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Cytogenetic abnormalities caused by extremely low frequency electromagnetic fields in canola

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ABSTRACT: A meiotic study was performed on *Brassica napus* exposed to electromagnetic fields. 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. A lower seed yield was produced in the plants grown from exposed grains. However, the weight of 1000 seeds were higher in the plants grown from exposed grains. The highest value of the weight of 1000 seeds occurred in the plants grown from pretreated dry seeds with 10 mT for 4 h. The size of yielding seeds of plants grown from dry pretreated seeds with 10 mT for 4 h were higher than that of the control seeds and they were of uniform size. A significant difference was observed for meiotic characters studied among genotypes. The mean value of total, terminal, and intercalary chiasmata seems to be reduced significantly in plants grown from exposed seeds, indicating that EM significantly reduces the mean value of the genetic recombination. Other meiotic characteristics including ring bivalent and quadrivalent formation were also reduced significantly in the plants grown from treated seeds. Cytological abnormalities observed are chromosome stickiness, laggards, and micronuclei formation which differed significantly among the genotypes indicating their genetic differences. Cytomixis occurred in treated plants, which led to the formation of aneuploid cells.

KEYWORDS: *Brassica napus*, cytogenetic chromosomal aberration, magnetotropism

INTRODUCTION

In the natural environment, plants are exposed to a continuous abiotic stress induced by electromagnetic (EM) fields. It has been shown that low frequency EM fields have an effect on microorganisms, plants, and animals. There are many studies showing that EM flux and exposure time affects different features of plants[1]. Plant species vary in their sensitivity and response to environmental stresses because they have various capabilities for stress perception, signalling, and response[2].

EM fields bring about oxidative stress, i.e., they increases in the activity, concentration and lifetime of free radicals[3]. Numerous effects of magnetic as well as electromagnetic fields on plants have been reported, including increased regeneration capability[4], delayed senescence, alterations in antioxidant enzyme levels, and stimulation of phosphoinositide breakdown[5]. EM cytological effects include changing the mitosis control mechanisms[6] and increasing the percentages of chromosomal aberrations such as stickiness, bridges, disturbances, fragments, lagging chromosomes, and micro nuclei[7–9]. Agronomic characteristics of plants exposed to EM fields are known to change too. For example, enhancement of growth under magnetic conditions[10–13] and increase in seed vigour, seedling growth, and yield[14–16] have been reported. A reduction in wheat and corn yield in fields near high voltage lines[17] has also been reported.

Magnetic fields have been widely used as a pretreatment for seeds for root development in plant cuttings[18]. Several studies have tried to determine the effect of electromagnetic fields on plant growth, development rates of cell division[4,19,20], and enhancement or inhibition of flowering[21]. Senescence is delayed and antioxidant enzyme levels changed in *Cucumis sativus* etiolated seedlings[3]. However, not much is known about the exact mechanism of action of EM fields in inhibiting or inducing plant growth.

Oilseed rape/canola (*Brassica napus* L.) is an important oil-producing plant cultivated in Europe, North America, China, and Iran. It is an amphidiploid species with 19 pairs of chromosomes. *B. napus* and has been evolved by crossing between *B. campestris* (2n=2x=20) and *B. oleracea* (2n=2x=18). Some basic cytogenetic information has been reported in *B. napus* cultivars available in Iran[22–24]. The present research

examines the effects of EM fields on cytogenetic and morphological characteristics of this species.

MATERIALS AND METHODS

Electromagnetic field exposure

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

Calibration of the system as well as tests for the accuracy and uniformity of the EM fields were performed using a tesla meter (516 62, LEYBOLD, Germany) with a Hall effect based B-probe. The B-probe dimensions without the stand rod were 40 mm $\times$ 35 mm $\times$ 340 mm.

An electrical current of 60 Hz was used to generate the magnetic field in the coil. The magnitude of the magnetic field was calculated from $B = \mu_0 nI$ and agreed well with the value from the probe. The applied magnetic field was varied from 1 to 10 mT and was uniform across the samples. A fan was employed to avoid any increase in temperature. The temperature was measured with a thermometer to be 22 ± 1 °C. The power to the coils was 15 min on and 15 min off.

Seeds of *Brassica napus* (Zarfam genotype) plants were grown in the field as control and also after treatments. For the wet seed treatment, the seeds were first grown on moist filter paper in Petri dishes and then planted in the field. Treatments used were selected according to Ref. 13, namely, three replicates were used in the experiment with 30 seeds in each treatment. For the wet seeds treatment, the seeds were spread on moist filter paper in Petri dishes and then placed in the middle of a horizontally fixed coil. Untreated seeds were used as a control under similar conditions (placed in the coil but with no power). The wet and dry seeds were exposed to EM fields of 1, 3, 5, 7 and 10 mT and in each case for 1, 2, 3, and 4 h. The differences among the seedlings grown was determined by ANOVA and Duncan's multiple range test.

