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Effects of extremely low frequency electromagnetic fields on growth and genetic diversity of (*Satureja hortensis* L.)

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ABSTRACT

Electromagnetic fields are an important environmental factor that can influence the growth and development of plants. Exposure to EMF was performed by a locally designed EMF generator. Different treatments for dry and wet seeds (from 2 mT to 4 mT for 30 to 120 minutes exposure time) were done. All the treatments showed significant difference in comparison with control plants. The highest fresh and dry weight in both root and shoot had occurred in plants grown from wet and dry pretreated seeds with 2 mT for 30 min, 2 mT for 120 min and the least amount had occurred in plants grown from wet and dry pretreated seeds with 4 mT for 120 min (Respectively). For genetic diversity DNA extraction and PCR with 12 RAPD primers were done. A total of 404 bands had generated by the 12 primers and out of that 110 bands were polymorphic bands. The primer showing maximum number of polymorphic bands was UBC100. The RAPD analysis indicated that plants grown from wet and dry pretreated seeds with 4 mT for 120 min showed genetic variation compared to control plants.

Keywords: *Satureja hortensis* L., Electromagnetic fields, fresh and dry weight, genetic diversity, RAPD primers

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INTRODUCTION

Satureja hortensis L., a well-known medicinal plant belong to Lamiaceae family, is used as a spice and traditional herb in Iran. It has shown antispasmodic, antioxidant, sedative and antimicrobial properties [23,9,6,8]. The oil of *S. hortensis* are extensively used as antioxidant and antimicrobial agents in food and pharmaceutical industries [19].

In the natural environment, living things are exposed to abiotic stress induced by electromagnetic fields (EMFs) due to distribution of different kinds of instruments and equipments[18]. All the electric devices that we use produce low-frequency electromagnetic field. Especially those who live near the electricity systems is quite low frequency and can be effective at different intensity depending on the distance to the system and power of system. It is known that biological systems give different biological responses to applications of EMF at different frequencies and intensities [6]. Various living organism are differently affected from EMF, and these effects vary according to the region applied and they occur at level of cell.

Many studies have reported the effects of magnetic field on variety of agriculturally important plants [2,16] such as studying effects of EMFs on seeds germination and seedlings growth and seed vigor [3,13,21,17]. In another research, it was found out that there were positive effect on the rooting percentage and plant growth of *Komatsuda* depending on EMF [12].

Learning about the molecular mechanisms by which plants tolerate environmental stresses is necessary for genetic engineering approaches to improve crop performance under stress. Different methods are available to investigate the effect of mutagens on plants. Molecular markers allow direct comparisons of the effects on genotypes at the DNA level. A variety of molecular techniques has been developed and is widely used in many fields such as agriculture and biology [1]. Random amplified polymorphic DNA (RAPD) may potentially form the basis of novel biomarker assays for the detection of DNA damage and mutational events (*e.g*. rearrangements, point mutation, small insert or deletions of DNA and ploidy changes) in cells of bacteria, plants, invertebrate and vertebrate animals [4].

In this study, the effects of extremely low frequency electromagnetic fields as an abiotic stress on plant growth and genetic diversity of summer savory as an important medicinal plant (*Satureja hortensis* L.) were compared to fields free plants.

MATERIALS AND METHODS

Electromagnetic field exposure

Exposure to EMFs was performed using a locally designed EMF generator. The electrical power was provided by a 220 V AC power supply (ED-345BM, China) with a variable voltage, current and fixed frequency (60 Hz). This system consisted of one handmade coil, cylindrical in form, made of polyethylene 12 cm in diameter and 50 cm in length. The coil was not shielded for electrical field and the seeds were exposed to both magnetic and electric fields generated by the coils.

Seed treatment was carried out during day light. Seeds of summer savory (*Satureja hortensis* L.) were obtained from seed and plant improvement institute, Karaj, Iran, which were selected for a uniform size, shape and equal average weight. Three replicates 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 papers in Petri dishes and then placed in the middle of a horizontally fixed coil. Untreated seeds were used as control under similar condition. The wet and dry seeds were exposed to EMFs by a magnitude of 2 to 4 mT, for 30 to 120 min. Then difference among the seedlings grown from treated seeds as well as control seedlings was determined by Analysis of Variance Test (ANOVA), followed by Duncan's multiple range test. Treated and untreated seeds(control) were grown in the pot. The most and the least significant growth of plants were chosen for genetic diversity in summer savory plant under the field condition.

