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Identification of sources of resistance to damping-off and early root/hypocotyl damage from *Rhizoctonia solani* in common bean (*Phaseolus vulgaris* L.)

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Abstract

Rhizoctonia solani causes economically important root and hypocotyl diseases in common bean throughout the world. Root health is a vital factor in plant development and root diseases would negatively influence water and nutrient uptake as well as cause direct stand reduction and root rot damage to the crop. An efficient common bean screening method to evaluate damping-off and early root/ hypocotyl damage from *R. solani* was developed and used to identify dry bean lines with levels of resistance to this disease. Two sets of 163 and 111 lines previously evaluated for drought tolerance in Nebraska and Puerto Rico were evaluated for damping-off resistance and early root/hypocotyl damage under greenhouse conditions. Disease severity on plants was identified based on above-ground symptoms, seedling survival and root lesions using a rating scale of 1 (resistant) to 9 (susceptible). In the first set of lines representing commonly grown dry bean cultivars, germplasm and sources of damping-off resistance, the Rhizoctonia mean rating ranged from 1.7 to 3.9; *Phaseolus vulgaris* lines PI 310668 and PI 533249 had the highest damping-off resistance. In the second set of the best lines from a drought tolerance shuttle breeding program the Rhizoctonia mean rating was between 2.6 and 5.7. The availability of drought tolerant dry bean lines allowed the testing of the hypothesis that there was a correlation between selecting for drought tolerance and *R. solani* damping-off resistance. No correlation between mean disease rating and drought tolerance was found, but adapted dry bean lines such as NE14- 08-176 released as SB-DT1, and NE14-08-225 were identified with moderate damping-off resistance and drought tolerance. Lines with both traits and other attributes will facilitate development of resistant bean cultivars to manage damping-off caused by *R. solani*.

Keywords: Root health, Drought tolerance, Fungal disease, Screening method, Isolates

1. Introduction

The most important economic species of the genus *Phaseolus* sp is the common bean (*Phaseolus vulgaris* L.) which is widely grown and arguably the most important grain legume for direct human consumption (Islam et al., 2002). Different factors affect bean production including the presence of the basidiomycete fungus *Rhizoctonia solani* Kühn, which is one of the most economically important causes of root and hypocotyl diseases in the world (Nerey et al., 2010; Sikora, 2004). This pathogenic fungus has a very wide host range that includes common bean (Venegas, 2008), sugar beet (Bolton et al., 2010), corn, rice (Gonzalez-Vera et al., 2010), potato (Aliferis and Jabaji, 2012), tomato (Baker and Martinson, 1970), cauliflower (Pannecoucque and Hofte, 2009) and cotton (Howell et al., 2000), causing damping-off, root rot, crown blight, fruit rot and leaf blights such as web blight (Godoy-Lutz et al., 2003). *R. solani* represents a threat to the dry bean and snap bean growers in the USA, especially because in some production areas such as the central high plains and North Dakota, Michigan and Minnesota dry beans are grown in a rotation with sugar beets (Franc et al., 2001; Miller et al., 2004). This crop is also attached by *R. solani* and thus may increase the amount of soil inoculum over time since the same AG groups infect both hosts (Engelkes and Windels,1996; Ruppel, 1985; Sumner, 1985). This threat may also occur in other bean production areas of the world where other hosts are grown in rotation with beans. Under favorable environmental conditions *R. solani* can reduce plant

stands by causing damping-off and early root/hypocotyl damage (D'Aes et al., 2011). Infection can occur in a range of 25–100% MHC (moisture-holding capacity) and a temperature range of 16 °C–25 °C (Bolton et al., 2010; Baker and Martinson, 1970). However, some isolates can be highly virulent up to 32 °C (Schultz and Bateman, 1969). Furthermore, with increasing concerns of availability of water from irrigation or in rainfed bean production areas, more attention has been given to increasing drought tolerance in *P. vulgaris* (Blair et al., 2012; Cortes et al., 2012; Rosales et al., 2013; Singh, 2007;Urrea et al., 2009). Without healthy roots the efficacy of germplasm that has been selected for production using less water would be challenged. A shuttle breeding program between Nebraska and Puerto Rico has been developed to improve drought tolerance (Urrea and Porch, 2010) and now these drought tolerant lines have been tested for levels of *R. solani* resistance.

Methods of screening for resistance to *R. solani* in beans have been reported for field trials (Abawi et al., 2006; Abawi and Pastor-Corrales, 1990). However, most fields will have a mixture of root rotting organisms even when inoculated with a single pathogen, and although they are more relevant to overall production losses, they make breeding for a trait like damping-off and Rhizoctonia root rot resistance more complicated (Abawi et al., 2006). Greenhouse screening can simplify the selection of resistance for damping-off and early root/hypocotyl damage. A single pathogen isolate can be tested by using pasteurized soil. Various methods for infesting soil to evaluate root rot in common bean have been reported (Abawi et al., 2006), but these methods are not as useful for evaluating root damage on seedlings. Thus, an efficient and inexpensive screening method is needed to identify germplasm with levels of resistance to damping-off and early root/hypocotyl damage caused by *R. solani* at early stages of bean development.

