

# GENE EXPRESSION INDUCED IN FABA BEAN (*Vicia faba* L.) BY *Orobanche crenata* AND ITS IMPACT ON THE FIELD LEVEL

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The holoparasitic angiosperm *Orobanche crenata* is considered an important constraint to legume crops in the Mediterranean area Rubiales *et al.* (2006). *Orobanche crenata* devoid of chlorophyll and consequently dependent on its host for a heterotrophic supply of organic resources and energy. Tolerance to *O. crenata* in legumes is a multigenic character with very low heritability, which makes breeding for *Orobanche* tolerance a difficult task Rubiales *et al.* (2003). Chemical control methods of *Orobanche spp.* are difficult to apply because both host and parasite are flowering plants (Eplee and Norris, 1995). Indeed, whatever the crop, none of the developed control methods is wholly successful. For this reason, new control methods, which are more selective and active at the early stages of subterranean development of *Orobanche spp.*, have to be developed. This has been approached through the engineering of new tolerant cultivars, but this tolerance was rapidly overcome Melero-Vara *et al.* (2000). Consequently,

a better understanding of mechanisms involved in the host–parasite interaction is needed. Although many physiological and biochemical studies have been carried out on both partners, most molecular studies have been focused on the parasite (O’Malley and Lynn, 2000) and not updated.

Morphologically, there are limited studies on the effect of broomrape on the faba bean growth and yield. Soliman *et al.* (2012) used directional selection in faba bean under infestation of *O. crenata* for relative seed yield per host plant to that of the most resistant cultivar “Giza843” under heavy natural infestation with broomrape. Recently, Zakaria *et al.* (2016) evaluated eight faba bean (*Vicia faba* L.) genotypes under heavily natural infested soil with *O. crenata* seeds. Their morphological results proved that shoot dry weight, root weight, shoot length and root length traits in addition to the chlorophyll content significantly increased in the broomrape-tolerant genotypes (“Misr1” and

“FAB476”) while the volume of root size and number of haustoria of faba bean plants traits were significantly increased in the broomrape-susceptible genotypes (“Karra”, “Nubaria1”, “FAB124” and “Vattholoma”).

Expression changes of some genes known to be involved in defence reactions to pathogens. Such genes were investigated in the *Arabidopsis thaliana*-*Phelipanche ramosa* (L.) Pomel system (Santos *et al.*, 2003a and b). The expression patterns of some host plant genes selected among genes known to be involved in metabolic pathways and resistance mechanisms activated during several plant–pathogen interaction including ethylene (*ACC2*), isoprenoid (*HMG*), phenylpropanoid (*C4H*), and jasmonate biosynthesis pathways, oxidative stress responses, and pathogenesis-related proteins (*PR*). Westwood *et al.* (1998) demonstrated the activation of tomato *hmg2* promoter in transformed tobacco as early as one day after root penetration by *O. aegyptiaca*. This could be considered as a defense reaction because tomato *hmg2* encodes a 3-hydroxy-3-methylglutaryl CoA reductase (*HMGR*), a protein involved in isoprenoid biosynthesis pathway, and is activated specifically during defense responses associated with phytoalexins and sesquiterpenes production. Delavault *et al.* (2002) found that the kinetic gene expression was assayed from 1h to 7 days after *O. ramosa* germinations were placed. The results indicated that, no salicylic acid dependent defense has been detected whereas jasmonate- and ethylene

dependent pathways were induced. Thus, to date, few molecular approaches have been undertaken, which is very little in view of the complexity of patterns of defense against parasitic plants. In addition, none used fully genetically characterized host plants. The aim of this study was to characterization of some stress related genes involved in the faba bean tolerance to the broomrape infestation such as thionin (*Thio*), cinnamate-4-hydroxylase (*C4H*), 3-hydroxy-3-methylglutaryl-coA reductase (*HMG*), 1-aminocyclopropane-1-carboxylate synthase (*ACC2*), pathogenesis related proteins (*PR*). The infection effect on the growth and yield of faba bean has been targeted also to monitor the impact of those genes on the morphological level.

## MATERIALS AND METHODS

### *Plant material*

Twenty five faba bean (*Vicia faba* L.) genotypes were used for assess the effects of broomrape (*Orobanche crenata* L.) on their growth and productivity through yield related traits as well as the gene expression of some genes conferring broomrape tolerance in faba bean. The genotypes were selected from different origins. The Egyptian faba bean genotypes were obtained from the Egyptian Agriculture Research Centre (ARC) while the foreign genotypes were imported from both the gene bank of the International Plant Genetics (IPK), Gatersleben, Germany and the Nordic Gene bank (NordGen), Alnarp, Sweden (Table 1).

### **Field evaluation**

The field evaluation has been carried out at Sakha Agricultural Research Station (SARS)'s farm, Agriculture Research Centre (ARC), Kafr El Sheikh, Egypt during the growing seasons 2013/2014 and 2014/2015. Seeds of the genotypes were planted in two different plots in the field. The first plot of soil was uninfected with the broomrape spores (seeds) representing the control treatment while the other plot was naturally infested with the broomrape seeds representing the infection treatment. Seeds were planted in 50 cm spaced rows (1.5 m in length) and 30 cm between the holes. Three rows of each genotype were planted and the samples and data records were collected from the middle row. Growth parameters of faba bean plants were collected from five plants after two growth periods 36 days from sowing (the first stage of broomrape seeds germination) and 50 days (the effective stage of broomrape infestation and faba bean tolerance), respectively. Ten yield related traits were measured through the growth season representing the effect of broomrape on faba bean growth and yield. The examined traits were: Flowering date, Plant height (cm), Number of branches/plant, Number of *Orobanche* spikes/plant, Number of *Orobanche* spikes/row, Number of pods/plant, Number of seeds/pod, Number of seeds/plant, Seed yield/plant (g) and 100-seed weight (g).

