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Original Article

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Comparing cytogenetic effects of extremely low frequency electromagnetic fields in Brassica napus L and Zea mays L

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Abstract

Many biological effects of exposure to extremely low frequency electromagnetic fields (ELF-EMFs) have been documented, but little work carried out on plants. A meiotic study was performed on Brassica napus L as C3 plant and Zea mays L as a C4 plant exposed to electromagnetic fields. Our investigations were focused on plants grown from wet pretreated seeds with 3 and 10 mT for a 4 h exposure time and compared with the control plants of maize. Also our investigation used plants grown from dry pretreated seeds with 10 mT for 4 h, wet pretreated seeds with 10 mT for 2 h, and a control line of canola. A significant difference was observed for meiotic characters studied among treatments. In canola the mean value of total, terminal, and intercalary chiasmata reduced significantly in plants grown from exposed seeds, indicating that EMFs caused reduction in the mean value of the genetic recombination. Other meiotic characteristics including ring bivalent and quadrivalent formation reduced significantly as well. In maize the mean value of total, terminal and intercalary chiasmata changed significantly in the plants grown from exposed seeds to 3 mT intensity, indicating increase in genetic recombination. Moreover, formation of ring bivalent and quadrivalent increased significantly.

All results suggested these plants react differently in some aspects against EMFs as abiotic stress.

Key words: Mutagenesis, cytogenetic, chromosomal aberration, electromagnetic fields

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1. Introduction

There is a controversial discussion if electromagnetic fields (EMFs) cause induction the biological effects, which might be harmful to organisms' health. Plants have been exposed to a continuous abiotic stress produced by EMFs. Various researchers proved that low frequency EMFs have biological effects on different organisms. Different species of plants show alteration in response to environmental stresses as a result of having various capabilities for stress perception and sensitivity (Bohner et al., 1995). Researchers believe that EMFs lead to oxidative stress and lifetime and concentration of free radicals increase under effect of this stress (Accorsi, 2001). Subsequently, EMFs have the potential to alter gene expression, protein biosynthesis and enzyme activity. Also, EMFs affect on cell reproduction and cellular metabolism (Nirmala and Rao, 1996). There are many reports on EMFs effects on plants , including cytological effects and changing the mitosis control mechanisms (Pavela et al., 2005) Also, some researchers that EMFs cause increase in the different chromosomal aberrations such as bridges, stickiness and lagging chromosomes (Pittman, 1977; Patil and Bhat, 1992; Selga and Selga, 1996). However, not much is known about the exact mechanism of action of EMFs in plant growth induction or reduction. The present study examines the effects of EMFs on cytogenetic of higher plants including Brassica napus L(C3 plant) and Zea mays L(C4 plant). This would help to improve a general knowledge on the mechanisms and response of higher plants to EMFs.

2.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. Calibration of the system as well as tests for the accuracy and uniformity of EMFs (60 Hz) were performed using a tesla meter (516 62, LEYBOLD, Germany) with a B-probe type of hall sound. Applied magnetic field was measured to be from 1 to 10 mT, uniform at all points of samples. A normal fan was employed to avoid any increase in temperature. The temperature was measured with a thermometer to be 22+1°C. The power was 15 min on and 15 min off. The temperature was measured with a thermometer to be 22+1°C. We measured the temperature of the middle of coil, where the samples were placed and the room temperature was 22°C. When the power was on, the fan worked in order to lower the temperature, which increased up to 1°C. The B-probe dimensions without the stand rod was 40 × 35 × 340 mm (516 60, LEYBOLD, Germany). An electrical current of 60 Hz was used to generate the magnetic field in the coil and the
magnitudes of magnetic fields calculated from $B = \mu^{\circ} n I$ and measured by probe were in very good agreement. Seed treatment was carried out during day light.

In the preliminary study both wet and dry seeds of canola (B. napus) and maize (Z. maize), were treated with 1, 3, 5, 7 and 10 mT in 1-4 h exposure time. Seeds 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. Then significant 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 tests.

