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Role of biochemical changes of highly resistant and susceptible bean cultivars in the physiology of seedling damping-off and/or root rot disease resistance

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Abstract

Seventeen fungal isolates obtained from diseased bean plants showing seedling damping-off (D-O) and root rotsymptoms collected from different locations in Sohag Governorate were identified as *Fusarium oxysporum* Schlecht. (5 isolates), *Fusarium solani* (Mart.) Sacc.(5 isolates), *Macrophomina phaseolina* (Tassi) Goid.(4 isolates), *Rhizoctonia solani* Kühn (2 isolates), and *Sclerotium rolfsii* Sacc. (Only isolate). Moreover, *F.oxysporum* and *F.solani* were the most frequently isolated fungi (29.41%, each), followed by *M. phaseolina* (23.52%). In contrast, *S.rolfsii* and *R.solani* were the lowest. Pathogenicity tests showed that all fungal isolates tested were pathogenic to the bean and significantly caused pre- and post-emergence seedling D-O and RR disease, except the isolates of *F. solani* did not cause seedling D-O but infecting old seedlings and induced only RR symptoms. The response of 5 common bean cultivars to infection by *F. solani* and *M. phaseolina*, causing D-O and/or RR disease, was studied under greenhouse and field conditions during the growing seasons of 2021 and 2022. Biochemical changes in the common bean plant root showed that highly resistant cultivar Kobo roots contained higher total protein contents than highly susceptible cultivar Nebraska after 21,28, 35, and 42 days of planting. The activity of peroxidase and polyphenol oxidase enzymes increased in the plant roots of common bean HR Kobo and HS Nebraska cultivars infected by *M. phaseolina* and *F. solani* after different planting intervals compared with the control plants. Also, the total phenolic contents gradually increased in the plant roots of common bean Kobo (HR) and Nebraska (HS) cultivars infected by *M. phaseolina* and *F. solani* after planting intervals.

Keywords: Common bean, damping-off, root rot (RR), *F. solani*, *M. phaseoloina*, resistance, cultivars

INTRODUCTION

Common or dry or French bean (*Phaseolus vulgaris* L.) is an important food legume crop in Egypt and worldwide. In Egypt, a total yield of 139607.12 tonnes was produced from 42857 Ha of area harvested (FAOSTAT, 2022). The RR disease is widespread and a major limiting factor to bean crop production, decreasing yield and productivity worldwide. Depending on the early infection with the causative fungal pathogen, general RR symptoms might include any mixture of the subsequent features: poor seedlings formation, D-O, rough growth, early defoliation, death of severely infected bean plants, and low yield. Several soilborne fungal pathogens, including *F. oxysporum*, *F. solani*, *M. phaseoloina*, *Pythium* spp., *R. solani*, *Sclerotinia sclerotiorum*, and *S. rolfsii* are known to cause seedling D-O and/or RR diseases on bean plants worldwide, but their occurrence and injury vary from one production area to another (Naseri and Mousavi, 2015; Binagwa *et al.*, 2016; Sabaté *et al.*, 2017; Abd-El-Kareem *et al.*, 2018; Paparu *et al.*, 2018; El-Kholy *et al.*, 2021; El-Mougy Nehal *et al.*, 2022; Tilahun *et al.*, 2023). Unlike the above-referred root-rotting fungi, *F. solani* do not cause seed rot and seedling D-O on bean plants, and the RR symptoms do not appear until two or three weeks after planting (Román-Avilés *et al.*, 2003). However, these fungi can infect bean roots and act either independently or in a complex manner depending on the cultivated soil and common environmental conditions (Rusuku *et al.*, 1997; Opio *et al.*, 2000; Schwartz *et al.*, 2005).

Moreover, their interaction might cause a higher rating of RR disease occurrence and harshness than when they are done individually. Traditional control methods for managing seedling D-O and/or RR disease on beans by fungicide applications harm humans and the environment. Therefore, searching for safe, eco-friendly alternative approaches to managing seedling D-O and/or RR disease has become necessary. Using resistant cultivars of beans is the most effective management approach against RR diseases (Nzungize *et al.*, 2012). Several investigators worldwide have evaluated various cultivars, varieties, lines, and genotypes of beans for the infection with the same D-O and/or RR pathogens *F. solani*, and *M. phaseoloina*, and other fungal pathogens, including the isolated fungi with the lowest frequency in this study, which caused bean D-O and/or RR disease and have been excluded. When a pathogen invades a plant, the host plant may activatevarious biochemical processes as part of the disease-resistance system (Moharam, 2013). In this regard, many investigators reported an increase in the total protein contents, the activity of oxidative enzymes peroxidase (PO), and polyphenol oxidase (PPO), as well as total phenolic contents in host tissues in their response to infection by the biotic pathogen (Kumari *et al.*,2015; Pareek and Varma, 2015; Poornima *et al.*, 2016; Belkar *et al.*, 2018; Tagele *et al.*, 2019). Therefore, the vulnerability of common bean cultivars to infection by *F. solani* and *M. phaseoloina* causing D-O and/or RR disease, was studied. Also, the role of biochemical changes from total protein and phenolic contents and the activity of PO and PPO in plant roots of highly resistant and susceptible bean cultivars in the physiology of seedling D-O and/or RR disease resistance were investigated.

MATERIALS AND METHODS

1. Isolation and identification of the causal pathogen of bean seedling D-O and RR disease:

The causal pathogen of bean seedling D-O and RR disease was isolated from roots of diseased plant samples showing D-O and RR symptoms collected from different locations in the Sohag Governorate. The roots of diseased plant samples were carefully washed through tap water to remove soil residues. Then,the roots were cut into small pieces, dipped into 0.5% NaOCl solution for 5 min, washed twice with sterile distilled water (SDW), and left to dry between two sterile filter papers. The root pieces were then placed on 9.0 cm Petri dishes containing potato dextrose agar (PDA) medium supplemented with chloramphenicol (200 mg L^{-1} medium). Then,dishes were incubated at 28±1 °C for 7 days. During incubation, colonies of the growing fungi were checked daily, transferred to PDA plates, and then left for growth at the same

conditions. The fungal isolates were purified using single spore and hyphal tip techniques by re-culturing on new PDA plates in the same growth conditions. The pure fungal isolates obtained were identified according to the morphological features of the growing colony, mycelia, spores, and sclerotia defined by Leslie and Summerell (2006). The frequency (%) of isolated fungi from bean plants' D-O and rotting root samples in all locations was calculated. Pure fungal cultures of all obtained isolates were then preserved at 5 °C in slants of the PDA medium until use.