Two replicates of each trait were recorded and were used for statistical

analysis of variance (ANOVA). LSD values were used to compare the means of the genotypes, treatments and growing seasons. The statistical analysis of the morphological traits was performed using JMP software.

### **Gene expression of assessment of some genes conferring broomrape tolerance in faba bean**

Four faba bean genotypes (two Egyptian including "Misr1"; "Nubaria1"; and two foreign including "FAB476", "Vattholma") were used to study the gene expression effect of broomrape infection on faba bean (Table 1). The genotypes have been selected depending upon their tolerance to the broomrape. "Nubaria1" and "Vattholma" genotypes were susceptible to the infection with broomrape while the genotypes "Misr1" and "FAB476" were tolerant to the broomrape.

### **Total RNA isolation and cDNA synthesis**

Total RNA was isolated from the leaves and roots of the selected genotypes at two different growth stages (after 36 and 50 days of sowing) using easy-RED™ Total RNA Extraction Kit (iNtRON Biotech.). The samples were collected from both infected and non-infected plants. The non-infected samples were considered as control samples and given signs (C1 and C2 for samples after 36 and 50 days, respectively) and the infected samples were given signs (T1 and T2, respectively). First-strand cDNA was synthesized through Reverse Transcription (RT-PCR)

reaction from the isolated mRNA using TIANScript RT Kit (TIANGEN Co.) according to the manufacturer's instructions.

### **PCR reaction**

PCR was performed using equal aliquots (2 µl) of the synthesized cDNA using gene-specific primers. Five genes were selected to study their expressions according to the references. The selected genes were thionin (*Thio*), cinnamate-4-hydroxylase (*C4H*), 3-hydroxy-3-methylglutaryl-coA reductase (*HMG*), 1-aminocyclopropane-1-carboxylate synthetase (*ACS2*), pathogenesis related proteins (*PR*). Primer pairs for each gene were designed using the oligo analyzer tool which is available only one: <http://eu.idtdna.com/calc/analyzer> (Table 2). The other components of the PCR reaction were 1 mM dNTPs, 1 U *Taq* DNA polymerase and 1 X PCR buffer. The reaction was completed to 25 µl using ddH<sub>2</sub>O. The PCR program consisted of 35 cycles at 95°C for 45 seconds, and 72°C for 1 minute. Annealing temperature was performed to 50 seconds and was adjusted according to the amplified gene. It was 53°C for *ACS2* gene, 50°C for *C4H* gene and 49°C for *HMG* gene. The above mentioned steps were preceded by a denaturation step at 95°C for 5 minute and followed by a final extension step at 72°C for 3 minutes. The products were separated on 2% agarose gel electrophoresis stained with ethidium bromide.

### **Fragments sequencing and analysis**

*C4H* gene fragment were extracted and recovered by their elution from the

agarose gel using a sharp and sterile scalpel blade. The elution was done by using kit MEGA quick-spin fragment DNA purification Kit (iNtRON Biotech.). The purity of the eluted cDNA was tested on 1.5% agarose gel electrophoresis. The eluted fragments were sequenced in Macrogen Company, Korea. The cDNA nucleotide sequence was analyzed using BioEdit software (Carlsbad, CA. 92008), DNA Dynamo software ([bluetractorsoftware.co.uk](http://bluetractorsoftware.co.uk)), and ORF Finder ([ncbi.nlm.nih.gov/gorf/gorf.html](http://ncbi.nlm.nih.gov/gorf/gorf.html)). The homology searches were performed with BLASTN and BLASTP programs on the basis of their homologies with the published sequences in GenBank/EMBL DNA databases which are available at <http://www.ncbi.nlm.nih.gov/BLAST> (Altschul *et al.*, 1997).

## **RESULTS AND DISCUSSION**

### ***Field evaluation of faba bean tolerance to O. crenata***

Analysis of variance of the morphological trait was carried out in order to detect the significant differences among the genotypes for all the morphological traits. The data revealed highly significant differences among genotypes for all the studied traits. Moreover, all traits revealed highly significant differences among season and treatment except flowering date and 100 seeds weight traits in which no significant differences were obtained for both seasons and treatments; and number of pods/plant and seeds yield/plant traits for seasons (Table 3). On the other hand, no significant differences between the

replications were obtained for all traits except for 100 seeds weight trait. The interaction between genotypes and season was significant for all traits except for flowering date, number of seeds/ pod and seed yield/plant traits. Similarly, the interaction between genotypes and treatment revealed highly significant differences for all the studied traits except for number of *O. crenata* spikes/ plant, number of seeds/ pod and 100 seeds weight traits in which no significant differences were obtained (Table 3).

EL-Harty *et al.* (2008) found highly significant differences among six faba bean genotypes for yield and its components characteristics. The same was reported by Ouji *et al.* (2011) in nine Tunisian faba bean.

