

Extremely low frequency electromagnetic field generator suitable for plant *in vitro* studies

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Abstract

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The extremely low frequency electromagnetic field (ELF-EMF) occurs naturally from the earth and artificially as a human invention. The objectives of this study were to develop a suitable ELF-EMF generator for *in vitro* plants culture studies and to determine the effect of ELF-EMF exposure on *in vitro* tobacco (*Nicotiana tabacum*) growth and chlorophyll content. An ELF-EMF generator, the coGEM 1,000 was constructed using four coils of copper wires that were connected to a transformer, multimeter and rheostat. The coGEM 1,000 suitable for tissue culture plants is able to produce stable and uniform 6 and 12 mT 50Hz ELF-EMF in the four coils of the ELF-EMF generator. The tobacco *in vitro* plantlets were exposed to 6 and 12 mT of 50 Hz ELF-EMF for a period of 0.5, 1, 2 and 4 hours. The exposure to 12 mT ELF-EMF for an hour increased plant growth (shoot height); whereas the exposure to 6 mT Elf-EMF for an hour increased chlorophyll *a*, chlorophyll *b* and the total chlorophyll content.

Keywords: chlorophyll content; coGem 1,000; ELF-EMF; growth; tobacco plant

Electromagnetic field (EMF), a physical field constructed by a combination of an electric field and magnetic field, influences the performance of a charged object in the surrounding area (CHRYSIKOPOULOS 2009; MROZYNSKI, STALLEIN 2013). The EMF produced from 30–300 Hz electric current is generally referred as extremely low frequency EMF (ELF-EMF). The effects of the ELF-EMF on plants,

particularly on seed germination have been reported in several plants, viz. *Arabidopsis thaliana*, *Prunus maritime*, *Phaseolus vulgaris*, *Cucumis sativus* (PIETRUSZEWSKI et al. 2007; PAZUR, RASADINA 2009; YAN et al. 2009). Investigations on the effect of exposure of these plants to ELF-EMF were performed in the presence of a wide range of electromagnetic strength, ranging from 0.7 μ T to

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60 MT at a short duration of 10 min to an extended duration of 56 days (CALESTINO et al. 1998; MONSELISE et al. 2003; ODHIAMBO et al. 2009; YAN et al. 2009). Furthermore, the frequency of ELF-EMF applied in these studies were also varied, viz. 50 Hz, 60 Hz, 65 Hz, 75 Hz and 100 Hz (REED et al. 1993; MONSELISE et al. 2003; PAZUR, RASSADINA 2009).

Compared to the research of the ELF-EMF effect on animals and humans, to date, only limited studies on the effects of ELF-EMF on *in vitro* plants have been documented (GOLDSWORTHY 2006), where the stability and uniformity of the ELF-EMF applied to the *in vitro* plant subjects were not clearly described. The ELF-EMF generators commercially available at present are generally based on the Helmholtz coil concept. This rather bulky device is often able to negate external magnetic field surrounding it while being able to create the electromagnetic field within the coil. Using a similar principle, this study developed an ELF-EMF generator that can be used as a small-scale generator for *in vitro* plants studies. This generator, named coGEM 1,000, was built using several copper wire coils, which is upgradable, and was connected to other electrical components to ensure accuracy, stability, reproducibility of the ELF-EMF generated and safety of the system developed.

As a model plant for biological research and plant biotechnological applications (GANAPATHI et al. 2004; BUDZIANOWSKA 2009), *Nicotiana tabacum* (tobacco plant) was used in this study to assess *in vitro* plant response to the ELF-EMF exposure at specific strength known to have a positive effect on plants (DARDENIZ et al. 2006, HUANG, WANG 2007; YAN et al. 2009; SHABRANGI et al. 2013). The present study reports on the growth, morphological development and chlorophyll content of the tobacco plant in response to the ELF-EMF generated by the coGEM 1,000.