2. Pathogenicity tests:

The pathogenic abilities of 17 fungal isolates of *F.* oxysporum (5 isolates), *F. solani* (5 isolates), *M. phaseoloina* (4 isolates), *R. solani* (2 isolates), and *S. rolfsii* (only isolate) were determined on the bean cv. Nebraska under greenhouse conditions during the 2020 growing season at the Experimental Farm, Faculty of Agriculture, Sohag University, El-Kawamel, Sohag. The sowing date in this experiment was the 15th of February. For preparing fungal inocula, the stored pure cultures of all tested fungal isolates were re-cultured on PDA Petri plates of 9.0 cm in diameter at 28±1 °C for 7-10 days. Agar 0.6 cm growth discs in diameter were made using a sterile cork borer from the growth plates of each tested fungal isolate under aseptic conditions. Sorghum grains were used for colonizing and producing fungal inocula, according to Tolêdo-Souza *et al.* (2009), with some modifications. Auto-cleavable polythene bags containing sorghum grains and washed sand (SG&WS) medium (300 g grains, 100 g sand, and 400 ml distilled water) were autoclaved at 120 ºC for 1.0 h, left to cool, then re-autoclaved the next day after the same process, and allowed to cool before inoculation. The bags were inoculated with 3 agar growth discs of each fungal isolate tested under aseptic conditions and then incubated at 28±1 ºC for 14 days to form inoculum, according to Mukamuhirwa *et al.* (2018 a and b). Formalin-sterilized 30 cm plastic pots were filled with formalin-sterilized clay-loam soil (5 kg per pot), infested with 3% inoculum of each tested fungal isolate (150 g per pot), and then slightly irrigated every other day for 7 days. Pots treated with the same amount of uninoculated SG&WS medium were served as control. Bean seeds were disinfected by dipping in 0.5% NaOCl solution for three minutes, rinsed three times in SDW for 3 minutes, and then sowed at a rate of 5 disinfected seeds per pot. Four pots as replicates of each tested fungal isolate were used in a completely randomized experimental design. All pots were checked daily and irrigated when necessary. The number of pre- and post- emergence seedling D-O caused by each tested fungal isolate was recorded 14 and 28 days after sowing, respectively, and the percent of each was then calculated (Abd El-Hai and Ali, Abeer 2018). Likewise, after 42 days, the survival plants were carefully uprooted without damaging roots and washed with tap water. Then, the RR disease severity (RRDS) induced by *Fusarium*, *Macrophomina*, and *Rhizoctonia* isolates was assessed using a scoring scale of 1-9, described by Mukamuhirwa *et al.* (2018 a and b). Where 1= No visible symptoms. 2= Light discoloration with no necrotic lesions. 3= Light discoloration with approx. 10% of the root tissues are covered with lesions. 4= Light discoloration with more than 10 to 25% of the root tissues covered with lesions. 5= Approx. 25% of the root tissues are covered with lesions, but tissues remain firm with the weakening of the root system. 6= More than 25 to 50% of the root tissues are covered with lesions and remain firm with the decline of the root system. 7= More than 50 to 75% of the root tissues are covered with lesions joined with substantialunstiffening, rotting, and decrease of the root system. 8= More than 75% of the root tissues are affected without progressive stages of rotting, and a severe decrease in the root system. 9= More than 75% of the root tissues are affected with progressive stages of rotting and a severe decrease in the root system. On the other hand, the RRDS induced by *Sclerotium* isolates was assessed based on a 1-5 scoring scale, described by Abawi and Pastor-Corrales (1990). Where 1= A healthy plant root. 2= A plant root with rotting symptoms without fungal growth. 3= A plant root with rotting symptoms with fungal growth. 4= A wilted plant. 5= A plant is completely dead. The percentage of RRDS in each replication of each tested fungal isolate was calculated according to the formula

used by Filion *et al*. (2003) as follows: RRDS % = Sum (a×b)/ (A×K) × 100

Where a= Number of diseased plants with the same scale degree of RR infection. B = Scale degree of RR infection. A= Total No. of examined plants. K= Highest scale degree of RR infection.

The casual pathogens of bean seedlingsD-O and RR were also re-isolated from the root tissues of the diseased seedlings and plants at the same conditions as mentioned before.

3. Response of some common bean cultivars to infection by *F. solani* and *M. phaseolani*:

The most frequent and highly pathogenic fungal isolates, *F. solani* (No. 8) and *M. phaseolani* (No. 14) causing bean seedling D-O and/or RR disease, were selected in this study for all greenhouse and field experiments in further studies.

3.1. Greenhouse experiments:

These experiments were conducted in the greenhouse at the Exper. Farm, Fac. of Agric., Sohag Univ., El-Kawamel, Sohag,during the 2021 and 2022 growing seasons to evaluate some common bean cultivars' susceptibility to infection by *F. solani* and *M. phaseolani* The sowing date in both experiments was the 15th of February. The common bean cultivars used were Giza 3, Giza 6, Karank, Kobo, and Nebraska, kindly supplied by the Field Crop Research Institute of Agricultural Research Center, Giza, Egypt. As mentioned before, the inocula of *Fs* and *Mp* were prepared using SG&WS medium. Formalin-sterilized 30 cm plastic pots filled with sterilized clay-loam soil were infested with 3% inoculum of each fungus and then a littlewatered every other day for 7 days, as mentioned before. Pots treated with the same amount of uninoculated SG&WS medium were served as control. Seeds of each tested cultivar were sterilized by dipping in 0.5% NaOCl solution for three min, rinsed three times in SDW for 3 min, left for drying at room conditions, and then sowed at a rate of 5 seeds per pot as mentioned before. Four pots as replications of each cultivar were used in a completely randomized experimental design. All cultivated pots were checked daily and watered when necessary. The number of pre- and post-emergence seedling D-O in each replication was recorded 14 and 28 days after planting, respectively, and the percent of each was then calculated. Likewise, after 42 days, the survival plants in each replicate of each tested cultivar were carefully uprooted, washed with tap water, and used for rating the RRDS. Then, the RRDS% was calculated using a scoring scale of 1-9 and the formula, as mentioned before. The reaction of each tested cultivar was classified based on the RRDS% values as the following: 0.0%= immune (I), 0.1 -20.0% = highly resistant (HR), 20.1-30.0%= resistant (R), 30.1-50%= susceptible (S), and more than 50.1%= highly susceptible (HS) according to Deng *et al*. (2022) with some modifications.

3.2. Field experiments:

Under field conditions and artificial infestation with *F. solani* and *M. phaseolani*, the following experiments were conducted in the Exper. Farm, Fac. of Agric., Sohag Univ., El-Kawamel, Sohag, during the 2021 and 2022 growing seasons. The planting date in both experiments was the 21st of February. In each experiment, the sterilized seeds of each tested common bean cultivar were sown in hills on rows of plots in a randomized complete block experimental design. Each row was 3.0 m long, with 0.6 m and 20 cm between hills within rows, and 12 opposite hills were per row. Three rows represented each tested cultivar as replications in each plot. A 3% inoculum (approx. 40 g) of each fungus was added to each hill with two seeds at the same time of planting and covered with soil. All cultural practices recommended for bean production were followed. As previously mentioned, pre- and post- emergence seedling D-O percentages were calculated 14 and 28 days after planting. Also, after 42 days, 15 random symptomatic plant samples per row were selected, carefully uprooted, washed with tap water, and used for rating the RRDS. The RRDS% in each replicate was calculated using a scoring scale of 1-9 and the formula, as mentioned before. The reaction of each tested cultivar to infection by *F. solani* and *M. phaseolani* was also classified based on the RRDS% values, as mentioned before.

4. Biochemical changes in the plant root of common bean highly resistant (Nebraska) and highly susceptible (Kobo) cultivars inoculated with *F. solani* and *M. phaseolani*:

Plant roots of the common bean highly resistant Nebraska and highly susceptible Kobo cultivars inoculated with *F. solani* and *M. phaseolani* were sampled after 21, 28, 35, and 42 days of planting to determine the total protein contents and also to estimate the oxidative enzyme activity of peroxidase (PO) and polyphenol oxidase (PPO). The intended experiment was done twice, with three replicates of each age of the root sample tested.

4.1. Total protein contents:

The total protein contents of the root samples of each tested cultivar were assessed according to the method defined by Bradford (1976) and using crystalline bovine serum albumin (BSA) as standard. Each cultivar's sampled roots (1.0 g of each) were heated at 85 °C with 1.0 N NaOH; the hydrolyzed protein was then assessed using Bio-Rad assay dye, and the developed color was measured at 595 nm. The total protein content in each tested root sample was then calculated as mg g^{-1} fresh weight from the standard curve of BSA.

