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USING THE STATIC MAGNETIC FIELD TO GENERATE MUTATIONS IN THE *VICIA FABA* GENOME AND MOLECULAR DETECTION WITH RAPD-PCR MARKER

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Abstract

Mutation is a sudden variation in the components of DNA, it was more important factor in evolution and development of genetic variations which improves the field groups. Magnetic field was one of the methods which leads to an increase in the rate of mutation. In this study, static magnetic field induced DNA mutations in the genome of *Vicia faba* L., were investigated by using Random Amplified Polymorphic DNA (RAPD). The two cultivars of *Vicia faba* L. (local cultivar, Aquadulced) were established and magnetic field potential was applied to the plants with different exposure periods (0, 15, 30, 45, 60) days and DNA was extracted from fresh of apical meristem to detect the mutation. The results of RAPD profiles of magnetic field-treated plants showed difference in banding patterns compared to the control and there is no significant differences between two cultivars treatments. It was observed that mutation is increased with the increase of exposure to magnetic field. It was concluded that DNA mutation detected by RAPD-PCR thus could be used as a potential molecular marker for the assessment of magnetic field-induced mutation effect in plants and different treatments.

Key words: Mutation, RAPD-PCR, *Vicia faba*, Static Magnetic Field.

Introduction

Mutation is a sudden variation in the components of DNA, which leads to a change in the traits of individual compared to the natural, this mutation remains genetically stable and passed on to offspring unless it undergoes a change which leads to another new mutation (Van Harten., 1998). Mutation was occurred naturally and continuously, it was the most important factor in evolution and development of genetic variations, mutations are improved the field crop. Therefore, plant breeders should pay attention to the methods which are not available in the natural sources and that lead to an increase in the rate of mutation, thus increase the yield (Bourgis *et al.*, 2008; Pathirana *et al.*, 2009). Sex cells are responsible for the inheritance of genetic traits, except if the mutation is fatal. While the somatic cells can transfer mutations to offspring through vegetative propagation only (Brunner., 1995). Mutations are the raw materials which are necessary for the emergence of genetic diversity and evolution. There are several effects of mutations, some of them may have any effect, or may improve genetic traits, or work to inhibit the genes (Brunner., 1995).

The magnetic field is the force by which electric currents affect other electrical currents. Magnetism is generated by moving the electrons into atoms of certain substances which are called magnets, they have the most common forms of bars, thick disk, squares and rectangles (Martinez., 2002). Some studies reported that fixed magnetic field have influenced on the life cycle of plant, genetic material, metabolism and growth of plants (Hirota., 1999; Penuelas *et al.*, 2004).

Studies made on various plants have shown that magnetic field was effective on the seed germination, the seeds of phaseolus when exposed to magnetic field the length of branches and roots were increased 24% and 33% respectively (Kiatgamjom *et al.*, 2003). While in the seeds of Maize which exposed to different periods of magnetic field of 1500 gauses the electrical conductivity of seeds was increased 15% and the absorption of compound, wet weight and length of branches increased 72.25% compared to non- exposed seeds (Hussain., 2016).

Vicia faba (broad bean) belong to the fabaceae, is an important grain legume in world agriculture due to its high essential nutrients in its seeds that is rich in protein, starch, fiber and vitamins (AL-Suhaibani *et al.*, 2013).

The evolution of the molecular markers are distinguished by the speed, accuracy and shorten time and effort to select the best of genetic performance (Bierwerth *et al.*, 1992). The discovery of PCR-technique in the last more than two decades has helped many researchers to develop various markers to study the genetic diversity, fingerprinting and gene detection which are responsible for many important qualities in plant breeding, among these markers is RAPD-PCR (Duc *et al.*, 2009; Al-adjadjyan., 2002). RAPD is a quite reproducible DNA fingerprinting technique that yields information on a large number of markers, generate more number of polymorphic bands, numerous polymorphism and the observation of the band pattern from each primer (Taryono *et al.*, 2011). RAPD has been commonly used for different study such as genetic diversity, construction of phylogenetic relationship and for evolution genotoxic and mutagenic effect on plants particularly when the sequencing of nucleotide is not known. (Taryono *et al.*, 2011; Selvi *et al.*, 2007).

