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BIOCHEMICAL MECHANISMS OF RESISTANCE IN SOME BEAN CULTIVARS AGAINST CHARCOAL ROT DISEASE

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ABSTRACT

Charcoal rot disease affects some morphological characters of three bean cultivars with different degrees of resistance. Carbohydrates, phenols and free amino acid content were determined in roots and stems of these three bean cultivars, (Nebraska, Morgan, and Xera) to study the nature of disease resistance among bean cultivars accompanied with their chemical components. The low susceptible cultivar Nebraska showed the lowest amount of reducing, non-reducing, total sugars, insoluble and total carbohydrates and exhibited the highest amount of free, conjugated total phenols and free amino acids compared with cv. Xera (highly susceptible cv.). Charcoal rot disease decreased the carbohydrate contents and increased the phenolic and amino acid contents. Cobalt nitrate and ethylene diamine decreased the disease incidence in plants, treated as seed soaking or soil drenching. Cobalt nitrate and potassium chloride increased the oxidative enzyme activity in the cotyledons treated as seed soaking or soil drenching.

Key words: *bean, charcoal rot disease, induced systemic resistance, Macrophomina phaseolina, oxidative enzyme activity.*

1. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the main source of protein in the diet of more than 100 million people in Latin America and Africa. In Egypt ,the cultivated area of bean is limited and the country needs to increase the production to meet the increment of consumption. The common bean is considered one of the most promising vegetable crops for local consumption and exportation. The infection with charcoal rot disease affects many morphological characters and chemical constituents of various cultivars according to their degree of resistance and susceptibility.

Faris *et al.,* (1992) indicated that cvs. Bronco, Morgan, Magum and XPB 201 gave the best total green pod yield, while Gazonil cv. was moderately in this respect, whereas the cvs. Massai and Mercur gave the lowest yield. As for total dry seed yield, cvs. Morgan, Giza 6, Bronco and XPB 201 gave highly yield, while cvs. Forum, Mognum, Primil, Massai, Baslin, Tilla and Mercure gave the lowest total dry seed yield. Songa *et al.,*(1997) reported that yield of common bean lines resistant to *M. phaseolina* ranged from 135 to 1051 kg/ha compared with 55 kg/ha for the susceptible ones. On the other hand, Jadeja and Patel (1989) showed that the content of phenols was higher in the resistant *Phaseolus lunatus* cultivar PLJ-1 than in the susceptible one *i.e.* PLJ-5. Phenols and amino acid contents were increased in the diseased stems of some bean cultivars infected by *M. phaseolina* than in healthy ones. The lowest share of acidic amino acids in root exudates and the highest share of aromatic and alkaline amino acids were found in the resistant cultivars (Pieta 1994).

Siegrist *et al.*, (1997) induced a systemic acquired resistance (SAR) in bean leaves by spraying with some chemical agents or BTH as a new developed form of application. The seeds of bean were allowed to germinate in the induced solution. A minimal period of 4 days was necessary to obtain resistance against some bacterial and fungal pathogens. Eisa, Nour-Jehan (1998) demonstrated that the total sugars in root exudates of cv. Giza 3 (highly susceptible) were higher than those observed in the root exudates of cv. Bronco (less susceptible one). Free and total phenol contents in the root exudates of the less susceptible bean cultivar *i.e.*, Bronco were much greater than those obtained from the root of highly susceptible cultivar.

The aim of the present work was to provide additional information on the nature of resistance of some bean cultivars against charcoal rot disease caused by *M. phaseolina* by studying some morphological and biochemical changes associated with this disease in the roots and stems of three bean cultivars under artificial infection. Also, the use of abiotic and synthetic compounds as the inducers of systemic resistance and their effects on some oxidative enzymes was investigated.

2. MATERIALS AND METHODS

The infection with *M. phaseolina* affects some morphological and biochemical changes of bean cultivars.

2.1. Morphological characters

This experiment was carried out to throw some light on the effect of *M. phaseolina* on the morphological characters of 3 bean cultivars namely, Nebraska, Morgan and Xera. Barley medium inoculated with the fungus, previously isolated from sweetmelon roots at Eltal Elkabeer. It was added to the soil in clay pots (25 cm in diameter) at the rate of 3 barley seeds/ bean seed. Five seeds were grown in each pot and five replicates were used for each treatment. Similar numbers of pots, containing non-inoculated soil were used as control. Fresh and dry weight of root (gm), shoot and whole plant were examined. Leaf area (cm^2), stem length (cm), number of branches and pods/plant, weight of fresh yield/ plant (gm) and weight of 100 dry seeds (gm) were also, calculated.

2.2. Biochemical characters

To study the nature of resistance, some chemical components were estimated in 3 cultivars of bean plants, *i.e.* Nebraska, Morgan and Xera as less susceptible, moderately and highly susceptible cultivars, respectively.

This experiment was carried out to determine some chemical constituents such as carbohydrates, phenolic compounds and free amino acids due to infection with *M. phaseolina.* These chemical constituents were determined in stems and roots of infected and healthy of the aforementioned three bean cultivars. Samples for analysis were taken twice *i.e.*, 10 and 20 days after inoculation. Control treatment (non-inoculated seedlings) was also run in the same manner.

