



Minia J. of Agric. Res. & Develop.
Vol. (32) No. 0 pp 833 - 849,
2012

FACULTY OF AGRICULTURE

EFFECT OF OROBANCHE PARASITISM ON THE BEHAVIOR OF FOUR FABA BEAN CULTIVARS (*Vicia Faba* L.)

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Received 22 Sept. 2012 Accepted 24 Dec. 2012

ABSTRACT

Four faba bean (*Vicia faba* L.) cultivars i.e. Giza 429, Misr 1 (tolerant to *Orobanche*) and Giza 40, Nubaria 1 (susceptible to *Orobanche*) were evaluated in *Orobanche* naturally infested soil and *Orobanche* free soil at Giza Research Station, Agricultural Research Center, in 2010/2011 and 2011/2012 seasons. Results revealed the superiority of Giza 429 in plant height, number of pods/plant, number of seeds/plant and seed yield/plant on the *Orobanche* - free soil, mean while Misr 1 was superior number of pods and seeds/plant and seed yield/plant on the *Orobanche*-infested soil. Giza 40 possessed a high level of infestation with the highest number and dry weight of *Orobanche* spikes/ m². The two tolerant genotypes Giza 429 and Misr 1 had the low number of *Orobanche* infested soil. Results of chemical compositions revealed significant differences among cultivars under the two soils. The two cultivars Giza 429 and Misr 1 gave higher values of protein and carbohydrates, respectively, while Giza 40 gave the lowest values. The analysis of protein banding pattern is an important tool, where absence of these bands from total root protein belongs to

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susceptible cultivars could be as a result of the effect of *Orobanche* parasitism. Therefore, these polymorphic bands can be considered as a positive marker for tolerant cultivars under *Orobanche* infection. In addition, another difference was found in the expression profile among the studied cultivars. Where, the intensity of each band was used as an indicator for its level of protein expression. The tolerant cultivars, Giza-^{٤٢٩} and Misr-^١, showed the highest values for protein expression.

INTRODUCTION

Faba bean (*Vicia faba* L.) is still the backbone of the legume crops grown in Egypt. It is an important source of high quality and inexpensive protein supplement for the majority of the Egyptian population through various popular dishes. Also, it is known as an efficient atmospheric nitrogen fixer depending on availability of *Rhizobium* spp. that colonizes the legume plants which keeping the Egyptian soil fertile and benefits for the subsequent crop. The average cultivated area devoted to faba bean during the last three seasons ٢٠٠٨/٠٩ – ٢٠١٠/١١ represented ١٧٨٣٣٣ feddans with an overage seed yield equal ٨.٦ ardab/fed.

Orobanche spp. is root holoparasitic plants causing severe yield and quality losses in a wide range of dicotyledonous crops. As most contemporary methods aimed at controlling *Orobanche* have resulted in limited success, the search for tolerant genotypes and understanding the tolerance mechanisms are considered important.

The parasitic weed (*Orobanche crenata* Forsk) is a major pest of faba bean (*vicia faba* L.) in Egypt. For example, in Behera Governorate (North Delta), Zaitoun et al.(^{١٩٩١}) reported that the percentage of *Orobanche* infestation amounted to ٦٥.٥% of faba bean cultivated area, with a total yield losses of about ١٩,٠٠٠ tons.

The food legume breeding program, FCRI, ARC resulted in releasing three cultivars (Giza ^{٤٢٩}, Giza ^{٨٤٣} and Misr ^١) having a

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higher level of resistance to *Orobanche* (Khalil et al ١٩٩٤, Saber et al ١٩٩٩ and ٢٠٠١)

The evaluation of tolerant materials under *Orobanche* -infested and *Orobanche*-free infested fields were investigated by Darwish et al (١٩٩٩) Abdalla and Darwish (٢٠٠٢) Morsy and Attia, (٢٠٠٢) El-Harty.E.H. (٢٠٠٥) Abdalla et al (٢٠٠٦) Darwish et al (٢٠٠٧) Abbas et al (٢٠٠٨) Abdalla and Darwish (٢٠٠٨) and Ashrie et al (٢٠١٠). They concluded that there were significant differences among genotypes in most traits under study.

