Silvio/VAX 6
14	Amadeus 77	Small red	Tio Canela 75/DICTA 105
15	Carrizalito	"	"
16	Talamanca	Black	A cultivar from Costa Rica reported to have moderate levels of resistance to web blight
17	VAX 6	Small red	A complex interspecific cross. The common bacterial blight resistance was derived from *Phaseolus acutifolius* L.

Figure 2. Web blight severity scale[1] visual key according to the *Centro Internacional de Agricultura Tropical* (CIAT)



[1]Evaluation scale from 1-9 where 1=no visible symptoms of the disease, 3=5-10% foliage area with symptoms, 5=20-30% foliage area with symptoms, 7=40-60% foliage area with symptoms and 9=>80% foliage area with symptoms (Van Schoonhoven and Pastor-Corrales, 1991).

Root rot greenhouse evaluation

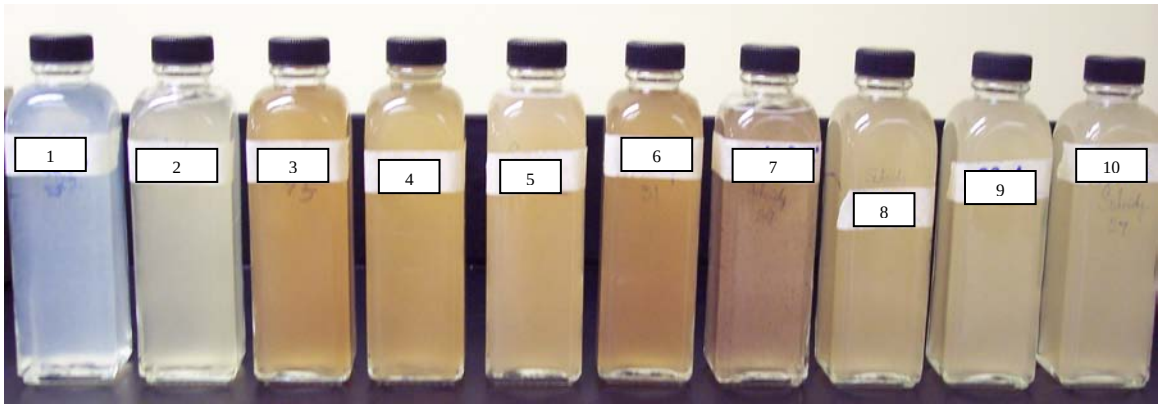
Root rot greenhouse trials were planted during the months of October 2007, February 2008 and June 2008. After the first and third fully expanded trifoliolate leaves were excised from the twelve breeding lines and five cultivars of common bean for the WB assay, they were immediately inoculated with liquid mycelia suspension of the nine *R. solani* isolates used in the detached leaf technique for WB evaluation. The plants were kept in the same greenhouse on the University of Puerto Rico, Mayagüez Campus in which they were originally planted.

Infections were induced by inoculating each bean line, using a disposable pipette and inserting the tip just under the top layer of the planting mix near the hypocotyls, with 10 ml with one of nine different mycelia suspensions of *R. solani* web blight isolates AG1-IA, AG 1-IE, AG 1-IF and a series of AG 4s which corresponded to web blight isolates 1, 2 and 3, and root rot isolates 1, 2 and 3 (Figure 3). The isolates were the same used for the WB assay. The seventeen lines had a completely randomized design with three repetitions and were all assigned the nine different fungal isolates. There were nine pots established for each bean line with three seeds per pot; and one pot for each isolate. A positive root rot control group was inoculated with a mycelia suspension from the root rot isolate AG 4 (RR1). A negative root rot control group was not administered any type of agar and mycelia suspension.

To prepare the liquid inoculum, five PDA Petri dishes (95x15mm) of each fungal isolate were first inoculated with a 6-mm *R. solani* disk, excised from pure colonies, and allowing to incubate for 14 days at 27ºC without light. The Petri dishes for the root rot test were covered in aluminum foil and kept in dark to simulate the lack of sunlight in an underground environment (Büttner et al., 2004). After 14 days the contents of the five Petri dishes per isolate were macerated to a smooth liquid in a Waring blender to which 1L of $_{dds}H_2O$ was added to dilute the agar/mycelia stock (Büttner et al., 2004). The final concentration for the liquid inoculum was of 10^3 mycelia fragments/1 ml. A dilution plating technique was used to quantify the inoculum density (Paulitz and Schroeder, 2005). A colony forming unit (CFU) count was established to estimate the number of viable microorganisms present in 1ml of the stock solution which consisted of five PDA plates inoculated with *R. solani* (Cappuccino and Sherman, 1999). One milliliter of the

R. solani AG 1-IA 10^{-3} dilution was sampled in 25 plates using the pour-plate technique. The plates were incubated for 3 days at 27ºC. Colonies were then counted and multiplied by the dilution factor, which is the reciprocal of the dilution (Cappuccino and Sherman, 1999). An estimate of $6x10^4$ CFU/ml was established in 1ml of the stock solution.

Figure 3. Mycelia suspension of *R. solani* root rot and web blight isolates used in root rot greenhouse evaluations



1=agar, 2=AG 1-IA, 3=AG 1-IF, 4=AG 4(WB1), 5=AG 4(WB2), 6=AG 4(WB3), 7=AG 1-IE, 8=AG 4(RR1), 9=AG 4(RR2), 10=AG 4(RR3)

The plants and their respective inoculi were allowed to incubate in the greenhouse for 21 days (Büttner et al., 2004; Cardosa and Echandi, 1986; Dorrance et al., 2003; Li et al., 2004; Park et al., 2008). After the incubation period, the aerial portion of the plants were excised and discarded. The root/planting mix mass was removed from the containers, bagged, labeled and transported immediately to the laboratory. Each root mass was rinsed for 15-20 minutes in running tap water (Dorrance et al., 2003; Wen et al., 2005).

Root disease severity was visually rated using an amended 0-7 subjective root rot severity scale (Table 2 and Figure 4) (Büttner et al., 2004; Dorrance et al., 2003). Then each root was scanned in an EPSON flatbed scanner, EPSON Perfection V700/V750 3.4 model, (Epson America, Inc., Long Beach, CA) and fresh root surface (cm^2) was analyzed using the WinRhizo Basic 2008a software program (Régent Instruments Canada, Inc 1993-2008©, Quebec, Canada). The experimental unit for root rot was the pot containing a plant with or without *Rhizoctonia* infection. There were three replicates, and the experiment was repeated three times. To verify the presence of *R. solani* as the infectious agent in the root systems and confirm Koch's postulate, the tips of the roots and the area nearest the stem lesions were cut, washed in 20% sodium hypochlorite for 4 minutes and 70% ETOH for 1 minute followed by two rinses of $_{dd}H_2O$ (Carling et al., 2002). The plant material was then inoculated in PDA and incubated for 4 days at 27ºC. Later, presence of *R. solani* was confirmed by preparing a semi-permanent slide of *Rhizoctonia*-like mycelium present in the plates and observing under light microscope at 40X magnification.

Table 2. Root rot severity evaluation scale modified from Büttner et al. (2004) & Dorrance et al. (2003)

Assigned severity degree	Visible root damage percentage
0	No visible disease symptoms*
1	1-5% root surface with visible lesions†
2	5-10% root surface with visible lesions
3	10-25% root with visible lesions and/or dry-rot cankers††
4	25-50% root with visible lesions and/or dry-rot cankers
5	50-75% roots showing dry-rot cankers, damaged or rotted roots
6	> 75% roots showing dry-rot cankers, damaged or rotted roots
7	Pre-emergence damping-off** and few if any roots

* Root lesions or rot.

** Hypocotyls girdled by coalescence of several cankers, resulting in pre-emergence or post-emergence of damping off.

† **Lesions:** Reddish brown lesions on hypocotyls and roots, usually early disease development symptoms.

†† **Cankers:** Larger sized sunken lesions; red in color.

Figure 4. *R. solani* root rot 0-7 severity degree visual key based on root damage

Data analyses

ANOVAs were calculated using the STATISTIX statistical package (Analytical Software, Tallahassee, FL). Analysis of variance was conducted on disease rating data from a WB detached leaf technique using a randomized complete block design and root rot greenhouse trials using a completely randomized design with three repetitions. The factorial arrangement consisted of inoculum and bean lines. If treatment effects in the ANOVA were significant, Least Significant Differences (LSD) among means for each parameter was determined at P= 0.05.