4.2. Activity of oxidative enzymes peroxidase (PO) and polyphenol oxidase (PPO):

The PO and PPO enzyme extraction was performed according to the method defined by Maxwell and Bateman (1967). Root tissue samples (1.0 g fresh weight) were ground in a sterile mortar with 10 ml of 0.1 M phosphate buffer (pH= 7.0) and strained through layers of sterile muslin cloths. The extract of root tissues of each sample was filtrated by centrifuging for 10 min at 2500 g and four °C, and the supernatant was then used as enzyme extract. A reaction mixture contained 0.5 ml of freshly dissolved 0.5% Catechol, 1.0 ml of 0.1 M phosphate buffer, 4.5 ml SDW, and 0.2 ml of enzyme extract. The activity of PO and PPO enzymes was assessed by measuring the absorbance at 470 and 480 nm for PO and PPO, respectively, after 15 min. Then, the PO and PPO activity was expressed as absorbance g^{-1} fresh weight 15 min^{-1}.

4.3. Total phenolic contents:

Fresh roots (1.0 g) of each tested sample of each cultivar were ground and extracted in 50% methanol (12 v:v) for 90 min at 80 °C to extract and assess the total phenolic contents. The extract was centrifuged at 14.000 g for 15 min, and then the supernatant was used to determine free and cell wall-bound phenolics using the Folin–Ciocalceus (FC) reagent according to the technique described by Kofalvi and Nassuth (1995). The pellet was saponified with 2.0 ml of 0.5N NaOH for 24 h at room temperature to release the bound phenolics, neutralized with 0.5 ml 2N HCl, and then centrifuged at 14.000g for 15 min. The supernatant was used for bound phenolic determination by FC assay. Extracts of the methanol and NaOH (100 µL) were diluted to 1.0 ml with distilled water and mixed with 0.5 ml of 2N FC reagent and 2.5 ml of 20% Na_2 CO_3. The mixture was allowed to stand in the dark for 20 min at room temperature, and the absorbance of samples was then measured at 725 nm by spectrophotometer. A stock solution (1.0 mg ml^{-1}) of gallic acid was prepared in distilled water. Then, various concentrations ranging from 1.0 to 10 µg ml^{-1} were prepared. To each used concentration, 1.5 ml of FC reagent was added and kept for 5 min, and then 4 ml of 20% Na_2 CO_3 solution was added and completed up to 10 ml with distilled water. Then, the mixture was kept for 20 min, and absorbance was measured at 725 nm. The samples' total phenolic concentration (µg ml^{-1}) was extrapolated from a standard curve constructed using gallic acid as a standard. Then, the absorbance values were converted to mg of total phenolics g^{-1} of fresh weight.

5. Statistical analysis:

In this study, data obtained were statistically analyzed by the MSTAT-C program version 2.10. Duncan's multiple range tests for means comparing and the least significant difference (L.S.D.) at the *P*= 0.05 probability level was used as described by Gomez and Gomez (1984). Also, the values in the drawn figures are the means, and the bars show the standard error.

RESULTS

1. Isolation and identification of the causal pathogen of bean seedling D-O and root-rot disease:

Table 1 shows that 17 fungal isolates were isolated from ailing bean plants showing seedling D-O and RR symptoms collected from diverse locations in Sohag Governorate. All fungal isolates were identified based on their morphological features of the colony, mycelia, spores, and sclerotia. The fungi were identified as *F. oxysporum* Schlecht. (5 isolates), *F. solani* (Mart.) Sacc.(5 isolates), *M. phaseolani* (Tassi) Goid. (4 isolates), *R. solani* Kühn (2 isolates), and *S.rolfsii* Sacc. (only isolate). *F. oxysporum* and *F. solani* were the most frequently isolated fungi from bean plants' D-O and rotting root samples, recording 29.41% of each, followed by *M. phaseolani* (23.52%). In contrast, *R. solani* and *S.rolfsii* were the lowest frequently isolated fungi, recording 5.89% and 11.77%, respectively.

Table 1: Identification and frequency of fungi isolated from roots of diseased common bean plant samples showing seedling damping-off and root-rot symptoms collected from different locations in the Sohag Governorate.

Identification of isolated fungi	Isolates							
	Location						Total	Frequency (%)
	El Monshah	Baliana	Girga	El Maragha	Tahta	Johenna		
Fusarium oxysporum Schlecht.	1	2	3	4	-	5	**5**	**29.41**
Fusarium solani (Mart.) Sacc.	6	7	8	-	9	10	**5**	**29.41**
Macrophomina phaseolina (Tassi) Goid.	-	11	-	12	13	14	**4**	**23.52**
Rhizoctonia solani Kühn	-	15	-	-	-	16	**2**	**11.77**
Sclerotium rolfsii Sacc.	-	-	17	-	-	-	**1**	**5.89**
Total							**17**	**100**

1 - 17 = Isolates number.
- = Absent

2. Pathogenicity tests:

The pathogenic abilities of 17 fungal isolates of *F. oxysporum* (5 isolates), *F. solani* (5 isolates), *M. phaseolani* (4 isolates), *R. solani* (2 isolates), and *S. rolfsii* (only isolate) were determined on the common bean cv. Nebraska under greenhouse conditions in the 2020 growing season. Results in Table 2 and Figs. 1 & 2 show that all fungal isolates tested were pathogenic to the bean and significantly caused pre- and post- emergence seedling D-O and RR disease, except the isolates of *F. solani* do not cause seedling D-O, but infecting old seedlings and inducing only RR symptoms. The most dangerous effects of *M. phaseolani*, *R. solani*, *S. rolfsii* and *F. oxysporum* have occurred at the early seedling stage, where they produced pre- and post- emergence seedlings D-O. In this regard, both *M. phaseolani* isolate No. 14 and *R. solani* isolate No. 15 were the most virulent pathogens and caused the highest percentage of total seedling D-O (55% of each), followed by *S. rolfsii* isolate No. 17 (50%), isolate No. 3 of *F. oxysporum*, and *M. phaseolani* isolates No. 12 and 13 (45% of each), *F. oxysporum* isolate No. 4 and *R. solani* No. 16 (40% of each). In contrast, *F. oxysporum* isolate No. 5 was the less virulent pathogen and caused the lowest percentage of total seedling D-O (30%), followed by isolates No. 2 and 5 (35% of each). On the other hand, *F.solani* isolate No. 8 was the most RR aggressive pathogen, which infects old bean seedlings and caused the highest percentage of RR disease severity (66.10%), followed by *R. solani* isolate No. 16 (49.54%), and *M. phaseolani* isolate No.14 (48.61%). In contrast, *F.oxysporum* isolate No. 2 was the lowest virulent pathogen, which caused the lowest percentage of root rost disease severity (17.50%), followed by isolates No. 5 (18.75%) and No. 4 (19.91%). At the same time, other pathogenic fungal isolates caused RRDS% ranging from 23.30 to 42.78%.

Table 2: Pathogenic abilities of 17 fungal isolates determined on common bean cv. Nebraska under greenhouse conditions in the 2020 growing season.

