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Evaluation of faba bean genotypes for yield and resistance to Fusarium root rot under greenhouse and field conditions

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Abstract

Faba bean is a paramount grain legume crop, which plays an important role in the food security for humans and animals, in addition to its vital role in sustainable agriculture. However, this crop is prone to yield losses due to infection with *Fusarium* root rot. We evaluated 16 faba bean genotypes for yield and resistance to *Fusarium* root rot as well as study the enzymes activity, including total phenolics, peroxidase, catalase and ascorbate, associated with resistance to *Fusarium. solani*. We found that some genotypes, including Giza-2, Giza-843 and Nobaria-2, showed high seed yield per plant under normal field conditions as well as high values of the total phenols, Guaiacol-dependent peroxidase activity, Catalase activity and Ascorbate peroxidase activity content. Furthermore, Nobaria-1 and Sakha-4 showed high resistance to *Fusarium* root rot with modest seed yield per plant, along with high values of the aforementioned enzymes. Our results imply the role of high content of phenolics as well as the other enzymes activities in the host-plant resistance against root rot caused by *F. solani*.

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Introduction

Faba bean (*Vicia faba* L.) is considered a low-cost source of protein (25%–40%) either as food or feed worldwide. Furthermore, it plays an important role in sustainable agriculture. However, faba bean is exposed to numerous pathogenic fungi worldwide. Faba bean is known to be attacked by *Fusarium solani*, which attacks both roots and stem base; resulting in dramatic losses in germination percentage (Abdel-Kader *et al.*, 2011). This pathogen is also known for its capability to survive long in the infested soil. The fungus can survive in different shapes either as mycelium or as spores in infected plant tissues (Agrios, 2005). *Fusarium solani* is one of the most commonly isolated soil borne pathogens causing root rot and black root rot in faba bean worldwide (Yu and Fang, 1948; Helsper *et al.*, 1994; Bekele *et al.*, 2003; Belete *et al.*, 2015; Habtegebriel and Boydom, 2016), with yield reduction of up to 45% (PPRC, 1996). *Fusarium solani* was recorded by Ibrahim and Hussein, 1974 to be the causal agent of root rot of faba bean in Sudan. *Fusarium solani* is the causal pathogen of root rot on faba bean fields in Egypt (Abdel-Kader *et al.*, 2002; Elwakil *et al.*, 2009; El-Shennawy, 2011).

Defense-related enzymes, e.g. elucidated the importance of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, play a vital role in the host-plant resistance (Xie *et al.*, 2017). These enzymes have been comprehensively investigated as part of plant defense against pathogens (Sun *et al.*, 2013; Ye *et al.*, 2013; Qin *et al.*, 2015; Han *et al.*, 2016). superoxide dismutase, peroxidase (POD) and polyphenol oxidase are demonstrative antioxidant enzymes and considered as vital constituents of defense against membrane lipid peroxidation and oxidative stress during pathogen invasion (Foyer and Noctor, 2003). Phenoxidase is an oxidoreductive enzyme that contributes in cell wall polysaccharide paths, i.e. oxidation of phenols, suberization, and lignification, of host-plant cells during defense response against pathogenic causes (Chittoor *et al.*, 1999). Polyphenol oxidase is engaged in the oxidation of polyphenols into quinones and lignification of plant cells during pathogen invasion (Mohammadi and Kazemi, 2002). Álvarez *et al.* (2010) explained the role of phenylalanine ammonia-lyase, which is the major enzyme in phenylpropanoid metabolism, in the production of numerous defense-related secondary components including phenols and lignin. These enzymes are responsible for tissue browning and erecting physical barriers against pathogens in case of invasion; in addition, correlation was observed between phenols accumulation and disease-resistant plants (Mohammadi and Kazemi, 2002).

The main goals of the current study were to: (1) identify root-rot resistant genotypes, (2) investigate the relationship between the resistance to root-rot and enzyme activity and biochemical changes in infected faba bean genotypes with *F. solani*.

Materials and methods

Plant material

A set of 16 faba bean genotypes (Table 1) were evaluated for two years (2016/2017 and 2017/2018) to investigate their resistance to Fusarium root rot, caused by *Fusarium solani* (Mart.) Sacc. These genotypes were developed to adapt different regions across Egypt.

