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Original Article

Exposure to 2100 MHz electromagnetic field radiations induces reactive oxygen species generation in *Allium cepa* roots

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ABSTRACT

During the last few decades there has been an enormous increase in the usage of cell phones as these are one of the most convenient gadgets and provide excellent mode of communication without evoking any hindrance to movement. However, these are significantly adding to the electromagnetic field radiations (EMF-r) in the environment and thus, are required to be analysed for their impacts on living beings. The present study investigated the role of cell phone EMF-r in inciting oxidative damage in onion (*Allium cepa*) roots at a frequency of 2100 MHz. Onion roots were exposed to continuous wave homogenous EMF-r for 1, 2 and 4 h for single day and generation of reactive oxygen species (ROS) in terms of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet -}$) content and changes in the activities of antioxidant enzymes- superoxide dismutases (SOD) and catalases (CAT) were measured. The results showed that EMF-r exposure enhanced the content of MDA, H_2O_2 and $O_2^{\bullet -}$. Also, there was an upregulation in the activity of antioxidant enzymes– SOD and CAT– in onion roots. The study concluded that 2100 MHz cell phone EMF-r incite oxidative damage in onion roots by altering the oxidative metabolism.

1. Introduction

Last couple of decades have witnessed unprecedented growth in communication sector with an exponential increase in the number of wireless devices based on non-ionizing radiations [1]. Of the various wireless devices, mobile phones are the ones that are being most widely used and have become part and parcel of modern day life. According to International Telecommunication Union (ITU) report, there are about 7.377 billion mobile cellular telephone subscriptions globally, with an average of 99.7 subscriptions per 100 inhabitants [2]. With an upsurge in the usage of such devices there has been a tremendous increase in the non-ionizing radio frequency electromagnetic fields in the environment and hence, an enhanced level of exposure of electromagnetic field radiations (EMF-r) to the living beings [3]. Notwithstanding, the accelerated and widespread use of these gadgets, it has become a topic of utmost concern among the scientists owing to the health implications of EMF-r on living beings [3,4]. Studies have documented various biological effects like induction of lesions in vital organs [5], and alterations in gene expression [6], cell cycle [7], enzyme activity [8], protein expression [9,10], hormone levels [11] oxidative metabolism [12], cell membrane permeability [13], and genotoxicity [14] on exposure to EMF-r. However, most of these findings have been derived from studies on animal system and less attention has been paid to explore the effect on plants [4]. Moreover, amongst the scanty publications on plants, few have reported EMF-r to enhance plant growth [15–17], whereas, others have documented the inhibitory effects of EMF-r. For example, EMF-r cause reduction in seed germination [18,19], impair root growth, early seedling growth and rhizogenesis [19–21] and induce biochemical changes [20,21] in plants. EMF-r at 900 MHz affect transcription, translation, calcium transport and energy charge in tomato (*Lycopersicon esculentum* Mill.) [22]. Monselise et al. [23] reported production of alanine (as a metabolic response to stress) in duckweed plants (*Spirodela oligorrhiza*) exposed to low-frequency electromagnetic fields, whereas, no accumulation was reported in unexposed control. EMF-r alter mitosis, and increase mitotic index, frequency of mitotic and chromosomal abnormalities, and micronucleus frequency [19,24–26].

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Of late, 900 EMF-r have been reported to induce ROS-mediated oxidative damage in plant roots [27,28].

With the ongoing increase of the electromagnetic field radiations in the environment and the advent of new technology like 3G and 4G, it is pertinent to evaluate the impact of high frequency (2100 MHz) EMF-r exposure. Till date, most of the studies have evaluated the impacts of 900–1800 MHz EMF-r on plants; however, not much is known about biological impacts of 2100 MHz EMF-r in plants. With this background, we hypothesized that 2100 MHz EMF-r may also inhibit plant growth and induce abiotic stress. We, therefore, investigated the role of 2100 MHz EMF-r in inciting oxidative damage in *Allium cepa* (onion) roots. For this, we evaluated reactive oxygen species (ROS) generation both, biochemically as well as histochemically, along with the alterations in the activities of antioxidant enzymes.