Fungal isolates No.	Emergence seedling damping-off %**			RRDS%***
	Pre-	Post-	Total	
F. oxysporum				
1	15.00	20.00	35.00	23.30
2	15.00	20.00	35.00	17.59
3	20.00	25.00	45.00	29.16
4	15.00	25.00	40.00	19.91
5	10.00	20.00	30.00	18.75
F. solani				
6	0.00	0.00	0.00	36.10
7	0.00	0.00	0.00	42.78
8	5.00	0.00	5.00	66.10
9	0.00	0.00	0.00	37.21
10	0.00	0.00	0.00	32.22
M. phaseolina				
11	20.00	20.00	40.00	22.45
12	25.00	20.00	45.00	26.39
13	20.00	25.00	45.00	34.26
14	25.00	30.00	55.00	48.61
R. solani				
15	30.00	25.00	55.00	49.54
16	15.00	25.00	40.00	38.89
S. rolfsii				
17	40.00	10.00	50.00	27.50
General control*	0.00	0.00	0.00	0.00
L.S.D. at 0.05	11.99	12.87	16.50	4.92

* Pots soil treated with uninoculated SG&WS medium.
** Pre- and post- emergence seedling damping-off were assessed on bean seedlings after 14 and 28 days of planting, respectively.
*** Root rot disease severity of bean plants evaluated after 42 days of planting.

Figure 1: Symptoms of damping-off on infected seedlings common bean cv. Nebraska; pre-emergence seedling damping-off (A) and post-emergence seedling damping-off (B).

Fig. 2: Symptoms of root rot disease on seedlings of common bean cv. Nebraska caused by (A) F. solani, (B) M. phaseolina, and (C) R. solani after 42, 28, and 28 days from planting, respectively.

3. Response of some common bean cultivars to infection by *F. solani* and *M. phaseolani*:

3.1. Greenhouse experiments:

Data in Table 3 and Fig. 3 show the response of 5 common bean cultivars to infection by *F. solani* and *M. phaseolani*, causing D-O and/or RR disease under greenhouse conditions during the 2021 and 2022 growing seasons. Results show no significant pre- and post- emergence seedling D-O occurred on young seedlings of all tested common bean cultivars after inoculating the soil with *Fs*. However, the tested cultivars varied meaningfully in their reaction to infection by *Mp*, causing pre- and post- emergence seedling D-O. The common bean cv. Nebraska exhibited the highest total seedlings D-O (55%), followed by Giza 3 cv. (50%). In contrast, the common bean cv. Kobo exhibited the lowest percentage of total seedling D-O (25%), followed by Giza 6 and Karank cultivars with 30 and 35%, respectively. Results also show that the Nebraska cv. was highly susceptible (HS) to infection by *F. solani* and *M. phaseolani* causing RR at 68.33% and 51.39%, respectively. In contrast, the Kobo cv. was highly resistant (HR) to infection by *F. solani* and *M. phaseolani*, causing RR at 18.34 and 13.43%, respectively. Moreover, the Karank and Giza 6 cultivars were resistant (R), and Giza 3 was susceptible (S) to infection by RR fungi *F. solani* and *M. phaseolani*.

Table 3: Reaction of some common bean cultivars to infection by *F. solani* and *M. phaseolina*, causing seedling damping-off and/or root rot disease under greenhouse conditions in the 2021 and 2022 growing seasons. Mean over the two growing seasons.

Cultivars	*F. solani*					*M. phaseolina*				
	Emergence seedling damping-off %*			RRDS%**	Reaction	Emergence seedling damping-off %*			RRDS%**	Reaction
	Pre-	Post-	Total			Pre-	Post-	Total		
Giza 3	2.50***	0.00	2.50	48.34	S	25.00	25.00	50.00	41.67	S
Giza 6	0.00	2.50	2.50	28.89	R	20.00	10.00	30.00	26.39	R
Nebraska	2.50	0.00	2.50	68.33	HS	20.00	35.00	55.00	51.39	HS
Karank	2.50	2.50	5.00	25.84	R	15.00	20.00	35.00	23.18	R
Kobo	0.00	0.00	0.00	18.34	HR	10.00	15.00	25.00	13.43	HR
Mean	**1.50**	**1.00**	**2.50**	**37.95**	**-**	**18.00**	**21.00**	**39.00**	**31.11**	**-**
L.S.D. at 0.05	NS	NS	NS	3.86		12.31	14.03	16.04	8.38	

* Pre- and post- emergence seedling damping-off were assessed on bean seedlings after 14 and 28 days of planting, respectively.
** Root rot disease severity of common bean plants evaluated after 42 days from planting.
*** The values are the means over the two growing seasons.
NS means no significant difference.
The reaction based on RRDS% values, where 0.0%= immune (I), 0.1 -20.0% = highly resistant (HR), 20.1-30.0%= resistant (R), 30.1-50%= susceptible (S), and more than 50.1%= highly susceptible (HS).

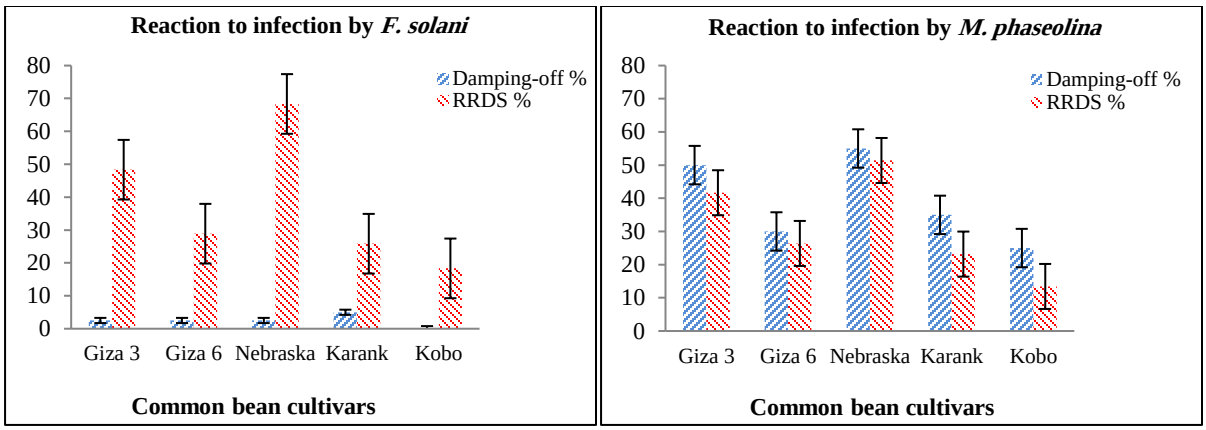

Fig. 3: Reaction of five common bean cultivars to infection by *F. solani* and *M. phaseolina*, causing seedling damping-off and/or root rot disease under greenhouse conditions.

3.2. Field experiments:

Data represented in Table 4 and Fig. 4 show the reaction of 5 common bean cultivars to infection by *F. solani* and *M. phaseolani*, causing D-O and/or RR disease under field conditions during the 2021 and 20222 growing seasons. Results show no significant pre- and post-emergence seedling D-O occurred on the young seedlings for all tested common bean cultivars after inoculating with *F. solani*. However, the cultivars varied significantly in their response to infection by *M. phaseolani*, causing pre- and post- emergence seedling D-O. Nebraska cv. has the highest percentage of total seedling D-O (55.56%), followed by Giza 3 cv. (53.57%). In contrast, the Kobo cv. has the lowest percentage of total seedling D-O (26.39%), followed by Giza 6 and Karank cultivars with 29.86 and 37.44%, respectively. Results also show that the Nebraska cv. was highly susceptible to infection by *F. solani* and *M. phaseolani*, causing RR at 67.65% and 52.59%, respectively. In contrast, the Kobo cv. was highly resistant (HR) to infection by *F. solani* and *M. phaseolani*, causing RR at 19.25 and 14.56%, respectively. Moreover, the Karank and Giza 6 cultivars were resistant (R), and Giza 3 was susceptible (S) to infection by RR pathogens *F. solani* and *M. phaseolani*.

Table 4: Reaction of some common bean cultivars to infection by *F. solani* and *M. phaseolina*, causing seedling damping-off and/or root rot disease under field conditions in the 2021 and 2022 growing seasons. Mean over the two growing seasons.

