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GENETIC BEHAVIOR AND ITS RELATION TO SDS-TOTAL PROTEIN AND ANATOMICAL TRAITS OF SOME TOLERANT *FABA BEAN GENOTYPES TO O. CRENATA* IN EGYPT

BY

Ola A. M.El-Galaly, *; El-Rodeny,W. M. * and Amal H. Selim, **

* Food Legumes Res. Sec. Field Crops Res. Inst., Agric. Res., Center(ARC).

** Seed Technology Research Dep., Field Crops Res. Inst., Agric. Res., Center(ARC), , Egypt

ABSTRACT

The present investigation was conducted to study the inheritance of some quantitative traits in faba bean of promising inbred lines. Randomized Complete Block Design (RCBD) with three replicates was utilized under natural infested soil with *Orobanche crenata* seeds at Sakha Agricultural Research Station, Kafr El Sheikh, Egypt during 2006/07 and 2007/08 seasons. Seven faba bean lines derived from hybridization between resistant cultivars to broomrape (Misr 1, Misr 2, Giza 843, Giza 429 and Giza 674) and resistant cultivar for foliar diseases Nubaria 1 were compared with three checks Misr 1, Giza 843 and Nubaria 1. The results revealed that the genotypes were significantly differed for studied traits indicating the existence of genetic variability among studied genotypes particularly for reaction to *Orobanche*. The crosses were selected according to their high performance in seed yield under heavy natural infestation with *Orobanche* compared with three checks Misr1, Giza 843 and Nubaria 1. The selected lines showed significantly lower levels of *Orobanche* infestation than their resistant parents Misr 1 while, Nubaria 1 revealed the highest level of infestation. The selected lines significantly exceeded their parent in *Orobanche* resistance and seed yield. The results indicated that selection for higher yield under heavy infestation with *Orobanche* is probably the most feasible approach for developing *Orobanche*-resistant faba bean genotypes. The analyses of parameters of variability showed little difference between GCV and PCV for pod yield per plant, no. of branches/ plant, seed yield /plant and traits of *Orobanche* reaction indicating a negligible environmental effect on these characters. While, PCV was higher than GCV for seed yield/plot and no. of seeds/pod, suggesting a significant environmental effect, which is not unexpected for such agronomic characters. High heritability in the broad sense estimates for *Orobanche* reaction no. of *Orobanche* spikes/plot (92.3, 97.2%) and (h^2_{bs}=98%, 96.4) for spikes dry weight and no. of pods/plant(97.2, 91.6%) action in its inheritance. These indicate that selection for higher yield under heavy infestation with *Orobanche* is probably the most feasible approach for developing *Orobanche*-resistant faba bean genotypes. Reaction to *Orobanche* appeared to be genetically and phenotypically negative correlated with yield and its components. It could be concluded that the recurrent selection is the proper method for developing desirable lines in advanced generations for their tolerance to *Orobanche* infestation. The patterns of seed protein were studied by using SDS-PAGE and the total protein analysis patterns revealed obvious differences in the various protein fractions. It may be concluded that the protein electrophoresis may be useful for identifying *V. faba* accessions. The histological investigation detected an incompatible interaction of *O. crenata* with the resistant faba bean genotypes (C.1675, Misr 1 and Giza 843x G.674). The intrusive cells of the parasite are stopped in the host cortex, before reaching the endodermis. In some cases, the parasite is scarcely able to pierce the epidermis lignification of host pericycle and endodermis has been observed in incompatible interactions between faba bean and *O. crenata*, which seems to prevent penetration of the parasite to the root vascular cylinder, therefore the parasite died before

a tubercle had formed. In contrast, on the roots of the susceptible genotypes (Nubaria1), the infection developed normally, with the intrusive cells reaching the central cylinder and the host vascular tissues.

Key words: *Vicia faba* - *Orobanche crenata*- Genetic Behavior – Biochemical - Histological

INTRODUCTION

Faba bean (*Vicia faba* L.) crop is one of the most cultivated pulses in the Arabian region. Total cultivated area was approached 25 million hectares with 18.4 million tones of seed yield production in the world (FAO, 2004). In Egypt and North Africa, faba bean together with lentils and chickpea provide an inexpensive source of high quality protein in the diets of people with low and middle incomes. Faba bean has been known, for many years, as the principal meal for more than 75% of the Egyptian families. Faba bean is subject to many biotic and abiotic stresses, which reduce yield and affect yield stability. (Various stresses also affect the pollinators of this food legume). Most of conducted research has been directed towards developing resistance to biotic stresses (Nuessly *et al.*, 2004). In the Mediterranean region, the most important biotic stress on faba bean is caused by broomrape (*Orobanche crenata* Forsk). This is an obligate parasitic weed which severely stresses faba bean plants, often leading to complete crop loss. The extent of the damage caused by *Orobanche* is often hidden because many farmers who have lost their crop in the past simply stop growing faba bean. Therefore, the most important factor limiting faba bean production throughout the Mediterranean region is broomrape (*O. crenata*) (Grenz *et al.*, 2005). The distribution rate of these pathogens is so high and it became as the most important constrains in Nile delta and the actual area of faba bean cultivation decreased by 30% in one decade (between 1968 and 1978) because of *O. crenata* infestations (Sauerborn 1991). In the last few years, plant breeders have tried to identify and select resistant or tolerant plant to broomrape. Different parameters have been used by different authors to evaluate the susceptibility/resistance of *Vicia faba* and other crop plants to *Orobanche* spp. (Boorsma, 1980; Perrino *et al.*, 1988; Radwan *et al.*, 1988 and Perez-de-Luque *et. al.*, 2005a). Electrophoretic techniques offer an exceptional opportunity to study the substructure differences in protein among different genotypes. Nevertheless, SDS-PAGE was used to differentiate between *V. faba* cultivars (Stegemann *et al.*, 1983) and to identify inbred lines (Gates and Boulter 1979). On the other hand, several researchers have found a greater amount of variation in seed storage protein fractions, legumin and vicilin (Gatehouse *et al.*, 1980). Histological studies have revealed that initial vascular connections are established and tubercles develop, but they then become dark and the parasite dies at an early developmental stage (Dorr *et al.*, 1994; Pe´rez-de-Luque *et al.*, 2005b). The presence of substances inside host vessels has been associated with the darkening of broomrape tubercles (Labrousse *et al.*, 2001; Zehhar *et al.*, 2003; Pe´rez-de-Luque *et al.*, 2005b) and it is possible that these substances block the vessels and interfere with the nutrient flux between host and parasite (Pe´rez-de-Luque *et al.*, 2005b). In this study, histological aspects related to the resistance of some faba bean genotypes to *Orobanche crenata* have been investigated in order to determine which types of resistance responses are involved in the unsuccessful penetration of *O. crenata* which reflect on the type of defense mechanism.

