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Efecto del campo electromagnético en el contenido mineral y grupo químico durante el establecimiento *in vitro* y la multiplicación de fases en las plántulas de café

Effect of electromagnetic field on mineral content and chemical group during in vitro establishment and multiplication phases of coffee seedlings

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Resumen

Se investigó la influencia de los campos electromagnéticos de frecuencia extremadamente baja en contenido de minerales y grupos químicos durante el establecimiento *in vitro* y la multiplicación de las fases en las plántulas de café. Plantas de café sin tratar se consideraron como control mientras que las plantas tratadas se exponen a un campo magnético sinusoidal de 60 Hz de 2 mT de inducción magnética, durante tres minutos. Los resultados revelaron que las plantas tratadas, respecto a su control muestran un aumento significativo en el calcio (55 %), de aluminio (73 %) y de manganeso (43,2 %) los niveles en fase de establecimiento. Se obtuvo el aspecto de cuatro selecciones sensibles en los embriones intentado lo que respecta a controlar embriones. Se observó la presencia de un doble enlace carbono-carbono, ion nitrito y aldehídos. En aumento fase de multiplicación en el calcio (17,9 %), hierro (29,2%), magnesio (5,7 %) y zinc (26 %) los niveles se refiere, en las plantas tratadas respetan control. En conclusión, los resultados sugieren que la aplicación de un campo magnético de 60 Hz a plantas de café *in vitro* puede mejorar la calidad de las plántulas de café mediante la modificación del contenido de iones de movilidad garantizar una mejor calidad de las plantas.

Palabras clave: iones, clorofilas, plántulas de café, embriones, campos electromagnéticos.

Abstract

Influence of extremely low frequency electromagnetic fields on minerals content and chemical groups during in vitro establishment and multiplication phases of coffee seedlings was investigated. Untreated coffee plants were considered as control whereas treated plants were exposed to a 60 Hz sinusoidal magnetic field of 2 mT of magnetic induction, during 3 minutes. The results revealed that treated plants, regard to their control shown a significant increase on calcium (55 %), aluminium (73 %) and manganese (43,2 %) levels in establishment phase. The appearance of four sensitive picks was obtained in the embryos tried regards to control embryos. The presence of carbon-carbon double bound, nitrit ion and aldehydes was noted. In multiplication phase increase on calcium (17,9 %), iron (29,2 %), magnesium (5,7 %) and zinc (26 %) levels was regards in treated plants respect control. In conclusion, the findings suggest

that the application of 60 Hz magnetic field to in vitro coffee plants may improve the coffee seedlings quality by modifying of ions mobility content ensuring a better quality plants.

Keywords: ions, chlorophylls, coffee seedlings, embryos, electromagnetic fields.

Introduction

Several studies have reported different positive effects on seed germination, shoot development, plant length, fresh weight, fruit production and mean fruit weight when extremely low frequency magnetic fields (ELF-MFs) are applied [1 -8].

The application of ELF-MFs can increase the ion mobility close to receptor places and/or through membrane ionic channels, mainly calcium ion or cluster in cytoplasm [9]. Others authors consider the cyclotron paramagnetic resonance like the main effects of ELF-MFs for membrane disorder related with calcium ion flux [10].

Polk and Postow [11] reported that ELF-MFs action on some organism occur through two mechanism. The first related to physical-chemical process and tissue energy interaction. The second related with ELF-MFs and chemical reaction balance and speed of biochemical process.

Nevertheless, for tissue plant this mechanism is not defined. Moreover, ELF-MFs have positive effects on some plant process like germination, seedling growing, photosynthetic process, enzymatic activity, and others [4, 5, 8].

Coffee is the second most commercialized product in the world posing as an important source of income and jobs in several countries. Coffee trees may be obtained from the coffee seeds which remain viable for three months. Coffee in vitro culture has been successfully established, and consequently high quality coffee seedlings during many years has been generated [13].

Even, the common way to produce coffee seedling is botanic seed, the loss of germination during storage is a problem for propagation and the conservation of genetic resources. Actually, embryos culture is effective to rescue the plant material in seed with low viability. This technique constitutes an alternative to coffee propagation during all year.

In this investigation, we attempted determinate the relation between 60 Hz ELF-MFs on ionic mobility and the component structural formation in in vitro seedling coffee.

Material and methods

Plant growth conditions

Micropropagation and biochemical identification of coffee variety "Catuai" were performed according to the methodology established [14]. The embryos of *Coffea arabica* var. Catuai seeds were grown during 6 weeks in Murashige-Skoog (MS) medium [15], supplemented with 30 g/L of sucrose, 6 g/L of agar, 25 mg/L of cysteine, 0.5 mg/L of casein, and pH was adjusted to 5,6. Test tubes of 25 x 150 mm were used, each one containing 10 mL of culture medium for establishment phase. The plants were maintained under temperatures of 24 ± 1 ºC, relative humidity of 70 ± 5 %, and a photoperiod of 24 h of illumination and 80 μmol/m^2s of photosynthetic photon flux (PPF) with fluorescent lamps.

