



[Science Archives \(ISSN:2582-6697\)](http://www.sciencearchives.org)

Journal homepage: www.sciencearchives.org



<http://dx.doi.org/10.47587/SA.2022.3206>

Research Article



Detecting the effects of Wi-Fi waves on phenotypic and molecular markers of *Vicia faba* L

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Received: May 10, 2022/ Revised: June 18, 2022 / Accepted: June 21, 2022

Abstract

The study objective was to evaluate the effects of exposure *Vicia faba* to Wi-Fi waves on growth and molecular levels. The seeds of the *Vicia faba* plant were soaked for 48 hours, then the seeds were divided into five groups, four for treatments and one for the control group, phenotypic characteristics were recorded, while the molecular markers were evaluated using RAPD-PCR markers to detect the effect of waves on DNA sequence. Morphological study, the highest plant height recorded in treatment B that was 123.9 followed by C, D and E. As for the bean pod number, the treatment E was 13.6 that showed the highest number that followed by D, C and B. For the seed number, the highest was E 34.4 seed that followed by D, B and C treatments. The number of leaves in the plants, the highest was in treatment B that was 251 followed by C, D and E treatments respectively. The leaf measurements recorded, the highest leaf area was in the treatment E that was 3.2-6.1 followed by D, C and B treatments respectively. Most of the treatments were distinguished by unique bands and absent of all mutant bands (51), unique bands (33) and absent bands (18), the treatment E (60) day got a higher number of unique bands that were (12), and the treatment B (15) day have got (4) bands, while the absent bands the treatment D (45) day got a higher number of the absent bands that were (6), and the treatment B (15) day got (4) bands. There was a difference in the results between the morphological and molecular markers because of the difference in mutation frequency on the genome of their coding and noncoding DNA. The Wi-Fi waves induced mutations in the genome of *Vicia faba* L. and the mutations increased with the period of exposure of the plant to the Wi-Fi waves. The efficiency of the RAPD markers was high in detecting the producing mutation in the genome of *Vicia faba* L

Keywords Phenotypic, Molecular Markers, *Vicia faba*, Wi-Fi Waves

Introduction

Plants exposed to different environmental stresses such as drought, salinity and temperature that affect plant growth and developments, plants grown under natural environmental conditions and exposed to different abiotic stresses throughout their life cycle that induces physiological and metabolic changes in plant body to adapt and cope with the stress (Zhang et al., 2018).

Multiple devices such as computers, smartphones and tablets connected to nearby networks that transmitted in space via Wi-Fi radio signals using router devices in order to translate and transfer the signal into data used by human beings (Sivani, 2012).

Advancement in technologies that led to increased usage of wireless and devices connected to networks by human have greatly increased the level of radiofrequency electromagnetic field radiation (EMF-r) in the environment surrounding organisms, plants one of the organisms that strongly affected by abiotic stresses since it is sessile and introduced to external environments (Saleh et al., 2020).

Last couple decades witnessed fast advancement in various technological fields. Cell phones and other various devices produced by the global communication industry that require wireless networks increased significantly. These contributed to an evident increase in the electromagnetic radiation field in the environment since it is used in most people (Zamanian et al., 2005). Many studies proved that plants react and respond to

wide types of EMP-r in different frequencies that are reported to have the ability of inducing developmental and molecular changes in plants (Roux et al., 2006; Vian et al., 2016; Tkalec et al., 2007; Beaubois et al., 2007 Singh et al., 2012).

Vicia Faba (broad bean) that belongs to the family of *fabaceae*, is considered as an important grain legume in the world since it has a high nutritional value in its seeds that are rich in starch, protein, vitamins and fiber (Al-Sugmiany et al., 2010). Faba bean, considered a grain legume crop originated in the Middle East and used traditionally as a main nutritional source for human and animal (Multari et al., 2015). A study conducted by researchers concluded that the effects of Wi-Fi waves on plants are influenced by some factors such as, the frequency of waves that increase the thermal effect on organisms, surface area of the organism that are exposed to waves of the Wi-Fi and the water content of the tissues of the organisms (Liptai et al., 2017). The high frequency influence led to increasing the internal temperature of the plant that caused metabolic and physiological effects that have effects on plant growth and development. (Rivero et al., 2022).

