Effects of electromagnetic field radiation on inducing physiological and biochemical changes in *Satureja bachtiarica* L.

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Abstract

As organisms exposed to various abiotic and biotic environmental impacts, plants are able to recognize and respond to their surrounding environment with high specificity. Electromagnetic field radiation is an important effective stress factor on growth and development of plants. Our research focused on plants grown from wet pretreated seeds with low frequency electromagnetic field exposure comparing them with the control. Three replicates were used in the experiment with 30 seeds in each sample. The treatment wet seeds, were spread on the moist filter paper in Petri dishes before they were placed between parallel coils of electromagnetic radiation generator and were exposed by a magnitude of 1mT, for 2hr. Control seeds were placed between coils under similar conditions but not connected to the power. Morphological comparison of the treated and control samples showed that the percentage of seed germination and average root length of the treatment plants increased, but the difference in root length was not significant. A significant decrease in the mean shoot length, leaf area, and fresh and dry weight was observed. Also, electromagnetic radiation exposure caused significant decrease in the rate of Chlorophylla and chlorophyll b. However, carotenoid and activity of non-enzymatic antioxidant content in treatment samples significantly increased in comparison with control plants.

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Introduction

Plants are able to recognize and respond to their surrounding environmental stresses. When plants are subjected to environmental stress condition, the balance between the production of reactive oxygen species (ROS) and the quenching activity of antioxidants is upset, often resulting in oxidative damage (Spychalla and Desborough, 1990). ROS are produced within cells as a consequence of normal metabolic processes, but the production of ROS often increases when cells are under stress (Smirnoff, 1993). ROS not only participate in signal transduction, but also modify cellular components and cause damage to them . Abiotic

stress results in the formation of ROS in plants which creates a condition called oxidative stress that can damage cellular components (Apel and Hirt, 2004). Oxidative stress occurs when there is a serious imbalance between the production of ROS and antioxidative defense (Smirnoff, 1995). Plants have developed efficient antioxidant system that can protect them from this disaster (Mittler et al. 2004). In plants affected by stress, a response is induced by changes in the plant metabolism, growth and general development (Mittler, 2002). The production of ROS is inevitable under stress; hence, plants are equipped with an array of enzymatic and non-enzymatic antioxidant molecules to alleviate cellular damage caused by ROS (Foyer and Noctor, 2000; Apel and Hirt, 2004). In fact, electromagnetic field radiation (EMFr) causes an oxidative stress that is, it increase the activity, concentration and lifetime of free radicals (Sen Gupta et al., 1993; Allen, 1995; Tkalec et al., 2005). EMF alters protein biosynthesis, enzyme activity, cell reproduction and cellular metabolism (Nirmala and Rao, 1996). Exposure to electromagnetic field can lead to cell death as a result of increase in free oxygen radicals and DNA damage (Cossarizza et al., 1993; Benov et al., 1994; Ivancsits et al., 2003; Bediz et al., 2006). Several studies have been conducted to find out the effect of EMFr on the growth and physiology of the plants (Angel et al., 2005; Yao et al., 2006; Shabrangi and majd, 2009), such as studying effects of EMFr on seeds germination and seedlings growth and seed vigor (Bhatnagar and Deb, 1977; Pittman, 1977; Moon and Chung, 2000). Plants produce a high diversity of secondary metabolites and antioxidant defense with a prominent function in the protection against stresses on the basis of their defense reactions. Secondary metabolites are to be involved in plant chemical defense systems. High concentrations of secondary metabolites for example phenols and flavonoids, might result in a more resistant plant (Mittler, 2002; Singh et al., 1999; Wuyts et al., 2006). Electromagnetic radiation stress induces proline accumulation in plants (Verbruggen and Hermans, 2008; Kostal et al., 2011). Proline accumulation is believed to be very important as part of the physiological adaptation of plants to stress (Chu et al., 1978; Alia and Saradhi, 1991; Saradhi et al., 1995; Hare et al., 1999; Siripornadulsil et al., 2002).

This study asserts that low frequency electromagnetic radiation causes abiotic stress on the growth parameters and activity of defense mechanisms of Satureja plant (*Satureja bachtiarica* L.). This would help us to improve general knowledge about mechanisms of the response of plants to EMF.