#### ***Least significant differences (LSD) among genotypes***

For the plant growth traits, the highest plant height (130.6 cm) was obtained for the genotype “Misr3” and the least plant height (80.7 cm) was assigned for the genotype “Vaksalatorg”. The highest significant trait was number of branches/plant (8.7 branches) which was recorded for the genotype “FAB579” while the lowest number (2 branches) was assigned for the genotype “Solberga”. The genotype “FAB579” produced 50% of their flowers after 82.1 days while the fastest genotype produced flowers (after 54.1 days) was “Sigvard” (Table 4). For the tolerance to broomrape infestation traits, “Nubarial” genotype was worst genotype to defeat broomrape growth in which the

highest number of *O. crenata* spikes/row and number *O. crenata* spikes/plant was obtained (39.8 and 3.98, respectively). On the other hand the best genotype in such traits was “Aunuksenkanta”; whereas; the lowest values of the above mentioned traits were obtained (8.2 and 0.82, respectively, Table 4). For the yield traits, the best results were obtained from the genotypes “Giza843”, “Misr1” and “Misr3” for the traits number of pods/plant (15.1, 15.3 and 17.3, respectively), number of seeds/pod (4.1, 4.1 and 4.8, respectively) and number of seeds/plant (37.1 for “Misr3”). Moreover, the genotype “FAB476” gave the highest value for the seed yield/plant trait (3.32 g) and “Nubarial” gave the highest value for the weight of 1000 seeds trait (99.2 g, Table 4). On the other hand, the lowest values were noted from the genotypes “Aunuksenkanta” for number of pods/plant (5.4), “FAB42” for number of seeds / pod (1.1) and number of seeds per plant (7.7) traits, “FAB42” for seed yield/plant (1.32 g) “Solberga” for the 100 seed weight trait (38 g, Table 4).

Soliman *et al.* (2012) found that the highest yielding ability under heavy broomrape infestation was shown by the F4 selection of three crosses involving the most resistant parental cultivar “Giza843” with a mean seed yield per plant of 60 g. Zakaria *et al.* (2016) proved that shoot dry weight, root weight, shoot length and root length traits significantly increased under heavily natural infested soil with *O. crenata* seeds in the broomrape-tolerant genotypes

(“Misr1” and “FAB476”) while the volume of root size and number of haustoria of faba bean plants traits were significantly increased in the broomrape-susceptible genotypes (“Karra”, “Nubarial”, “FAB124” and “Vattholoma”). The same criteria was reported by Trabelsi *et al.* (2015) where they studied performance of faba bean genotypes with *O. foetida* Poir and *O. crenata* (Forsk) infestation in Tunisia.

The results of season 2013/2014 were better than the results of season 2014/2015 for all the significant traits. The non significant traits were flowering date, seed yield/plant and 100 seeds weight traits (Table 5). No significant differences were obtained between both treatments for the 100 seeds weight trait, while the control treatment statistically significant surpassed the infestation treatment for the other traits. The infested treatment surpassed the control treatment just for the *Orobanche* infestation traits (e.g. number of *O. crenata* spikes/row and number of *O. crenata* spikes/plant, Table 5).

These results agree with those reported by Shafik *et al.* (2014) where they studied individual vs. bulk selection in faba bean variety “Cairo5” grown under free and *Orobanche* infestation. They found significant differences between individual and bulk selections for many characters.

### **Two-way hierarchical cluster analysis**

Dendrogram of hierarchical clus-

tering using Ward’s method was constructing using program of JMP software. According to this analysis, the faba bean genotypes were distributed into seven clusters (Fig. 1). The first cluster contained the genotypes “Misr1”, “Misr3” and “Giza843”; while the second cluster included the genotypes “Sakha2” and “Nubarial”. It is clear that the above mentioned clusters included the Egyptian genotypes; the first cluster included the most resistant to broomrape genotypes while the second cluster included the susceptible genotypes. The third cluster contained the genotypes “Vattholma” and “FAB551” while the fourth cluster included the genotypes “FAB579”, “FAB476” and “FAB124”. The fifth cluster contained the genotypes “Sigvard”, “Solberga”, “Karra”, “FAB42” and “FAB406”. The sixth cluster included the genotypes “FAB6313”, “FAB422”, “FAB322”, “FAB56” and “FAB178” and the seventh cluster contained the genotypes “Vaksalatorg”, “FAB6171”, “FAB6315”, “FAB6275” and “Aunuksenkanta” (Fig. 1).

Similar findings were noted by El-Absawy *et al.* (2012) found that hierarchical cluster analysis divided the varieties into three clusters. The first consists of “Giza3” and “Misr1” varieties. The second cluster consists of “Sakha1”, “Sakha2” and “Giza716” varieties while the third cluster consists of “Nubarial”, “Sakha3” and “Giza843” varieties.

RNAs used for reverse transcription were extracted from whole roots and

whole leaves from 35 (C1 and T1) and 50 days (C2 and T2). Amplifications of five genes expressed in both leaves and roots including different growth stages for control and infected samples are illustrated in Fig. (2).