Isolation and identification of the causal pathogen

Samples were collected from naturally diseased faba bean plants, showing typical symptoms of root rot. Samples were collected during 2015-2016 growing season from fields of farmers in Assiut Governorate, Egypt. The infected parts were then sliced into tiny pieces of 0.5 cm and surface disinfected with 2% sodium hypochlorite solution for 2 min, washed couple of times in sterile distilled water and dried between sterile filter paper sheets. Then sited in Petri dishes containing Potato Dextrose Agar (PDA) modified with chloramphenicol (250mg L^{-1}) and incubated at 25±2°C in the dark. The developed fungal colonies were purified by single-spore isolation techniques after three days incubation period. Identification of the isolates was carried out on 5-12 days old culture based on the morphological features of mycelia and spores as well as culture appearances,

the gotten isolates were recognized as *Fusarium solani* (*Martius*) *Saccardo* (Booth, 1977; Nelson *et al.*, 1983; Domsch, *et al.*, 2007).

Greenhouse experiment

Experiment was conducted during two growing seasons in 2016/2017 and 2017/2018 in the greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University, Egypt. Inoculums of *F. solani*, for infection test, was prepared by growing *F. solani* in sterilized Erlenmeyer flask (500 ml) containing barley medium (100g barley, supplemented with 2g glucose + 1g yeast extract + 100 ml distilled water). Inoculated flasks were incubated at 25±2°C for two weeks. Sterilized pots (20 cm in diameter) were filled with sterilized sandy-loam soil which mixed thoroughly with equal amounts of *F. solani* inoculums at the ratio of 2% soil weight. Seeds were surface sterilized by soaking in 0.5 % solution of sodium hypochlorite for 2-3 min., then the seeds were removed, washed three times in distilled sterilized water, and air dried on the top layer of two sterilized filter paper sheets before sowing. Soil infestation was conducted three days before sowing seeds. Four pots were used as replicates and randomly distributed on the greenhouse benches, 10 seeds were planted in each pot. The minimum and maximum temperatures during the infection test were 15±2°C and 22±2°C, respectively.

Disease assessment

Appearance and development of disease symptoms were observed daily. The root rot infection percentage at the pre- emergence damping-off (PRD) was recorded as the number of absent plants relative to the number of sown seeds, after 15 days from planting date. While, the root rot infection percentage at the post- emergence damping-off (POD) was recorded as the number of absent plants relative to the number of emerging seedlings, after 30 days from planting date. At 60 days faba bean plants were carefully pulled out from pots after being flooded with water in order to leave the root system undamaged. Root rot severity (RRS) was determined according to Mahmoud, 2016 based on the incidence of infected plants and severity of disease symptoms. Fusarium root rot was evaluated based on the type of symptoms using a numerical scale, which ranged from 0 to 5 as follows:

0 indicated no visible symptoms;

1. represented symptoms appear only on the cotyledons or first leaf of plant;

2. showed symptoms appear above the first true leaf of the plant, and 25-<50 % the root systems are brown;

3. revealed that whole plant starts to wilt and 50-<75 % of root systems turn dark brown showing root rot symptoms;

4. indicated that whole plant starts to wilt, 75-≤100 % of root systems turn dark brown showing root rot symptoms;

5. expressed that the whole plant was completely dead.

$$\text{Disease severity (\%)} = \frac{\sum [(N\times 0)+(N\times 1)+\ldots..(N\times 5)]}{5\,T}\times 100$$

Where: the number of plants for each of the numerical scale component is denoted as N, while denominator expressed the sum of plants (T) multiplied by the numerical categories (5).

Phenolic and oxidative enzymes assessment

Analyses of total phenolics and oxidative enzymes were carried out at the laboratory of medicinal plants, Department of Ornamental plants and Landscape Gardening, Faculty of Agriculture, Assiut University, Egypt. All Spectrophotometric measurements were done on Uv-vis Spectrophotometer (Optizin Pop, Mecasys, Korea) at the correspondent wave length of each analysis.

Enzymes extraction

Faba bean leaves (1g) collected from each treatment were homogenized separately in a mortar along with 0.1 M sodium phosphate buffer at pH 7.1 at the rate of 2 ml/g fresh weight leaves for 1 min.

Then these triturated tissues were strained through four layers of cheesecloth and the filtrates were centrifuged at 3000 rpm for 15 minute at 5 °C. The

clear supernatant was collected and considered as a crude extract for enzymes assay.

