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## Impact of controlled microwave radiation in enhancing the productivity of *Abelmoschus esculentus* seedlings (L.) Moench

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### ABSTRACT

The study describes the impact of microwave radiation on the germination rate and growth of *Abelmoschus esculentus* (L.) Moench (okra). The two okra varieties, viz. Meenaxi-371 and Soniya-1402, were used for the experiment. Their seeds were exposed to 0–14 h (h) of microwave radiation (9.3 GHz). Non-irradiated seeds (0 h exposure) served as control. We found that microwave radiation on seeds resulted in an increase in their germination indices and also stimulated the concentration of  $\beta$ -1,3-glucanase enzyme. The results also indicate a remarkable increase in bioactive compounds such as flavonoids, polyphenols, carotenoids, chlorophyll, lutein, protein, and  $\beta$ -carotene in okra seedlings exposed to 8–10 h of radiation. A further dose escalation in the microwave radiation caused a decline in the germination and growth of the okra plant. The expression level of *ACS4*, *ETR2*, and *ACS6* showed up-regulation under the influence of microwave irradiated conditions. Therefore, it is evident that a well-controlled and defined irradiation has the potential to enhance the overall growth and yield of selected okra varieties.

**Abbreviations:** RNA: ribonucleic acid; qRT-PCR: quantitative real-time polymerase chain reaction; EMF: electromagnetic field; ACS: amino cyclo synthase; ETR: ethylene transport receptor

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### KEYWORDS

Microwave radiation;  $\beta$ -galactosidase; protein content; photosynthetic pigments; okra seeds

## 1. Introduction

Globally, about 7 billion people need sufficient food to survive in a scenario of declining crop productivity (Samir and Lutz 2017). Consequently, about 2 billion people are facing nutrient deficiencies, and millions are faced with acute food shortages (Holdsworth and Bricas 2016). Vegetables are one of the most preferred food sources required daily. *Abelmoschus esculentus* (L.) Moench. (Okra) is one of the most edible vegetables that provide many useful nutrients and fatty acids needed in the human diet (Habtamu et al. 2014). This vegetable is native to Africa and Asia, and it is estimated that about 120 million metric tons is produced worldwide (Naveed et al. 2009). Anthropogenic activities are a major constraint for agronomic practices resulting in an alarming drawback for crop productivity (Kiaya 2014). Therefore, in order to meet the great demand for improved crops, it is necessary to adopt innovative technologies that can ensure the production of important crops. Azadi and Ho (2010) reported a decade ago that the development of genetically modified crops is necessary to fulfill the demand of ameliorating food shortage, but later it became a social, legal, and ethical issue for humans. Therefore, scientists are now developing innovative and socially acceptable technologies that can increase crop productivity in environmentally friendly and economical ways without compromising the nutritional value of agricultural products. Kumari et al. (2018) reported the use of microwave radiation in food processing as an important method, particularly for the purpose of drying to inactivate the growth of bacteria and other microorganisms. Later, the application of radiation

treatment in the case of food crops became vital to increase the nutritional value and productivity of crops. Senavirathna and Asaeda (2014) have also reported that electromagnetic fields (EMFs) cause physiological changes in the cell structure of plants and increase yields. Therefore, interest has developed to test the effect of artificial electromagnetic radiation on the productivity of agricultural crops.

There are several signaling pathways that work in different parts of the plants such as *Rosa hybridus* which respond to external stimuli (Grémiaux et al. 2016).

The response of plant tissues in the EMF is very complex and differs in its biological processes due to external stimuli. Apart from some efforts in this direction, there is a paucity of data regarding the effects of radiation on economically important plants. There is a need to investigate effects of EMF as a biotechnology tool on plants selected in order to assess its potential in plant production.

Therefore, the objective of this study is to use wireless communication as a medium to influence the effect of electromagnetic radiation in the form of microwaves on *A. esculentus* (Okra). We hope that this is the first study on the effect of microwaves radiation on two okra plants.

## 2. Material and methods

### 2.1. Plant materials and application of radiation treatment

The study used okra seeds of two selected varieties, namely Meenaxi-371 and Soniya-1402, obtained from Green Field

Seed company, Gujarat, India. Meenaxi-371 is known to be resistant to the yellow vein mosaic virus and grows best in the Rabi season, while Soniya-1402 is widely cultivated at any time of the year for market purposes due to its fertility. A total of 40 okra seeds of two varieties were irradiated with microwave at 0 (non-irradiated), 10, 20, 30, 40, 50, 60 min, and till 14 h at an interval of 2 h. Okra seeds were first sterilized with 0.1% (m/v) of mercury (II) chloride for 2 min and carefully washed thrice with double-distilled water. After irradiation, okra seeds were grown in Petri dishes, then the seeds were sown in Petri plates in the growth chamber for 6 days, containing 1/4 volume of nutrient solution. In addition, okra seedlings of 12 cm were then transplanted in earthen clay pots in six replicates each, about 300 g of soil rite was added and watered regularly. Every week the location where the plants were placed in the growth facility was arranged to minimize the adverse effects of variations due to heat generated from light and temperature. A complete randomized block pattern was used for the experiment setup. The photoperiodic periods of 8 h darkness and 16 h light were applied. The recorded temperature was maintained at  $23 \pm 2$  and  $14 \pm 3^\circ\text{C}$  and relative humidity of 70/80% for the day/night period, respectively.

The facility that housed the exposure system comprises an artificial faraday cage, Klystron tube, variable attenuator, detector mount, meter, functional generator, isolator, and frequency meter. A chamber protected by a 2-mm aluminum coating was meant to expose okra seeds that are randomly distributed over moist cellophane paper inside Petri plates housing the radiation that is capable of creating a homogenous electromagnetic force and to preserve from an external electric force. The values of specific absorption rates used in this study for both Meenaxi-371 and Soniya-1402 okra varieties were followed according to Szasz et al. (2016).

## 2.2. Activity of degrading enzyme

Leaf samples of irradiated and non-irradiated okra plants were homogenized in 4 ml of 0.05M potassium acetate buffer solution, in a cold mortar and pestle having pH 5.5. The homogenate was filtered, and the collected filtrate was centrifuged for 10 min at 10,000 rpm for  $4^\circ\text{C}$ . After centrifugation, the supernatant extract was then stored at  $-20^\circ\text{C}$  and used to prepare enzyme assay such as  $\beta$ -1,3-glucanase. The evaluation of  $\beta$ -1,3-glucanase assay was performed using a substrate called *Laminaria digitata* laminarin (Sigma) according to the protocol of Abeles and Forrence (1970).

