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Original Research Article

Responses of *Vicia faba* L. Seedlings Exposed to Microwave Seed Priming: Morphological and Molecular Analysis

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Abstract: Microwaves have emerged as a promising technology with wide range of applications across various fields, due to inconsistent results across various mutagenicity studies, the research intends to detect the impact of microwave radiation at 2450 MHZ on the morphological traits and molecular characteristics of *Vicia faba L.* seedlings exposed to different microwave exposure periods. Six treatments in three replicates were performed and the plant seeds were exposed to microwaves radiation at (0, 10, 20, 30, 40, 50 seconds) respectively, and then planted in the greenhouse and the morphological traits were recorded are (plant height, number of pods, number of seeds, weight of seeds, number of leaves and length and width of leaves). RAPD technique was used to evaluate the molecular variations among treatments. The results revealed the morphological traits (plant height, number of pods, leaf length and width) were positively affected and directly correlated with exposure time and were significantly increased compared with control sample while other traits (number of seeds, seeds weight and number of leaves) were negatively affected and inversely correlated with control where the measurements decreased with the increase of exposure time and were insignificant compared with the control sample except number of leaves which was significant. RAPD analysis revealed the occurrence of 67 unique and absent bands which reflected on growth performance. The study revealed the promising effect of microwaves on the vegetative traits at short term exposure, while long term exposure could inhibit the growth performance.

Keywords: Microwaves, Morphological, Molecular Analysis, Seed Priming, *Vicia faba* L.

INTRODUCTION

Plants are immobile, unable to avoid unfavorable climate factor like drought, flood, heat, salinity, heavy metals, and radiation in addition to physical stressors (Hameed *et al*., 2023; Katiyar *et al*., 2022). Climatic changes and deterioration in ecosystems that considered as abiotic stress negatively affected plant flourishing, development and production (Adeniyi *et al*., 2022; Dinler & Cetinkaya, 2024; Gamar *et al*., 2023). Microwaves have emerged as a promising technology with varied applications across various fields. Microwaves are electromagnetic waves bands that ranging (300 MHZ to 300 GHZ), these radiations are absorbed at molecular level and pose changes in vibrational energy of heat or molecules, the mechanical consequence of microwave bands has been explained as the rotation of the electrical field, which generates thermal energy in the presence of water. Moisture content is a crucial factor in the success of applying microwave technology (Kaur *et al*., 2021). Microwave radiations affects living organisms through causing DNA damage, involve oxidative stress and direct interaction with DNA molecules, protein structure and function and cell membrane integrity (Alkis *et al*., 2021; Mumtaz *et al*., 2022). *Vicia Faba* L. named as (faba house, faba beans or broad beans) and positioned as the fourth most important and widely grown legume. Its protein content is higher than other common food legumes (Maalouf *et al*., 2019). Nevertheless, they have provided a large portion of the population in developing countries with an affordable source of protein, thus compensating for the significant shortage of animal protein (Salehi *et al*., 2021). In addition to containing a high percentage of protein, it contains a high percentage of carbohydrate 48% in the form of starch and about 2% fat, it was participate in animal feed as an alternative to the meal of soybean in developed countries, and it's very important in the industrialized countries (Rahate *et al*., 2021). The effect of microwave and radiation on plant growth and development are complex and varied, with some studies reporting stimulatory responses, such as enhanced seed germination, accelerated plant growth, and improved biomass production, while others have observed inhibitory effects,

including stunted growth, reduced yield, and morphological abnormalities (Nadir & Khan, 2024). Due to inconsistent results across various mutagenicity studies on the microwaves effects. This research intends to detect the impact of microwave radiation on the phenotypic traits and molecular characteristics of *Vicia faba* L. seedlings.

MATERIALS AND METHODS

Plant Seedlings and Experimental Design

The experiment was carried out during the growing season 2023-2024 at the department of biologic green house, Faculty of science, university of Tirkit. Three replicates of each treatment in addition to control sample was sown. The seeds of *Vicia Faba* L. were exposed to different times of microwaves prior the planting which were (10, 20,30,40 and 50 seconds), the control was seeds that not exposed to microwaves.

