Molecular and Biochemical Markers of Some *Vicia faba* L. Cultivars in Response to Broomrape Infestation

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Abstract: This study was conducted to compare some faba bean (*Vicia faba*) cultivars (Giza 843, Nubaria 1 and Misr 1) on their reaction to *Orobanche* infestation by analyzing M1 generation using ISSR-PCR and protein electrophoresis techniques. Gamma ray at 25 Gy was selected, based on preliminary and experimental results, to create possible beneficial effects on the host-parasite relationship between the tested cultivars and *Orobanche*. Data obtained by ISSR and protein electrophoresis showed that there were considerable genetic variation and different genetic responses between the three studied cultivars, irradiation and cultivars and cultivar /infection /irradiation. Some clear reproducible polymorphic products per ISSR primers were detected, such bands could be considered cultivar specific markers, and sequencing of these bands is the aim in future studies. On the other hand, the results obtained showed that it should be considering genetic structure and cultivar typing on designing the crop for increasing faba bean yield and also the importance of gamma-rays to induce distinctive genetic effects for breeding proposes.
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Key words: faba bean, ISSR, protein, gamma rays, mutation, broomrape.

1. Introduction:

Parasitic plants of the genus *Orobanche* (broomrapes) connects to dicotyledonous host plants using a special intrusive multicellular organ, the haustorium, and deprive water and nutrients from them. They are holoparasitic, devoid of leaves and totally dependent on their hosts. Survival of the parasite depends on its ability to establish contact with a host and develop a haustorium. Infestation of the crop by *Orobanche* is considered one of the most important factors reducing faba bean yields.

Broomrape (Orobanche *crenata* Forsk.) is a major root- parasite faba bean (Vicia *faba* L), that seriously limits crop cultivation in the whole Mediterranean area. This parasitic weed is difficult to control, difficult to evaluate and the resistance identified so far is of polygenic nature Diaz-Ruiz et al (2009).

Various techniques for controlling parasites are used including: mechanical control, physical control, biological control and integrated control. Chemical control can be developed into two groups: on one hand the controls of the vegetative stages through herbicides and on the other hand, the control of the generative stage, namely the seeds through soil decontamination agents, which kill the seed directly, and synthetic germination stimulants. Physical control, including cleaning of crop seeds, hand weeding, burning of weeded plants, deep ploughing and solarization. Integrated control of parasitic weed means to combine and to integrate different preventive measures and control instruments into the given farming system. Several studies showed that single methods are not sufficient to control parasitic weeds effectively.

The possibility to obtain a desired effect on faba bean by physical mutagens may provide an indirect role in controlling *Orobanche* through the induction of benefit mutations. Addai and Safo-Kantanka (2006) subjected three soybean genotypes to (0, 50, 100, 150, 200, 250 and 300 Gy) of gamma irradiation. The 250 Gy dose reduced both percentage emergence and seedling height by about 50% relative to the 0 Gy dose which served as the control and was therefore, used as the dosage appropriate for induced mutation for the genotypes.
Román *et al.* (2002) used Inter Simple Sequence Repeat markers to assay the variation among and within populations of the parasitic weed *Orobanche crenata* from Spain and Israel. Fahmy *et al.* (2006) used ISSRs-PCR technique to get molecular markers for blast resistance. Fifteen primers were used to obtain ISSRs markers for blast disease, only 12 succeeded in the DNA amplification. As a general conclusion, ISSRs are considered good molecular markers for blast disease study, especially when comparing parents, F_1 and F_2 individual plants.

The aim of this study was to compare some faba bean cultivars on their reaction to Orobanche infestation by analyzing M1 generation using ISSR-PCR and protein electrophoresis techniques. On the other hand, gamma rays at 25 Gy were used to create possible beneficial effects on the host – parasite relationship between the tested cultivars and *Orobanche crenata.*

2. Materials and Methods

The present investigation was carried out during the two successive seasons of 2006-2007 in greenhouse of Botany Department, National Research Center, Dokki, Giza Egypt.Three cultivars (Giza 843, Nubaria 1 and Misr 1) differing in resistance phenotype against *O. crenata* were selected to carry out the resent study. Smaller doses of γ-rays were selected ranging from 3 Gy to 100 Gy. A sample of 100 uniforms, healthy dry seeds (10% of moisture) from each cultivar were arranged in monolayer in polyethylene bags and subjected to non lethal doses 3, 6, 12, 25, 50 and 100 Gy. The exposure time was exactly adjusted to allowing the seeds to receive the predetermined dose. Irradiation was achieved at the National Center for Radiation Research Technology, Cairo, Egypt.