## 2.3. Determination of photosynthetic pigments

The method of Lichtentaler and Buschman (2001) was used to determine photosynthetic pigments (total chlorophyll and carotenoid content). Samples of non-irradiated and irradiated okra plant leaves were recorded by homogenizing 5 ml of the sample with (80%) acetone. Next, the grounded substrate was then centrifuged at 3000 rpm at  $4^\circ\text{C}$  for 10 min. After centrifugation, the absorbance of the supernatant was recorded at 480, 645 and 663 nm. The carotenoid and chlorophyll pigments were estimated by the following formula below:

$$\text{Total Chlorophyll content (mg/l)} = 20.2 (A_{645}) + 8.02 (A_{663})$$

$$\text{Carotene content (mg/l)} = A_{480} \times 200, \text{ where } A - \text{absorbance.}$$

## 2.4. Determination of secondary metabolites in okra

Flavonoid and polyphenol contents were estimated according to the procedures of Chang et al. (2002) and McDonald et al. (2001), respectively. Polyphenol extract was prepared on 14, 28, and 40 d old okra plants by homogenizing 1 mg leaf sample with 5 ml of 50% methanol. Gallic acid was used to examine the standard for polyphenol calculation and expressed as a ratio of  $\text{mg g}^{-1}$  fw. The aluminum chloride colorimetric method was used to determine the flavonoid content. About 1-g leaf sample was homogenized with 25 ml of 95% ethanol. The solution was then stored for 30 min at room temperature, and the spectrophotometer absorbance reading was observed at 415 nm. The flavonoid content was expressed as  $\text{mg g}^{-1}$ . The protocol of Lowry et al. (1951) was used to estimate the soluble protein in two varieties of okra under irradiated and non-irradiated on 14, 28, and 42 days. The use of a modified protocol of Pocock and Krol (2004) to evaluate  $\beta$ -carotene and lutein + zeaxanthin in irradiated okra plants was investigated. A 0.1-g leaf sample was cut from irradiated leaf, then kept in liquid nitrogen, and further preserved at  $-80^\circ\text{C}$ . High-performance liquid chromatography was used to measure the solvents, and thin-layer chromatography was now used to calculate zeaxanthin and lutein fractions.

## 2.5. Gene expression analysis

The total ribonucleic acid (RNA) was isolated from control and irradiated plants using a protocol adapted from Sabzevari and Hosseini (2014). Nuclease-free water was used to dilute isolated RNA, and the purity was determined using an absorbance ratio at 260 and 280 nm ( $A_{260}/A_{280} > 1.8$ ). The transcript expression levels of *ACS4* and *ACS6* synthase and ethylene receptor (*ETR2*) genes were observed in okra fruits (Meenaxi-371 and Soniya-1402) under all conditions, i.e. microwave irradiated and non-irradiated conditions at 14, 28, and 42 days were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) (Sharma et al. 2014). The pair of primer pairs used were specific to *Abelmoschus esculentus ACS4* with Accession number JF508505 (F, 5' CAGAGTGAAGGGCTTGCTCA-3'; R, 5'-TCCATCTCGGCTGCAAATGT -3'), *ACS6* with Accession number DQ122174 (F, 5'GAGGTGGAGAACAGGGGTTG-3'; R, 5'-CTGCTCCTTGCTCTCCACAA-3'), and *ETR2* with Accession number XM\_016859813 (5'GCTTCAGATGCCGAGTTAG -3'; R, 5'- GACTGGCTTTCGAACGACTC -3') (Neta et al. 2016), and the internal reference gene is Ubiquitin (Chen et al. 2013) with Accession number AT3G52590 (Ubiquitin -F, 5'-TTCCTTGATGATGCTTGCTC -3'; Ubiquitin -R, 5'- TTGACAGCTCTTGGGTGAAG -3'). The qRT-PCR analysis was conducted by adding 10  $\mu\text{L}$  of SYBR green PCR master mix with 2  $\mu\text{L}$  cDNA and the addition of 0.5  $\mu\text{L}$  of forward and reverse primer of each gene. The transcript analyses were carried out with six biological replicates. The formulas of Livak and Schmittgen (2001) was used to measure gene expression of relative fold change:  $\text{Fold Change} = 2^{-\Delta(\Delta C_t)}$ , where  $\Delta C_t = C_t, \text{target} - C_t, \text{normalizer}$  and  $\Delta(\Delta C_t) = \Delta C_t, \text{stimulated} - \Delta C_t, \text{control}$ .

## 2.6. Statistical analysis

Data were analyzed using a two-way analysis of variance (ANOVA) data variables: okra variety (two levels),

microwave treatment replicated six times. Tukey's post hoc was used for multiple comparisons across treatments. The level of statistical significance was set at  $P \leq 0.05$ . All statistics were done using SPSS 16.0 and R.3.5.0. Statistical software package and sigma plot package version (12.0) were used to draw bar charts and regression lines.

### 3. Results

#### 3.1. Measurements of photosynthetic pigments

The concentration of carotenoid and chlorophyll content at '0' h non-irradiated and different 2–14 h irradiation conditions was determined at 14, 28, and 42 days old okra variety. The result showed an increase in chlorophyll content of 2.1–3 fold in 14, 28, and 42 days old okra plants of the two okra varieties (Figure 1(A,B)). An increase in chlorophyll content at 10 h after radiation and a decrease in radiation were observed continuously (Figure 1(A,B)). However, carotenoid content in both varieties under control and irradiated microwave condition showed an increase even as the duration of radiation exposure increases (Figure 1(C, D)). The pigments (chlorophyll and carotenoid) in irradiated okra variety were 2.1–3 times more abundant in okra plants for 6–10 h under non-irradiated conditions.