2.2.1. Determination of reducing, non reducing, total sugars and total carbohydrates: A weight of 0.5 gm of dry plant material powder was extracted using 50 ml of ethanol and incubated in a water bath at 70 °C for 3 hours, then the mixture was filtered. The filtration was made up to 100 ml with distilled water. Reducing, non-reducing and total sugars as well as total carbohydrates were measured at 550 nm according to the methods described by Bernfeld (1955). The data were expressed as mg glucose/gm dry weight from the standard curve of glucose.

2.2.2. Determination of phenolic compounds: Phenolic compounds (free, conjugated and total phenols) in inoculated and non-inoculated roots and stems were determined using the calorimetric method as suggested by Snell and Snell (1953). Color density was detected spectrophotomtrically at 520 nm and determined as mg catechol/gm dry weight from the standard curve of catechol.

2.2.3. Determination of free amino acids: Paper chromatograms for quantitatively separation of the individual amino acids was used with extraction of inoculated and non-inoculated samples of bean cultivars at two period intervals. The solvent system was prepared according to Block (1958). Spots were made visible by means of 0.2% ninhydrin in acetone (w/v).

2.3. Abiotic and synthetic compounds as inducers of systemic resistance

Potassium chloride, cobalt nitrate, ethylene diamine and sodium carbonate were evaluated as abiotic or chemical inducers at the rate of 20 ppm. The enzyme activities of peroxidase and polyphenol oxidase were determined in the cotyledons. Enzyme activity of peroxidase and polyphenol oxidase was measured at 10 and 20 days after inoculation as follows:

2.3.1. Seed treatment: Sterilized seeds of Xera bean cultivar were soaked in water suspension of abiotic inducers at 20 ppm for 1, 5 and 10 hr. The seeds were sown in the infested soil at the rate of 5 seeds/pot. Three replicates were employed for each treatment. Three pots were left without treatment and used as a control. The percentages of pre- post- emergence damping-off, charcoal rot and surviving plants were recorded as mentioned before. Also, peroxidase and polyphenol oxidases as mechanisms of induced resistance were determined in the cotyledons of Xera bean cultivar at 10 and 20 days after inoculation.

2.3.2. Soil treatment: Soil drench was carried out in clay pots during the sowing time with water suspension of abiotic inducers at 20 ppm (250 ml/pot). Other pots were left without treatment as a control. The percentage of pre- post - emergence damping-off, charcoal rot and surviving plants were determined as described before.

For enzymatic activity (peroxidase and polyphenol oxidases), several clay pots were divided into four groups as follows: 1) Pots filled with infested soil and treated with inducers. 2) Pots filled with uninfested soil and treated with inducers. 3) Pots filled with infested soil and untreated. 4) Pots filled with uninfested soil or amended with inducers.

Also, peroxidase and polyphenol oxidases as mechanisms of induced resistance were determined in the cotyledons of Xera bean cultivar at 10 and 20 days after inoculation. The enzyme extracts from the infected and healthy cotyledons of Xera bean cultivar were prepared as recommended by Maxwell and Bateman (1967). The supernatant fluids were used for enzyme assay.

The peroxidase and polyphenol oxidase assay was colorimetrically determined using Specol Spectrocolourimeter (Carl Zeiss, Jana) at 27 ± 2°C. The readings of the colorimeter were recorded every 30 sec. for 5 min. The control cuvette contained the same solution except that the substrate solution was replaced by distilled water.

Peroxidase activity was estimated according to the method of Allan and Hollis (1972) by measuring the oxidation of Pyrogallol to Pyrogallin in the presence of H_2O_2 at 425 nm, whereas, the activity of polyphenol oxidase was determined by the calorimetric method of Maxwell and Bateman (1967). The activity of polyphenol oxidase was expressed as the change in absorbance/1.0 ml of extract per min. at 495nm.

3. RESULTS

3.1. Morphological changes

Charcoal rot disease affected some morphological characters of the bean cultivars (Nebraska, Morgan and Xera). This effect varied from one cultivar to another (Table 1). Data show that the fresh and dry weights of the roots, shoots and whole plant decreased in the infected plants than the healthy ones for the three tested bean cultivars. Nebraska was less affected compared with Morgan and Xera, which exhibited highly response to infection.

Leaf area of the infected plants was less than the healthy ones for the three bean cultivars being 43.26 and 68.30 cm^2 (for cv. Nebraska), 50.76 and 61.93 cm^2 (for cv. Morgan), 30.33 and 34.33 cm^2 (for cv. Xera), respectively. Stem length in the infected plants was shorter than the healthy ones. Number of branches /plant of the infected plants was less if compared with the healthy ones. Number of pods/plant in the infected plants was less than the healthy ones being 18.00, 18.33,15.76,16.67, 11.86 and 15.69 pods/plant for the three bean cultivars, respectively.

Weight of fresh yield/ plant exhibited the lowest amounts in the infected plants compared with the healthy ones for the three tested bean cultivars. The same trend was true concerning the weight of 100 dried seeds, being 89.2 and 90.1 gm (Nebraska), 33.1 and 34.23 gm (Morgan), 34.56 and 36.23 gm (Xera) for infected and healthy plants Nebraska, Morgan and Xera, respectively.

3.2. Biochemical changes

Carbohydrates, phenols, free amino acid contents were determined in the infected, healthy roots and stems of the aforementioned bean cultivars, to study the nature of resistance among these cultivars including their chemical components.