Genetic diversity and pathogenicity of *R. solani* anastomosis groups (AGs) in dry bean production fields in Latin America (Argentina, Costa Rica, Cuba, Dominican Republic, Panama, Honduras, Puerto Rico) and the United States (Minnesota, North Dakota, New York, Nebraska) have been reported (Godoy-Lutz et al., 2003; Engelkes and Windels, 1996; Ohkura et al., 2009; Venegas, 2008). Among 59 isolates collected from 60 to 75-day-old plants in western Nebraska dry bean fields, 34% were AG-4 and 34% were AG-2-2 (Harveson, R. M. Personal communication; Venegas, 2008). These two AGs were highly associated with bean root and crown rot, especially the subgroups AG2-2 IIIB and AG4-HG III. Isolates from AG2-2 IIIB cause diseases in rice, ginger, corn (Sneh et al., 1991), sugar beet (Bolton et al., 2010), and cauliflower (Pannecoucque and Hofte, 2009) and AG4 including HG III isolates cause diseases in pine, sugar beet, common bean, soybean, alfalfa (Anderson,1982), tomato, potato, onion, snap bean, cotton and peas (Sneh et al., 1991). Isolates from these groups collected in western Nebraska were selected as screening isolates for damping-off and early root/hypocotyl damage in common bean germplasm. Furthermore, drought tolerance or increased water use efficiency is a component of the Nebraska and other national dry bean breeding programs. Crop productivity is highly disturbed by drought, it affects bean yield on 60% of the world bean production fields (Urrea et al., 2009), and in western Nebraska, recent water shortages for irrigation have demonstrated the need for bean cultivars with improved water uptake in this semi-arid environment. Drought also may aggravate development of certain diseases associated with plant stress and a healthy root system free from severe root diseases will be necessary for improving water and nutrient uptake (Ogoshi, 1996; Miklas et al., 2006). For instance, drought tolerant genotypes have been associated with Fusarium root rot, as well as charcoal rot, a disease caused by *Macrophomina phaseolina* in common bean (Miklas et al., 2006; Pastor-Corrales and Abawi, 1988; Frahm et al., 2004). However, associations between root diseases caused by *R. solani* and drought tolerant genotypes in common bean have not been reported.

The objectives of this research were to (1) develop an efficient and inexpensive greenhouse screening method to identify sources of resistance to damping-off and early root/hypocotyl damage caused by *R. solani* at early stages of development in common bean, (2) determine if there is a correlation between drought tolerance and damping-off resistance, and (3) identify sources of resistance to damping-off and early root/hypocotyl damage in lines from a dry bean breeding program, the National Plant Germplasm System, and a shuttle breeding program between Nebraska and Puerto Rico previously evaluated for drought tolerance.

2. Materials and methods

2.1. Development of screening method

In order to develop an efficient screening method for selecting damping-off and early root/hypocotyl damage resistance, preliminary studies were conducted to determine the inoculum substrate and concentration of initial inoculum. Substrates evaluated were water agar in petri dishes or small containers, and soil in pots. Seeds of bean lines were placed on Difco potato dextrose agar (PDA) and inoculated with a 6 mm plug of *R. solani* mycelium from a 4- day-old water agar plate placed in the middle of the agar surface surrounded by four seeds equally spaced. A method published by Abawi et al. (2006) using autoclaved potato pieces as inoculum also was tested in soil. The two types of initial inoculum were 100 g of potato pieces and 20 plugs of the *R. solani* isolate grown on PDA in 1000 cc of soil. Concentration of initial inoculum was 100 cc of the soil/inoculum mixture placed in clean pasteurized sandy loam soil in the following proportions 1:5, 1:10, and 1:100.

Five bean lines were selected to test the screening method. Two of the lines, Pinto UI 114 and Morales were susceptible, and three were previously reported to have root rot resistance: TARS 08107- 3299, TARS 08109-3409, TARS 08114-4351 from USDA-ARS Tropical Agriculture Research Station, Puerto Rico (Porch, personal communication).

2.1.1. Pathogen isolates

Three isolates of *R. solani*, WN-11 (AG2-2 IIIB), WN-116 (AG2-2 IIIB) and WN-293 (AG4-HG III) collected by R. Harveson from 60 to 75-day-old bean plants in western Nebraska bean fields and characterized by Venegas and verified by sequence in GenBank as FJ492114.2, FJ492146.3 and JQ66932.1, respectively, were selected for this study (Harveson, R.M. personal communication; Venegas, 2008). The isolates were stored in sugar beet seeds, and activated by placing seeds on water agar (Difco, Detroit, MI, USA) to induce fungus growth and detect contamination by bacteria or other fungi. After 3–5 days of fungal growth at 22 ±1 °C, a 6mm agar plug with mycelium was transferred to a Petri dish of PDA and incubated for 3–5 days at 22 ±1 °C to increase the amount of inoculum for each isolate. A mixture of 100 cc of pasteurized soil (3 parts soil and 1 part sand to simulate sandy loam field soil in western Nebraska), 30 ml of autoclaved Difco potato dextrose broth and 20 6mmplugs of *R. solani* taken from the margins of the 4-day-old PDA culture were placed in a deep Petri dish. After 15 days of incubation at 22 ±1 °C the contents of each deep Petri dish was mixed with pasteurized soil in a ratio 1:10 (one petri dish per 900 cc of soil). Five seeds of each bean line were planted in a 10 cm pot and covered with 100 cc of the infested soil inoculum. Two sets of host plants were grown at two different environments, a growth chamber at 19 ±1 °C and a greenhouse at 26 ±1 °C, to determine the appropriate environment to promote damping-off and root damage and to identify

