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SENSITIVITY OF PIGMENT CONTENT OF BANANA AND ORCHID TISSUE CULTURE EXPOSED TO EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELD

Sensitivitas Kandungan Pigmen pada Kultur Jaringan Tanaman Pisang dan Anggrek yang Terpapar Medan Elektromagnetik Frekuensi Sangat Rendah

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ABSTRACT

Natural exposure of extremely low frequency electromagnetic field (ELF-EMF) occurs in the environment and acts as one of the abiotic factors that affect the growth and development of organisms. This study was conducted to determine the effect of ELF-EMF on the tissue cultured banana and slipper orchid chlorophyll content as one of the indicators in measuring plant photosynthetic capacity. Four days old banana (*Musa* sp. cv. Berangan) corm and seven days old slipper orchid (*Paphiopedilum rothschildianum*) cultures were exposed to 6 and 12 mT ELF-EMF generated by controllable ELF-EMF built up machine for 0.5, 1, 2 and 4 hours. After exposure, the banana and orchid cultures were incubated at 25° C for 8 and 16 weeks, respectively. The results showed that the ELF-EMF exposure had different effects on banana and slipper orchid cultures though both plant species belong to monocotyledon. The highest increase in chlorophyll content on banana was resulted by the high intensity and long duration of ELF-EMF exposure (12 mT for 4 hours), whereas on slipper orchid the modest and short duration of ELF-EMF exposure produced the most excessive chlorophyll content. Different ELF-EMF exposures (12 mT for 4 hours and 6 mT for 30 minutes) had potential to be applied on each plant to improve *in vitro* plant (banana and slipper orchid, respectively) growth. The increased chlorophyll and carotene/xanthophyll content on banana indicated that the banana was more tolerant to ELF-EMF exposure compared to slipper orchid.

[***Keywords***: Banana, orchid, carotene, chlorophyll, electromagnetic field]

ABSTRAK

*Paparan alami medan elektromagnetik frekuensi sangat rendah (ME-FSR) terdapat di lingkungan dan merupakan salah satu faktor abiotik yang memengaruhi pertumbuhan dan perkembangan makhluk hidup. Penelitian bertujuan untuk mengetahui pengaruh paparan ME-FSR pada kandungan pigmen kultur jaringan pisang dan anggrek sliper sebagai indikasi kapasitas fotosintesis. Kultur pisang (*Musa *sp. cv. Berangan) dan anggrek sliper (*Paphiopedilum rothschildianum*) dipapar dengan ME-FSR 6 dan 12 mT selama 0,5; 1, 2, dan 4 jam. Hasil penelitian menunjukkan bahwa paparan ME-FSR berpengaruh berbeda terhadap kultur jaringan pisang dan anggrek sliper, meskipun keduanya termasuk ke dalam monokotiledon. Kandungan klorofil dan karoten/xantofil pada pisang yang terpapar ME-FSR meningkat seiring dengan peningkatan intensitas dan durasi paparan ME-FSR. Sebaliknya, kandungan klorofil dan karoten/xantofil pada anggrek sliper berkurang seiring dengan peningkatan intensitas dan durasi paparan ME-FSR. Perbedaan paparan ME-FSR (12 mT selama 4 jam dan 16 mT selama 30 menit) berpeluang untuk diaplikasikan pada kedua tanaman tersebut untuk meningkatkan pertumbuhan tanaman. Penemuan ini mengindikasikan bahwa eksplan pisang lebih toleran terhadap paparan MF-FSR dibandingkan dengan anggrek sliper.*

[***Kata kunci***: Pisang, anggrek, klorofil, karoten, medan elektromagnetik]

INTRODUCTION

Earth, as a habitat for many living organisms, is controlled by natural physical forces to support life continuously (Mrozynski and Stallein 2013). One of the Earth's fundamental interactions is electromagnetism, an interaction between electrically charged particles. The electromagnetic field (EMF) is naturally produced by the Earth, but it can also be released from human inventions in the form of electrical and electronic devices, and through transmission and distribution of power lines (Frija *et al.* 2006; Kostoff and Lau 2013). The electric distribution usually operates at frequencies of 50 and 60 Hz. The EMF produced from 30–300 Hz electric current is generally referred to as extremely low frequency-EMF (ELF-EMF) (Hanninen *et al.* 2011; Lambrozo and Souques 2012).