#### 3.2. Analysis of $\beta$ -1,3-glucanase activity and bioactive compounds

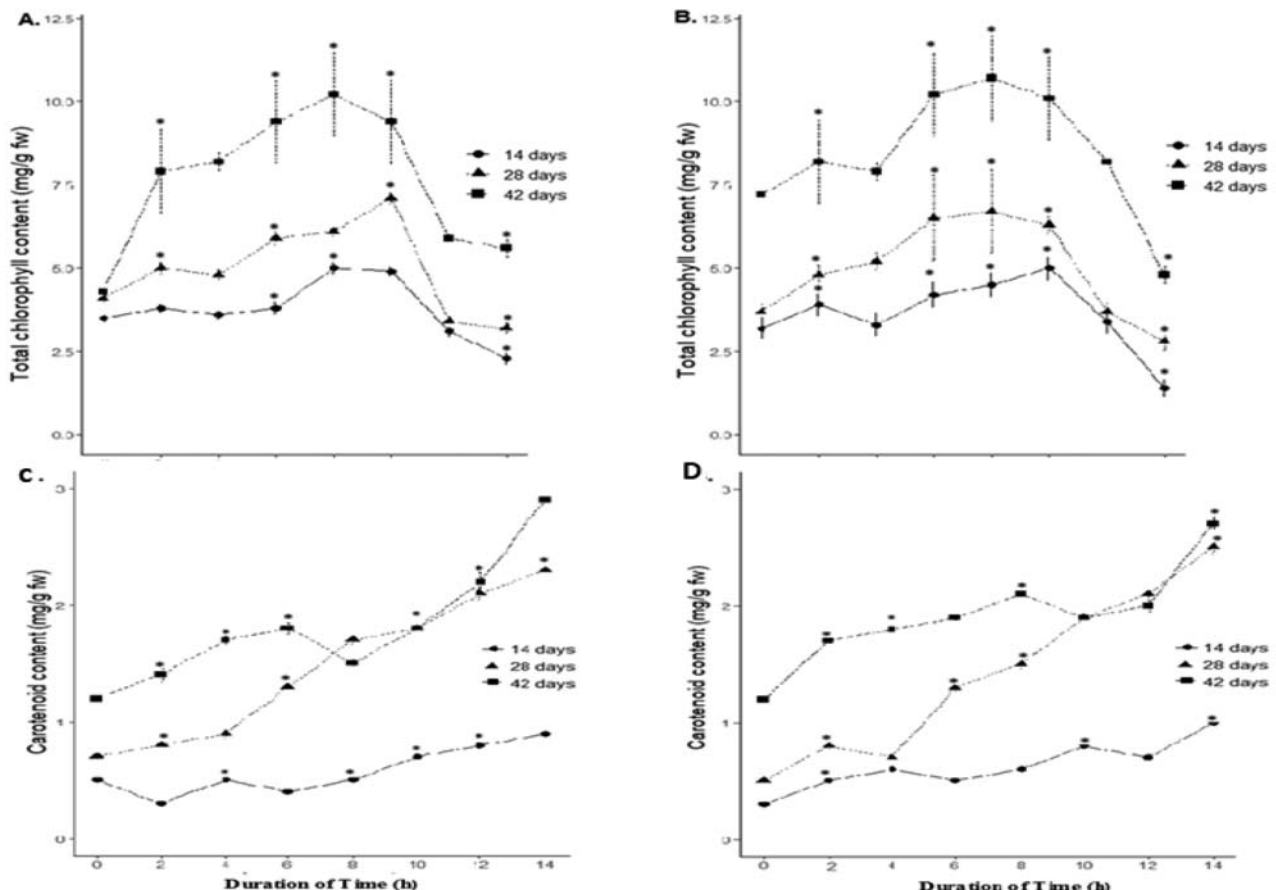
The amount of polyphenol showed a significant increase more than in the control (non-irradiated) since the two

varieties had longer exposure (Figure 2). The highest content of polyphenols was recorded in the Sonia-1402 variety exposed for six more periods. (Figure 2(B)). In the Meenaxi-371 okra plant, an increase in polyphenol content was recorded at values of 51, 43, and 57%. Similarly, the highest increase was observed in the Soniya-1402 variety to 45% at 8 and 10 h exposure to radiation.

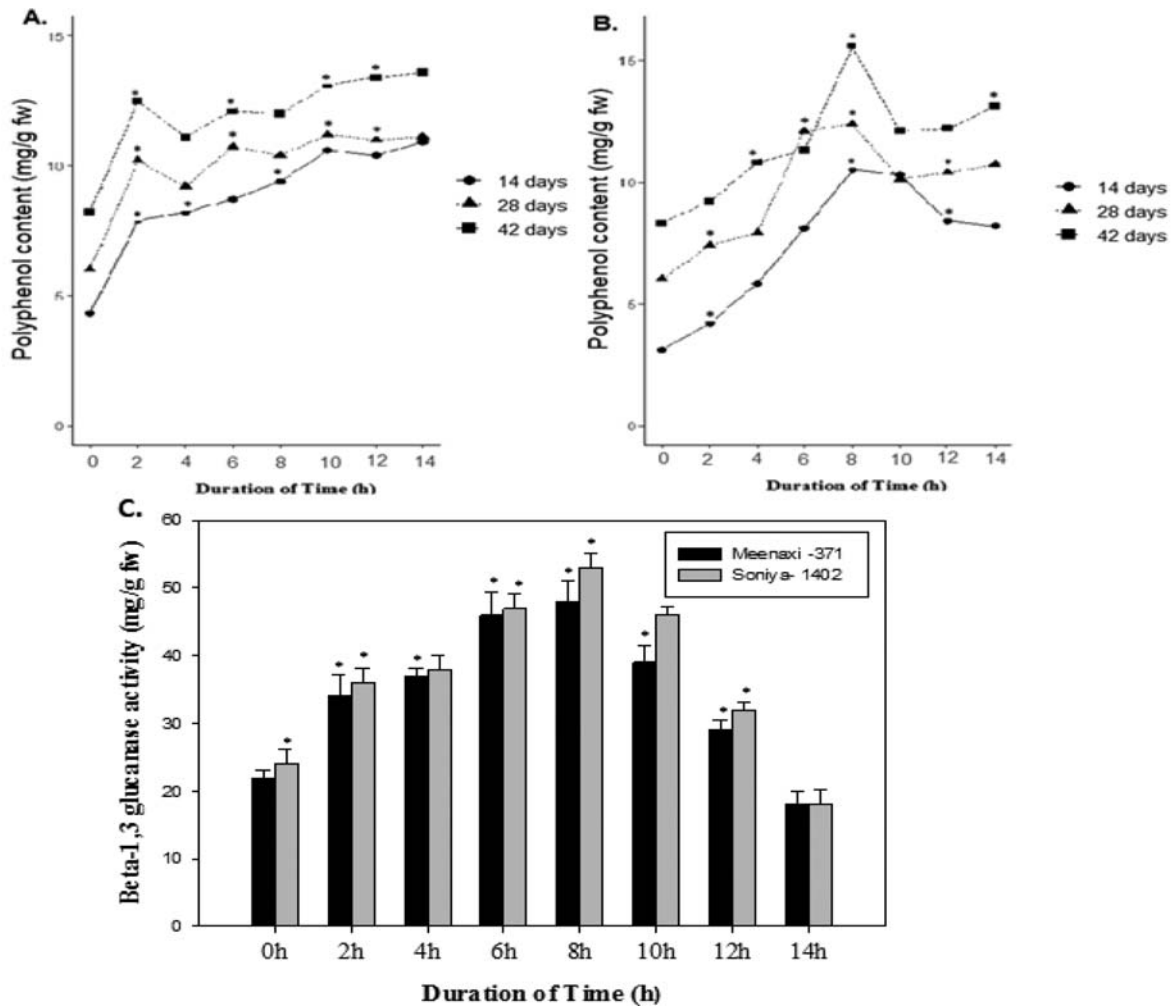
The results in Figure 2 show the concentration levels in the enzyme  $\beta$ -1, 3-glucanase present in the two okra varieties, i.e. Meenaxi-371 and Soniya-1402. A significant increase in the activity of  $\beta$ -1, 3-glucanase was shown in irradiated okra seedlings compared to non-irradiated okra seedlings of both varieties. When exposed to microwave radiation in both Meenaxi-371 and Soniya-1402 seedlings, a 2-fold increase and a reduction of 32 and 44% after 14 h were recorded (Figure 2(A,B)). A significant reduction in the activity of  $\beta$ -1, 3-glucanase was observed at 14 h, while the highest level of activity was recorded at 6–10 h after exposure to microwave radiation.