Total phenol contents

The content of soluble phenols was measured using as per Folin and Ciocalteu (1927) method, using the reduction of a phosphowolframate–phosphomolybdate complex to blue products by phenolic compounds. 10 mg of each plant total extract were dissolved in 10 ml of distilled water with the aid of sonication for 15 min at room temperature and centrifuged for 10 min at 7000 rpm. Briefly, an aliquot (0.5 ml) of the extract, blank or standard was sited in a 25 ml flask, where the Folin-Ciocalteu reagent (0.5 ml) was added and the mixture was permitted to react for 3 min under continuous stirring before a solution of sodium carbonate (150 g/l, 10 ml) was added and mixed well. The volume was then completed to 25 ml with distilled water and left standing at room temperature for 60 minutes. The absorbance was then measured at 750 nm. The results were expressed as gallic acid equivalents (GAE), using a calibration curve over the range of 25-200 ppm.

Assay of guaiacol-dependent peroxidase activity

The activity of Guaiacol-dependent Peroxidase was assayed by direct spectrophotometric approach of Hammerschmidt *et al.*, 1982 using guaiacol as common substrate for peroxidase. The reaction mixture (3 ml) contained of 0.2 ml crude enzyme extract and 1.40 ml of a solution containing guaiacol, hydrogen peroxide (H_2O_2) and sodium phosphate buffer pH 7.1, (0.2 ml 1% guaiacol+0.2 ml 1% H_2O_2+1 ml 10 mM potassium phosphate buffer), was kept at 25°C for 5 min and the initial rate of increase in absorbance was measured over 1 min at 470 nm using spectrophotometer. Peroxidase activity was expressed as units of PO/min/g protein**.**

Assay of catalase activity

The activity of catalase was specified as per Aebi (1974). Enzyme extract (100 µl) was mixed with 2.9 ml of a reaction mixture comprising 20 mM H_2O_2 and 50 mM sodium phosphate buffer (pH 7.1). The activity of CAT was recorded by monitoring the reduction in the absorbance at 240 nm as a result of H_2O_2 consumption. The quantity of consumed H_2O_2 was calculated by using a molar extinction coefficient of 0.04 cm^{-2} μmol^{-1}. Catalase activity was expressed as units mg^{-1} protein min^{-1}. One unit of enzyme activity was defined as the decomposition of 1 µmol of H_2O_2 per min.

Ascorbate peroxidase activity

Ascorbate peroxidase activity was assessed as per Nakano and Asada (1981) and Asada (1992), with slight modifications. Ascorbate peroxidase was assessed by measuring the decrease in optical density due to ascorbic acid at 290 nm in a reaction mixture. The 3 ml reaction mixture contained 0.2 M TRIS/HCl buffer (pH 7.8), 5.625 mM ascorbic acid, 0.1 mM EDTA, 11.25 mM H_2O_2, 50ul enzyme extract. The reaction was run for 3 min at 25 °C. Enzyme activity was measured utilizing the extinction coefficient of 2.8 mM^{-1} cm^{-1}, and was calculated as the amount of enzyme required to oxidize 1µmol of ascorbate min^{-1} g^{-1} tissue.

Field experiment

During the same two growing seasons (2016/2017 and 2017/2018), the 16 faba bean genotypes were grown under field conditions, in Agronomy Research Station, Assiut University, to assess plant height, number of pods per plant and seed yield per plant of these genotypes. The 16 genotypes were grown using a randomized complete block design with 3 replications. Each genotype was seeded in three rows of 3 m long and 0.6 m inter-row spacing. The inter-plant spacing was 0.3 m, giving 30 plants per plot. Plant height (cm) was measured from the soil surface to the top of the plant. At harvest, pods were collected from each plot and number of pods per plant was calculated by dividing the number of pods by number of plants per plot. Similarly, seed yield per plant (g) was calculated by dividing the whole seed yield per plot by the number of plants per plot.

Statistical analysis

Data were checked for normality using Shapiro-Francia normality test (Shapiro and Francia 1972),

Data were transformed using log10(x+2) for traits that violated the normality test, i.e. PRD and POD. The remaining traits did not violate the assumptions of normality test; therefore, they were not transformed. Both separate and combined analyses were accomplished with GLM procedure in SAS v9.0 (The SAS Institute Inc., Cary, NC, USA). Variances were homogeneous based on Bartlett's test ($P \leq 0.05$).