In fact, breeding for tolerance and identification of genes responsible for it is one of the most promising methods to increase the tolerance of Faba bean towards *Orobanche* parasitism. Biotechnology tools such as marker-assisted breeding, tissue culture and genetic engineering can be useful in achieving this goal. However, only limited success has been gained. So we need to increase our efforts to get more progress in this area of research.

The current study was aimed to detect proteins involved in the tolerance of Faba bean for the parasitism of *Orobanche* spp. using SDS-PAGE protein analysis.

MATERIALS AND METHODS

The present investigation was conducted at the farm of Giza Research Station, ARC, Egypt in ٢٠١٠/١١ and ٢٠١١/١٢ seasons to study the performance of four faba bean cultivars (Giza ٤٢٩, Misr ١ tolerant to *Orobanche* and Giza ٤٠, Nubaria ١ susceptible to *Orobanche*) under both *Orobanche*-free and *Orobanche*-naturally infested soils. The pedigree and reaction of the materials used to *Orobanche* are shown in Table (١).

Table 1. Pedigree and reaction to *Orobanche* of four faba bean genotypes under study.

Genotype	Pedigree	Reaction to <i>Orobanche</i>
Giza 29	An individual selected plant from Giza 2	Tolerant to <i>Orobanche</i>
Misr 1	G.3x 123A/20/16	Tolerant to <i>Orobanche</i>
Giza 2	An individual plant selection from Rebaya 2	Susceptible to <i>Orobanche</i>
Nubaria 1	An individual selected plant from Spanish variety Reina Blanca	Susceptible to <i>Orobanche</i>

A Randomized Complete Block Design (RCBD) with three replications was used, in both infested and non infested *Orobanche* soils. The experimental plot consisted of three ridges 3 m long, 10 cm apart, with single seeded hills, 10 cm apart on both sides of ridges. Cultivation conditions were followed as normally recommendation for *Vicia faba* L.. In turn, five random plants were taken from each replicate every interval time, after 30, 40 and 50 days of sowing date, and inspected for the presence of *Orobanche* tubercles. At harvest time, ten guarded plants were taken at random from each experimental plot. The following data were recorded :-

1 - Agronomic traits:

Plant height (cm), number of branches/plant, number of pods/plant, number of seeds/plant, seed yield /plant (g), 100-seed weight (g), number of *Orobanche* spikes and dry weight of *Orobanche* spikes (g/m 1) were recorded at maturity of faba bean plants for all accession. The susceptible check variety Giza 2 and Nubaria 1. In Giza 2 and Nubaria 1, *Orobanche* spikes were collected just when its plants started to death, and their data were recorded immediately.

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٢- Chemical composition analysis:

Protein and carbohydrate percentages were determined according to the procedures outlined by AOAC, (٢٠٠٠). Percentage of protein was obtained by multiplying nitrogen percentage by ٦.٢٥ as stated by **Sadasium and Manickam** (١٩٩٦).

٣- SDS- PAGE analysis:

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS – PAGE), was used to investigate different protein banding profiles among studied cultivars under both infected and non infected conditions. Analysis of protein banding patterns was performed according to methods reported by **Laemmli** (١٩٧٠) using the Mini-Protein Electrophoresis Cell, manufactured by Bio-Rad Corporation.

٤-Statistical Analysis

Statistical analyses were performed using the SPSS software (version ٧.٥ for windows) and as outlined by Gomez and Gomez (١٩٨٤).

RESULTS AND DISCUSSION

Agronomic traits

The mean performance of four faba bean cultivars under *Orobanche*-infested and free soils is presented in Table (٢). Results showed significant differences among cultivars for plant height, number of branches/plant, number of pods/plant, number of seeds/plant, seed yield /plant, ١٠٠-seed weight. number and dry weight of *Orobanche* spikes under infested soil. However, significant differences were found among cultivars for plant height, number of branches/plant, number of pods/plant, number of seeds/plant, , and ١٠٠-seed weight were observed under *Orobanche*- free soil, On the other hand differences between seed yield /plant of both sides were insignificant.