The ELF-EMF potential effects on organisms have become a subject to many extensive studies (Furse *et al.* 2008; Grandolfo 2009; Waite *et al.* 2011). Although the data from these studies have shown different results, the World Health Organization (WHO) recommends that environmental ELF-EMFs are harmless to human health. However, high density ELF-EMF (more than 12 mT) may potentially induce cancer, leukaemia, depression, cardiovascular disorder, and other physiological dysfunctions as shown from studies on laboratory animals (Ravazzani 2008).

Most of the studies on the impacts of ELF-EMF on various plants showed varied results (Pietruszewski *et al.* 2007; Pazur and Rassadina 2009). The ELF-EMF may induce positive effects on plants, such as increasing seed germination percentages of cucumber (*Cucumis sativus*) and mung bean, and improving shoot growth of *in vitro* plum (*Prunus maritime*) (Piacentini *et al.* 2001; Huang and Wang 2007). Since plant responses to ELF-EMF exposure were varied, the effects of ELF-EMF exposure on some important tropical horticulture plants need investigation. Both banana and slipper orchid were chosen on this study because of their high economical value. In addition, these two horticulture species were representing plants with different characteristics; banana represents fruiting plants and orchid represents flowering plants. In addition, both species require different growth conditions. Banana adapted in relatively higher range of temperature (16–38° C), humidity (75–85%) and altitude (low to high land up to 1,300 m asl) (Muas 2010) than orchid (15–25° C, 40–50%, only highland, respectively) (Guan *et al*. 2010).

Biochemical property usually measured to determine the growth and development of *in vitro* plant is the chlorophyll content (Kadlecek *et al.* 2003; Liu *et al.* 2015). Due to its function to absorb solar radiation, chlorophyll content could be directly estimated using a photosynthetic potential and primary production. Furthermore, chlorophyll content can also be used for resolving indirect determination of the nutrient level since most of the nitrogen is integrated in chlorophyll. Chlorophyll content is also associated with physiological stage, such as stress and senescence (Gitelson *et al.* 2003; Damaraju *et al.* 2011; Suzuki *et al.* 2014). Therefore, this study was conducted to determine the effect of ELF-EMF on the tissue cultured banana and slipper orchid chlorophyll content as one of the indicators to measure plant photosynthetic capacity.

MATERIALS AND METHODS

Plant Materials

Banana plantlets cv. Berangan (8 weeks old) were bought from Hexagon Sdn. Bhd., a commercial tissue culture laboratory in Selangor, Malaysia. The corm explants (1 cm) were cut under sterile conditions and cultured for 4 days on a modified Murashige and Skoog (1962) (MS) basal salt medium with addition of 2 mg l^{-1} BAP, 1 mg l^{-1} IAA, 30 mg l^{-1} sucrose, and 2 g l^{-1} Gelrite prior to exposure to ELF-EMF.

The orchid mother plants were derived from *in vitro* established culture of *P. rothschildianum*. Single buds with three leaves (0.5 g) were used as explants for the treatment. The explants were cultured on a modified ½ MS basal salt medium with addition of 30 g l^{-1} marsh potato, 2 g l^{-1} peptone, 30 g l^{-1} sucrose, and 3 g l^{-1} Gelrite for 7 days prior to exposure to ELF-EMF. There were 20 samples for each treatment and control. The whole experiment was repeated three times.

ELF-EMF Generator

The ELF-EMF generator used in this study was a built up machine comprised of electrical elements including four copper coils (10 cm in diameter and 1,000 turns each), a transformer, two variable resistors, a multimeter and a power supply. Those elements were connected in both series and parallel in such a way that each coil produced uniform ELF-EMF from the same amount of electrical current. The uniformity and stability of the ELF-EMF generator had been determined (data not shown).