The Pearson's coefficient of correlation was performed to compare the aforementioned traits using CORR procedure in SAS v.9 (The SAS Institute Inc., Cary, NC, USA).

Table 1. List of faba bean cultivars.

Cultivar	Developed by
Sakha-1	ARC†
Giza-2	ARC
Giza-843	ARC
Wadi-5	ARC
Sakha-4	ARC
Nobaria-1	ARC
Roomy-3	ARC
Misr-1	ARC
Misr-3	ARC
Roomy-80	ARC
Assiut-143	AUN‡
Nobaria-3	ARC
Marut-2	ARC
Assiut-215	AUN
Giza-40	ARC
Nobaria-2	ARC

†ARC= Field Crops Research institute, Agriculture Research Center, Ministry of Agriculture, Egypt

‡AUN= Department of Vegetables, Assiut University, Egypt.

This study was established to evaluate reaction of sixteen faba bean cultivars against *Fusarium solani*, the causal pathogen of Fusarium root rot under greenhouse conditions. Data was collected on seedling emergence and root rot severity for two seasons in 2016/2017and 2017/2018.

The results varied somewhat depending on faba bean cultivar and year.The percentage of disease severity ranged from (14.25%) for the resistant cultivar Nobaria-1 to (60.25%) for the susceptible cultivar Roomy-80 (Table 2). None of the faba bean cultivars presented reaction similar to immunity to *F. solani*; however, five cultivars (Nobaria-1, Nobaria-2, Nobaria-3, Giza-2 and Sakha-4) performed as highly resistant.

Most of the tested cultivars were moderately resistant, while four cultivars (Roomy-80, Roomy-3, Wadi-5 and Misr-3) were highly susceptible. The

Results

Reactions of faba bean cultivars for resistance to Fusarium root rot under greenhouse conditions

Fusarium root rot adversely affected faba bean production in many ways. Infect the seedlings prior to or shortly after emergence resulting in damping-off and death of the seedlings.

The root systems of adult plants may become severely diseased, resulting in yellowing of the basal leaves, stunting of the plants and lower yields. Occasionally there are no above-ground symptoms, but a red discoloration can be seen on the root exterior and in the vascular system.

evaluation of cultivars for pre- and post- emergence damping-off was done after 2 and 4 weeks respectively. The percentage of dead plants at pre-emergence damping-off ranged from the least (2.5 %) for the resistant cultivars (Nobaria-1, Nobaria-2 and Giza-2).to the maximum (25 %) for the susceptible cultivars (Roomy-3 and Wadi-5). Whereas, the percentage of dead plants at post-emergence damping-off ranged from the least (0.0 %) for the resistant cultivar Nobaria-1 to the maximum (10 %) for the susceptible cultivars (Roomy-80 and Roomy-3).

Table 2. Reactions of faba bean cultivars for resistance to *Fusarium* root rot under greenhouse conditions.

Genotype	Seedling Damping Off %				Root rot severity % (RRS)	
	pre-emergence damping-off (PRD)		post-emergence damping-off (POD)			
	2016- 2017	2017- 2018	2016- 2017	2017- 2018	2016- 2017	2017- 2018
Sakha-1	10	7.5	5	2.5	33.25	37.47
Giza-2	7.5	2.5	2.5	7.5	22.25	22.25
Giza-843	20	10	5	5	40.25	39.75
Wadi-5	25	15	7.5	7.5	55.25	49.25
Sakha-4	10	5	5	5	28.25	24.25
Nobaria-1	2.5	2.5	0	5	14.25	19.75
Roomy-3	25	15	5	10	58.25	51.75
Misr-1	10	10	7.5	5	44.25	39.25
Misr-3	20	15	7.5	5	52.25	43.25
Roomy-80	20	15	10	10	60.25	51.5
Assiut-143	10	5	5	5	40.25	35.5
Nobaria-3	5	5	5	2.5	20.25	20.00
Marut-2	22.5	12.5	5	5	48.25	45.5
Assiut-215	12.5	7.5	7.5	7.5	38.25	35.75
Giza-40	25	15	5	2.5	44.25	44.00
Nobaria-2	5	2.5	2.5	2.5	18.25	19.05

Data were not transformed.