Finally, treatments used for cytogenetics studies were selected which showed the most significant differences in growth parameters including the length of root and shoot, biomass and dry seeds weight (Shabrangi and Majd, 2009). In continues of our previous work, in the present study we focused on wet seeds of canola treated with 10 mT for a 2 h and dry seeds of canola treated with 10mT for a 4h exposure time, and wet seeds of maize treated with 3mT and 10mT both for 4h exposure time. These pretreated seeds were planted in the open agricultural fields.

Cytogenetic studies

For meiotic analysis, flower buds were collected from 10 randomly selected plants from each treatment and the control. Young flower buds were collected during 09:00/12:00 and fixed in acetic acid absolute ethanol (1:3 v/v) for 24 h. After fixation the flower buds were stored in 70% ethanol at 4 °C. For cytological analysis, six replicates were performed for each treatment and control; the content of the flower buds anthers were squeezed out onto slides and stained using the aceto-orsein smearing technique (Sheidai et al., 2003). Chromosome pairing and chiasma frequency was determined by using a minimum of 100 meiocytes showing diakinesis/metaphase-I stages, while chromosome segregation was studied in a minimum of 100 anaphase-I and anaphase-II stages. ANOVA followed by Duncan's multiple range tests was used to indicate significant differences in meiotic characteristics among different treatments used. Cytological abnormalities were studied by using the x2 test.

3.Results

Meiotic analyses in Brassica napus L

Chiasma frequency and distribution as well as chromosome associations among control plants and plants grown from exposed seed are given in Table 1. ANOVA test followed by Duncan's multiple range test revealed significant difference ($p < 0.01$) for all meiotic characters studied among genotypes. The mean value of total, terminal, and intercalary chiasmata seems to be reduced significantly in the plants grown from exposed seeds. This means that EMFs significantly reduce the mean value of genetic recombination.

Table 1: Meiotic characters in B. napus genotypes studied

Sample	TX	IX	TOX	RB	ROD	IV	I	M1S	L1	A1S	M2S	L2	A2S	M
1	17.67	4.71	22.79	9.54	2.75	3.33	3.25	0.083	0	0	0	0	0	0
2	16.68	3.42	20.45	9.35	3.48	2.39	4.74	0.067	0.093	0	0.053	0.09	0.053	0.29
3	14.85	2.82	17.68	7.53	2.91	2.38	6	0.073	0.12	0.097	0.0137	0.03	0.097	0.26

Significant from control at 0.05 level (t-test).1 = Control plants, 2 = Plants raised from dry seed treated, and 3 = Plants raised from wet seed treated.

Abbreviations: N = Sample size, TX = Total chiasmata, IX = Intercalary chiasmata, TOX = Total chiasmata, RB = Ring bivalents, ROD = Rod bivalents, IV = Quadrivalents, I = Univalents, M1S =Metaphase-I stickiness, L1 = Anaphase-I laggards, A1S = Anaphase-I stickiness, M2S = Metphase-II stickiness, L2 = Anaphase-II laggards, A2S = Anaphase-II stickiness, M = Micronuclei formation. Sample size = 100.

Other meiotic characteristics including ring bivalent and quadrivalent formation were also significantly reduced in the plants grown from treated seeds. The genotypes studied formed mainly ring and rod bivalents in metaphase of meiosis-I. However, a low value of univalents and quadrivalents were observed in all of them (Fig. 1a,c). The recorded abnormalities are mostly stickiness, laggards and micronuclei formation (Fig. 1a–e). Micronuclei formation was the most pronounced phenomenon observed in meiotic cell divisions. Lagging chromosomes were recorded at anaphase-I and II stages in both treatments (Table 1). Chromosome bridges resulting from stickiness were observed in anaphase-I and II as well as telophase-I and II stages.

