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Identification of soil-borne pathogens in a common bean root rot nursery in Isabela, Puerto Rico[1,2]

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ABSTRACT

Limited research has been completed on the root rot complex of common bean (*Phaseolus vulgaris* L.) in the Caribbean, while yield losses of over 50% due to root rot disease have been reported worldwide. In this study, the predominant root rot pathogens in a 43-year old common bean root rot nursery in Isabela, Puerto Rico, were identified using standard and molecular diagnostic techniques over four planting periods. Evaluations were conducted from Dec. 2009 to Sept. 2012. The most prevalent fungi identified were *Fusarium solani*, causal agent of, Fusarium root rot; *Macrophomina phaseolina*, causal agent of charcoal rot; and *Sclerotium rolfsii*, which causes southern blight. *Pythium aphanidermathum* and *Pythium graminicola* were also identified during the 2012 evaluation, which cause damping-off and

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root rot. Other fungi, such as *Rhizoctonia solani*, were isolated from root and hypocotyl tissue with less frequency. The incidence of the predominant soil-borne pathogens was largely correlated across seasons. Low nitrogen levels in the soil, and low nitrogen, phosphorus, potassium, and magnesium in leaf tissue were identified in the nursery and have been achieved through no application of fertilizer. Knowledge of the prevalence of soil borne pathogens and fertility conditions will be used for targeting the selection of breeding materials at the Isabela root rot nursery and in other testing locations.

Key words: *Fusarium* spp., *Macrophomina phaseolina, Sclerotium rolfsii*, root rot complex, *Phaseolus vulgaris* L.

RESUMEN

Identificación de los patógenos predominantes en un vivero de pudrición radicular del frijol en Isabela, Puerto Rico

La investigación sobre el complejo de la pudrición radicular del frijol (*Phaseolus vulgaris* L.) ha sido muy limitada, mientras que mundialmente se han informado pérdidas en el rendimiento superiores al 50% debido a pudrición de raíz. En este estudio se identificaron los patógenos predominantes en un vivero de Isabela de 43 años de monocultivo de frijol, utilizando métodos de diagnóstico estándares y moleculares en cuatro ciclos de cultivo. Las evaluaciones se realizaron desde diciembre 2009 hasta septiembre 2012. Los hongos prevalentes identificados fueron: *Fusarium* spp., siendo *F. solani* el causante de la pudrición de raíz por Fusarium; *Macrophomina phaseolina*, que causa la pudrición carbonosa, y *Sclerotium rolfsii,* que causa el tizón sureño. *Pythium aphanidermathum* y *Pythium graminicola* también se identificaron durante la evaluación del 2012, causando el "damping-off" y pudrición de raíz. Otros hongos, como *Rhizoctonia solani*, se aislaron desde las raíces y el tejido del hipocótilo con menor frecuencia. La incidencia de los patógenos de suelo predominantes estuvo correlacionada a través de los ciclos evaluados. En el vivero se identificaron bajos niveles de nitrógeno en el suelo, y bajo nitrógeno, fósforo, potasio y magnesio en el tejido foliar; estos bajos niveles se debieron a que no se realizaron aplicaciones de fertilizantes. El conocimiento de la prevalencia de los hongos de suelo y las condiciones de fertilidad serán utilizados para la selección de materiales de fríjol para mejoramiento en el vivero de pudrición de raíz en Isabela, Puerto Rico, y en otros lugares de evaluación.

Palabras clave: *Fusarium* spp., *Macrophomina phaseolina, Sclerotium rolfsii*, pudrición radicular, *Phaseolus vulgaris* L.

INTRODUCTION

Soil-borne pathogens cause root rot disease in common bean (*Phaseolus vulgaris* L.), and are a major constraint to bean production worldwide (Abawi, 1989). Root rot diseases cause seedling death that leads to poor plant stands, and symptoms such as chlorosis and defoliation of leaves, reduced biomass and plant stunting, finally resulting in reduced seed yield (Abawi and Pastor Corrales, 1990; Estevez de Jensen et al., 2002; Park and Tu, 1994). Poor seedling emergence and disease incidence during flowering and pod fill result in the most significant

yield reduction. In addition, low or high temperatures, drought, and flooding can lead to more severe root rot. There have been limited efforts to identify the soil-borne pathogens present in production areas or in root rot nurseries at experiment stations in Puerto Rico. In addition, there has been little research on the root rot complex in common bean in the Caribbean.