Cultivars	*F. solani*					*M. phaseolina*				
	Emergence seedling damping-off %*			RRDS%**	Reaction	Emergence seedling damping-off %*			RRDS%**	Reaction
	Pre-	Post-	Total			Pre-	Post-	Total		
Giza 3	2.08***	1.39	3.47	47.40	S	23.41	30.15	53.57	43.94	S
Giza 6	1.39	1.39	2.77	28.64	R	18.75	11.11	29.86	29.38	R
Nebraska	2.08	2.08	4.16	67.65	HS	18.05	36.81	55.56	52.59	HS
Karank	0.69	0.69	1.38	26.17	R	15.22	22.22	37.44	24.44	R
Kobo	1.39	0.69	2.08	19.25	HR	10.42	15.97	26.39	14.56	HR
Mean	**1.53**	**1.25**	**2.77**	**37.82**	**-**	**17.17**	**23.25**	**40.56**	**32.98**	**-**
L.S.D. at 0.05	NS	NS	NS	2.31		3.66	2.40	4.43	2.15	

* Pre- and post- emergence seedling damping-off were assessed on bean seedlings after 14 and 28 days of planting, respectively.
** Root rot disease severity of common bean plants evaluated after 42 days from planting.
*** The values are the means over the two growing seasons.
NS means no significant difference.
The reaction based on RRDS% values, where 0.0%= immune (I), 0.1 -20.0% = highly resistant (HR), 20.1-30.0%= resistant (R), 30.1-50%= susceptible (S), and more than 50.1%= highly susceptible (HS).

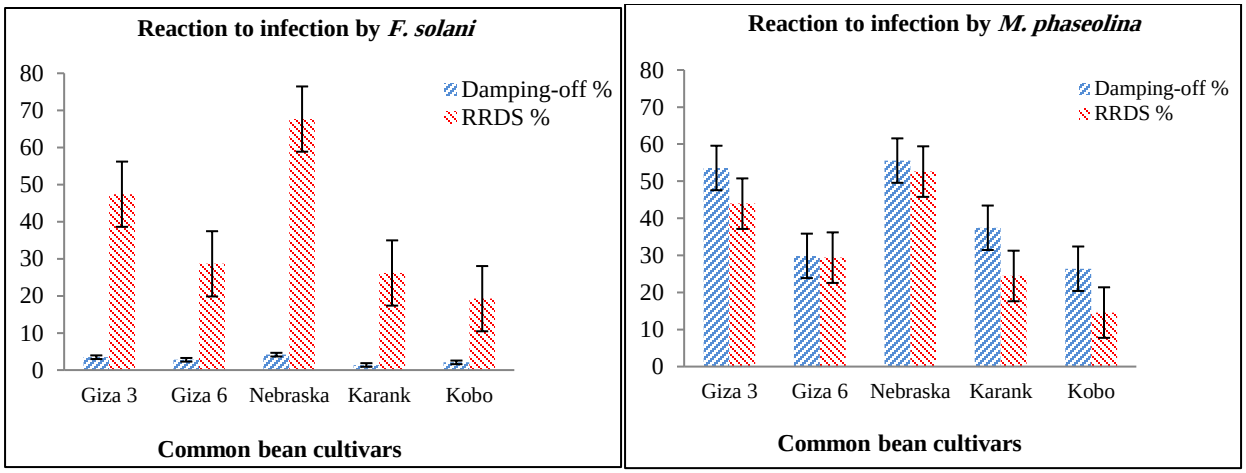

Fig. 4: Reaction of five common bean cultivars to infection by *F. solani* and *M. phaseolina*, causing seedling damping-off and/or root rot disease under field conditions.

3. Biochemical changes in the plant root of common bean highly resistant (Nebraska) and highly susceptible (Kobo) cultivars inoculated with *F. solani* and *M. phaseolani*:

3.1. Total protein contents:

Results in Table, 5 show that the total protein contents increased in the plant roots of common bean Kobo (HR) and Nebraska (HS) cultivars infected by *M. phaseolani* and *F. solani* after 21, 28, 35, and 42 days of planting compared with the control of healthy (non-infected) plants. The roots of the HR cultivar Kobo contained higher total protein contents than the HS cultivar Nebraska after 21, 28, 35, and 42 days of planting, where it recorded 1.471±0.01, 1.601±0.02, 1.741±0.02 and 1.787±0.01 mg g^{-1} fresh weight, respectively with mean of 1.650±0.02 mg g^{-1} fresh weight in roots of plants inoculated with *M. phaseolani* and 1.497±002, 1.522±0.01, 1.641±0.03 and 1.693±0.01 mg g^{-1} fresh weight, respectively with mean of 1.588.02 mg g^{-1} fresh weight in roots of plants inoculated with *F. solani*.

Table 5: Total protein contents in the plant roots of the common bean highly resistant (HR) Nebraska and highly susceptible (HS) Kobo cultivars inoculated with *F. solani* and *M. phaseolina*

Fungal pathogen	Total protein content (mg g-1 fresh weight) in the plant roots of common bean cv. Nebraska (HS) after					Total protein content (mg g-1 fresh weight) in the plant roots of common bean cv. Kobo (HR) after				
	21 days	28 days	35 days	42 days	Mean	21 days	28 days	35 days	42 days	Mean
F. solani	1.481±0.08**	1.515±0.033	1.623±0.25	1.631±0.03	**1.563±0.17**	1.497±0.02	1.522±0.01	1.641±0.03	1.693±0.01	**1.588±0.02**
M. phaseolina	1.502±0.08	1.653±0.31	1.721±0.34	1.737±0.03	**1.654±0.19**	1.471±0.01	1.601±0.02	1.741±0.02	1.787±0.01	**1.650±0.02**
Control*	1.476±0.03	1.489±0.33	1.502±0.93	1.514±0.17	**1.495±0.36**	1.408±0.01	1.435±0.02	1.462±0.03	1.476±0.02	**1.445±0.02**
Mean	**1.483±0.06**	**1..552±0.32**	**1.615±0.50**	**1.627±0.08**	**1.571±0.24**	**1.458±0.01**	**1.519±0.02**	**1.614±0.03**	**1.652±0.01**	**1.561±0.02**

* Uninoculated common bean plants with fungal pathogens.

** Values are the means (mg g-1 fresh weight ±standard deviation) over three replicates from the standard curve of BSA.

3.2. Activity of oxidative enzymes peroxidase (PO) and polyphenol oxidase (PPO):

Results represented in Tables, 6 and 7 show that the activity of PO and PPO increased in the plant roots of common bean HR Kobo and HS Nebraska cultivars infected by *Mp* and *Fs* after 21, 28, 35, and 42 days of planting compared with the control of healthy (non-infected) plants. The roots of the HR cultivar Kobo exhibited higher activity of PO and PPO than the HS cultivar Nebraska after 21, 28, 35 and 42 days of planting, where it recorded (0.351±0.16, 0.401±0.22, 0.451±0.12 and 0.467±0.21, respectively) and (0.341±0.15, 0.391±0.14, 0.433±0.14, 0.459±0.22, respectively) absorbance g^{-1} fresh weight 15 min^{-1}, respectively with mean of (0.417±0.17 and 0.405±0.13 absorbanceg^{-1} fresh weight 15 min^{-1}, respectively) in roots of plants inoculated with *M. phaseolani* and (0.335±12, 0.398±0.13, 0.441±0.15 and 0.451±0.21, respectively) and (0.243±0.17, 0.389±0.21, 0.421±0.13, and 0.453±0.18, respectively) absorbance g^{-1} fresh weight 15 min^{-1}, respectively with mean of (0.405.12 and 0.376±0.10 absorbanceg^{-1} fresh weight 15 min^{-1}, respectively) in roots of plants inoculated with *F. solani*.