an isolate that causes a similar disease reaction in both environments. Plants were watered daily to maintain field capacity. No nutrients were added. Plants were removed from the pots 15 days after inoculation and the roots were washed gently. Each plant was scored for damping-off severity and early root/hypocotyl damage using the CIAT 1–9 rating scale based on plant symptoms and root lesions (Fig. 1) with ratings 1–3 resistant, 4–6 intermediate and 7–9 susceptible (Venegas, 2008).

2.1.2. Experimental design

The experiment was designed to measure the effect of three fixed factors: environment (2), bean line/cultivar (5) and pathogen isolate (3) where the number of levels for each factor is given in parenthesis. The experiment was conducted as a randomized complete block design with a 5 × 3 line-by-isolate factorial arrangement with four blocks in each environment. For each block, there were 15 pots of five plants each. The response measured was the mean of the Rhizoctonia damping-off severity and early root/ hypocotyl damage score of five plants per pot.

All the main effects, two-way and three-way interactions were analyzed starting from the highest level of interactions to the main effects. If there were no interaction effects, the main effects means were separated with an LSD. If any interaction was significant, simple effects were tested with *t*-tests. The analysis of variance was done using SAS® 9.2 Software.

2.2. Identification of sources of resistance

2.2.1. Plant material

Two sets of bean germplasm, lines from the National Plant Germplasm System (NPGS) and lines from the Nebraska and other USA breeding programs, were analyzed separately to identify sources of resistance to Rhizoctonia damping-off severity and early root/hypocotyl damage. The first set was coded as NE-08, which contained 164 dry bean lines; composed of 79 experimental dry bean lines from different USA bean breeding programs, 24 current dry bean commercial cultivars, 29 tepary beans (*Phaseolus acutifolius* A. Gray), and 31 dry bean germplasm accessions from the NPGS. Pinto UI-114 was used as the susceptible control. These NPGS accessions were previously selected for photoperiod insensitivity in Puerto Rico and Nebraska. The lines were also screened for drought tolerance under terminal drought stress conditions at Mitchell, NE in 2008 and Fortuna, PR in 2009, where irrigation was stopped at the flowering stage (Urrea and Porch, 2010). The second set coded as NE-14 was composed of 111 lines from an on-going shuttle-breeding program between Nebraska and Puerto Rico, initiated in 2007. These lines were also screened for drought tolerance under the same conditions used for the NE-08 (Urrea and Porch, 2010).

2.2.2. Inoculum preparation and inoculation

Isolate WN-11 (AG2-2 IIIB) of *R. solani* was increased from storage in sugar beet using the same method and conditions as previously described. The inoculum preparation and the inoculation procedure also was the same as described in the previous section. Plants were grown in the greenhouse at 24 ±3 °C for 15 days, and were watered daily to maintain field capacity, without fertilizer. Fifteen days after inoculation, plants were removed from the pots and the roots were washed gently. Each plant was scored for root rot using the 1-9 CIAT scale (Van Schoonhoven and Pastor- Corrales, 1987).

2.2.3. Experimental design

Each set of lines planted in the greenhouse was arranged and analyzed independently in an alpha lattice design. For the NE-08 set, the 164 treatments were each of the 163 lines plus the control, one treatment per pot of five seeds and four pots per incomplete block for a total of 41 incomplete blocks for each of four replications. For the NE-14 set, there were 112 treatments with 111 lines and one control (Pinto UI-114), with one treatment per pot of five seeds and four pots per block for a total of 28 incomplete blocks with four replications. The first two replications for NE-08 and NE-14 were conducted during Fall 2009 and the last two replications during Spring 2010. In each set of lines, all the experimental units

Fig. 1. Screening scale from 1 to 9. 1. No visible symptoms. Normal plant development. 2. 10% root infection. Small (3 mm) superficial root lesions. Normal plant development. 3. 10– 20% infection. Small (3–5 mm). superficial lesions surrounding hypocotyls or roots. Normal plant development. 4. 20–35% infection. Small (3–5 mm) deep lesions surrounding hypocotyls or roots. Normal plant development. 5. 35–50% infection. Deep (3–5 mm) lesions surrounding hypocotyls or roots. Secondary roots and plant development reduced. 6. 50–65% infection. Deep (5–10 mm) lesions surrounding hypocotyls or roots. Few secondary roots visible. Plant development highly reduced. 7. 65–80% infection. Deep (10 mm) lesions surrounding hypocotyls or roots. Few or no secondary roots visible. Elongation of hypocotyl, and no formation of first trifoliolate leaf. 8. 80–95% infection. Emergence followed by loss of cotyledon and absence of secondary roots. 9. 95–100% infection. Seed dead. No emergence (Based on Van Schoonhoven and Pastor-Corrales, 1987).