In common bean, the primary diseases and pathogens associated with root rot include: Fusarium root rot, caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W. C. Snyder & H. N. Hans; southern blight, caused by *Sclerotium rolfsii* Sacc.; Rhizoctonia root rot, caused by *Rhizoctonia solani* Kuhn; Pythium root rot, caused by several species of *Pythium*; and Aphanomyces root rot, caused by *Aphanomyces euteiches* f. sp. *phaseoli* and f. sp. *pisi* (Abawi and Pastor Corrales, 1990). Stem diseases, often found in the same areas as these root-rot pathogens, include Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *phaseoli*; charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goidanich; whereas root-knot nematodes from the genus *Meloidogyne* can also occur. Specific pathogens are also associated with certain agroecological zones, with southern blight and charcoal rot occurring in tropical and sub-tropical areas (Beebe et al., 1981), and Aphanomyces root rot found mostly in temperate areas, whereas Fusarium root rot is the most pervasive disease and is found in both temperate and tropical areas (Schneider et al., 2001).

Pathogen composition of the bean root rot complex varies across locations due to many factors including differences in the soil, in the environment and in plant genotype. The effects of root rots are amplified when environmental and soil conditions, such as temperature, humidity, drought, excess water, and/or soil compaction occur with biotic sources of stress, such as insects and other diseases (Burke and Hall, 1991). Surveys of plant material or soil samples have been conducted in common bean in order to identify root rot pathogens across seasons and locations (e.g., Estevez de Jensen, 2000; Pieczarka and Abawi, 1978; Rusuku et al., 1997). These evaluations have identified differences in incidence and severity of pathogens across sites within a geographical zone. Sequential plantings and reduced soil fertility can increase root rot symptoms (Burke and Kraft, 1974; Rusuku et al., 1997), whereas production practices and soil conditions can result in considerable year to year differences (Abawi and Widmer, 2000). Variations in pathogen composition may also occur in the same field (Estevez de Jensen, 2000), across seasons where beans are planted after beans each year, and in locations where multiple plantings are conducted within a year.

In order to effectively evaluate the potential results from screening and selection efforts of common bean germplasm for root rot resistance, the identification of soil-borne pathogens present in root rot nurseries is important. The objective of this research was to identify the root rot pathogens, and to characterize the soil elemental composition and environmental conditions in a root rot nursery in Isabela, Puerto Rico.

MATERIALS AND METHODS

Nursery description

The root rot nursery at the Tropical Agriculture Experiment Station (TARS) in Isabela, Puerto Rico, is located at a longitude of 67.3°, a latitude of 18.3° and at 128 masl (meters above sea level), and was established in 1971 as a common bean root rot nursery through inoculation with a complex of pathogens including *F. solani*, *Pythium* spp., *R. solani*, and *Verticilium* spp. The soil type is an Oxisol series Coto. After inoculation of the approximately 0.3 ha field in 1971, the field has been maintained in almost constant common bean monoculture and without fertilization, in order to approach conditions present in marginal, low-input agricultural production environments. In addition, during the wetter summer season, the field is often waterlogged. Use of the field for screening and selection of common bean breeding lines resistant to root rots and abiotic stress conditions, has resulted in the recent releases of TARS-PT03-1 (Smith et al., 2005), TARS-SR05 (Smith et al., 2007), SB-DT1 (Porch et al., 2012), and TARS-LFR1 (Porch et al., in press).

Plant and soil analyses

A root rot susceptible kidney bean, packaged as Delmejor (Sucesores de Esmoris & Co., Inc., Mayagüez, PR), was planted in the first evaluation conducted in Nov. 2008, and diverse Mesoamerican cultivars, germplasm and breeding populations were planted and sampled for the Jan. 2009, Apr. 2009, and Sept. 2012 evaluations at the root rot nursery in Isabela, Puerto Rico. Root and stem samples presenting root rot symptoms were collected from 267 selected common bean plants between Dec. 2008 and Sept. 2012. A total of 267 samples were evaluated during the experiments. In Dec. 2008, 60 samples were collected; in Feb. 2009, 36 samples were collected; in May 2009, 41 samples were collected and in Sept. 2012, 130 samples were collected. Root and stem samples between the V1 to V3 stage were dug up, and placed in labeled plastic bags. For the first three evaluations, samples were transported on ice to the laboratory at TARS in Mayagüez, Puerto Rico, for processing. In 2012, samples were collected during the V3 stage and processed

at the Plant Disease Clinic at the Juana Diaz, University of Puerto Rico, Agricultural Experiment Station.