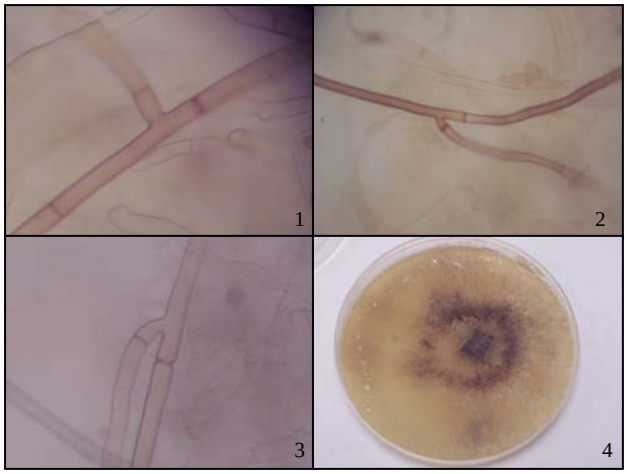
RESULTS AND DISCUSSION

Molecular rDNA-ITS analysis of uncharacterized root rot field *R. solani* isolates

For years mycologists have used cultural, which rely heavily on morphological characterization, and biochemical methods to identify *Rhizoctonia* into its different species and subgroups. Because the use of selective media alone cannot discriminate between *Rhizoctonia* spp. and *Rhizoctonia*-like fungi, researchers also need to have a considerable level of taxonomic expertise (Thornton et al., 2004). To reduce the probabilities of identifying incorrectly the three root rot isolates (RR1, RR2 and RR3) obtained from infected common bean roots harvested from Isabela, Puerto Rico, we proceeded to do polymerase chain reactions using specific rDNA-ITS primers.

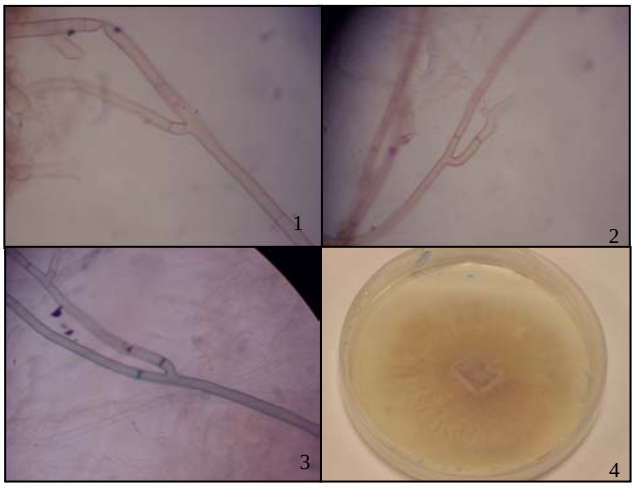
Previously characterized web blight isolates and the root rot isolates were first compared morphologically and microscopically to observe similarities (Figures 5 and 6). Then the isolates were paired on PDA media to observe if hyphal fusion occurred. By using these techniques we were able to observe similarities between the three AG 4 web blight isolates and the three root rot isolates. The hyphae width and colony coloration were similar. Young colonies of both AG 4 (WB) and root rot isolates had an off-white to crème color, and turned slightly brownish as they matured. Neither of these isolates formed sclerotia, nor did they grow abundant aerial mycelium as did the AG1-IA, -IE and -IF isolates.

Figure 5. Macro and microscopic view of *R. solani* isolates used in this study



1. AG 1-IF, 2. AG 1-IA, 3. AG 4(WB), 4. AG 4(WB) colony

Figure 6. Macro and microscopic view of *R. solani* AG 4 root rot isolates used in this study

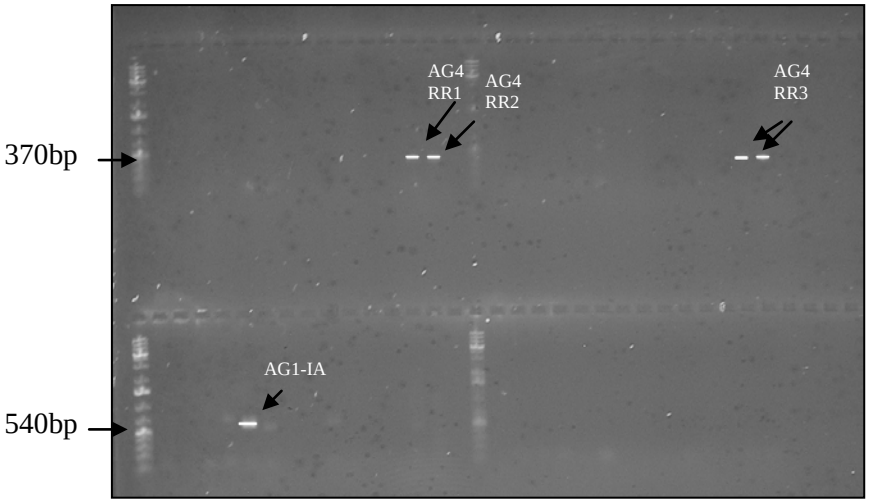


1. AG 4(RR1), 2. AG 4(RR2), 3. AG 4(RR3), 4. AG 4(RR1)

Based on these cultural and morphological similarities the three root isolates were initially considered to belong in anastomosis group 4. Five repetitions using a specific ITS-5.8S-rDNA region primer pair for AG 4 were conducted to confirm the cultural

anastomosis group classification of the root rot isolates. Isolate AG 1-IA and its respective primer pair were used as a positive control. The AG 4 specific primers amplified a region of approximately 370 bp, which confirmed a positive classification of root isolates RR1, RR2 and RR3 in AG 4 (Figure 7). This result was compared with the check isolate AG 1-IA, which amplified a region of approximately 540 bp. The empty lanes in the PCR gel belong to DNA and primer combinations of AG subgroups 1-IB, -IC, -ID, -IE and 2-2, 2-IIIB, 2-IV, 2-LP and 2-23, which did not amplify.

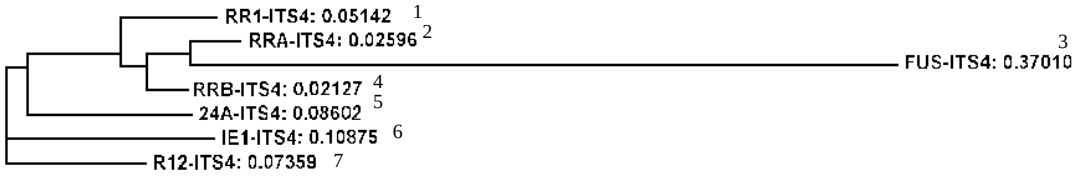
Figure 7. PCR image of *R. solani* root rot isolate DNA amplified with ITS-5.8S region specific primers



Sequence alignment and phylogenetic analysis of rDNA using basidiomycete specific primers ITS1-F and ITS4-B were conducted to provide further confidence of correct AG classification and phylogenetic relationship among root rot and web blight isolates. Root rot AG 4 isolate sequences matched with web blight AG 4 isolates from the NCBI GenBank. In the phylogram image we can observe that the root rot isolates

belong to the *R. solani* group (Figure 8). The AG 4 root rot isolates used in this study are closely related phylogenetically among each other because their points of origin are located closer in the phylogram branches. They distance themselves from another root rot sample, *R. solani* AG 2-2, isolated from diseased common bean roots at the Isabela region. Web blight isolates AG 1-IA and AG 1-IE were also used in the construction of the phylogram and are observed to be the most genetically distant of the *R. solani* isolates. The most divergent fungal sample is *Fusarium* spp. It was a root rot pathogen also isolated from common bean at Isabela. This genus was chosen as an outgroup because it belongs to the phylum Ascomycota whereas *Rhizoctonia* belongs to the phylum Basidiomycota.

Figure 8. Phylogram based on ITS-rDNA sequence data from six *R. solani* isolates and other root rot pathogens isolated from diseased common bean roots at Isabela, Puerto Rico



1. AG 4RR1, 2. AG 4RR2, 3. *Fusarium* spp., 4. AG 4RR3, 5. AG 2-2, 6. AG 1-IA, 7. AG 1-IE

The specific primers used in this study were accurate in classifying *R. solani* isolates in their respective anastomosis groups. The PCR method also proved to be a faster and easier approach to fungal identification. Cultural and molecular methods complement each other in fungal species identification and anastomosis group classification of the *R. solani* complex.

Virulence determination for web blight and root rot

Web blight greenhouse evaluation

Twelve lines and five cultivars of *P. vulgaris* were screened to detect differential reactions by inoculating them with web blight and root rot isolates of *R. solani*. Using a detached leaf technique leaflets were evaluated at 24, 48 and 72 hours. At 24h leaves presented few, if any, signs of the pathogen, which in this case is the visible observation of mycelium on the host surface. No disease symptoms were observed at this hour reading (Appendix 1). The observations were consistent throughout all 24h evaluations of the three planting dates. For this reason they were not taken into consideration in the identification of common bean lines with moderate levels of resistance to WB.

Although we used the CIAT Van Shoonhoven and Pastor-Corrales (1991) 1-9 scale to evaluate disease severity, we assigned a value of cero to leaves that presented no sign of the pathogen on leaf surface. The scale designates a severity degree of 1 to leaves with no *symptoms* of disease. In this study we separated visible sign from symptom since sign of the pathogen, i.e. mycelia presence, was observed throughout the evaluation of some screened lines without it ever leading to a manifestation of disease symptoms. While other lines never had mycelia grow on leaf surface.

In the overall mean WB scores of the 48h evaluation for the three planting dates we can observe that PR0401-259 and PR0650-32 had the lowest scores for all the pathogenic isolates with an overall line mean of 1.3 (Table 3). The line PR0650-27 had an overall line mean of 2.1, which was considered the highest WB severity mean score for any line screened in this evaluation. The lines PR0518-10, PR0518-16 and the cultivar Talamanca had an overall mean score of 2.0, which is the second highest severity

score reported. The rest of the lines and cultivars evaluated had mean scores which ranged between 1.5 and 1.9. In general terms the 48h evaluated lines expressed a high level of resistance to WB.