#### 3.3. Analysis of $\beta$ -carotene, lutein + zeaxanthin, flavonoid, and total protein content

It was also shown that under radiation, results of  $\beta$ -carotene content showed a reduction of 6.2–4 fold at 14, 28, and 42 days okra seedlings (Table 1). There was an increase in  $\beta$ -carotene levels after 10 h during radiation exposure and furthermore a decrease in levels was observed as the exposure time increased. The lutein content of okra plant exposed to non-irradiated and irradiated conditions showed an increase with



**Figure 1.** Changes in total chlorophyll and carotenoid content on Meenaxi-371 (A & C) and Soniya-1402 (B & D) varieties of okra exposed to a different time of radiation. 0 h denotes control, while 2–14 h denotes radiated. All data denotes mean  $\pm$  standard deviation of six replicates, while '\*' indicates  $p \leq 0.05$  across the values of mean between 14, 28, and 42 days.



**Figure 2.** Changes in polyphenol content on Meenaxi-371 (A) and Soniya-1402 (B), while (C) shows  $\beta$ -galactosidase activity on the two varieties of okra exposed to a different time of radiation. 0 h denotes control, while 2–14 h denotes radiated. All data denotes mean  $\pm$  standard deviation of six replicates, while “\*” indicates  $p \leq 0.05$  across the values of mean between 14, 28, and 42 days.

a value of 5.11 and 3.28-folds, as there was a continuous increase in exposure to the radiation treatment in Table 1. In Figure 3(A,B), the variety Meenaxi-371 showed an increase in flavonoid content to 2.1–3 folds (Figure 3(A)), and a rapid increase of  $2.5 \pm 1.2$  fold was observed in Soniya-1402 (Figure 3(B)). The necessary stimulating effect is therefore dependent on different parameters that are evaluated under the conditions of microwave radiation. Both Meenaxi-371 and Soniya-1402 okra varieties recorded a remarkable increase of 58 and 47% in polyphenol content within 42 days. Also, there was 2.1–3 folds and  $2.5 \pm 1.2$  folds in flavonoid content in the two okra varieties, viz. Meenaxi-371 and Soniya-1402. The total protein content in *Abelmoschus esculentus* was determined under microwave radiation exposure for 0–14 h in 14, 28, and 42 days in the two varieties, i.e. Meenaxi-371 and Soniya-1402 (Figure 3). There was an increase in the protein content in irradiated conditions, as the duration of exposure time increased compared with those under non-irradiated (0 h) conditions in the two okra varieties (Figure 3). The highest content of protein in okra varieties exposed to radiation was observed in 6 and 10 h, which is in contrast to other duration of exposure time. In Meenaxi-371 and Soniya-1402 okra plants, there was an increase with a value of 42, 31, and 42% and 23, 32 and 43% in irradiated okra plants, respectively (Figure 3(C,D)). The two okra varieties recorded an increase of 62% in protein content for 6–10 h duration of microwave radiation at 42 d.