MATERIAL AND METHODS

Construction of the ELF-EMF generator. Setup of the ELF-EMF generator was started by connecting 1,000 turns of copper wire (AW16) that was coiled around the PVC core (coil 1) to the power supply, a transformer, and a multimeter (Tektonix DMM 4040; Tektonix Inc., USA) (referred as circuit design 1). The design was gradually expanded by adding another coil into the design, one at a time. The circuit also modified the series connection, parallel connection and combination between series

and parallel. Overall there were five designs that were tested. For each design, the functional test was carried out to determine the capacity and uniformity of the ELF-EMF strength that can be generated from each coil on the circuit. The best design was the one that had capacity to produce a uniform ELF-EMF strength up to 12 mT. Following that, a stability test was conducted on the best circuit to determine the stability of the ELF-EMF produced by each coil when the generator operated for 5 hours. The Gauss meter logger data were employed to record the ELF-EMF that was produced by each coil. The observation data of the ELF-EMF strength versus time were translated into graphs using the EMCALC computer software (Enertech Inc., USA).

Preparation of plant material. The tobacco mother plants as the explants source for this experiment were germinated from seeds of *Nicotiana tabacum* on a MS medium (MURASHIGE, SKOOG 1962). The stem explants (3 cm) were obtained from 4 weeks old mother plants. Four-day-old cultures were placed in a vessel (a glass jar with diameter of 7 cm and height of 12 cm). The *in vitro* tobacco plants were then subjected to two different strengths of ELF-EMF (6 and 12 mT) at four different durations of exposure (0.5, 1, 2 and 4 hours). The plants of similar age which were not exposed to ELF-EMF, but were grown under similar condition, were chosen as controls. After the exposure, the *in vitro* cultures were incubated in the growth chamber with a 16 h photoperiod at 25°C.

Assessment of growth parameters and chlorophyll content. The growth parameters were measured to find the effect of the ELF-EMF on plant growth and developments. The chosen parameters were namely number of shoots, shoot height and number of leaves. The number of shoot and leaves was counted manually, while shoot height was determined by measuring the stem length from the points where roots occur until the end of the stem.

The chlorophyll content of the plants was analysed using the modified method proposed by NI et al. (2009), which can be described as follows: approximately 100 mg of fresh leaves were ground into a fine powder in the presence of liquid nitrogen. The powdered leaves were transferred into a 1.5 ml microcentrifuge tube covered with aluminum foil and added with 1 ml of 80% acetone. The microcentrifuge tube was then centrifuged at 4°C for 15 min (3,000 g), and the supernatant was transferred to a new centrifuge tube and kept in the dark. The ab-

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sorbance (A) of chlorophyll content in the mixture was measured using a spectrophotometer at 663, 645 and 470 nm wavelength; and 80% acetone was used as a blank control. The chlorophyll concentration of each sample was then calculated as follows:

Chlorophyll a content (mg/g) = $[(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V/1,000 \times W$

Chlorophyll b content (mg/g) = $[(22.9 \times A_{645}) - (4.86 \times A_{663})] \times V/1,000 \times W$

Total chlorophyll content (mg/g) = $[(8.02 \times A_{663}) + (20.20 \times A_{645})] \times V/1,000 \times W$

Carotene/xanthophyll content (mg/g) = $[(1,000 \times A_{470}) - (3.27 \times C_a) - (1.04 \times C_b)]/229$

Ratio of chlorophyll a and b = C_a/C_b

A_{663} – absorbance on 663 nm; A_{645} – absorbance on 645 nm; A_{663} – absorbance on 470 nm; V – volume of the extract (ml); W – weight of fresh leaves (g); C_a – concentration of chlorophyll a content (mg/g); C_b – concentration of chlorophyll b content (mg/g)

Experimental design and statistical analysis. All *in vitro* experiments were arranged in the Randomly Complete Block Design (RCBD) which included 8 treatments and one control. There were 20 samples for each treatment; the whole experiments were replicated three times. The data were analysed using a multivariate analysis of variance (MANOVA) that was based on the Wilks's Lambda to identify the significance between groups of treatment ($P < 0.05$). Statistical analyses were done using the computer software of SPSS ver. 18.0 (SPSS Inc. 2009).