Twenty pots (25 cm in diameter) filled with clay, and fine sand (2:1) were subjected for each mutagen treatment for each cultivar. The pots were prefertilized with 2.2 g super phosphate, 1.1 g potassium sulphate and 0.55 g calcium nitrate. Pots were infected with (0.1 g seed/pot) *Orobanche crenata* beneath the soil surface. Seeds of faba bean were sown at 3 cm from the soil surface (7 seeds/pot). The pots were grouped for the following gamma rays treatments, 3, 6, 12, 25, 50 and 100 Gy for each tested cultivar. Each treatment was replicated 4 times. Another two groups of pots, one group was infected with Orobanche crenata seeds and sown with faba bean seeds being used as infected control and the second one was sown with faba bean seeds, served as non-infected unirradiated control Pots, were watered as required. Seeds (M1) of the previous groups were gathered, at physiological maturity, and were kept separately in sacks (bags) for the next investigation after dried in the sun for one week until seed moisture content reached 10%.

Biochemical and molecular studies

1. SDS- protein electrophoresis

Sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the banding patterns of water-soluble protein of the studied cultivars, including the highly tolerant and sensitive cultivars for *Orobanche crenata*. Protein fraction was performed on a vertical slab (16.5 cm x 18.5 cm x 0.2 cm) Hoefer E600, Amersham Pharmacia biotech according to the method of Laemmli (1970) as modified by Studier (1973).

Seeds of M1 produced from the previous pot experiments of the three tested cultivars with their controls were used for this experiment.

Seeds were planted for 2 weeks, 0.5g fresh tissue was taken from different plantlets was homogenized in 2 ml of total protein extraction buffer & 2 ml of water-soluble protein extraction buffer.

- Samples were transferred to Eppendorf tubes and left in the refrigerator overnight.
- Samples were then centrifuged at 12.000 rpm at 4°C for 10 min.
- The supernatants containing total protein and water-soluble protein were transferred to new Eppendorf tubes and kept at deep freezer until use for electrophoretic analysis.

2. Inter simple sequence repeats (ISSRs)

This technique was done at Genetic Dept, Faculty of Agricultural, Zagazig University.

Other groups of M1 seed, infected and irradiated (25 Gy) with their controls were germinated for two weeks. DNA for ISSR analysis was isolated from the obtained plantlets according to the mini-premodified protocol described by Dellaporta *et al.* (1983). Such variants were chosen for this experiment because they exhibited remarkable dosage-infection responses for the three tested cultivars as shown from data obtained from morphological studies (Abdel-Hady *et al*. 2008).

DNA isolation

For each studied sample, 0.1 g of leaves was grinded in liquid nitrogen, 400µl extraction buffer plus 10 µl B-mercabtoethanol were added just before the extraction. Incubation in water bath at 65 °C for 10 min was done. Three µl RNase was added, incubated for 15 min at 37 °C. Samples were cooled to room temperature for 5 min, 400 µl of 5 M NaCl was added then incubated in (-20 °C) for 10 min. Supernatant of each sample, after centrifugation at 10.000 rpm for 5 min, was transferred to new tube and 600 µl iso-propanol was added. Centrifugation was done again for 10 min at 13.000 rpm. One ml of 70% ethanol was added to pellet and centrifugation for 1 min at 13.000 rpm was carried out. The pellet was air dried after ethanol aspiration. The obtained pellet was dissolved in 50 µl TE buffer, dehydrated at 65 °C for one hour or over night at 4°C.

A number of ISSR primers (Sigma) were initially screened to identify well amplified polymorphic

bands among the studied samples. Four primers that had a high level of polymorphism and the best readability were used for polymerase chain reaction amplification Table. (1) shows the code numbers and sequence of the ISSR primers of oligonucleotide.