Table 4 shows the mean score lines received during each planting date and gives more detailed information on each line x isolate interaction. We observe that although some lines had low scores representing a higher degree of resistance to the disease, other line x isolate interactions resulted in moderate resistance with a 4.0-5.5 mean score. The highest score received by the lines is an indication of the level of disease expression that could manifest in the field if exposed to the pathogen under favorable conditions. The highest score PR0401-259 received occurred in January 2008 and was 4.0. It was caused by the root rot isolate AG 4RR1. The highest severity score PR0650-32 received was a 3.0 and occurred during the months of October 2007 and January 2008. They were caused by root rot isolates AG 4RR3 and web blight isolates AG 4WB1 and AG 4WB2 respectively. The highest score value assigned during the 48h screening was a 5.5 which represents 30% of leaf surface damage. This value is considered as moderate resistance to web blight. High severity scores of 5.0-5.5 among the different lines occurred during the month of October 2007. The grand mean for the second planting date was 2.0, whereas the first and third planting date had a grand mean of 1.6.

Although PR0650-27 had the highest overall severity score, when observed by planting date the cultivar Talamanca received more high scores of 5.0-5.5 with different isolate interactions than other lines or cultivars evaluated. In October 2007 it received high scores of 5.5 caused by AG 4WB2 and AG 1-IE, and in January 2008 by AG 4WB3. In the 48h analysis of variance for WB severity score, July and October 2007 readings

were more consistent with a 37.4 and 38.4% (Table 5).

Table 3. Overall 48 hour web blight score means of bean lines inoculated *in vitro* in July and October, 2007 and January 2008 with different isolates of *Rhizoctonia solani*

		Overall 48 hour web blight score[1] means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG 1-IA**	**AG 1-IF**	**AG 4 (WB1)**	**AG 4 (WB2)**	**AG 4 (WB3)**	**AG 4 (RR1)**	**AG 4 (RR2)**	**AG 4 (RR3)**	**AG 1-IE**
1	PR0401-257	2.0	0.8	0.8	2.2	1.0	1.0	1.7	1.8	2.2
2	PR0401-259	1.0	0.7	1.2	2.5	0.6	1.7	1.5	1.0	1.8
3	PR0401-277	2.0	1.3	2.3	3.7	1.3	0.7	1.8	3.0	1.3
4	PR0518-10	1.8	1.5	1.7	3.7	2.0	0.7	3.0	1.8	2.2
5	PR0518-15	3.0	1.3	1.0	3.5	1.5	1.5	1.3	2.5	1.8
6	PR0518-16	1.5	2.7	1.7	3.7	1.8	0.3	2.7	2.7	1.2
7	PR0650-27	2.0	1.7	2.5	4.5	1.5	0.8	2.0	2.7	0.8
8	PR0650-31	1.2	0.5	1.3	2.5	1.0	1.2	1.5	1.7	1.8
9	PR0650-32	0.8	0.8	1.0	2.2	1.2	1.0	1.8	1.7	0.8
10	PR0650-34	1.7	1.3	1.0	2.5	1.7	1.5	2.5	1.2	2.2
11	PR0650-41	0.8	1.7	1.0	3.0	1.5	2.0	2.5	1.2	2.3
12	Morales	1.5	2.2	2.2	2.8	1.3	1.3	2.0	1.3	2.7
13	Verano	1.0	1.8	0.5	3.0	1.3	1.0	2.0	1.5	1.5
14	Amadeus 77	0.8	1.5	0.5	3.0	1.5	1.8	2.2	1.5	2.5
15	Carrizalito	0.5	2.5	1.2	2.7	1.7	0.7	1.7	1.3	3.0
16	Talamanca	1.3	1.8	1.0	3.2	3.3	1.8	1.5	1.2	2.5
17	VAX 6	1.8	1.3	1.3	2.8	0.8	1.5	1.8	1.7	2.5
	LSD (0.05)	14.2	14.9	12.5	8.4	12.6	16.0	15.8	7.8	8.5
	CV (%)	58.5	61.0	54.2	36.9	50.8	64.8	69.9	35.0	34.3

[1]Evaluated on a scale from 1-9 where 1=no visible symptoms of the disease, 3=5-10% foliage area with symptoms, 5=20-30% foliage area with symptoms, 7=40-60% foliage area with symptoms and 9=>80% foliage area with symptoms (Van Schoonhoven and Pastor-Corrales, 1991).

Table 4. Web blight 48 hour score means of bean lines inoculated *in vitro* in July and October, 2007 and January 2008 with different isolates of *Rhizoctonia solani*

		Web blight 48 hour score[1] means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG 1-IA**	**AG 1-IF**	**AG 4 (WB1)**	**AG 4 (WB2)**	**AG 4 (WB3)**	**AG 4 (RR1)**	**AG 4 (RR2)**	**AG 4 (RR3)**	**AG 1-IE**
1	PR0401-257	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	**4.5**
		3.0	**1.0**	1.0	2.0	0.0	0.0	**2.5**	**2.5**	2.0
		2.0	1.0	**1.5**	**3.5**	**2.0**	**2.0**	1.5	2.0	0.0
2	PR0401-259	1.0	0.0	1.0	1.0	**1.0**	1.0	1.0	1.0	**3.5**
		2.0	0.5	0.0	**3.5**	0.0	0.0	**2.5**	**1.0**	2.0
		0.0	**1.5**	**2.5**	3.0	0.7	**4.0**	1.0	1.0	0.0
3	PR0401-277	**3.5**	1.0	**4.0**	**4.5**	**4.0**	**1.0**	2.5	**4.5**	**3.0**
		2.5	1.0	0.0	3.5	0.0	0.0	**2.5**	3.5	1.0
		0.0	**2.0**	3.0	3.0	0.0	1.0	0.5	1.0	0.0
4	PR0518-10	1.5	**2.0**	1.5	2.5	**4.0**	0.0	2.0	1.0	**2.5**
		3.0	1.0	1.0	**4.5**	0.0	0.0	**2.0**	**3.5**	2.0
		1.0	1.5	**2.5**	4.0	2.0	**2.0**	1.0	1.0	2.0
5	PR0518-15	**5.0**	1.5	1.5	3.0	**2.5**	1.0	1.5	3.0	**3.5**
		3.0	1.0	0.0	**4.0**	0.0	0.0	**1.5**	**3.5**	1.0
		1.0	**1.5**	**1.5**	3.5	2.0	**3.5**	1.0	1.0	1.0
6	PR0518-16	2.0	**3.5**	2.0	2.5	**2.5**	0.0	**3.5**	**4.5**	**2.5**
		2.5	1.0	0.0	4.0	1.0	0.0	3.0	2.5	1.0
		0.0	3.5	**3.0**	**4.5**	2.0	**1.0**	1.5	1.0	0.0
7	PR0650-27	**2.5**	1.5	3.0	4.5	**3.5**	0.0	2.5	3.0	1.0
		2.0	1.0	0.0	**4.5**	0.0	0.0	**2.5**	**4.5**	**1.5**
		1.5	**2.5**	**4.5**	4.5	1.0	**2.5**	1.0	0.5	0.0
8	PR0650-31	1.0	0.0	1.0	0.0	1.0	1.0	1.0	1.0	**3.5**
		2.5	**1.0**	0.0	3.5	0.0	0.0	**2.0**	**2.5**	1.5
		0.0	0.5	**3.0**	**4.0**	**2.0**	**2.5**	1.5	1.5	0.5
9	PR0650-32	**1.5**	0.0	0.0	1.0	1.0	1.5	1.5	1.0	**1.5**
		1.0	1.0	0.0	2.5	0.0	0.0	**2.0**	**3.0**	1.0
		0.0	**1.5**	**3.0**	**3.0**	**2.5**	**1.5**	2.0	1.0	0.0
10	PR0650-34	1.0	0.0	0.0	2.0	**2.0**	1.5	1.0	1.0	1.5
		1.5	**3.0**	0.0	**3.5**	1.0	0.0	**5.5**	1.0	**5.0**
		2.5	1.0	**3.0**	2.0	2.0	**3.0**	1.0	**1.5**	0.0
11	PR0650-41	1.0	0.0	0.5	1.5	1.5	2.5	1.0	0.5	3.0
		1.5	**4.0**	0.0	**4.5**	**1.5**	0.0	**5.0**	**2.5**	**5.0**
		0.0	1.0	**2.5**	3.0	1.5	**3.5**	1.5	0.5	0.0
12	Morales	1.0	0.0	1.0	2.0	1.0	2.0	1.5	0.0	2.5
		2.5	**5.0**	1.5	**4.5**	**1.5**	0.0	**3.5**	**3.0**	**5.5**
		1.0	1.5	**4.0**	2.0	1.5	**2.0**	1.0	1.0	0.0
13	Verano	1.5	1.0	0.0	1.5	1.5	**2.0**	1.5	1.0	0.5
		1.5	**3.0**	0.5	**4.5**	1.0	0.0	**3.5**	**2.5**	**4.0**
		0.0	1.5	**1.0**	3.0	**1.5**	1.0	1.0	1.0	0.0
14	Amadeus 77	**1.4**	0.5	0.0	1.0	**2.0**	2.0	2.0	1.0	2.5
		1.0	**3.0**	0.0	**5.0**	1.5	0.0	**4.5**	**2.5**	**5.0**
		0.0	1.0	**1.5**	3.0	1.0	**3.5**	0.0	1.0	0.0
15	Carrizalito	0.0	0.0	0.5	1.0	2.0	**1.0**	1.0	1.0	3.5
		1.5	**4.5**	1.0	**4.5**	0.0	0.0	**3.0**	**2.0**	**3.5**
		0.0	3.0	**2.0**	2.5	**3.0**	1.0	1.0	1.0	2.0
16	Talamanca	1.0	0.0	1.0	1.0	1.0	1.5	1.0	1.0	2.0
		0.5	**4.5**	0.0	**5.5**	4.0	1.0	**3.0**	**2.0**	**5.5**
		2.5	1.0	**2.0**	3.0	**5.0**	**3.0**	0.5	0.5	0.0
17	VAX 6	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	**4.5**
		0.0	**3.0**	0.0	**4.0**	0.0	**2.0**	**3.5**	**3.0**	3.0
		4.5	1.0	**3.0**	3.5	**1.5**	1.5	1.0	1.0	0.0