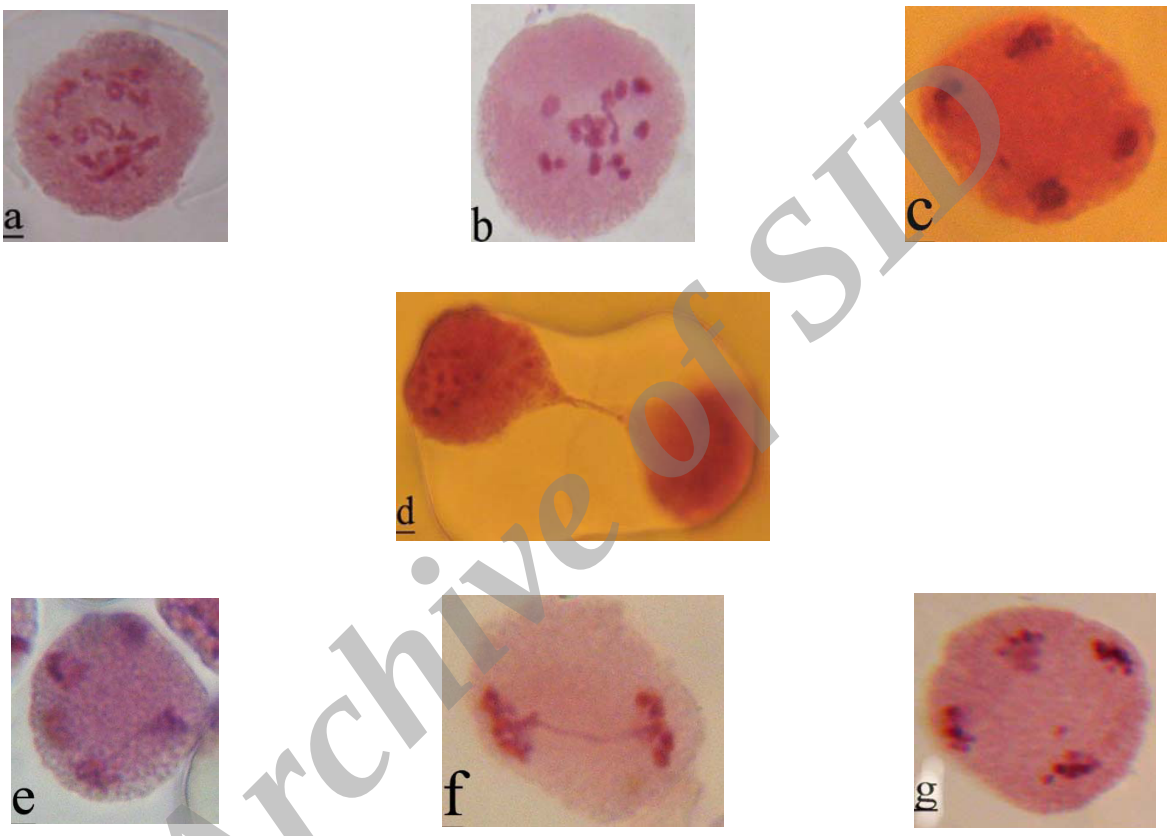

Figure 1: Representative meiotic cells in canola treatments: (a)chromosome stickiness in control plants of canola; (b) aneuploid cell showing reduction in chromosome number in plants raised from treated dry seeds;(c) Laggard chromosome in plants raised from treated dry and wet seeds, respectively; (d) cytomictic cells in plants raised from treated dry seeds; (e) multipolar cell in plants raised from treated wet seeds, respectively;(f) Anaphase-I bridge in plants raised from treated wet seeds; (g) micronuclei formation in plants raised from treated dry seeds.

Meiotic analyses in Zea mays L

Meiotic analysis concerning chiasma frequency and distribution as well as chromosomes association among control plants and plants grown from exposed seed are given in Table 2. ANOVA test revealed significant difference ($p<0.01$) for all meiotic characters studied among genotypes.

Table 2: Meiotic characters in Z. mays tratments studied.