Table (1). Inter simple sequence repeat primers used in the amplification reaction.

Primer	Nucleotide sequence 5`—3`
17899A	CACACACACACAAG
17899B	CACACACACACAGG
HB8	GAGAGAGAGAGAGG
HB10	GAGAGAGAGAGACC

PCR and gel electrophoresis were carried out as described in (Williams *et al.*, 1990)

Amplification condition consists of pre denaturation at 94 °C for 5 min., followed by 40 cycles for 30 sec. at 94°C (denaturation), 45 sec. at 42°C (annealing), 1.5 min. at 72°C (elongation), 1 cycle of 10 min at 72 °C (final extension) and 4 °C (infinitive). Annealing temperature was calculated as : Tm = [4(C+G) + 2 (A+T)].

The amplified products were visualized in 2% agarose gel containing 0.5 µg ml^{-1} of ethidium bromide. The bands were scored by the molecular weight marker and as present (1) or (0) for each studied sample. Weak bands were excluded. The possible induced effect of γ-rays-infection between the studied cultivars was measured by the percentage of the polymorphic band, calculated by dividing the number of polymorphic bands for each studied cultivar by the total number of band surveyed.

A dendrogram was constructed by an Unweighted Paired Group Method of cluster analysis using arithmetic averages (UPGMA). The relationships among cutivars & treatments as revealed by dendrograms were done using NTSYSpc21 windows program.

3. Results and Discussion

Biochemical genetic markers

Water-soluble protein

SDS-PAGE patterns of water soluble protein fractions for the M1 plantlets of the progeny of the three studied cultivars were shown in Tables (2-4).

The cultivar Nubaria 1 showed the banding patterns (Table 2), band number 4 at molecular weight 143.95 appeared under the infected condition. This band may be referred to the infection stress. The infected irradiated at 25 Gy treatment showed the presence of two bands at number 6 and 9 at molecular weight 86.72and 27.5 KDa. These bands may be linked with *Orobanche* tolerance. Another unique band (with MW 95.1) was recorded in case of the infected irradiated at 50 Gy treatments only. On the other hand, band number 4 (143.95KDa) was disappeared due the irradiated effect in comparison with infected one (Table 2).

Table (2). Densitometric analysis for water-soluble protein profile of Nubaria 1 cultivar under the different treatments of infection and irradiation.

Band No.	MW KDa	Treatments						
		1*	2*	3*	4*	5*	6*	7*
1	175.54	0	0	0	0	0	0	0
2	158.43	1	1	1	0	0	0	0
3	150.12	0	0	0	0	0	0	0
4	143.95	0	1	0	0	0	0	0
5	95.1	0	0	0	0	0	0	1
6	86.72	0	0	0	0	0	1	0
7	64.5	0	0	0	0	0	0	0
8	28.1	0	0	0	0	0	0	0
9	27.5	0	0	0	0	0	1	0

1*= Control 2*= Infected 3*= 3 Gy with infection 4*= 6 Gy with infection
5*= 12 Gy with infection 6*= 25 Gy with infection 7*= 50 Gy with infection

The cultivar Giza 843 showed the different banding patterns in Table (3). The presence of band number 3 at molecular weight 119.02 KDa under the infected treatment may be due to the infected stress, which was not found under control conditions. On the other hand, band numbers 5 and 14 at molecular weights 108.55 and 12.33 KDa, were absent under the infected condition. Band number 7 at molecular weight 61.21 KDa appeared under the infected irradiated treatment, while this band absent under the control and the infected condition. This band could

be considered as a positive marker for the infected irradiated treatments. High doses of gamma-rays induced specific band with MW 7.0 in the infected treatment.

Table (3). Densitometric analysis for water-soluble protein of Giza 843 cultivar under the different treatments of infection and irradiation.