In 2009, sixteen leaf samples for elemental analysis were collected randomly in a "W" pattern from the root rot field; the samples were processed individually at the USDA-ARS in Mayagüez, Puerto Rico using the dry ash method (Perkin-Elmer, 1994) and inductively coupled plasma optical emission spectrometry (ICP-OES). The bulked soil sample was also collected in a "W" pattern and elemental analysis was conducted in a commercial laboratory (A&L Eastern Laboratories, Richmond, VA)[7].

Fungal isolation and identification

The common bean tissue samples from individual plants with root rot symptoms were processed by washing roots with tap water to remove soil and then cutting small pieces of roots and stems near visible areas of necrosis or lesions. Plant tissue was then surface sterilized in three treatments of one minute each. The first solution was 20% commercial bleach, the second was 70% ethanol, and a final wash was with sterile distilled water. The excised root and stem segments were transferred to Petri dishes containing potato dextrose agar (PDA) (Bacto™, Difco Laboratories, Maryland, USA) amended with 25% lactic acid. Plates were incubated at 27° C for seven days. Fungal colonies and hyphae growing from the root and crown segments were later purified by subculturing them in media-miser dishes containing V8 juice agar and PDA to promote fungal sporulation. Plates were then incubated for 4 to 5 days at 27° C. In 2012, acidified PDA, water agar and corn meal agar amended with pimaricin (10 mg/L), ampicillin (250 mg/L), rifampicin (10 mg/L) and pentachloronitrobenzene CNB (100 mg/L) were used (Singleton et al., 1993). The fungal isolates were identified based on cultural and morphological characteristics using taxonomic keys (Barnett and Hunter, 2006; Nelson et al., 1983; Watanabe, 2002).

Fungal specific protocols were conducted for specific isolates. During the 2012 evaluation, *Fusarium* spp. were single spored and transferred to fresh PDA media. For *Fusarium* spp. identification, cultures were grown in carnation leaf agar incubated at 22° C in 12 hour light/dark for 15 days. To observe fungal reproductive structures, semi-permanent microscope slides were prepared from each pure fungal colony using lactophenol and/or a 1:1 ratio of lactophenol:cotton blue. Microscopic fungal reproductive structures and hyphae were ob-

[7]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

served at 40X using a light microscope. In 2008 and 2009, an ELISA kit (Agdia, Elkhart, Indiana) was used to diagnose *Pythium ultimum* from roots. In the 2012 evaluation, *Fusarium* and *Pythium* species were confirmed using DNA sequence analysis. DNA of the pathogens was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Amplification of the internal transcribed spacer ribosomal DNA (ITS rDNA) of collected and representative *Fusarium* spp. isolate Fs-1748-2012 and sequencing of the internal transcribed spacer ribosomal DNA (ITS rDNA) region was carried out by the USDA-ARS Vegetable and Forage Crops Research Unit, Dry Bean Breeding and Genetics Program (Prosser, WA). For *Pythium* spp. representative isolates Py-1767-2012 and Py-1768-2012, DNA extraction and amplification of the ITS rDNA were conducted at the Plant Disease Clinic at the Juana Diaz Experiment Station of the UPR and sequencing was performed at a commercial facility (Macrogen Ltd., Rockville, MD). The resulting DNA sequences were queried on NCBI Genbank using the BLAST tool (www.ncbi.nih.gov/blast). Identification of the pathogen was based on nucleotide sequence similarity. Incidence of each pathogen was calculated as the percentage of samples collected, harboring a specific pathogen, during each evaluation. Correlation analysis was conducted to compare pathogen incidence (%) between each evaluation by calculating the Pearson correlation coefficient.

Pathogenicity tests

In 2013, pathogenicity tests were conducted at the UPR Agricultural Experiment Station in Juana Diaz on the *Fusarium* spp. and *Pythium* spp. pathogens collected in 2012. Representative *F. solani* single spore isolate Fs-1748-2012 was multiplied on PDA and then on sterilized millet seed. Millet was sterilized for two consecutive days for two hours after being submerged in water for one hour. Five hundred grams of sterilized millet was inoculated with 10 pieces of PDA from a two week old *F. solani* culture. The inoculated millet was incubated for one week at room temperature using a 12 hour light/dark cycle. Seeds were inoculated with millet grown inoculum mixed with sterile sand in a 1:10 ratio by volume. For *Pythium* spp., inoculum was multiplied in sterilized millet and the inoculum was mixed with sand in a 1:10 ratio by volume, as described above. Twenty common bean seeds of 'Verano' (Beaver et al., 2008) were planted in the inoculated substrate whereas ten healthy 'Verano' seeds were planted as the control. After germination, plants were observed daily and at 21 days, the plants were evaluated for symptoms and pathogens were reisolated on PDA to fulfill Koch's Postulates.