***Orobanche*- free field**

The cultivar Giza ٤٢٩ recorded the highest values of plant height (١٠٦.٣), number of pods/plant (٢٥.٥), number of seeds/plant (٧٤.٢) seed yield /plant (٥٩.٠), Nubaria ١ recorded the highest number of branches/ plant (٥.٥), and ١٠٠-seed weight (١٠٤.٤), under free soil.

***Orobanche*- infested field**

The tolerant cultivar Misr ١ recorded the highest values of plant height (١٠١.٨), number of pods/plant, (١٤.٠), number of seeds/plant, (٣٩.٥) seed yield /plant, (٣١.٢), followed by Giza ٤٢٩ under infested soil .

These findings are clearly supported by those finding obtained by Darwish *et al* (١٩٩٩), Abdalla and Darwish (٢٠٠٢), Morsy and Attia, (٢٠٠٢), El-Harty.E.H. (٢٠٠٥) Abdalla *et al* (٢٠٠٦), Darwish *et al* (٢٠٠٧), Abbas *et al* (٢٠٠٧), Abdalla and Darwish (٢٠٠٨), and Ashrie *et al* (٢٠١٠) who reported that faba bean genotypes differed from each other in their yield and yield components regarding *Orobanche* infestation.

Number and dry weight of *Orobanche* spikes/ m^٢

Results in Table (٣) showed that Nubaria ١ cultivar possessed the highest level of infestation with high number and dry weight of *Orobanche* spikes/ m^٢ . The two tolerant cultivars Misr ١ and Giza ٤٢٩ had the lowest number of *Orobanche* spikes , and the lowest dry weight of *Orobanche* spikes, followed by Misr ١ and Giza ٤٢٩. The similar results were obtained by Morsy and Attia, (٢٠٠٢) , Abbas *et al* (٢٠٠٧) and Ashrie *et al* (٢٠١٠).

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Table ٢. Performance of the four faba bean genotypes under *Orobanche* –infested and - free soils in ٢٠١٠/٢٠١١ and ٢٠١١/٢٠١٢ seasons.

Genotype	Plant height (cm)						No. of branches /plant					
	Infested			Free			Infested			Free		
	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined
Giza ٤٢٩	١٠٣.٧	١٠٠.٠	١٠١.٨	١٠٨.٣	١٠٤.٣	١٠٦.٣	٥.٢	٤.٠	٤.٦	٣.٧	٤.٣	٤.٠
Misr ١	١٠٥.٠	٩٨.٣	١٠١.٧	١٠١.٧	١٠٢.٧	١٠٢.٢	٤.٥	٤.٨	٤.٧	٤.٤	٤.٧	٤.٧
Giza ٤٠	٧٥.٠	٦٨.٣	٧١.٧	١٠٠.٠	٩٨.٣	٩٩.٢	٤.٠	٣.٥	٣.٨	٣.٨	٤.١	٣.٩
Nubaria ١	٨١.٧	٧٦.٧	٧٩.٢	٩٥.٠	٩٠.٠	٩٢.٥	٥.٧	٦.٠	٥.٨	٥.٣	٥.٧	٥.٥
LSD ...٥	٧.٣٩	٥.٣٤	٣.٥٤	٧.٣٤	٩.٣٠	٧.٥٨	٠.٧٧	١.٢٢	٠.٧٦	٠.٨٤	١.٠١	٠.٦٤

Table ٢.Cont.