RESULTS

Development of the ELF-EMF generator

The electromagnetic field required for this study was 120 Gauss (equivalent to 12 mT). The data calibrated

showed that the electromagnetic field produced had a linear correlation with the current input. Several coils arrangements (series, parallel, and combination of series and parallel) were tested and the data obtained were plotted. The parallel and series circuit (fourth circuit) generated 60 Gauss of magnetic field from 2.6 A current and 120 Gauss from 5.3 A current in every coil. Furthermore, it was also revealed that all coils had similar current and electromagnetic field. Following the construction of the CoGEM 1,000 (Fig. 1), the stability of electromagnetic field was also analysed. The experiment was conducted in order to determine if there were any variations in electromagnetic field produced in each coil in the generator.

Effect of the ELF-EMF on the tissue-cultured tobacco plants

The statistical test of the effects of ELF-EMF on developmental parameters of tobacco plants revealed that ELF-EMF exposures to the tissue-cultured tobacco had significantly affected ($P < 0.05$; F Wilk's Lambda = 10.394) the shoot height of the plants, whereas the treatment did not cause any changes on plant's number of shoots and leaves. The treatment of the 12 mT ELF-EMF for 0.5 hours (3.9 ± 0.8 cm) increased shoot height by 44.4%; yet a higher and longer duration of ELF-EMF exposures (more than 12 mT 0.5 hour treatment) significantly reduced shoot height.

Effect of ELF-EMF on the chlorophyll content of tissue-cultured tobacco plants

The MANOVA result in an effort of finding out the effects of ELF-EMF exposure to the to-



Fig. 1. The CoGEM 1,000, an ELF-EMF generator consists of 4 coils connected in series and parallel with AC supply, ampere meter and rheostats