RESULTS

Plant and Soil Analysis

Based on the range of values reported by Mills and Benton Jones (1996), bean plant tissue samples from the root rot nursery had nutrient concentrations below the sufficiency range for nitrogen, phosphorus, potassium and magnesium (Table 1), thus indicating the presence of low fertility stress in addition to biotic stress. In addition, the bulked soil analysis showed low plant available levels of nitrogen and magnesium, and medium levels of phosphorus and potassium. Average soil pH was 6.7, close to neutral. In Isabela, Puerto Rico, the amount of precipitation varied seasonally, corresponding to the historical trend of drier and moderate temperature winters and wetter and high temperature summers with the first and fourth trials being the wettest. There was a small, 1° C, average temperature difference between the 2nd, and the 1st and 3rd evaluations, while the 4th evaluation was substantially warmer, averaging about 2.0 to 3.0° C higher than the others (Table 2).

Fungal isolation and identification

Using standard diagnostic techniques, *Fusarium* spp. was identified to the genus (Nelson et al., 1983; Watanabe, 2002) in the 2008 and 2009 evaluations, characterized by boat-shaped macroconidia with slightly curved apical cells. In 2012, *Fusarium* isolates were identified to the species, with *F. solani* isolates grown on PDA producing white-cream mycelia. Microconidia were slow growing, but abundant, and had an oval to kidney shape, borne on long monophialides, with an average size of 67.8 x 3.2 uM. Microconidia size averaged 12.7 x 4.2 uM. Macroconidia had thick walls with a round apical end and basal foot, with an average size of 30.1 x 4.2 uM.

Pythium ultimum was not detected in the second and third evaluation using ELISA tests, but using molecular diagnostics in 2012, *P. graminicola* Subraman and *P. aphanidermathum* Edson were identified. These two species would not have been identified using *P. ultimum* ELISA test. *Macrophomina* spp. pycnidia measured 0.5 to 0.8 mm after 7 to 10 days of incubation at room temperature. Isolate ISATARS-2011 was characterized by black globose picnidia with ostioles. Fungi isolated from common bean plants with girdling of the stem near the soil interface and mycelium around the stem were identified as *Sclerotium rolfsii*. These isolates on PDA media were characterized by a fast growing white hyphae and subsequent development of dark brown globose sclerotia measuring 1.5 to 3.3 mm after 5 to 7 days.

TABLE 1.—*Elemental analysis of leaf and soil samples[1] from the USDA-ARS root rot nursery in Isabela, Puerto Rico.*

	Elemental analysis of common bean tissue based on 16 samples							
Variable	N	P	K	Ca	Mg	Fe	Mn	Zn
	%					ug/g		
Mean	0.78	0.049	0.99	1.3	0.21	2833	97.2	53.8
SD	0.25	0.04	0.33	0.20	0.02	1633	34.5	3.2
Sufficiency range[2]	4.8-5.5	0.32-0.42	2.4-3.2	0.5-0.75	0.38-0.42	—[3]	—	—
	Elemental analysis of bulked soil sample							
	ENR[4] kg/ha	P	K	Ca	Mg	Fe	Mn	Zn
		mg/kg						
Bulked sample	67.3 L[5]	44 M	130 M	3012 VH	157 L	82 VH	332 VH	3.8 H

[1]Leaf samples evaluated at the USDA-ARS-Tropical Agriculture Research Station in Mayagüez, Puerto Rico, whereas the soil samples were evaluated at A&L Eastern Laboratories (Richmond, VA, USA).

[2]The sufficiency range indicates the level of each nutrient for normal common bean plant growth; values outside the range could affect growth (Mills and Benton Jones, 1996).

[3]Data not available for this element.

[4]ENR, Estimated nitrogen release.

[5]L, low; M, medium; H, high; VH, very high; ratings for each value based on plant available nutrients in the soil.