2. Material and methods

2.1. Materials

Onion bulbs of equal size were procured from local market and scrapped to remove old and dry scales and roots so that the apices of root primordia were exposed. The bulbs were placed in coplin jars with their basal ends dipped in distilled water, and were set for rooting at room temperature (25 ± 1 °C). All the reagents and chemicals used in the study were of analytical grade purchased from Sisco Research Laboratory Pvt. Ltd., India; Sigma Co., St. Louis, USA; Merck Ltd., India; and Loba-Chemie Pvt., Ltd., India.

2.2. EMF-r treatment

The exposure system consisted of RF signal generator (Agilent N9310A; Keysight Technologies, USA) that generates homogenous EMF-r, similar to mobile phone frequency in a range of 9KHz – 3 GHz. It was further attached with an RF power amplifier (Model No. ZHL-5W-2GX+; Minicircuits, USA) and a DC regulated power supply. The exposure system was placed in an empty room coated with YSHIELD® (HSF54) radiofrequency shielding paint, free from any external source of EMF-r. Once the roots attained the length of 3–4 cm, they were subjected to exposure. No EMF-r treatment was given to group 1 and group 2, which served as control and Sham control, respectively. Group 1 was placed in an empty room similar to the one of exposure system without any EMF-r source. Group 2 was placed in the exposure room only but without any EMF-r exposure. Group 3,4 and 5 were exposed to EMF-r for 1, 2 and 4 h. The output power density was measured using ScanEM®-C Probe (Model No. CTK015; 3 M Technologies, USA) attached to RF power density meter (Spectran, HF-4060, range 100 MHz to 6 GHz; Aaronia AG, Germany). The average power density received at a distance of 5 cm from antenna was 489.7 ± 18.15 mW m^{-2} with a specific absorption rate (SAR) of 2.82 ± 0.12 × 10^{-1} W kg^{-1}. SAR value was determined roughly as it is relatively difficult to measure SAR values on exposed tissues directly [29]. It was calculated by taking the values of electrical conductivity (σ) and tissue density (ρ) for the dielectric properties of tissue at 2100 MHz (σ = 1.57 S m^{-1} and ρ = 1030 kg cm^{-3}) from IFAC (Institute of Applied Physics, Sesto Fiorentino, Italy) database [30].

2.3. Appraisal of the oxidative stress in terms of ROS generation

EMF-r induced oxidative stress was measured in terms of lipid peroxidation and ROS– hydrogen peroxide (H_2O_2) and superoxide anions ($O_2^{\bullet -}$)– accumulation in the onion roots.

Lipid or membrane peroxidation was estimated in terms of malondialdehyde (MDA) content [31]. Briefly, 100 mg of roots were homogenized in 10 ml of 0.1% (*w/v*) trichloroacetic acid and centrifuged at 12,000*g* for 15 min at 4 °C. To 1 ml of supernatant, 4 ml of 0.5% of thiobarbituric acid (prepared in 20% trichloroacetic acid, TCA) was added. The reaction mixture was then heated at 95 °C for 30 min, cooled immediately in an ice bath and then again centrifuged at 10,000*g* for 10 min. The absorbance of the supernatant thus obtained was read at 532 nm and corrected for non-specific absorbance at 600 nm. The concentration of MDA was calculated using extinction coefficient (ε) of 155 mM^{-1} cm^{-1} and expressed as nanomoles per gram fresh weight (nM g^{-1} f. wt.).

H_2O_2 content was determined as per the method described by Velikova et al. [32]. The roots (100 mg) were homogenized in 0.1% TCA (10 ml) and centrifuged at 12,000*g* for 15 min. To 0.5 ml of the supernatant, 0.5 ml of 10 mM PO_4^{3-} buffer (pH 7) and 1 ml of 1 M potassium iodide were added and absorbance of the reaction mixture was read at 390 nm. The H_2O_2 content was quantified using ε = 0.28 μM^{-1} cm^{-1} and expressed as nM g^{-1} f. wt. [28].