ELF-EMF Exposure to Plant Materials

To ensure that all plants used in this study receive consistent and uniform exposure to ELF-EMF, four days old banana and seven days old orchid explants collected after subculture were placed in each vessel (a diameter of 7 cm and height of 12 cm, two explants for each vessel). The *in vitro* cultures were then subjected to two different intensities of ELF-EMF (6 and 12 mT) at four different durations of exposure (0.5, 1, 2 and 4 hours). Banana and orchid explants were placed at similar conditions but were not exposed to ELF-EMF as controls.

After the exposure, the *in vitro* cultures were incubated in the growth chamber at photoperiod of

16/8 hour day/night and 25° C. Due to differences in the growth rates, the two different plant species were incubated at different durations; the banana cultures were incubated for eight weeks, whereas the slipper orchid plantlets, due to its slow growth rate, were grown for 16 weeks before the data collection was done.

Total Chlorophyll Content

In order to reveal the changes in a photosynthetic rate of the plants in response to the different doses and durations of ELF-EMF exposure, chlorophyll content of the plants was analyzed using the modified method proposed by Ni *et al.* (2009). Approximately 100 mg fresh leaves was ground into a fine powder using a mortal and a pestle in the presence of liquid nitrogen. The powdered leaves were transferred into a 1.5 ml microcentrifuge tube, then 1 ml of 80% acetone was added and the tubes were covered with aluminium foil. The samples were then centrifuged at 4° C for 15 minutes (3,000xg) and kept in the dark. The absorbance (A^o) of chlorophyll content in the mixture was measured using a spectrophotometer (Genesys 10uV Thermo Scientific) 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:

Chl a content (mg/g) =

$$[(12.7 \times A663) - (2.69 \times A645)] \times \frac{V}{1{,}000} \times W$$

Chl b content (mg/g) =

$$[(22.9 \times A645) - (4.86 \times A663)] \times \frac{V}{1{,}000} \times W$$

Total chl content (mg/g) =

$$[8.02 \times A663) + (20.20 \times A645)] \times \frac{V}{1{,}000} \times W$$

$$\text{Ratio chl a/b} = \frac{[Ca]}{[Cb]}$$

Carotene/xanthophyll content (mg/g) =

$$\frac{[(1{,}000 \times A470) - (3.27 \times Ca) - (1.04 \times Cb)]}{229}$$

Where: A_{663} is absorbance at 663 nm; A_{645} is absorbance at 645 nm; A_{470} is absorbance at 470 nm; V is volume of the extract (ml); W is weight of fresh leaves (g); C_a is concentration of chlorophyll a content (mg g^{-1}); C_b is concentration of chlorophyll b content (mg g^{-1}).

Statistical Analysis

The data obtained were subjected to multivariate analysis of variance (MANOVA) and continued with Duncan's multiple range test (DMRT) at $P = 0.05$ using the SPSS ver. 18.0 (SPSS Inc. 2009) computer software.

RESULTS AND DISCUSSION

The ELF-EMF exposure to plants resulted varying effects; some plants showed better growth performance and others experienced poor growth. Furthermore, the effect of ELF-EMF was also varied with electromagnetic field intensities and duration of exposure (Apasheva *et al.* 2006).

The effect of ELF-EMF exposure on the chlorophyll and carotene/xanthophyll contents of tissue cultured banana and slipper orchid is presented in Table 1. The results showed that the ELF-EMF exposure had a significant effect both on banana and slipper orchid cultures. In detail, the ELF-EMF exposure on banana cultures affected the chlorophyll a, chlorophyll b, total chlorophyll, the ratio of chlorophyll a/b, and carotene/xanthophyll content. On slipper orchid culture, the ELF-EMF exposure significantly affected

Table 1. Multivariate analysis of variance (MANOVA) of pigment content response of tissue cultured banana and slipper orchid to extremely low frequency electromagnetic field (ELF-EMF).

MANOVA result	Banana	Slipper orchid
F Wilk's Lambda	8.117	3.463
P value	0.000	0.000
Developmental parameters that are significantly affected by ELF-EMF treatment ($P < 0.05$)	Chlorophyll a content Chlorophyll b content Total chlorophyll content Ratio of chlorophyll a/b Carotene/xanthophyll content	Chlorophyll a content Chlorophyll b content Total chlorophyll content Carotene/xanthophyll content

chlorophyll a, chlorophyll b, total chlorophyll, and carotene/xanthophyll content, whereas the statistical analysis showed that the ratio of chlorophyll a/b in orchid cultures was not affected by the ELF-EMF exposure.