Genotype	No. of pods / plant						No. of seeds / plant					
	Infested			Free			Infested			Free		
	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined
Giza ٤٢٩	١٣.٣	١١.٧	١٢.٥	٢٤.٣	٢٦.٧	٢٥.٥	٣٨.٣	٣٣.٣	٣٥.٨	٧١.٧	٧٦.٧	٧٤.٢
Misr ١	١٤.٣	١٣.٧	١٤.٠	٢١.٠	٢٢.٧	٢١.٨	٤٠.٠	٣٩.٠	٣٩.٥	٦١.٧	٦٢.٣	٦٢.٠
Giza ٤٠	١.٧	١.٣	١.٥	٢٤.٣	٢٦.٣	٢٥.٣	٤.٠	٣.٣	٣.٧	٧١.٣	٧٦.٧	٧٤.٠
Nubaria ١	١.٣	٢.٠	١.٧	١٤.٧	١٥.٠	١٤.٨	٥.٠	٧.٣	٦.٢	٥١.٧	٥٥.٠	٥٣.٣
LSD ...٥	٢.٤٣	٢.٣٥	١.٩٩	٥.٤٥	٥.٧٠	٣.٠٧	٧.٢١	٦.٠٢	٦.١٥	NS	NS	١١.٢ ٧

Ns: non significant

Table ٢. Cont.

Genotype	Seed yield / plant (g)						100-seed weight (g)					
	Infested			Free			Infested			Free		
	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined
Giza ٤٢٩	٣١.٧	٢٦.٧	٢٩.٢	٥٢.٧	٥٧.٣	٥٩.٠	٨٢.٢	٧٩.٩	٨١.٠	٨٠.٣	٨١.١	٨٠.٧
Misir ١	٣٢.٠	٣٠.٣	٣١.٢	٤٧.٧	٤٩.٣	٤٨.٥	٧٩.٤	٧٧.٨	٧٨.٨	٧٧.٢	٧٩.٤	٧٨.٣
Giza ٤٠	٢.٧	٢.١	٢.٤	٤٥.٣	٤٩.٣	٤٧.٣	٦٦.٠	٦٣.٩	٦٥.٠	٦٣.٥	٦٤.٥	٦٤.٠
Nubaria ١	٥.٢	٧.٧	٦.٣	٥٤.٠	٥٧.٣	٥٥.٧	١٠٣.٩	١٠٢.٣	١٠٣.١	١٠٤.٦	١٠٤.٢	١٠٤.٤
LSD ...	٦.٥٩	٥.٧٦	٥.٦٩	NS	NS	NS	٥.٣٢	٥.٣٥	٤.٥٧	٢.٧٨	٢.٧١	١.٩٠

Ns: non significant

Table ٣. Mean number and dry weight of *Orobanche* spikes under infested soils during ٢٠١٠/١١ and ٢٠١١/١٢ seasons.

Genotype	No. of <i>orobanche</i> /m ^٢			<i>Orobanche</i> dry weight(g/m ^٢)		
	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined
Giza ٤٢٩	٣٨.٣	٤٣.٣	٤٠.٨	١٦٣.٣	١٦١.٧	١٦٢.٥
Misir ١	٣٤.٧	٤١.٠	٣٧.٨	١٧٣.٣	١٨٠.٠	١٧٦.٧
Giza ٤٠	١٤٦.٠	١٢٦.٧	١٣٦.٣	٢٨٣.٣	٢٠١.٧	٢٤٢.٥
Nubaria ١	١٥٠.٧	١٢٥.٧	١٣٨.٢	٢٨٣.٣	٢٥٦.٧	٢٧٠.٠
LSD ...	١٢.٣٤	١٩.٣٠	١١.١٢	٢٦.٦٥	NS	٦٦.٧٨

Chemical analysis

The mean performance of the studied faba bean cultivars under *Orobanche* - infested and *Orobanche* - free field conditions is presented in Table ٤. The results showed significant differences among

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cultivars in protein and carbohydrates. The percentage of protein and carbohydrates in Giza ٤٢٩ were higher in the *Orobanche* - free field than those in the *Orobanche* - infested field.

Table ٤. Protein and carbohydrates content of the four studied faba bean genotypes under *Orobanche* –infested and –free fields in ٢٠١٠/١١ and ٢٠١١/١٢ seasons.