[1]Evaluated on a scale from 1-9 where 1=no visible symptoms of the disease, 3=5-10% foliage area with symptoms, 5=20-30% foliage area with symptoms, 7=40-60% foliage area with symptoms and 9=>80% foliage area with symptoms (Van Schoonhoven and Pastor-Corrales, 1991).

Table 5. Analysis of variance of 48h web blight severity evaluation of *P. vulgaris* lines

Source of Variation	Degrees of Freedom	48h Web Blight Severity Mean Squares		
		July 2007	October 2007	January 2008
Line	16	7.84	5.60	1.28
Isolate	8	10.90	61.13	28.70
Line x Isolate	128	1.60*	1.97*	1.50*
Error	153	0.34	0.58	0.66
Total	305			
CV (%)		37.4	38.4	49.6

*Significance of 0.05 probability

The 72h inoculation results provided the best separation among lines in web blight reactions. This evaluation showed more severe scores, yet these observations helped in selecting PR0401-259 as the line with the highest level of disease resistance (Table 6). This screening also demonstrated an increased level of variability between the isolates and their respective host interactions. Although, by planting date, the line PR0401-259 received the highest scores of 6 and 7 from AG 4RR1, AG 4RR2 and the WB isolate AG 1-IE respectively, its overall line mean score was 2.8 (Table 7). Line PR0650-32 had an overall line score of 3.1, and its highest severity score was 6.0 by planting date. Line PR0650-27 had the highest overall line score of 4.4, with a high severity score of 7.5 by planting date. We can also observe how the lines interacted differently to each of the nine isolates. Whereas a line could express a resistant to moderate resistant response to certain isolates it was infected with, it was susceptible to other isolates. In the 72h analysis of variance for WB severity score, July and October 2007 readings were more consistent with a 27.5 and 27.2% (Table 8).

Table 6. Overall 72 hour web blight score means of bean lines inoculated *in vitro* in July and October, 2007 and January 2008 with different isolates of *Rhizoctonia solani*

		Overall 72 hour web blight score[1] means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG 1-IA**	**AG 1-IF**	**AG 4 (WB1)**	**AG 4 (WB2)**	**AG 4 (WB3)**	**AG 4 (RR1)**	**AG 4 (RR2)**	**AG 4 (RR3)**	**AG 1-IE**
1	PR0401-257	3.3	2.5	2.8	4.5	3.2	1.3	3.8	3.0	4.2
2	PR0401-259	2.3	1.8	1.7	4.0	2.7	2.3	3.2	2.8	4.0
3	PR0401-277	4.2	3.2	3.8	5.3	3.3	2.0	4.2	4.8	3.5
4	PR0518-10	3.3	5.0	2.8	5.3	3.8	2.7	4.3	2.3	4.8
5	PR0518-15	5.0	4.0	2.5	5.8	3.8	2.3	4.3	4.7	4.2
6	PR0518-16	2.7	4.7	3.0	6.2	3.5	1.8	5.0	6.3	3.2
7	PR0650-27	4.0	3.8	5.2	6.8	5.7	2.0	4.8	5.2	2.2
8	PR0650-31	2.2	3.5	3.3	4.2	3.3	2.8	3.2	3.0	4.5
9	PR0650-32	2.3	3.3	2.0	4.3	3.0	3.0	4.2	3.2	2.5
10	PR0650-34	3.2	3.0	2.5	4.3	4.3	2.7	4.2	2.8	4.2
11	PR0650-41	2.3	3.7	3.0	4.5	4.0	4.5	4.8	3.7	4.0
12	Morales	2.8	4.2	4.0	4.3	3.3	3.7	3.3	3.2	4.3
13	Verano	1.7	3.5	2.2	5.0	3.2	1.8	3.7	3.6	2.7
14	Amadeus 77	1.5	3.7	3.2	5.0	3.2	3.8	2.8	3.3	4.7
15	Carrizalito	2.2	4.5	2.5	4.2	3.3	1.5	4.0	2.2	4.3
16	Talamanca	2.7	3.7	3.2	4.7	4.7	2.9	3.2	3.3	4.2
17	VAX 6	3.2	2.7	2.8	4.2	2.8	4.8	3.0	3.8	4.2
	LSD (0.05)	14.4	12.2	17.4	10.2	16.7	13.1	14.0	14.0	18.9
	CV (%)	55.2	32.2	65.6	42.1	61.9	41.2	52.9	44.1	69.7

[1]Evaluated on a scale from 1-9 where 1=no visible symptoms of the disease, 3=5-10% foliage area with symptoms, 5=20-30% foliage area with symptoms, 7=40-60% foliage area with symptoms and 9=>80% foliage area with symptoms (Van Schoonhoven and Pastor-Corrales, 1991).

Table 7. Web blight 72 hour score means of bean lines inoculated *in vitro* in July and October, 2007 and January 2008 with different isolates of *Rhizoctonia solani*

		Web blight 72 hour score[1] means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG 1-IA**	**AG 1-IF**	**AG 4 (WB1)**	**AG 4 (WB2)**	**AG 4 (WB3)**	**AG 4 (RR1)**	**AG 4 (RR2)**	**AG 4 (RR3)**	**AG 1-IE**
1	PR0401-257	2.0	1.5	1.5	2.0	3.0	1.0	2.5	1.5	**6.5**
		5.0	**5.0**	**3.5**	**6.0**	3.0	0.0	**6.5**	**4.5**	6.0
		3.0	1.0	3.5	5.5	**3.5**	**3.0**	2.5	3.0	0.0
2	PR0401-259	1.5	0.0	1.0	2.0	2.5	1.0	1.0	1.0	**7.0**
		3.5	**3.5**	1.0	**5.5**	2.3	0.0	**6.0**	**4.5**	5.0
		2.0	2.0	**3.0**	4.5	**3.3**	**6.0**	2.5	3.0	0.0
3	PR0401-277	**5.5**	3.0	5.0	5.5	**4.0**	1.5	5.0	**7.0**	**5.5**
		4.0	**3.5**	1.0	**6.5**	3.0	0.0	**5.5**	5.5	4.0
		3.0	3.0	**5.5**	4.0	3.0	**4.5**	2.0	2.0	1.0
4	PR0518-10	3.5	**5.5**	3.0	4.5	**4.5**	0.5	3.0	1.0	**5.5**
		5.0	4.0	2.0	**6.5**	4.0	0.0	**6.5**	**5.0**	5.5
		1.0	5.5	**3.5**	5.0	3.0	**7.5**	3.5	1.0	3.5
5	PR0518-15	**8.5**	3.5	3.0	5.0	3.5	1.0	4.0	**5.5**	**6.5**
		4.0	**5.0**	1.5	**7.5**	3.0	0.0	3.5	5.0	5.0
		2.5	3.5	**3.0**	5.0	**5.0**	**6.0**	**5.5**	3.5	1.0
6	PR0518-16	**4.0**	4.5	2.5	**6.6**	**4.0**	0.5	**5.5**	**7.5**	**5.5**
		4.0	4.0	1.5	6.5	3.5	0.0	5.0	5.5	4.0
		0.0	**5.5**	**5.0**	5.5	3.0	**5.0**	4.5	6.0	0.0
7	PR0650-27	**5.5**	2.5	**7.0**	**7.5**	**6.5**	1.0	3.5	5.0	1.0
		4.0	4.0	2.0	7.0	5.5	0.0	**7.0**	**7.5**	**5.5**
		2.5	**5.0**	6.5	6.0	5.0	**5.0**	4.0	3.0	0.0
8	PR0650-31	1.5	2.0	2.5	1.0	2.0	2.0	1.0	1.0	**7.5**
		4.0	**5.5**	1.5	**6.5**	2.5	0.0	**4.5**	**5.0**	4.5
		1.0	3.0	**6.0**	5.0	**5.5**	**6.5**	4.0	3.0	1.5
9	PR0650-32	**4.0**	3.0	0.0	4.0	3.0	3.5	**5.0**	2.0	**3.5**
		3.0	**4.0**	3.0	**6.0**	2.5	0.0	3.5	**5.5**	3.5
		0.0	3.0	**3.0**	3.0	**3.5**	**5.5**	4.0	2.0	0.5
10	PR0650-34	2.0	2.5	2.0	4.5	**5.5**	4.0	2.5	2.5	6.0
		3.5	**5.5**	1.5	**5.5**	3.5	0.0	**7.5**	2.5	**6.5**
		4.0	1.0	**4.0**	3.0	4.0	**4.0**	2.5	**3.5**	0.0
11	PR0650-41	3.0	2.0	1.0	4.0	**4.5**	6.5	2.5	2.5	**6.0**
		4.0	**7.0**	4.0	4.5	4.0	0.0	**7.0**	**5.0**	6.0
		0.0	2.0	**4.0**	**5.0**	3.5	**7.0**	5.0	3.5	0.0
12	Morales	2.5	3.0	3.0	5.0	2.0	5.5	3.0	2.0	5.5
		5.0	**7.5**	2.5	**5.5**	**5.0**	0.0	**5.0**	**6.0**	**7.5**
		1.0	2.0	**6.5**	2.5	3.0	**5.5**	2.0	1.5	0.0
13	Verano	2.0	2.0	2.0	5.0	2.5	**4.5**	4.0	3.5	2.0
		3.0	**5.5**	**3.0**	**6.0**	**4.0**	0.0	**5.0**	**5.0**	**6.0**
		0.0	3.0	1.5	4.0	3.0	1.0	2.0	2.5	0.0
14	Amadeus 77	**2.5**	2.0	2.5	4.0	3.5	**6.0**	2.0	3.5	**7.0**
		2.0	**6.0**	3.0	**6.5**	**3.5**	0.0	**5.0**	**4.0**	7.0
		0.0	3.0	**4.0**	4.5	2.5	5.5	1.5	2.5	0.0
15	Carrizalito	1.0	1.0	0.5	2.0	3.5	1.5	2.5	1.0	**5.0**
		4.0	**7.5**	**4.0**	**7.5**	**3.5**	0.0	**6.5**	**3.5**	4.5
		1.5	5.0	3.0	3.0	3.0	**3.0**	3.0	2.0	3.5
16	Talamanca	2.5	1.0	2.5	2.0	2.0	2.0	2.5	2.0	5.0
		2.0	**7.0**	3.0	**8.0**	4.0	1.5	**5.5**	**5.5**	**7.5**
		3.5	3.0	**4.0**	4.0	**8.0**	**5.0**	1.5	2.5	0.0
17	VAX 6	1.0	0.0	1.0	2.0	2.5	4.5	2.5	2.0	**7.5**
		2.0	**6.0**	3.0	**6.5**	3.0	3.5	**5.0**	**6.5**	5.0
		6.5	2.0	**4.5**	4.0	**3.0**	**6.5**	1.5	3.0	0.0