$O_2^{\bullet -}$ content was estimated according to the method given by Misra and Fridovich [33]. The root tissue (100 mg) was homogenized in 10 ml of 0.1 M PO_4^{3-} buffer (pH 7), and centrifuged at 12,000*g* for 15 min at 4 °C. To 1.8 ml of 1 mM adrenalin solution (prepared in 75 mM PO_4^{3-}buffer; pH 7.4), 0.2 ml of supernatant was added. The change in the absorbance was read at 480 nm over a time period of 5 min and $O_2^{\bullet -}$ content was calculated using ε = 4.02 mM^{-1} cm^{-1} and expressed as μM g^{-1} f. wt.

2.4. In situ ROS detection

In situ ROS accumulation in onion roots was determined histochemically in terms of lipid peroxidation, $O_2^{\bullet -}$, H_2O_2 and loss of membrane integrity. Histochemical detection of lipid peroxidation was performed using Schiff's reagent [34]. Freshly excised root tips were stained in Schiff's reagent for 1 h, followed by rinsing with potassium metabisulphite solution (0.5%, *w/v*). Loss of membrane integrity was determined using Evans blue as per Yamamoto et al. [35]. Freshly excised root tips were stained in Evan's blue solution (0.025%, *w/v*, in 100 μM $CaCl_2$, pH 5.6) for 30 min. Diaminobenzidine (DAB) solution was used for the histochemical detection of H_2O_2 [36]. Freshly harvested roots were stained in DAB solution (0.3 mg/ml) for 10 min, followed with rinsing with distilled water. Root tips were then boiled in 90% ethanol (*v/v*) for 10 min for decolorization. *In situ* localization of $O_2^{\bullet -}$ was done as per the method given by Doke [37]. Freshly cut roots were incubated in nitrobluetetrazolium (NBT; 0.05%, *w/v*, prepared in 100 mM PO_4^{3-}buffer, pH 7.8) for 20 min, then transferred to 96% ethanol for 30 min to stop the reaction. The excess stain was washed out by immersing the root tips in distilled water, followed by observation under the Trinocular Stereo Zoom microscope. *In situ* ROS detection in roots was done on the basis of colour intensity.

2.5. Assays of antioxidant enzymes

For the estimation of activities of antioxidant enzymes, root tissue (100 mg) was homogenized in 10 ml of 100 mM PO_4^{3-} buffer (pH 7) and centrifuged for 25 min at 15,000 g. The supernatant thus obtained was used for the assay of activities of superoxide dismutases (SOD; EC 1.15.1.1) and catalases (CAT; EC 1.11.1.6). SOD was assayed for its ability to inhibit the photochemical reduction of NBT at 560 nm [38]. The amount of enzyme required to inhibit the photoreduction of NBT by 50% at 25 °C was defined as one unit of SOD. CAT activity was determined in terms of disappearance rate of H_2O_2 at 240 nm and calculated using ε = 2.8 mM^{-1} cm^{-1} [39]. One unit of CAT is the amount of enzyme required to decompose 1 μM of H_2O_2 per min at 25 °C.

Table 1
Effect of cell phone EMF-r at 2100 MHz on the contents of (a) MDA, (b) H_2O_2, and (c) $O_2^{\bullet-}$ in onion roots.

Treatment/ Exposure Period (h)	MDA content (nM g^{-1} f. wt.)	H_2O_2 content (nM g^{-1} f. wt.)	Superoxide anions (mM g^{-1} f. wt.)
Control	11.2 ± 1.14 a	3.57 ± 0.41 a	0.30 ± 0.03 a
Sham Control	13.8 ± 0.57 a	5.24 ± 0.63 ab	0.35 ± 0.03 a
1 h	14.4 ± 0.42 b	7.86 ± 0.41 b	0.41 ± 0.04 a
2 h	15.5 ± 0.37 b	10.48 ± 0.63 c	0.61 ± 0.04 b
4 h	19.4 ± 0.99 c	18.33 ± 0.86 d	0.70 ± 0.03 c

Different letters in a column represent significant difference at $p \leq 0.05$ according to Tukey's Post hoc test.