In spite of many research done by scientists on the effect of wireless radiations on living organisms, but most of the studies performed on animal and human, fewer studies conducted in the field of plant. The study aimed to evaluate the effect of Wi-Fi Waves on the *Vicia Faba L* growth parameters and molecular changes and rate of mutations.

Materials and Methods

Design of the experiment

Vicia faba L. seeds (Aquadulce) have been planted in pots and divided into 5 groups, each group includes 10 pots. 3 seeds per pot. The first group was (control) that was planted far away from the Wi-Fi source, the other treatments exposed to Wi-Fi radiation at different periods (0, 15, 30, 45 and 60 days respectively) and pots distanced 1m from the Wi-Fi source. After the end of the period the plants moved the field, the growth measures recorded, and DNA extracted from all samples and RAPID PCR analysis performed at the laboratory of the college of science at Tikrit university. As symbolled in Table (1).

Table 1. Treatments for one product, symbols used and duration of exposure to Wi-Fi Waves

Treatment	Control A	Treatment			
Treatment	Control A	1 st B	2 nd C	3 rd D	4 th E
Exposure time	non days	15 days	30 days	45 days	60 days

Sample collection

Samples were collected from plants after 60 days of cultivated under waves range (60 days after 4th treatment exposed to Wi-Fi Waves), 4-5 fresh leaves were taken from apical meristem that placed in labeled sacks to transfer to the laboratory to isolate DNA from them.

Extracting DNA samples

DNA samples were extracted from fresh leaves using CTAB methods as described by (Weigand et al., 1993; Huang et al., 2013).

DNA purification

DNA was purified according to the method of (Al-Sugmany and Mukhlif, 2017).

Measuring DNA concentration and purity

Concentration and purity of DNA was determined by using Nano Drop, the sample was diluted to have the concentration of 50 ng/μL and stored in freeze until use.

Agarose gel electrophoresis

Materials, solutions, gels and samples were prepared for the electrophoresis according to the method of (Al-Sugmany and Mukhlif, 2017; Sambrook et al., 1989).

RAPD-PCR markers

RAPD-PCR makers were carried out for all treatments of *Vicia faba* using 6 primers shown in table (2) according to the procedure recommended by (Williams et al., 1990).

Table 2. The RAPD-PCR primers used in the study.

Primer name	Primer Relay
OPD -09	GTCTTGCGGA
OPG -14	GGATGAGACC
OPJ -12	CAGCTCACGA
OPC -16	CACACTCCAG
OPB- 20	GGACCCCTTAC
OPD- 08	GTGTGCCCA

Methodology

Mixed solutions (Master Reaction) were prepared by mixing the reaction elements into sterilized Eppendorf tube (2ml), then centrifuged in Microfuge for 3-5 sec. This work was done inside a sterilized hood. The solutions and materials used to show in table (3).

Table 3. The RAPD markers solutions

C	Components	Volume
1	Green Master mix	12.5 μ l
2	Primer	2 μ l
3	Nuclease free water	8.5 μ l
4	DNA template	2 μ l
5	Total Volume	25 μ l

RAPD-PCR was performed according to the following steps: First denaturation 1 cycle at 94 °C for 5 min, 40 cycle (denaturation 93 °C for 45 Sec; annealing 36 °C for 45 Sec; extension 72 °C for 1 min), and the final extension 1 cycle at 72 °C for 5 min. After the PCR amplification was completed, 4 μ L of PCR products were separated using gel electrophoresis in a concentration of 1.5% with DNA marker, after electrophoresis visualized under UV- trans illuminator.

Detection of mutations

Genetic variation in DNA material that can be obtained from RAPD-PCR markers, can be adopted to identify variations in treatments compared with control, and that is done by converting the bands which appeared in the gel in the description table, by putting 1 when there is a bands and 0 at the absence of the bands, that appears in the treatments (Treviño-Castellano et al., 2003; Al-Ghamdi and Hammoud 2009).

Results and discussion

Morphological traits

After the *Vicia faba* plants maturation, growth parameters were measured that included (plant high, bean pods per plant, seeds per plant, leaves number and the dimension of leaves).

Table 4. The Morphological traits of *Vicia faba*.