Genotype	Protein (%)						Carbohydrates (%)					
	Infested			Free			Infested			Free		
	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined	٢٠١٠/١١	٢٠١١/١٢	Combined
Giza ٤٢٩	٢٥.٧	٢٦.٤	٢٦.١	٢٥.٠	٢٥.٥	٢٥.٣	٦٤.٧	٦٤.٦	٦٤.٦	٦٤.٢	٦٤.٩	٦٤.٦
Misr ١	٢٤.٢	٢٤.٠	٢٤.١	٢٥.٢	٢٥.٧	٢٥.٥	٦٤.٨	٦٥.٨	٦٥.٣	٦٦.٢	٦٥.٠	٦٥.٦
Giza ٤٠	٢٤.٠	٢٤.٠	٢٣.٨	٢٣.١	٢٤.٢	٢٣.٧	٦٤.١	٦٤.٩	٦٤.٥	٦٣.٢	٦٣.٧	٦٣.٤
Nubaria ١	٢٣.٩	٢٣.٢	٢٣.٦	٢٤.٢	٢٤.٣	٢٤.٣	٦٤.٥	٦٤.٧	٦٤.٦	٦٤.٤	٦٤.١	٦٤.٣
LSD ...	١.٢٢٩	٠.٥٧٩	٠.٦٣٦	١.٢٥	١.٠٢٧	٠.٧٢٥	٠.٨٩١	NS	٠.٧٥٤	٠.٧٢٥	NS	٠.٠٦٢

Ns: non significant

The susceptible cultivar (Giza ٤٠) gave the lowest values for all studied traits, while the tolerant genotypes (Giza ٤٢٩ and Misr ١) gave the highest values for protein and carbohydrates, respectively. It could be concluded that seeds from *Orobanche* – free field were better than those of *Orobanche* – infested field and had higher protein content.

These findings were clearly confirmed by those obtained by Frejnage *et al* (١٩٩٧), Megahed (٢٠٠٠), Morsy and Attia (٢٠٠٢) and Ashrie *et al.*, (٢٠١٠).

٢- Analysis of Total Root Protein:

Total root protein of the four *Vicia faba* cultivars Giza- ٤٢٩, Misr-١, Nubaria-١ and Giza-٤٠ were determined to indicate the differences due to *Orobanche* parasitism. The data shown in Table (٥)

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and Figure (1) revealed that infected samples for tolerant cultivars, Giza-29 and Misr-1 showed two polymorphic bands at 70 and 75 kDa, while un-infected plants of the same cultivars were similar to each other in the protein banding pattern except for Giza-2, which missed a band at 75 kDa. Absence of these bands from the total root protein banding patterns is due to DNA sequence differences between the studied cultivars which in turn are transcribed to different peptides which are involved in certain changes due to the effect *Orobanche* spp. parasitism on them (Sakr *et al.* 2010). Therefore, these polymorphic bands can be considered as a marker for tolerant cultivars under *Orobanche* infection (Abd El-maksoud *et al.* 2007 and Gadalla *et al.* 2010).

Non Infected Plants				Infected Plants			
		G2	G29			G2	
G2	N1	E1	29	2	E1	N1	2 M

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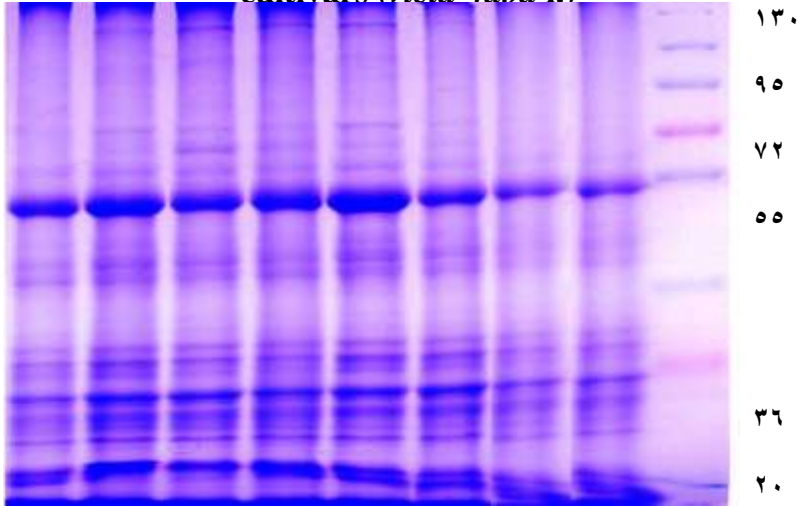