Petri dishes were placed in a seed germination chamber at 23 °C, relative humidity 20%, photo period of 14 h day/10 h dark, and watered daily. The number of germinated seeds was recorded on the 5th day after watering and 7–10 day old seedlings from each replicate were randomly chosen for shoot and root length as well as fresh weight measurements. Subsequently, seedlings were dried in an oven at 62 °C for 48 h and dry weights were measured. Treated and untreated (control) seeds were grown in the field. Wet seeds were treated with 3 and 10 mT for 4 h.

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[23]. 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 II stages. ANOVA followed by Duncan's multiple range test was used to indicate significant differences in meiotic characteristics among different treatments used. Cytological abnormalities were studied by using the χ^2 test.

Morphological study

For the morphological study, the plants from each treatment were sampled at the harvest, and the pod length, number of pods per main shoot, number of seeds per pod, number of shoots per plant, and 1000 seed weight were measured. ANOVA followed by Duncan's multiple range test was used to indicate significant difference among different treatments used. For morphological and cytogenetic studies a minimum of 20 randomly selected plants were analysed.

RESULTS AND DISCUSSION

Morphological Characters

The mean values of the morphological characters is given in Table 1. ANOVA followed by Duncan's multiple range test showed significant difference among the genotypes/treatments used. A lower seed yield was produced in the plants grown from exposed grains, while the weight of 1000 seeds were higher in the plants grown from exposed grains. The highest value of the weight of 1000 seeds occurred in the plants grown from dry seeds pretreated with 10 mT for 4 h. (Table 1).

The size of yielding seeds of plants grown from dry pretreated seeds were higher than for the control

Table 1 Mean values (±SE) of morphological characters in canola genotypes.

Genotypes	Pod No.	Pod Length	Shoot No.	Seed weight
1	22.6 ± 1.1	6.3 ± 0.2	2.7 ± 0.2	3.5 ± 0.8
2	15.7 ± 0.8	5.7 ± 0.1	2.3 ± 0.3	5.5 ± 1.0
3	14.8 ± 1.1	6.0 ± 0.2	2.3 ± 0.1	3.7 ± 0.9

1 = Control plants, 2 = Plants raised from treated dry seeds, and 3 = Plants raised from treated wet seeds. Sample size = 32.

seeds and they were uniform in size. We observed no uniformity in size of seeds from plants grown from wet pretreated seeds with 10 mT for 2 h.

The morphological results obtained show that exposure of the seeds to EM fields resulted in a significant inhibition in all the parameters including number of pods per main shoot, number of seeds per pod, length of pods, and number of shoots per plant (Table 2, $p < 0.05$). EM fields also caused delay in flowering and senescence of pretreated plants, especially in plants grown from wet seeds pretreated with 10 mT for 2 h.

Meiotic analyses

Chiasma frequency and distribution as well as chromosome associations among control plants and plants grown from exposed seed are given in Table 2. 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 EM fields significantly reduce the mean value of genetic recombination. 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). Domesticated *B. napus* exhibits predominantly bivalent chromosome pairing, while resynthesized *B. napus*, formed from interspecific crosses between *B. campestris* and *B. oleraceae*, exhibits multivalent formation that probably reflects pairing between homologous chromosomes[25]. Therefore it may be suggested that residual homologous recombination in the genotypes studied may be the reason for quadrivalent formation in the cultivars studied. This has also been suggested to be

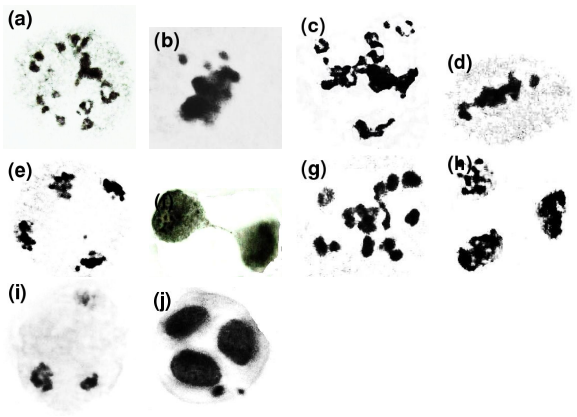

Fig. 1 Representative meiotic cells in canola genotypes: (a),(b) chromosome stickiness in control plants of canola; (c),(d) chromosome stickiness in plants raised from treated dry and wet seeds, respectively; (e) micronuclei formation in plants raised from treated dry seeds; (f) cytomictic cells in plants raised from treated dry seeds; (g) aneuploid cell showing reduction in chromosome number in plants raised from treated dry seeds; (h),(i) tripolar cells in control plants of canola and plants raised from treated dry and wet seeds, respectively; (k) multipolar cell in plants raised from treated wet seeds.

the casein other *B. napus* domesticated cultivars[22–24]. Reduction of quadrivalent values in treated plants may also show the effect of EM fields in such residual homologous recombination.