Microwave Seeds Priming

Seeds were soaked in water for 48 h in dark place and exposed to 2450 MHZ microwave for different times (10, 20, 30, 40 and 50 seconds) respectively. Seeds were exposed to microwaves in the water to avoid thermal effects and embryo deterioration. The seeds were sown in the soil in the green house after marking the samples and preparing table of measurements.

Morphological Traits

The morphological traits that were evaluated are (plant height, number of pods, number of seeds, weight of seeds, number of leaves and length and width of leaves) that were taken after the seedlings maturation. All traits were in three replicates and compared with the control samples value.

Extraction of DNA and Gel Electrophoresis

DNA deoxyribonucleic acid was extracted from fresh *Vicia Faba* L. younger and healthy seedlings shoots after 60 days of planting to ensure the stability of genetic variations using CTAB procedure (Essa *et al*., 2023). DNA purity and concentration was measured using NanoDrop. Quality and yield of DNA was assessed using 0.8% agarose gel-electrophoresis stained with ethidium bromide (Creager, 2020; Maniatis *et al*., 2001). The bands were analyzed using a gel documentation system and compared to those from a standard lambda DNA marker. Subsequently, the extracted DNA was diluted with distilled deionized water. The DNA concentration in all samples was standardized to 50 ng/µl and was stored at -20°C for further use.

DNA amplification by PCR using RAPD-PCR Markers

Primary reaction mixture RAPD-PCR (Bioneer Company) was tested for RAPD fragments production. 20 µl reaction mixture as shown in Table (1). The amplification was applied as follows: (94 ºC - 7 min) as starting denaturation subsequently by 40 cycle at (93 ºC - 45 sec). 36 ºC for 1 min., and (72 ºC -1.5 min). The prepared PCR was left at (72 ºC - 10 min) for final addition. The PCR products were screened using 1.5% agarose gel. The electrophoresis images recorded using high resolution camera and the images saved for further use.

Table 1: RAPD reaction mixture components

NO	Material	Quantity
1	Master Mix	10 µl
2	Primer	2 µl
3	Nuclease free water	4 µl
4	DNA template	4 µl
5	Total Volume	20 µl

Table 2: primers used in the RAPD-PCR markers

NO	Primers	Seq.5'___3'
1	P1 OPQ_15	GGGTAACGTG
2	P2 OPG_15	ACTGGGACTC
3	P3 OPJ_12	GTCCCGTGGT
4	P4 OPJ_14	CACCCGGATG
5	P4 OPJ_16	CTGCTTAGGG

Statistical Analysis

The data were analyzed statistically using ANOVA analysis of variance using GraphPad prism software. Significance determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Morphological Traits Results

Morphological results were recorded at maturation stage of *Vicia Faba* L. The table (3) illustrates the traits measurements of the plant that were include (plant height, pods number, leaves number, seeds number, seeds weight and length and width of the leaves), values in the table are average of 3 replicates. The highest height was 123.9cm at 10 seconds exposure and the lowest was 105.3cm at 40 second exposure, all treatments were insignificant compared with control sample. The highest number of pods produced was 19 at 50 sec and the lowest 9.4 at 20 seconds which was significantly different from the control sample. The highest number of seeds produced was 3.01 at control sample and the lowest was 1.91 at 20 seconds which was statistically different from the control treatment. Seed weight highest was 2.77g and the lowest was 1.88g which was significantly different from control sample. The number of leaves produced was 289 at the control sample which is the highest and the lowest was 150 at 50 seconds exposure time which was significantly different from control sample. The leaf length highest measure was 7.3cm at 50 second exposure and the lowest was 5.4cm at 20 second exposure which was significantly different compared with the control sample. The leaf width highest value was 3.2cm at 40 second and the lowest was 2.36cm at the control sample which was significantly different with the control sample.