Treatment	High cm	No. bean pod	No. seed	No. leaves	Leaf dimension cm
A (control)	110.7	10.9	32.9	289.8	2.36-5.7
B	123.9	9.6	18.6	251	2.4-5.5
C	114	9.4	18.	202	2.36- 5.4
D	113	13.3	30.4	182	2.63-6.4
E	105.3	13.6	34.4	175	3.2-6.1

The following morphological characteristics studied and recorded for the treatments and control for *Vicia faba* cultivar, (plant height, bean pod number, of seeds in pods, leaves number and dimensions of leaves) and the exposure period to Wi-Fi waves were (no exposure for control samples, B 15 days, C 30 days, D 45 days and E 60 Days).

Plant height measures taken from the soil surface to the plant top shoot that measured in centimeter cm. The highest plant recorded in treatment B that was 123.9 followed by C, D and E. As for the bean pods number, the treatment E was 13.6

that showed highest number followed by D, C and B. For the number of seeds, the highest was E 34.4 seed followed by D, B and C. The leaves number in the plants, the highest was in treatment B that was 251 followed by C, D and E respectively. The leaf dimensions recorded, the highest leaf area was in the treatment E that was 3.2-6.1 followed by D, C and B respectively.

Molecular markers

Most of the treatments were distinguished upon unique bands and absent bands as shown in (table 4, 5), all the mutant bands which were produced in this study were (51), unique bands (33) and absent bands (18), the treatment E got a higher number of unique bands was (12), and the treatment B got (4) bands, while the absent bands in the treatment C got a higher number of absent bands that were (6), and the treatment D got (4) bands, those bands (absent & present were considered as a discriminatory and diagnostic characteristics about treatments that have been indicated to the effect of magnetic field on DNA because the appearance of unique bands in a treatment without the others were due to the produced mutations at the annealing site of primers, as well as the occurrence of the mutations in the primer annealing site leads to failure of the primer to recognize the sites and the disappearance of bands (absent band), these results were correspond to the results of (Blair et al., 2009; Treviño-Castellano et al., 2003; Al-Zuhiri and Ali, 2014).

The molecular weight of amplification bands ranged between (100- 2000) pb, the minimum weight was (125) pb for P-1 primer and the maximum weight was (2000) pb for P-6 primer

Discussion

Growth indicators were including the plant height, leaves and leaves dimension, plant height and number of leaves is inversely proportional to exposure period, the high measures of plant height recorded at the treatment B where the exposure were for 15 days. The leave dimension is directly proportional to exposure period, the highest measures recorded at the exposure period 60 days. The influence of the Wi-Fi field increases the internal temperature of plants and can cause physiological and metabolic changes that influence plant growth and productivity (Al-Qaisi and Khader 2014; Liptai et al., 2017).

The productivity indicators included pods and seeds number, the highest productivity of pods and seeds that were recorded at the treatment E where the plants were exposed to the highest period to Wi-Fi waves. The productivity parameters were directly proportional to exposure period where it was 60 days of exposure (Gorbāns and Jurenoks, 2019). The delay in plant physiological, metabolic and growth changes and genetic mutations could lead to plant deterioration and death. Long period exposure to Wi-Fi waves induced genetic

mutations and metabolic changes that may lead to switching from vegetation to reproductive growth (Arluis et al., 2021; Ahloowalia & Maluszynski, 2001).

This 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 Wi-Fi waves had an effect on the genome of *Vicia faba* L. in all treatments and the high efficiency of RAPD-PCR markers in the detection of mutations with the dispersion of few primers (Vian et al., 2016) primers on the reverse of the 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 (Laskar et al., 2015; Hussein et al., 2016; Mustafa et al., 2020; Al-doury et al., 2019; AL-Samarraie et al., 2019; AL-Samarraie et al., 2021).

The treatment showed a difference in the quality and number of mutant bands with the different exposure period for the studied cultivar. In the local cultivar, treatment D which has

classic breeding methods which need two or three generations to track the mutations (Al-Sugmiany et al., 2020)

The occurrence of genetic mutations in the genome of *Vicia faba* L. and the genetic variation between control and treatments may have positive and/or negative impact on the phenotypic and crop traits of the plant for several reasons, the so-called Amber mutation a change in DNA by replacing the genetic code elsewhere, these mutations produced additional copies of the gene or motivate a mutant gene to the Intermediate period of (45) days, it obtained the highest number of mutant bands (17), while the lowest number of mutant bands (8) for treatment B which was exposed to the least period of waves field was (15) days. This proved that increasing the exposure of apical meristem to the Wi-Fi waves increases the incidence of mutation.