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 2). Chromosome bridges resulting from stickiness were observed in anaphase-I and II as well as telophase-I and II stages. Stickiness occurred among two pairs of chromosome and more, leading to the formation of a complete clump (Fig. 1a–d). The percentage of cells showing stickiness differed among the cultivars studied. A χ^2 test showed significant difference in the mean values of meiotic abnormalities observed among the genotypes indicating their genetic differences.

Micronuclei originate either from centric fragments or from laggards. Micronuclei are indicators of mutagenic aspects which may lead to the loss of genetic material and have been regarded as an indication of the mutagenicity of their inducers[17]. Genetic as well as environmental factors have been considered as the reason for chromosome stickiness. The dif-

Table 2 Meiotic characters in *B. napus* genotypes studied (±SE).

Samples	TX	IX	TOX	RB	ROD	IV	I	M1S	L1	A1S	M2S	L2	A2S	M
1	17.6 ± 0.7	4.7 ± 0.4	22.8 ± 0.9	9.5 ± 0.4	2.7 ± 0.4	3.3 ± 0.3	3.3 ± 0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0
2	16.6 ± 0.7	3.4 ± 0.4	20.4 ± 1.0	9.3 ± 0.6	3.5 ± 0.3	2.4 ± 0.2	4.7 ± 0.4	0.1	0.1	0.0	0.1	0.1	0.1	0.3
3	14.8 ± 0.4	2.8 ± 0.3	17.7 ± 0.5	7.5 ± 0.3	2.9 ± 0.3	2.4 ± 0.2	6.0 ± 0.3	0.1	0.1	0.1	0.0	0.0	0.1	0.2

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.

ference observed in the percentage of the stickiness among the genotypes studied may also indicate the effect of genomic background on this phenomenon. However, genomic-environmental interaction cannot be ruled out as also suggested for a similar situation in *B. napus*[22–24] and *Avena sativa* cultivars[26]. Other meiotic abnormalities observed were the occurrence of cytomixis in treated plants, which led to the formation of aneuploid cells (Fig. 1f), as well as tripolar and multipolar cell formation (Fig. 1h–j). The earlier cytogenetic studies in *B. napus* cultivars as well as their hybrids did not show the occurrence of aneuploidy[22–24]. Therefore, EM fields may be the reason for the occurrence of aneuploidy in this study.

Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic connections originating from the pre-existing systems of plasmodesmata formed within the anther tissues. The plasmodesmata become completely obstructed by the deposition of callose, but in some cases they persist during meiosis and increase in size forming conspicuous inter-meiocytes connections or cytomictic channels that permit the transfer of chromosomes[27]. Cytomixis has been reported in several plant species leading to the formation of aneuploid as well as polyploid meiocytes[22]. Cytomixis leads usually to aneuploidy and reduction in fertility of plants. It is therefore considered to be of lower evolutionary significance. However, it may bring about new genetic variability by producing aneuploid gametes and new phenotypic characters as reported in other plants[22–24]. In some cases, cytomixis may lead to the migration of the whole chromatin material among the neighbouring meiocytes and to the formation of unreduced gametes.

Tripolar cells can form unreduced gametes as also reported in canola cultivars[22–24]. The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in chromosome alignment during the metaphase. Any distortion or breakage in the spindle may result in random sub-grouping of the chromosomes which function independently[28]. In several instances, spindle abnormalities have led to the production of aneuploid gametes, e.g., in polyploidy hybrids and derivatives of *Aegilops* × *Triticum* hybrids, amphiploid Triticineae, and amphiploids of *Solanum* hybrids. Different reasons have been suggested for the occurrence of spindle abnormalities including duality of nucleus in foreign cytoplasm, environmental influence, and disharmonious gene interaction[28].

Promila and Bhattacharya[29] studied the effect of static and EM fields on the mitotic and meiotic divisions and nucleoli of root meristematic cells of *Allium cepa*, reporting a significant fall in mitotic indices associated with various anomalies including stickiness, clumping of chromosomes, anaphase chromosome bridge, and disturbed spindles. Various meiotic abnormalities were also recorded. Therefore, it may be suggested that EM fields bring about changes in genetic recombination of the plants and may lead to aneuploidy production and also unreduced gamete formation. Moreover, the genomic changes occurring in canola as a result of EM fields caused some morphological changes in this crop plant. Hence EM fields may be used as a putative biotechnological tool in agricultural plant culture[30].

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