MATERIALS AND METHODS

Field exprement was carried out during 1st November 2006/2007 and 5th November 2007/08 growing seasons at Sakha Agricultural Research Station. The seeds of ten faba bean genotypes included three check cultivars, Misr1, Giza 843 and Nubaria 1 (Table 1) were design with three replication of each genotype. Each sown under natural

infested soil with Orobanche crenata using a randomized complete block design with three replications of each genotype. Each plot consisted of 3 ridges, three meters long with 60 cm apart (plot size =5.4 m^2). Recommended cultural practices for growing faba bean were followed. Data recorded in each season included plant height (cm), no. of branches/plant, no. of pods/plant, no. of seeds/ plant, seed yield/plant(g), 100-seed weight(g), no. of seeds/pod, seed yield/plot (g) and reaction to *Orobanche* (no. of *Orobanche* spikes/plot and spikes dry weight(g)).

Table (1):Origin, pedigree, sources and some features of the faba bean genotypes.

Genotypes	Origin	Pedigree	Some features	
			Reaction to *Orobanche*	Seed size
Line 1	FCRI	Misr 1 x G.843	tolerant	medium
Line 2	FCRI	L.383	tolerant	medium
Nubaria 1 (No.3)	FCRI	Egyptian variety originated and selected from Spanish genotype	susceptible	large
Line3	FCRI	C.1675	tolerant	medium
Line 4	FCRI	Misr 1 x Nubaria	tolerant	large
Line 5	FCRI	C. 957/501	tolerant	medium
Misr 1 (No.7)	FCRI	(123A/45/76 x G.3) x (Romixhabashi)	tolerant	medium
Line6	FCRI	(G.674 x G.429)	tolerant	medium
Line. 7	FCRI	Giza 843 x G.674	tolerant	medium
Giza 843 (No,10)	FCRI	Cross 461 x Cross 561	tolerant	medium

Data for the two growing seasons were Statistically analyzed using Mstatc Computer Program, 1994). The combined analysis of variance was carried out according to Steel and Torrie (1980). Broad sense heritability (h^2) was estimated by using variance components method (Fehr, 1987) as shown in Table (2). The genotypic and phenotypic variances (δ^2 g and δ^2 ph) were calculated from the partitioning mean squares expectation (Table 2) as follows:

Table (2): Expectation of mean squares (M.S) for the analysis of variance for separate and combined analysis.

Source of variation	Degree of freedom	Mean Squares	Expectation of MS
	Separate		
Replications	(r-1)	M_g	$\delta^2_e + \delta^2_g$
Genotypes	(a-1)	M_a	δ^2_e
Error	(r-1) a-	M_e	
	Combined		
Season (S)	(*S-1*)		
Reps/season.	*S*(r-1)		
Genotypes	(a-1)	M_a	$\delta^2_e + \delta^2_{ga} + \delta^2_g$
G x S	(a-1) (*S-1*)	M_{as}	$\delta^2_e + \delta^2_{gu}$
Error	*S*(r-1) (a-1)	M_e	δ^2_e

δ^2**g** = (Ma – Me)/r and δ^2 ph = (δ^2 g + δ^2 e/r, where δ^2 e = Me However, those over seasons is calculated from the pertinent mean squares expectation (Table 2) as follows:
δ^2 **g** = (Ma – Mas)/rs, and δ^2 ph = (δ^2 g + δ^2 gs/r + δ^2 e/rs, where δ^2 e = Me . Broad sense heritability (h^2 B) was calculated as follows: $\mathbf{h^2\ B} = (\delta^2\ g/\ \delta^2\ ph)\ ^*100$

Data regarding the above mentioned traits were averaged and subjected to analysis of variance (Panse and Sukhatme, 1984). The genotypic and phenotypic coefficients of variation (GCV and PCV) for each character were calculated by using the following formula:

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100 \text{ and}$$

$$PCV = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100$$

Where:

σ^2_g = the genotypic variance.

σ^2_p = the phenotypic variance, and

X = the grand mean of the trait

Biochemical identification:

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Total seed proteins were extracted from the ten *Vicia faba* genotypes. These protein extractions were analyzed using SDS-PAGE according to the method of Laemmli (1970).

Anatomical structure:

A minimum of 20 samples of each genotype was taken at random. The sampled material was fixed in FAA (50 % ethanol + 5 % formaldehyde + 10 % glacial acetic acid in water) for 48 h. Fixed samples were then dehydrated in an ethanol series (50 %, 80 %, 95 %, 100 %, 100 %; 12 h each) and transferred to an embedding solvent(through a xylene–ethanol series 30 %, 50 %, 80 %, 100 %, 100 %; 12 h each) and finally saturated with paraffin Sections (10 μm) were cut with a rotary microtome. After removal of paraffin with xylene (20 min twice) and rehydration with an ethanol-water series (100 %, 100 %, 95 %, 70 %, 50 %, 30 %, 0 %: 20 min each), sections were stained with alcian green–safranin (AGS) (Joe, 1983). With this staining method, carbohydrates (including cell walls and mucilage) appeared green, yellow or blue, while lignified, cutinized and suberized walls, as well as tannin and lipid material inside cells appeared red. Sections were observed using a transmission microscope magnification 100 to 400 and photographed using a digital camera.

RESULTS AND DISCUSSION

The purpose of the present investigation also aimed to study genetically, biochemical, and histological traits on faba bean plants grown under broomrape parasitism, to use them not only in classical plant breeding programs but also to make them more amenable to genetic engineering techniques. The significance mean squares of ten genotypes for studied traits are presented in Table (3). The results cleared the presence of highly significant differences were recorded among all genotypes (seven lines and three checks) for all studied traits in two seasons. This finding indicated the existence of genetic variability among studied genotypes, particularly for reaction to *Orobanche*. Similar results were obtained by Darwish *et al.* (1999) Kalia and Sood (2004) and El-Rodeny (2006).