The aim of this study assessment effect of fixed magnetic field on two cultivars of broad bean through detecting the produced mutation by using RAPD-PCR markers and to determine the ideal period to occur the mutation by exposing the plant to different periods of magnetic field.

Materials and Methods

Design of the experiment

Seeds of *Vicia faba* L. (Aquadulce, Local cultivar) have been cultivated in the farm of Agriculture College of Tikrit University at 25th of October 2017, seeds of the two varieties were divided into two parts, the first was considered as control that cultivated into pot planting directly, while the second part was germinated in disposable plastic bags containing peat moss at three

Table 1: Transactions for the two products, symbols used and duration of exposure to the magnetic field.

The treatment	sample control	First treatment	second treatment	third treatment	Fourth treatment	sample control	First treatment	second treatment	third treatment	Fourth treatment
Code	E1	A1	B1	C1	D1	E2	A2	B2	C2	D2
Exposure time	non days	15 days	30days	45days	60days	non days	15 days	30 days	45days	60 days

Table 2: Primers and their sequences used in this study.

Primer name	Primer Sequence	Primer name	Primer Sequence	Primer name	Primer Sequence
OPB-12	CCTTGACGCA	OPM-09	GTCTTGCGGA	0PD-08	GTGTGCCCCA
OPB-04	GGACTGGAGT	OPG-14	GGATGAGACC	OPA-04	AATCGGGCTG
OPB-10	CTGCTGGGAC	OPG-12	CAGCTCACGA	OPH-01	GGTCGGAGAA
OPB-14	TCCGCTCTGG	OPC-16	CACACTCCAG	OPF-16	GGAGTACTGG
OPD-02	GGACCCAACC	OPB-20	GGACCCTTAC	OPA-11	CAATCGCCGT

Table 4: Number loci and types, number produced bands number and type of special bands.

No.	Primer Number	Loci number	Monomorphic loci	Polymorphic loci number	Bands number	Monomorphic bands number	Polymorphic band number	Unique bands	Absent bands	Variation Ratio
1	OPB-12	7	1	6	43	10	24	4	2	85
2	OPB-04	10	-	10	49	-	49	7	1	100
3	OPB-10	8	-	8	30	-	30	5	4	100
4	OPB-14	5	-	5	17	-	17	2	-	100
5	OPD-02	2	2	2	20	20	-	-	-	0
6	0PD-08	8	3	5	54	30	24	3	1	62
7	OPA-04	6	-	6	22	-	22	3	1	100
8	OPH-01	8	-	-	29	-	29	4	1	100
9	OPM-09	10	1	9	35	10	25	6	-	90
10	OPG-14	7	1	6	30	10	20	4	1	85
11	OPG-12	11	-	11	32	-	32	3	-	100
12	OPC-16	10	-	10	55	-	55	5	3	100
13	OPB-20	9	1	8	54	10	44	3	3	100
Summation		101	9	92	470	90	380	49	17	90%

replications and exposure to magnetic field 1500 Gauses which designed like Hollow Cylindrical Diameter 6 cm, then these seeds were grown in pot planting while apical meristems continue to be exposed to different periods of magnetic field, thus, each cultivar of broad bean has four replicates. As shown by symbols in table 1.

Sample collection

Samples were collected from plants after 120 days of cultivated (60 days after the last treatment of fixed magnetic field) of all treatments and control of the two studied cultivars, 4-5 fresh leaves were taken from apical meristem and put in labeled bags to transfer to the laboratory to isolate DNA from them.

Genomic DNA extraction

Genomic DNA was extracted from fresh leaves using CTAB methods as described by (Weigand *et al.*, 1993; Huang *et al.*, 2013). DNA was purified according to the method of (AL-Sugmany., 2017). DNA concentration and purity were determined by using Nno Drop, the sample was diluted to concentration of 50 ng/µL and stored frozen until further use. Intgreity of DNA was assessed by Agarose gel electrophoresis according to the method of (AL-Sugmany., 2017; Sambrook *et al.*, 1989).