Band No.	MW KDa	Treatments						
		1*	2*	3*	4*	5*	6*	7*
1	182.9	0	0	0	0	0	0	0
2	131.19	0	0	0	0	0	0	0
3	119.02	0	1	0	0	0	1	1
4	113.97	0	0	1	0	1	0	0
5	108.55	1	0	0	1	0	0	0
6	85.19	0	0	0	0	0	0	0
7	61.21	0	0	1	1	1	1	0
8	59.01	0	0	0	0	0	0	0
9	53.44	0	0	0	0	0	1	0
10	49.43	0	0	1	1	1	0	0
11	44.87	0	0	0	0	0	1	0
12	38.34	0	0	0	0	0	0	0
13	26.4	0	0	0	0	0	0	0
14	12.33	1	0	0	0	0	0	0
15	7.99	0	0	1	1	0	0	0
16	7.0	0	0	0	0	1	1	1

1*= Control 2*= Infected 3*= 3 Gy with infection 4*= 6 Gy with infection
5*= 12 Gy with infection 6*= 25 Gy with infection 7*= 50 Gy with infection

Banding pattern of water-soluble protein of the bulked leaves collected from the M1 progeny of Misr 1 was shown in Table (4). This cultivar exhibits band number 2 under the infected condition as compared with control. Bands 4, 8 and 9 with molecular weights 89.15, 49.43 and 42.77 KDa respectively were appeared in both infected and control samples. Band number 3, at molecular weight 93.44 KDa appeared only under the infected irradiated treatment at 6 Gy, as compared with the infected treatment and control one, this may be referred to the irradiated effect. Band number 6 at molecular weight 65.03 KDa induced only under the infected irradiated treatment at 12 Gy. This band and the previous one could be considered as a positive marker for this dose. The disappearance of band number 8 at molecular weight 49.43 KDa in all the infected irradiated treatment as compared with the control and the infected condition. The absence band under these treatments due to the irradiated effect.

Table (4). Densitometric analysis for water-soluble protein of Misr 1 cultivar under the different treatments of infection and irradiation.

Band No.	MW KDa	Treatments						
		1*	2*	3*	4*	5*	6*	7*
1	149.91	0	0	0	0	0	0	0
2	117.71	0	1	0	0	0	0	0
3	93.44	0	0	0	1	0	0	0
4	89.15	1	1	1	0	1	1	0
5	82.39	0	0	0	0	0	0	0
6	65.03	0	0	0	0	1	0	0
7	51.52	0	0	0	0	1	1	0
8	49.43	1	1	0	0	0	0	0
9	42.77	1	1	0	0	1	1	0

1*= Control 2*= Infected 3*= 3 Gy with infection 4*= 6 Gy with infection
5*= 12 Gy with infection 6*= 25 Gy with infection 7*= 50 Gy with infection

The results of the SDS-protein banding pattern of water-soluble proteins illustrated that the three cultivars showed different responses under the tested factors. The results indicated also that the combination treatments of infection and irradiation specially the infected irradiated at 25 Gy showed new bands in some cases, which were not found under the infected condition. Such bands may be responsible for *Orobanche* tolerance.

The results were agreed with Al Shabi (2002) who detected some specific protein bands associated with iron deficiency stress in sensitive Sorghum cultivars. El-naggar (2005) who found that the F1 plants of the sensitive parent had more bands than the tolerant parent. Also, the sensitive F2 plants had more bands under iron deficiency treatment compared with the tolerant F2 plants.

Castillejo *et al.*, (2004) in their study to identify the response of *P. sativum* to the *Orobanche* infection. Their results revealed that the parasite infection led to a decrease in the amount of proteins matching with enzymes involved in the energetic metabolism in the susceptible genotype. They discussed their results in terms of changes in the carbohydrate and nitrogen metabolism and induction of defense protein in response to broomrape parasitism.

Azzam *et al.*, (2007a) reported that irradiation caused several types of reaction on proteins. One type of reaction leads to the breaking of a small number of peptide bonds to form polypeptides of shorter length than the original protein. Radiation can also lead to aggregation or cross linking of individual polypeptide chains resulting in protein denaturation. The third type of reaction involves the reaction of amino acids in the polypeptide chain with the free radicals from water without the breaking of peptide bonds. The authors build their opinion according to the previous ones reported by Diehl (1995) and Choi and Hwang (1997).