The analyses of parameters of variability (Table 4) showed little difference between GCV and PCV for no.of pod per plant, no. of branches/plant, seed yield /plant and traits of *Orobanche* reaction, no. of *Orobanche* spikes/plot and spikes dry weight indicating a negligible environmental effect on these characters. However, PCV was higher than GCV for seed yield/plot and no. of seeds/pod, suggesting a significant environmental effect, which is not unexpected for such agronomic characters. Heritability in broad sense ranged from (59.0 , 67.6%) for plant height to (98.9, 96.4%) for spikes dry weight in the first and second seasons, respectively. High heritability estimates for *Orobanche* reaction no. of *Orobanche* spikes/plot (92.3, 97.2%) and (h^2bs=98.9%, 96.4) for spikes dry weight and no. of pods/ plant (97.2, 91.6%) indicate an additive gene action in its inheritance.

Table (3): Significance of mean squares duet to different source of variation for all studied traits in 2006/07, 2007/08 & combined over them.

	2006/2007		2007/2008	
Source of variation df	Genotypes	Error	Genotypes	Error
Plant height (cm)	127.22	23.91	492.53	67.74
No. of branches/ plant	8.61	0.08	6.97	0.19
No. of pods/plant	21.81	0.92	69.79	2.08
No. of seeds/plant	249.42	3.68	629.15	8.27
Seed yield / plant(gm)	137.14	2.25	476.84	9.86
100-seed weight(gm)	190.39	14.75	242.68	17.92
No. of seeds/Pod	0.79	0.07	0.44	0.07
Seed yield / plot (gm)	130326.30	17431.85	263016.30	12144.07
No. of *Orobanche* spikes/plot	192.67	5.19	570.52	5.39
Spikes dry weight(gm)	10363.39	38.86	5073.84	62.11
	Combined			
Source of variation df	Season(S)	Genotypes(G)	S x G	Error
Plant height (cm)	416.59	344.46	275.34	45.80
No. of branches/ plant	2.60	12.59	2.99	0.13
No. of pods/plant	133.80	61.44	30.14	1.49
No. of seeds/plant	2277.97	558.70	319.97	5.98
Seed yield / plant(gm)	1991.81	398.62	215.33	6.06
100-seed weight(gm)	462.04	361.46	71.64	16.32
No. of seeds/Pod	2.13	0.60	9.27	0.07
Seed yield / plot (gm)	543401.67	343744.63	49597.96	14787.96
No. of *Orobanche* spikes/plot	135.00	685.90	65.74	5.25
Spikes dry weight(gm)	135.00	14898.56	538.67	45.82

*, ** Significant at 0.05 and 0.01 levels of probability respectively.

Parameters of variability and heritability (%) for agronomic traits of ten faba bean genotypes are presented in Table (4)

Table (4): Estimate of parameters of variability and heritability for agronomic traits of ten faba bean genotypes during season 2006/07 and 2007/08

Traits	2006/07			2007/08		
	GCV	PCV	h^2	GCV	PCV	h^2
Plant height (cm)	5.2	6.7	59.0	10.0	12.2	67.6
No. of branches/ plant	65.8	66.7	88.4	50.5	52.5	92.4
No. of pods/plant	26.0	27.6	97.2	36.1	37.7	91.6
No. of seeds/plant	37.8	38.6	95.7	39.7	40.4	96.2
Seed yield / plant(gm)	36.5	37.4	95.2	41.7	43.0	94.0
100-seed weight(gm)	9.8	11.0	79.9	10.4	11.6	80.7
No. of seeds/Pod	21.1	24.0	77.4	13.0	16.2	64.5
Seed yield / plot (gm)	14.9	18.0	68.3	19.3	20.7	87.3
No. of *Orobanche* spikes/plot	55.2	57.4	92.3	75.3	76.3	97.2
Spikes dry weight(gm)	124.1	124.8	98.9	92.3	94.0	96.4

GCV= Genotypic coefficient of variation and PCV= phenotypic coefficient of variation and (h^2) = heritability

The results indicated that selection for higher yield under heavy infestation with *Orobanche* is probably the most feasible approach for developing *Orobanche*-resistant faba bean genotypes. The relationship between seed yield and its components would be of considerable value to breeders for screening breeding materials and selecting donor parents for breeding programs. These results are in agreement with those of Mahmoud *et al.* (1984), Kumar and Dubey (2001), Ramgiry (1997), El-Sayed *et al.* (2003), El-Rodeny (2006) and AL-Ghamdi (2007). Seven lines of faba bean derived from hybridization between resistant cultivars to broomrape (Misr 1, Misr 2, Giza 843, Giza 429 and Giza 674) with resistant cultivar for foliar diseases Nubaria1. The crosses were selected according to their high performance in seed yield under heavy natural infestation with *Orobanche*. The selected lines compared with three checks Misr1, Giza 843 and Nubaria1 were evaluated for *Orobanche* resistance and seed yield under heavy natural infestations with *Orobanche* for two years. These results confirmed the potential value of selected lines in reducing the damage to yield caused by *Orobanche* and quantify of herbicide needed (Saber *et al*, 1999). It could be concluded that the recurrent selection is the proper way for selecting resistant faba bean types to *Orobanche*, infestation.

Mean performance of genotypes for the studied traits are presented in Table (5). Significant differences were observed among most genotypes for measured traits. Judging by the level of yield and its components, the studied genotypes compared with resistant check (Misr1& Giza 843)could be arranged into three groups. The first group, contains the genotypes of line1, line6 and line7, which considered to be the most tolerant group to *Orobanche*. These genotypes accompanied with high yielding (28.07, 35.75 and 32.22g, respectively.), and least number of *Orobanche* (11.17, 9.667 and 9.167g, respectively) in the combined data. The second group of parents, includes line2, line3, line4 and line5 is partial tolerant to *Orobanche* which parasitized by moderate levels of *Orobanche* and accompanied with medium seed yield/plant (21.88, 15.72, 26.17 and 24.43, respectively.) and small seeds size in the combined analysis. These two groups of genotypes exhibited high percentage of podded plants accompanied by reliable characters of the individuals in addition to lower rate of *Orobanche* infestation (as number of spikes per plant) reflected in a better seed yield per plant compared to the other genotypes. Third group includes Nubaria 1 which may be considered as susceptible parents. This group is parasitized by high number of *Orobanche* accompanied with low yielding stocks (7.683), with high number of branches, large seeds and highly susceptible to *Orobanche*. The low parasitism is generally accompanied by relatively better host growth, a higher percentage of fruiting plants and higher seed yield as reported by Abdalla and Darwish (1996).

The phenotypic (r_{ph}) correlations were determined and are presented in Table (6). The results showed that the magnitudes of phenotypic correlations for the reaction to *Orobanche* which represented by *Orobanche* spikes and spikes dry weight appeared to be and phenotypically negative correlated with yield and its components. These results in agreement with the results obtained by many investigator, among them Abdalla and Darwish (1994), Ahmad *et al.* (2001) and Belen *et al.* (2002).