RAPD-PCR amplification

RAPD-PCR amplification was carried out for all treatments of *Vicia faba* using 15 primers which shown in table 2 following the procedure recommended by (Williams *et al.*, 1990).

The PCR reaction was carried out by using GoTaq Green Master mix Kit purchased from Promega Company (U.S.A). The work was done inside a sterilized hood, 25 mg of genomic DNA and 10 Pico mole of random primer were added in each tube total, volume made up to 25ìl with distilled water. Vortex the tubes and briefly spin centrifuge then RAPD-PCR program performed by the following: first denaturation 1 cycle at 94ºC for 4 min, 40 cycle (denaturation 92ºC for 30 sec; annealing 36ºC for 30 sec; extension 72ºC for 1 min) and final extension 1 cycle at 72ºC for 7 min. After the PCR amplifications, 5µL of PCR products were separated using 1.5% agarose gel electrophoresis with DNA ladder stained by ethidium bromide for 60 min and visualized under UV-trans illuminator using gel documentation system.

Band scoring and RAPD product analysis

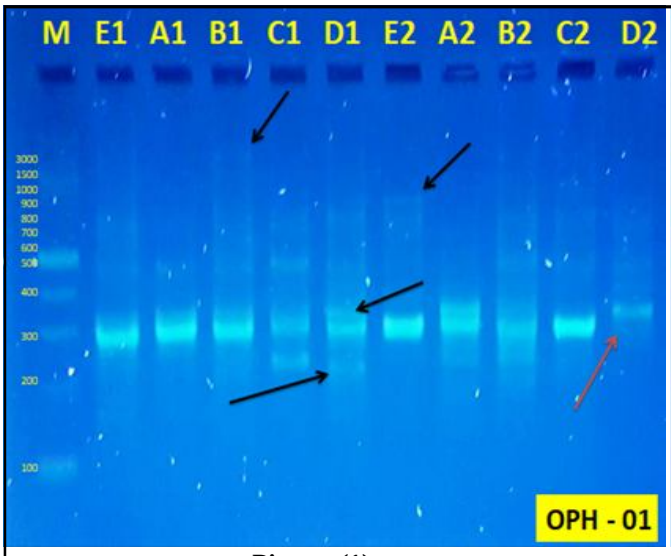
The variation in genetic material DNA which can obtained from RAPD markers can be adopted to identify mutations in treatments compared with control and that is done by converting the bands which appeared in the gel to description table, by putting 1 when there is a band and 0 at the absence of the band, that is, the band which appears in the treatment and dose not appear in the control considered as mutation varieties (AL-Gamdi., 2009; Blair *et al.*, 2009).

Results and discussion

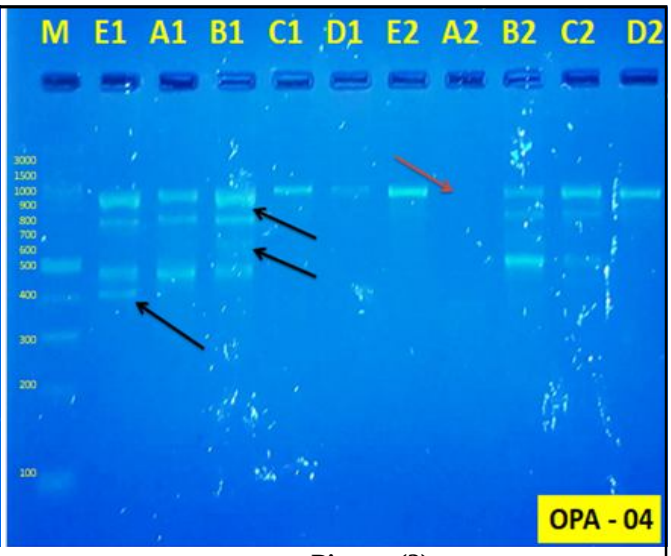
Most of treatments were distinguished with unique bands and absent bands as shown in table 4, 5, the all mutant bands which were produced in this study was

Table 5: Characteristic mutations of coefficients in the two species compared to the control sample.