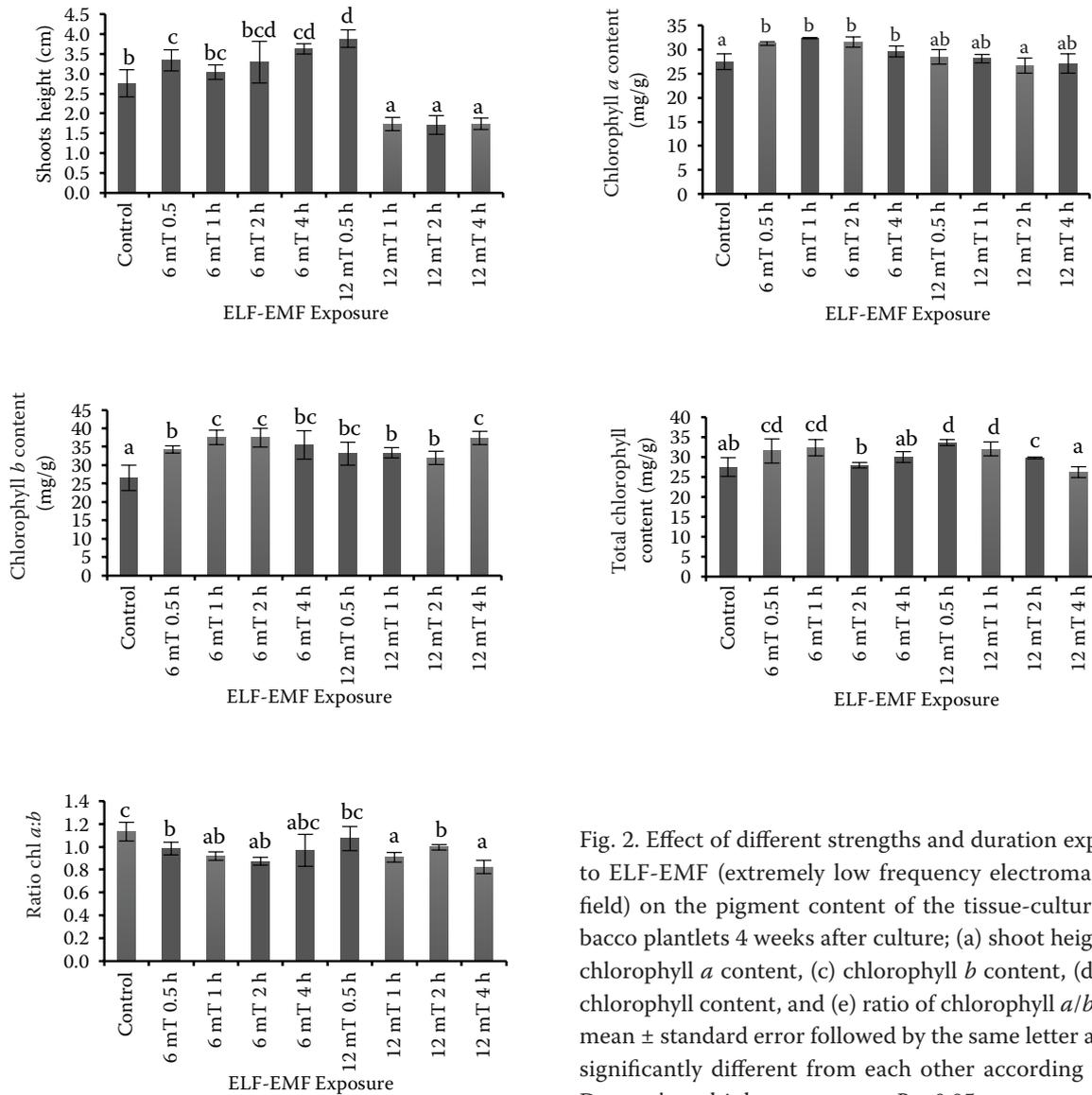


Fig. 2. Effect of different strengths and duration exposure to ELF-EMF (extremely low frequency electromagnetic field) on the pigment content of the tissue-cultured tobacco plantlets 4 weeks after culture; (a) shoot height, (b) chlorophyll *a* content, (c) chlorophyll *b* content, (d) total chlorophyll content, and (e) ratio of chlorophyll *a/b* mean \pm standard error followed by the same letter are not significantly different from each other according to the Duncan's multiple range test at $P = 0.05$

bacco plant biochemical properties indicated that the ELF-EMF exposures had a significant effect ($P < 0.05$, F Wilk's Lambda = 6.203) on the content of chlorophyll *a*, chlorophyll *b*, total chlorophyll and ratio of chlorophyll *a/b*. The exposure to the 6 mT ELF-EMF for an hour on tobacco plantlets produced the highest content of chlorophyll *a* (32.3 ± 0.14 mg/g), chlorophyll *b* (37.6 ± 0.98 mg/g) and total chlorophyll (20.4 ± 0.27 mg/g) or increased by 17.9%, 41.7% and 17.8%, respectively. However, the ratio chlorophyll *a/b* was decreased since the increment of chlorophyll *b* content was higher than the increment of chlorophyll *a* content (Fig. 2).

DISCUSSION

Influence of the circuit design on capacity and stability of the generated ELF-EMF

In order to carry out this study, an ELF-EMF generator that can produce the specific ELF-EMF strength needed to be developed. Several factors were necessary to be considered in the development of the ELF-EMF generator, namely the number of turns on the coils, number and size of the coils, the amount of electric current needed and safety of the equipment to the operator and the environment.