(pots) were randomly assigned to treatments in an alpha lattice design. The 15 most resistant lines identified from the NE-08 and NE-14 sets were tested again in a randomized complete block design with four replications to confirm the damping-off severity and early root/hypocotyl damage ratings during Fall 2010. Data was analyzed using a mixed model analysis of variance assuming lines as fixed and incomplete blocks as random.

Since both sets of lines were previously screened for drought tolerance in Nebraska and Puerto Rico (Urrea and Porch, 2010), the root rot variable was also correlated with the geometric mean of the drought variable (defined as the square root of the product of mean seed yield of a line under drought stress by the mean seed yield of a line under non-stress) using Pearson's correlation. The correlations included the combined data from the analysis of drought tolerance as well as per location. The analysis of variance and the correlations were conducted using SAS® 9.2 Software.

3. Results

3.1. Development of screening method

Growing bean seeds in inoculated soil were more successful than growing bean seeds in plates. This was due to high levels of contamination, limited space available for growing the seeds on agar, and high variability between plates; thus this method was not used for screening. Soil inoculum placed in 10 cm pots was selected for all screening experiments.

Potato dextrose broth was used in the soil mixture instead of potato pieces in preparation of the final inoculum because it produced more uniform and consistent infection. The 1:100 ratio of inoculum to soil failed to produce disease on any of the five cultivars inoculated with the three isolates, while the 1:5 inoculum to soil ratio caused severe to lethal damage to the seedlings. Thus, the 1:10 inoculum to soil ratio that produced a consistent range of disease severities was used for evaluation of the lines.

The analysis of the data from the bean line, isolate and environment effect experiment indicated no significant three-way interactions (P = 0.3526). There was, however, a significant interaction between lines and isolates (P < 0.0001), which was expected due to the differences between their genetic backgrounds, origins and damping-off severity and early root/hypocotyl damage resistance. There also was a significant interaction between environment and isolates (P = 0.0002). Isolate WN-11 (AG2-2 IIIB) displayed a wider range of disease reactions among the cultivars compared to isolates WN-116 (AG2-2 IIIB), and WN-293 (AG4-HG III) (data not shown). WN-11 also produced similar disease reactions in both environments (data not shown).

3.2. Identifying sources of Rhizoctonia damping-off resistance

All 164 lines from the NE-08 experiment were screened for damping-off and early root/hypocotyl damage resistance (data not shown). The analysis of variance of the alpha lattice design indicated that there were significant effects of lines and replications (P < 0.0001). The overall mean of the damping-off/early root/hypocotyl damage rating was 7.0, indicating that these 164 lines were generally susceptible to this pathogen. Lines with partial resistance were identified based on the rating and most of them were germplasm accessions from the NPGS (Table 1). The lines with the lowest ratings for damping-off exhibited mostly intermediate resistance with ratings ranging from 3.0 to 5.0 and were from Middle America with a growth habit type III or II. Black seed color was the predominant seed class followed by cream, red and light brown classes. The fifteen lines with the lowest scores were tested again in a RCBD to confirm their intermediate resistance reaction (Table 1). The most resistant *P. vulgaris* lines were PI 310668 and PI 533249 (Table 1). The overall mean rating (3.3) was nearly one-half the mean from the 164 lines experiment since there were no susceptible lines in this experiment. There were significant differences between lines but not between replications. Ten of the 164 lines were identified as sources of partial resistance to damping-off with drought tolerance (Table 2). In the second test PI 311807 had the lowest rating.

Table 1. Origin, growth habit, seed color and Rhizoctonia damping-off score of *Phaseolus vulgaris* partially resistant lines from set NE-08.

Line	Species/country	Origin[a]	Growth habit[b]	Seed color	RRR score Mean[c]	Mean[d]
PI 310668	*P. vulgaris*/Guatemala	NPGS	II	Black	3.0	2.7
PI 533249	*P. vulgaris*/Mexico	NPGS	III	Black	3.1	3.5
PI 200967	*P. vulgaris*/Guatemala	NPGS	III	Black	3.7	3.2
PI 311807	*P. vulgaris*/Guatemala	NPGS	III	Dark red	4.0	1.7
BAT 477	*P. vulgaris*/Colombia	CIAT	III	Cream	4.2	3.7
PI 207279	*P. vulgaris*/Colombia	NPGS	III	Light brown	4.3	2.4
PI 313701	*P. vulgaris*/Mexico	NPGS	II/III	Black	4.3	3.2
A774	*P. vulgaris*/Brazil	CIAT	III	Cream	4.6	3.1
PI 288016	*P. vulgaris*/Nicaragua	NPGS	II	Black	4.7	2.6
PI 477037	*P. acutifolius*/USA	NPGS	IV	Speckled brown	4.8	2.4
SER 22	*P. vulgaris*/Colombia	CIAT	II	Small red	4.8	2.6
ICA PIJAO	*P. vulgaris*/Colombia	CIAT	II	Purple black	4.8	2.4
PI 313727	*P. vulgaris*/Mexico	NPGS	III	Cream	4.9	2.4
PI 533312	*P. vulgaris*/Mexico	NPGS	II	Black	5.0	3.9
PI 311853	*P. vulgaris*/Guatemala	NPGS	III	Red	5.0	3.1

a. NPGS = National Plant Germplasm System; CIAT = International Center for Tropical Agriculture.
b. Growth habit: I = determinate bush; II = indeterminate upright; III = indeterminate semiprostrate vine; IV = indeterminate climbing vine.
c. Mean of root rot score from initial (Alpha 25 lattice design) screening based on a scale 1–9; overall mean: 7.02.
d. Mean of root rot score only retesting 15 best lines (RCBD) based on a scale 1–9; overall mean: 3.26, LSD (From RCBD) (0.05*); 1.66, CV%: 36.33. 1–3 = resistant; 4–6 = intermediate; 7–9 = susceptible (Van Schoonhoven and Pastor-Corrales, 1987).