Results revealed that the resistant cultivars were least affected by root rot disease at harvest, and they produced surviving plants at rate more than 85% and disease severity less than 30%. The susceptible cultivars was highly affected by root rot disease at harvest, and they produced surviving plants at rate less than 70% and disease severity more than 50%. While, the moderately resistant cultivars produced surviving plants at rate of 70% to less than 85% and disease severity of 33% to less than 50%. No symptoms were observed in non-inoculated plants (control).

Means of studied traits

Means of all studied traits along with $LSD_{0.05}$ in the two growing seasons 2016/2017 and 2017/2018 and combined over the two seasons were shown in Tables 3 and 4. These tables showed that there were differences among genotypes in all studied traits. Briefly, this experiment was carried out to study enzymes activity and biochemical changes associated faba bean cultivars after infection by *F. solani* under greenhouse conditions. Obtained results presented in tables 3 and 4 showed that, in general, with few exceptions, the total phenol contents, Guaiacol-dependent peroxidase activity, Catalase activity and Ascorbate peroxidase activity were increased in infected plants than in the healthy ones (control). Furthermore, the total phenol contents and oxidative enzymes were increased in the most resistant faba bean cultivars than the most susceptible cultivars.

Table 3. Means of studied traits (PRD= pre-emergence damping-off, POD= post-emergence damping-off, RRS= Root rot severity, PH= plant height, NPP= number of pods per plant, SYP= seed yield per plant (g), PHC= total phenolics (mg GAE g^{-1} DW[f]) under control condition, PHI= total phenolics (mg GAE g^{-1} DW[d]) under infected condition, PERC= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW min^{-1}) under control condition, PERI= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW min^{-1}) under infected condition, CATC= Catalase activity (Unit g^{-1} FW min^{-1}) under control, CATI= Catalase activity (Unit g^{-1} FW min^{-1}) under infected condition, ASC= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under control and ASI= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under infected condition) in two seasons 2016 and 2017 for 16 faba bean genotypes.

						2016								
Gen	PRD§	POD§	RRS	PH	NPP	SYP	PHC	PHI	PERC	PERI	CATC	CATI	ASC	ASI
Sakha-1	0.460	0.418	0.370	82.333	38.500	47.100	1.077	4.927	0.209	0.570	7.167	21.500	3.537	17.680
Giza-2	0.418	0.360	0.349	107.667	42.200	42.433	2.323	4.560	0.364	1.090	35.833	16.500	8.143	12.430
Giza-843	0.634	0.360	0.383	94.333	42.100	36.400	1.037	1.853	0.621	1.036	28.667	60.667	7.930	6.643
Wadi-5	0.667	0.360	0.409	89.667	19.733	27.733	0.760	2.720	1.346	0.310	7.167	60.667	11.787	19.610
Sakha-4	0.460	0.418	0.361	99.000	30.767	35.567	1.763	3.153	0.155	0.570	21.500	50.167	14.357	18.320
Nobaria-1	0.360	0.301	0.334	98.333	19.233	67.802	1.790	4.103	0.260	1.144	50.167	28.667	4.180	8.357
Roomy-3	0.667	0.360	0.414	124.000	42.133	23.110	1.353	3.127	0.466	0.881	21.500	28.667	11.787	10.610
Misr-1	0.519	0.418	0.390	106.333	44.467	47.033	1.013	2.827	0.570	0.415	43.000	21.500	11.463	5.143
Misr-3	0.634	0.418	0.404	102.000	37.733	55.433	0.880	5.720	1.036	0.520	10.333	5.260	15.213	2.037
Roomy-80	0.593	0.460	0.418	126.333	50.800	22.821	1.310	3.910	1.346	0.466	21.500	14.333	20.143	11.463
Assiut-143	0.418	0.418	0.383	114.000	47.200	18.204	3.333	2.700	0.675	0.466	14.333	21.500	22.180	17.357
Nobaria-3	0.360	0.360	0.345	100.333	42.833	46.241	2.000	3.700	0.260	0.364	28.667	14.333	18.537	23.787
Marut-2	0.634	0.418	0.397	105.000	34.033	33.033	1.603	2.600	1.555	0.779	7.167	7.167	12.430	23.463
Assiut-215	0.519	0.418	0.379	106.667	54.200	14.234	2.400	2.143	0.881	0.570	12.590	14.333	11.037	14.890
Giza-40	0.667	0.360	0.390	96.000	35.833	38.400	2.153	4.153	0.310	0.260	10.340	12.653	3.110	2.787
Nobaria-2	0.418	0.301	0.342	100.000	38.633	69.783	3.537	8.563	0.520	0.830	11.287	14.333	21.213	19.070
Mean	0.527	0.384	0.379	103.250	38.775	39.083	1.771	3.797	0.661	0.642	20.701	24.515	12.315	13.353
LSD0.05	0.153	0.170	0.002	11.969	13.579	3.791	0.138	0.274	0.122	0.047	3.060	6.890	1.017	1.166