Table 3: Morphological traits measurements of *Vicia Faba* L. under different exposure times of microwaves

Treatment/time Sec	Plant Height cm	Number of pods	Number of seeds	Weight of Seeds g	Number of Leaves	Leaf length cm	Leaf width cm
Control 0 sec	110.7^{ab}	10.9^{c}	3.01^{a}	2.43^{ab}	289^{a}	5.7^{bc}	2.36^{c}
10 sec	123.9^{a}	9.6^{c}	2.06^{b}	2.19^{bc}	251^{b}	5.5^{c}	2.4^{c}
20 sec	114^{ab}	9.4^{c}	1.91^{b}	1.88^{c}	202^{c}	5.4^{c}	2.36^{c}
30 sec	114.5^{ab}	13.3^{b}	2.28^{ab}	1.97^{c}	182^{cd}	6.4^{b}	2.63^{b}
40 sec	105.3^{b}	13.6^{b}	2.52^{ab}	2.77^{a}	175^{d}	6.1^{b}	3.2^{a}
50 sec	113.9^{ab}	19^{a}	2.28^{ab}	2.68^{a}	150^{e}	7.3^{a}	2.72^{b}

The data presented in the table are the mean of three replicates, values with different letters within the same column are significantly different at P≤0.05.

RAPD-PCR Markers

RAPD analysis showed the presence of (unique bands & absent bands) in the five primers used, it illustrated in table (2). The exposure of the plants to different periods of microwave radiations demonstrated different presence of unique bands and disappear of bands that referred as absent bands. The most affected samples that M2 and M4, it was (9 and 10) unique bands respectively. The unique bands almost increased with the exposure time and absent bands decreased with exposure time except M1 and M3 that were equal in the accumulative results of unique and absent bands. Absent mutations could have led to loss of essential functions that impair the growth and development (Hameed *et al*., n.d.; Sun *et al.*, 2024)

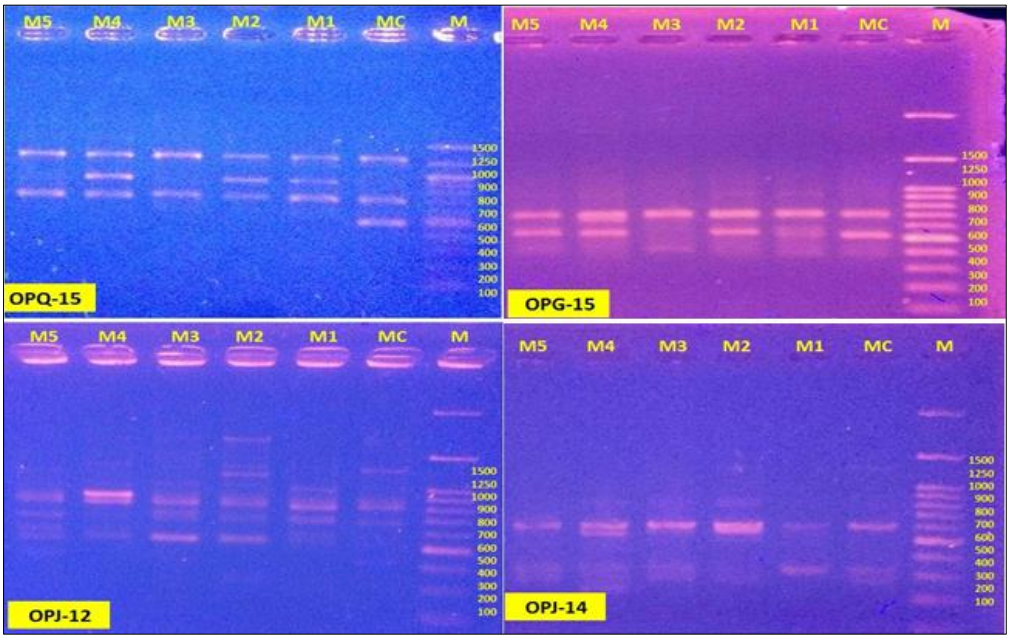

Fig. 1: Representing the electrophoresis results produced from primers bands of *Vicia Faba* L. exposed to microwaves on agarose 1.5% markers.