Primer name	Molecular weightbp	Mutations in the first classE1 for control								Mutations in the second classE2 for control							
		A1		B1		C1		D1		A2		B2		C2		D2	
		Unique	absent	unique	absent	unique	absent	Unique	absent	Unique	absent	Unique	absent	Unique	Absent	unique	Absent
OPB-12	400-2750	-	1	-	-	-	-	1	-	1	1	-	-	1	-	-	-
OPB-04	250-1000	-	-	-	-	1	1	1	-	1	-	1	-	1	-	1	-
OPB-10	250-650	-	-	1	-	2	2	-	1	-	1	1	-	1	-	1	-
OPB-14	400-900	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
OPD-02	500-1100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0PD-08	325-1500	-	-	1	-	-	1	-	-	-	-	-	-	2	-	-	-
OPA-04	400-1000	-	-	2	-	-	-	-	-	-	1	-	-	-	-	-	-
OPH-01	400-1500	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	1
OPM09	200-1500	-	-	1	-	-	-	1	-	-	-	4	-	-	-	-	-
OPG-14	200-800	-	-	-	-	-	-	1	1	-	-	1	-	1	-	1	-
OPG-12	300-2000	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
OPC-16	300-3250	-	-	-	-	-	-	3	1	1	-	-	-	-	-	1	1
OPB-20	125-3000	-	1	-	-	1	-	1	-	-	-	-	-	1	1	-	1
Summation	60	2	2	6	0	4	3	10	3	3	3	9	0	7	1	4	3



Picture (1)



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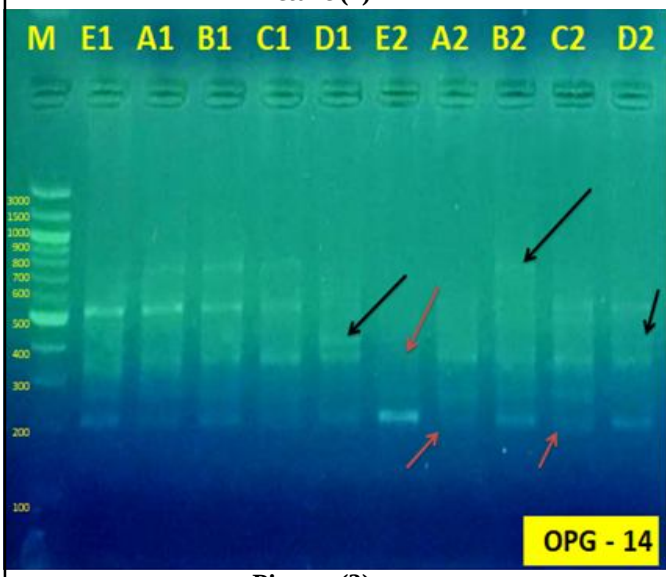

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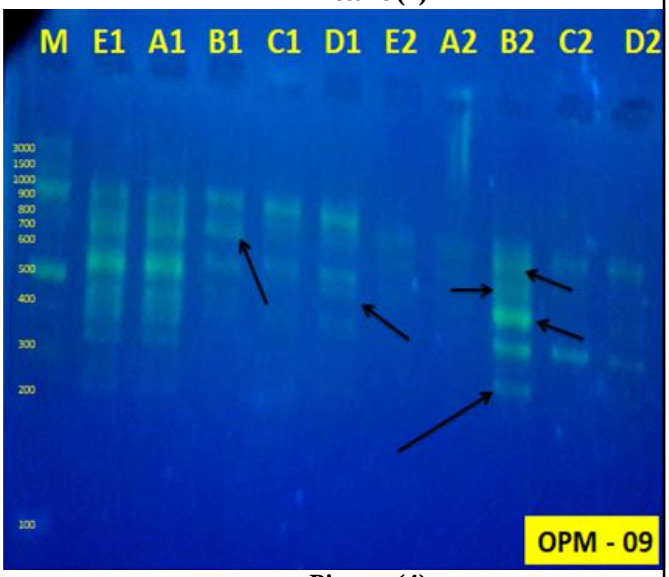