Physiological Study

30 plants for each treatment were chosen for measurement of fresh and dry weight in both root and shoot tissues and shoot diameter assay.

DNA extraction and RAPD analysis

The DNA extraction method used was a slightly modifies version of that of Tsumura et al. [22]. 12 primers (10-mer oligonucleotides) from University of British Colombia (UBC) series, were selected according to the number and consistency of amplified fragments (Table 2). PCR reactions were carried out in a 25 µl volume containing 1 U of Taq polymerase, 25 ng of genomic DNA template, 0.2 µmol of primer, 2 µm of each dATP, dCTP, dGTP and dTTP and 2.5 µl of 10× PCR reaction buffer. Amplifications were performed in a DNA Thermo-cycler (Eppendorf) programmed for 45 consecutive cycles each consisting of 1 min at 92 ºC, 1 min at 37 º C and 2 min at 72 ºC. Following amplification, the samples were subjected to electrophoresis in 1% agarose gels in 1% TAE buffer. After electrophoresis, the gel was soaked in to ethidium bromide for 15 minutes. The amplified fragments were detected by UV light. UPGMA clustering and principle coordinating analysis was performed by NTSYS 2.2 software.

RESULTS

Physiological characters

The mean values of physiological characters are given in Table1.

ANOVA test, showed significant difference among the treatments used. There was no significant difference in shoot diameter in plants grown from wet and dry pretreated seeds in comparison with control plants.

The highest fresh and dry weight of both root and shoot were three occurred in plants grown from wet and dry pretreated seeds with 2 mT for 30 min , 2 mT for 120 min (Respectively) and the least were in plants grown from wet and dry pretreated seeds with 4 mT for 120 min.

Table1:Physiological trait for plants grown from dry and wet pretreated seeds in comparison with control plants. means of three replicates, numbers followed by the same are not significantly different (p>0.05)

Treatment	Shoot diameter	Fresh shoot weight	Dry shoot weight	Wet root weight	Dry root weight
Control	1.708a	1.8ab	0.56b	0.29b	0.0652ab
Dry2-30	1.682a	1.76ab	0.504bc	0.16bc	0.0432ab
Dry2-120	2.386a	2.92a	1.02a	0.386a	0.156a
Dry4-30	1.62a	1.475ab	0.501bc	0.14bc	0.04ab
Dry4-120	1.614a	1.1b	0.12c	0.08c	0.0084b
Wet	1.942a	2.64ab	1.14b	0.46b	0.096a
wet 2-30	2.336a	4.422a	1.5a	0.56a	0.1084a
wet 2-120	2.09a	1.68ab	0.916b	0.3bc	0.0845ab
wet 4-30	2.21a	1.66ab	0.96b	0. 36bc	0.072ab
wet 4-120	1.702a	0.8b	0.52c	0.14c	0.0332b

RAPD analysis

12 primers were used to analyze PCR products and they generated 404 bands. The total number of bands, the number of polymorphic bands and the percentage of polymorphism for each treatments were given in Table2.

Out of total 404 bands, 110 were polymorphic bands and the size of amplification ranged between 250 to 10000bp. The primer showing maximum number of polymorphic bands was UBC100 (16bands) in Fin.1. The polymorphism shown by different primers ranged from 5.8 to 80%. The UPGMA clustering were shown in Fig.2.

The accessions were grouped by subjecting the Jaccard's analysis similarity values to UPGMA clustering. The treatments were grouped in to two major clusters and the genetic distance between two clusters was 0.69. Cluster A included 2 treatments (S3=Plants from dry pretreatment seeds grown in 4 mT for 120 min, S6= Plants from wet pretreatment seeds grown in 4 mT for120 min). Cluster B had 4treatments (S1=Control plants from dry seeds,S2=Plants from dry pretreatment seeds grown in 2 mT for 120 min, S4=Control plants from wet seeds, S5= Plants from wet pretreatment seeds grown in2 mT for 30 min).