3.2.1. Determination of carbohydrate contents: Soluble carbohydrates (reducing, non- reducing and total sugars), insoluble carbohydrates and total carbohydrate contents of the infected and healthy plants of three bean cultivars twice (10 and 20 days after

Table (1): Growth parameters of three tested bean cultivars (Nebraska, Morgan and Xera) as affected by charcoal rot disease under greenhouse conditions.

Cultivar		Dry weight (gm)			Fresh weight (gm)			Leaf area (cm^2)	Stem length (cm)	No. branches/ plant	No.pods/ plant	Weight of fresh yield/ plant	Weight of 100 dried seeds
		Root	Shoot	Whole plant	Root	Shoot	Whole plant						
Nebraska	*I.	6.7	243.3	250.0	84.4	497.8	532.2	43.26	22.16	2.66	18.0	142.53	89.2
	U.	19.9	256.0	275.9	38.0	509.2	547.2	68.3	22.56	3.96	18.33	155.19	90.1
	% ±	66.33	4.96	9.38	9.47	2.23	2.72	36.66	40.0	32.82	1.8	8.15	0.99
Morgan	I.	4.4	138.9	143.3	34.4	260.8	295.2	50.76	29.86	2.46	15.76	139.69	33.1
	U.	17.8	143.9	161.7	39.3	331.4	370.7	61.93	30.13	3.86	16.67	144.64	34.23
	% ±	75.28	3.47	11.37	12.46	21.3	20.36	18.03	0.89	36.26	5.45	3.42	3.30
Xera	I.	3.5	18.4	21.9	18.3	129.6	139.9	30.33	18.93	2.33	11.86	133.26	34.56
	U.	8.1	39.1	47.2	12.1	204.8	216.9	34.33	21.63	2.66	15.69	136.93	36.23
	% ±	56.79	52.94	53.6	14.87	36.57	35.5	11.65	12.48	12.4	24.41	2.68	4.6

*I. = Plants sown in infested soil with the fungus. U. = Plants sown in uninfested soil (control).

% increase (+) or decrease (-) relative to the control.

inoculation), were estimated. It was found from the data in Table (2) that the infected stems and roots of the less susceptible cultivar (Nebraska) exhibited the lowest amounts of reducing sugars. On the contrary, the infected stems and roots of the highly susceptible cultivar (Xera) had higher levels of this component at 10 and 20 days.

The infected stems and roots of the moderately susceptible cultivar Morgan contained (2.6 and 3.5, 0.7 and 0.8 mg glucose/gm dry weight) at 10 and 20 days, respectively compared with the healthy stems and roots of the three tested cultivars.

In general, the infected plants of all tested cultivars showed a lower amount of reducing sugar contents than that noticed in the healthy ones. Reducing sugar content increased with the age of plants.

Results in Table (2) indicate that non-reducing sugar content in the stems and roots of the infected plants was low when compared with the healthy plants. The infected stems and roots of the highly susceptible cultivar *i.e.* Xera had high amount of non-reducing sugars being, 3.5, 6.4 in stems and 1.6, 2.1 mg glucose/gm dry weight in roots at 10 and 20 days, respectively. The moderate susceptible cultivar (Morgan) contained 2.5, 5.0 in stems and 1.6, 1.5 mg glucose/gm dry weight in roots) at 10 and 20 days, respectively, followed by the less susceptible cultivar (Nebraska).

Data in the same Table show that total sugar contents in the healthy plants were the highest at all growth stages compared with the infected plants. Total sugar content was high in stems and roots of cv. Xera than in cv. Nebraska and increased with the increasing of plant age in both cases (uninfested and infested soil). Concerning the infected stems and roots of highly susceptible cultivar (Xera) contained the highest amount of this component at 10 and 20 days, followed by the moderate susceptible cultivar (Morgan). The less susceptible cultivar (Nebraska) contained lower amounts in both stems and roots at 10 and 20 days after inoculation. Total sugar content increased with the plant age.

Table (2): Effect of charcoal rot disease on carbohydrate content (mg glucose/gm dry weight) in the stems and roots of three bean cultivars 10 and 20 days after inoculation.

Plant parts	Cultivars	Treatments	Soluble carbohydrates						Insoluble carbohydrates		Total carbohydrats	
			Reducing sugars		Non reducing sugars		Total sugars					
			10 days	20 days	10 days	20 days	10 days	20 days	10 days	20 days	10 days	20 days
Stems	Nebraska	I.*	1.2	2.9	1.6	2.4	2.8	5.3	8.6	13.6	11.4	18.9
		U.	3.1	3.2	2.0	3.0	5.1	6.2	11.7	16.5	16.8	22.7
		% ±	61.29	9.37	20.0	20.0	45.09	14.51	26.49	17.57	32.14	16.74
	Morgan	I.*	2.2	2.8	2.5	5.0	4.7	7.8	5.2	9.6	9.9	17.4
		U.	2.6	3.5	2.6	5.9	5.5	10.4	13.1	15.9	18.6	26.3
		% ±	15.38	20.0	3.84	27.53	14.54	25.0	60.3	39.62	46.77	33.84
	Xera	I.*	2.8	3.3	3.5	6.4	6.3	9.7	4.9	8.8	11.2	18.5
		U.	3.6	4.1	4.5	9.4	8.1	13.5	12.6	15.7	20.7	29.2
		% ±	22.22	19.51	22.22	31.91	22.22	28.15	61.11	43.95	45.89	36.64
Roots	Nebraska	I.*	0.6	0.8	1.2	1.6	1.8	1.8	2.9	2.9	4.7	5.3
		U.	0.8	1.2	1.6	1.7	2.4	2.4	3.2	5.8	6.3	8.7
		% ±	25.0	33.33	25.0	5.88	58.33	58.33	9.38	50.0	25.4	39.08
	Morgan	I.*	0.7	0.8	1.6	1.5	2.3	2.3	2.8	5.9	5.1	8.5
		U.	1.1	1.4	1.7	1.7	2.8	2.8	3.7	8.3	9.6	11.4
		% ±	36.36	42.86	5.88	11.76	17.86	17.86	24.32	28.92	26.09	25.44
	Xera	I.*	0.9	0.9	1.6	2.1	2.5	3.0	3.3	6.8	7.4	9.8
		U.	1.3	1.8	1.8	2.1	3.1	3.9	4.7	9.3	11.1	13.2
		% ±	30.77	50.0	11.11	100	19.35	23.08	29.79	26.88	33.33	25.76