### 3.4. Gene expression pattern of ACS4 and ACS6 oxidase and ETR2 synthase gene

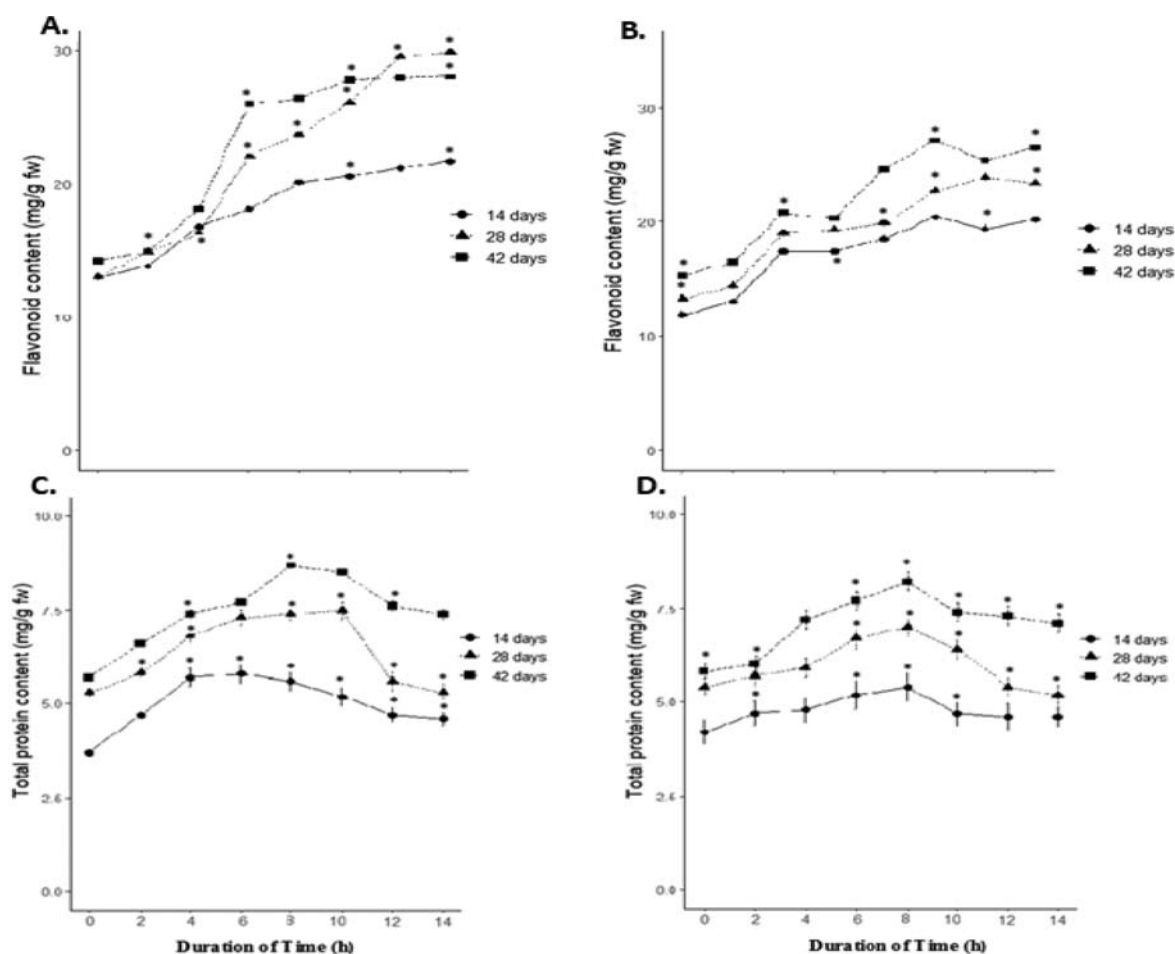
Gene expression studies of ACS4 and ACS6 synthase and ETR2 receptors were carried out in the two okra varieties, i.e. Meenaxi-371 and Soniya-1402 (Figures 4 and 5) on 14, 28, and 42 days. The exposure to microwave radiation showed a gradual reduction in the transcriptome expression level of ACS4 gene in variety Meenaxi-371 on the 14th day in comparison to non-irradiated plants (Figure 4(A)). There were a reduction in a fold change value of 1.0 from 0 to 6 h and an increase in a fold change value of 1.4–1.0 from 8 to 14 h in comparison to the non-irradiated conditions with a value of 1.8, while on the 28 and 42 days, the expression level was reduced in a fold change value of 0.6 from 0 to 8 h and increased in a fold change value of 1.0 from 8 to 14 h and 10 to 14 h, respectively (Figure 4(A)). Likewise, in Soniya-1402, there were a significant reduction in a fold change value of 0.8 in the transcript expression pattern of ACS4 on the 28 and 42 days from 0 to 6 h and 0 to 8 h and subsequently an increase in a fold change value of 1.0 to 2.0 from 10 to 14 h and 1.1 from 10 to 14 h, respectively (Figure 4(B)). Also, for ACS6, the transcript expression level on the 14th day in Meenaxi-371 displayed a remarkable reduction in a fold change value of 0.7–0.5 from 0 to 4 h and showed an increase in a fold change value of 0.8–1.9 from 6 to 14 h and 0.9 to 1.7 from 8 to 14 h at 14, 28, and 42 days,

**Table 1.** Changes in  $\beta$ -carotene and lutein on two (Meenaxi-371 and Soniya-1402) varieties of okra on 14, 28, and 42 days exposed to a different time of radiation.

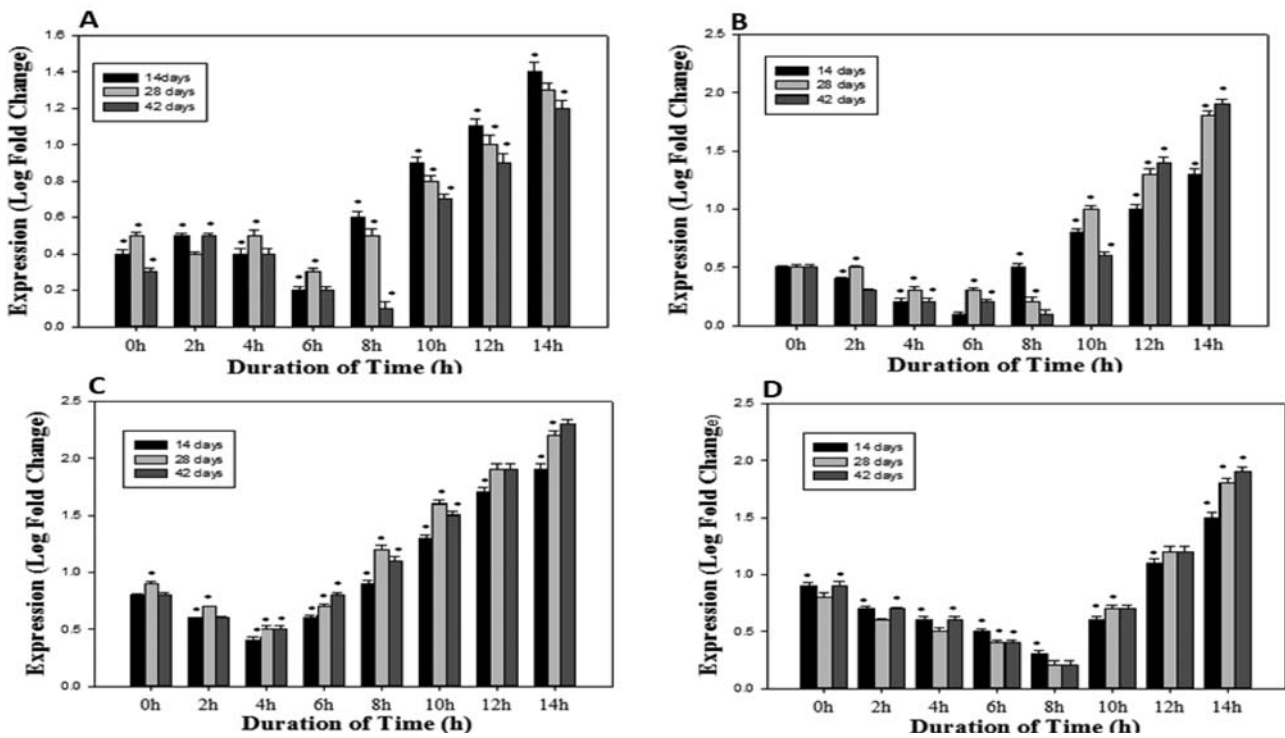
Duration of exposure Time (h)	$\beta$ -carotene ( $\mu\text{g g}^{-1}$ )		L+Z ( $\mu\text{g g}^{-1}$ )	
	Meenaxi-371	Soniya-1402	Meenaxi-371	Soniya-1402
14 d				
0	16.2abc $\pm$ 3.5	12.2bc $\pm$ 2.5	15.7e $\pm$ 3.26	16.7c $\pm$ 4.9
2	18.1abc $\pm$ 2.3	15.3bc $\pm$ 4.7	19.7*bcd $\pm$ 4.5	21.5bc $\pm$ 6.2
4	23.4abc $\pm$ 4.2	19.3abc $\pm$ 6.5	26.4*cd $\pm$ 7.7	27.5bc $\pm$ 5.4
6	32.5ab $\pm$ 7.1	24.1*a $\pm$ 9.8	31.5*a $\pm$ 5.8	34.5*b $\pm$ 7.8
8	49.8ac $\pm$ 10.3	29.5*a $\pm$ 8.6	39.4*bc $\pm$ 9.5	39.2*b $\pm$ 9.6
10	59.9abc $\pm$ 11.3	36.4*a $\pm$ 7.6	45.6*b $\pm$ 10.3	45.4*a $\pm$ 10.4
12	61.2bc $\pm$ 10.4	42.1c $\pm$ 11.7	51.4de $\pm$ 11.6	52.9*b $\pm$ 11.8
14	69.3c $\pm$ 19.3	51.9*d $\pm$ 12.4	58.1de $\pm$ 13.8	61.4c $\pm$ 13.7
28 d				
0	75.4e $\pm$ 5.9	57.6d $\pm$ 8.9	65.3c $\pm$ 9.1	69.5d $\pm$ 6.7
2	79.2bc $\pm$ 6.1	63.7de $\pm$ 7.2	70.5bc $\pm$ 8.9	7.8*de $\pm$ 7.5
4	84.6*d $\pm$ 7.5	69.5*e $\pm$ 5.2	78.6de $\pm$ 8.6	83.2*cd $\pm$ 6.9
6	89.3*c $\pm$ 4.7	74.8*de $\pm$ 6.1	85.3*b $\pm$ 7.5	89.4*c $\pm$ 7.6
8	95.1*b $\pm$ 5.8	79.1*cd $\pm$ 5.1	92.4*b $\pm$ 8.6	94.4*b $\pm$ 6.1
10	102.4*de $\pm$ 4.9	84.1*b $\pm$ 6.5	99.1*c $\pm$ 6.1	103.7*b $\pm$ 8.4
12	113*de $\pm$ 7.9	91.6*f $\pm$ 5.9	109.5*cd $\pm$ 7.5	113.6*ef $\pm$ 9.9
14	121.7*f $\pm$ 9.4	98.1g $\pm$ 6.6	128.7bc $\pm$ 9.1	125.2ef $\pm$ 8.6
42 d				
0	127.9d $\pm$ 6.9	111.8f $\pm$ 8.6	136.2d $\pm$ 10.6	129.7e $\pm$ 7.9
2	131.7d $\pm$ 7.3	116.6ef $\pm$ 5.7	143.6*c $\pm$ 8.7	132.6*d $\pm$ 9.1
4	139.5cd $\pm$ 8.4	127.6f $\pm$ 7.9	149.14*d $\pm$ 9.2	139.3*f $\pm$ 12.4
6	147.8*cd $\pm$ 7.9	139.7*ef $\pm$ 8.1	161.7*b $\pm$ 7.9	156.2*c $\pm$ 11.3
8	152.6*b $\pm$ 8.2	146.8*cd $\pm$ 9.3	172.6*c $\pm$ 8.5	161.4*b $\pm$ 12.4
10	159.4d $\pm$ 7.9	155.4*b $\pm$ 10.4	181.5*c $\pm$ 9.4	172.6*d $\pm$ 11.2
12	164.6*c $\pm$ 11.5	161.8*d $\pm$ 12.3	196.6*d $\pm$ 11.8	184.5*e $\pm$ 12.7
14	171.8*f $\pm$ 10.7	170.9*g $\pm$ 11.2	202.5*e $\pm$ 13.1	195.6gh $\pm$ 11.2