Materials and Methods

Electromagnetic field exposure

Exposure to EMF was performed using a locally designed EMF generator. The magnetic field was provided by a parallel pair of identical circular coils spaced one radius apart and wound so that the electrical current flew through both coils in the same direction. Magnetic field was produced at uniform low frequency and homogeneously. This system consisted of a 21 cm in diameter handmade coil, cylindrical in form and with 100 roll of winding. The parallel coils of electromagnetic radiation generator produced a power with intensity of 1 mT.

The coil was not shielded for electrical field and the seeds were exposed to both magnetic and electric fields generated by the coils. The winding results in a very uniform magnetic field between the coils with the primary component parallel to the axes of the two coils (Fig. I). Samples were placed in the middle of a horizontally fixed coil and were exposed to EMF radiation. During the study period, the mean temperature ranged between 22 and 26 °C.

Fig. I. Electromagnetic field exposure arrangement

Experimental condition, growth and treatment condition

Uniform in size and shape seeds of *S. bachtiarica* L. were obtained from Seed and Plant Improvement Agriculture Institute, Karaj, Iran. Three replications were used in the experiment with 30 seeds in each treatment. In case of wet seeds treatment, the seeds were spread on the moist filter paper in Petri dishes and then placed in the middle of a horizontally fixed coil and were exposed to EMF by a magnitude of 1 mT, for 2hr in the EMF generator apparatus. Untreated seeds were used as control under similar condition. It means they were placed in the similar coil but not connected to the power. Then difference in growth parameters including seed germination, root length, and shoot length between the seedlings grown from treated seeds and control was recorded. Leaf samples of 30-day old seedlings were chosen for measurement of fresh weight, dry weight, leaf area, photosynthetic pigments and antioxidant activity assay.

Determination of photosynthetic pigments

Rate of photosynthetic pigments estimated according to the method of Lichtenthaler et al., (1987). Fresh leaves (0.2 g) were homogenized in 80% acetone and centrifuged at 10,000×g for 10 min. The supernatant was subjected to spectrophotometeric analysis of 646.8, 663.2 and 470 nm respectively. Chlorophyll a, chlorophyll b, total Chlorophyll and carotenoid content was determined and expressed in mg g^{-1} fw.

Chl. a, Chl. b, total Chl. And carotenoids contents were calculated using the following formulas:

Chl. a = (12.25A663.2 − 2.79A646.8) × volume of supernatant (ml) × dilution factor/sample weight (g)

Chl. b = (21.21A646.8 − 5.1 A663.2) × volume of supernatant (ml) × dilution factor/sample weight (g)

Car. = [(1000A470 - 1.8 Chl. a - 85.02 Chl. b) /198] × volume of supernatant (ml) × factor /sample weight (g)

Aluminum chloride colorimetric method was used for flavonoids determination (Change et al., 2002). Each extract of the plant material (0.5 ml of 1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The extract remained at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm with UV-VIS spectrophotometer. The calibration plot was generated using quercetin solution. Total flavonoid values were expressed in mg g^{-1}dw.

Determination of total phenol

Total phenol content was determined by Folin Ciocalteu reagent (McDonald et al., 2001). A dilute solution of extract (0.5 ml of 1:10 g ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml ,1:10 diluted with distilled water) and aqueous Na2CO3 (4 ml,1 M). The mixture was allowed to stand for 15 min and the phenols were determined by colorimetry at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg ml^{-1} solutions of gallic acid in methanol: water (50:50, v/v).Total phenol values were expressed in terms of gallic acid equivalent (mg g^{-1}dw).

Determination of proline content

Free proline content in the leaves was determined following the method of Bates et al., (1973). Leaf samples (0.5 g) were homogenized in 5 mL of sulphosalicylic acid (3%) using mortar and pestle. 2 ml of the extract was taken in a test tube and 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added to it. The reaction mixture was boiled in a water bath at 100° C for 30 min. After cooling the reaction mixture, 6 ml toluene was added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorption was read at 520 nm. Toluene was used as blank. Concentration of proline was estimated by referring to a standard curve of proline. The absorbance of the diluted sample was calculated and converted to µM g^{-1} fw.