Further statistical test showed that the exposure to the 12mT ELF-EMF for 4 hours increased gradually chlorophyll a, chlorophyll b, total chlorophyll, and carotane/xanthophylls content of the plant. Chlorophyll content of the plants exposed to ELF-EMF increased with increasing ELF-EMF intensities and exposure duration.

Effects of ELF-EMF on Chlorophyll and Carotene/Xanthophyll Contents of Tissue Cultured Banana

For banana, the ELF-EMF exposure gradually reduced the ratio of chlorophyll a/b concomitant with the increase in ELF-EMF intensity and duration of exposure (Fig. 1). The changes in pigment content associated with colour symptoms of plant illness (Parekh *et al.* 1990). The increase in chlorophyll a, b, and total chlorophyll on banana explants exposed to 12 mT ELF-EMF for 4 hours indicated significant

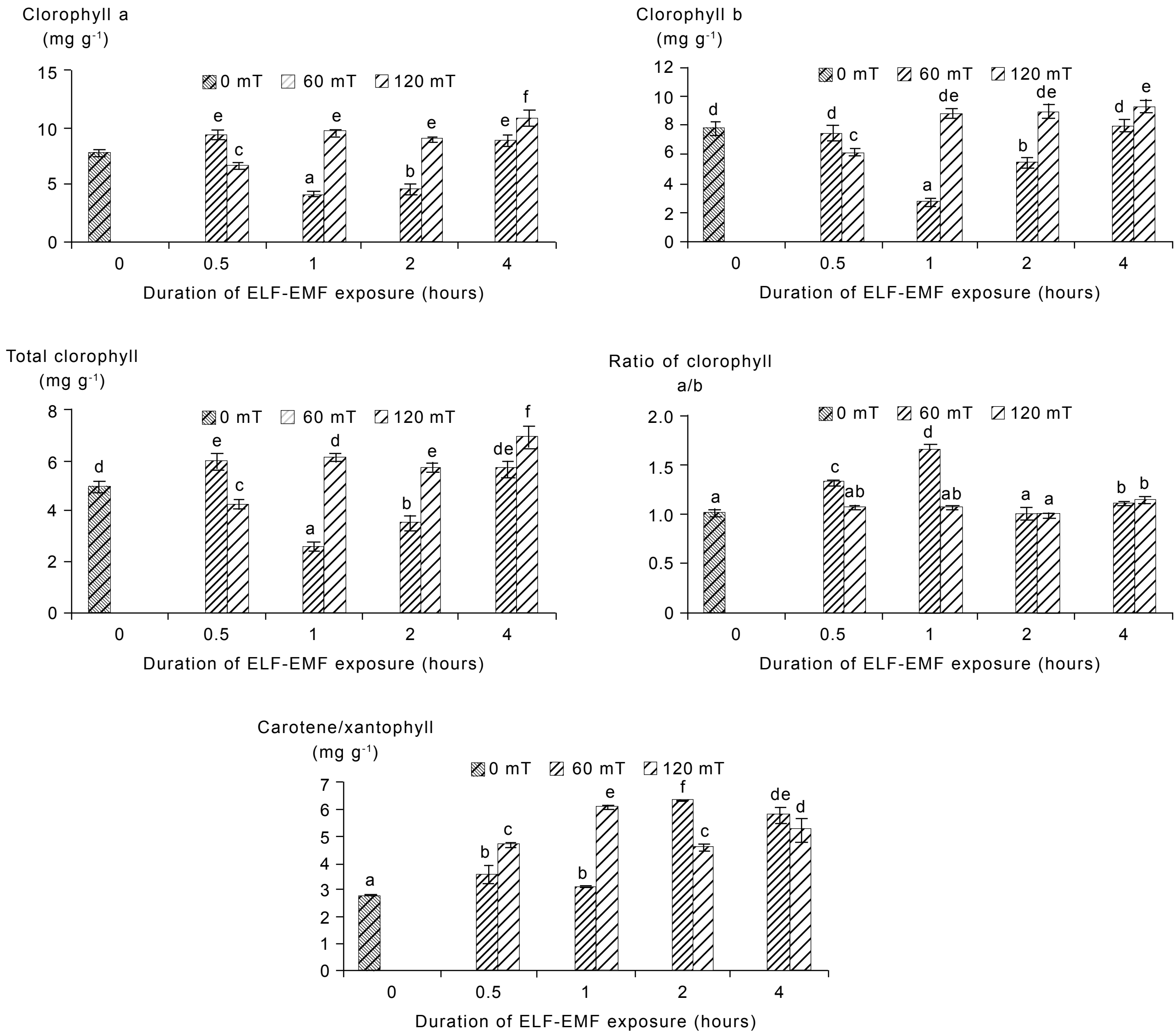

Fig. 1. Effect of intensities and duration of exposure to extremely low frequency electromagnetic field (ELF-EMF) on the pigment content of tissue cultured banana at 8 weeks after treatment. Means ± standard error followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

stimulation of leaf growth and plant photosynthetic potential. A similar result was shown in the growth of *Spirulina* exposed to 0.25 T electromagnetic field. The increase in chlorophyll content indicated more efficient ability of plant to absorb light, which resulted in stimulated plant growth (Li *et al.* 2007). In spite of fluctuative carotene content in the present study, the higher carotene was found in every exposed plantlet. In addition, a gradually reduced chlorophyll a/b ratio demonstrated that in the presence of ELF-EMF, chlorophyll b was more sensitive to degradation compared to chlorophyll a.

Chlorophyll degradation was influenced by the phenolic-peroxidase-H_2O_2 system (Kato and Shimizu 1987). More recent study found that chlorophyll degradation was also mediated by abscisic acid. Some genes that regulate the chlorophyll degradation in *Arabidopsis* had been described, including NYE1 and STAY GREEN genes (Gao *et al.* 2016). Chlorophyll degradation is a crucial process during leaf senescence and fruit ripening because it helps plants to recycle nitrogen and other nutrients (Hortensteiner 2006). It is also a protective mechanism from the build-up of phototoxic chlorophyll that intermediates and participates in the signalling of other physiological process (Hoetensteiner and Krautler 2011).