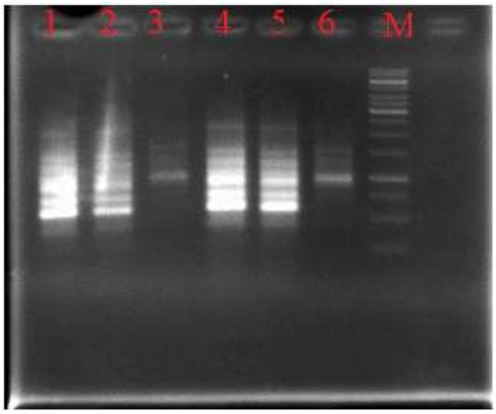

Fig1.Amplification profiles of savory treatments employing RAPD primer UBC100. 1:Dry control, 2:Seed under 2mT for 120 min, 3:Seed under 4mT For 120 min , 4:Wet control, 5:Wet seed under 2mT for 30 min 6:Wet seed under 4mT for 120 min, M:Marker

Table2: Results of RAPD analysis for plants pretreated with electromagnetic field and control plants

Primer name	Base sequence5'-3'	Total band	Polymorphic band	% polymorphic band
UBC1	CCTGGGCTTC	34	2	5.88
UBC3	CCTGGGCTTA	34	2	5.88
UBC5	CCTGGGTTCC	34	8	23.52
UBC9	CCTGCGCTTA	37	17	45.94
UBC13	CCTGGGTGGA	32	16	50
UBC66	GAGGGCGTGA	36	2	5.55
UBC76	GAGCACCAGT	36	2	5.55
UBC77	GAGCACCAGG	36	18	50
UBC84	GGGCGCGAGT	42	10	23.80
UBC89	GGGGGCTTGG	36	2	5.55
UBC95	GGGGGGGTTGG	27	15	55.55
UBC100	ATCGGGTCCG	20	16	80

The UPGMA dendrogram showed considerable genetic distance in individuals grown from wet and dry pretreated seeds with 4 mT and 120 min exposure time in comparison with control plants, but there was no significant difference among other treatments compared with control plants.

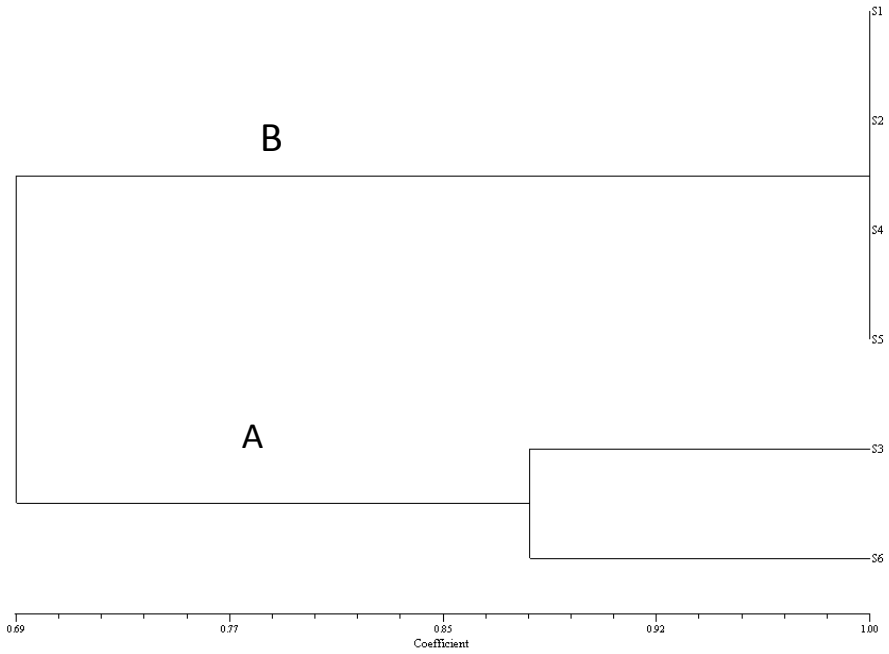

Fig2:UPGMA dendrogram of treated and control plants

DISCUSSION

Numerous biological effects of extremely low frequency electromagnetic fields were recorded in the last decades. They were observed at various cellular or molecular levels in living tissues. However no clear interaction mechanisms yet proved [13]. Magnetic field is known as an environmental factor, which mostly effects on gene expression [11].