I.* = Plants sown in infested soil with the fungus. U. = Plants sown in uninfested soil (control).
% Increase (+) or decrease (-) relative to the control.

Results obtained in this study (Table 2) indicate that the infected stems and roots of the highly susceptible cultivar (Xera) exhibited the highest amount of insoluble carbohydrate being, 4.9, 8.8

and 3.3, 6.8 mg glucose/gm dry weight in both stems and roots 10 and 20 days, respectively, followed by moderately susceptible cultivar (Morgan). The less susceptible cultivar (Nebraska) showed the lowest amount of this component in both stems and roots 10 and 20 days. The healthy plants had the highest amount of insoluble carbohydrates if compared with the infected plants. On the other hand, insoluble carbohydrates increased with the plant age.

In the same Table (2) data show that total carbohydrates were increased in the stems and roots of all tested cultivars with the plant age. Total carbohydrate content was higher in the healthy plants than in the infected ones. The infected stems and roots of the highly susceptible cultivar (Xera) contained the highest level of total carbohydrates, being 11.2, 18.5 in stems and 7.4, 9.8 mg glucose/gm dry weight in the roots after 10 and 20 days, respectively. The moderately susceptible one (Morgan) contained 9.9, 17.4 and 5.1, 8.5 mg glucose/gm dry weight in both stems and roots at 10 and 20 days, respectively. Stems and roots of the less susceptible cultivar (Nebraska) contained the lowest amount of this component.

From the data in Table (2) it could be concluded that the healthy roots and stems of the less susceptible bean cultivar (Nebraska) contained the lowest amounts of reducing sugars, non reducing sugars, total sugars, insoluble carbohydrates and total carbohydrates, followed by the moderately susceptible cultivar (Morgan) and the highly susceptible one (Xera). Generally, charcoal rot disease infection caused a decrease of carbohydrate content at all the examined periods, if compared with the healthy plants.

3.2.2. Determination of phenolic compounds: Free, conjugated and total phenols were determined in the stems and roots of infected and healthy plants of the previous three bean cultivars at 10 and 20 days after inoculation. Data presented in Table (3) show that the charcoal rot disease caused an increase of free phenol contents in the stems and roots of the infected plants almost at all examined growth stages. The infected stems and roots of less susceptible cultivar (Nebraska) had the highest values of free phenols, being 7.45, 12.24 in the stems and 8.80, 10.62 mg catechol/gm fresh weight in the roots after 10 and 20 days, respectively.

Table (3): Effect of charcoal rot disease on phenolic contents (mg catechol/ gm fresh weight) in the stems and roots at two periods (10 and 20 days after inoculation).

Cultivars	Treatment	Free phenols				Conjugated phenols				Total phenols			
		Stem		Root		Stem		Root		Stem		Root	
		10 day	20 day	10 day	20 day	10 day	20 day	10 day	20 day	10 day	20 day	10 day	20 day
Nebraska	I.*	7.45	12.24	8.8	10.62	2.98	3.07	1.09	5.09	10.43	15.31	9.89	15.71
	U.	5.52	10.43	6.25	10.27	1.11	1.04	1.02	4.09	6.64	11.47	7.27	14.36
	% ±	-34.96	-17.35	-40.8	-3.4	-168.46	-195.19	-6.86	-24.44	-57.07	-33.47	-36.03	-9.4
Morgan	I.	5.93	9.86	5.21	10.39	0.85	1.71	2.02	1.05	6.78	11.57	7.23	11.44
	U.	5.19	6.32	4.78	9.63	0.42	1.06	1.0	1.01	5.61	7.38	5.78	10.64
	% ±	-14.25	-56.01	-8.99	-7.89	-102.38	-61.32	-102.0	-3.96	-20.85	-43.22	-25.08	-7.51
Xera	I.	2.53	4.51	5.43	8.23	1.14	1.05	1.06	1.64	3.67	5.56	6.49	9.87
	U.	1.89	3.35	3.73	7.59	1.01	1.01	1.05	1.01	2.9	4.36	4.78	9.6
	% ±	-33.86	-34.62	-45.5	-8.43	-12.87	-3.96	-0.95	-62.23	-26.55	-27.52	-35.77	-2.81

I.* = Plants sown in infested soil with the fungus. U. = Plants sown in uninfested soil (control).
% Increase (+) or decrease (-) relative to the control.