2.6. Statistical analysis

The experiments were conducted in a completely randomized design. There were five replications for each treatment, with each replication consisting of single onion bulb. For biochemical analysis, there were five replicated (independent) tissue samples. The data were analysed by one-way ANOVA followed by the comparison of mean values at $p \leq 0.05$ using post hoc Tukey's test. The statistical analyses were done using SPSS ver. 16.

3. Results and discussion

In the present study, exposure to cell phone EMF-r incited a ROS-mediated oxidative stress in the onion roots. Upon exposure, the levels of ROS like MDA, H_2O_2 and $O_2^{\bullet-}$ were enhanced as compared to the control in a time-dependent manner. To quantify the EMF-r induced damage to the membranes, lipid peroxidation analysis was done. Exposure to 2100 MHz EMF-r enhanced MDA content (by ~23–73%), a measure of lipid peroxidation, as compared to the control (Table 1). MDA is a free radical generated as a byproduct of lipid peroxidation and enhanced levels of MDA upon exposure advocates that EMF-r increased lipid peroxidation, caused damage to the membrane and induced oxidative stress in the roots. It was confirmed by staining of roots with Evans blue that showed the damage to the membrane in a time-dependent manner (Fig. 1a). Enhanced lipid peroxidation upon EMF-r exposure was confirmed by the *in situ* histochemical localization with Schiff's reagent, wherein the roots exposed to cell phone EMF-r stained darker than those of the control (Fig. 1b). Parallel to MDA accumulation, H_2O_2 and $O_2^{\bullet-}$ levels were increased upon exposure to EMF-r (Table 1). H_2O_2 content increased by ~5.1 times upon exposure for 4 h, compared to the control (Table 1). *In situ* detection of H_2O_2 with DAB also supported the greater accumulation of H_2O_2, exhibiting greater colour intensity with increase in the exposure duration (Fig. 1c). $O_2^{\bullet-}$ content was enhanced by ~2.3 times over the control on EMF-r exposure for 4 h (Table 1). It was confirmed by *in situ* detection with NBT (Fig. 1d). The observations made in the present study are corroborated by earlier reports that EMF-r act as an abiotic stress for plants [23,40,41], and induce ROS (H_2O_2, $O_2^{\bullet-}$) generation, resulting in enhanced lipid peroxidation and damage to membranes [27,28,42]. Monselise et al. [23] reported that low intensity magnetic field exposure cause oxidative stress in duck weed plants, as evidenced by accumulation of alanine, another stress signal.

The cells have an inherent mechanism to overcome stress induced by overproduction of ROS and one such strategy is the upregulation/greater production of antioxidant enzymes that scavenge free radicles [12,43]. In the present study, an upregulation in the activities of antioxidant enzymes- SOD and CAT- in a dose-dependent manner was also observed upon EMF-r exposure (Table 2). EMF-r exposure for 1 h enhanced the SOD activity