§ Data were transformed using log10(x+2)

[f] Fresh Weight.

Table 4. Means of studied traits (PRD= pre-emergence damping-off, POD= post-emergence damping-off, RRS= Root rot severity, PH= plant height, NPP= number of pods per plant, SYP= seed yield per plant (g), PHC= total phenolics (mg GAE g^{-1} DW[d]) under control condition, PHI= total phenolics (mg GAE g^{-1} DW) under infected condition, PERC= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW[f] min^{-1}) under control condition, PERI= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW min^{-1}) under infected condition, CATC= Catalase activity (Unit g^{-1} FW min^{-1}) under control, CATI= Catalase activity (Unit g^{-1} FW min^{-1}) under infected condition, ASC= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under control and ASI= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under infected condition) combined over the two growing seasons for 16 faba bean genotypes.

Gen	PRD§	POD§	RRS	PH	NPP	SYP	PHC	PHI	PERC	PERI	CATC	CATI	ASC	ASI
Sakha-1	0.469	0.389	0.369	85.833	38.083	48.495	1.037	4.933	0.215	0.589	7.048	20.875	3.545	17.723
Giza-2	0.389	0.410	0.346	101.333	40.267	43.991	2.298	4.575	0.379	1.058	35.717	16.917	7.780	12.198
Giza-843	0.577	0.389	0.380	98.000	42.383	35.053	1.025	1.888	0.641	1.046	28.808	59.967	7.548	7.105
Wadi-5	0.614	0.389	0.406	94.333	21.200	27.550	0.780	2.710	1.382	0.323	7.052	61.717	11.343	19.172
Sakha-4	0.410	0.389	0.357	99.167	29.550	35.812	1.715	3.202	0.166	0.575	21.445	50.033	14.078	18.410
Nobaria-1	0.360	0.330	0.337	97.167	20.950	66.237	1.752	4.152	0.270	1.155	50.017	29.067	4.450	8.828
Roomy-3	0.614	0.418	0.409	120.833	40.900	23.622	1.360	3.188	0.487	0.880	21.160	28.750	11.577	10.672
Misr-1	0.469	0.389	0.385	105.667	43.733	47.176	1.022	2.838	0.594	0.426	42.467	21.273	11.448	5.622
Misr-3	0.577	0.418	0.399	101.833	38.533	56.590	0.855	5.727	1.061	0.518	10.600	5.212	14.977	2.368
Roomy-80	0.577	0.469	0.412	124.000	49.567	21.902	1.245	3.882	1.373	0.468	21.010	13.967	19.913	11.548
Assiut-143	0.389	0.389	0.380	111.500	48.433	19.004	3.292	2.692	0.692	0.469	14.245	20.953	21.923	17.612
Nobaria-3	0.389	0.360	0.346	101.667	40.750	45.202	1.983	3.717	0.265	0.371	28.417	14.067	18.618	23.310
Marut-2	0.577	0.389	0.395	102.833	35.183	33.374	1.610	2.585	1.533	0.778	7.252	7.178	11.882	23.315
Assiut-215	0.489	0.439	0.377	99.833	53.100	14.118	2.400	2.168	0.850	0.572	12.582	13.800	10.952	15.212
Giza-40	0.614	0.360	0.392	99.167	36.250	37.844	2.143	4.167	0.299	0.272	10.553	12.692	3.122	2.977
Nobaria-2	0.360	0.330	0.341	101.000	40.983	68.813	3.502	8.615	0.538	0.832	11.397	14.053	21.057	19.552
Mean	0.492	0.391	0.377	102.760	38.742	39.049	1.751	3.815	0.671	0.646	20.611	24.408	12.138	13.476
LSD0.05	0.104	0.120	0.007	9.262	8.482	3.311	0.107	0.204	0.087	0.050	2.222	4.819	0.804	1.050

§ Data were transformed using log10(x+2)
f Fresh Weight
d Dry weigh.