The infected stems and roots of the highly susceptible cultivar (Xera) contained the lowest values of free phenols, being 2.53, 4.51 and 5.43, 8.23 mg catechol / gm fresh weight in both the stems and roots after 10 and 20 days of inoculation, respectively. Free phenols increased with increasing of plant age.

The results in the same Table indicate that the infection caused an increase of the conjugated phenols in the stems and roots at the two tested periods, but this increase was limited. The infected stems and roots of the less susceptible cultivar (Nebraska) had higher values of this component, while, the infected stems and roots of the highly susceptible cultivar (Xera) exhibited lower values of the conjugated phenols. The conjugated phenols varied between increasing or decreasing with the increasing of plant age.

Meantime, charcoal rot disease caused an increase of total phenol. Stems and roots of the highly susceptible cultivar (Xera) exhibited the lowest values of total phenols, being 2.09, 4.36,4.78 and 9.6 mg catechol / gm fresh weight at 10 and 20 days after inoculation, respectively. The infected stems and roots of the less susceptible cultivar (Nebraska) had higher values of total phenols, being 10.43, 15.31, 9.89 and 15.71 mg catechol/gm fresh weight after 10 and 20 days of inoculation, respectively. The total phenols increased with the plant age.

It could be concluded from Table (3) that the less susceptible cultivar (Nebraska) exhibited the highest free phenols, conjugated phenols and total phenols in the healthy roots and stems, followed by the moderate cultivar (Morgan) and the highly susceptible cultivar (Xera).

3.2.3. Determination of free amino acids: Data in Tables (4) and (5) indicate that the infection caused an increase of amino acid contents in all cultivars after 10 and 20 days of inoculation. The amino acid concentrations were higher in the infected roots of the less susceptible cultivar (Nebraska) compared with the highly susceptible one (Xera) after 10 and 20 days of inoculation.

Data also show that the acidic amino acids group (Aspartic and Glutamic) was found in high concentrations in both the infected and healthy roots of the tested cultivars compared with other amino acids, followed by Arginine, Phenylalanine and Leucine. Sulphoric amino acid group (Cystine, Cysteine and Methonine) was found in moderate concentrations in the roots of tested cultivars, but it increased in the infected roots of the highly susceptible cultivar (Xera) compared with the less susceptible one (Nebraska) at 10 and 20 days after inoculation.

Data in the same table indicate that the less susceptible cultivar (Nebraska) exhibited the highest free amino acids in the healthy roots followed by the moderate cultivar (Morgan) and the highly susceptible cultivar (Xera). In general, amino acid content increased with growth stages.

3.3. Induction of systemic resistance in bean plants by abiotic compounds and its effect on some oxidative enzymes

3.3.1. Seed soaking: Data in Table (6) show that seed treatment at all

Table (4): Effect of charcoal rot disease on the free amino acid level in the roots of three bean cultivars after 10 days of inoculation.

Cultivars	Treatments	Sulphuric group		Acidic group		Non-polar Alphatic group			Aromatic group			Emino	Basic group		
		Cystine + Cysteine	Methonine	Aspartic Acid	Glutamic acid	Leucine	Alanine	Glycine	Tyrosine	Phenyl alanine	Trptophan	Prline	Lysine	Arginine	Histidine
Nebraska	*I.	22.37	24.92	291.9	129.0	103.06	52.02	59.89	62.49	130.07	35.05	15.17	23.71	162.5	11.16
	U.	19.58	55.33	247.06	123.1	70.76	41.21	43.12	49.11	85.84	23.49	10.37	14.26	125.0	9.8
	% ±	-14.24	54.96	-18.14	-4.79	-45.64	-26.23	-38.89	-27.24	-51.52	-49.21	-46.28	-66.26	-30.0	-13.87
Morgan	I.	18.87	61.57	206.71	119.15	51.13	69.6	45.81	46.13	61.6	22.68	77.84	20.33	125.9	26.04
	U.	7.26	49.17	186.16	116.89	48.93	38.43	42.18	36.77	52.59	16.78	54.16	12.28	90.68	18.37
	% ±	-159.9	-25.21	-11.03	-1.93	-4.49	-81.1	-8.6	-25.45	-17.13	-35.16	-43.72	-65.55	-38.83	-41.75
Xera	I.	9.48	22.36	181.72	104.85	47.02	18.24	68.51	19.46	59.45	11.41	15.01	14.42	132.83	17.92
	U.	5.73	18.52	123.88	99.47	38.19	11.31	42.04	15.53	33.43	9.03	6.09	11.13	89.27	5.18
	% ±	-65.44	-20.73	-46.69	-5.4	-23.12	-61.27	-62.96	-25.3	-77.83	-26.36	146.46	-26.55	-48.79	245.94

*I = Plants sown in infested soil with the fungus. U. = Plants sown in uninfested soil (control).

%Increase (+) or decrease (-) relative to the control.

Table (5): Effect of charcoal rot disease on the free amino acid level in the roots of three bean cultivars after 20 days of inoculation.

