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Effect of Flame Treatment and Radiofrequency Electromagnetic Radiations on phenolic content in *in vitro* cultures of *Ipomoea batatas* (L.) Lam.

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Abstract

In vitro grown callus cultures of *Ipomoea batatas* (L.) Lam. were exposed to flame treatment and electromagnetic radiations generated by mobile phone. The cultured tissues responded to the treatments as evidenced by the significant reduction of phenolic contents compared to controls. Even though the growth of the tissues was normal, there was a change in the phenolic content of the tissues. There exhibited not much significant variation among the treatments regarding the growth rate. The morphology and texture of the callus also remained the same. It has been concluded that like animal cells, plant cells also respond to non-ionizing radiations like electromagnetic radiation emitted by mobile phones.

Keywords

Callus culture, flame treatment, *Ipomoea batatas*, Radio frequency electromagnetic radiation

Introduction

Mobile phone uses and emits non-ionizing radiation known as radio-frequency electromagnetic radiations (RF-EMR) (1). The use of the mobile phone has raised concerns in the environment (2), human health (3) and cognitive functions (4). It also affects animal behavior (5). Similar to humans and animals, it also affects plants (6). Few reports available indicates that plants respond to high-frequency electromagnetic radiations (7). These responses include altered growth (8) and biochemical changes (6).

Plants respond to environmental changes. Such changes involve altered flowering and vegetative development (9). All these changes occur because of the altered metabolism due to stress-responsive genes (10). This is reflected in the accumulation of secondary metabolites through stress and defensive molecular signaling (11). Phenolic compounds represent a class of secondary metabolites whose accumulation differs according to the stress faced by the plant. Therefore, phenolic compounds are often used as stress markers in plants (12). Hence, it is interesting to analyse the phenolic acid accumulation in plants when exposed to non-ionizing radiations. The present study is aimed at analyzing the effect of mobile phone radiation on growth and phenol accumulation using *in vitro* cultured plant tissues of *Ipomoea batatas.*

Materials and Methods

Stock plants of *Ipomoea batatas* were grown under the green-house conditions at the Department of Biotechnology, Manipal Institute of Technology, Manipal. Young green leaf segments were taken from this plant, served as explants and washed with running tap water for 30 min. Chemical sterilization was carried out with 0.1% (w/v) mercuric chloride for eight min. Post surface sterilization was done by washing the explants two times with sterile double distilled water. The media used for the experiment is woody plant medium (13) containing 2 mg/l Benzyl Adenine and 1 mg/l naphthalene acetic acid. The pH of the media was adjusted to 5.80 and solidification was achieved with 8 g/l agar. Autoclaving was done under 12 psi for 15 min at a temperature of 121 ^{0}C. The cultures were allowed to grow for 21 days and sub cultured into fresh medium. These cultures were used for treatments immediately after subculture and the readings were measured on 42nd day.

Exposure with RF-EMR was done by using an android mobile phone hand-set with a specific absorption rate of 0.38 W/Kg. The phone was placed in close proximity to the culture tubes and was exposed to radiation by using a call receive mode for fixed time exposures such as 5, 15, 25 and 35 min daily for 5 days. For the flame exposure, the culture tubes were exposed to a Bunsen burner flame for 3 separate treatments such as (i) 3 seconds (ii) 6 seconds (iii) 3+3+3 seconds exposure with a gap of 3 seconds each and (iv) 3+3+3+3 seconds exposure with a gap of 3 seconds each. This was done for each culture daily for 5 days. The cultures were harvested for growth and biochemical analysis on completing the experimental cycle.

The callus growth was expressed as dry wt. after drying the callus tissue at 30 ^{0}C for 48 hrs. The total phenolic content was expressed as gallic acid equivalents (GAE) and was analyzed by using the standard protocol (14). Results were expressed as mean $\pm$ standard error of six replications, and comparisons of means were performed by Duncan's multiple range test. Untreated cultures served as the controls.

Results and Discussion

The callus tissue's growth didn't statistically differ when compared to controls on exposure to mobile phone radiation except in the case of 25 min RF-EMR exposure (Table 1). However, there exhibited a variation in the accumulation of phenolic acids. Accumulation of phenolic compounds was less in the treatments. The lowest accumulation of phenolic content was noted when the callus tissue was exposed to 5 and 25 min of mobile phone radiation. Even though the results exhibited a significant difference of phenolic content accumulation with respect to controls; there was no significant difference in the growth of the biomass among the treatments. Highest biomass accumulated was noted in cultures treated with 25 min of RF-EMR exposure (0.204 g dry wt , Fig. 1c). A 15 min of RF-EMR exposure yielded a biomass of 0.151 g dry wt (Fig. 1b). Callus nature, texture and morphology didn't distinguish between the treatments and controls (Fig. 1).