Statistical analyses

Analyzes of variance (ANOVA) followed by Duncan's multiple range test were performed using the SPSS 18.0 for Windows statistical software package. Differences were considered significant at P<0.05.

Results

Growth characteristics

Morphological observations in our study showed that, in the irradiation samples the percentage of seed germination and average root length increased in comparison with control, but this difference in root length was not significant. EMF exposure caused significant increase in mean shoot length (Fig. II). A significant decrease in leaf area, fresh and dry weight was observed in comparison with control (Fig. III).

Photosynthetic pigments assays

The photosynthetic pigments (chlorophyll a, b, total and carotenoids) were measured in this research. Chlorophyll a and b content decreased in irradiation plants in comparison with the control plants. Total chlorophyll content showed the same pattern as chlorophyll and a b. Carotenoids concentration was significantly increased in irradiation plants in comparison with control (Fig. IV).

Non-enzymatic antioxidant activity assays

Figure (V) shows that exposing *S. bachtiarica* plants to EMF caused significant increase in the activities of non-enzymatic antioxidants such as phenol and flavonoids. Increase in the level of phenol and flavonoids is considered as an important response of EMFr. Our study showed that the content of proline significantly increased in irradiation plants. In fact electromagnetic radiation exposure induced an increase in the content of this compound as compared with the control plants (Fig VI).

Discussion

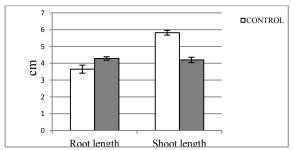ROS are usually kept in balance by the antioxidative mechanisms that exist in all living

Fig. II. Effect of low frequency electromagnetic radiation on growth parameters in *S.bachtiarica*. Results are means ±SE of 3 replicates. Significant level for Student test is shown at P<0.05.
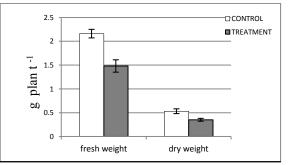

Fig. III. Effect of low frequency electromagnetic radiation on fresh and dry biomass weight in *S.bachtiarica*. Results are means ±SE of 3 replicates. Significant level for Student test is shown at P<0.05.
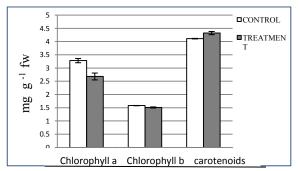

Fig. IV. Effect of low frequency electromagnetic radiation on photosynthetic pigments in *S. bachtiarica*. Results are means ±SE of 3 replicates. Significant level for Student test is shown at P<0.05.
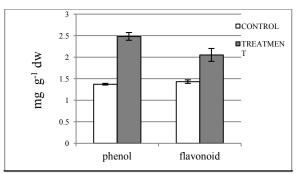

Fig. V. Effect of low frequency electromagnetic radiation on flavonoid and phenol *in S. bachtiarica*. Results are means ±SE of 3 replicates. Significant level for Student test is shown at P<0.05.

beings. Because ROS have an important signaling role in plants, their concentration must be carefully controlled through adequate pathways (Mittler, 2002). Thus, oxidative stress can be defined as inducing physiological and biochemical changes in the plant (Burritt and Mackenzie, 2003; Lesser, 2006; Burritt, 2008).

Treatment samples in our study experienced an increase in the percentage of seed germination in comparison with control. The possible reason for intensification of germination may be increasing metabolism in irradiation seeds and increase in substance consumption and more water absorption under effect of EMFr (Shabrangi and Maid, 2009).

In the treatment samples, reduction of shoot length had a destructive effect on the growth regulator indol-3-acetic acid (IAA). Practically, inhibition of elongation in EMF irradiation plants might also be due to the action of peroxidases working as IAA-oxidase and causing a decrease in cell wall extensibility (Ros and Tevini, 1998; Hosseini et al., 2011). EMF significantly decreased leaf area in the exposed plants (Noguse et al., 1998; Yao et al., 2006). Reduction of leaf area under EMF radiation is a photomorphogenic response that can limit the damage to leaf tissue caused by radiation (Jansen, 1998). Reduction of leaf area is a response to reduce extent of cell division and elongation. EMF radiation decreased the proportion of mitotically active cells and increased the time taken for cell division (Hopkins, 2002). Rate of fresh and dry weight and Leaf area in irradiation samples significantly decreased in comparison with control. The possible reason for reduction of fresh and dry biomass weight might be due to reduction of the leaf area (Noguse et al., 1998).