Cultivars	Treatments	Sulphuric group		Acidic group		Non-polar Alphatic group			Aromatic group			Emino	Basic group		
		Cystine + Cysteine	Methonine	Aspartic Acid	Glutamic acid	Leucine	Alanine	Glycine	Tyrosine	Phenyl alanine	Trptophan	Prline	Lysine	Arginine	Histidine
Nebraska	*I.	11.65	70.1	252.34	119.34	190.35	20.68	80.93	27.41	110.23	43.74	15.46	7.84	180.92	72.78
	U.	7.45	55.91	248.32	119.65	181.42	41.76	42.11	39.03	87.21	25.36	12.53	9.37	154.18	41.76
	% ±	-56.37	-25.38	-1.61	-0.25	-4.62	50.47	-92.18	-29.77	-26.39	-72.47	-23.38	16.32	-17.34	-74.28
Morgan	I.	10.64	68.32	212.83	120.23	72.87	54.93	43.26	37.67	64.28	22.68	75.12	6.27	119.23	38.96
	U.	6.35	50.12	192.27	117.21	43.67	41.28	41.51	37.65	55.71	14.75	50.44	10.61	95.21	19.58
	% ±	-76.55	-36.31	-10.69	-2.57	-66.86	-33.06	-4.21	-0.05	-15.38	-53.76	-48.92	40.90	-25.22	-98.97
Xera	I.	9.48	32.73	154.2	115.72	45.68	17.69	75.84	26.09	53.37	13.86	12.47	10.56	117.57	14.67
	U.	5.47	20.26	141.91	99.16	39.75	11.85	43.37	18.89	33.62	9.57	6.63	11.54	84.64	5.65
	% ±	-73.3	-61.54	-8.66	-16.7	-14.9	-49.28	-74.86	-38.11	-58.74	-44.82	-88.08	8.49	-38.9	-159.64

*I = Plants sown in infested soil with the fungus.
U. = Plants sown in uninfested soil (control).
%Increase (+) or decrease (-) relative to the control.

Table (6): Effect of some chemical inducers as seed soaking on the percentage of damping off, % charcoal rot and % survival plants of cv. Xera bean under greenhouse conditions.

Treatments (A) / seed soaking (hrs)	% damping-off								% Charcoal rot (60 days)				% Survival plants (60 days)			
	Pre emergence (15 days)				Post emergence (30 days)											
	1	5	10	Mean	1	5	10	Mean	1	5	10	Mean	1	5	10	Mean
Potassium chloride	13.33	13.33	6.66	11.1	6.66	6.66	0.0	4.44	32.26	22.2	11.84	22.1	80.0	80.0	93.33	84.44
Cobult nitrate	6.66	0.0	0.0	2.22	0.0	0.0	0.0	0.0	17.02	6.66	5.55	9.74	93.33	100.0	100.0	97.77
Ethylene diamin	6.66	6.66	0.0	4.44	0.0	0.0	0.0	0.0	27.23	11.84	8.14	15.74	93.33	93.33	100.0	95.55
Sodium carbonate	20.0	13.33	13.33	15.55	6.66	6.66	6.66	6.66	37.3	17.2	11.84	22.05	73.33	80.0	80.0	77.77
Control	47.25	46.8	45.93	46.66	15.56	13.13	11.3	13.33	46.1	44.1	43.2	44.46	38.0	40.0	42.0	40.0
Mean	18.78	16.02	13.18		5.77	5.29	3.59		31.98	20.4	16.11		75.59	78.66	83.06	

L.S.D at 5% for		% damping-off Pre emergence	% damping-off Post emergence	% Charcoal rot	% Survival plants
	A	13.67	18.05	5.45	13.14
	hrs	4.56	2.32	4.69	4.55
	A x hrs	10.19	5.18	10.48	10.17

tested times of Xera bean seed soaking with chemical inducers reduced pre- post- emergence damping off and percentage of charcoal rot, with different degrees. Cobalt nitrate, after 10 hr. soaking controlled the disease incidence, being 0.0, 0.0 and 5.55% of pre- post- emergence damping off and charcoal rot, respectively while, after 5 hrs. showed 0.0,0.0 and 6.66% of pre, post emergence damping off and charcoal rot, respectively. Ethylene diamine after 10 hrs diminished pre- post- emergence damping off and charcoal rot to 0.0, 0.0 and 8.14%, respectively. Sodium carbonate and potassium chloride were less effective.

Generally, seed soaking with abiotic agents, *i.e.* cobalt nitrate and ethylene diamine, were superior in controlling pre, post- emergence damping-off and charcoal rot compared with control.

Data in Table (7) show that the chemical inducers increased the activity of peroxidase and polyphenol oxidase. The enzyme activity was determined in the treated cotyledons compared with the control. Cobalt nitrate was more effective for increasing the activity of both enzymes (peroxidase and polyphenol oxidase) at all times of soaking and at all examined periods, followed by potassium chloride than the other ones. Meantime, ethylene diamine was less effective for increasing the activity of both enzymes. Data also indicate that after 10 hr. of seed soaking was the best period for inducing and increasing the activity of enzymes compared with one and 5 hr.

3.3.2. Soil drenching: Data presented in Table (8) reveal that soil treatment with chemical inducers reduced the disease incidence in the plants sown in infested soil compared with the control. Cobalt nitrate controlled disease incidence being 6.66,0.0 and 6.66% of pre- post- emergence damping off and charcoal rot, respectively, followed by ethylene diamine, sodium carbonate and potassium chloride.

Table (7): Peroxidase and polyphenol oxidase activity in the cotyledons of Xera bean cultivar soaked in water suspension of some chemical inducers under greenhouse conditions.