Table 4: Representing the mutation bands (unique and absent) resulted from exposing *Vicia faba* L. to microwaves

Number	Primer name	Sequence 5'-3'	Molecular weight	Microwaves Treatments									
				M1		M2		M3		M4		M5	
				Unique bands	Absent bands	Unique bands	Absent bands	Unique bands	Absent bands	Unique bands	Absent bands	Unique bands	Absent bands
1	P1 OPQ_15	GGGTAACGTG	1300-500bp	1	1	1	1	-	1	1	1	-	1
2	P2 OPG_15	ACTGGGACTC	850-400bp	2	-	2	-	1	1	2	-	1	-
3	P3 OPJ_12	GTCCCGTGGT	1400-550bp	3	1	4	1	2	1	2	2	3	2
4	P4 OPJ_14	CACCCGGATG	600-250bp	-	1	2	2	1	-	4	-	1	1
5	P4 OPJ_16	CTGCTTAGGG	700-350bp	1	1	-	2	2	2	1	3	3	2
		Summation mutant bands (unique and absent)		7	4	9	6	6	5	10	6	8	6
				11		15		11		16		14	
				67									

DISCUSSION

The results obtained revealed direct correlation of plant height, number of pods, leaf length and width with the exposure time which were significantly different with control sample except plant height that was insignificant. Number of seeds produced, seeds weight and number of leaves measurements were inversely correlated with the control sample which decreased with exposure time increase that was insignificant compared with the control.

The variations in results due to different exposure times due to enhancement of the enzymatic activity, cellular processes, cell division and membrane permeability that could led to enhancing nutrient uptake level and that reflected on growth and developments (Kohli *et al*., 2023). On contrary, the long exposure times could cause protein denaturation and membrane damage due to generated heating (Zhong *et al*., 2021). Exposure to high level could generate reactive oxygen species ROS that damage at the cellular level and metabolic activities (Tauffenberger & Magistretti, 2021).

Provoked mutations previously harnessed to improve economically important crops such as rice, barley, cotton, wheat, and beans. Subjecting seeds to chemical and physical factors caused changes in DNA sequences that reflected on the morphology of plants that called mutations. These in turn would lead to a rapid enhancement of crop yields and quality (Mishra *et al*., 2024). All the exposure samples were notable with unique and absent bands that illustrated in table (2), the mutant bands which were resulted from microwaves exposure were (67), unique bands were (40) and absent bands were (27). The M4 treatment have got the highest number of unique bands resulted (10) bands and the M2 treatment resulted (9) bands. The absent bands have appeared in most treatments which were approximately (6) bands except M1 treatment which was (4) bands. The molecular weight of amplification bands ranged between (500-1400) bp, the minimum weight was (500) bp for P1 OPQ-15 primer and the maximum weight was (1400) bp for P3 OPJ-12 primer. The (unique and absent bands) were considered as a differentiation features among samples which attributed to the effect of microwaves bands on DNA (Mishra *et al*., 2024). The correlation between growth performance and molecular markers were approximately inversely, the increased frequency of unique and absent bands decrease the growth parameters such as the length, number of pods and leaves while the decreased number of bands increased the growth parameters compared to the control treatments. The results showed that the microwave bands field had an effect on the genome and the frequency of mutations induction in *Vicia faba* genome and the genetic variation between the control and treatments may have negative or positive impact on the phenotypic and plant traits due to the change DNA by replacing the genetic code that called Amber mutation, these mutations triggered mutant genes or produced multiple copies of genes or stimulates cross-over in mitosis phases, irregular transcription may affects stop codons and activating silent genes (Hussein, 2016). The results obtained from the study matches the fact in the literature that exposing plants to low duration of microwaves radiation increased seed germination and growth and significantly inducing genetic variation (Balint *et al*., 2016; Paparella *et al*., 2015; Shabrangi *et al*., 2015).

CONCLUSION

The priming of *Vicia faba* L. seeds with microwaves revealed promising enhancement in growth performance of vegetative traits and the induction of mutations that reflected morphologically. The results obtained revealed that short term exposure durations to microwaves has positive potential on *Vicia faba* L. vegetative morphological parameters where most of the growth parameters were increased compared with the control treatment. The molecular markers tested in the

study revealed the existence of unique and absent bands that reflected on the growth performance which indicate that some mutations led to positive growth and enhanced growth and productivity. Microwave radiation could harm the plants that exposed to radiations at high bands and long term exposure exceeds 60 seconds.

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