3.3. Correlation between Rhizoctonia damping-off resistance and drought tolerance, NE-08

There were no significant correlations between damping-off resistance and yield under drought stress and non-stress conditions from the combined data of both drought test locations (data not shown). However, when a single location was considered, a negative correlation was found between damping-off resistance and yield under the non-stress treatment in Fortuna, PR (P = 0.0264). This data set was also analyzed independently by groups of bean lines from similar sources, e.g. cultivars, tepary beans, experimental lines, NPGS germplasm (Table 3). Again, among lines, the only significant correlation (P = 0.013) was between damping-off resistance and yield under non-stress conditions in Fortuna, PR, with a coefficient of –0.51 (Table 3). Similar significant damping-off resistance and drought tolerance correlations were found in experimental lines from dry bean breeding programs and the NPGS (Table 3).

3.4. Identification of sources of resistance from NE-14 lines

The ongoing shuttle breeding program between NE and PR developed 111 lines that were screened for Rhizoctonia damping-off and root/hypocotyl damage resistance (data not shown). As with the NE-08 experiment, the analysis of variance of the alpha lattice design indicated that there were significant effects of replications and lines (P < 0.0001). The overall mean of the ratings of damping-off was 6.4,

Table 2. Source, origin, growth habit, seed color, Rhizoctonia damping-off score and yield under drought stress and non-stress conditions for partially resistant and drought tolerant lines from set NE-08.

Line	Source[a]	Origin	Growth habit	Seed color	*R* mean[b]	NSC[c] kg ha^{-1}	DSC[d] kg ha^{-1}	GMC[e]
A774	Cultivar CIAT	CIAT	III	Cream	4.6	2194	1429	1771
SEA 15	Exp. lines	CIAT	III	Red	5.3	2146	1517	1804
LEF 2RB	Exp. lines	Colorado, US	III	Cream striped	5.3	2430	1725	2047
PI 310739	NPGS	Guatemala	III/IV	Black	5.4	1714	1416	1558
VAX 1	Exp. lines	CIAT	III	Carioca	5.6	2327	1404	1807
NE25-07-17	Exp. lines	Nebraska, US	II	Pinto	5.7	2476	1574	1974
PI 476751	NPGS	Guatemala	III	Dark red	6.0	1624	1543	1583
Maverick	Cultivar	ND, US	II	Pinto	6.0	2438	1519	1925
Stampede	Cultivar	ND, US	II	Pinto	6.4	2321	1674	1971
NE25-07-18	Exp. Lines	Nebraska, US	II	Pinto	6.5	2179	1715	1933

a. CIAT = Center for Tropical Agriculture, Colombia; NPGS = National Plant Germplasm System.
b. *R* mean = Mean of damping-off score based on a scale 1–9. 1–3 = resistant; 4–6 = intermediate; 7–9 = susceptible (Van Schoonhoven and Pastor-Corrales, 1987).
c. NSC = Non-stressed combined from Fortuna, PR and Mitchell, NE.
d. DSC = Drought stress combined from Fortuna, PR and Mitchell, NE.
e. GMC = Geometric mean combined from Fortuna, PR and Mitchell, NE. Growth habit: I = determinate bush; II = indeterminate upright; III = indeterminate semiprostrate vine; IV = indeterminate climbing vine.

indicating overall susceptibility of these lines to *R. solani*. However, some lines from this nursery were selected as sources of partial resistance (Table 4). These lines were tested again in a RCBD experiment to confirm the intermediate resistance. There were no significant differences between replications.

3.5. Correlation between Rhizoctonia damping-off resistance and drought tolerance, NE-14

All of the data from the damping-off screening of the NE-14 lines was tested for correlations with the data of the drought analysis (Table 5). There was a significant positive correlation between damping-off and drought tolerance for the combined data and from the field trials conducted at Mitchell, NE (Table 5). This correlation was significant also for the two non-stress and drought stress treatments.

The data was also analyzed by pedigree of lines (Table 6). All the 111 lines could be placed in 15 pedigree backgrounds. Out of the 15 pedigrees, 13 had no significant correlation by any treatment or drought test location (Table 6). However, certain drought tolerant pedigrees did correlate with damping-off rating at one location. Lines from the pedigree DOR 364/TLP 19//A774 correlated with drought stress in Mitchell, NE (P = 0.0038). The pedigree BAT 477/ B98311 had a significant correlation between drought stress and the geometric mean at Mitchell. Lines from the pedigree Matterhorn/ SEN 10 (P = 0.048) also correlated with the geometric mean of the combined experiments and with the non-stress treatment in Scottsbluff (P = 0.0139). Matterhorn and BAT 477 are sources of tolerance to drought. A 774 has broader adaptation to Brazil. Despite these correlations, there were no significant correlations over all the lines, but some lines were selected for partial resistance to Rhizoctonia damping-off and root/hypocotyl damage, drought tolerance and adaptation to two agro-eco regions (data not shown).