Figure 1: SDS PAGE banding pattern for total root protein of the four studied *Vicia faba* L. cultivars.

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Also, another difference was found in the expression profiles among the studied cultivars. Where, the intensity of each band can be used as an indicator for its level of protein expression. Both tolerant cultivars, Giza-429 and Misr-1, showed the highest values for protein expression, which are represented in the values indicated for each band as shown in figure (2).

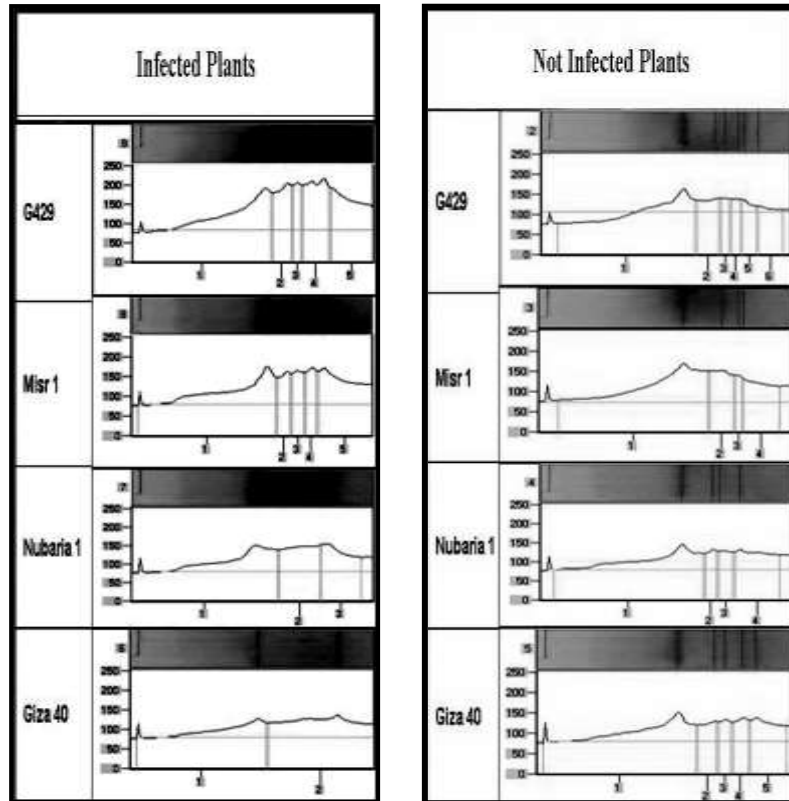


Figure 2: Protein expression profiles among infected and un-infected plants.

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Table ٥: SDS-PAGE banding pattern for total root proteins of the four *Vicia faba* L. Cultivars under infected and uninfected conditions of *Orobanche* spp.

Band #	Total RF	Total MW	Non Infected Plants				Infected Plants			
			G٤٠	N١	M١	G٤٢٩	G٤٢٩	M١	N١	G٤٠
١	٠.٢٤	٧٠	+	+	+	+	+	+	-	-
٢	٠.٢٩	٦٤	+	+	+	+	+	+	-	-
٣	٠.٣٣	٥٩	+	+	+	+	+	+	+	+
٤	٠.٣٨	٥٣	+	+	+	+	+	+	+	+
٥	٠.٤٩	٤٣	+	+	+	+	+	+	+	+
٦	٠.٥٣	٣٩	+	+	+	+	+	+	+	+
٧	٠.٦٧	٣٠	+	+	+	+	+	+	+	+
٨	٠.٧	٢٨	+	+	+	+	+	+	+	+
٩	٠.٧٢	٢٧	+	+	+	+	+	+	+	+
١٠	٠.٧٦	٢٥	+	+	+	+	+	+	+	+
١١	٠.٧٩	٢٣	+	+	+	+	+	+	+	+
١٢	٠.٨٤	٢١	+	+	+	+	+	+	+	+
١٣	٠.٨٦	٢٠	+	+	+	+	+	+	+	+