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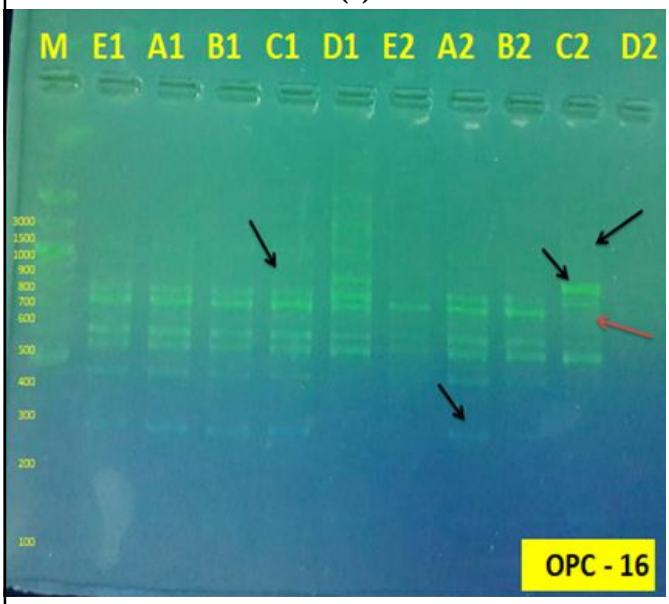

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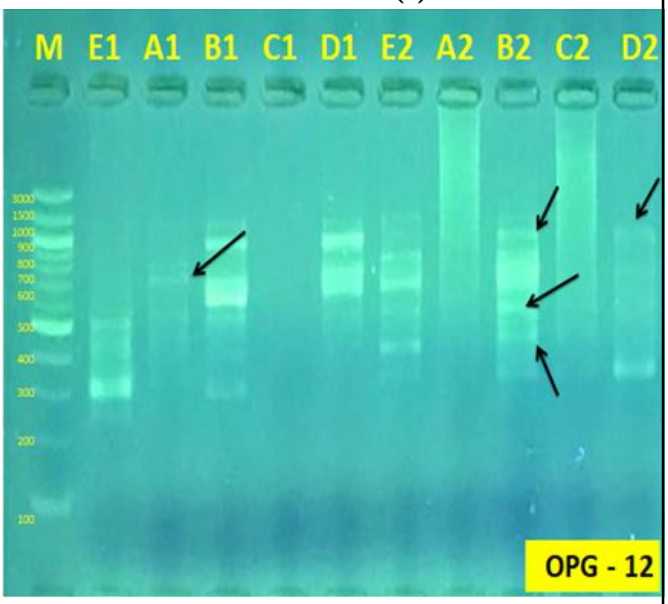

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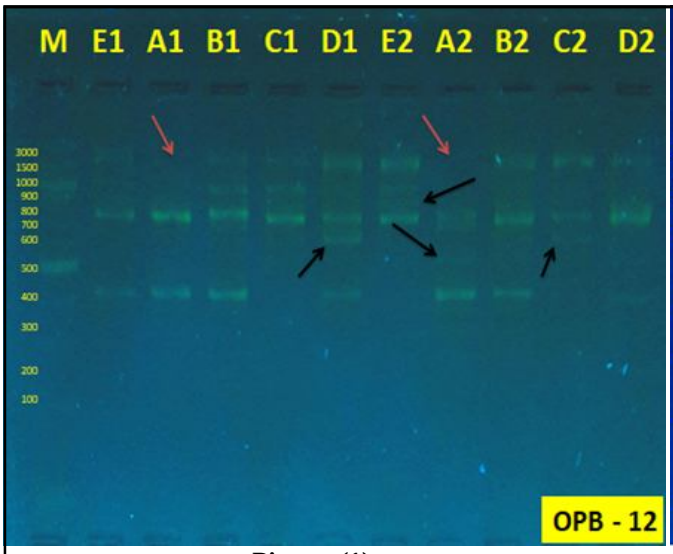