[1]Evaluated on a scale from 1-9 where 1=no visible symptoms of the disease, 3=5-10% foliage area with symptoms, 5=20-30% foliage area with symptoms, 7=40-60% foliage area with symptoms and 9=>80% foliage area with symptoms (Van Schoonhoven and Pastor-Corrales, 1991).

Table 8. Analysis of variance of 72 h web blight severity evaluation of *P. vulgaris* lines

Source of Variation	Degrees of Freedom	72h Web Blight Severity Mean Squares		
		July 2007	October 2007	January 2008
Line	16	14.90	4.00	6.39
Isolate	8	31.50	124.52	62.16
Line x Isolate	128	4.92*	2.05*	3.44*
Error	153	0.80	1.30	1.25
Total	305			
CV (%)		27.5	27.2	34.9

*Significance of 0.05 probability

Based on the results of this research the bean line that provided useful levels of web blight resistance to the greatest number of *R. solani* isolates was PR0401-259 (Table 9). It expressed resistance to AG 1-IA, -IF, AG 4WB, AG 4WB3 with overall web blight severity scores ranging between 1.7 and 2.7 for each isolate, and intermediate resistance to AG 4WB2 with an overall web blight severity score of 4.0 for that isolate throughout the three trials. Other lines that provided useful levels of resistance to specific strains were Carrizalito to AG 4RR1 and AG 4RR3, and PR0650-32 to AG 1-IE and AG 4WB3. This is important information to plant breeders because they can cross these lines in order to develop ones with useful levels of resistance to a greater number of *R. solani* isolates.

Table 9. Web blight reactions of bean lines at 72 hours after inoculation

	Rhizoctonia Anastomosis Group								
	AG 1-IA	AG 1-IF	AG 4 (WB1)	AG 4 (WB2)	AG 4 (WB3)	AG 4 (RR1)	AG 4 (RR2)	AG 4 (RR3)	AG 1-IE
Line	PR0401-259	PR0401-259	PR0401-259	PR0401-259	PR0401-259	Carrizalito	PR0650-31	Carrizalito	PR0650-32
Highest mean WB score for each AG	3.5	3.5	3.0	5.5	3.3	3.0	4.5	3.5	3.5
Rank of highest mean score among lines (lowest to highest) for each AG	2nd (tie)	1st	1st (tie)	2nd (tie)	2nd (tie)	1st (tie)	1st	1st (tie)	1st (tie)
Mean WB score over 3 trials	2.3	1.8	1.7	4.0	2.7	1.5	3.2	2.2	2.5

	Rhizoctonia Anastomosis Group	
	AG 4 (WB3)	AG 4 (WB3)
Line	PR0650-32	Carrizalito
Highest mean WB score for each AG	3.5	3.5
Rank of highest mean score among lines (lowest to highest) for each AG	3rd (tie)	3rd (tie)
Mean WB score over 3 trials	3.0	3.3

Through the WB evaluation we also established that root rot *R. solani* isolate were capable of inducing web blight symptoms on leaf surface of common bean. The root rot isolates responded very similar to the web blight isolates throughout the three hour screenings of each planting date. The most aggressive RR isolates were AG 4RR2 and AG 4RR3. The most aggressive WB isolates were AG 1-IE, AG 4WB2 and AG 4WB3. Yet AG 4WB2 was more aggressive than any of the other isolates used in this evaluation with a 48h overall score of 2.8 compared to AG 1-IE (1.19) and AG 4RR2 and RR3 (1.8). AG 4 isolates also varied in aggressiveness within the group. At 48h screenings WB1 and RR1 had overall scores of 1.2, WB2 had 2.8, WB3 had 1.5, and RR2 and RR3 had scores of 1.8. It is interesting to observe these differences not only within a group, but also within group specificity. Even within WB and RR specific isolates variations in disease aggressiveness occurred. Montoya et al. (1997) and Polanco et al. (1993) reported that web blight isolates collected in Puerto Rico vary in levels of virulence to beans. When studying the phylogram of the identified of AG 4 RRs, a minor divergence could be observed between isolate RR1 and isolates RR2 and RR3. This difference was also observed in their level of host WB aggressiveness where RR1 was the least aggressive amongst its group. Balali and Kowsari (2004) suggest that heterokaryosis could directly influence AG 4 pathogenicity and that isolates which have heterokaryons with genetically diverse nuclei could result in the more pathogenic AG 4 isolates.

Root rot greenhouse evaluation

The twelve breeding lines and five cultivars of common bean were tested for root rot resistance using a mycelia suspension method adapted from a sugarbeet method

(Büttner et al., 2004). The lines were infected with the same WB and RR isolates used in the previous evaluation. Twenty one days after inoculation, the roots were harvested and scored using a 0-7 severity scale. In table 10 we can observe the overall means of the host x isolate interactions scored between 5.0 (50% damage) and 6.5 (>75% damage). Yet some lines seemed to be moderately resistant to specific *R. solani* isolates. Line PR0401-257 had a score of 4.3-4.9, which represents over 25% root damage, from WB isolates AG 1-IA, AG 1-IF and RR isolates AG 4RR1 and RR3. Its most severe scores were caused by WB isolates AG 4WB1-3 and AG 1-IE, ranging from 50-75% root damage.

The line PR0401-259 had its lowest severity score from isolate AG 4WB1, which was 3.9, representing 10% damage. Morales was susceptible to eight out of nine isolates, with 75% damage in most cases. Yet it was resistant to isolate AG 4WB2, with a low score of 2.7. This represents 5-10% root damage and it had 189.2 cm^2 overall root surface area recovered in the evaluation for all three planting dates (Table 11). Its result was very similar to the WB evaluation in which it had a 2.8 score for the same isolate. We believe that although the inoculation test may have been too severe there is evidence which suggests that some of the lines have moderate resistance to specific isolates, mainly to those which cause WB. The lines PR0401-257 and PR0650-27 had the lowest overall severity score of 3.5, which represents 50% of root damage. Verano and Amadeus 77 had the highest overall severity score in this evaluation of 6.2, which is >75% root damage. Based on the results of this study the bean lines that provided useful levels of root rot resistance to *R. solani* isolates were PR0401-257 and PR0650-27 (Table 12).