4. Discussion

In this study an effective and simple screening method was developed to select bean lines that display Rhizoctonia damping-off and root/hypocotyl damage resistance. Even though, damping-off has been studied in several crops, methods of screening for resistance to *R. solani* have varied according to the hosts and techniques used (Keijer et al., 1997). From previous work, the selection of inoculum density was found to be important in determining disease severity depending on the host (Sneh et al., 1991). In the bean system the lower ratio (1:100) of inoculum to soil of each of the three different isolates did not cause disease on any of the five bean cultivars. While the higher ratio (1:5) caused severe to lethal damage to the bean seedlings, the inoculum ratio 1:10 produced a wider range of disease reaction among the cultivars evaluated. These results are similar to those reported by Schroeder and Paulitz (2008) who studied the effects of inoculum density on the development and severity of Rhizoctonia root rot; and also found that the higher the inoculum density the higher the disease severity.

Table 3. Correlation between Rhizoctonia damping-off and drought tolerance for beans that are cultivars, experimental lines, tepary beans and lines from the National Plant Germplasm System of the NE-08 set.

	NSC[a]	DSC[b]	GMC[c]	NSM[d]	DSM[e]	GMM[f]	DSF[g]	NSF[h]	GMF[i]
Mean/cultivars *N* = 23	–0.19	0.12	–0.02	0.01	0.05	0.04	0.21	–0.51*	–0.19
Mean/exp. lines *N* = 79	–0.12	–0.12	–0.13	0.02	–0.08	–0.04	–0.17	–0.30*	–0.27**
Mean/tepary *N* = 29	0.09	–0.20	–0.08	–0.04	–0.22	–0.16	0.21	0.05	0.16
Mean/NPGS *N* = 31	–0.24	–0.10	–0.22	–0.07	–0.21	–0.17	–0.36**	0.22	–0.12

*P < 0.01; **P < 0.05 Pearson's correlation coefficients, N = 23. Prob>|r| under H0:Rho = 0.
Drought tolerance data was provided by Dr. Carlos Urrea.
a. NSC: Non-stress combined from Fortuna, PR and Mitchell, NE.
b. DSC: Drought stress combined from Fortuna, PR and Mitchell, NE.
c. GMC: Geometric mean combined from Fortuna, PR and Mitchell, NE.
d. NSM: Non-stress at Mitchell, NE.
e. DSM: Drought stress at Mitchell, NE.
f. GMM: Geometric mean at Mitchell, NE.
g. DSF: Drought stress at Fortuna, PR.
h. NSF: Non-stress at Fortuna, PR.
i. GMF: Geometric mean at Fortuna, PR.

Table 4. Rhizoctonia damping-off scores and pedigrees of bean lines with partial resistance from the shuttle breeding program between Puerto Rico and Nebraska (NE-14 set).

Line code	Pedigree	Mean[a]	Mean[b]
NE14-08-278	Tacana/VAX6	4.1	2.7
NE14-08-9	BAT 477/TLP 19//BelMiDak RMR10/B01741	4.1	4.4
NE14-08-181	BAT 477/L88-63	4.2	3.1
NE14-08-310	P00646/'TARS PT03-1	4.2	5.7
NE14-08-176	Black Rhino/SEN 10 (released as SB-DT1)	4.3	3.7
NE14-08-33	BAT 477/L88-63	4.5	4.4
NE14-08-194	Tacana/VAX6	4.5	3.4
NE14-08-277	Tacana/VAX6	4.6	4.2
NE14-08-225	Tacana/VAX6	4.7	3.1
NE14-08-55	BAT 477/L88-63//BelMiDak RMR10/B01741	4.7	3.6
NE14-08-274	BAT 477/TLP 19	4.7	4
NE14-08-12	BAT 477/TLP 19//BelMiDak RMR10/B01741	4.7	2.6
NE14-08-286	DOR 364/TLP 19//A774	4.8	2.7
NE14-08-134	Merlot//05F-5055-1/98020-3-1-6-2	4.8	5.1
NE14-08-196	EAP 9503-32A/A774	4.9	2.9
NE14-08-7	BAT 477/TLP 19//BelMiDak RMR10/B01741	4.9	–
NE14-08-23	Black Rhino/SEN 10	5.1	–
NE14-08-13	BAT 477/TLP 19//BelMiDak RMR10/B01741	5.1	–
NE14-08-6	Morales/XAN 176//BAT 477/B98311	5.2	–

a. Mean of damping-off score from initial (Alpha lattice design) screening based on a scale 1–9; overall mean: 6.43.

b. Mean of damping-off score only retesting 15 best lines (RCBD) based on a scale 1–9; overall mean: 3.26, LSD (From RCBD) (0.05*); 1.66. CV%: 36.33 1–3 = resistant; 4–6 = intermediate; 7–9 = susceptible (Van Schoonhoven and Pastor-Corrales, 1987).