The results of gene expression indicated that the genes are exhibiting constitutive expression. A fragment of about 800 pb in length has been recognized for the gene *C4H* and it was clear enough to be recognized in roots rather than leaves. For the tolerant genotypes (such as “Misr1” this gene was not active in the control samples while its expression increased gradually for the different infected samples (Fig. 2). The opposite was recorded for the susceptible genotypes such as “Nubaria1” while its expression decreased gradually between the two treatment samples. The gene cinnamate-4-hydroxylase *C4H* was the only case of an mRNA accumulation induced and maintained throughout the experiment at early days (35 days). The genotype “Misr1” had unique pattern at 400bp in roots at 50 days (T2). On the other hand the genotype “Nubaria1” had two patterns in roots (C1) at 400-500 bp (Fig. 2). The same thing was obtained by Die *et al.* (2009), where they studied gene expression analysis of molecular mechanism of defense induced in *Medicago truncatula* parasitized by *O. crenata*.

For the gene *ACC2*, the amplification of its fragment (about 800 bp in length) was noticed only in the leaf control samples of the genotype “Misr1” and

in the (T1) sample of both roots of “Misr1” genotype and leaves of “FAB467” genotype (Fig. 2). These findings could suggest that broomrape infection could be accompanied by ethylene synthesis *ACC synthases* in faba bean tolerant genotypes such as “Misr1”. Santos *et al.* (2003b) studied defense gene expression analysis of *Arabidopsis thaliana* parasitized by *O. ramosa*. They found that the *acc2* gene, which encodes for the *ACC synthase*, is induced, suggesting an ethylene synthesis. This indicates that *A. thaliana* responds to *O. ramosa* parasitism by an induction of both ethylene and *ACC synthases*.

A fragment of about 300 bp in length for the gene *HMG* was amplified clearly in the root samples than leaves samples regardless the tolerance to the broomrape. The gene was expressed in the root samples C1, T1 of the genotype “Misr1”, T1 of the genotype “Nubaria1” and faintly in most samples of the other two genotypes. Very faint amplification was noticed in the leaves samples (Fig. 2). Griffiths *et al.* (2001) observed localized expression of *hmg2* and *hmg1* in tomato roots parasitized by *O. aegyptiaca*. The same thing was observed also by Labrousse *et al.* (2001).

For the gene *PR* a fragment of about 1400 bp in length was obtained only in the root sample T1 of “Misr1” genotype and another fragment of about 1500 bp in length was obtained in the root sample C1 of “Misr1” genotype and in the leaf samples C2, T1 and T2 of the genotype

“FAB476” in addition to C2 of the genotype “Vatholma” (Fig. 2). Santos *et al.* (2003b) found that four genes (*pdf1.2*, *PR-3*, *lox1* and *hmg2*) were not activated by wound but activated in *A. thaliana* roots after infestation by *O. ramosa* for 15 days which suggests that *O. ramosa* parasitism has more in common with pathogens than with wounding alone in *A. thaliana*.

For the gene *Thio*, also the amplification was clearer in the root samples than leaf samples. Very strong band was noticed in the sample T2 of the genotype “Misr1” at molecular weight of about 290 bp in length while a faint band was amplified in the other samples of the same genotype with different molecular weight sizes (Fig. 2). No constant trend was noticed for this gene corresponding to the broomrape tolerance. These results could suggest that either *Thio* not involved in the mechanism of broomrape tolerance or the collection time of the samples was not appropriate. In this regard, Santos *et al.* (2003b) found that the highest differential expression was obtained for the *thi2.1* assay, starting from mRNA accumulation in no infested roots and increasing up to a maximum after only 1 h. They reported also that the size of the amplified product was higher (800 bp) than expected (104 bp).

The co-expression of the above mentioned genes suggests that both general defense genes and specific tolerance to *Orobancha crenata* genes are participating in the mechanism of faba bean tolerance to broomrape. These results are in

agreements with Die *et al.* (2009), where they studied gene expression analysis of molecular mechanism of defense induced in *Medicago truncatula* parasitized by *O. crenata*. The induction of all of the analyzed transcripts significantly increased over a range from 2- to 321-fold higher than the control depending on the gene and time point. The transcriptional changes observed in response to *O. crenata* infection suggest that resistance could rely on both, the induction of general defense-related genes and more specific responses.

### ***Gene sequence analysis***

A band of molecular weight about 800 bp in length was excised from the amplification pattern of the genotype “Misr1” of the gene *C4H* and purified before sequencing. The sequence was subjected for alignment through the NCBI Blast tool. The sequence was aligned with *Glycine max coumarate 4-hydrrolase (C4H) mRNA* sequence accession number FJ770468.1 with 100% of identity.

The sequence was aligned with different accessions of the *Glycine max cinnamate* sequences including the accession numbers JN858958, IX92437, FJ770468, FJ968526 and HM036117 (Fig. 3). The sequence is considering the first attempt to isolate *C4H* gene for *Vicia faba*, so it was aligned with different degrees with the same gene of *Glycine max*. This sequence is being preparing to be submitted to the gene bank in the NCBI universal data base. This sequence is being preparing to be submitted to the gene bank in the NCBI universal data base.

Both morphological and molecular studies revealed that “Misr1” genotype is broomrape tolerant genotype while “Nubaria1” is broomrape susceptible genotype. The morphological results were proved by the co-expression of common defense genes (such as *HMG* gene) and broomrape specific defense genes (such as *C4H* gene). It can be concluded from our results that the morphological and the molecular results revealed that faba bean cultivar “Misr1” is broomrape tolerant cultivar and “Nubaria1” is broomrape susceptible cultivar. More studies still needed to understand the mechanism and mode of action of each of the above mentioned genes.