From the results of the studied cultivar, it is being noted that there is more certainty proved that the Wi-Fi waves have a significant impact on *Vicia faba* L. and the exposure period that was directly proportional to the number of bands.

Table 5. Number of loci and types, number of produced bands, number and type of special bands

C	Primer Number	Loci number	Monomorphic loci number	Polymorphic loci number	Bands number	Monomorphic bands number	Polymorphic band number	Unique bands	Absent bands	Variation Ratio
1	OPD -09	11	5	6	48	25	23	5	1	54%
2	OPG -14	8	3	5	44	15	19	3	2	62%
3	OPJ -12	7	1	6	23	5	18	7	3	85%
4	OPC -16	12	6	6	38	30	8	5	4	50%
5	OPB- 20	4	0	4	16	0	16	7	9	100%
6	OPD- 08	7	1	6	30	5	25	1	4	85%
summation										

Table 6. Characteristic mutations of coefficients in the *Vicia faba* compared to the control sample

Primer name	Molecular weight Bp	Mutations in the treatment Compared to control							
		B		C		D		E	
		unique	absent	unique	absent	unique	Absent	Unique	absent
OPD -09		1	-	1	1	2	-	1	-
OPG -14		-	-	-	-	-	1	3	1
OPJ -12		1	1	1	2	3	-	3	-
OPC -16		-	-	3	-	2	-	4	-
OPB- 20		2	3	1	2	3	2	1	2
OPD- 08		-	-	-	-	1	4	-	-
Summation		4	4	6	5	11	6	12	3
		8		11		17		15	
		51							

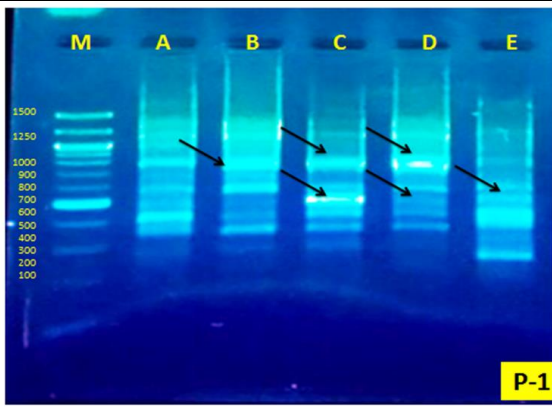


Image 1: Primer 2 products under Agarose gel electrophoresis with Marker

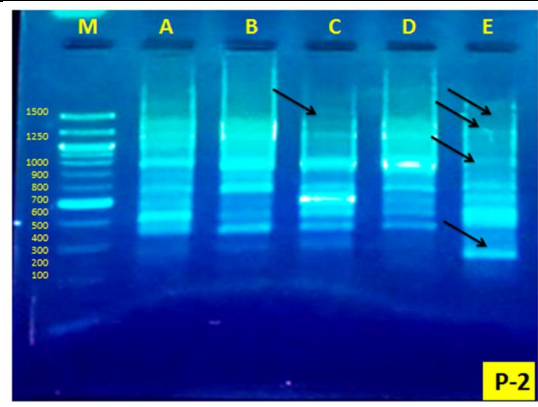


Image 2: Primer 2 products under Agarose gel electrophoresis with Marker

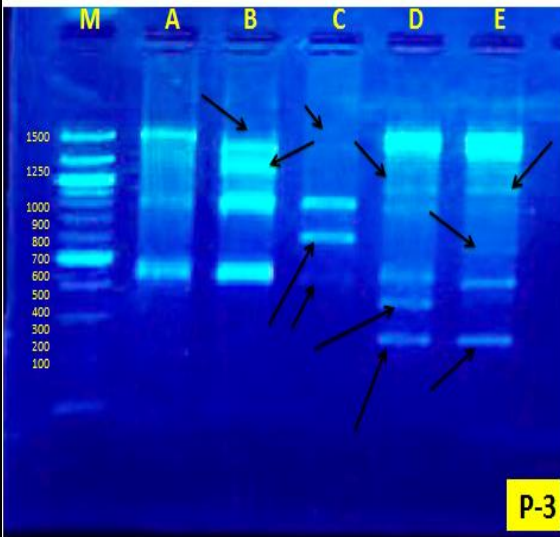


Image 3: Primer 3 products under Agarose gel electrophoresis with Marker.