Molacular markers

Inter-simple sequence repeat (ISSR) markers, in the present study, were used to identify the genetic diversity among three faba bean cultivars under 25 Gy irradiation and infection with *Orobanche*. Also, dendrogram tree was used to assess knowing the relationships among the three cultivars and their responses to the treatments. Four oligonucleotides primers were used in PCR amplification of inter simple sequence repeat regions.

Four selected primers developed PCR products, namely HB10, 17899A, 17899B and HB8. Amplification products of the four primers were recorded as present (1) or absent (0). The total number of bands which developed through each of these four primers were 4, 4, 2 and one respectively and the approximate size of the products ranged from 600-1251 bp Tables (5-8), Figs. (1-4). Detection of specific markers for *Orobanche* tolerance was recorded among the PCR products obtained.

Primer HB10 pattern showed different fragments with size ranging from 600 to 1251 bp (Table 5) which showed polymorphic patterns (100%). The cultivar (Misr 1) was characterized by the presence of three bands, which were not found in the sensitive cultivar (Nubaria 1) these three bands with numbers 2, 5 and 8 with molecular size 1221, 1004 and 805 bp respectively. These bands may be considered as specific markers for *Orobanche* tolerance.(Fig.1).

In contrast, in case of the sensitive one, HB10 primer could amplify 3 fragments (1, 9 and 12) with molecular size 1251, 786 and 678 bp respectively; such fragments could be considered as specific ones for these cultivars. On the other hand, HB10 failed to amplify fragments from extracted DNA of Giza 843 cultivar (control & infected).

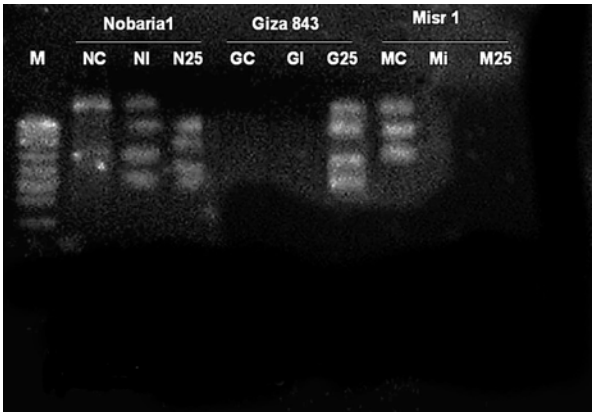


Fig. (1). Amplified fragments generated by PCR using ISSR primer (HB10) composed of (GA) 6CC.

M= marker; Nc =control; N1 infected; N25= infected irradiated – Gc= control ; GI= infected; G25= infected irradiated - Mc= control; Mi= infected ; M25= infected irradiated.

Application of 25 Gy γ-rays, HB10 primer could amplify 3 fragments from DNA extracted from Nubaria 1 progeny (7, 11 and 15); 4 fragments

from DNA extracted from Giza 843 planlets (2, 5, 10 and 15). No products in case of Misr 1 cultivar.

Table (5). DNA polymorphism among the three studied cultivars and under the different treatments with primer HB10.

Band No.	MW bP	Lane								
		Nubaria 1			Giza 843			Misr 1		
		1*	2*	3*	1*	2*	3*	1*	2*	3*
1	1251	1	1	0	0	0	0	0	0	0
2	1221	0	0	0	0	0	1	1	0	0
3	1054	0	0	0	0	0	0	0	0	0
4	1029	0	1	1	0	0	0	0	0	0
5	1004	0	0	0	0	0	1	1	0	0
6	888	0	0	0	0	0	0	0	0	0
7	867	0	0	1	0	0	0	0	0	0
8	805	0	0	0	0	0	0	1	0	0
9	786	1	1	0	0	0	0	0	0	0
10	746	0	0	0	0	0	1	0	0	0
11	712	0	0	1	0	0	0	0	0	0
12	678	1	0	0	0	0	0	0	0	0
13	630	0	0	0	0	0	0	0	0	0
14	615	0	0	0	0	0	0	0	0	0
15	600	0	0	1	0	0	1	0	0	0

1*= Control 2*= Infected 3*= 25 Gy infection

1*= Control 2*= Infected 3*= 25 Gy infection

Primer HB8

There was one polymorphic band at molecular size 1004 bp. There was one positive molecular marker which was appeared in the tolerant cultivar Misr 1 under the control conditions.

