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Early reaction of bean varieties to root rot under laboratory conditions

Reacción temprana de variedades de frijol a la pudrición de la raíz en condiciones de laboratorio

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Abstract

Root production (RP) is a disease caused by various pathogens and causes a reduction in bean (Phaseolus vulgaris L.) yield. The PR occurs before and after the emergence of the seedlings between the first and 5 to 9 days after sowing (dds). However, few works have been reported on the effect of this disease in early stages. The objective of the work was to determine the early reaction of bean varieties to root rot in different soils under laboratory conditions. 26 composite soil samples were taken in commercial bean plots in the state of Aguascalientes. Bean seeds of the Bravo, Centenario, Coloso, Raramuri, San Rafael and Negro Zacatecas pinto varieties were sown in plastic counters in duplicate of each of the soil samples. On average, 7 days after sowing, the roots of each seedling in all containers were collected to identify and classify the incidence and severity of the disease. Also, the pathogens present in the seedlings. The results indicate that only in eight plots was an incidence of the disease of 25% recorded and the varieties with the highest percentage of incidence values between 75.1 and 100% were Pinto Centenario, Pinto Coloso. Also, a higher incidence was observed in pinto bean varieties compared to black bean varieties. PR caused lesions ranging from trace to root death in seedlings of the six bean varieties evaluated from pre-emergence to six or eight days after emergence. Rhizoctonia spp., Fusarium spp. and Sclerotium rolfsii in lesions or on the root and hypocotyl of the plants of the six bean varieties.

Pathogens, Incidence, Soil health

Resumen

La producción de la raíz (PR) es una enfermedad causada por diverso patógenos y provoca una reducción de rendimiento de frijol (Phaseolus vulgaris L.). La PR se presenta previo y posterior a la emergencia de las plántulas entre los primeros y 5 a 9 días después de siembra (dds). Sin embargo, se han reportados pocos trabajos sobre el efecto de esta enfermedad en etapas tempranas. El objetivo del trabajo consistió en determinar la reacción temprana de variedades de frijol a la pudrición de la raíz en diferentes suelos bajo condiciones de laboratorio. Se tomaron 26 muestras compuestas de suelo en parcelas comerciales de frijol en el estado de Aguascalientes. Las semillas de frijol de las variedades pinto Bravo, Centenario, Coloso, Raramuri, San Rafael y Negro Zacatecas, fueron sembradas en contadores de plástico por duplicado de cada una de las muestras de suelo. En promedio a los 7 días después de siembra se recolectaron las raíces de cada plántula en todos los contenedores para identificar y clasificar la incidencia y severidad de la enfermedad. También, se los patógenos presentes en las plántulas. Los resultados indican que solo en ocho parcelas se registro una incidencia de la enfermedad del 25% y las variedades con mayor porcentaje de valores de incidencia entre 75.1 y 100% fueron Pinto Centenario, Pinto Coloso. También, se observó una incidencia superior en las variedades de frijol pinto con respecto a las variedades de frijol negro. La PR causó lesiones que variaban desde trazas hasta la muerte de raíces en plántulas de las seis variedades de frijol evaluadas desde pre emergencia hasta seis u ocho días después de la emergencia. Se identificó a Rhizoctonia spp., Fusarium spp. y Sclerotium rolfsii en lesiones o sobre la raíz e hipocotilo de las plantas de las seis variedades de frijol.