SDS-PAGE Electrophoresis of storage seed proteins:

SDS-PAGE studies using seed storage protein patterns in different genotypes revealed wide variation among them. Electrophoresis and other techniques have been widely used to group and identify cultivars. Identification of the seven lines and three checks under study was carried out using SDS-PAGE. Figure 1 represents the protein banding patterns of ten faba bean genotypes, while Table 7 & 8 shows the presence and/or absence of bands and their molecular weight, the protein fractions gave a total of 24 bands. The protein zymogram of all parental genotypes revealed that these bands had molecular weight ranged from 24 to 176 kilo Daltons (Table 7).

Table (5). Mean performance of ten faba bean genotypes for studied traits during 2006/07, 2007/08and combined data.

Genotypes	Plant height (cm)			No. of branches/ plant			No. of pods/plant			No. of seeds/plant			Seed yield / plant(gm)		
	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.
Line 1	113.6 AB	118.0 AB	115.8 ABC	3.5 B	3.7 B	3.6 B	10.40 BC	17.37 A	13.88 B	26.30 C	47.63 B	36.97 BC	18.93 BC	37.20 CD	28.07 C
Line 2	116.0 AB	116.0 B	116.0 ABC	1.2 D	1.2 E	1.2 D	14.27 A	8.667 E	11.47 C	32.20 B	25.03 D	28.62 E	25.10 A	18.67 F	21.88 EF
Nubaria 1	113.8 AB	85.83 C	99.80 D	6.0 A	6.0 A	5.9 A	4.57 E	3.600 F	4.083 D	10.13 F	5.833 E	7.983 G	9.500 DE	5.867 G	7.683 H
Line 3	101.0 C	118.2 AB	109.6 C	1.0 D	2.0 CD	1.5 D	7.367 D	13.43 C	10.40 C	11.20 F	35.07 C	23.13 F	7.967 E	23.47 F	15.72 G
Line 4	118.5 AB	119.2 AB	118.8 AB	3.9 B	1.5 DE	2.7 C	9.733 C	13.97 BC	11.85 C	29.00 BC	38.00 C	33.50 D	20.03 B	32.30 DE	26.17 CD
Line 5	115.3 AB	133.3 A	124.3 A	1.0 D	1.3 DE	1.2 D	12.03 B	9.667 DE	10.85 C	36.90 A	25.67 D	31.28 DE	27.20 A	21.67 F	24.43 DE
Misr 1	112.4 B	128.3 AB	120.4 A	3.6 B	4.0 B	3.8 B	10.80 BC	16.33 AB	13.57 B	20.90 D	47.50 B	34.20 CD	17.13 C	40.37 BC	28.75 C
Line 6	122.2 A	125.8 AB	124.0 A	2.9 C	2.5 C	2.7 C	12.17 B	19.00 A	15.58 A	28.47 C	53.33 A	40.90 A	24.93 A	46.57 A	35.75 A
Line 7	103.5 C	117.5 AB	110.5 BC	1.2 D	3.5 B	2.4 C	11.00 BC	17.77 A	14.38 AB	29.00 BC	49.33 AB	39.17 AB	21.43 B	43.00 AB	32.22 B
Giza 843	117.3 AB	124.2 AB	120.8 A	1.3 D	4.0 B	2.7 C	9.433 C	11.83 CD	10.63 C	15.40 E	35.33 C	25.37 F	11.77 D	30.13 E	20.95 F
Season (S)			ns			*			**			**			**
Genotypes (G)			**			ns			**			**			**
S x G			**			**			**			**			**

Genotypes	100-seed weight(gm)			No. of seeds/Pod			Seed yield / plot (gm)			No. of *Orobanche* spikes			Spikes dry weight(gm)		
	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.	1 st.	2 ed	Comb.
Line 1	71.90 DE	78.00 CD	74.95 E	2.500 C	2.767 A	2.633 ABC	1183. C	1393. C	1288. E	10.33 CDE	12.00 CD	11.17 EF	30.00 CD	30.33 BCD	30.17 B
Line 2	77.93 CD	74.57 D	76.25 DE	2.267 CD	2.933 A	2.600 ABC	1367. BC	1510. C	1438. CDE	14.33 BC	13.67 CD	14.00 DE	28.33 D	26.67 CD	27.50 B
Nubaria 1	94.23 A	101.0 A	97.63 A	2.200 CD	1.700 B	1.950 E	896.7 D	726.7 D	811.7 F	35.00 A	56.33 A	45.67 A	213.3 A	160.0 A	186.7 A
Line 3	71.50 DE	66.83 E	69.17 F	1.533 E	2.600 A	2.067 DE	1393. ABC	1603. BC	1498. BCD	17.00 B	14.67 C	15.83 CD	28.33 D	31.33 BCD	29.83 B
Line 4	68.83 E	84.90 BC	76.87 DE	3.033 AB	2.733 A	2.883 A	1347. BC	1530. C	1438. CDE	18.00 B	18.67 B	18.33 BC	41.67 B	29.67 BCD	35.67 B
Line 5	73.83 DE	84.30 BC	79.07 CDE	3.100 A	2.667 A	2.883 A	1623. A	1580. BC	1602. AB	12.67 CD	14.00 CD	13.33 DE	38.33 BC	41.33 BC	39.83 B
Misr 1	82.07 BC	84.77 BC	83.42 BC	1.900 DE	2.933 A	2.417 BC	1167. C	1547. C	1357. DE	10.67 CDE	12.33 CD	11.50 EF	25.00 DE	31.67 BCD	28.33 B
Line 6	87.60 B	87.20 B	87.40 B	2.367 CD	2.800 A	2.583 ABC	1333. BC	1760. AB	1547. ABC	9.000 DE	10.33 CD	9.667 F	17.67 E	26.00 D	21.83 B
Line 7	74.17 DE	87.13 B	80.65 CD	2.600 BC	2.800 A	2.700 AB	1550. AB	1803. A	1677. A	8.333 E	10.00 D	9.167 F	21.67 DE	23.33 D	22.50 B
Giza 843	76.37 CD	85.20 BC	80.78 CD	1.667 E	3.000 A	2.333 CD	1183. C	1493. C	1338. E	17.00 B	20.33 B	18.67 B	28.33 D	42.33 B	35.33 B
Season (S)			**			**			**			**			ns
Genotypes (G)			**			ns			**			*			*
S x G			**			**			**			**			**

Table (6): Correlation coefficients among studied traits of faba bean genotypes (Combined data).