The selection of one isolate representing an individual AG of *R. solani* also has been suggested in the screening of germplasm to identify useful *R. solani* resistance (Keinath and Farnham, 1997). Three isolates from two different *R. solani* AGs, WN-11 (AG2-2 IIB), WN-116 (AG2-2 IIIB) and WN-293 (AG4-HG III), were tested to determine isolate preference in the screening experiments. WN-11 was chosen because it produced a consistent range of disease reactions on the five host lines over two environments. The significant interaction between environments and isolates, which is likely to be due to the temperature difference between environments, indicates that *R. solani* isolates may cause more or less disease within their optimum temperature range of 20–30 °C (Schultz and Bateman, 1969). Isolate WN-293 produced a more severe reaction in the greenhouse (26 °C) than the growth chamber (19 °C), while isolates WN-116 and WN-11, in the same AG, produced a similar reaction in both environments.

In the analysis of the damping-off ratings for lines in sets NE-08 and NE-14, there was a significant difference in both treatments and replications. The coefficient of variation was slightly larger for the NE-14 lines with 19.9% compared to NE-08 lines which had 17.8%. However, the overall mean rating of damping-off reaction of NE-08 (7.0) was higher than NE-14 (6.4). The large size of the experiment and limited greenhouse space dictated that replications be planted at different times. The mean temperature in the greenhouse for the Fall planting was 21 ±3 °C (reps 1 and 2) and for the Spring planting (reps 3 and 4) was 25 ±3 °C. The *R. solani* isolate selected was the most stable pathogen in both spring and fall. However, temperature was confounded because in this experiment plants growing at 19 °C were in a growth chamber and plants at 26 °C were grown in the greenhouse. There was more root disease on bean plants at 19 °C than 26 °C. The similar results obtained in both nurseries where the overall means of the first two replicates were significantly lower than replicates 3 and 4, support the hypothesis that this particular isolate causes a more severe reaction at lower temperatures.

These results are consistent with other reports that the growth, distribution and severity of AG2-2 IIIB are increased at lower temperatures (Singh et al., 1999; Bolkan and Ribeiro, 1985). It is important to determine early and late season infections because of soil temperature differences and also to determine an efficient way to manage this pathogen until disease resistant cultivars are available. For example, the type of crop rotation can be an important factor in Nebraska and other Midwestern states where growers rotate beans with sugar beets. Sugar beets are also susceptible to most isolates from *R. solani* AG2-2 IIIB and AG4-HG III (Sikora, 2004; Venegas, 2008) and this may increase the chances of Rhizoctonia root rot and/or damping-off becoming a more serious economic disease in these areas.

Sources of partial resistance to Rhizoctonia damping-off and early root/hypocotyl damage were identified from some of the NE-08 lines and the shuttle breeding program (NE-14) between NE and PR and can now be used in crosses to improve damping-off resistance in dry bean lines. When the resistance was confirmed in these individual lines, damping-off ratings ranged from 1.7 to 5.7, and were lower than ratings from the initial screening experiment. Lower ratings could indicate higher resistance or could be due to the higher temperature in the greenhouse, compared to the initial screening experiment. Higher temperatures would promote faster germination and growth of plants thus allowing them to avoid damping-off and/or reflect the lower severity of *R. solani* normally found at higher temperatures. However, the control cultivar Pinto UI 114 rating in the same experimental conditions was 8.9 which indicated little temperature effect on susceptible reactions but confirmed the resistance shown by the resistant lines identified in NE-08 and NE-14. These lines are not only useful for damping-off resistance but also for heat tolerance in the case of lines in NE-08 and drought tolerance for lines in both sets.

The lack of significant correlations between damping-off resistance and drought tolerance among the lines could be due to the

Table 5. Correlation between Rhizoctonia damping-off and drought tolerance for all the bean lines of the NE-14 set.

	DSC[a]	NSC[b]	GMC[c]	DSM[d]	NSM[e]	GMM[f]	DSF[g]	NSF[h]	GMF[i]
Mean	0.2308**	0.1877**	0.22736**	0.28916*	0.26173**	0.28916*	0.13631	–0.0306	0.08151
N	111	111	111	94	94	94	111	111	111

*P < 0.01; **P < 0.05 Pearson's correlation coefficients. Prob>|r| under H0:Rho = 0.
Drought tolerance data was provided by Dr. Carlos Urrea.
a. DSC: Drought stress combined.
b. NSC: Non-stress combined.
c. GMC: Geometric mean combined.
d. DSM: Drought stress at Mitchell, NE.
e. NSM: Non-stress at Mitchell, NE.
f. GMM: Geometric mean at Mitchell, NE.
g. DSF: Drought stress at Fortuna, PR.
h. NSF: Non-stress at Fortuna, PR.
i. GMF: Geometric mean at Fortuna, PR.

Table 6. Correlation between Rhizoctonia damping-off and drought tolerance for all the bean lines of the NE-14 according to pedigree.