Similar to chlorophyll content, the carotene/xanthophyll content of banana plant exposed to ELF-EMF also increased with increasing intensities and exposure duration to ELF-EMF. In *Curcumis pepo,* the exposure to 50 Hz 10 mT electromagnetic field increased all chlorophyll content parameters and carotene content. It has been reported that carotene content highly correlated with chlorophyll a content (Racuciu and Creanga 2005).

Plants produce carotene/xanthophylls and xanthophylls as accessory pigments serve to absorb and transmit the sunlight that has not been absorbed by chlorophyll. Both pigments can scavenge ROS produced in response to stress (Apel and Hirt 2004). The increase in chlorophyll content of banana plantlets exposed to ELF-EMF was similar with that of cultured banana treated with high concentration (up to 30 μM) of cytokinins (Aremu *et al.* 2012). This finding suggests that the increase in chlorophyll content might be related with the increase in photo system II reaction center and the rate of electron transferred from H_2O to the terminal receptors (CO_2) (Wu *et al.* 2015).

By producing more carotene and xanthophylls, the banana plantlets exposed to ELF-EMF had a high survival capacity to grow in the environment exposed to ELF-EMF. The effect of ELF-EMF exposure on banana plantlets was similar with that of plant growth hormone on tissue culture media. Thus, ELF-EMF is potential to be applied to promote *in vitro* banana growth.

Effects of ELF-EMF on Biochemical Properties of Tissue Cultured Slipper Orchid

Overall, the ELF-EMF exposure to slipper orchid influenced the biochemical characteristics of the plants by reducing the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotene/xanthophylls, but did not affect the ratio of chlorophyll a/b (Fig. 2). On the contrary to the effect of ELF-EMF on banana, the increase in ELF-EMF intensities and duration of exposure reduced the chlorophyll content of the orchid, except for plants exposed to 6 mT for 0.5 and 1 hour which showed a slight increment of chlorophyll and carotene/xanthophyll contents compared to the non-exposed plants.