Traits	No. of branches/ plant	No. of pods/ plant	No. of seeds/ plant	Seed yield / plant(gm)	100- seed weight (gm)	No. of seeds/ Pod	Seed yield / plot (gm)	No. of Oro. spikes	Spikes dry weight(gm)
Plant height (cm)	-0.526	0.661*	0.689*	0.687*	-0.298	0.672*	0.605	-0.683*	-0.720*
No. of branches/ plant		-0.524	-0.526	-0.380	0.850**	-0.551	-0.849**	0.791*	0.803*
No. of pods/plant			0.986**	0.978**	0.634	0.852**	0.915**	-0.174	-0.888**
No. of seeds/plant				0.992**	0.958**	0.921**	0.945**	-0.754*	-0.609
Seed yield / plant(gm)					0.967**	0.883**	0.967**	-0.825*	-0.891**
100-seed weight(gm)						0.905**	0.989**	-0.931**	-0.688*
No. of seeds/Pod							0.887**	-0.776*	-0.933**
Seed yield / plot (gm)								-0.907**	-0.907**
No. of *Orobanche* spikes/plot									0.998**

* and ** indicate significant at 0.05 and 0.01 level of probability, respectively.

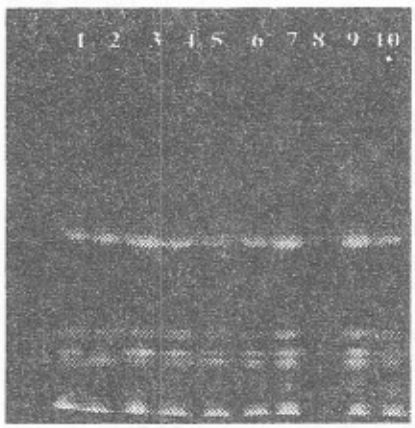

Figure (1): Electrophoretic patterns of SDS-PAGE for total soluble protein of *Vicia faba* for ten genotypes.

There were distinct differences in seed protein banding patterns between different genotypes. The band no. 1& 2 with molecular weight 167.0 and 163.5 kd was present in the resistant cultivar Giza 843 and it was absent in the rest of genotypes. The analysis of seed proteins by SDS-PAGE revealed that the heterotic effect in the F_1 protein banding pattern in the two lines; line 1 (20 bands) and line 4(22 bands) which exceed than the common parent Misr 1(18 bands). It could be identify the faba bean variety as following, seeds of line 5 characterized by protein molecular weight ranged from 141.6 to 24.0kd. in addition, protein detection in line 2 was ranged from 115.2 to 24.0 while, the susceptible cultivar Nubaria 1 which had 23 bands ranged from 141.6 to 25.02 kd. the protein fractions in line 3 gave 22 bands ranged from 141.6 to 28.6. On the other hand the high yielding and resistant line; line 7 to *Orobanche* showed a little number of bands (19) ranged from 119.7 to 29.0kd was most similarly to resistant cultivar Misr1 in both number of bands and molecular weight as well. This finding indicates clearly that the electrophoretic analysis is an important tool for the identification of faba bean varities.

These observations are compatible with those by Sammour (1992), Abd El-Halim, (1994), Zimniak and Przybyiska (1995) and Angeles *et al.* (2004) reported that the changes in the carbohydrate and nitrogen metabolism an induction of defense proteins in response to broomrape parasitism. Biometrical and total protein analysis on the importance of heterosis and its usefulness in the improvement of *Vicia faba* Abd EL-Maksoud *et al.* (2007). Total protein analysis content of the studied genotypes revealed obvious differences in the various protein fractions. It may be concluded that the protein electrophoresis may be useful for identifying *V. faba* accessions.

Table (7): Molecular weight of protein banding patterns by SDS-PAGE for total soluble protein of *Vicia faba* for ten genotypes.

Genotypes / Bands	M.W. k.d	Line1	Line2	Nubaria 1	Line3	Line4	Line5	Misr 1	Line6	Line7	Giza 843
1	176.0	135.	115	141.6	141.	141.6	115	115.	115.	119.	176
2	163.5	115.	114	115.4	115.	115.5	114	115.	115.	115.	163
3	141.6	114.	110	114.5	115.	114.9	111	111.	111.	112.	123
4	135.3	110.	106	111.6	111.	111.2	108	109.	109.	110.	115
5	12379	104.	103	110.4	110.	108.2	104	103.	104.	105.	112
6	115.3	101.	98.	107.7	108.	103.5	99.	93.2	100.	96.0	110
7	114.9	91.9	92.	102.5	102.	100.5	91.	82.4	97.0	92.6	109
8	111.6	84.9	85.	97.5	100.	93.5	83.	69.3	85.9	84.9	104
9	109.2	74.8	79.	86.5	91.5	80.8	66.	66.0	78.7	80.2	100
10	107.5	66.8	71.	80.2	82.7	71.8	63.	62.6	73.7	66.5	92.
11	104.4	63.9	65.	69.9	77.0	69.3	61.	61.0	67.0	63.4	84.
12	100.8	62.0	62.	65.5	71.1	65.3	61.	60.4	63.9	61.1	71.
13	97.40	61.0	61.	62.3	66.2	63.2	60.	60.1	62.1	61.0	67.
14	92.70	60.3	61.	61.3	62.6	61.0	59.	56.8	61.1	60.1	63.
15	85.40	58.7	60.	61.0	61.1	60.3	50.	52.1	60.5	51.3	61.
16	79.13	49.2	58.	60.0	60.1	59.4	46.	48.3	60.0	44.8	61.
17	71.40	41.7	48.	58.7	59.5	51.3	38.	41.2	51.7	37.4	60.
18	66.70	36.0	44.	49.2	49.6	46.0	36.	31.1	46.2	34.1	57.
19	63.50	32.3	37.	43.2	44.8	38.4	29.		41.2	29.1	53.
20	60.80	25.8	32.	37.8	38.4	35.1			37.0		47.
21	58.50		26.	33.3	34.7	30.7			31.7		40.
22	49.40		24.	30.5	28.7	24.0					36.
23	41.80			25.0							31.
24	35.00										26.
Total		**20**	**22**	**23**	**22**	**22**	**19**	**18**	**21**	**19**	**24**

Table (8): Analysis of protein banding patterns by SDS-PAGE for total soluble protein of *Vicia faba* for ten genotypes