Under infection with *Orobanche* and combination treatments HB8 primer could amplify such a fragment in case of the other two studied cultivars (Table 6) and (Fig. 2).

Primer 17899A pattern resulted in six fragments with molecular sizes ranging from 648 to 1008 bp Table7 (Fig. 3) which showed polymorphic patterns (100%). There was a specific band in the cultivar Misr 1 progeny. This band with a number (1) at molecular size 1008 bp. It could be considered as a positive marker for *Orobanche* tolerance. Brimer 17899A has the ability to amplify 2 specific fragments (2 &6) from the sensitive progeny (Nubaria 1).

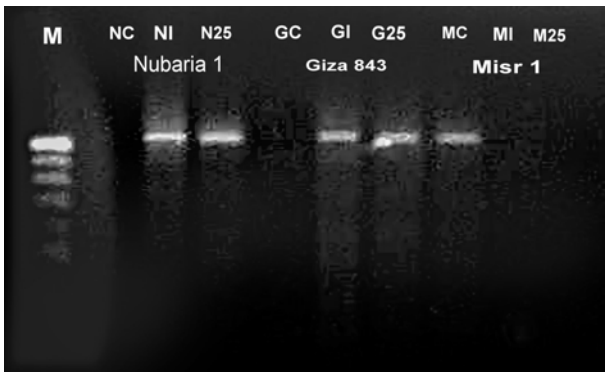


Fig. (2). Amplified fragments generated by PCR using ISSR primer (HB8) composed of (GA) 6GG.

M=marker; Nc=control; N1 infected; N25= infected irradiated – Gc=control; GI=infected; G25= infected irradiated - Mc=control; Mi=infected; M25= infected irradiated.

Table (6). DNA polymorphism among the three studied cultivars and under the different treatments with primer HB8.

Band No.	MW bP	Lane								
		Nubaria 1			Giza 843			Misr 1		
		1*	2*	3*	1*	2*	3*	1*	2*	3*
1	1004	0	1	1	0	1	1	1	0	0

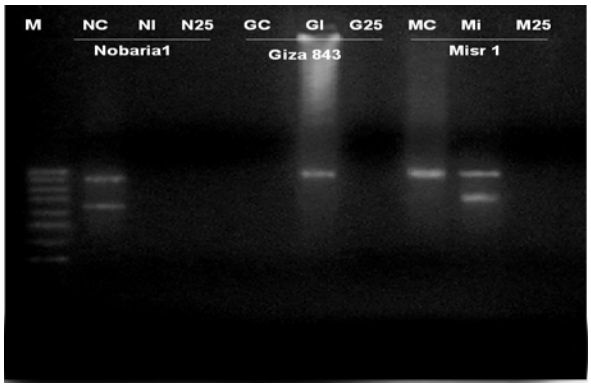


Fig. (3). Amplified fragments generated by PCR using ISSR primer (17899A) composed of (CA) 6AG.

M= marker; Nc=control; N1 infected; N25= infected irradiated – Gc=control; GI=infected; G25=infected irradiated - Mc=control; Mi=infected; M25= infected irradiated.

-Primer 17899A

Under *Orobanche* infection treatment, primer 17899A was able to produce amplified fragments with molecular size 708 bp (band 5) from DNA

extracted from Misr 1 progeny and another one with molecular size 923 bp (band 3) from DNA of Giza 843 progeny (Fig. 3).

Table (7). DNA polymorphism among the three studied cultivars and under the different treatments with primer 17899A.