Pedigree	DSC[a]	NSC[b]	GMC[c]	DSM[d]	NSM[e]	GMM[f]	DSF[g]	NSF[h]	GMF[i]	*N*
BAT 477/L88-63//BelMiDak RMR10/B01741	0.27	–0.08	0.15	0.55	0.27	0.55	0.07	–0.35	–0.22	9
BelMiDak RMR10/B01741//BAT 477/L88-63	0.25	0.12	0.24	0.16	0.09	0.16	0.47	0.33	0.60	6
Black Rhino/SEN 10 (released as SB-DT1)	–0.81	–0.65	–0.75	–0.77	–0.32	–0.77	0.68	0.09	0.70	5
DOR 364/TLP 19//A774	0.39	0.66	0.53	–0.99*	–0.31	–0.99*	–0.12	0.55	0.36	6
Matterhorn/SEN 10	0.79	0.74	0.81**	0.11	–0.41	0.11	0.49	0.13	0.40	6
Matterhorn/SER 21	–0.09	0.31	0.12	–0.01	0.44	–0.01	–0.55	0.00	–0.55	12
Morales//XAN 176//BAT 477/B98311	–0.25	–0.36	–0.30				–0.99	–0.77	–0.87	3
P00646/TARS PT03-1	–0.27	–0.48	–0.36	–0.37	–0.43	–0.37	0.86	–0.83	0.35	3
Tacana/VAX6	0.51	0.31	0.44	0.57	0.38	0.57	–0.18	–0.19	–0.23	9
USPT-ANT//Matterhorn/98078-5-1-5-1	0.49	0.39	0.57	0.54	0.25	0.54	0.59	0.49	0.58	6
XAN 176/Matterhorn//EAP 9503-32A	0.42	0.49	0.45	–0.23	–0.11	–0.23	0.02	0.62	0.34	6
BAT 477/B98311	–0.79	–0.26	–0.62	–0.92**	0.47	–0.92**	0.38	–0.90**	–0.20	6
Merlot//05F-5055-1/98020-3-1-6-2	0.36	0.20	0.29	0.62	0.01	0.62	0.34	0.16	0.39	9
Merlot//98020-3-1-6-2/Tacana	0.06	–0.27	–0.04	0.34	–0.25	0.34	0.31	–0.13	0.37	6
Merlot//Merlot/SER 16	0.47	0.22	0.39	0.50	0.33	0.50	–0.08	–0.29	–0.28	10

*$P < 0.01$; **$P < 0.05$ Pearson's correlation coefficients. Prob>|r| under H0:Rho = 0. Drought tolerance measured as yield (kg ha^{-1}) under stress and non-stress conditions.
N: Number of lines per pedigree.
Drought tolerance data was provided by Dr. Carlos Urrea.
a. DSC: Drought stress combined.
b. NSC: Non-stress combined.
c. GMC: Geometric mean combined.
d. DSM: Drought stress at Mitchell, NE.
e. NSM: Non-stress at Mitchell, NE.
f. GMM: Geometric mean at Mitchell, NE.
g. DSF: Drought stress at Fortuna, PR.
h. NSF: Non-stress at Fortuna, PR.
i. GMF: Geometric mean at Fortuna, PR.

previous selection of all the lines for drought tolerance and although both locations had the pathogen, the level of *R. solani* in the two fields is not known (Porch and Harveson, personal communication). This selection could reduce the probabilities of identifying correlations because there are not drought susceptible lines to be correlated with damping-off susceptible ones. However, some lines identified with damping-off resistance also had multiple desirable traits. For instance, lines that are not only adapted to two very different areas (western Nebraska and Fortuna, PR), have drought tolerance and moderate resistance to *R. solani*, and NE-14-08-187 also has rust resistance and is adapted to some regions in Mexico. One tested line, VAX 1, was derived from an interspecific cross between *P. acutifolius* and *P. vulgaris* that has common bacterial blight resistance. The lines NE-25-07-17 and NE-25-07-18 are two pinto bean lines from the Nebraska dry bean breeding program that are drought tolerant with rust resistance genes *Ur-3* and *Ur-6*, earliness, common bacterial blight resistance, and white mold avoidance due to upright plant architecture in addition to the partial resistance to Rhizoctonia damping-off. In the shuttle breeding program, the entry NE14-08-176 released as SB-DT1 (Porch et al., 2012) is a black bean with drought and heat tolerance, common bacterial blight resistance, brown spot resistance and partial resistance to Rhizoctonia damping-off. Another entry with multiple favorable alleles is NE14-08-314, which has the marker SW13 for bean common mosaic virus and the gene *Ur-3* for rust resistance. The other selected lines have similar characteristics that make them valuable germplasm for dry bean breeding programs.

5. Conclusions

A greenhouse screening test was developed to evaluate damping-off and early root/hypocotyl damage caused by *R. solani*. This test was used to screen 274 bean lines previously identified to have drought and in some lines heat tolerance. A number of elite breeding lines and diverse lines with other important biotic stress resistance were found exhibiting drought tolerance and damping-off resistance. However, no correlation between damping-off resistance and drought tolerance was found. Screening these germplasm lines with more isolates of *R. solani* AGs will determine their utility in dry bean production areas other than the High Plains. Damping-off resistance is the most cost effective strategy for disease management, especially in regions where inoculum will be a predisposing factor.

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