### SUMMARY

Twenty five faba bean (*Vicia faba* L.) genotypes were evaluated under broomrape (*Orobanche crenata* L.) natural infestation conditions for their growth and yield characteristics as well as the gene expression of some tolerance genes. Analysis of variance of the morphological traits revealed highly significant differences among the genotypes for all the morphological traits. The results of some yield related traits such as number of pods/plant, number of seeds/pod and number of seeds/plant were significantly increased in the Egyptian broomrape-tolerant genotypes “Giza843”, “Misr1” and “Misr3” and in the foreign genotype “FAB476”. On the other hand, “Nubaria1” genotype was worst genotype to defeat broomrape growth in which the highest number of *O. crenata* spikes/row and *O.*

*crenata* spikes/plant was obtained (broomrape-susceptible genotypes). Cluster analysis of yield related traits distinguished between broomrape tolerant and susceptible genotypes as well as between Egyptian and foreigner genotypes. Gene expression was assessed using Reverse Transcriptase PCR (RT-PCR) to study genes transcript accumulation during early (35 days) and late stages (50 days) of infestation. RT-PCR results revealed a kind of co-expression in common defense genes such as (*HMG* gene) and broomrape specific defense genes such as (*C4H* gene) in the most tolerant genotype “Misr1”. It is possible to conclude that yield related traits and the molecular results revealed that faba bean cultivar “Misr1” was the most broomrape tolerant cultivar and “Nubaria1” was the broomrape susceptible cultivar.

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Table (1): Pedigree or origin of faba bean genotypes and their tolerance to (*O. crenata* L.) according to the preliminary data.

No.	Genotype	Pedigree or Origin	Tolerance to <i>Orobanche</i>
1	Aunuksenkanta	NordGen	Susceptible
2	FAB 124	Marokko (MAR)	Tolerant
3	FAB 178	Italien (ITA)	Susceptible
4	FAB 322	Polen (POL)	Susceptible
5	FAB 406	Slowakei (SVK)	Susceptible
6	FAB 42	Niederlande (NLD)	Susceptible
7	FAB 422	Spanien (ESP)	Susceptible
8	FAB 476*	Sudan (SDN)	Tolerant
9	FAB 551	Libyen (LBY)	Susceptible
10	FAB 56	Deutschland (DEU)	Susceptible
11	FAB 579	Spanien (ESP)	Susceptible
12	FAB 6171	Rumanian (ROU)	Susceptible
13	FAB 6275	Großbritannien (GBR)	Susceptible
14	FAB 6313	Sowjetunion (SUN)	Susceptible
15	FAB 6315	Syrien (SYR)	Susceptible
16	Giza 843	561/2076/85x 461/845/83	Tolerant
17	Karra	NordGen	Susceptible
18	Misr 1*	(123A/ 45/76 x G.3) x (62/1570/66 x G.2)	Tolerant
19	Misr 3	Line 667 x ( Cairo 241 x Giza 461	Tolerant
20	Nubaria 1*	introduced from Spain	Susceptible
21	Sakha 2	Reina Blanca x461/845/83	Susceptible
22	Sigvard	NordGen	Susceptible
23	Solberga	NordGen	Susceptible
24	Vaksalatorg	NordGen	Susceptible
25	Vattholma*	NordGen	Susceptible

\* Genotypes used for gene expression study.

Table (2): Primers used for reverse transcription-polymerase chain reaction (RT-PCR) of some faba bean genes conferring resistance to *Orobanche crenata*.

No.	Name*	Forward primer (5'-3')	Reverse primer (5'-3')
1	ACS2-11	ATG GGA GTA ATG AAC TTG GAT CAA CA	TCA AGG CGG GGC TTT AAC
2	ACS2-2	TAC GCT GCA ACG GTT TTT AGC	CCG TGT CGT GTT CTA TTA TCT CAG
3	Thiol	ATG GGT GCT AAA TGG AAT TCA ATG	CTA TTT CTG CGC TTC CAC TTC
4	C4H-1	ATG GAT CTC CTC CTT CTG GAA	CTA AAA TGA CCT TGG CTT TGCC
5	C4H-2	ATT TGC AAG GAG GTG AAG GAG	AAA TGT GGT CAA TAG CGC ATT TAA G
6	HMG-1	ATG GAG GAT CTC CGT CGT AG	TCA CCT GTT GAC TTG AGA CG
7	HMG-2	GGA TTG CAG GAC CTT TGT TG	ACA CTA AAA GCA CCA CCA GA
8	PR	ATG GCG TCA TCA AGT GTT AGG	TAG CCG GTG TTC CTG AGT A

\*Genes names are: *Thio* (thionin), *C4H* (cinnamate-4-hydroxylase), *HMG* (3-hydroxy-3-methylglutaryl-coA reductase), *ACS2* (1-aminocyclopropane-1-carboxylate synthase), *PR* (pathogenesis related proteins).

Table (3): Analysis of variance of ten morphological traits of faba bean genotypes under *Orobanche* infestation condition.