The resistance of some faba bean cultivars may be due to the role of oxidative enzymes. Furthermore, for the seed yield per plant (SYP) under normal field conditions, the total phenol, Guaiacol-dependent peroxidase activity, Catalase activity and Ascorbate peroxidase activity content were high in the high yielding genotypes, e.g. Giza-2, Giza-843 and Nobaria-2. Furthermore, Nobaria-1, Nobaria-2, Giza-2 and Sakha-4 were highly resistant *Fusarium* root rot and showed moderate to high yielding ability, based on SYP, along with high values of total phenol contents, Guaiacol-dependent peroxidase activity, Catalase activity and Ascorbate peroxidase activity.

Table 5. Analysis of variance for studied traits (PRD= pre-emergence damping-off, POD= post-emergence damping-off, RRS= Root rot severity, PH= plant height, NPP= number of pods per plant,SYP= seed yield per plant (g), PHC= total phenolics (mg GAE g^{-1} DW^{d}) under control condition, PHI= total phenolics (mg GAE g^{-1} DW) under infected condition, PERC= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW^{f} min^{-1}) under control condition, PERI= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW min^{-1}) under infected condition, CATC= Catalase activity (Unit g^{-1} FW min^{-1}) under control, CATI= Catalase activity (Unit g^{-1} FW min^{-1}) under infected condition, ASC= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under control and ASI= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under infected condition) in two seasons 2016 and 2017 for 16 faba bean genotypes.

Year	SOV	DF	MS													
			PRD§	POD§	RRS	PH	NPP	SYP	PHC	PHI	PERC	PERI	CATC	CATI	ASC	ASI
2016	Rep	2	0.012	0.001	0.004	70.1888	296.766	7.113	0.508	2.314	0.020	84.03	46.355	41.337	24.268	28.502
	Gen	15	0.040**	0.006	0.002***	387.933***	275.471**	796.588***	2.057***	8.126***	0.598***	297.3***	536.523***	924.992***	111.318***	146.747***
	Error	30	0.008	0.010	9.3×10-7	51.521	66.310	5.168	0.007	0.027	0.005	0.001	3.367	17.071	0.372	0.489
2017	Rep	2	0.007	0.002	0.003	40.083	271.896	2.845	0.723	2.291	0.034	0.103	51.098	47.323	7.286	22.444
	Gen	15	0.024**	0.007	0.002***	206.943*	215.906***	768.342***	1.991***	8.244***	0.602***	0.224***	518.748***	949.150***	107.659***	132.277***
	Error	30	0.008	0.011	7.8×10-5	77.106	41.585	11.270	0.010	0.035	0.006	0.003	4.034	17.757	0.596	1.165

§ Data were transformed using log10(x+2)

*,**,***Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

f Fresh Weight

d Dry weigh.

Analysis of variance (ANOVA)

Genotypes showed significant differences (P<0.01 or 0.001) for all studied traits either in separate ANOVA for growing seasons 2017/2018 or combined ANOVA over the two growing seasons (Tables 5 and 6). However, in the combined ANOVA, neither years nor years × genotypes showed significant differences.

Discussion

Pearson's correlation coefficient

Cultivating disease-resistant genotypes is the best approach to help farmers control plant diseases that attacks faba bean such as Fusarium root rot. Plants producing various pathogenesis-related (PR) proteins for protect themselves against pathogen infection.