We compared these results with the data obtained from the root surface area evaluation and noticed that the highest overall measurement for the line x isolate interaction belonged to PR0650-27 with a 53.2 cm^2 (Table 11). Nonetheless, the data from root surface measurements, using WinRhizo Basic 2008© software, did not correlate with results obtained from root rot severity trials. It was not possible to observe a measurement decrease in root surface area as the severity levels increased in the root samples. Dry weight analysis of recovered root mass might be a more efficient confirmation of root damage when compared to a root severity scale in future studies.

Table 10. Overall mean root rot scores of bean lines inoculated in the greenhouse in October 2007, February and June 2008 with different isolates of *Rhizoctonia solani*

		Overall root rot score[1] means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG 1-IA**	**AG 1-IF**	**AG 4 (WB1)**	**AG 4 (WB2)**	**AG 4 (WB3)**	**AG 4 (RR1)**	**AG 4 (RR2)**	**AG 4 (RR3)**	**AG 1-IE**
1	PR0401-257	4.3	4.9	6.2	5.9	5.5	4.6	5.4	4.7	6.1
2	PR0401-259	6.0	5.3	3.9	5.5	6.0	6.1	6.1	6.0	6.1
3	PR0401-277	4.3	5.4	5.3	6.0	4.8	6.1	5.6	6.5	4.9
4	PR0518-10	6.3	5.9	5.9	5.4	5.7	5.5	5.9	5.2	5.3
5	PR0518-15	6.0	4.9	5.2	4.8	6.4	5.9	6.9	6.4	5.3
6	PR0518-16	4.5	5.8	5.6	6.0	5.9	5.4	5.9	6.2	5.8
7	PR0650-27	4.2	4.8	5.9	5.5	6.1	5.5	5.1	5.7	5.3
8	PR0650-31	5.6	6.5	5.7	6.3	5.8	6.3	5.4	6.5	6.1
9	PR0650-32	5.7	5.9	4.4	5.7	6.1	5.8	5.0	6.2	5.6
10	PR0650-34	6.4	6.2	6.0	5.8	6.4	5.7	6.4	6.7	5.6
11	PR0650-41	6.2	6.6	6.3	5.5	5.2	6.1	6.2	6.0	5.8
12	Morales	6.2	5.8	6.0	2.7	6.3	6.2	5.7	6.5	6.5
13	Verano	6.3	6.2	6.0	6.3	5.8	6.4	5.9	6.3	6.4
14	Amadeus 77	6.0	5.9	6.2	6.1	4.9	5.7	5.8	5.2	6.0
15	Carrizalito	5.4	5.6	6.5	5.8	5.7	6.2	6.0	5.0	6.1
16	Talamanca	5.1	5.8	5.3	5.9	6.1	6.2	5.4	5.4	4.7
17	VAX 6	5.3	5.6	5.8	5.8	5.5	6.1	5.0	5.6	5.7
	LSD (0.05)	15.7	11.0	19.0	14.3	19.0	13.7	19.8	16.6	12.4
	CV (%)	54.7	45.1	66.3	58.8	72.9	55.1	68.4	57.9	50.2

[1] Evaluated on a scale from 0-7 where 0= no visible symptoms of the disease, 1= 1-5% root surface with visible lesions, 2=5-10% root surface with visible lesions, 3=10-25% root with visible lesions and/or dry-rot cankers, 4= 25-50% root with visible lesions and/or dry-rot cankers, 5=50-75% roots showing dry-rot cankers, damaged or rotted roots, 6=>75% roots showing dry-rot cankers, damaged or rotted roots, 7=pre-emergence damping off and few if any roots modified from Büttner et al. (2004) and Dorrance et al. (2003).

Table 11. Mean root surface area (cm^2) of bean lines inoculated in the greenhouse in October 2007, February and June 2008 with different isolates of *Rhizoctonia solani*

		Root surface area[1] (cm^2) means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG 1-IA**	**AG 1-IF**	**AG 4 (WB1)**	**AG 4 (WB2)**	**AG 4 (WB3)**	**AG 4 (RR1)**	**AG 4 (RR2)**	**AG 4 (RR3)**	**AG 1-IE**
1	PR0401-257	47.4	93.9	51.4	70.1	69.5	89.9	85.8	104.0	54.8
		68.2	13.2	24.3	26.1	28.9	23.7	18.9	30.2	35.3
		6.4	11.4	9.0	7.1	7.5	8.6	8.2	6.8	6.3
2	PR0401-259	69.7	62.8	88.7	79.1	43.6	102.7	71.8	65.4	56.9
		20.9	22.8	66.7	34.0	29.2	17.0	23.6	19.6	20.6
		5.0	5.5	22.9	9.0	10.5	10.4	10.3	12.2	9.5
3	PR0401-277	117.7	75.4	81.3	61.2	113.4	84.0	71.9	44.4	93.7
		22.4	28.6	21.6	20.0	19.2	49.4	50.8	12.7	63.4
		13.9	6.7	8.5	11.9	6.0	3.6	5.6	12.3	13.8
4	PR0518-10	55.4	46.1	42.6	67.0	45.1	86.0	66.9	73.8	38.3
		15.1	50.6	19.7	19.2	73.8	31.8	28.7	32.4	73.5
		3.7	6.4	4.6	10.8	13.5	16.6	12.1	10.2	17.2
5	PR0518-15	102.0	101.2	125.9	160.7	37.4	78.0	33.4	44.7	86.1
		10.1	59.3	30.7	15.1	15.9	16.4	14.1	12.3	33.8
		7.0	5.4	10.4	8.7	7.0	6.2	5.3	4.8	11.3
6	PR0518-16	110.3	43.1	54.1	54.4	47.6	35.2	50.6	41.4	38.3
		32.2	18.0	41.6	13.1	16.4	33.9	34.7	24.8	41.7
		10.8	11.0	8.5	7.7	5.0	11.7	8.7	5.8	7.3
7	PR0650-27	109.6	90.3	175.2	159.6	103.9	49.1	95.2	97.4	113.3
		33.6	50.8	29.5	23.2	21.4	45.9	30.3	29.1	32.8
		31.7	16.5	15.0	11.5	7.7	9.5	23.0	12.7	20.2
8	PR0650-31	94.1	72.4	67.3	83.0	86.4	50.4	61.7	114.9	54.8
		23.6	17.6	31.7	19.7	24.2	25.3	33.6	23.4	19.3
		6.0	9.0	42.0	5.3	17.6	22.2	8.1	11.4	28.1
9	PR0650-32	47.2	44.5	67.8	88.0	65.7	57.3	112.7	90.1	60.9
		22.8	14.2	115.8	28.7	37.1	16.9	81.9	30.8	26.6
		7.9	17.5	17.4	18.6	13.9	11.9	15.2	12.8	18.7
10	PR0650-34	31.0	105.1	46.4	113.8	55.5	98.6	47.9	51.2	66.2
		27.4	16.3	37.6	33.6	16.0	34.3	13.9	10.3	97.6
		13.5	3.5	16.1	30.0	15.9	25.3	14.6	12.4	16.7
11	PR0650-41	44.2	48.8	42.5	121.3	89.5	76.4	83.3	76.5	78.8
		20.7	13.7	12.4	69.5	180.7	14.8	62.0	24.8	28.4
		9.0	10.5	14.7	15.1	11.7	11.2	13.8	10.7	22.7
12	Morales	28.4	52.4	92.7	267.0	57.8	78.6	83.0	93.9	39.8
		33.6	39.9	24.1	291.1	14.6	20.3	33.4	18.1	21.6
		8.4	12.1	8.8	9.4	12.9	14.8	6.8	10.3	17.6
13	Verano	97.6	90.3	46.0	33.2	87.9	25.8	59.6	79.9	40.5
		47.0	44.4	44.7	17.5	22.6	14.4	29.0	17.4	16.2
		14.5	5.4	6.5	20.9	12.6	11.0	13.3	16.1	9.4
14	Amadeus 77	58.2	48.8	90.1	53.9	78.4	118.7	72.9	90.7	63.9
		11.2	18.2	9.1	19.9	52.6	9.8	40.3	12.6	17.8
		11.0	8.7	13.2	15.6	8.9	9.4	5.6	8.5	13.2
15	Carrizalito	123.2	101.9	44.2	132.0	104.8	52.7	59.4	125.3	55.1
		19.5	15.3	22.4	11.5	12.7	12.6	12.3	23.5	16.1
		4.2	12.1	4.3	9.8	7.1	7.9	12.5	8.4	22.1
16	Talamanca	67.3	82.8	78.5	56.2	60.0	52.7	88.7	112.6	54.9
		25.2	37.3	28.3	21.3	25.0	21.7	29.7	16.9	43.9
		33.3	10.2	9.8	17.1	24.6	11.5	14.1	24.0	30.8
17	VAX 6	42.3	57.5	60.3	48.1	67.7	60.5	100.4	17.0	72.6
		18.8	16.3	28.7	25.6	45.9	11.3	16.4	18.3	23.8
		21.6	21.1	11.2	17.8	8.2	12.2	17.0	11.4	10.3

[1]Digital root analysis evaluated using WinRhizo Basic 2008 © Copyright Regent Instrument Canada Inc.