Table 6: Peroxidase (PO) activity in the plant roots of the common bean highly susceptible (HS) Nebraska and highly resistant (HR) Kobo cultivars inoculated with *F. solani* and *M. phaseolina*.

Fungal pathogen	PO activity in the plant roots of common bean Nebraska cv. (HS) after					PO activity in the plant roots of common bean Kobo cv. (HR) after				
	21 days	28 days	35 days	42 days	Mean	21 days	28 days	35 days	42 days	Mean
F. solani	0.243±0.17**	0.389±0.21	0.421±0.13	0.453±0.18	**0.376±0.17**	0.335±0.12	0.398±0.13	0.441±0.15	0.451±0.21	**0.405±0.15**
M. phaseolina	0.341±0.15	0.391±0.14	0.433±0.14	0.459±0.22	**0.405±0.13**	0.351±0.16	0.401±0.22	0.451±0.12	0.467±0.21	**0.417±0.17**
Control*	0.188±0.13	0.211±0.13	0.223±0.13	0.234±0.21	**0.214±0.15**	0.201±0.17	0.220±0.22	0.248±0.13	0.259±0.21	**0.232±0.18**
Mean	**0.257±0.15**	**0..330±0.13**	**0.359±0.13**	**0.382±0.20**	**0.332±0.15**	**0.296±0.15**	**0.339±0.19**	**0.380±0.13**	**0.392±0.21**	**0.351±0.17**

* Uninoculated common bean plants with fungal pathogens.
** Values are the means (absorbance ±standard deviation g-1 fresh weight 15 min-1) over three replicates.

Table 7: Polyphenol oxidase (PPO) activity in the plant roots of the common bean highly susceptible (HS) Nebraska and highly resistant (HR) Kobo cultivars inoculated with *F. solani* and *M. phaseolina*.

Fungal pathogen	PPO activity in the plant roots of common bean Nebraska cv. (HS) after					PPO activity in the plant roots of common bean Kobo cv. (HR) after				
	21 days	28 days	35 days	42 days	Mean	21 days	28 days	35 days	42 days	Mean
F. solani	0.227±0.11**	0.239±0.13	0.259±0.15	0.267±0.12	**0.248±0.13**	0.227±0.12	0.239±0.13	0.258±0.12	0.266±0.11	**0.247±0.12**
M. phaseolina	0.230±0.12	0.243±0.11	0.266±0.14	0.279±0.13	**0.254±0.19**	0.229±0.11	0.240±0.12	0.261±0.13	0.279±0.11	**0.252±0.12**
Control*	0.200±0.12	0.210±0.13	0.219±0.13	0.228±0.12	**0.214±0.13**	0.217±0.11	0.231±0.12	0.245±0.13	0.255±0.12	**0.237±0.12**
Mean	**0.219±0.12**	**0..231±0.12**	**0.248±0.14**	**0.258±0.12**	**0.239±0.15**	**0.224±0.11**	**0.237±0.12**	**0.255±0.13**	**0.267±0.11**	**0.245±0.12**

* Uninoculated common bean plants with fungal pathogens.
** Values are the means (absorbance ±standard deviation g-1 fresh weight 15 min-1) over three replicates.

3.3. Total phenolic contents:

Results in Table8 indicate that the total phenolic contents gradually increased in the plant roots of common bean Kobo (HR) and Nebraska (HS) cultivars infected by *M. phaseolani* and *F. solani* after 21, 28, 35, and 42 days of planting compared with the control of healthy (non-infected) plants. The roots of the HR cultivar Kobo contained higher total phenolic contents than the HS cultivar Nebraska after 21, 28, 35, and 42 days of planting, where it recorded 8.229±0.11, 10.240±0.12, 12.261±0.13 and 14.279±0.11 mg g^{-1} fresh weight, respectively with mean of 11.252±0.12 mg g^{-1} fresh weight in roots of plants inoculated with *M. phaseolani* and 8.227±012, 10.239±0.13, 12.258±0.12 and 14.266±0.11 mg g^{-1} fresh weight, respectively with mean of 11.248.12 mg g^{-1} fresh weight in roots of plants inoculated with *F. solani*. While Nebraska cv. recorded 6.230±0.12, 8.243±0.1, 9.266±0.14 and 10.279±0.13 mg g^{-1} fresh weight, respectively, with a mean of 8.505±0.19 mg g^{-1} fresh weight in roots of plants inoculated with *Mp* and 6.227±011, 8.239±0.13, 9.259±0.15 and 10.267±0.12 mg g^{-1} fresh weight, respectively with mean of 8.498.13 mg g^{-1} fresh weight in roots of plants inoculated with *F. solani*.

Table 8: Total phenolic contents in the plant roots of the common bean highly susceptible (HS) Nebraska and highly resistant (HR) Kobo cultivars inoculated with *F. solani* and *M. phaseolina*.

Fungal pathogen	Total phenolic contents (mg of phenolics g^{-1} of fresh weight) in the plant roots of common bean cv. Nebraska (HS) after					Total phenolic contents (mg of phenolics g^{-1} of fresh weight) in the plant roots of common bean cv. Kobo (HR) after				
	21 days	28 days	35 days	42 days	Mean	21 days	28 days	35 days	42 days	Mean
F. solani	6.227±0.11**	8.239±0.13	9.259±0.15	10.267±0.12	**8.498±0.13**	8.227±0.12	10.239±0.13	12.258±0.12	14.266±0.11	**11.248±0.12**
M. phaseolina	6.230±0.12	8.243±0.11	9.266±0.14	10.279±0.13	**8.505±0.19**	8.229±0.11	10.240±0.12	12.261±0.13	14.279±0.11	**11.252±0.12**
Control*	6.200±0.12	6.210±0.13	7.219±0.13	7.228±0.12	**6.714±0.13**	6.217±0.11	6.231±0.12	7.245±0.13	7.255±0.12	**6.737±0.12**
Mean	**6.219±0.12**	**7.564±0.12**	**8.581±0.14**	**9.258±0.12**	**7.906±0.15**	**7.558±0.11**	**8.903±0.12**	**10.588±0.13**	**11.930±0.11**	**9.745±0.12**

* Uninoculated common bean plants with fungal pathogens.

** Values are the means (mg g-1 fresh weight ±standard deviation) over three replicates from the standard curve of Gallic acid.

Discussion

In the current study, seventeen fungal isolates were obtained from naturally ailing plant samples of common beans showing seedlings D-O and RR symptoms collected from diverse locations in Sohag Governorate. The isolated fungi were identified as *Fusarium oxysporum* Schlecht. (5 isolates), *Fusarium solani* (Mart.) Sacc. (5 isolates), *M. phaseolani* (Tassi) Goid. (4 isolates), *Rhizoctonia solani* Kühn (2 isolates), and *Sclerotium rolfsii* Sacc. (only isolate). Identification of all obtained fungal isolates was perfo rmed based on their morphological features of the colony, mycelia, spores, and sclerotia described by Leslie and Summerell (2006). Concerning the frequency of fungi obtained from diseased bean plant samples showing seedling D-O and RR symptoms, *F. oxysporum* and *F. solani* were the most frequent, recording 29.41% of each, followed by *M. phaseolani* (23.52%). In contrast, *S. rolfsii* and *R. solani* were the lowest frequencies, registering 5.89% and 11.77%, respectively. These findings could be interpreted in light of similar results in Egypt and worldwide reported by Abd El-Hai and Ali, Abeer (2018), Al-Juboory and Al-Bindawy (2018), Mukamuhirwa *et al.*(2018 a and b), Paparu *et al.* (2018), Koçak and Erper (2019), Khalifa *et al.* (2021), Omar *et al.* (2021), Rahkhodaei *et al.* (2021), Ahanger *et al.* (2022),