Values denote mean  $\pm$  standard error (SE) ( $n = 6$ ). 0 h denotes control, while 2 to 14 h denotes radiated. Nonsimilar alphabets denote significant values following the test by Tukey's post hoc at  $p < 0.05$ .

\*Significant values following the test by Tukey's post hoc at  $p < 0.05$ .



**Figure 3.** Changes in flavonoid and total protein content on Meenaxi-371 (A & C) and Soniya-1402 (B & D) varieties of okra exposed to a different time of radiation. 0 h denotes control, while 2–14 h denotes radiated. All data denotes mean  $\pm$  standard deviation of six replicates, while '\*' indicates  $p \leq 0.05$  across the values of mean between 14, 28, and 42 days.



**Figure 4.** Changes in relative gene expression levels of *ACS4* and *ACS6* gene on Meenaxi-371 (A & C) and Soniya-1402 (B & D) varieties of okra exposed to a different time of radiation. 0 h denotes control, while 2–14 h denotes radiated. All data denotes mean  $\pm$  standard deviation of six replicates, while  $^{**}$  indicates  $p \leq 0.05$  across the values of mean between 14, 28, and 42 days.

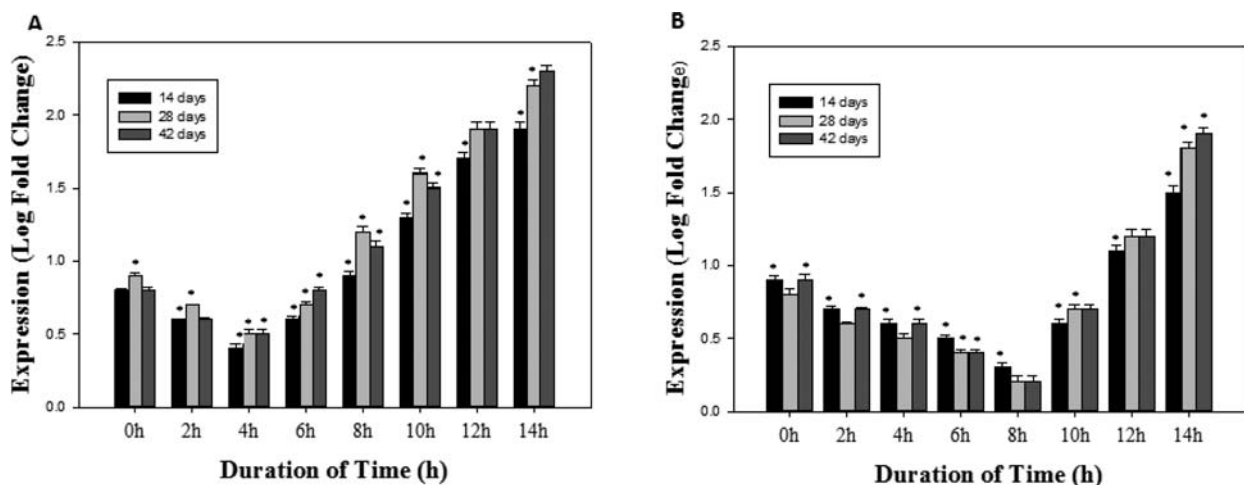
respectively (Figure 4(C)). In the case of Soniya-1402, there was no significant increase from 0 to 8 h throughout all the days exposed to radiation, but an increase was observed in a fold change value of 0.7–1.4 from 10 to 14 h in 14, 28, and 42 days, respectively (Figure 4(D)). There was a reduction in the expression of *ETR2* in Meenaxi-371 on the 14th day when exposed to radiation from 0 to 8 h and increased with a value of 1.3–1.8 from 10 to 14 h as compared to the okra plant in non-irradiated conditions (Figure 5(A)). Similarly, a decrease in the transcript expression level was evident in 0–8 h and later increased till 14 h from 1.6 to 2.2 on the 28 and 42 days, respectively.