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تأثير تطفل الهالوك على سلوك أربعة أصناف من الفول البلدي

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أجرى هذا البحث تحت ظروف أرض موبوءة وأخرى خالية من الهالوك بمحطة بحوث الجيزة - مركز البحوث الزراعية خلال موسمي ٢٠١١/٢٠١٠ و ٢٠١١/٢٠١٢ وذلك بهدف دراسة سلوك أربع أصناف من الفول البلدي في كلا الترتيبين. وقد اشتملت الدراسة صنفين متحملين للإصابة بالهالوك (جيزة ٤٢٩، مصر ١) وصنفين حساسين للإصابة بالهالوك (جيزة ٤٠، نوبارية ١). قد أوضحت النتائج تفوق صنف جيزة ٤٢٩، في صفات طول النبات، عدد القرون/نبات وعدد البذور/نبات ومحصول بذور النبات في الأرض الخالية من الهالوك، كما تفوق الصنف مصر ١ في صفات عدد القرون/نبات، عدد البذور/نبات، ومحصول بذور النبات تحت ظروف الإصابة بالهالوك. كما انخفض عدد ووزن شمراخ الهالوك /م^٢ في حالة صنف جيزة ٤٢٩، مصر ١ مقارنة بالصنفين جيزة ٤٠ ونوبارية ١.

وأظهرت النتائج وجود اختلافات معنوية للصفات الكيميائية و البيوكيميائية بين الأصناف تحت الدراسة في كلا الترتيبين (الخالية والموبوءة)، وقد سجلت على الترتيب الأصناف جيزة ٤٢٩ و مصر ١ أعلى قيم لنسبة البروتين، الكربوهيدرات، في حين سجل الصنف جيزة ٤٠ أقل القيم. وقد تم تحليل نمط حزم البروتين باستخدام التفريد الكهربى لوصف الفروق بين أصناف الفول البلدي تجاه التطفل من الهالوك وتفقد شكل

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البروتين الكلي لجذور أصناف من الفول البلدي من الجيزة ٤٢٩ و مصر ١ والنوبارية
والجيزة ٤٠ لفحص الاختلافات الناتجة بسبب تطفل الهالوك. وأظهرت النتائج من العينات
المصابة للأصناف المتحملة من الجيزة ٤٢٩، ومصر ١ ظهور حزمتين مميزتين في الوزن
الجزئي ٧٠ و ٦٤ كيلو دالتون، في حين أن النباتات غير المصابة لنفس الأصناف لم
تظهر أي اختلافات. عدم وجود هذه الحزم في البروتين الكلي من الأصناف الحساسة
للإصابة يمكن أن يعتبر نتيجة لتأثير تطفل الهالوك عليها. ولذلك، يمكن اعتبار هذه الحزم
كمعلومات جزيئية ايجابية للأصناف المتحملة للإصابة تحت عدوى الهالوك.

كما أظهرت النتائج وجود اختلافات أخرى على مستوى نمط التعبير الجيني وإنتاج
البروتين بين الأصناف المدروسة حيث أن شدة وضوح كل حزمة يمكن أن تستخدم كمؤشر
لمستوى تعبير البروتين. وبالمقارنة بين الاختلافات في شدة وضوح الحزم أظهرت النتائج أن
الأصناف المتحملة (الجيزة ٤٢٩ و مصر ١) لها قيم أعلى في التعبير للبروتين.