Band No.	MW bP	Lane								
		Nubaria 1			Giza 843			Misr 1		
		1*	2*	3*	1*	2*	3*	1*	2*	3*
1	1008	0	0	0	0	0	0	1	1	0
2	965	1	0	0	0	0	0	0	0	0
3	923	0	0	0	0	1	0	0	0	0
4	809	0	0	0	0	0	0	0	0	0
5	708	0	0	0	0	0	0	0	1	0
6	648	1	0	0	0	0	0	0	0	0

1*= Control 2*= Infected 3*= 25 Gy infection

-Primer 17899B

Primer 17899B pattern resulted in eight fragments with molecular sizes ranging from 676 and 1030 bp (Fig. 4). There was a positive marker for *Orobanche* tolerant. This band number 8 at molecular size 793 bp which was found in the tolerant cultivars Misr 1 and Giza 843, while this band was absent in the sensitive cultivar (Nubaria 1). On the other hand, there were four bands in sensitive cultivar (1, 3, 7 and 11) under Orobanche infection. These four bands at molecular size 1030, 930, 804 and 676 bp respectively could be negative markers for such a cultivar progeny.

Fragment number 4 at molecular size 903 bp appeared only in Giza 843, this fragment considered as specific marker for this tolerant cultivar using such tested primer. Fragment number 6 at molecular size 865 bp appeared only in Giza 843 under the irradiated treatment at 25 Gy, this fragment considered as positive marker for this tolerant cultivar under this treatment.

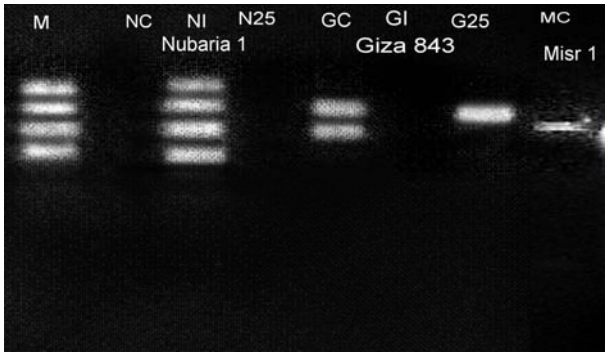


Fig. (4). Amplified fragments generated by PCR using ISSR primer (17899B) composed of (CA) 6GG.

M=marker; Nc=control; N1 infected; N25= infected irradiated – Gc=control; GI=infected; G25=infected irradiated - Mc=control; Mi= infected; M25= infected irradiated.

Table (8). DNA polymorphism among the three studied cultivars and under the different treatments with primer 17899B.

Band No.	MW bP	Lane								
		Nubaria 1			Giza 843			Misr 1		
		1*	2*	3*	1*	2*	3*	1*	2*	3*
1	1030	0	1	0	0	0	0	0	0	0
2	1000	0	0	0	0	0	0	0	0	0
3	930	0	1	0	0	0	0	0	0	0
4	903	0	0	0	1	0	0	0	0	0
5	890	0	0	0	0	0	0	0	0	0
6	865	0	0	0	0	0	1	0	0	0
7	804	0	1	0	0	0	0	0	0	0
8	793	0	0	0	1	0	0	1	0	0
9	759	0	0	0	0	0	0	0	0	0
10	706	0	0	0	0	0	0	0	0	0
11	676	0	1	0	0	0	0	0	0	0

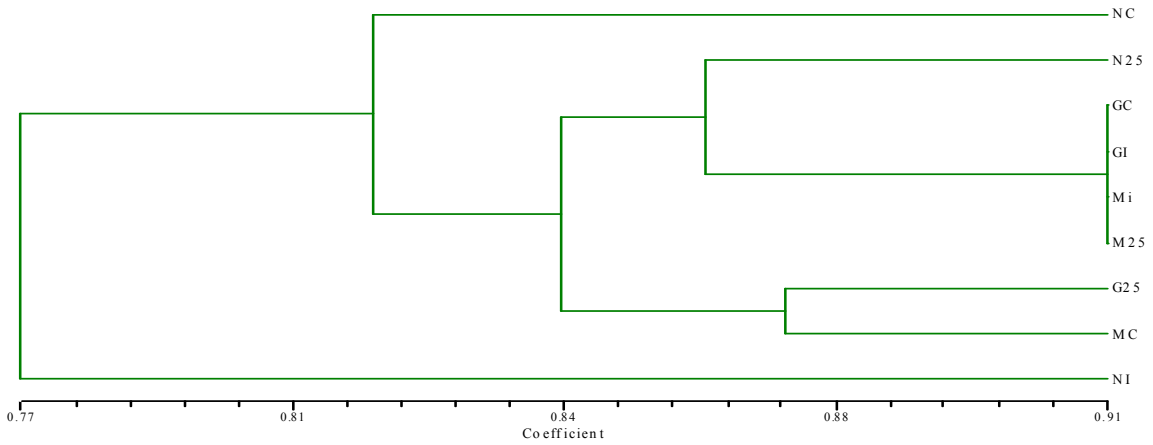
1*= Control 2*= Infected 3*= 25 Gy infection