Sample	TX	IX	TOX	RB	ROD	IV	I	DC	L1	B	C	M	IP
1	9.703	2.781	12.487	5.405	3.162	0.135	2.476	0	3.7	3.33	6.3	0	0.4
2	11.271	4.104	15.375	7.188	1.479	0.063	1.250	25	25	25	7.7	11	14
3	9.262	2.905	12.167	5.667	1.810	0.071	1.270	29.7	30	12.3	18	12	49.7

**Significant from control at 0.05 level (t-test). 1 = Control plants, 2 = Plants raised from seed pre-treated with 3 mT for 4 h and 3 = Plants raised from seed pretreated with10 mT for 4 h.*
TX = Total chiasmata, IX = Intercalary chiasmata, TOX = Total chiasmata, RB = Ring bivalents, ROD = Rod bivalents, IV = Quadrivalents, I = Univalent, DC =disorganized chromosome, L1 =Anaphase-I laggards, B= Anaphase-I Bridges, M = Micronuclei formation, C= Clumping, IP= infertile pollen.

The mean value of total, terminal and intercalary chiasmata increased significantly in the plants
grown from exposed seeds to 3 mT for a 4 h exposure time, while the same values were
reduced in the plants grown from exposed seeds to 10 mT for a 4 h exposure time. The mean
number of ring bivalent also increased significantly in the plants grown from treated seeds, while the mean values of rod bivalent, quadrivalents and univalents reduced significantly in the plants grown from treated seeds. Meiotic abnormalities observed in the plants grown from exposed seeds include: Chromosome stickiness, bridges, laggards and disorganized chromosomes (Figure 2A to G). Disorganized chromosomes were the most phenomenons observed in meiotic cell divisions. Lagging chromosomes were recorded at anaphase-I stage in both treatments. Chromosome bridges resulting from stickiness were mostly observed in anaphase-I as well as telophase-I stage.

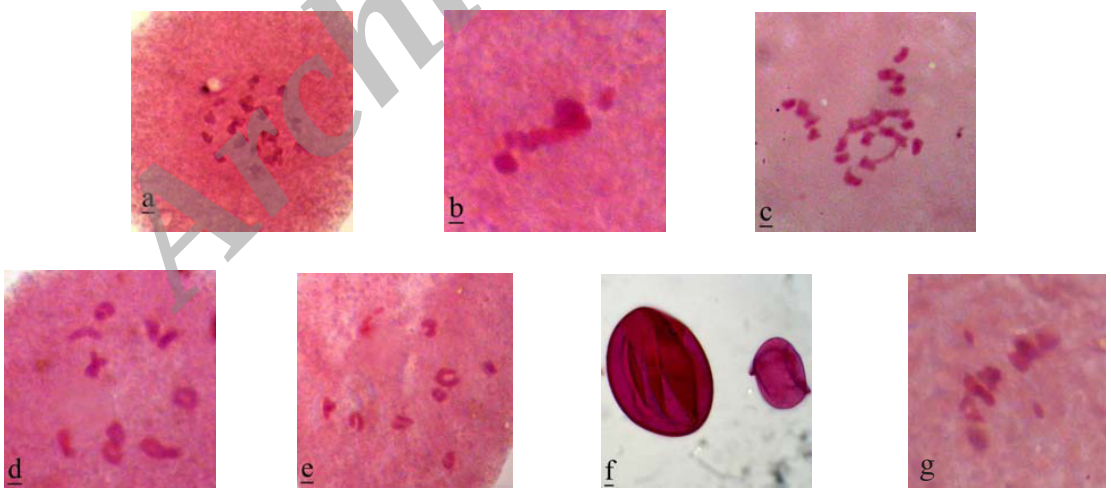

Figure 2: Representative meiotic cells in Maize treatments studied.a: Meiocyte showing extra chromosomes in pretreated plants; b,d : Meiocyte showing quadrivalent , in plants raised from pretreated plants; c: Anaphase-I bridge in pretreated plants; e: Meiocyte showing aneuploidy (chromosome number reduction, 2n-1) in plants raised from seed pretreated with10 mT for 4 h; f: :Infertile pollen grains (smaller size pollen grain) in plants raised from seed pretreated with10 mT for 4 h and the last one is disorganized chromosome.