Treatments	Seed soaking (hrs)	Peroxidase		Polyphenol oxidase	
		10 days	20 days	10 days	20 days
Potassium chloride	1	*0.79	1.87	0.12	0.23
	5	0.88	1.94	0.16	0.28
	10	0.92	1.98	0.21	0.33
Cobalt nitrate	1	0.78	1.80	0.12	0.22
	5	0.91	1.95	0.18	0.31
	10	0.98	2.02	0.25	0.36
Ethylene diamine	1	0.78	1.81	0.12	0.22
	5	0.81	1.86	0.15	0.25
	10	0.87	1.9	0.19	0.28
Sodium carbonate	1	0.78	1.81	0.13	0.22
	5	0.84	1.89	0.18	0.27
	10	0.87	1.92	0.22	0.3
Control		0.78	1.8	0.09	0.18

* Enzyme's activity per minute as optical density.

Table (8): Effect of some chemical inducers as soil drench on the percentage of damping off, % charcoal rot and % survival plants of Xera bean cultivar under greenhouse conditions.

Treatments	% damping off		% Charcoal root rot (60days)	% Survival plants (60 days)
	Pre emergence (15days)	Post emergence (30 days)		
Potassium chloride	20.0	6.66	27.23	73.33
Cobalt nitrate	6.66	0.0	6.66	93.33
Ethylene diamin	13.33	6.66	11.84	80.0
Sodium carbonate	20.0	6.66	22.2	73.33
Control	46.66	13.33	44.4	40.0
L.S.D at 5 %	16.12	12.86	11.17	20.62

Data in Table (9) show that the enzyme activity of peroxidase and polyphenol oxedase had increased in the plants sown in the treated soil if compared with those sown in the untreated soil. Data also show that peroxidase and polyphenol oxidase were higher in the infected cotyledons of Xera bean compared with the healthy ones. Cobalt nitrate and potassium chloride increased peroxidase and polyphenol oxidase activity more than other treatments. Peroxidase and polyphenol oxidase activities were increased with the increasing of plant age.

Generally, peroxidase and polyphenol oxidase activities were increased in the cotyledons produced from seedlings grown in the soil treated with chemical inducers compared with those grown in the untreated soil.

Table (9): Peroxidase and polyphenol oxidase activity in the cotyledons of (Xera) bean cultivar treated with some chemical inducers as soil drench at 10 and 20 days after inoculation.

Treatments		Peroxidase		Polyphenol oxidase	
		10 days	20 days	10 days	20 days
Infested soil	**Potassium chloride**	*0.95	1.89	0.39	0.46
	Cobalt nitrate	0.99	1.98	0.48	0.55
	Ethylene diamin	0.82	1.84	0.16	0.3
	Sodium carbonate	0.86	1.94	0.24	0.41
Infested soil		0.78	1.3	0.09	0.18
Uninfested soil	**Potassium chloride**	0.92	1.87	0.36	0.45
	Cobalt nitrate	0.98	1.96	0.45	0.53
	Ethylene diamin	0.79	1.83	0.15	0.29
	Sodium carbonate	0.81	1.92	0.22	0.38
Uninfested soil		0.69	1.18	0.06	0.14

* Enzyme's activity per minute as optical density.

4. DISCUSSION

The infection with charcoal rot disease caused remarkable changes in the diseased plants. This disease affects many morphological characters and chemical constituents, which are changed by infection according to the resistance and susceptibility of the bean cultivars. This effect varied with different degrees from one cultivar to the other and from growth parameter to the other. Fresh and dry weight of roots, weight of shoots and whole plant were decreased in the infected plants than the healthy ones for the three tested bean cultivars, but cv. Nebraska was less affected compared with Morgan and Xera which exhibited high response to the infection. Leaf area of infected plants was less than of healthy ones for the three tested bean cultivars. Stem length in the infected plants was shorter than the healthy ones. No. of branches and pods/plant in the infected plants were less compared with the healthy ones. Weight of fresh yield/ plant and weight of 100 dried seeds exhibited the lowest amounts in the infected plants compared with the healthy ones for the three bean cultivars.These results are confirmed by many investigators *e.g.*, Tohamy(1977), Faris *et al.*(1992) and Songa *et al.*(1997).

Total carbohydrates, soluble carbohydrates (reducing, non-reducing and total sugars) and insoluble carbohydrates were higher in the healthy stems and roots compared with the infected ones. Generally, the infection almost decreased the carbohydrate contents in all the examined periods if compared with the healthy plants. These results are in agreement with those of Farahat (1980) who recorded that this could be attributed to the consumption of the reducing sugars in the growth of the pathogen, and/or conversion of non-reducing sugar as a result of infection.

Free and total phenols have increased by infection in the stems and roots of all tested cultivars at all tested periods (10 and 20 days) comparing with the healthy ones. This increase was major in the less susceptible cultivar compared with the highly susceptible one. Jadeja and Patel (1989) and Mandavia and Parameswaran (1993) reported similar results. Kosuge (1969) noted that this might be attributed to the phenols synthesized by plant tissues after infection appears to have a broad antifungal spectrum.

Free amino acids exhibited high levels in the healthy roots of the less susceptible cultivar (Nebraska) which exhibited the highest level of acidic amino acids, followed by aromatic amino acids, both of them were increased in the roots, as well as, non polar-aliphatic amino acids. Sulfuric amino acids were decreased within plant growth and increased in the roots but they were found in low concentrations, followed by basic amino acids. Proline was found in the lowest concentrations and increased in the roots. Amino acids were higher in the less susceptible cultivars comparing with the highly susceptible ones.