Summer savory plants grown from dry and wet pretreated seeds with 2 mT for 120 min, 2 mT for 30 min showed the highest fresh and dry weight in comparison with controls. Similar experiment was reported that 16 Hz electromagnetic field of low intensity (20 microT) caused significant increase in the fresh weight of young plantlets of *Helianthus annuus* [7] .

Plants from dry and wet pretreated seeds with 4 mT for 120 min, showed the least fresh and dry weight in comparison with controls. Also, Ramezani et al. (2012) stated that ***Satureja bachtiarica*** from wet pretreated seeds with 1mT for 2 hr showed significant decrease in mean shoot length, leaf area and fresh and dry weight compared with control plants [15]. Additionally, Tkalec et al. (2005) found out that *Lemna* from pretreated seeds with 400,900,1900 MHz electromagnetic fields showed significantly reduction in growth by increasing the time of exposure and frequencies of electromagnetic fields in compared with control plants [20].

Plants grown from dry and wet pretreated seeds with 4 mT for120 min showed genetic variation in comparison with control plants. This result indicated that may be in this frequency of electromagnetic field and in this duration, the DNA fragmentation could be due to the leaks in the membranes surrounding lysosomes which release digestive enzymes like DNAase and may explain the damage done to DNA after exposing to electromagnetic field [5 ,14]. Some workers have found that the damage of one base pair in the target sequence of the genome may result in a completely different RAPD profile, since each 10 bp oligonucleotide primer only covers a very limited part of the genome [10].

REFERENCES

[1] A Abdolhamid. Genomics at a Glance , Lambert Academic Publishing, Germany, **2011**, 107.
[2] A De Souza, D Garcia, L Sueiro, L Licea, E Porras. *Spanish j.Agri.Res.*, **2005**,3,1,113-122.
[3] D Bhatnagar, *AR Deb. Seed Res*, **1977**, 5,129-137.
[4] D Sawa. *Toxicology and Ecotoxicology*,**1996**, 3, 110-114.

[5] E Diem, C Schwarz, F Adlkofer, O Jahn, H Rüdiger. *Mutatation Research*, **2005**, 583, 178-183.
[6] G Eugene, G Ben, M Michael. *Int.Reu.Cytol*,**1995**,158,297-338.
[7] G Fischer, M Tausz, M Kock, D Grill. *Bioelectromagnetics*, **2004**, 25, 638–641.
[8] G Ulvi, G Mervi, G Orhan, C Lutfu, K Eija, U Veli Jukka. *Anaerobe*, **2009**, 15, 164–167.
[9] H Valliollah, S Hassan, G Alireza, M Mandana. *J. Ethnopharmacol*, **2000**, 71, 187–192.
[10] H Zhang, F Hashinaga. *Journal of the Japanese Society for Horticultural Science*, **1997**, 347-152.
[11] J C Scaiano, FL Cozens, N Mohtat. *Photochemistry and Photobiogy*, **1995**,l 62, 818-829.
[12] K Namba, A Sasao, S Shibusawa.*Acta Horticulture* , **1995**,399, 143-148.
[13] M Jae Duk, C Hwa. *Electrostatics*, **2000**, 48,103-114.
[14] P Dimitris, C Evengelia, N Loannis, M Lukas. *Mutation Research*, **2007**, 10, 69-78.
[15] R Fariba, M Ahmad, N Taher ,A Sedighhe. Iranian *Journal of Plant Physiology*, **2012**, 2, 4, 509 - 516.
[16] S Abdul R.H, A Reyad, A J Waleed, F H Hussain.*J.Nat.Prod.Plant Resour*.,**2012**,2,3,456-459.
[17] S Azita, M Ahmad. *Progress In Electromagnet. Res*.,**2010**,10,46,5-8
[18] S Azita, M Ahmad, Sh Masoud. *African Journal of Biotechnology*,**2011**,10 ,46, 9362-9369
[19] S Pack. *Int. J. Aromath*, **2003**, 13, 196–202.
[20] T Mirta, M Kresimir,P K Branka.*Bio Electro Magnetic*, **2005**, 26,3, 165-193
[21] U J Pittman. Canad. J. Plant Sci.,**1977**, 57, 37-45.
[22] Y Tsumura, K Yushimura, N Tomaru, K OHBA. *Theor Appl. Genet*, **1995**,91,1222-1236.
[23] Z Ali. Medicinal Plants, 4th edn. Tehran University Publications, Tehran, Iran,**1990**,325.