Table 1. Effect of mobile hand-set radiation on the growth and phenolic content of callus cultures in *I. batatas*

Treatment (Exposure in min.)	Growth of callus (g dry wt)*	Phenolic content (mg GAE/100 g dry wt)*
Control	$0.092^{a} \pm 0.01$	$280^{c} \pm 52$
5 min.	$0.187^{a} \pm 0.07$	$103^{ab} \pm 32$
15 min.	$0.151^{a} \pm 0.04$	$47^{a} \pm 14$
25 min.	$0.204^{b} \pm 0.14$	$72^{ab} \pm 18$
35 min.	$0.073^{a} \pm 0.03$	$154^{b} \pm 34$

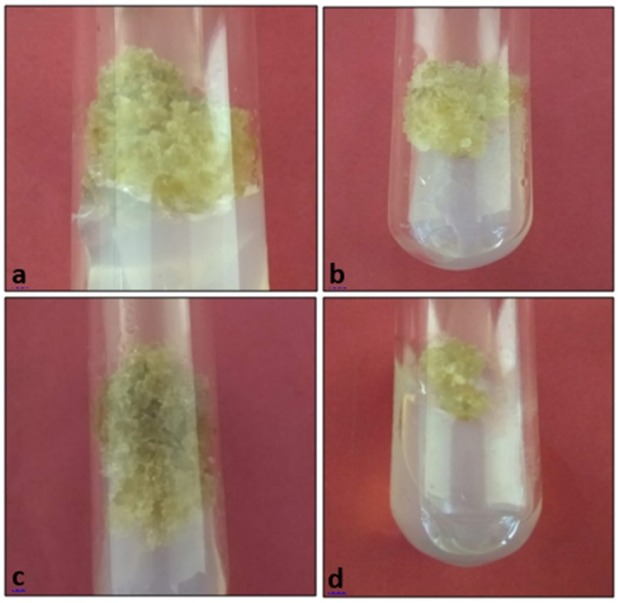

Fig. 1. Response of callus on exposure to Radio Frequency Electro Magnetic Radiation and flame treatment (a): Control (b): 15 min. of EMF exposure (c): 25 min. of exposure daily for five days (d): flame treatment of 3+3+3 sec. The calls texture and morphology remains the same in all the cases, similar to control.

Flame treatment also didn't resulted in the difference of biomass accumulation (Table 2). Callus growth was lowest when the cultures were exposed to 3+3+3 second treatment with a gap of 3 seconds (Fig. 1d). Accumulation of phenolic compounds exhibited a significant variation from the controls; however, there was no significant difference in callus growth among the treatments. The fire exposure lowered the phenolic content in all the treatments. Thus, it is possible to draw out a negative relationship with the exposure duration of flame.

Table 2. Effect of flame treatment on the growth and phenolic content of callus cultures in *I. batatas*

Treatment (Exposure in sec.)	Growth of callus (g dry wt)*	Phenolic content (mg GAE/100 g dry wt)*
Control	$0.092^{ab} \pm 0.01$	$280^{a} \pm 52$
3 sec.	$0.082^{ab} \pm 0.01$	$143^{b} \pm 23$
6 sec.	$0.123^{b} \pm 0.21$	$93^{b} \pm 20$
3+3+3 sec.	$0.076^{a} \pm 0.01$	$91^{b} \pm 16$
3+3+3+3 sec.	$0.120^{ab} \pm 0.02$	$82^{b} \pm 10$

Living cells respond to the electromagnetic field. There are several reports available regarding the hazardous effects of electromagnetic field (EMF) on living systems. However, only a few studies have been conducted in

plants. Earlier it has been demonstrated that high tension lines were capable of altering the growth of rice (15), wheat and corn (16) plants. Experiments conducted on *Vigna radiata* indicate that cell phone EMF indicates a negative correlation with length of seedlings and biomass accumulation and altered biochemical pattern (6). Results of the current study also confirm that *in vitro* cultured plant tissues respond to EMF. Apart from affecting biomass yield, there is also a change in the phenolic acid content. Experiments conducted in previous reports proved that fire can be utilized to stimulate shoot organogenesis in *Vellozia pyrantha* under *in vitro* conditions (17).

Plants respond to both biotic and abiotic stress. This response is exhibited as altered pattern of expression of metabolites. In the present study mobile phone radiation acted as a stress as evidenced from the enhanced phenolic content. Similar reports of enhanced accumulation of phenolic acids in response to stress has been found in *Silene littorea* in response of UV irradiation (18). However, in some cases, stress reduces the accumulation of phenolic compounds (19). Hence, it is evident that plants vary in such responses and such metabolic expressions can't be generalized. Another report reveals altered enzymatic profiles on EMF exposure in the leaf tissues (20). High-frequency EMF exposure is capable of altering even distantly located tissues and has been proposed to consider EMF as a non-injurious factor that is able to alter metabolism (7).

Conclusion

Developments in the field of electronics and communication technology resulted in accelerated exposure of plants to electromagnetic field. EMF and flame exposure acted as a stress agent on plant tissues. The current study on *Ipomea batatas* (L.) Lam. confirmed that such an exposure significantly alter phenolic accumulation. The results generated in this study are significant because it confirms that EMF and flame exposure can alter plant metabolism under *in vitro* conditions. However, the impact of EMF and flame exposure on plants remains largely unexplored. It is also not clear that such changes are reversible or permanent. Therefore, future work identifying the molecular signatures generated and the nucleic acid damage in plant cells when exposed to EMF is highly interesting.

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Authors contributions

The work plan was supervised by NS and BS. Experiments were performed by UB and NS. The manuscript writing and statistical calculation were done by NS and BS. The corrections of manuscript were done by UB, NS and BS. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interests to declare.

Ethical issues: None.

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