Patogenos, Indicencia, Salud del suelo

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Introduction

During the production process, common bean (*Phaseolus vulgaris* L.) plants are affected by various pathogens that reduce their yield and quality. With some regularity a wide number of pathogens destroy or disable the aerial part of the plants, however, diseases caused by soil-dwelling organisms are a constant yield drain; root rot (RP) has been reported as a severe threat in some American countries such as Brazil, Mexico, Peru, Nicaragua and the United States (Naseri, 2014). Yield losses of up to 100% have been reported in Uganda and up to 70% in Rwanda and Kenya; elsewhere bean planting has been halted due to severe outbreaks of the disease (Diaz et al., 2021). Globally, RP is recognised to be caused by several pathogens including Pythium spp., Fusarium spp., Rhizoctonia solani, and Sclerotium rolfsii (Paparu et al., 2017; Mayo-Prieto et al., 2020); in north-central Mexico the pathogens most commonly associated with this disease are Fusarium spp., Rhizoctonia spp, Colletotrichum spp, Thielaviopsis spp, Pythium spp, as well as the nematodes Meloidogyne spp. and Nacobbus aberrans (Groenewold-Labrada et al., 2003; Velásquez-Valle et al., 2022). RP can occur pre- or post-emergence causing lesions in the root or hypocotyl during the first weeks of growth (Araya and Hernández, 2006; Conner et al., 2014). Usually the severity of PR and its impact on plant development are assessed at stages close to flowering or harvest (Naseri 2014; Jacobs et al., 2019), although it is known that some pathogens such as Fusarium spp. can cause temporary symptoms between five and nine days after artificial inoculation of bean plants (Abawi and Pastor, 1990) but, in general, little is known about the reaction of bean plants to the disease in the first days after emergence, consequently, the aim of the work was to determine the early reaction of bean varieties to root rot in different soils under laboratory conditions.

Materials and method

During April 2023, soil was collected from 26 commercial plots in the state of Aguascalientes; in each plot, three subsamples of approximately 500 g each were taken from the first 20 cm of the soil profile. The soil subsamples were homogenised and two samples of approximately 400 g were taken from the resulting mixture and placed in a plastic pot; the soil was moistened to allow bean sowing.

Seed of the pinto bean varieties Bravo, Centenario, Coloso, Raramuri and San Rafael released by INIFAP and Negro Zacatecas (public domain) provided by MC José Ángel Cid Ríos (Campo Experimental Zacatecas, INIFAP) was used. Prior to sowing, the seed was surface disinfested with a commercial chlorine solution (0.2%) allowing it to dry before sowing. The fungal colonies were counted in each soil using the dilution technique where an aliquot of the 10-6 dilution was sown in a box of PDA culture medium; after five days of incubation, the number of fungal colonies was counted.

For each variety, two containers with each soil type were used; 10-12 seeds of each bean variety were placed in each container. After sowing, the containers were kept at room temperature (16 to 26°C) in the laboratory.

At 6 - 8 days after emergence the plants were removed from the soil, excess soil was removed from the root and the incidence (presence of one or more lesions) of disease in the hypocotyl was assessed and classified as low (0 - 25%), medium (25.1 - 50.0%), high (50.1 - 75.0%) and extreme (75.1 - 100.0%). In addition, the severity or degree of PR damage to the hypocotyl was determined according to the following damage categories: 0 (healthy): hypocotyl with no visible external damage; 3 (slight damage): hypocotyl with traces of longitudinal lesions; 5 (moderate damage): hypocotyl with circular or oval lesions, but without necrosis; 7: (severe damage): hypocotyl with necrotic lesions and/or longitudinal or circular lesions advancing into the neighbouring portion of the stem. The severity of PR for each variety was calculated by multiplying the number of plants with damaged hypocotyl by the value of each category and dividing the result by the number of damage categories present; the result was classified as mild (0 - 25%), medium (25.1 - 50.0%), severe (50.1 - 75.0%) and extreme (≥ 75.1%). At the time of assessment, the foliar symptoms present on each plant were recorded. Pathogens present in randomly selected seedling lesions were identified morphologically to genus level using the taxonomic keys provided by Barnett and Hunter (1972) and Watanabe (1994).

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Results and discussion

In only three of the bean varieties (Pinto Bravo, Pinto Raramuri and Negro Zacatecas) planted in soil from eight plots (1, 7, 14, 15, 18, 20, 23 and 25) was disease incidence less than 25%. In 71.1% of the soils, PR incidence ranged from 25.1 to 70%, regardless of bean variety or type. The highest disease incidences (75.1 - 100%) were reached in 21.8% of the soils irrespective of bean variety or type (Figure 1).