Ali, Mai *et al*. (2022), Elagamey *et al*. (2022), Muhanna *et al*. (2023), and Tilahun *et al*.(2023). Koch's postulates of the obtained 17 fungal isolates were fulfilled by the pathogenicity tests performed on the bean cv. Nebraska under greenhouse conditions in the 2020 growing season. This study demonstrated that all 17 fungal isolates testedcould early infect young bean seedlings and significantly cause pre- and post- emergence seedling D-O and RR disease, except the *Fs* isolates do not induce seedling D-O but induce only RR symptoms on old seedlings after 42 days of bean planting. *M. phaseolani*, *R. solani* and *S. rolfsii* and *F. oxysporum* caused the most dangerous effects at the seedling stage, where they caused pre- and post- emergence seedling D-O. *M. phaseolani* isolates No.14 and *R. solani* isolate No.15 were found to be the most virulent pathogens with total seedling D-O (55% of each). In contrast, isolate No.5 of *F. oxysporum* was found to be the less virulent pathogen with total seedling D-O (30%). On the other hand, *F. solani* isolate No.8 was the most RR aggressive pathogen, which caused RR disease severity of 66.10% after 42 days of planting, followed by *R. solani* isolate No.16 (49.54%), and *M. phaseolani* isolate No.14 (48.61%). In contrast, *F. oxysporum* isolate No.2 was the lowest virulent pathogen, which caused RR disease severity of 17.50%. At the same time, the other tested fungal isolates caused RR disease severity ranging from 23.30 to 42.78%. These findings could be also interpreted in light of similar results reported by Abd El-Hai and Ali, Abeer (2018), Adesemoye *etal.* (2018), Al-Juboory and Al-Bindawy (2018), Mukamuhirwa *et al*. (2018 a and b), Paparu *et al*. (2018), Koçak and Erper (2019), Khalifa *et al*. (2021), Omar *et al*. (2021), Rahkhodaei *et al*. (2021), Ali, Mai *et al*. (2022), Ahanger *et al*. (2022), Elagamey *et al*. (2022), Muhanna *et al*. (2023), and Tilahun *et al*. (2023), who reported dissimilar levels of virulence between the tested fungal isolates of *F. oxysporum*, *F. solani*, *M. phaseolani*, *R. solani* and *S. rolfsii*, which may be due to the difference in the genetic structure of each fungal isolates. It is known that early infection with these fungal pathogens often reduces bean seedling emergence, where they cause seedlings' death (D-O) before or shortly after appearance from the soil, resulting in irregular field plant stands and severe loss in the plant yield and death of severely affected plants (Opio *et al*., 2000; Harveson *et al*., 2005). In the case of bean infection by *F. solani*, unlike the other root-rotting fungi, including *F. oxysporum*, *M. phaseolani*, *R. solani*, and *S. rolfsii*, the fungus *F. solani* does not cause seed rot and seedling D-O, and the RR symptoms do not appear until two or three weeks after bean planting (Román-Avilés *et al*., 2003). In the current study, the RR pathogen *F. solani* did not cause seedling D-O on the young seedlings of bean cv. Nebraska in which disagree with the results obtained by El-Mohamedy and Abd Alla (2013), Shahda, Wafaa *et al*. (2017), Abd El-Hai and Ali, Abeer (2018), Al-Juboory and Al-Bindawy (2018) and Ali, Mai *et al*. (2022), who showed that *Fs* caused pre- and post- emergence seedling D-O on the young seedlings after 15 and 45 days, respectively of planting when they tested the same bean cv. Nebraska and/or other cultivarsin different districts than Sohag in Egypt. In these studies, the investigators considered the bean seedling at 45 days old as a young seedling. Likewise, Abd-El-Khair *et al*. (2010) reported that *F. solani* and *R. solani* were the common causal pathogens of seedling D-O and RR disease of common beans, and the fungus *Fs* can attack young bean seedlings (after 45 days from planting), and it is most severe on bean plants growing under stressful conditions. No available information worldwide, except in Egypt, has reported *Fs* as the causal pathogen of bean D-O. Only a previous report in Canada regarding *F. solani* f. sp. *phaseoli* causing white bean post-emergence RR (after 4 weeks from planting) was documented (Reddy *et al*., 1994). Using resistant bean cultivars is the most effectivecontrolapproach against RR diseases (Nzungize *et al*., 2012). In this study, the vulnerability of common bean cultivars to infection by *F. solani* and *M. phaseolani* causing D-O and/or RR disease, varied significantly under artificial inoculation in the greenhouse and field experiments during the 2021 and 2022 growing seasons. Results of both greenhouse and field experiments are comparable and showed no significant infection by pre- and post- emergence seedling D-O occurred on the young seedlings of all tested common bean cultivars after inoculating the soil with *F. solani*. However, the tested five cultivars varied significantly in their reaction to infection by *M. phaseolani*, causing pre- and post-

emergence seedling D-O. Nebraska cv. exhibited the highest percentage of total seedling D-O, followed by Giza 3 cv. In contrast, the Kobo cv. showed the lowest percentage of total seedling D-O, followed by Giza 6 and Karank cultivars, respectively. These findings could be interpreted in light of similar results of Baraka *et al.* (2004), Rashad *et al.* (2012), Shahda, Wafaa *et al.* (2017), Ali, Mai *et al.*(2022), and Muhanna *et al.* (2023), who reported the difference in the response levels of the same or other common bean cultivars to infection by *M. phaseolani* causing bean seedling D-O in other districts than Sohag in Egypt. In this study, the common bean cv. Nebraska was found to be highly susceptible to RR disease caused by *F. solani* and *M. phaseolani* In contrast, the Kobo cv. was highly resistant to infection by RR pathogens *F. solani* and *M. phaseolani*. Moreover, the Karank and Giza 6 cultivars were resistant, and Giza 3 was susceptible to infection by both RR pathogens. These results agree with those obtained by Shahda, Wafaa *et al.* (2017), Ali, Mai *et al.*(2022), and Muhanna *et al.*(2023), who reported the difference in the response levels of the same or other common bean cultivars to both RR pathogens in different districts than Sohag in Egypt. In addition, many studies worldwide have been applied by which the susceptibilities of bean cultivars, varieties, lines, and genotypes to infection by the same RR pathogens *F. solani* and *M. phaseolani* and/or other fungi were evaluated and showing different response levels (Adesemoye *etal.*, 2018; García *et al.*, 2019; Mohamed and Atallah, 2020; Zanella *et al.*, 2020; Haus *et al.*, 2021; Hesami, Nafiseh *et al.*, 2021; Omar *et al.*, 2021; Rahkhodaei, 2023; Tilahun *et al.*, 2023). In an earlier study, *Fusarium* RR was controlled by root genotype, and root vigor played an essential part in resistance (Cichy *et al.*, 2007). Likewise, Wang *et al.* (2018) established that bean-resistant lines had a little higher root biomass. These lines hypothesized that the quantitative trait locus (QTL) of a region of the DNA associated with *Fusarium*RR resistance is more likely related to root biomass. In previous studies, six QTL were identified for resistance to *Fusarium* RR using a RIL population derived from a cross between the RR susceptible snap bean 'Eagle' and 'Puebla 152', a small black seeded *Fusarium* RR resistant dry bean (Navarro *et al.*, 2004). Most of these QTL were located on LGs B2 and B3 of the integrated bean map (Freyre *et al.*, 1998) close to a region where the defense response genes Pgip and ChS to *Fusarium*RR and pathogenes-related proteins, PvPR-1 and PvPR-2, have been identified (Schneider, Kristin, *et al.*, 2001). Several studies on resistance to *Fusarium*RR have reported and confirmed about two to four resistance genes (Hassan *et al.*, 1971; Mukankusi *et al.*, 2011). Regardingthe susceptibility of bean cultivars to infection by *M. phaseolani*, the different resistance levels expressed in the experiments and the variation in resistance between the two seasons also suggest that resistance to the RR pathogen is polygenic (García *et al.*, 2019). Furthermore, the variation in the resistance expressed in the two seasons is comparable with the broad variation in the results reported for resistance among genotypes of common bean to *M. phaseolani* in Brazil (García *et al.*, 2019). However, further studies are still needed to define if the resistance to both pathogens is due to root architecture, biochemical changes, or some other feature. The disease-resistance mechanism is a complex phenomenon in response to invasion by a pathogen, by which the host plant may produce various biochemical reactions (Moharam, 2013). Therefore, the biochemical changes such as pathogenesis-related protein, including the activity of oxidative enzymes PO and PPO, as well as phenolic contents in roots of the common bean highly resistant (Kobo) and highly susceptible (Nebraska) cultivars, were investigated following infection by the D-O and/or RR pathogens to understand their role in the physiology of disease resistance. In this study, results showed a gradual increase in the total protein contents, the activity of PO and PPO, as well as total phenolic contents occurred in the plant roots of common bean Kobo (HR) and Nebraska (HS) cultivars infected by *M. phaseolani* and *F. solani* after 21, 28, 35, and 42 days of planting compared with the control of non-infected plants. However, the roots of the HR cultivar Kobo contained higher total protein contents, PO and PPO activities, as well as total phenolic contents than the HS cultivar Nebraska inoculated with *M. phaseolani* and *F. solani*. These results could be interpreted in light of similar other findings reported by Leite *et al.* (2014), Kumari *et al.* (2015), Pareek and Varma (2015), Poornima *et al.* (2016), Belkar *et al.* (2018), and Tagele *et al.*