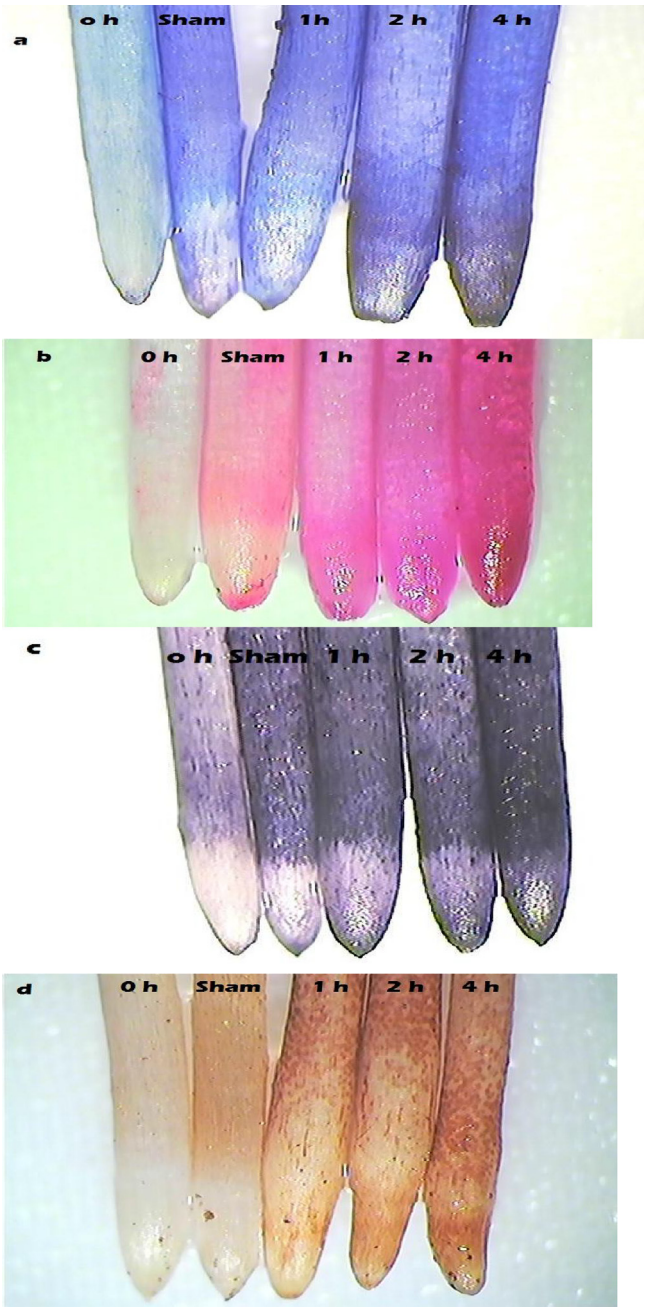

Fig. 1. Photographs showing in situ histochemical localization depicting (a) loss of membrane integrity, (b) lipid peroxidation, and accumulation of (c) H_2O_2 and (d) $O_2^{\bullet-}$ in *Allium cepa* roots after exposure to 2100 MHz EMF-r.

Table 2
Effect of cell phone EMF-r at 2100 MHz on the specific activities of (a) SOD and (b) CAT in onion roots.

Treatment/Exposure Period (h)	SOD activity (EU mg^{-1} protein)	CAT activity (EU mg^{-1} protein)
Control	3.51 ± 0.09 a	1.28 ± 0.04 a
Sham Control	3.90 ± 0.02 ab	1.44 ± 0.08 ab
1 h	4.12 ± 0.06 b	1.73 ± 0.03 b
2 h	5.21 ± 0.13 c	2.13 ± 0.06 c
4 h	8.51 ± 0.41 d	3.17 ± 0.15 d

Different letters in a column represent significant difference at $p \leq 0.05$ according to Tukey's Post hoc test.

by ~1.17 times, which further increased by ~1.5 and 2.42 times over control, on exposure for 2 h and 4 h, respectively (Table 2). Likewise, CAT activity increased by ~1.66 and ~3.5 times as compared to the control on exposure for 2 h and 4 h, respectively (Table 2).

SOD is a first line of defence of plants against the elevated levels of ROS and mitigates the excessive $O_2^{\bullet -}$ generation. Likewise, CAT also plays an important role in plants under stress and decompose H_2O_2 into H_2O and O_2 [42]. Hence, the upregulated activities of SOD and CAT could be attributed to excessive ROS generation upon EMF-r exposure. These observations are in agreement with the earlier studies documenting the elevated activities of SOD and CAT in response to EMF-r [12,27,28,44].

4. Conclusions

The study concludes that 2100 MHz EMF-r alter the oxidative metabolism in plants. It incites the enhancement of ROS generation and alters the activities of antioxidant enzymes in a dose-dependent manner. This study implies that plants perceive EMF-r as an abiotic stress and it induces oxidative damage to the plants. Nevertheless, further studies are required to explore the mechanism of action of EMF-r on plants at the molecular levels to understand impacts of EMF-r on cellular metabolism and develop certain strategies to undermine its harmful effects.

Conflict of interest

Authors declare that they have no conflicts of interest.

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