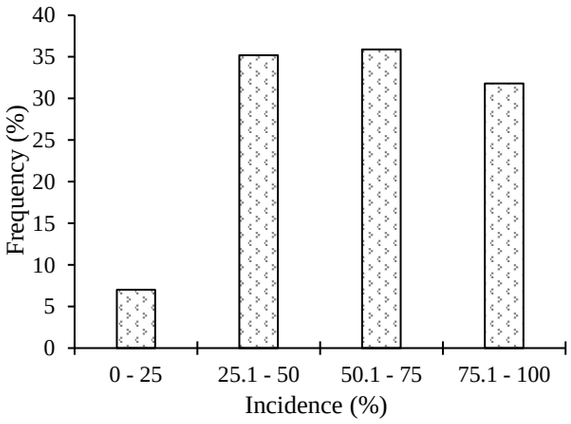

Figure 1. Distribution of bean root rot incidence (%) in soil from 26 plots.

The distribution of RP incidence in four categories (0-25, 25.1-50, 50.1-75 and 75.1-100%) revealed that in the first category (0-25%) only three varieties (Pinto Bravo, Pinto Raramuri and Negro Zacatecas) recorded disease incidence in that range and that the percentage of soils where those values were obtained was not higher than 20%. On the contrary, the varieties Pinto Centenario, Pinto Coloso and Pinto San Rafael, did not obtain RP incidence values in this category (Table 1).

Variety	Advocacy (%)			
	0 - 25	25.1 - 50	50.1 – 75	75.1 – 100
Pinto Bravo	20.0	40.0	20.0	20.0
Pinto Centenario	0.0	30.7	42.3	28.9
Pinto Coloso	0.0	44.0	32.0	24.0
Pinto San Rafael	0.0	20.8	50.0	29.1
Pinto Rarámuri	9.1	31.8	40.9	18.1
Negro Zacatecas	17.4	52.2	17.4	13.0

Table 1 Distribution of root rot incidence of six bean varieties in soil from 26 plots

Most of the disease incidence values were recorded in the intermediate categories (25.1-50 and 50.1-75%) in all varieties; the combined values of both categories ranged between 60 (Pinto Bravo) and 73% (Pinto Centenario). At the other extreme, the varieties with the highest percentage of incidence values between 75.1 and 100% were Pinto Centenario, Pinto Coloso and Pinto San Rafael which, coincidentally, did not obtain incidence values between 0 and 25%.

The number of soils where the incidence was extreme (75.1 - 100%) varied between five and seven for the pinto varieties while in the black bean variety it was three. The Pinto Centenario and San Rafael varieties stand out for the high incidence in seven soils (Table 2). It should be noted that the soil of some plots, such as plot 3, was consistently associated with extreme incidence of RP in four of the five pinto bean varieties and in the black bean variety.

Variety	Soil/Incidence (%)						
P. Bravo	3 (76.9)	5 (100)	16 (90)	19 (90)	26 (85.7)	-	-
P. Centenario	1 (80)	3 (100)	5 (100)	11 (83.3)	12 (92.3)	19 (90.5)	23 (86.7)
P. Coloso	1 (78.6)	3 (86.7)	17 (90)	18 (81.8)	20 (78.6)	25 (100)	-
P. San Rafael	6 (93.7)	8 (88)	12 (100)	20 (100)	22 (83.3)	23 (100)	24 (94.1)
Pinto Rarámuri	3 (77.8)	5 (76.9)	8 (88)	23 (93.9)	26 (80)	-	-
Negro Zacateas	3 (92.3)	9 (92.3)	23 (100)	-	-	-	-

Table 2 Soils with higher incidence of RP in six bean varieties under laboratory conditions

The average extreme incidence values of PR ranged between 83.3 and 94.9% in Pinto Raramuri and Negro Zacatecas, respectively; Pinto Raramuri and Pinto Coloso stood out with mean extreme incidence values of 83.3 and 85.9% in five and six soils, respectively (Figure 2). It is interesting to note that Negro Zacatecas and Pinto San Rafael with similar mean extreme incidence values (94.9 and 94.1%, respectively) had extreme incidence values in three and seven soils, respectively, but the values obtained in these soils exceeded 90% in most cases (Table 2).