Electromagnetic radiation exposure, caused significant decrease in chlorophyll a, b, and total chlorophyll content, but carotenoid significantly increased. Jordan et al. (1991) explained that the decline in chlorophyll level might be due to inhibition of cab gene, which codes for chlorophyll protein. Carotenoids are known to have protective roles in safeguarding the photosynthetic apparatus and sub-cellular organelles from EMF damage. The biosynthetic pathway of carotenoids might be less influenced
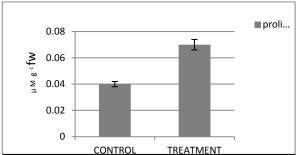

Fig. VI. Effect of low frequency electromagnetic radiation on proline in *S. bachtiarica*. Results are means ±SE of 3 replicates. Significant level for Student test is shown at $P<0.05$.

by EMFr than that of chlorophyll. The carotenoids are involved in light harvesting and photoprotection of chloroplast from the effects EMF radiation (Tevini et al., 1981).

EMF exposure caused significant increase in activities of non-enzymatic antioxidant. The increase in ROS scavenging capacity brought about by increased intracellular non-enzymatic antioxidant levels could be a key mechanism in reducing cellular damage. Plants are able to prevent the dangerous effects of EMFr by synthesizing flavonoids, a class of radiation absorbing compounds located mainly in the epidermis and acting as an internal filter. In fact, flavonoids are one of the largest classes of plant phenolic compounds and perform very different functions such as defense (Kondo et al., 1992). In higher plants, flavonoids accumulate in large quantities in the vacuoles of epidermal cells of leaves and stems and absorb EMF radiation (Flint et al., 1985; Mazza et al., 2000). Theses compounds may offer a measure of protection by screening out harmful EMF radiation (Caldwell et al., 1983; Saviranta et al., 2010). According to Tevini et al (1991), flavonoid accumulation is regarded as a defense mechanism in higher plants to provide protection against radiation; hence, it is concluded that the EMF treated seedlings may activate a defense mechanism against EMF damage by increasing flavonoid content.

Plants produce a large variety of secondary metabolites that contain a phenol group, a hydroxyl functional group on an aromatic ring called phenol, a chemically heterogeneous group. Phenols accumulate in plant tissues during stress and due to oxidant

damage. Phenols concentration also depends on the competition for the allocation of photosynthetically fixed carbon to growth or defense (Sakihama et al., 2002; Wuyts et al., 2006). Phenols could also be an important part of the plants defense system against biotic and abiotic stresses (Mittler, 2002 ; Singh et al., 1999; Wuyts et al., 2006).

EMF exposure caused significant increase in proline content. Accumulation of proline to high levels in plant cells under stress could greatly increase the ROS scavenging capacity of the cells and reduce the potential for oxidative damage. Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism (Smirnoff and Cumbes, 1989). Proline could potentially act as storage reserve of carbon and nitrogen, a compatible osmolyte, a buffer for cytosolic pH, a scavenger of reactive oxygen species (ROS), and as an aid to balancing cellular redox status (Hare and Cress, 1997; Smirnoff and Cumbes, 1989). Proline could act as a molecular chaperon, helping to stabilize the structure of proteins, and as part of the signal transduction chain alerting plant cells in the presence of a stressor and hence triggering adaptive responses (Maggio et al., 2002). In fact, proline has the potential to reduce ROS levels and could help reduce oxidative damage to vital cellular macromolecules and hence stabilize proteins (Anjum, 2000) DNA (Iakobashvil, 1999) and lipid membranes (Alia, 1991). The increase in ROS scavenging capacity brought about by increased intracellular proline levels could be a key mechanism by which proline helps reduce the cellular damage. Proline protects protein monomers and oligomeric protein complexes from denaturation and dissociation. The accumulation of proline could also be a mechanism to store energy as the oxidation of a single proline molecule can produce up to 30 ATP equivalent (Hare et al., 1997; Hare and Cress, 1999).

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