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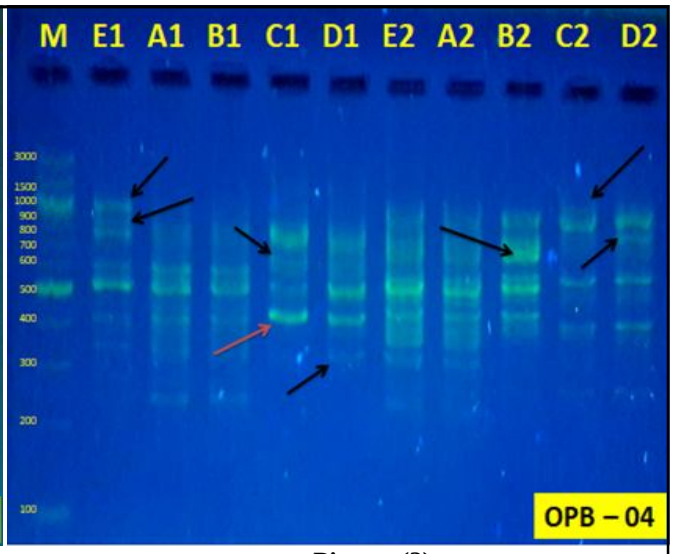

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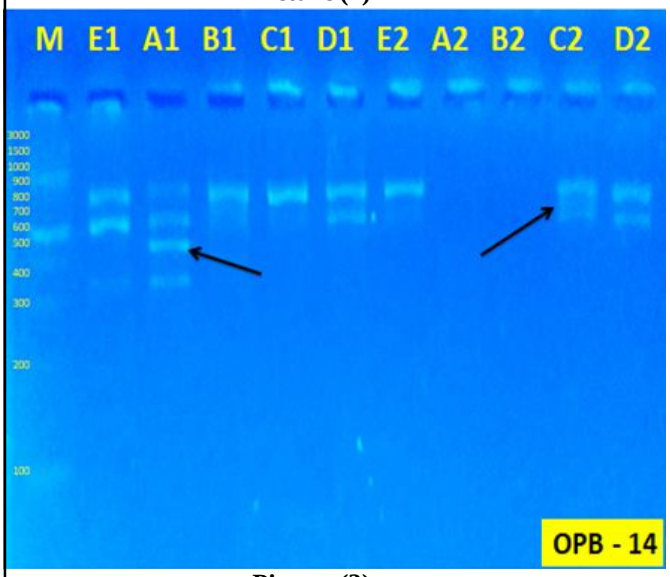

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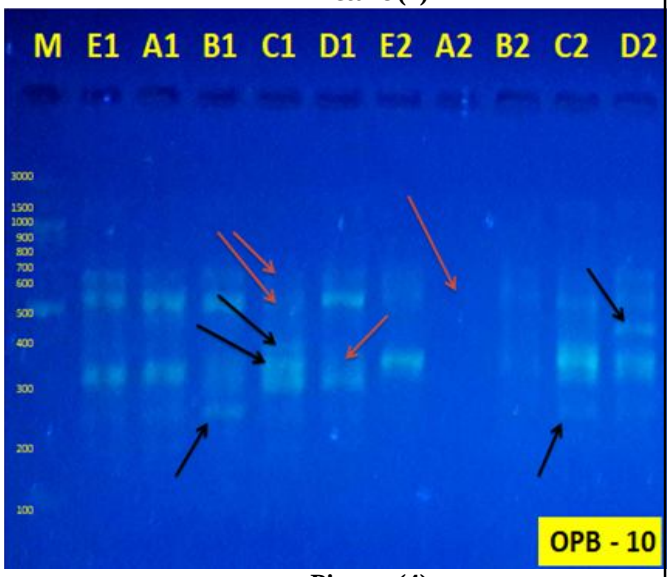