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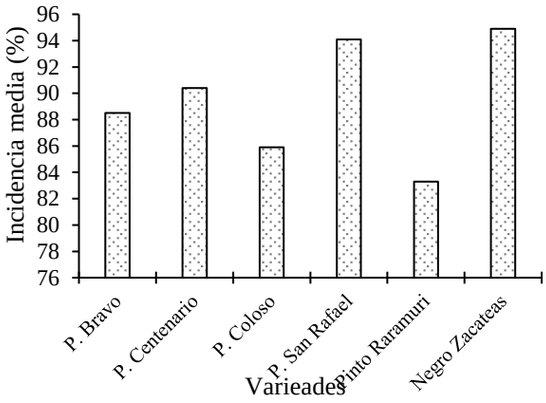

Figure 2 Average incidence of RP in soils with extreme incidence in six bean varieties under laboratory conditions

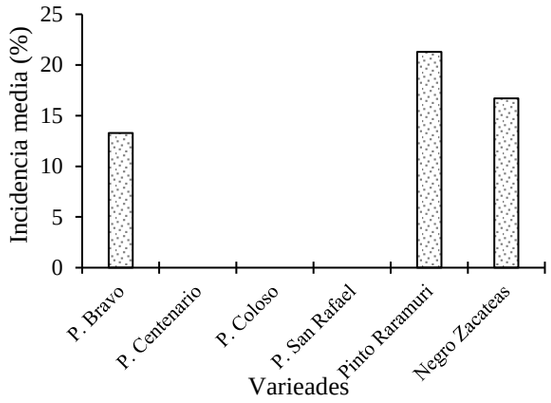

Figure 3 Average incidence of RP in soils with mild incidence in six bean varieties under laboratory conditions

In only three of the six bean varieties were recorded PR incidences lower than 25%; such incidence was recorded in five soils planted with the Pinto Bravo variety with values ranging between 0.0 and 21.4%; in the Negro Zacatecas variety the minimum incidence values were obtained in four soils where the incidence values were between 0.0 and 21.7%. The Pinto Rarámuri variety obtained minimum incidence values of the disease in two soils with values of 17.6 and 25%. It is important to note that no incidence values less than or equal to 25% were recorded in any of the soils planted with the varieties Pinto Centenario, Pinto Coloso and Pinto San Rafael (Table 3).

Variety	Soil/Incidence (%)						
P. Bravo	14 (7.6)	18 (11.7)	20 (12.5)	23 (21.4)	25 (0.0)	14 (7.6)	18 (11.7)
P. Centenario	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0
P. Coloso	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0
P. San Rafael	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0
Pinto Rarámuri	15 (25)	18 (17.6)	0/0.0	0/0.0	0/0.0	15 (25)	18 (17.6)
Negro Zacateas	1 (11.7)	7 (0.0)	10 (16.7)	14 (21.7)	0/0.0	1 (11.7)	7 (0.0)

Table 3 Soils with RP incidence less than 25% in six bean varieties under laboratory conditions

The average of mild PR incidence values ranged between 0.0 in the soil from plots 25 and 7 for Pinto Bravo and Negro Zacatecas, respectively, and 25% in the soil from plot 15 planted with Pinto Raramuri. The Pinto Bravo variety obtained the lowest average of slight incidence of the disease, 13.3%, followed by the varieties Negro Zacatecas, 16.7% and Pinto Raramuri 21.3%; it is opportune to remember that in the varieties Pinto Centenario, Pinto Coloso and Pinto San Rafael no incidences of RP lower than 25% were registered (Figure 3).