TABLE 2.—*Rainfall, and average, average maximum, and average minimum temperatures at the USDA-ARS root rot nursery in Isabela, Puerto Rico, in four field evaluations in 2008, 2009 and 2012 based on two-month cumulative rainfall and daily temperature averages.*

	Trial Period			
Characteristic	1 Nov. to Dec. 2008	2 Jan. to Feb. 2009	3 Apr. to May 2009	4 Aug. to Sept. 2012
Rainfall (mm)	139.7	55.9	106.7	175.0
Average Temp. (°C)	23.6	22.6	23.8	25.7
Average Max. Temp. (°C)	27.0	26.1	27.1	29.7
Average Min. Temp. (°C)	20.5	19.2	20.7	22.2

Rhizoctonia solani was characterized by a pale brown/beige color and the right-angle branching of the hyphae (Watanabe, 2002), and identified to the species and anastomosis group level (AG4) using AG specific PCR primers (Godoy-Lutz et al., 2008). DNA sequence similarity analysis of the ITS rDNA region using Genbank's BLAST tool identified the Fusarium isolate Fs-1748-2012 as *F. solani*, whereas *Pythium* spp. isolates Py-1767-2012 and Py-1768-2012 were identified as *P. graminicola* and *P. aphanidermathum*, respectively.

Pathogenicity Tests

Pathogenicity testing of the ISA-TARS-2011 isolate, identified as *M. phaseolina* using standard diagnostic techniques, was completed in 2013 on three common bean genotypes: BAT 477 (resistant), 'Verano' (intermediate), and G122 (susceptible). Based on the resulting symptoms, the isolate was confirmed as *M. phaseolina*. Pathogenicity tests showed that *Fusarium* and *Pythium* species isolates obtained during 2012 were able to infect seedlings of 'Verano' with different degrees of severity. Symptoms were observed five days after inoculation as reddish specks in the hypocotyl that later developed into large lesions that coalesced. Isolates identified as *F. solani* were found to be most virulent on 'Verano' seedlings, producing plant death 21 days after inoculation. The isolates identified as *P. graminicola* produced root rot while the isolates identified as *P. aphanidermathum* produced damping-off symptoms.

DISCUSSION AND CONCLUSIONS

Fusarium spp., *M. phaseolina*, and *S. rolfsii* were found with the greatest frequency in the samples collected from the root rot nursery over four evaluation periods from 2008 to 2012 (Table 3). Although not identified

TABLE 3.—*Fungi identified in the USDA-ARS root rot nursery in Isabela, Puerto Rico, using diagnostics as the percentage incidence in the total root samples evaluated from common bean plants in four field evaluations in 2008, 2009, and 2012.*

	Trial period (Sample collection date)			
	1 Dec. 2008	2 Feb. 2009	3 May 2009	4 Sept. 2012
Fungi	%	%	%	%
Acremonium spp.	0	11	0	0
Alternaria spp.	2	0	0	0
Aspergillus spp.	2	8	7	0
Colletotrichum spp.	0	8	0	0
Curvularia spp.	5	17	0	0
Fusarium spp.	10	75	43	65
Macrophomina phaseolina	2	47	50	11
Nigrospora spp.	5	3	0	0
Penicillium spp.	0	3	10	0
Phoma spp.	2	0	0	0
Pythium spp.[1]	—	0	0	15
Rhizoctonia solani	5	3	2	2
Sclerotium spp.	23	19	36	0
Trichoderma spp.	2	22	7	0
Unknown	5	14	0	0

[1]*Pythium ultimum* was evaluated in 2009 using ELISA (Agdia, Indiana, USA); in 2012, *Pythium* spp. were isolated from diseased tissue.

to the species level in the 2008 and 2009 trials, on the basis of 2012 findings the prevalent pathogen was *F. solani*, in 26% of the samples isolated. Fusarium root rot is one of the most prevalent diseases of common bean in the world and it is particularly common and severe in bean productions countries in South America, Central America and Mexico, the Caribbean, and Eastern and Southern Africa (Abawi and Pastor-Corrales, 1990). Charcoal rot disease, caused by *M. phaseolina*, is also prevalent in many bean production countries in Latin America and Africa and particularly in areas with periods of drought and high temperatures. Similarly, southern blight, caused by *S. rolfsii*, occurs most often in warm tropical and subtropical bean-producing areas (Abawi and Pastor-Corrales, 1990).