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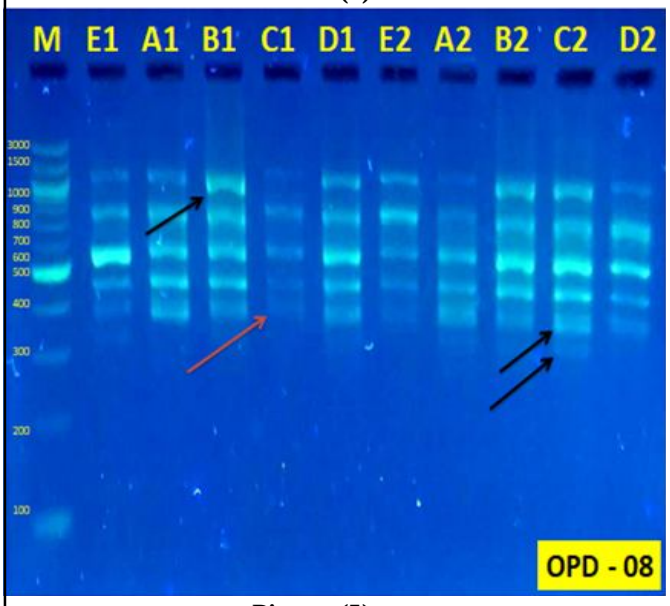

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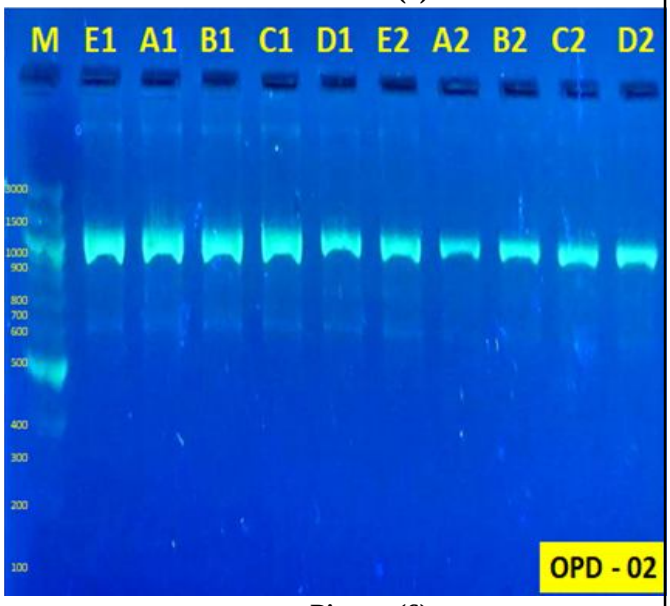

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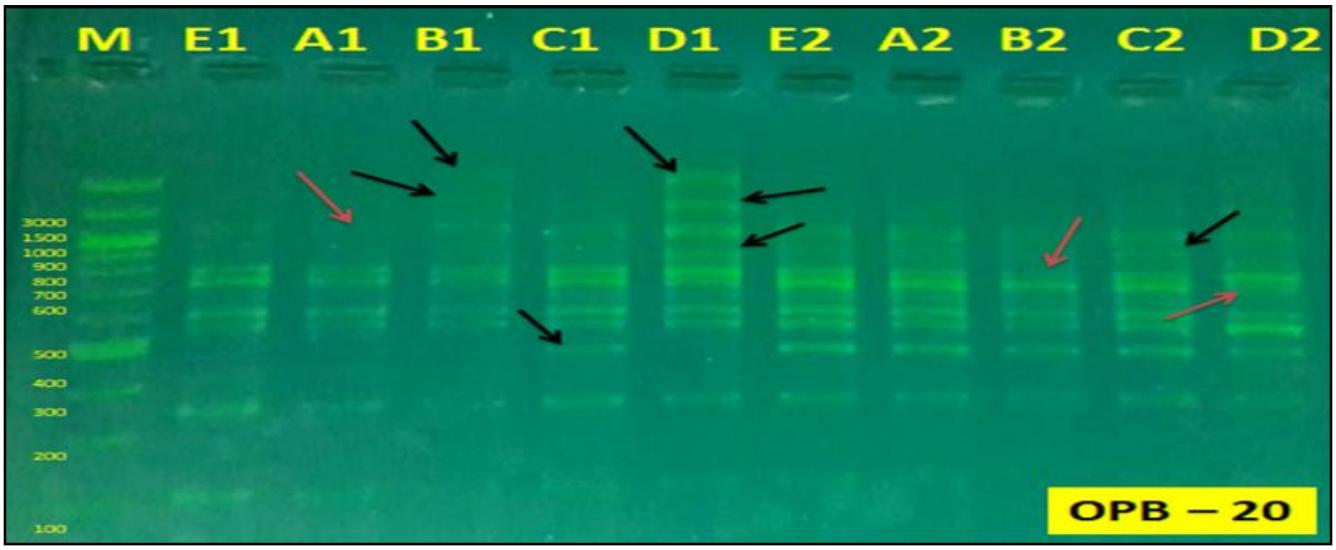