Genotypes / Bands	M.W. k.d	Line1	Line2	Nubaria 1	Line3	Line4	Line5	Misr 1	Line6	Line7	Giza 843
1	176.00	-	-	-	-	-	-	-	-	-	+
2	163.53	-	-	-	-	-	-	-	-	-	+
3	141.61	-	-	+	+	+	-	-	-	-	-
4	135.37	+	-	-	-	-	-	-	-	-	-
5	123797	-	-	-	-	-	-	-	-	-	+
6	115.32	+	-	+	+	+	-	+	+	+	+
7	114.98	+	-	+	+	+	-	-	-	-	-
8	111.68	+	-	+	+	+	+	-	+	+	+
9	109.20	+	+	-	-	-	+	+	+	-	+
10	107.52	-	-	+	+	-	+	-	-	-	-
11	104.40	+	+	+	+	+	+	+	+	+	+
12	100.80	-	-	-	-	+	+	-	+	-	+
13	97.40	-	-	+	-	-	-	-	+	-	-
14	92.70	+	+	-	+	+	+	+	-	+	+
15	85.40	+	+	+	-	-	-	-	+	+	+
16	79.13	+	+	-	+	+	-	-	+	-	+
17	71.40	-	+	-	+	+	-	+	+	+	+
18	66.70	+	+	+	+	+	+	+	+	+	+
19	63.50	+	+	+	+	+	+	+	+	+	+
20	60.80	+	+	+	+	+	+	+	+	+	+
21	58.50	+	+	+	+	+	-	-	+	-	+
22	49.40	+	+	+	+	-	-	-	+	-	-
23	41.80	+	-	-	-	-	-	+	+	-	+
24	35.00	+	-	-	-	+	+	-	+	+	+
25	31.7	+	+	+	+	-	+	-	+	-	+
26	24.01	-	+	-	-	+	-	-	-	-	-
Total		17	13	14	15	15	11	9	17	10	19

Histological behavior of some selected faba bean genotypes under *Orobanche* infestation:

In the present work there are differences in resistance to broomrape between faba bean genotypes line3, Misr 1 and line7. Analysis of fig.1&2 show that histological studies were performed on sections of infected roots of faba bean genotypes. These sections demonstrated that, accumulation of a brown substance was observed around the penetration pathway of abortive haustoria in both the cortex and the central cylinder of the host root Fig.2A and Fig.2B. The substance covered the whole tissue and looked dark brown, almost black in thick sections, resembling the appearance of necrotic tissue. These results were in agreement with Pe´rez-de-Luque *et al.*, 2005b who found that resistance does not necessarily include the death of host cells or tissues. The data point to a different mechanism and it is suggested that the darkening of the tissue is a secondary symptom; it developed as a result of the operation of a different type of resistance mechanism that stopped the development of the parasite. A more detailed observation during the incompatible interaction of *O. crenata* with the resistant faba bean geno-types (line3, Misr 1 and line7), the intrusive cells of the parasite are stopped in the host cortex, before reaching the endodermis (Fig. 2A). In some cases, the parasite is scarcely able to pierce the epidermis (Fig.1B) Lignification of host

pericycle and endodermis has been observed in incompatible interactions between faba bean and *O. crenata*, which seems to prevent penetration of the parasite to the root vascular cylinder therefore, the parasite died before a tubercle had formed. (Fig. 2 (A,B,C)). In contrast, on the roots of the susceptible faba bean accession, the infection developed normally, with the intrusive cells reaching the central cylinder and the host vascular tissues(Fig.2D). Previous reports described lignification of host cortex and xylem elements (Dorr *et al.*, 1994and Pérez-de-Luque *et al.*, 2006) This may explain why, in some cases, the haustorium developed in the cortex around the vascular cylinder but could not penetrate the cylinder itself as was previously shown in the *O. aegyptiaca–Vicia* spp. interaction (Goldwasser *et al.*, 1999). Cross-sections of incompatible (A–B-C) show the blue arrow indicate Lignification of endo-dermal and pericycle host cells seems to prevent parasite intrusion into the root vascular cylinder at early infection stages HC:host cortex, VC: host vascular cylinder, Pic: Parasite. The secretion found in vessel elements was of two kinds, one stained red with safranin, the other stained green with alcian dyes one substance, possibly a carbohydrate (mucilage), as it stained with alcian green, accumulated inside host vessels away from the core of the haustorium.