Table 12. Root rot reactions of bean lines at 21 days after inoculation

	Rhizoctonia Anastomosis Group								
	AG 1-IA	AG 1-IF	AG 4 (WB1)	AG 4 (WB2)	AG 4 (WB3)	AG 4 (RR1)	AG 4 (RR2)	AG 4 (RR3)	AG 1-IE
Line	PR0401-257	PR0650-27	PR0401-259	Morales	PR0401-257	PR0401-257	PR0650-27	PR0518-10	PR0401-277
Highest mean RR score for each AG	5.3	5.0	5.0	5.5	5.7	5.7	5.3	6.0	5.3
Rank of highest mean score among lines (lowest to highest) for each AG	1st (tie)	1st	1st	1st	1st	1st	1st	1st (tie)	1st
Mean RR score over 3 trials	4.3	4.8	3.9	2.7	5.5	4.6	5.1	5.2	4.9

	Rhizoctonia Anastomosis Group								
	AG 1-IA	AG 1-IA							AG 1-IE
Line	PR0650-27	PR0518-16							PR0650-27
Highest mean RR score for each AG	5.3	5.3							5.5
Rank of highest mean score among lines (lowest to highest) for each AG	1st (tie)	1st (tie)							2nd (tie)
Mean RR score over 3 trials	4.2	4.5							5.3

The grand means per planting date for RR severity score increased as the ambient and greenhouse temperature increased. October 2007 and February 2008 had a grand mean of 5.6 and June 2008 had a grand mean of 6.0. Root surface area measures

decreased as the greenhouse temperature increased with grand means ranging from 74.6 cm^2 in October 2007, 31.1 cm^2 in February 2008 and 12.3 cm^2 in June 2008. This resulted in less root mass recovered as the weather got warmer. It was possible to observe that the environment can affect disease development and severity (Román-Avilés and Kelly, 2005). Also environmental conditions and disease pressure can influence variations in disease expression (Büttner et al., 2004). The analysis of variance for root rot scores showed more consistent readings during the months of October 2007 and February 2008 with 13.18 an 13.79% (Table 13). Morales and isolate AG 4RR1 were used as a positive control group with an overall grand mean score of 6.3 of RR severity and non-inoculated Morales were used as a negative control group with a grand mean of 2.9 RR severity (Figure 9).

Figure 9. Root rot greenhouse experiment control group

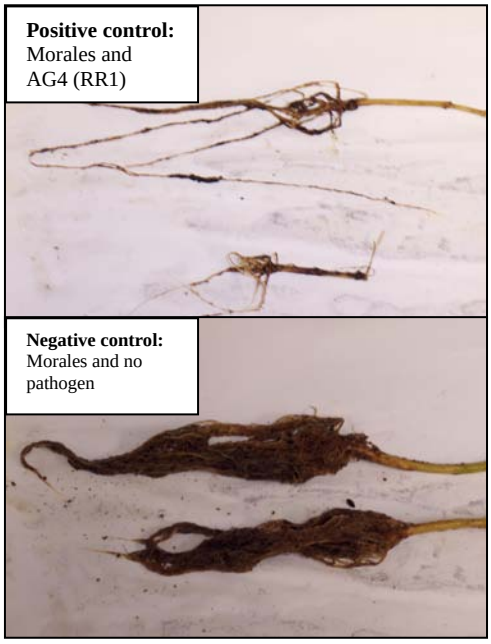


Table 13. Analysis of variance of root rot severity evaluation of *P. vulgaris* lines

Source of Variation	Degrees of Freedom	October 2007 Mean Squares	DF	February 2008 Mean Squares	DF	June 2008 Mean Squares
Line	16	10.80		3.17		2.99
Isolate	8	3.74		2.01		1.10
Line x Isolate	128	2.86*		2.38*		0.67*
Error	255	0.55	242	0.60	200	0.26
Total	407		394		352	
CV (%)		13.18		13.79		8.55

*Significance of 0.05 probability

In this evaluation we also ascertained that WB and RR isolates were equally effective in causing root rot symptoms. Isolates AG 1-IA was the least aggressive among the lines, with a score an overall score of 5.5. Host specificity may have influenced its interaction with the evaluated bean lines since this isolate is considered as the fungus responsible for sheath blight disease of rice plant (Matsumoto et al., 1997). In 2005, Windels and Brantner reported AG 4 as one of the most aggressive root rot isolate in different hosts. In this research, isolates AG 4RR1 and RR3 had the highest score of 5.9. It is interesting to observe that isolate AG 4RR1 had the lowest WB score (1.2) in the 48h leaf screening and one of the highest scores for root infection. This data suggests that there might be specialization within certain strains. In figure 10 we observe how a root rot isolate caused web blight symptoms after roots were inoculated with the liquid mycelia suspension. This could have occurred if mycelium which grew up towards the soil surface was carried by air from the ventilator or during watering, then adhering itself to the plant's aerial portion and developing WB symptoms.

Figure 10. Common bean leaves with web blight symptoms after being inoculated with root rot

The amended sugarbeet inoculation technique seemed too severe for bean root infection. This might be due to the difference in overall root mass between species. This technique proved to be capable of infecting the roots and could be reconsidered for future use with further modifications. Such as producing an inoculum with a higher mycelia dilution, reducing the amount of liquid inoculum added per plant, or reducing the time of disease incubation in the greenhouse.

CONCLUSIONS

By using molecular techniques we were able to characterize field root rot isolates of *R. solani* into anastomosis group 4. The process was faster and more accurate than by using cultural methods alone. We also observed how different isolates varied in aggressiveness among common bean lines. We were able to identify bean lines with high and moderate levels of resistance to web blight and root rot. The line PR0401-259 had the highest level of resistance to this disease and that isolate AG 4WB2 was the most aggressive. In the RR evaluation we used disease severity scores and root surface area data to conclude that line PR0650-27 had a moderate level of disease resistance. We were also able to observe that some of the lines tested had more resistance to specific *R. solani* isolates, and that AG 4RR1 was the most aggressive isolate in the RR evaluation. In both WB and RR evaluations isolate AG 1-IA seemed to be the least virulent strain. Finally, we established that WB and RR isolates were equally capable of causing both diseases.

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APPENDICES

Appendix 1. Web blight 24 hour score means of bean lines inoculated *in vitro* in July and October, 2007 and January 2008 with different isolates of *Rhizoctonia solani*

		Web blight 24 hour score means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG-1-IA**	**AG-1-IF**	**AG-4 (WB1)**	**AG-4 (WB2)**	**AG-4 (WB3)**	**AG-4 (RR1)**	**AG-4 (RR2)**	**AG-4 (RR3)**	**AG-1-IE**
1	PR0401-257	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**1.5**
		1.0	**0.0**	0.0	**1.0**	**0.0**	0.0	**0.5**	0.5	1.0
		0.0	0.0	**0.5**	1.0	0.0	**1.0**	0.0	**1.0**	0.0
2	PR0401-259	0.0	0.0	**1.0**	0.0	**1.0**	0.0	0.0	0.0	**1.5**
		1.0	0.0	0.0	**1.0**	0.0	0.0	**1.0**	**0.5**	1.0
		0.0	**0.5**	0.5	1.0	0.0	**1.0**	0.0	0.0	0.0
3	PR0401-277	1.0	0.0	0.0	1.0	**1.0**	0.0	0.0	0.0	**1.0**
		1.0	0.0	0.0	1.0	0.0	0.0	**1.0**	**1.0**	0.5
		0.0	**0.5**	**0.5**	**1.0**	0.0	**0.5**	0.0	0.0	0.0
4	PR0518-10	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	**1.0**
		1.0	**0.0**	0.0	1.0	0.0	0.0	**0.5**	**0.5**	1.0
		0.0	0.0	**0.5**	**1.0**	**0.5**	**1.0**	0.5	0.5	0.0
5	PR0518-15	1.0	0.0	0.0	1.0	**1.0**	0.0	0.0	0.0	**2.0**
		1.0	**0.0**	**0.0**	**1.0**	0.0	0.0	0.5	**1.0**	0.5
		0.0	0.0	0.0	1.0	0.5	**1.0**	**1.0**	0.0	0.0
6	PR0518-16	1.0	0.0	0.0	1.0	**1.0**	0.0	0.0	0.0	**1.5**
		1.0	0.0	**0.0**	**1.0**	0.0	0.0	**1.0**	**0.5**	0.5
		0.0	**1.0**	0.0	1.0	0.5	**1.0**	1.0	0.5	0.0
7	PR0650-27	1.0	0.0	0.0	1.5	**1.0**	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.5	0.0	**0.0**	**1.0**	**1.0**	**0.5**
		1.0	**0.5**	**1.5**	**1.5**	0.0	0.0	0.0	0.0	0.0
8	PR0650-31	0.0	0.0	1.0	0.0	0.0	**1.0**	1.0	1.0	**1.5**
		0.5	0.0	0.0	0.0	0.0	0.0	**1.0**	**1.0**	0.5
		0.0	**0.5**	**1.0**	**1.0**	**1.0**	0.5	0.5	0.0	0.5
9	PR0650-32	**1.0**	0.0	0.0	0.0	0.5	0.0	1.0	0.0	0.0
		0.5	**0.0**	**0.0**	1.0	0.0	0.0	**1.0**	**0.5**	**0.5**
		0.0	0.0	0.0	**1.0**	**0.5**	**1.0**	1.0	0.5	0.0
10	PR0650-34	0.0	0.0	0.0	1.0	0.5	0.0	1.0	0.0	0.0
		0.0	**0.0**	0.0	1.0	0.0	0.0	**1.0**	**0.0**	**1.0**
		1.0	0.0	**1.0**	**1.0**	**1.0**	**1.0**	0.0	0.0	0.0
11	PR0650-41	**1.0**	0.0	0.0	1.0	**1.0**	1.0	1.0	0.0	**1.5**
		0.5	**1.0**	0.0	1.0	0.0	0.0	**1.0**	**1.0**	1.0
		0.0	0.0	**1.0**	**1.0**	0.0	**1.0**	0.0	0.0	0.0
12	Morales	0.0	0.0	0.0	1.0	**1.0**	0.0	0.5	0.0	0.0
		0.0	**0.0**	0.0	1.0	0.0	0.0	**1.0**	**1.0**	**1.0**
		0.0	0.0	**1.0**	**1.0**	0.0	**0.5**	0.0	0.0	0.0
13	Verano	0.0	0.0	0.0	**1.0**	**1.0**	0.0	0.5	0.0	0.0
		0.0	**1.0**	0.0	1.0	0.0	**0.0**	**1.0**	**0.5**	**0.5**
		0.0	0.0	**1.0**	1.0	0.0	0.0	0.0	0.0	0.0
14	Amadeus 77	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.5	0.5
		0.0	**1.0**	0.0	**1.0**	**0.0**	0.0	**0.0**	**0.5**	**1.0**
		0.0	0.5	**0.5**	0.5	0.0	**0.5**	0.0	0.0	0.0
15	Carrizalito	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	**1.0**
		0.0	1.0	0.0	1.0	0.0	**0.0**	**1.0**	**0.5**	0.5
		0.0	**1.0**	**0.5**	**1.0**	**1.0**	0.0	0.0	0.5	1.0
16	Talamanca	0.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0
		0.0	**0.5**	0.0	**1.0**	**1.0**	0.0	**1.0**	**0.0**	**1.0**
		1.5	0.0	**1.0**	1.0	1.0	**1.0**	0.0	0.0	0.0
17	VAX 6	0.0	0.0	0.0	0.0	**0.5**	0.0	0.0	0.0	1.0
		0.0	**1.0**	0.0	**1.0**	0.0	0.0	**1.0**	**1.0**	**1.0**
		2.0	0.0	**0.5**	1.0	0.0	**1.5**	0.0	0.0	0.0