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An ELF-EMF generator which consisted of four coils (which refer to 4 chambers) was built in order to investigate the effects of ELF-EMF on tissue-cultured plants. The currently available ELF-EMF generators were usually not appropriate for this study because their size was too big, thus it required more expensive materials to be constructed, whereas the ELF-EMF generated by such generator would be less uniform and constant. The common ELF-EMF generator also required high electrical voltage and current input, making it unsafe to be operated in the plant tissue culture laboratory.

Initially, only a single chamber or coil was constructed. After that, the second and the third coils were added in series. When there was only one coil, the result showed that the coil could produce 12 mT ELF-EMF. However, in the presence of the two coils in series, the ELF-EMF produced by both coils was not uniform. The second coil had different resistance compared to the first coil, thus causing the variation in the electromagnetic field produced by the second coil.

When three coils were connected in series, the three coils generated different and smaller electromagnetic fields compare to single and two coils arrangements. Connecting third coil resulted in the formation of a weak electromagnetic field. Even with the highest current possible to be generated from the circuit (1.8 A), coil 1 only emitted 94.08 Gauss, coil 2 emitted 89.8 Gauss, while coil 3 emitted 76.54 Gauss. It was clear that the circuit had some flaws, and therefore it could not generate the uniform magnetic field which had different resistance among the three coils.

Thus, a parallel connection of the coils was proposed to overcome the electromagnetic field inconsistency produced by the generator. Coils 1 and 2 were connected in parallel with the AC power supply and multimeter. The magnetic field produced inside both coils from 0.4 to 6.0 A (in 0.4 A steps). The data were recorded, and the graph was plotted.

The main consideration on the construction of the ELF-EMF generator was to ensure that the flow of the current in each coil was uniform so that it could generate the same electromagnetic field in all four coils. To achieve that, the following circuit was designed and included the additional apparatus known as rheostat (variable resistor). The four coils were connected in parallel connection of two series coils with the AC supply, a multimeter and two rheostats. The circuit was calibrated by measuring the electro-

magnetic field inside each coil when the current of 0.5 to 5.3 Ampere (at 0.5 A steps) was applied.

The coGEM 1000

The functional and stability tests for the parallel and series circuit (fifth circuit) showed that the ELF-EMF generated from the coGEM 1,000 was up to 12 mT and it can be operated for 5 hours steadily, continuously and safely. This means that the circuit is an excellent ELF-EMF generator and it is capable of producing high and uniform electromagnetic field in every coil. This generator was then named coGEM 1,000 and was used for the following experiment to find out the effects of ELF-EMF on the rate growth and chlorophyll content of tobacco plants.

This coGEM 1,000 is a suitable ELF-EMF generator that could be applied in the tissue culture study since it has many advantages. First of all, the coGem 1,000 is a user friendly as it is easy to operate; the ELF-EMF strength can be varied by setting up the current input. It was also easy to calibrate it by measuring the ELF-EMF produced by each coil with the common Gauss meter and the intact software. The coGEM 1,000 built is a basic equipment which can be upgradable by adding another coil into design and by modifying the circuit to construct larger generators for mass application.

Effect of the ELF-EMF on the tissue-cultured tobacco plants

Most studies on the impacts of ELF-EMF on various crop plants have been conducted using native plants that are grown under natural conditions. However, it is difficult to measure the true impacts of ELF-EMF on these plants since other abiotic factors contribute to the measurement. In order to eliminate these factors so that the true impacts of ELF-EMF on the plants can be precisely measured, *in vitro* culture also known as “tissue culture system” was selected for this research. In this study, selected plants which had been propagated through the tissue culture technique were used to study the impacts of ELF-EMF on plants.

High shoots were also induced in cucumber and chilli seedlings when exposed to lower ELF-EMF strength (0.1 mT and 62 μ T, respectively). Exposures of etiolated *Cucumis sativus* seedlings to 50 Hz 1 Gauss ELF-EMF produced higher shoots

(500%) compared to the unexposed seedlings (PIACENTINI et al. 2001).