(2019), who reported the increasing in the total protein contents, the activity of PO, and PPO as well as the total phenolic contents in host tissues in their reaction to infection by the causal pathogen. In this regard, the expected increase in total protein levels in roots of protected plants of HR cultivar Kobo, after inoculation with D-O and/or RR pathogens, could lead to increased activities of defense-related lignification enzymes PO and PPO. It is well known that the oxidative enzymes PO and PPO play an essential role in the defense mechanism of plants towards attacking fungal pathogens by oxidation of phenols to toxic quinones, which limit and stop fungal progress inside tissue cells (Melo *et al.*, 2006; Shimzu *et al.*, 2006; Khatun *et al.*, 2011). Moreover, the PO enzyme itself was reported to inhibit spore germination and mycelial growth of certain fungi (Joseph *et al.*, 1998) and also catalyze the final polymerization stage of lignin synthesis in plant tissue cells, which increases the capability of the tissue to lignify, which may also lead to restriction of fungal penetration and protect the plant root against infection and invasion (Barilli *et al.*, 2010). It is also well known that phenolic compounds play an important role in the defense mechanism against fungal infection by inhibiting the penetration of fungi and extending their growth and attack in plant tissues (Kalaichelvan and Flangovan, 1995).

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دور التغيرات البيوكيميائية لأصناف الفاصوليا شديدة المقاومة والحساسة للإصابة في فسيولوجيا مقاومة مرض موت البادرات و/أو عفن الجذور

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الملخص العربي

في هذه الدراسة تم التعرف على سبع عشرة عزلة فطرية مأخوذة من نباتات الفاصوليا المريضة التي يظهر عليها أعراض سقوط البادرات وأعفان الجذور التي تم جمعها من مواقع مختلفةٍ في محافظة سوهاج على أنها *Fusarium oxysporum* Schlecht. (5 عزلات) ، *Fusarium solani* (Mart.) Sacc. (5 عزلات) ، *Macrophomina phaseolina* (Tassi) Goid (4 عزلات) ، *Rhizoctonia solani* Kühn (2 عزلة) ، و *Sclerotium rolfsii* Sacc. (عزلة واحدة فقط). علاوةً على ذلك ، كان الفطر *F. oxysporum* (*Fo*) و *F. solani* (*Fs*) أكثر الفطريات عزلًا بنسبة 29.41٪ لكلٍ منهما ، يليهما 23.52 (Mp)٪ . في المقابل ، كان *S. rolfsii* (*Sr*) و *R. solani* (*Rs*) أقل الفطريات المعزولة بشكل متكرر. أظهرت اختبارات القدرة المرضية أن جميع العزلات الفطرية التي تم اختبارها كانت مسببة لتعفن البذور وتسببت بشكل كبير في سقوط البادرات قبل وبعد الظهور ومرض تعفن الجذور ، باستثناء عزلات *Fs* لا تسبب موتاً للبادرات ، ولكنها تصيب البادرات الكبيرة في العمر وتسبب فقط أعراض تعفن الجذور. تمت دراسة استجابة 5 أصناف من الفاصوليا الشائعة للعدوى بواسطة *Fs* و *Mp* ، مما تسبب في سقوط البادرات و/أو مرض تعفن الجذور ، تحت ظروف الدفيئة والحقل خلال موسمي النمو لعامي 2021 و 2022. وأظهرت النتائج أنه لم يحدث موتاً للبادرات الصغيرة من جميع أصناف الفاصوليا المختبرة بعد تلقيح التربة بـ *Fs*. اظهر صنف نبراسكا أعلى نسبة لموت البادرات. في المقابل ، فإن صنف كوبوأظهر أقل نسبة من الموت الكلي للبادرات، صنف نبراسكا كان شديد الحساسية (HS) للعدوى بواسطة Fs و *Mp* ، مما تسبب في مرض تعفن الجذور. بينما كان صنف كوبو شديد المقاومة (HR) للعدوى بواسطة *Fs* و *Mp*. علاوةً على ذلك ، فإن الصنفين الكرنك وجيزة 6 كانا مقاومين (R) ، وصنف جيزة 3 كان عرضة (S) للإصابة بفطري تعفن الجذور *Fs* و *Mp*. أظهرت التغيرات البيوكيميائية في جذر نبات الفاصوليا أن صنف كوبو (HR) يحتوي على محتوى بروتيني أعلى من الصنف (HS) نبراسكا بعد 21 و 28 و 35 و 42 يومًا من الزراعة. زاد نشاط إنزيمات البيروكسيديز (PO) والبوليفينول أوكسيديز PPO في جذور نباتات الفاصوليا كوبو(HR) و(HS) نبراسكا المصابة بـ *Mp* و *Fs* بعد 21 و 28 و 35 و 42 يومًا من الزراعة مقارنةً بالكنترول في الأصناف السليمة (غير المصابة). أظهرت جذور صنف كوبو (HR) نشاط PO و PPO أعلى من صنف HS نبراسكا بعد 21 و 28 و 35 و 42 يومًا من الزراعة. كما زاد إجمالي محتويات الفينول تدريجيًا في جذور نباتات الفاصوليا (HR) كوبو (HS)المصابة بـ *Mp* و *Fs* بعد 21 و 28 و 35 و 42 يومًا من الزراعة مقارنةً بنباتات الكنترول السليم (غير المصاب). احتوت جذور الصنف كوبو (HR) على إجمالي محتويات فينوليه أعلى من الصنف (HS) نبراسكا بعد 21 و 28 و 35 و 42 يومًا من الزراعة.