At the level of ISSRs molecular markers, four primers showed specific markers for the studied traits. These results confirmed the useful applying of ISSRs-PCR analysis to detect the genetic variability between genotypes, which agreed with Blair *et al.* (1999) and Joshi *et al.* (2000) who demonstrated that ISSR markers are a valuable method for detecting genetic variability among rice varieties and for rapidly identifying cultivars. Ratnaparkhe *et al.* (1998) indicated that ISSRs markers can be used as highly informative markers for genome mapping and gene tagging linked to a disease resistance.

El-naggar (2005) detected some specific ISSR molecular markers associated with iron deficiencies to tolerance in maize. Furthermore, Ghareeb (2006) indicated some molecular markers for burchid resistant in faba bean for parents, F1 and F2 individual genotypes.

Dendrogram for the genetic relationships among three faba bean cultivars under control, infection with *Orobanche crenata* and the infected irradiated with 25 Gy was carried out as in Fig. (5). The three faba bean cultivars under treatments were separated into two clusters; cluster one included Nubaria 1 under the infection. Cluster two divided into three subclusters, the first one contained Nubaria 1 cultivar (control). The second subcluster Nubaria 1 under the infected with 25 Gy, Giza 843 (control), Giza 843 under the infection, Misr 1 under the infection and Misr 1 under the infected irradiated with 25 Gy. The third subcluster

contained Giza 843 under the infected irradiated with 25 Gy and Misr 1 (control).



Nc= Nubaria 1 (control), NI= Nubaria 1 (infected), N25= Nubaria 1 (under infected irradiation)
Mc=Misr 1 (control), MI= Misr 1 (infected), M25= Misr 1 (under infected irradiation) Gc= Giza 843 (control), GI= Giza 843 (infected), G25= Giza 843 (under infected irradiation)

Fig. (5). Dendrogram for the genetic distance between three faba bean cultivars under the infected and irradiated with gamma rays treatments based on ISSR-PCR analysis.

Mahmoud (2004) stated that the correlation between induced resistance and some biochemical changes in plant tissues like increased the activity of enzymes and appearance of new polypeptides protein has become a model in the study of plant disease resistance, this biochemical change become a marker to inducer resistance.

From the obtained data, in our study, we can consider that 25 Gy became a marker to inducer resistance to Orobanche in some faba bean cultivars.

ISSR-PCR markers provide a quick, reliable and highly informative system for DNA fingerprinting. ISSR markers were used in the present study to detect genetic responses of the studied cultivars towards the broomrape infestation and to illustrate the effect of 25 Gy doses on the genome. The amplified fragments revealed 11 fragments of different sizes were detected. Percentage of polymorphic bands was 100%. Primer HB10 indicated three bands presence in the sensitive cultivar (Nubaria 1) and absence in tolerance cultivar (Giza 843 and Misr 1) with molecular weight 1251, 786, 678 bp.

Primer 17899A showed that a positive molecular marker for *Orobanche* tolerance at 1008 bp which was generated in the tolerant cultivar Misr 1.

ISSR primer 17899B could generate a specific fragment of 793 bp which in the tolerant cultivars Misr 1 and Giza 843.The obtained bands in the present study could be considered cultivar-specific markers. Direct sequencing of the unique bands may be useful for future studies Several. authors (Guo *et al.*, 2005; Khaleifa *et al.*, 2006 and Azzam *et al.*, 2007b) used molecular marker in peanut to identify the molecular marker$_{(s)}$ associated with resistance to TSWV; leaf spots and pod rot resistance/susceptibility. (Torogood and Hiscock 2010) suggested that difference in signal responsiveness and perception by the parasites, as well as, qualitative differences in signal production by the host, may elicit hots specificity in Orobanche.

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