Effect of ELF-EMF on the chlorophyll content of tissue-cultured tobacco plants

Due to its function to absorb solar radiation, the chlorophyll content could be directly estimated using a photosynthetic potential and primary production. Furthermore, chlorophyll content can also be used to resolve indirect determination of the nutrient level, since most of the nitrogen is integrated in chlorophyll (GITELSON et al. 2003). Chlorophyll content is also associated with the physiological state, such as stress and senescence (DAMARAJU et al. 2011).

On the one hand, the chlorophyll content affects the carbon dioxide exchange rate of photosynthesis, and thus it is also affected by other physiological processes, such as mitochondrial respiration and photorespiration (TANAKA, TANAKA 2011). On the other hand, the chlorophyll content can be stimulated by endogenous cytokines, irradiance, temperature and drought stress (OLAH, MASAROVIKOVA 1998; AFREEN 2005).

Chlorophyll contents, including chlorophyll a, chlorophyll b, total chlorophyll a and b and carotene were used in several studies as biochemical parameters. For instance, tobacco leaves from a plantlet with high irradiance treatment showed a decrease in total chlorophyll content and chlorophyll *a/b* ratio, but the contents of carotene and xanthophylls pigment were increased (KADLECEK et al. 2003). In *in vitro* plant culture, chlorophyll content might not be affected by media concentration, but increasing the amount of air exchange in the culture vessel would increase the chlorophyll contents (AFREEN 2005).

The induced chlorophyll concentration in the tobacco plants exposed to the ELF-EMF might be triggered by the increased activity of the chlorophyll synthesis-related enzymes. The chlorophyll biosynthesis started with glutamate that was oxidized into several intermediate compounds before converted into chlorophyll *a* or *b*. The pathway involves many specific enzymes, coenzymes and cofactors, such as photochlorophyllide oxidoreductase, nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) (RUDIGER 2009). The increment of chlorophyll content in plant exposed to ELF-EMF indicated the increment of photosynthetic capacity and resulted in promoted shoot growth.

Similar to the ELF-EMF, approximately a two-fold increase in the concentration of chlorophyll a and total chlorophyll content was observed when PGR cytokinins (0.4 μ M 4-PU-30 [*N*-phenyl-*N'*-(4-chloro-4-pyridyl) urea], urea and TDZ) were added in the MS growth medium of 40-day-old of *Dianthus caryophyllus* plantlets (GENKOV et al. 1997). Thus, the application of ELF-EMF on tissue culture technique can potentially replace the function of plant hormone as a plant growth inducer.

In an *in vitro* system, plants are often characterized by a low photosynthetic rate since the availability of sucrose on the media allows plants to use the carbon source from the medium instead of producing carbohydrate through photosynthesis (KADLECEK et al. 2003). However, the increase of chlorophyll content of the *in vitro* plantlets might be an advantage since it can increase the survival rate of the plants in the acclimatization stage (AFREEN 2005).

CONCLUSION

The device used to generate the ELF-EMF was successfully constructed. The complete set of the generator is called coGEM 1000 and consists of four chambers, each made up of copper wire coils connected to the transformer, multimeter and rheostat. The four chambers or coils generator can achieve a maximum of 120 Gauss or 12 mT ELF-EMF at 5.3 A current when the coils were connected in a parallel connection of two series coils. The ELF-EMF produced by the equipment was tested for its stability and uniformity. The result showed that it can produce stable 6 and 12 mT ELF-EMF for 5 hours. The result obtained showed that the ELF-EMF exposures had a significantly increasing shoots height of tobacco cultures. It was also observed that the exposure increased chlorophyll *a*, chlorophyll *b*, and the total chlorophyll content of the plant. Thus, these results implied that the ELF-EMF exposure had a potentially positive effect to increase growth and chlorophyll content of plant *in vitro* culture.

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