Appendix 2. Analysis of variance of infected root surface area (cm^2) evaluation of *P. vulgaris* lines

Source of Variation	Degrees of Freedom	October 2007 Mean Squares	DF	February 2008 Mean Squares	DF	June 2008 Mean Squares
Line	16	4140.47		1657.91		234.15
Isolate	8	3332.44		17774.96		97.84
Line x Isolate	128	2070.02		1776.88		57.42
Error	255	681.96	242	241.52	200	24.48
Total	407		394		352	
CV (%)		35.00		49.89		24.38

Appendix 3. Mean root rot scores of bean lines inoculated in the greenhouse in October 2007, February and June 2008 with different isolates of *Rhizoctonia solani*

		Root rot score means of bean lines inoculated with *Rhizoctonia solani* isolates								
Accession Number	**Identification**	**AG-1-IA**	**AG-1-IF**	**AG-4 (WB1)**	**AG-4 (WB2)**	**AG-4 (WB3)**	**AG-4 (RR1)**	**AG-4 (RR2)**	**AG-4 (RR3)**	**AG-1-IE**
1	PR0401-257	4.7	3.0	6.0	5.0	5.0	3.0	4.7	3.7	6.0
		3.0	5.7	6.0	5.7	**5.7**	**5.7**	5.3	4.0	5.7
		5.3	**6.0**	**6.5**	**7.0**	5.7	5.0	**6.3**	**6.3**	**6.7**
2	PR0401-259	5.0	3.7	3.3	5.0	6.0	5.0	5.3	5.7	5.0
		6.3	5.3	3.3	**6.3**	**6.0**	**7.0**	6.3	**6.7**	**6.7**
		6.7	**7.0**	**5.0**	5.3	6.0	6.3	**6.7**	5.7	6.7
3	PR0401-277	1.7	4.0	3.3	6.0	1.7	6.0	6.0	6.7	**5.3**
		6.3	6.0	**6.5**	**6.7**	**7.0**	6.0	4.0	**7.0**	4.3
		5.0	**6.3**	6.0	5.0	5.7	**6.3**	**6.7**	5.7	5.0
4	PR0518-10	5.3	6.0	5.7	5.3	6.0	5.5	**6.3**	**6.0**	**7.0**
		6.7	5.5	**6.0**	**6.0**	**6.0**	**6.0**	5.7	4.0	4.0
		7.0	**6.3**	6.0	5.0	5.0	5.0	5.7	5.7	5.0
5	PR0518-15	5.0	4.7	3.7	2.0	7.0	5.5	7.0	6.3	5.5
		6.3	3.4	5.7	**6.3**	**7.0**	6.0	6.7	6.3	5.0
		6.7	**6.7**	**6.3**	6.0	5.3	**6.3**	**7.0**	**6.5**	**5.5**
6	PR0518-16	3.7	6.0	6.3	**6.3**	**6.3**	5.0	6.0	**6.7**	**6.7**
		4.5	**6.3**	4.0	6.3	5.3	5.3	5.5	6.0	4.3
		5.3	5.0	**6.5**	5.3	6.0	**6.0**	**6.3**	6.0	6.3
7	PR0650-27	3.3	**5.0**	5.0	4.5	6.0	**6.7**	5.0	**7.0**	5.5
		5.3	4.5	6.3	6.0	6.0	4.0	**5.3**	5.0	5.0
		4.0	5.0	**6.5**	**6.0**	**6.3**	5.7	5.0	5.0	**5.5**
8	PR0650-31	5.3	**7.0**	**7.0**	**6.7**	**7.0**	**7.0**	**6.3**	6.5	**7.0**
		5.5	6.5	5.0	6.0	5.3	6.3	4.0	6.3	5.7
		6.0	6.0	5.0	6.3	5.0	5.7	6.0	**6.7**	5.5
9	PR0650-32	6.0	**6.3**	6.0	4.3	**6.7**	6.0	5.5	5.7	**6.0**
		5.0	5.3	1.2	6.3	5.7	5.0	3.5	**7.0**	5.7
		6.0	6.0	**6.0**	**6.5**	6.0	**6.3**	**6.0**	6.0	5.0
10	PR0650-34	**7.0**	5.0	6.0	6.0	6.3	5.7	**7.0**	**7.0**	**7.0**
		5.7	6.7	6.0	5.5	6.3	5.3	6.3	7.0	3.5
		6.5	**7.0**	**6.0**	**6.0**	**6.5**	**6.0**	6.0	6.0	6.3
11	PR0650-41	**7.0**	**7.0**	**6.3**	5.0	**6.5**	5.3	**7.0**	6.0	**6.0**
		5.5	6.3	6.3	5.0	3.0	**6.7**	5.0	5.0	5.5
		6.0	6.5	6.3	**6.5**	6.0	6.3	6.5	**7.0**	6.0
12	Morales	**7.0**	**6.3**	5.0	2.0	6.3	5.7	5.3	6.0	**7.0**
		6.0	5.0	6.0	0.5	5.5	6.0	5.0	6.5	6.5
		5.5	6.0	**7.0**	**5.5**	**7.0**	**7.0**	**6.7**	**7.0**	6.0
13	Verano	**7.0**	6.5	**7.0**	**7.0**	**6.0**	**7.0**	5.7	**6.5**	**7.0**
		5.5	5.5	4.5	6.0	5.3	6.3	5.5	6.3	6.3
		6.5	**6.7**	6.5	6.0	6.0	6.0	**6.5**	6.0	6.0
14	Amadeus 77	5.5	**6.7**	6.0	**6.3**	4.7	4.0	5.3	4.3	5.7
		6.3	5.0	6.3	6.0	4.0	6.5	6.0	5.0	6.0
		6.3	6.0	**6.3**	6.0	**6.0**	**6.5**	**6.0**	**6.3**	**6.3**
15	Carrizalito	3.0	3.7	6.3	3.7	4.5	5.3	5.3	3.0	**6.3**
		6.3	**6.7**	6.3	6.7	6.0	6.3	6.3	6.0	6.0
		7.0	6.3	**7.0**	**7.0**	**6.7**	**7.0**	**6.3**	**6.0**	6.0
16	Talamanca	5.5	6.0	5.0	**7.0**	**7.0**	6.3	5.0	5.3	**6.0**
		5.7	5.0	5.0	5.7	5.7	5.7	5.3	**6.0**	4.0
		4.0	**6.3**	**6.0**	5.0	5.5	**6.5**	**6.0**	5.0	4.0
17	VAX 6	**6.0**	**6.5**	**6.0**	6.0	**6.0**	**6.3**	4.0	5.3	5.7
		6.0	6.3	5.5	**6.3**	5.5	6.3	**6.0**	**6.5**	**6.3**
		4.0	4.0	6.0	5.0	5.0	5.7	5.0	5.0	5.0