The slipper orchid plants exposed for short time to low intensity of ELF-EMF (6 mT for 30 minutes) showed a positive response by an increment of the chlorophyll and carotene/xanthophyll contents, whereas the exposure at higher than 6 mT and longer than 30 minutes did not promote the pigment production. The similar case occurred on black locust exposed to high frequency electromagnetic field. Electromagnetic exposure longer than 2 hours decreased the chlorophyll content (Sandu *et al.* 2005). On *Zea mays*, exposure to electromagnetic field for 30 minutes also reduced the photo assimilatory pigments. It was assumed that the electromagnetic field exposure produces a process similar to hyperthermia. A local heating occurred due to the electromagnetic field energy is absorbed by the electromagnetic nanoparticles and internalized in the vegetal tissue. Local heating of the vegetal tissue affected the redox reactions which implicated in the photosynthesis process (Racuciu *et al.* 2009).

The increment of chlorophyll content occurred on orchid exposed to 40 and 80 kV/m electric field; whereas higher intensity of electric field reduced the chlorophyll a, chlorophyll b, total chlorophyll, carotene/xanthophylls, and total soluble protein of the orchid (Mahmood *et al.* 2011). The decline in chlorophyll content was illustrated in *Cicer arietinum* (Mafakheri *et al.* 2010) and *Triticum aestivum* (Nyachiro *et al.* 2001) suffering of drought stress. The low concentration of chlorophyll in ELF-EFM