Least Squares FitSource	Plant height (cm)	No. branches/ plant	Flowering date	No. <i>O. cre</i> spikes/row	No. <i>O. cre</i> spikes/ plant	No. pods/ plant	No. seeds/ pod	No. seeds/ plant	Seed yield/ plant (g)	100-seed weight (g)
Genotypes (G)	17372.3**	248.3**	4204.4**	7922.0**	79.2**	1278.2**	33.0**	12886.9**	8758.3**	21049.5**
Season (S)	8990.8**	34.4**	0.0 ns	12432.3**	124.3**	24 ns	6.3**	466.8**	202.2 ns	160.2 ns
Treatment (T)	65075.4**	54.3**	10.6 ns	5720.0**	57.2**	685.5**	6.0**	5635.0**	3422.4**	12.5 ns
Rep (R)	3.9 ns	0.3 ns	1.0 ns	9.6 ns	0.1 ns	1.1 ns	0.3 ns	0.15 ns	0.15 ns	821.4**
(G)*(S)	5715.9**	17.4**	0.0 ns	6486.8**	64.7**	35.9**	58.4**	0.20 ns	57.2 ns	88.9*
(G)*(T)	25590.9**	117.1**	8421.3**	1982.8**	17.7 ns	275.3**	123.3**	2.40 ns	2157.6**	6.6 ns

\*\* statistically highly differences, ns: statistically not significant.

Table (4): LSD values among 25 faba bean genotypes grown under *Orobanche* infestation condition of ten morphological traits.

Genotype	Plant height (cm)	No. branches /plant	Flowering date	No. <i>O. cre</i> spikes/ row	No. <i>O. cre</i> spikes/plant	No.Pods/ plant	No. seeds/ pod	No. seeds/ plant	Seed yield / plant (g)	100-seed weight (g)
Aunuksenkanta	88.1HIJK	4.1DEFGH	63.1BCDEF	8.2J	0.82J	5.4C	2.29CDEFG	12.6DE	7.9G	48.5IJ
FAB 124	102.7DEFGHI	5.5BC	66.7BC	17.2EFGHIJ	1.72FGHIJ	6.9C	2.62BCDEF	18.1CDE	13.6EFG	66.8DEFG
FAB 178	97.2DEFGHIJ	4.3CDEFGH	60.5BCDEF	18.6EFGHI	1.86EFGHI	5.6C	2.01FGHI	13.1DE	9.0FG	60.3FGHI
FAB 322	104.6CDEFG	2.3JK	64.9BCD	14.0HIJ	1.40HIJ	5.6C	1.88GHI	12.2DE	9.0FG	64.8EFG
FAB 406	104.1CDEFGH	3.4EFGHIJ	55.9DEF	26.2BCDEF	2.62BCDEF	6.0C	1.68GHI	11.8DE	8.7GH	68.6DEFG
FAB 42	113.2BCD	3.0HIJK	55.5EF	24.2DEFG	2.42DEFG	6.0C	1.59HI	11.3E	7.7H	59.1GHI
FAB 422	88.3GHIJK	3.3FGHIJK	66.3BC	15.8GHIJ	1.58GHIJ	6.4C	1.65GHI	11.8DE	9.4FG	81.9BC
FAB 476	111.4BCDE	3.9EFGH	60.9BCDEF	23.2DEFGH	2.32DEFGH	10.0B	3.32A	29.6B	21.4CDE	69.0DEFG
FAB 551	96.0EFGHIJK	5.3BCD	67.9B	27.2BCDE	2.72BCDE	7.6BC	1.76GHI	15.3DE	11.1FG	66.6DEFG
FAB 56	96.2EFGHIJK	2.2JK	60.7BCDEF	24.6CDEFG	2.46CDEFG	5.7C	1.95FGHI	12.8DE	9.0FG	58.8GHI
FAB 579	99.8DEFGHIJ	8.7A	82.1A	19.4EFGHI	1.94EFGHI	5.7C	1.82GHI	12.2DE	9.2FG	72.0CDEF
FAB 6171	99.3DEFGHIJ	3.4EFGHIJ	61.3BCDEF	15.0GHIJ	1.50GHIJ	7.3BC	1.32I	11.6DE	8.2G	61.6FGH
FAB 6275	86.1JK	3.1HIJK	63.9BCDE	13.6HIJ	1.36HIJ	6.7C	1.77GHI	13.3DE	8.5G	52.2HI
FAB 6313	100.9DEFGHIJ	3.4EFGHIJ	60.9BCDEF	20.2EFGHI	2.02EFGHI	7.5BC	1.72GHI	14.3DE	11.0FG	74.8BCDE
FAB 6315	105.7BCDEF	4.6BCDEF	55.9DEF	12.8IJ	1.28IJ	7.7BC	2.06FGH	17.2CDE	12.3EFG	70.3CDEFG
Giza 843	119.7ABC	4.6BCDEF	55.5EF	20.2EFGHI	2.02EFGHI	15.1A	2.79ABCDE	41.3A	32.6AB	78.2BCD
Karra	93.5FGHIJK	2.3IJK	60.9BCDEF	20.4EFGHI	2.04EFGHI	5.3C	1.95FGHI	11.9DE	8.4G	59.1GHI
Misr 1	121.7AB	3.3FGHIJK	56.3DEF	17.0FGHIJ	1.70FGHIJ	15.3A	2.82ABCD	41.1A	28.9ABC	69.0DEFG
Misr 3	130.6A	4.7BCDE	55.9DEF	12.2IJ	1.22IJ	17.3A	2.93ABC	47.5A	37.1A	77.1BCD
Nubaria 1	86.5IJK	5.6B	65.9BC	39.8A	3.98A	8.1BC	3.03AB	25.9BC	25.5BCD	99.2A
Sakha 2	91.7FGHIJK	4.4BCDEFG	57.9CDEF	31.4ABCD	3.14ABCD	6.9C	2.82ABCD	21.1BCD	18.4DEF	85.6B
Sigvard	101.4DEFGHIJ	3.2GHIJK	54.1F	36.2AB	3.62AB	6.6C	2.29CDEFG	15.9DE	9.1FG	49.0IJ
Solberga	100.1DEFGHIJ	2.0K	59.1BCDEF	18.6EFGHI	1.86EFGHI	7.1C	2.15DEFGH	15.5DE	8.5G	38.J
Vaksalatorg	80.7K	2.4IJK	58.9BCDEF	11.4IJ	1.14IJ	6.3C	2.10EFGH	15.4DE	8.3G	49.4IJ
Vattholma	81.6 HIJ	3.7EFGHI	64.7BCDE	34.6ABC	3.46ABC	5.6C	1.93FGHI	12.9DE	9.6FG	66.3DEFG