Picture (13)

(66), unique bands (49) and absent bands (17), the treatment D1 have got a higher number of unique bands was (10) and the treatment B2 have got (9) bands, while the absent bands have appeared in most of treatments were (3) bands, those bands (absent & present were considered as a discriminatory and diagnostic characteristics about treatments which have been indicated to the effect of magnetic field on DNA because the appearance of unique bands in a treatment without the other was due to the produced mutation at the annealing site of primers, as well as the occurrence of the mutation in the primer annealing site leads to inability of the primer to recognize the site and the disappearance of bands (absent band), these results were corresponded to the results of (Trevino-Castellano *et al.*, 2003; AL-Zuhiri., 2014; AL-Qaisi., 2014).

The molecular weight of amplification bands ranged between (125- 3250) bp, the minimum weight was (125) bp for OPB-20 primer and the maximum weight was (3250) bp for OPC-16 primer.

Application of this study was done for the first time at Tikrit University, this can open the way for many studies in the field of fixed magnetic field, the results showed that the magnetic field had an effect on the genome of *Vicia faba* L. in all treatments and the high efficiency of RAPD-PCR markers in the diagnosis of mutation with the dispersion of few primers (13) on the reverse of the classic breeding methods which need two or three generations to track the mutations (Hussein., 2016).

The occurrence of genetic mutations in the genome of *Vicia faba* L. and the genetic variation between the control and treatments may have negative or positive impact on the phenotypic and crop traits of the plant for several reasons, the so-called Amber mutation a change in the DNA by replacing the genetic code elsewhere, these mutations produced additional copies of the gene or motivate a mutant gene to return to wild style, or stimulates the crossing- over in mitosis, it may affect the stop codons due to an irregular transcription and also affect silent genes and turn them into active (Elsahookei., 2007; Hussein., 2016).

The treatment showed a difference in the number and quality of mutant bands with different exposure period for the two studied cultivars. In the local cultivar, treatment D1 which has the longest exposure period of (60) days, it obtained the highest number of mutant bands (10), while the lowest number of mutant bands (2) for treatment A1 which was exposed to the least period of magnetic field was (15) days. This proved that increasing the exposure of apical meristem to the magnetic field increases the incidence of mutation.

In the second cultivar (Aquadulce) treatment B2 which has a (30) days exposure period has (9) bands and minimum number of bands (3) for treatment A2 which has a (15) days exposure period, this corresponds to the local cultivar because there is a significant effect of the exposure period. The absent mutant bands in the local cultivar were obtained by C1, D1 on (3) bands for each, while the treatment B1 did not get any band. On the other hand, in the Aquadulce cultivar the treatments A2, D2 have got a higher number of absent mutant bands (3) while the treatment B2 did not receive any band.

From the results of the two cultivars, it is been noted that there is more certainity than the difference and conclusive proof that the magnetic field has a significant impact on *Vicia faba* L. and the exposure period is

directly proportional to the number of bands.

Conclusion

There is a difference in the RAPD profile between the two cultivars because of the difference in the genome of them due to different sources. The magnetic field creates mutations in the genome of *Vicia faba* L. and the mutation increases with the period of exposure of the plant to the magnetic field. The efficiency of the RAPD markers was high in detecting the producing mutation in the genome of *Vicia faba* L.

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