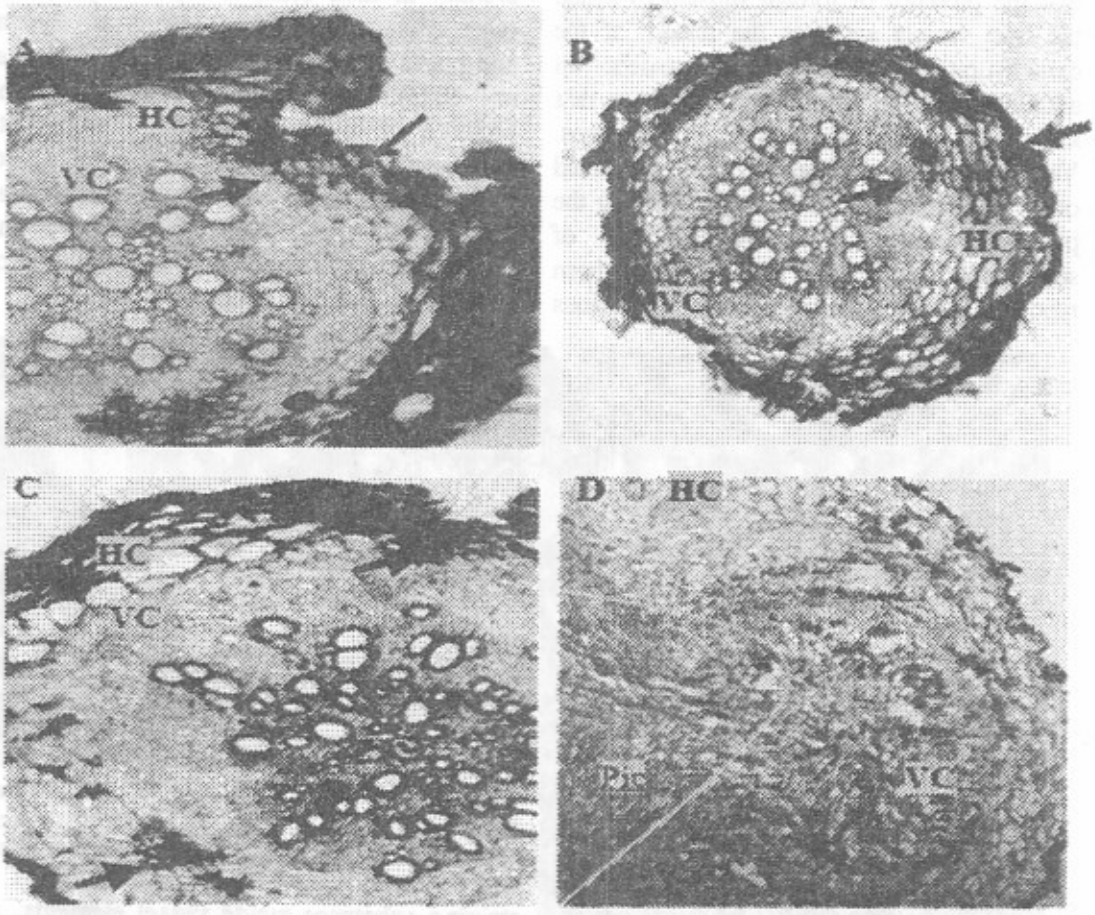

Fig. (2): Cross-sections of incompatible (A–B-C) and compatible (D) interactions of broomrape on faba bean stained with AGS. Incompatible interactions were collected on C.1657, Misr1and Giza 843x G.674 and compatible interactions Nubaria 1 (resistant and susceptible faba bean, respectively). Arrows indicate (A) AGS staining showing how the parasite is stopped after penetrating the cortex and before reaching the endodermis. (B) AGS staining showing parasite arrest just after piercing the epidermis. (D) AGS staining showing a successful penetration attempt on Nubaria 1.

The other substance that stained red with safranin was usually present close to the haustorium, within xylem vessels and also in intercellular spaces and cell walls in the central cylinder of the root. the host cells in contact with the parasite intrusive cells presented a thickening of their walls that stained intensely red with safranin. This reddish coloration was also observed at the intercellular spaces. All this indicated the presence of polyphenols, pectins, and lignins. (Fig. 3 A,B) substance was only found in vessel elements, and only away from the haustorium, not in the host–parasite interface itself, one may suppose that this carbohydrate is derived from the host. It may be possible that the presence of foreign substances inside the host vessels activates the release of the carbohydrate into the infected vessels, an additional defense mechanism, similar to that known to be associated with wilt diseases. Vascular sealing of similar type prevented other pathogens from further spread along the plant (Beckman, 2000). Accumulation of substances inside host tissues have been reported by authors in the case of various parasitic plants: Dorr *et al.* (1994), Goldwasser *et al.* (1999). Usually these substances are regarded as defensive materials from the host, but it would be possible to differentiate several substances in the *Orobanche*–faba bean interaction that may play different roles and seem to originate from both the host and the parasite. In conclusion, the death of *O. crenata* seedlings during unsuccessful penetration into the studied resistant faba bean cannot be attributed to cell death in the host. It seems to be associated with lignification of host endodermis and pericycle cells at the penetration site. mucilage production can be considered as a quantitative defensive reaction taking place against *O. crenata* in faba bean, and probably also in other legumes (Pe´rez-de-Luque *et al.*, 2005a) and plants (Labrousse *et al.*, 2001; Zehhar *et al.*, 2003). It seems to be activated by the presence of foreign substances (i.e. parasite secretions) and host-degraded products (i.e. carbohydrates from cell walls) inside host vessels, and leads to the obstruction of the parasite supply channel and to the death of established *Orobanche* tubercles.

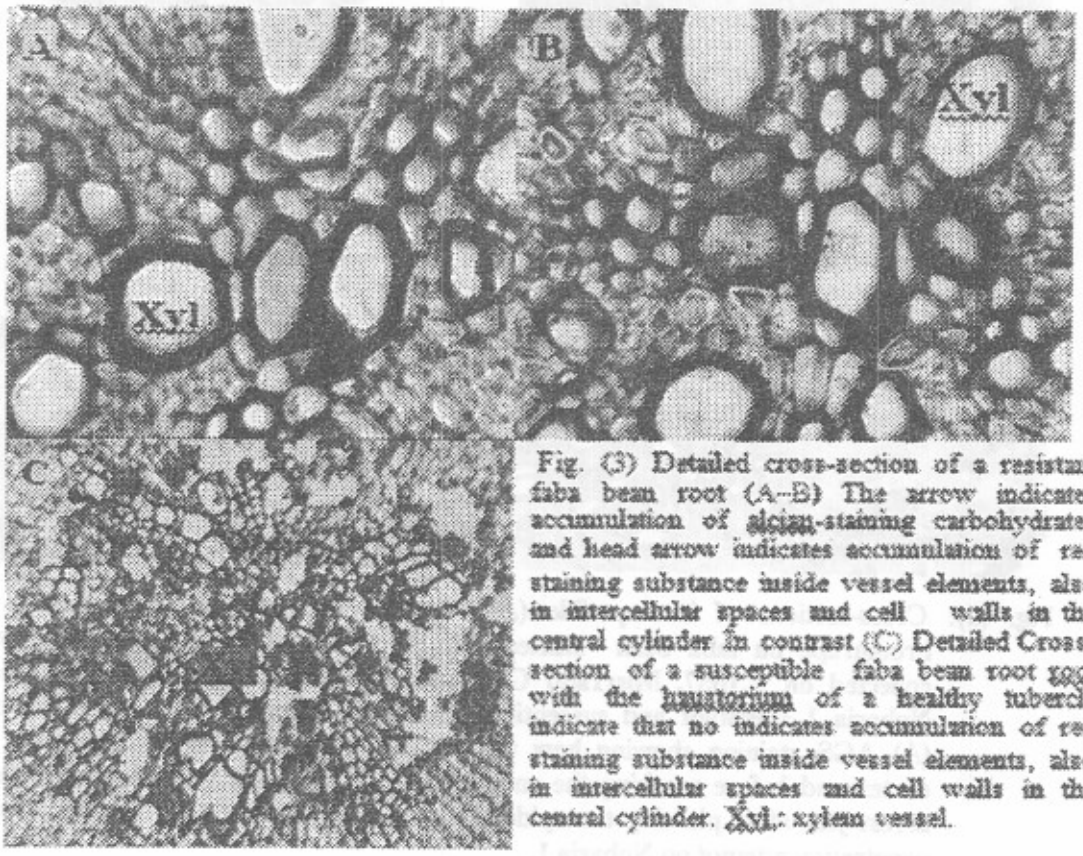

Fig. (3) Detailed cross-section of a resistant faba bean root (A–B) The arrow indicates accumulation of alcian-staining carbohydrates and head arrow indicates accumulation of red staining substance inside vessel elements, also in intercellular spaces and cell walls in the central cylinder in contrast (C) Detailed Cross-section of a susceptible faba bean root xxxx with the haustorium of a healthy tubercle indicate that no indicates accumulation of red staining substance inside vessel elements, also in intercellular spaces and cell walls in the central cylinder. Xyl.: xylem vessel.