Levels not connected by same letter are significantly different.

Table (5): LSD values between seasons and treatments of ten morphological.

Seasons and Treatments	Plant height (cm)	No. branches/plant	Flowering date	No. <i>O. cre</i> spikes/ row	No. <i>O. cre</i> spikes/plant	No. pods/ plant	No. seeds/ pod	No. seeds/ plant	Seed yield /plant (g)	100-seed weight (g)
Seasons										
2013/2014	109.8 A	4.5 A	61.5 A	32 A	3.2 A	8.2 A	2.4 A	20.8 A	15.1 A	64.6 A
2014/2015	90.9 B	3.3 B	61.5 A	9.7 B	1 B	7.3 B	1.9 B	16.5 B	12.3 A	67.1 A
Treatments										
Control	134.1 A	4.9 A	61.9 A	10.8 B	1.1 B	11.2 A	2.5 A	28.6 A	21.4 A	66.3 A
Infested	66.6 B	2.9 B	61.0 B	30.9 A	3.1 A	4.3 B	1.8 B	8.7 B	6 B	65.4 A

Levels not connected by same letter are significantly different.

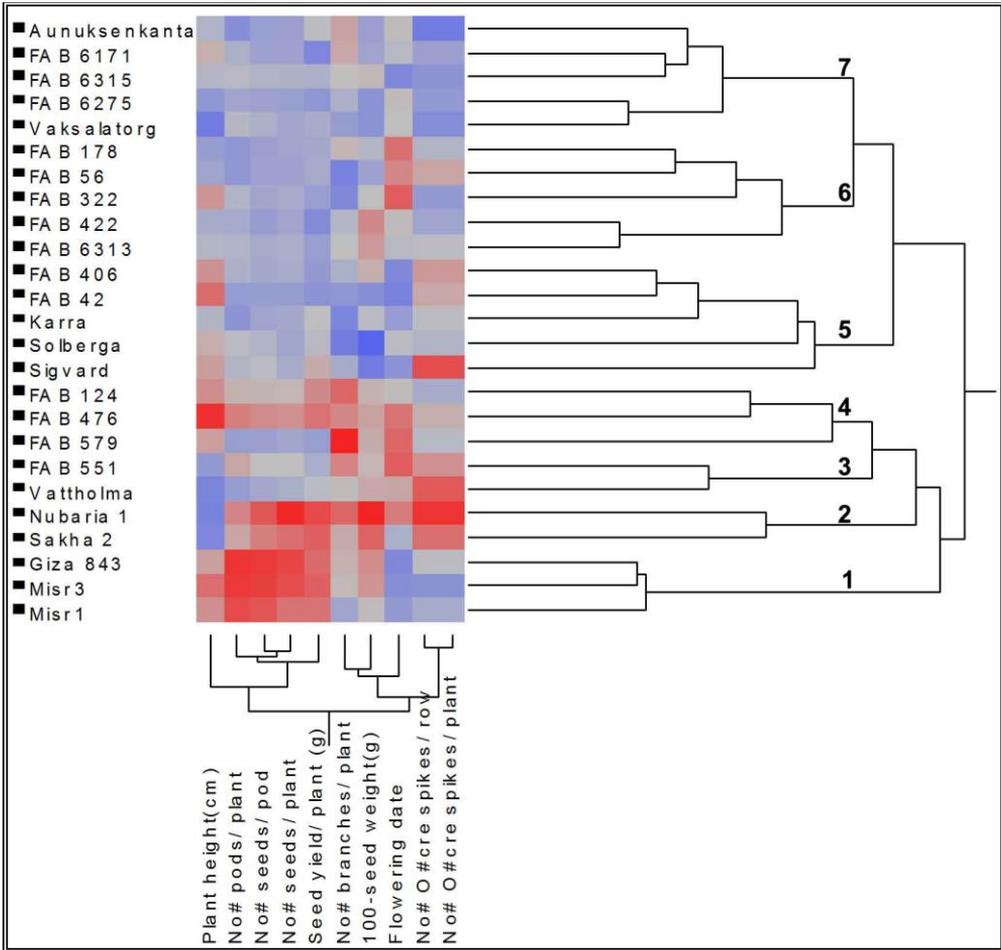


Fig. (1): Two-way hierarchical cluster analysis of 25 faba bean genotypes and ten morphological traits collected from two growing seasons under broomrape infestation condition. Gene expression pattern