4. Discussion

It is assumed that 50/60-Hz EMFs do not have sufficient amounts of energy transferring to cells in order to damage DNA directly, which lead to genotoxic effects (Sander et al., 1982). However, cellular processes alteration by exposure to EMF, like generation of free radicals, affect the structure of DNA, which would cause strand breaks and other chromosomal aberrations. Also, they may lead to cytotoxic effects inducing cell death (Piacentini et al., 2001). Genotoxic effects of EMF exposure like chromosomal aberration, micronucleus formation (Hanafy et al., 2006), and chromatid breaks was reported by different researchers (Nirmala and Rao, 1996; Ruediger, 2009).One of these chromosomal aberration is bridges that cause gene duplication at one pole. Deletion at the other pole would occur, while laggards chromosomes would cause pole producing such as monosomic or trisomic cells (Galland and Pazur, 2005). A x2 test on the results showed significantly difference of the mean values of meiotic abnormalities that occurred among the treatments indicating their genetic differences. Ruediger reported chromosome stickiness in some species of plants under effects of EMFs (2009). It seems that the main reason for chromosome stickiness, occurring in pretreated plants, is genomic-environmental interaction such as EMFs.

Some meiotic aberrations such as occurrence of cytomixis in pretreated plants would cause the formation of aneuploid cells (Fig. 1f). Also, tripolar and multipolar cell formation would be the result of cytomixsis (Fig. 1h–j). The previous cytogenetic studies in B. napus cultivars and some of their hybrids did not show any aneuploid cell (Sheidai et al., 2001; Sheidai et al., 2003) . All these results suggested EMFs may be the reason for the formation of aneuploid cell in this study.

Cytomixis seems to form aneuploid and polyploid meiocytes and leads to the reduction in the fertility of plants (Sheidai et al., 2001) . Also, cytomixis may cause the formation of unreduced gametes, in condition that the whole chromatin material migrate among the neighbouring meiocytes. In this study, we observed significantly increase in the percentage of infertile pollen due to EMFs. Furthermore, cytomixsis may cause the formation of meiocyte with missing chromatin materials. Therefore, the formation of abnormal tetrads increased significantly in pretreated plants.

Promila and Bhattacharya studied the effect of static EMFs on the mitotic and meiotic divisions and nucleoli of root meristematic cells of Allium cepa. They reported a significant decrease in mitotic indices with many aberrations including clumping of chromosomes, disturbed spindles, Bridge, and stickiness (1991). All results suggested that EMFs cause changes in genetic recombination of the pretreated plants compared to control line and may cause aneuploidy production and also unreduced gamete formation.

Chiasma frequency and distribution are controlled genetically (Fadaei et al., 2010), and alteration in chiasma frequency indicates effects of EMFs on genetic recombination.

B-chromosomes, found in many species of plants, would affect the frequency and distribution of chiasmata. Therefore, B chromosomes may affect meiotic characters or affect genes controlling A-chromosomes meiosis (Sheidai and Inamdar, 1993). Meiotic study in pretreated plants with EMFs showed increase in the intercalary chiasmata and the mean number of ring bivalents, which would be the result of increase of B-chromosome. C4 plants such as maize are assumed to be more resistant than C3 plants(canola) against different stresses. In this study, we observed a significant increase in the intercalary chiasmata and mean number of ring bivalents of maize as a C4 plant. On the other hand, the mean value of total, terminal, and intercalary chiasmata reduced significantly in pretreated plants, indicating that EMFs significantly reduce the mean

value of the genetic recombination in canola as a C3 plant. Authors concluded genomic changes occurred by EMFs in maize caused increasing recombination values. Therefore, cytological alterations including aneuploidy production could be used in breeding of maize plant as suggested in some other crop plants (Pavela et al., 2005). Hence, EMFs would be a reasonable biotechnological tool in agricultural plant culture (Racuciu and Creanga, 2007).
Conflict of interests : None declared.