Free amino acid contents had increased by infection, in the roots of all tested cultivars at all tested periods (10 and 20 days) compared with the healthy plants. Amino acid concentrations were higher in the infected roots of the less susceptible cultivar (Nebraska) compared with highly susceptible one (Xera) after (10 and 20 days) after inoculation. The acidic amino acid group was found in high concentrations in both infected and healthy roots of all tested cultivars, followed by Arginine, Phenylalanine and Leucine. Sulphuric amino acid group was recorded to be the moderate concentrations in the roots of all tested cultivars. These conclusions are in complete agreement with Jadeja and Patel (1989) who found that there was an increase of amino acids in the diseased stems of some bean cultivars infected by *M. phaseolina* than the healthy stems. Mandavia and Parameswaran (1993) observed that at early infection with *M. phaseolina*, the resistant plants had a high levels of cystine and arginine + histidine.

The pesticide contamination of food, water reservoir and soil had become a fact of life but their residual toxic effects increased by the continuous use of potentially hazardous chemicals and are imposing on increasing environmental threat. Among these approaches, biological control to plant pathogens is becoming increasingly important and biocontrol agents have the ability to reproduce and to establish themselves in the soil ecosystem, as well as, to colonized seeds spermosphere, rhizosphere, rhizoplane and foliage. Furthermore, biocontrol strategies are highly compatible with sustainable agriculture practices required for conserving natural resources of agriculture (Sivan and Chet 1992).

Chemical inducers reduced pre- post- emergence damping-off and charcoal rot, with different degrees at all times of Xera bean seed soaking. Cobalt nitrate, after 10 hr.and 5 hr. of seed soaking controlled the disease incidence, followed by ethylene diamin efter 10 hr., while sodium carbonate and potassium chloride were less effective. On the other hand, soil treatment with chemical inducers decreased the disease incidence in the plants sown in the infested soil compared with the control. Cobalt nitrate controlled the disease incidence; ethylene diamine and sodium carbonate diminished infection of charcoal rot. These results are in harmony with those reported by Siegrist *et al.* (1997).

Enzymes activity of peroxidase and polyphenol oxidase had increased by soaking seeds of Xera bean cultivar in the chemical inducers. Cobalt nitrate was more effective for increasing the activity of both enzymes (peroxidase and polyphenol oxidase) at all times of soaking and at all examined periods, followed by potassium chloride than the other ones. Meantime, ethylene diamine was less effective for increasing the activity of both enzymes. Data also indicate that, 10 hr. of seeds soaking was the best period for inducing resistance and increasing the enzymes activity compared with one and 5 hr.

Enzyme activity of peroxidase and polyphenol oxidase was increased in the plants grown in the treated soil if compared with those grown in the untreated soil. Peroxidase and polyphenol oxidase were higher in the infected cotyledons of Xera bean compared with healthy ones. Cobalt nitrate and potassium chloride increased peroxidase and polyphenol oxidase activity more than other treatments. We have no explanation for the specificity of activity of the different studied inducers on the induction of these enzymes. However, it was suggested that these inducers could interfere with general amino acid metabolism, while the physiological roles of the inducers in the plant for enzyme induction have not been clearly established.

Agrios (1988) showed that the importance of polyphenol oxidase activity in disease resistance probably, was due to its property to oxidize phenolic compounds to quinones, which are often, more toxic to microorganisms than the original phenols. It is reasonable to assume that an increased activity of polyphenol oxidase results in high concentrations of toxic products of oxidation. Another oxidative enzyme (peroxidase) not only oxidizes phenolic compounds but also increases the rate of polymerization of such compounds into lignin like substances. The latter are deposited in cell walls and papillae and interfere with the further growth and development of the pathogen.Cardose and Echandi (1987) proved that one biochemical change that was detected in the tissue of treated bean plants (for induction) was the unusual accumulation of lignin, suberin, and phenolic compounds in epidermal cells.

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ميكانيكية المقاومة الكيموحيوية
لبعض أصناف الفاصوليا لمرض العفن الفحمي

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ملخص

يؤثر مرض العفن الفحمي على بعض الصفات المورفولوجية والبيوكيماوية لثلاث أصناف من الفاصوليا بدرجات مختلفة وفقا لطبيعة المقاومة . ولدراسة هذه الطبيعة تم تقدير المواد الكربوهيدراتية والفينولات والأحماض الأمينية لثلاث أصناف تختلف في درجة حساسيتها للإصابة بالممرض تحت ظروف العدوى الصناعية. يحتوى الصنف نبراسكا الأقل حساسية على كميات منخفضة من السكريات المختزلة وغير المختزلة والكلية وكميات مرتفعة من الفينولات الحرة والمرتبطة والكلية والأحماض الأمينية بالمقارنة بالصنف إكسيرا الأعلى حساسية . كما أدى غمر بذور الفاصوليا أو معاملة التربة قبل الزراعة بنترات الكوبالت وايثلين داى أمين كعوامل غير حيوية في استحثاث المقاومة الجهازية المستحثة إلى انخفاض نسبة حدوث المرض. أما نترات الكوبالت وكلوريد البوتاسيوم فقد أديا إلى زيادة الأنزيمات المؤكسدة البيروكسيديز والبوليفينول أوكسيديز في البادرات .

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