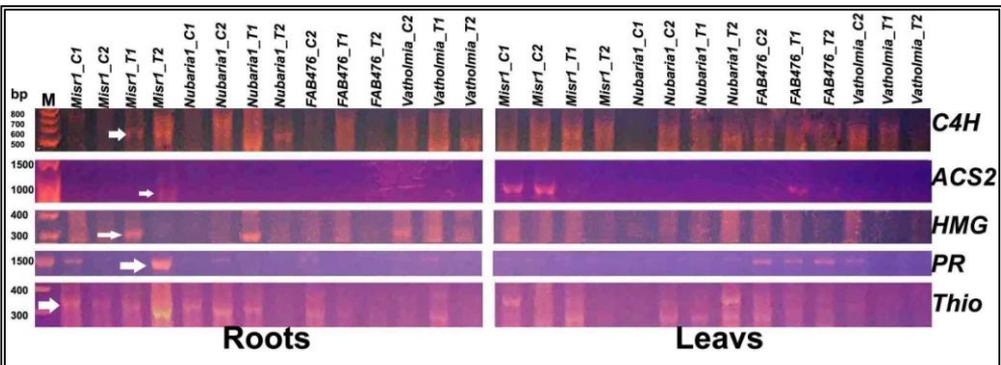


Fig. (2): RT-PCR amplification pattern of five different genes expressed in faba bean under broomrape (*O. crenata*) infestation condition.

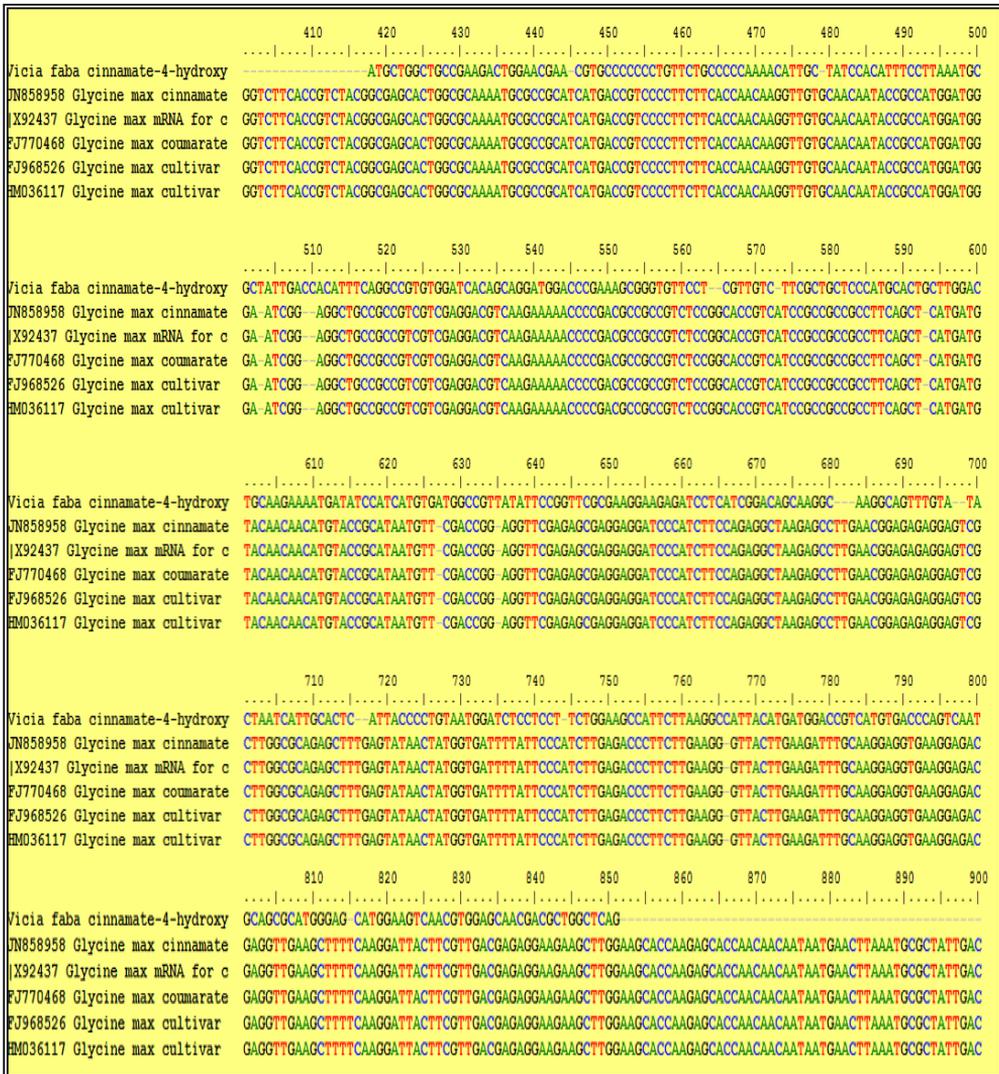


Fig. (3): Sequence alignment of *Vicia faba* C4H gene sequence with five accessions of *Glycin max* sequences previously submitted to NCBI database.