Exposed radiation condition showed a reduction in fold change in the Soniya-1402 variety from 0 to 6 h on the 14th day and further increased in a fold change of 0.7–1.3 from 8 to 14 h as compared to plant from non-irradiated

conditions (Figure 5(A)). Similarly, the expression level of *ETR2* from microwave-exposed condition showed a reduction in 0–8 h and increased remarkably in a value of 0.7–1.8 from 10 to 14 h at 28 and 42 days, respectively (Figure 5(B)).

#### 4. Discussion

In the modern day, a systematic and quantitative study is necessary to make a model for the useful practice of applying microwave exposure to agricultural experiment. Several scientists make the use of several types of lasers, coils, EMF generators, and magnets for the stimulation of EMF. The principle of altering the electric signaling pathway in the okra plant is the objective of this study. The effect of low EMF showed an increase in carotenoid and chlorophyll



**Figure 5.** Changes in relative gene expression levels of *ETR2* gene on Meenaxi-371 (A) and Soniya-1402 (B) varieties of okra exposed to a different time of radiation. 0 h denotes control, while 2–14 h denotes radiated. All data denotes mean  $\pm$  standard deviation of six replicates, while  $^{**}$  indicates  $p \leq 0.05$  across the values of mean between 14, 28, and 42 days.

content of *Zea mays* and subsequently decreases the duration of radiation exposure time (Kumari et al. 2018). Also, Radzevičius et al. (2013) found that the effect of microwave treatment on seeds has a positive effect on the concentration of carotenoid and chlorophyll. A two-fold significant increase in the enzyme  $\beta$ -1,3-glucanase was observed, which is responsible for the development of okra seedling vigor and the seed germination. Wei et al. (2010) found that an increase in activities of  $\beta$ -1,3-glucanase enhanced the penetration of the radicle in *Solanum lycopersicon* seeds and aids the early germination of *Lycopersicon esculentus* seeds. Similarly, the activity of  $\beta$ -carotene and lutein was reported on the potency of energy accumulators which is known to have the capacity to quench chlorophyll and pigment molecules (Farooq et al. 2009).

Radhika and Rao (2015) describe that the protein content could serve as a reserve that is necessary to supply nutrients for tomato seed germination. Similarly, Wang et al. (2018) found that in various grass species like Tartary buckwheat, there is an increase in flavonoid content under the radiation exposure. In addition, Vian et al. (2016) recorded an increase in the total protein content in *Triticum aestivum* grains under radiation exposure of frequency 10.525 GHz and wavelength of 2.85 cm for 15, 45, and 75 min.

Harpaz-Saad et al. (2018) also reported on how the gene ACS 4 catalyzes S-adenosyl methionine to ACC in the synthesis pathway of ethylene.

Kumar et al. (2014) describe ethylene metabolism as the indicator which modulates senescence and delays ripening in tomato fruit. Bu et al. (2013) and Tiecher et al. (2013) found the positive response associated with the exposure of ultraviolet-C radiation on the synthesis of ethylene and ripening in *L. esculentum* fruit. Besides, Liu et al. (2011) reported on the gene expression studies in *S. lycopersicon* fruit and on the specific response that is induced under UVC radiation treatment such as the synthesis of ACS oxidase and the induction of ACC synthase. Vwioko et al. (2017) also studied the ability of waterlogged conditions to induce the expression of ethylene gene and antioxidant enzymes in the okra plant. However, the ethylene receptor gene (*ETR2*) is suppressed when there is an increase after irradiation treatment. Likewise, Severo et al. (2015) reported that a relationship exists in the application of UV-C and lateness in the ripening of okra fruit. The application of microwave irradiation to okra in the study showed a distinct result in the expression levels of *ETR2* and *ACS4* and *ACS6* synthase. The use of low and high gamma-irradiated treatment increases the hydrogen content in barley seeds, thereby lowering the gene expression level of ACC oxidase (Volkova et al. 2020). An et al. (2006) documented the transcriptome studies on the activities of ACC oxidase and synthase in *L. esculentum* leaves under UV-C treatment.

## 5. Conclusion

In conclusion, the activity of  $\beta$ -glucanase showed an increase up to 3 folds in irradiated okra seeds. The photosynthetic pigments and protein content showed a rapid increase of 2.1–3 folds in okra plants at 10 h radiation exposure. The study has shown that the application of microwave irradiation treatment at 0–6 h suppressed the expression levels of amino cyclopropane (*ACS4* and *ACS6*) synthase and ethylene genes even as the duration of irradiation

treatment was increased. Therefore, the findings have revealed that microwave radiation treatment has a positive effect on the germination rate of the okra seeds.

## Author's contribution

EI and IA have designed and conceptualize the experiment, while JA, TA, OM and MP helped in the analysis and preparing of manuscripts.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## References