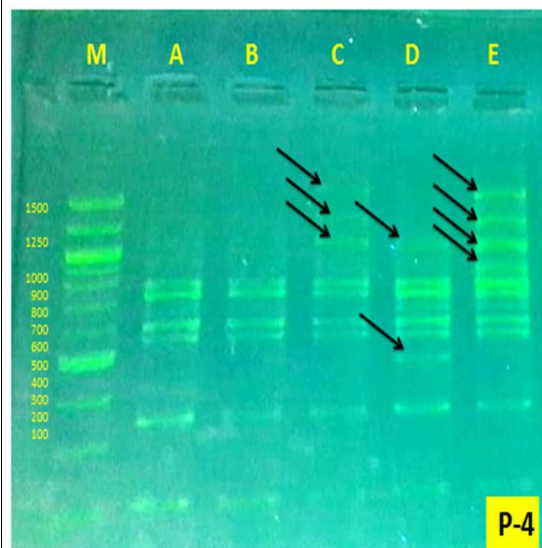


Image 4: Primer 4 products under Agarose gel electrophoresis with Marker.

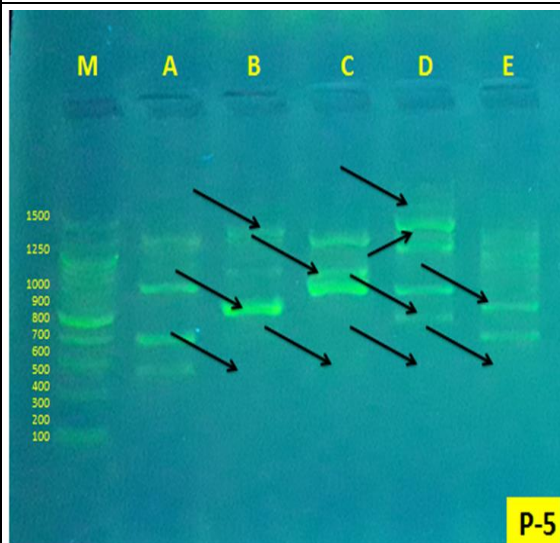


Image 5: Primer 5 products under Agarose gel electrophoresis with Marker

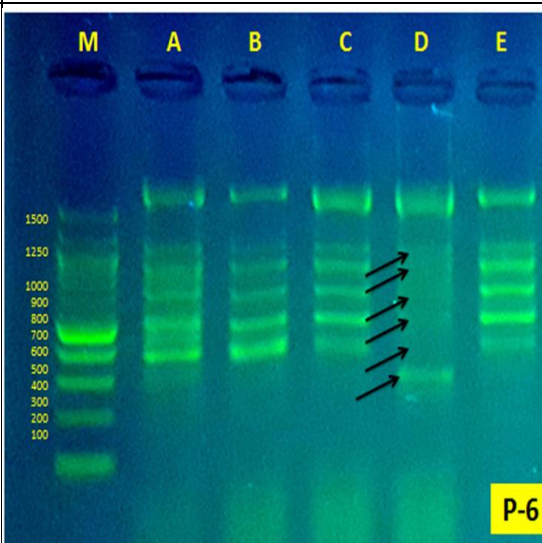


Image 6: Primer 6 products under Agarose gel electrophoresis with Marker

Conclusion

There is a difference in the results between the morphological and molecular markers because of the difference in mutations on the genome of them coding and noncoding DNA. The Wi-Fi waves induced mutations in the genome of *Vicia faba* L. and mutations increased with the period of exposure of the plant to the Wi-Fi waves. The efficiency of the RAPD markers was high in detecting the producing mutation in the genome of *Vicia faba* L.

Conflict of Interest

The author hereby declares no conflict of interest.

Funding support

The author declares that they have no funding support for this study

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How to cite this article

Hameed, R.K., Al-Sugmiany, R.J., Shlash, H.M., and Salih, M.H. (2022). Detecting the effects of Wi-Fi waves on phenotypic and molecular markers of *Vicia faba L.* *Science Archives* Vol. 3 (2), 1113--119. <http://dx.doi.org/10.47587/SA.2022.3206>

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