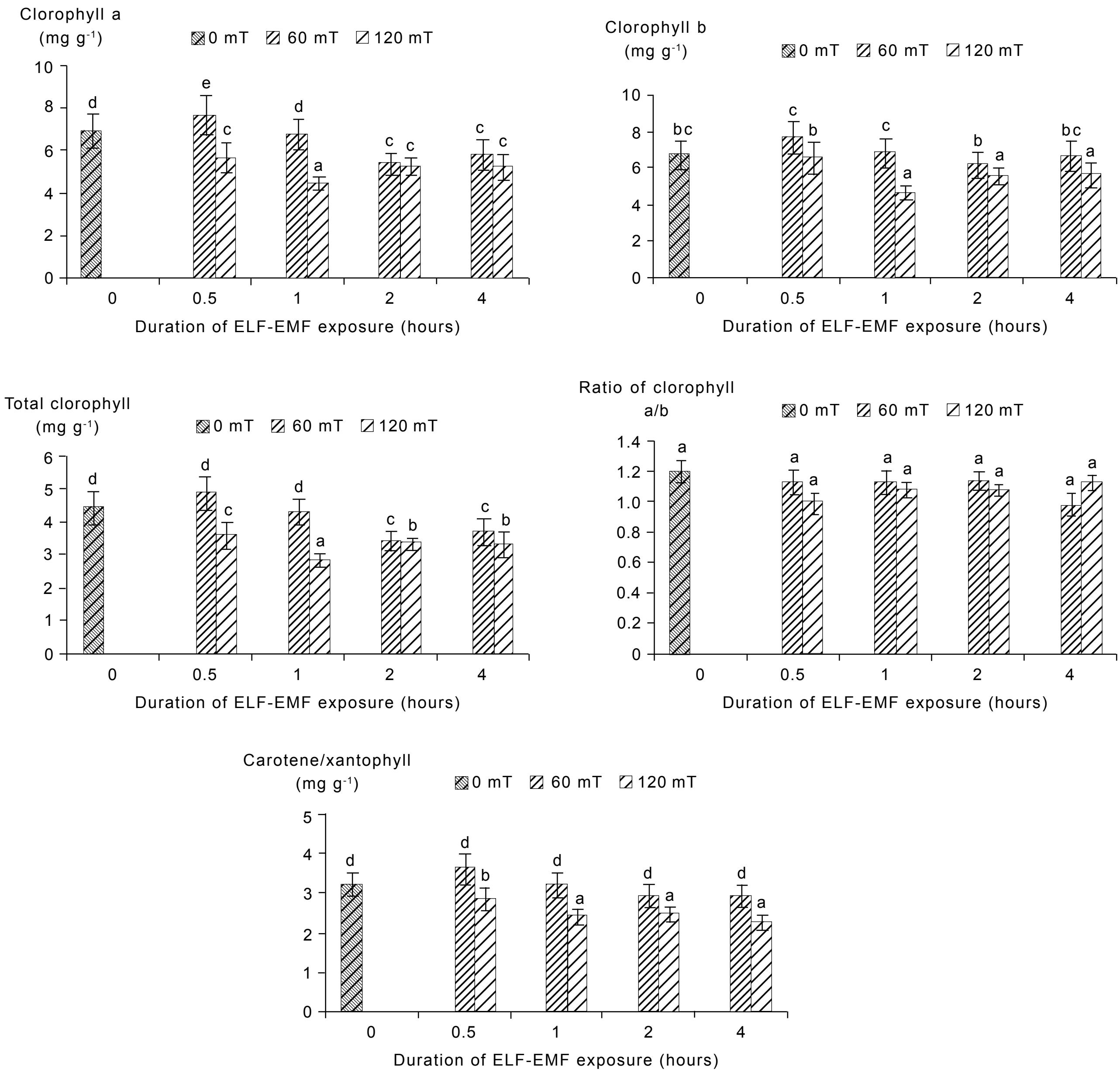

Fig. 2. Effect of intensities and exposure duration to extremely low frequency electromagnetic field (ELF-EMF) on pigment content of tissue cultured slipper orchid at 16 weeks after treatment. Means ± standard error followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

exposed orchid might be a feature of adaptive behavior of the plants to reduce photodynamic destruction of the chloroplast during the high exposure of ELF-EMF. The decrease in chlorophyll content implied a lower capability of the plant to harvest the light, thus reducing photosynthetic capacity. It also suggests that the decrease in chlorophyll content might be a plant adaptive system to the environment stress by regulating PSII reaction centers normalization and ROS metabolization (Wu *et al.* 2015).

The non-significant changes in the ratio of chlorophyll a/b indicated that the chlorophyll a and b had the same sensitivity to ELF-EMF exposure. The ELF-EMF treatment on slipper orchid decreased the chlorophyll a and b content at the same amount, which indicates that the ratio of chlorophyll a/b was not altered by the ELF-EMF treatment.

In comparison, chlorophyll and carotene/ xanthophyll contents of banana and slipper orchid exposed to the same ELF-EMF demonstrated a contrast result. Although both plant species belong to monocotyledon, both acted differently when exposed to ELF-EMF. The highest increase in chlorophyll content on banana was resulted by the high intensity and long exposure duration to ELF-EMF (12 mT for 4 hours), whereas on slipper orchid the modest and short exposure duration of ELF-EMF produced the most excessive chlorophyll content. Thus, the different ELF-EMF exposures (12 mT for 4 hours and 6 mT for 30 minutes) had a potential to be applied on each plant to improve *in vitro* plant (banana and slipper orchid, respectively) growth. The increase in chlorophyll and carotene/xanthophyll content on banana indicates the banana was more tolerant to ELF-EMF exposure compared to slipper orchid.

CONCLUSION

Exposure to ELF-EMF increased shoot height, chlorophyll a, and total chlorophyll content of banana plantlets. On slipper orchid, ELF-EMF exposure reduced the content of chlorophyll a, chlorophyll b, total chlorophyll and carotene. The significant increment of chlorophyll and carotene/xanthophyll content of banana exposed to the ELF-EMF showed an adaptive response of the plant to the exposure. The contrast response was measured on slipper orchid plants exposed to the same ELF-EMF. The different ELF-EMF exposures (12 mT for 4 hours and 6 mT for 30 minutes) had potential to be applied on each plant to improve *in vitro* plant (banana and slipper orchid, respectively) growth. Banana plants are more tolerant to ELF-EMF exposure compared to slipper orchid.

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