In this study defense-related enzymes such as peroxidase and catalase were determined for different faba bean genotypes in response to pathogen infection. Phenolic compounds play an important role in the plant defense mechanisms. In this study total phenolic compounds were determined in the tested genotypes; the results of total phenolic showed that accumulation of phenolic contents increased in resistant genotypes more than the susceptible genotypes. Naczk and Shahidi, (2004) mentioned that phenolic compounds are products of secondary metabolism of plants and are essential for growth and reproduction, as well as acting as an anti-pathogenic agent and contributing to pigmentation. Peroxidase enzyme is related to the oxidation of phenolic compounds, catalyzing the oxidation of phenols into quinones (Constabel and Barbehenn, 2008). Quinones have antimicrobial activity because they form irreversible complexes with proteins (Bruneton, 1995). Resistance in plant was associated with an

increase in PR- proteins as well accumulation of phenols, flavonoids and lignin in faba bean tissues (Mazen, 2004). Peroxidases are a well-known component of pathogenesis related protein and induced in host plant when infected by pathogen (Passardi *et al.*, 2005). Peroxidase oxidizes phenolics to quinines and generates hydrogen peroxide which prevents the further growth and development of the pathogen (Agrios, 2005). Peroxidases have been related with a number of physiological functions that may play an important role in the resistance to the plant pathogens (Thakker *et al.*, 2013). The high levels of peroxidase as a result of the induced systemic resistance, leads to cell death and inhibits pathogenic activities in the host plants (Halfeld-Vieira *et al.*, 2006).

Table 6. Analysis of variance of studied traits (PRD= pre-emergence damping-off, POD= post-emergence damping-off, RRS= Root rot severity, PH= plant height, NPP= number of pods per plant, SYP= seed yield per plant (g), PHC= total phenolics (mg GAE g^{-1} DW[d]) under control condition, PHI= total phenolics (mg GAE g^{-1} DW) under infected condition, PERC= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW[f] min^{-1}) under control condition, PERI= Guaiacol-dependent peroxidase activity(Unit g^{-1} FW min^{-1}) under infected condition, CATC= Catalase activity (Unit g^{-1} FW min^{-1}) under control, CATI= Catalase activity (Unit g^{-1} FW min^{-1}) under infected condition, ASC= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under control and ASI= Ascorbate peroxidase activity (Unit g^{-1} FW min^{-1}) under infected condition) combined over the two growing seasons for 16 faba bean genotypes.

		MS													
SOV	DF	PRD[§]	POD[§]	RRS	PH	NPP	SYP	PHC	PHI	PERC	PERI	CATC	CATI	ASC	ASI
Year (Y)	1	0.117	0.004	0.001	23.010	0.107	0.111	0.037	0.029	0.011	0.001	0.787	1.118	3.010	1.465
Rep(Year)	4	0.01	0.001	0.003	58.135	284.331	4.979	0.615	2.302	0.027	0.085	48.726	44.33	15.777	25.473
Gen	15	0.059^{***}	0.008	0.004^{***}	526.266^{***}	479.832^{***}	1559.22^{***}	4.045^{***}	16.366^{***}	1.198^{***}	0.463^{***}	1054.95^{***}	1872.912^{***}	218.704^{***}	278.431^{***}
Y × G	15	0.005	0.005	4.0×10^{-5}	68.610	11.545	5.711	0.003	0.004	0.002	0.001	0.321	1.231	0.273	0.593
Error	60	0.008	0.011	3.9×10^{-5}	64.313	53.947	8.219	0.009	0.0311	0.006	0.002	3.701	17.414	0.484	0.827

[§] Data were transformed using log10(x+2)

*,**,***Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

[f] Fresh Weight

[d] Dry weigh.

Regulation of reactive oxygen species (ROS) was found to be a necessary aspect in plant-pathogen interactions. Occurrence of the oxidative burst and subsequent hypersensitive cell death in response to pathogen invasion has been well characterized in resistant plants (Lamb and Dixon, 1997). Catalase expression and activity vary during plant-pathogen interactions. For example, decreases in plant catalase activity were occurred in resistant plants as response infection by viruses (Yi *et al.*, 2003). In contrast, in susceptible plants increases in catalase activity were observed (Pompe-Novaka *et al.*, 2006). In tomato infected with *B. cinerea*, catalase activity increases transiently but is then progressively inhibited as disease develops (Kużniak and Skłodowska, 2005), exogenously applied catalase can result in decreased hypersensitive cell death and increased penetration by pathogens of normally resistant hosts (Able *et al.*, 2000).

Conclusion

Our results suggested that growing resistant faba bean cultivars is a paramount step towards creating sustainability of disease resistance in Egypt. Furthermore, chemical compounds such as total phenolics and enzymes activities including peroxidase, catalase and ascorbate were responsible for host-pant resistance against root rot cause by *F. solani*.

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