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السلوك الوراثى وعلاقته بالصفات البيوكيماوية و التشريحية فى بعض التراكيب الوراثية من الفول البلدى المتحملة للهالوك فى مصر

علا أحمد مختار الجلالى*، وليد محمد الرضينى عبد الحليم البلقينى ** ، آمال حسن سليم**

* مركز البحوث الزراعية ــ معهد بحوث المحاصيل الحقلية ــ قسم بحوث المحاصيل البقولية.

** مركز البحوث الزراعية ــ معهد بحوث المحاصيل الحقلية ــ قسم بحوث تكنولوجيا البذور.

تهدف هذة الدراسة الى معرفة السلوك الوراثى لبعض الصفات وخاصة صفة المقاومة للهالوك وكذلك قياس التباين الوراثى و التباين البيئى ومعامل التوريث بالإضافة لمعامل الارتباط المظهري لبعض الصفات الاقتصادية فى الفول البلدي. أجريت الدراسة فى محطة البحوث الزراعية بسخا وذلك خلال موسمى الزراعة ٢٠٠٧ و حتى ٢٠٠٨ حيث تم تقييم سبعة سلالات من الفول البلدى مقارنة بثلاثة اصناف مصر ١ وجيزة ٨٤٣ مقاومة للهالوك وصنف نوبارية ١ قابل للإصابة وذلك تحت ظروف الإصابة الطبيعية الشديدة ببذور الهالوك فى تجربة ذات تصميم قطاعات كاملة العشوائية فى ثلاث مكررات لدراسة الصفات التالية :-طول النبات ، عدد الفروع للنبات ، عدد القرون للنبات ، عدد البذور للنبات ، محصول بذور النبات، وزن ١٠٠بذره ، عدد البذور فى القرن ، محصول بذور القطعة التجريبية ، عدد شماريخ الهالوك ، الوزن الجاف لشماريخ الهالوك. وبعد إجراء التحليل الإحصائي المناسب للبيانات المتحصل عليها أمكن تلخيص النتائج فى التالى:- كانت الاختلافات عالية المعنوية بين كافة التراكيب الوراثية محل الدراسة وهذا يدل على وجود مدى واسع من الاختلافات بين التراكيب الوراثية فى سلوكها الوراثى للمقاومة للهالوك. كان متوسط السلوك الوراثى للتراكيب الوراثية للصفات المدروسة معنوى وتتفوق الهجن المنتخبة فى مكونات المحصول عن أصناف المقارنة وتقل عنه فى شدة الإصابة بالهالوك. كما ظهر مدى ضيق من الاختلافات بين التباين الوراثى الكلى والتباين المظهري بين الصفات عدد القرن للنبات و عدد الفروع محصول البذور للنبات كذلك صفات المقاومة للهالوك. بينما كان الاختلاف بينهم عالى فى صفات طول النبات و محصول القطعة التجريبية و عدد البذور فى القرن فى دلالة على تأثر هذة الصفات بالبيئة. كانت قيم معامل التوريث لمعظم الصفات المدروسة بمعناها الواسع مرتفعه تراوحت قيمة من(٩٢,٣: ٩٧,٢%) لصفة عدد شماريخ الهالوك الى(٩٨,٠: ٩٦,٤%) لصفة وزن شماريخ الهالوك وكذلك من (٩١,٦: ٩٧,٢%) لصفة عدد القرون للنبات. مما يدل على أن الانتخاب للتراكيب الوراثية ذات المحصول العالى تحت ظروف الإصابة الشديدة ببذور الهالوك وسيلة فعالة فى تحسينها الوراثى. تم تقدير الارتباط بين الصفات المدروسة و أظهرت النتائج أن هناك ارتباط سالب بين عدد شماريخ الهالوك وعدد القرون

وعدد البذور ومحصول النبات كذلك أظهرت ارتباط موجب بين عدد شماريخ الهالوك وعدد الفروع. أجري التفريد الكهربائي (SDS-PAGE) على البروتينات المختزنة فى البذور والمستخلصة من عشرة تراكيب وراثية. وقد استخدم التعدد المظهري الوراثي للبروتينات المختزنة فى تقدير التباعد الوراثى بين التراكيب الوراثية المستخدمة فى الدراسة. وهذا التعدد المظهرى الوراثي للبروتينات المختزنة اظهر مدى واسعا من الاختلافات مما يجعله وسيلة فعالة فى تعريف التراكيب الوراثية المختلفة من الفول البلدى. كما أجرى فى هذا البحث أيضا بعض الدراسات التشريحيه لمعرفه الفروق التشريحيه بين اصناف الفول المقاومه للهالوك (مصر ١ ،سلالة٧ و سلالة٣) و الصنف الحساس نوبارية ١وقد وجد ان هناك عده فروق تشريحيه تتلخص فى فيما يلى :- اظهرت الدراسات التشريحيه وجود تجمع من مواد بنيه اللون تظهر حول مناطق اختراق الممصات الغير ناجحه لمنطقه القشره والاسطوانه الوعائيه فى الأصناف المقاومه مثل سلالة٧ و سلالة٣ وغير موجوده فى الأصناف الحساسه مثل صنف نوبارية ١ كما وجد ايضا انه فى بعض الأصناف المقاومه يحدث موت لخلايا الممصات قبل وصولها الى منطقه الاندودرمس فى القشره و فى بعض الأصناف المقاومه مثل مصر ١ يكون الطفيل غير قادر على اختراق طبقه البشره. كما وجد ايضا تجمعات من مواد معينه بداخل أوعيه الخشب في النبات العائل منها المواد الكربوهيدراتية التى تأخذ اللون الاخضرعند الصبغ بماده AGS والماده الأخرى هى فينول او بكتين او لجنين وهى تاخذ اللون الاحمر عند الصبغ باستعمال نفس الماده وهذه المواد تعمل على اعاقه نقل المواد الغذائيه بين النبات العائل والطفيل و عدم حدوث اتصال بين العائل والطفيل وفى النهايه يحدث موت للطفيل فى مراحل مبكره من الإنبات.