- Abeles FB, Forrence LE. 1970. Temporal and hormonal control of  $\beta$ -1,3-glucanase in *Phaseolus vulgaris* L. *Plant Physiol.* 45:395–400.
- An L, Xu X, Tang H, Zhang M, Hou Z, Liu Y, Zhao Z, Feng H, Xu S, Wang X. 2006. Ethylene production and 1-Aminocyclopropane-1-Carboxylate (ACC) synthase gene expression in tomato (*Lycopersicon esculentum* Mill.) leaves under enhanced UV-B radiation. *J Integr Plant Biol.* 48:1190–1196.
- Azadi H, Ho P. 2010. Genetically modified and organic crops in developing countries: A review of options for food security. *Biotechnol Adv.* 28:160–168.
- Bu J, Yu Y, Aisikaer G, Ying T. 2013. Postharvest UV-C irradiation inhibits the production of ethylene and the activity of cell wall-degrading enzymes during softening of tomato (*Lycopersicon esculentum* L.) fruit. *Postharvest Biol Technol.* 86:337–345.
- Chang CC, Yang MH, Wen HM, Chern JC. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 10:178–182.
- Chen D, Xu G, Tang W, Jing Y, Ji Q, Fei Z, Lin R. 2013. Antagonistic basic Helix-Loop-Helix/Bzip transcription factors form transcriptional modules that integrate light and reactive oxygen species signaling in arabidopsis. *Plant Cell.* 25:1657–1673.
- Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. 2009. Plant drought stress: effects, mechanisms and management. *Agron Sustain Dev.* 29:185–212.
- Grémiaux A, Girard S, Guérin V, Lothier J, Baluška F, Davies E, Bonnet P, Vian A. 2016. Low-amplitude, high-frequency electromagnetic field exposure causes delayed and reduced growth in *Rosa hybrida*. *J Plant Physiol.* 190:44–53.
- Habtamu F, Ratta N, Haki GD. 2014. Nutritional quality and health benefits of okra (*Abelmoschus esculentus*): a review. *J Food Sci Qual Manag.* 33:87–96.
- Harpaz-Saad S, Yoon GM, Mattoo AK, Kieber JJ. 2018. The formation of ACC and competition between polyamines and ethylene for SAM. *Annu Plant Rev Online.* 15:53–81.
- Holdsworth M, Bricas N. 2016. Impact of climate change on food consumption and nutrition. In: Torquebiau E., editor. *Climate change and agriculture worldwide*. Heibelberg: Springer; p. 227–238.
- Kiaya V. 2014. Post-harvest losses and strategies to reduce them. Technical Paper on Postharvest Losses, Action Contre La Faim (ACF). p. 1–25.
- Kumar M, Ahuja S, Dahuja A, Kumar R, Singh B. 2014. Gamma radiation protects fruit quality in tomato by inhibiting the production of reactive oxygen species (ROS) and ethylene. *J Radioanal Nucl Chem.* 301:871–880.
- Kumari N, Verma S, Sharma V. 2018. Manipulating tomato plant electric signaling system by microwave radiation to enhance crop productivity and nutritional value. *Comput Electron Agric.* 154:330–340.



- Lichtentaler HK, Buschman C. 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food and Analytical Chemistry* F4.3.1–F4.3.8.
- Liu C, Cai L, Han X. 2011. Temporary effect of postharvest UV-C irradiation on gene expression profile in tomato fruit. *Gene*. 486:56–64.
- Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2–CT method. *Method*. 25:402–408.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 193:265–275.
- McDonald AE, Niere JO, Plaxton WC. 2001. Phosphite disrupts the acclimation of *Saccharomyces cerevisiae* to phosphate starvation. *Can J Microbiol*. 47:969–978.
- Naveed A, Khan AA, Khan IA. 2009. Generation mean analysis of water stress tolerance in okra (*Abelmoschus esculentus* L.) *Pak J Bot*. 41:195–205.
- Neta ICS, Edila VDRVP, Viviane MDA, Heloisa ODS, Danielle RV, Renzo GVP, Maria LMDC. 2016. Enzyme activities and gene expression in dry maize seeds and seeds submitted to low germination temperature. *Afr J Agr Res*. 11:3097–3103.
- Pocock TM, Krol NPA. 2004. The determination and quantification of photosynthetic pigments by reverse phase high-performance liquid chromatography, thin-layer chromatography and spectrophotometry. In: R. Carpentier, editor. *Photosynthesis research protocols*. Totowa: Humana Press; p. 137–148.
- Radhika V, Rao VSH. 2015. Computational approaches for the classification of seed storage proteins. *J Food Sci Technol*. 52:4246–4255.
- Radzevičius A, Sakalauskienė S, Dagys M, Simniškis R, Karklelienė R, Bobinas Č, Duchovskis P. 2013. The effect of strong microwave electric field radiation on: (1) vegetable seed germination and seedling growth rate. *ZEMDIRBYSTE*. 100:179–184.
- Sabzevari AG, Hosseini R. 2014. A quick, efficient, and cost-effective method for isolating high-quality total RNA from tomato fruits, suitable for molecular biology studies. *Prep Biochem Biotechnol*. 44:418–431.
- Samir KC, Lutz W. 2017. The human core of the shared socioeconomic pathways: population scenarios by age, sex and level of education for all countries to 2100. *Glob Environ Change*. 42:181–192.
- Senavirathna MDHJ, Asaeda T. 2014. Radio-frequency electromagnetic radiation alters the electric potential of *Myriophyllum aquaticum*. *Biol Plant*. 58:355–362.
- Severo J, Tiecher A, Pirrello J, Regad F, Latché A, Pech J-C, Bouzayen M, Rombaldi CV. 2015. UV-C radiation modifies the ripening and accumulation of ethylene response factor (ERF) transcripts in tomato fruit. *Postharvest Biol Technol*. 102:9–16.
- Sharma I, Kumari N, Sharma V. 2014. Defense gene expression in *Sorghum bicolor* against *Macrophomina phaseolina* in leaves and roots of susceptible and resistant cultivars. *J Plant Interact*. 9:315–323.
- Szasz O, Szigeti G, Szasz A. 2016. Connections between the specific absorption rate and the local temperature. *Open J Biophys*. 6:53–74.
- Tiecher A, de Paula LA, Chaves FC, Rombaldi CV. 2013. UV-C effect on ethylene, polyamines and the regulation of tomato fruit ripening. *Postharvest Biol Technol*. 86:230–239.
- Vian A, Davies E, Gendraud M, Bonnet P. 2016. Plant responses to high frequency electromagnetic fields. *Biomed Res Int*. 2:1–13.
- Volkova PY, Duarte GT, Soubigou-Taconnat L, Kazakova EA, Pateyron S, Bondarenko VS, Bitarishvili SV, Makarenko ES, Churyukin RS, Lychenkova MA, et al. 2020. Early response of barley embryos to low- and high-dose gamma irradiation of seeds triggers changes in the transcriptional profile and an increase in hydrogen peroxide content in seedlings. *J Agron Crop Sci*. 206:277–295.
- Vwioko E, Adinkwu OA, El-Esawi M. 2017. Comparative physiological, biochemical, and genetic responses to prolonged water logging stress in Okra and Maize given exogenous ethylene priming. *Front Physiol*. 8:632.
- Wang S, Wang J, Guo Y. 2018. Microwave irradiation enhances the germination rate of Tartary buckwheat and content of some compounds in its sprouts. *Polish J Food Nutr Sci*. 68:195–205.
- Wei J, Ma F, Shi S, Qi X, Zhu X, Yuan J. 2010. Changes and postharvest regulation of activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit. *Postharvest Biol Technol*. 56:147–154.