Lower incidence of other pathogens, such as *R. solani*, may be due to lower prevalence since this species is favored by high moisture in the soil and moderate temperatures. Although the nursery was initially inoculated with *Pythium* spp. in 1971, *Pythium* spp. were not isolated on PDA, nor detected in the samples using ELISA (specific to *P. ultimum*)

TABLE 4.—*Correlation matrix, using the Pearson correlation statistic, based on the percentage of fungi identified in the USDA-ARS root rot nursery in Isabela, Puerto Rico, using diagnostics in four field evaluations in 2008, 2009, and 2012.*

Evaluations	1 Dec. 2008	2 Feb. 2009	3 May 2009	4 Sept. 2012
1 (Dec. 2008)	—	0.30	0.50	0.25
2 (Feb. 2009)		—	0.82	0.82
3 (May 2009)			—	0.58
4 (Sept. 2012)				—

in trials from 2008 to 2012. However, in 2012, *Pythium* was isolated from hypocotyl tissue. *Pythium* spp. are favored by high soil moisture. The effect of temperature varies depending on the species involved, with *P. ultimum* most prevalent at lower temperatures, and *P. myrotylum* and *P. aphanidermatum* at higher temperatures (Martin and Loper, 1999). Higher temperatures in Puerto Rico favored *P. graminicola* and *P. aphanidermathum*; both were found causing root rot and damping off, respectively.

Consistency in disease incidence was also noted in this study, particularly in the case of *Fusarium* spp. and *M. phaseolina*. The highest incidence of *Fusarium* spp. and *M. phaseolina* occurred in 2009 and 2012. Correlation was found across the four evaluations based on the percent of samples harboring each pathogen. Notable are particularly high correlations of 0.82 between the second and the third evaluation and the second and the fourth evaluations, even though the environmental conditions were variable (Table 4). Pathogen incidence did not appear to be associated with soil humidity or ambient temperatures, since there was a high correlation both between the cooler and drier evaluation periods (Evaluations 2, 3) and between these and the high temperature and the higher rainfall evaluation period (Evaluation 4). The first evaluation showed lower levels of correlation, however, when compared with the other trials. This result may be due to the use of a single common bean Andean host genotype in the first evaluation, whereas during the other evaluations a diverse set of Mesoamerican common bean genotypes were used. It is possible that different frequencies of pathogens could be identified on different germplasm depending on resistance gene composition, genetic diversity and/or gene pool or race background.

Fusarium spp. are widespread soil-borne pathogens of common bean production areas whereas *M. phaseolina* and *S. rolfsii* are most commonly found in dry and humid areas, respectively, of the tropics and subtropics. Thus, common bean material selected from this

nursery may need to be tested in other locations in order to screen for different root rot pathogens, such as *Pythium ultimum* and Aphanomyces root rot, and to ensure resistance under variable climatic conditions, as has been accomplished by some breeding programs (Smith et al., 2007). Screening of root rot resistant germplasm under abiotic stress conditions can result in the identification of multiple stress tolerant material (Porch et al., 2012). For example, selection for disease resistance may contribute to drought tolerance because of the importance of diseases caused by *M. phaseolina* and *F. solani* commonly observed under drought stress conditions (Singh et al., 2001). Thus, the nursery can be used in the dry season to select for tolerance to drought and resistance to root and stem diseases caused by *F. solani* and *M. phaseolina*. In addition, breeding lines in this nursery can be selected throughout the year for tolerance to low soil fertility, although additional testing would be required to confirm this trait.

Management of root rots can be achieved to some extent through crop rotation, improving soil tilth, use of cover crops, improving soil fertility (Abawi and Wilmer, 2000), fungicides applied to soil and seeds, adjusting planting time and depth, and use of biocontrol agents such as *Bacillus subtilis* (Estevez de Jensen et al., 2002). The development of cultivars resistant to root rot diseases is an important tool in the management of the root rot complex, as the disease can rarely be completely eradicated from a field, and chemical treatments can be expensive or unavailable for resource-poor farmers. Breeding beans for adaptation to specific environments should target the prevalent root rot diseases, especially considering their interaction with climatic and edaphic constraints. In addition to seasonal variability, long-term changes in temperature and rainfall patterns could have an effect on the incidence and severity of root rot disease in crops (Garrett et al., 2006). Few bean germplasm lines and cultivars have been developed that possess high levels of root rot resistance, thus continued crop improvement is needed for this important constraint. The evaluation of breeding lines for the root rot complex in the target environment should facilitate the improvement of common bean for specific agroecological zones.

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