5. REFERENCES

Accorsi A. Senescence delay and change of antioxidant enzyme levels in Cucumis sativus L. etiolated seedlings by ELF magnetic fields. Plant Sci 2001; 161:45–53.

Bohner H, Nelson D, Jenson R. Adaptation to environmental stresses. Plant Cell 1995; 7:1099–111.

Fadaei F, Sheidai M, Asadi M .Cytological study the genus Arenaria L. (Caryophyllaceae). Caryologia, 2010; 63(2):149-156.

Falistocco E, Tosti T, Falcinelli M . Cytomixis in pollen mother cells of diploid 5-Dactylis, one of the origin of 2n gametes. J Hered 1995; 89: 448–53.

Galland P, Pazur A . Magnetoreception in plants. J. Plant Res. 2005; 118(6): 371-389.

Nirmala A, Rao P N Genetic of chromosome numerical mosaism in higher plants. Nucleus 1996; 39: 151-175.

Nirmala A, Rao PN . Genesis of chromosome numerical mosaism in higher plants. Nucleus 1996; 39:151–75.

Pavela A, Dorina-Emilia C . Chromosomal aberrations in plants under magnetic fluid influence. J Magn Magn Mater 2005; 289: 469–72.

Patil BC, Bhat GI. A comparative study of MH and EMS in the induction of chromosomal aberration on lateral root meristem in Clitoria ternatea L. Cytologia 1992; 57: 295–64.

Pittman UJ Effect of magnetic seed treatment on yields of barley, wheat, and oats in southern Alberta. Can J Plant Sci 1977; 57:37–45.

Selga T, Selga M. Response of Pinus Sylvesteris L. needles to electromagnetic fields cytological and ultrastructural aspects. Sci Total Environ 1996; 180: 65–73.

Sheidai M, Manossorniya MR, Ahmadi MR Cytomorphological study of some rapeseed (Brassica napus L.) cultivars in Iran. Nucleus 2001; 44: 42–5.

Sheidai M, Noormohammadi Z, Kashani N, AhmadiM .Cytogenetic study of some rapeseed (Brassica napus L.) cultivars and their hybrids. Caryologia 2003; 56: 387–97.

Sheidai M, Noormohammadi Z, Mirabdolbaghi- Kashani N, Ahmadi MR .Cytogenetic study ofsome rapeseed (Brassica napus L.) cultivars and their hybrids. Cytology 2003; 56: 386–97.

Promila R, Bhattacharya S. Effect of magnetic field on the living cells of Allium cepa L. Cytologia 1991; 56: 63–72.

Racuciu M, Creanga D. Cytogenetic changes induced by aqueous ferrofluids in agricultural plants. J Magn Magn Mater 2007; 311: 288–90.

Pavela A, Creanga D, Dorina-Emilia C. Chromosomal aberrations in plants under magnetic fluid influence. J. Magnetism Magnetic Materials J. 2005; 289: 469-472.

Piacentini MP, Fraternale D, Piatti E, Ricci D,

Vetrano F, Dachà M, Accorsi A . Senescence delay and change of antioxidant enzyme levels in Cucumis sativus L. etiolated seedlings by ELF magnetic fields. Plant Sci. 2001; 161(1): 45-53.

Ruediger HWR . Genotoxic effects of radiofrequency electromagnetic fields. Pathophysiology, 2009; 16: 89-102.

Sander R, Brinkmann J, Ku¨hne B. Laboratory studies on animals and human beings exposed to 50 Hz electric and magnetic fields. CIGRE, International Cong. Large High Voltage Electric Syst. September 1982; 1-9, Paris, Abstract number 36-01.

Shabrangi A, Majd A . Comparing Effects of Electromagnetic Fields (60 Hz) on seed germination and seedling development in Monocotyledons and Dicotyledons. Progress In Electromagnet. Res. Symp. Proceed. Moscow, Russia, August 2009; 18-21.

Sheidai M, Inamdar AC. B-chromosomes in Asparagus L. Nucleus, 1993; 44: 141-144.