When considering separately the average incidence of RP in pinto and black bean varieties, it was observed that in 16.7% of the soils the average incidence of the disease was in the range of 0 to 25.0%, in contrast to the pinto varieties, whose average incidence was higher than 25.0%. Furthermore, in 66.7% of the soils, the average incidence of PR in the black variety reached values between 0 and 50.0%, while in the pinto varieties this incidence was obtained in only 26.9% of the soils.

When combining the values of PR incidence in both bean types, it was observed that the higher incidence of the disease in pinto bean varieties in soils where the black bean variety obtained values lower than 25.0% eliminates the influence of the latter, so that no incidence values are reported for that range of incidence when the combined values of pinto and black bean varieties are used (Table 4).

Varieties	Incidence (%)			
	0 – 25.0	25.1 - 50	50.1 – 75.0	75.1 – 100.0
Black Type	16.7	50	20.8	12.5
Pinto Type	0.0	26.9	57.7	15.4
Black + Pinto	0.0	34.6	50	15.4

Table 4 Distribution of the average incidence (%) of RP in six varieties of pinto and black bean in 26 soils under laboratory conditions

In general, the average incidence of PR exceeded 40%, regardless of variety and soil origin, however, in Pinto Bravo and Negro Zacatecas plants the incidence of the disease was 44 and 49%, respectively, while in the rest of the varieties it exceeded 50%, highlighting the high incidence of PR in Pinto San Rafael with 69% (Figure 4).

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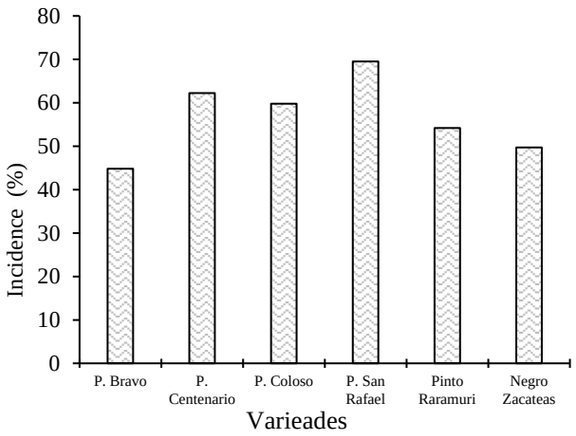

Figure 6 Average incidence of RP under laboratory conditions on six bean varieties in 26 soils collected in Aguascalientes

One of the main factors by which the incidence and severity of RP is variable is the soil; the specific physicochemical and biological conditions of each soil will determine, at least partially, the phytopathological behaviour of the host, common bean in this case. In the soil collected in 16 plots, the highest incidence values were recorded, that is, without considering the bean variety; however, the soils from plots 3, 5 and 23 stand out as having high incidence values for the disease in the six varieties evaluated. These three soils represent 30% of the cases where there were high incidences of RP. In 90% of the soils with high disease incidence, the previous crop was maize (Table 5).

Variety					
Pinto Bravo	Pinto Centenario	Pinto Coloso	Pinto San Rafael	Pinto Raramuri	Negro Zacatecas
5[x] (100.0)[y]	3 (100.0)	5 (100.0)	12 (100.0)	25 (93.9)	23 (100.0)
16 (90.0)	5 (100.0)	17 (90.0)	20 (100.0)	8 (88.0)	3 (92.3)
19 (90.0)	12 (92.3)	3 (86.7)	23 (100.0)	26 (80.0)	9 (92.3)
26 (85.7)	19 (90.5)	18 (81.8)	24 (94.1)	4 (77.8)	6 (73.7)
3 (76.9)	23 (86.7)	20 (78.6)	6 (93.7)	5 (76.9)	25 (58.8)
x Plot number; and Incidence (%) of RP.					

Table 5 Soils with maximum incidence of PR with six bean varieties under laboratory conditions

Among the soils with lower incidence of RP, those collected in plots 7, 9, 10, 14, 18 and 20 stand out, which together represent 63.3% of the 30 soils with lower incidence of the disease, however, the range of minimum incidence separates the varieties in two groups; in the first one, Pinto Bravo and Negro Zacatecas could be located, which presented similar ranges of minimum incidence; 0 - 21. 4 and 0 - 28%, respectively, while in the second group would be Pinto Raramuri (17.6 - 36.7%), Pinto Coloso (30 - 42.8%), Pinto Centenario (33.3 - 41.2%) and Pinto San Rafael (40 - 51.7%) (Table 6).

Variety					
Pinto Bravo	Pinto Centenario	Pinto Coloso	Pinto San Rafael	Pinto Raramuri	Negro Zacatecas
5x (0.0)y	14 (33.3)	7 (30.0)	13 (40.0)	18 (17.6)	7 (0.0)
14 (7.6)	20 (41.2)	4 (30.7)	2 (43.7)	15 (25.0)	1 (11.7)
18 (11.7)	22 (41.2)	9 (33.3)	18 (43.7)	20 (27.3)	10 (16.7)
20 (12.5)	7 (41.7)	10 (42.8)	7 (47.6)	9 (31.8)	14 (21.7)
23 (21.4)	10 (42.1)	11 (42.8)	9 (51.7)	19 (36.7)	2 (28.0)
x Plot number; and Incidence (%) of RP.					

Table 6 Soils with minimum incidence of RP with six bean varieties under laboratory conditions

Symptoms and associated pathogens

No foliar symptoms were observed on most of the plants assessed, irrespective of variety; wilting or yellowing was occasionally observed. In contrast, longitudinal or circular reddish-brown lesions on the hypocotyl or a dry rot of the main root, often associated with *S. rolfsii* infection, were observed on most of the damaged roots (Figures 7 and 8).

Figure 7 Oval, reddish-brown lesions on the hypocotyl of bean seedlings

Figure 8 Dry rot of the main root of bean seedlings associated with *Sclerotium rolfsii* infection

Fusarium spp. and *Rhizoctonia* spp. were identified in most of the oval to circular reddish-brown lesions on the hypocotyl of asymptomatic seedlings; *Sclerotium rolfsii* was also identified forming a thick, white to greyish mycelium on the root and hypocotyl between which sclerotia formed initially white and eventually turned brown.

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Most of the seedlings infected with this fungus germinated but did not emerge; when the attack occurred after emergence, the affected seedlings wilted and died. Occasionally the fungus continued its mycelial development on the soil surface where it also formed sclerotia (Figure 9).

Figure 9 Sclerotia and mycelium of *S. rolfsii* developing on the soil surface.

These fungi (*Fusarium* spp., *Rhizoctoni*a spp. and *Sclerotium rolfsii*) have been globally mentioned (Abawi, 1989) as a cause of bean root rot. It has been mentioned (Saremi et al., 2011) that severe infections of pathogens such as *Fusarium* can lead to plant death because damage to the root tip causes numerous rootlets to be emitted providing inadequate water supply; however, in the current study, the greatest amount of damage was observed in the hypocotyl and was associated with Rhizoctonia spp. lesions mainly, with most of the roots being healthy. When S. rolfsii occurred, destruction of the entire root system, including the hypocotyl, was present.

Conclusions

Root rot caused lesions ranging from trace to dead roots on seedlings of the six bean varieties evaluated from pre-emergence to six to eight days after emergence. The average natural incidence of the disease ranged from 44.8 (Pinto Bravo) to 69.5% (Pinto San Rafael).

Rhizoctonia spp., *Fusarium* spp. and *Sclerotium rolfsii* were identified in lesions or on the root and hypocotyl of the plants of the six bean varieties.

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