Coffee seedlings with one pair of leaves were subcultivated in same culture medium and maintained under temperatures of 24 ± 1 ºC, relative humidity of 70 ± 5 %, photoperiod of 16 h and 80 μmol/m^2s of PPF, reaching the multiplication phase after six weeks.

Magnetic field exposure

The 60 Hz MF treatment was performed using the BioNaK-03-1 electromagnet, manufactured at National Centre of Applied Electromagnetism (CNEA), Santiago de Cuba, Cuba. This electromagnet was located in a place away from other electromagnetic sources and ferromagnetic materials. It is formed by two parallel coils (dimensions 50384 cm) as depicted in figure 1a. These measurements were made with a Vernier caliper with clamping screw (0,05mmof precision; Model 5 30-104; Mitutoyo, Japan). The coils were connected in series and coupled to a signal generator of sinusoidal wave form. The coils were connected in series and coupled to a signal generator of sinusoidal waveform.

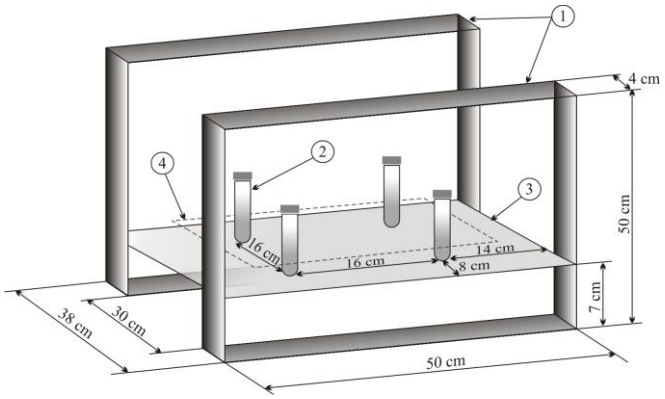

Figure 1a. The schematic representation of the experimental electromagnet setup (BioNak-03-1 electromagnet): (1) two parallels coils; (2) tubes with samples are placed inside of the air gap of coils (16 cm in distance from each other) to expose seedlings to electromagnetic field (60 Hz MF, 2mT during 3min); (3) tray; (4) horizontal plane of electromagnetic induction received by samples.

A non-uniform variable magnetic field was generated inside of the coil when a sinusoidal electric current passed through it. The magnetic induction was 2 mT. It was measured with a digital gauss meter (0,01mT of resolution; basic accuracy: DC ± 3% (without probe), AC: ±2 % (Magnet-Physics, Model FH 54; CWIEME, Berlin, Germany). The measurements were taken at 24 ± 1^{o}C. The magnetic field homogeneity was 0,01 % in the work volume (20 x 20 x 4 cm) inside of the coils. It was computed by (Bi–Bo)/Bo, where Bi and Bo are the magnetic inductions at any point inside of the coil and in the center of magnetic system, respectively (figure 1b).

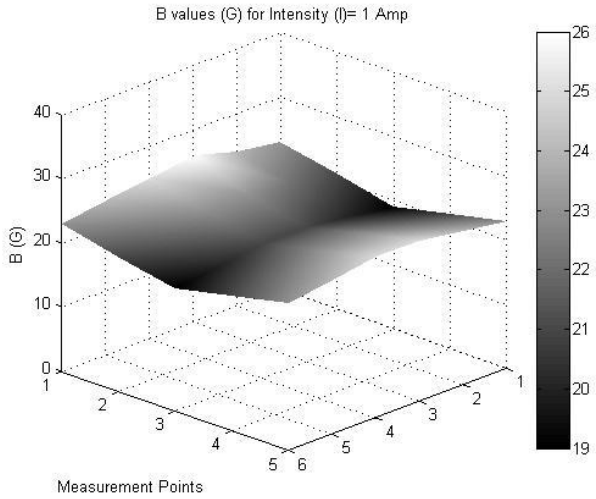

Figure 1b: Distribution map of horizontal component (B) generated in the region occupied by the samples

Although, there were electric fields induced in the seedlings by 60 Hz magnetic fields, these were not calculated because the electric vector decreased 10^5times in intensity and changed the distribution when it crossed the biological tissue, while the magnetic vector did not change for EFL-MF. No magnetic field other than that of the geomagnetic field was detected within the experimental electromagnet when it was switched off.

The local geomagnetic field within the coils was approximately 61mT, measured previously by Gilart [16]. One week after establishment phase, two experimental groups were formed: treated (60 Hz MF, 2mT during 3 min) and control (exposure to the local geomagnetic field only inside of the BioNaK-03-1electromagnet for 3 min) taking into account the results published by [17]. After 6 weeks, seedlings were transferred to the multiplication phase and after 1 week, treatment was applied as the establishment phase for each group (treated and control).

Determination of chemical groups and mineral content

Mineral content determination was made for Inductively Coupled Plasma mass spectrometry (ICP-MS). Six grams of fresh foliar tissue was weighed and dry during 48 hours to 70^{o}C. One grams of dry tissue was introduced in muffle at 250ºC±25ºC and the temperature was increased gradually, (50ºC/hour), until 450ºC ± 25ºC to obtain ahs. Subsequently 1 ml of nitric acid and 2 ml of distilled water for laundering ash was added.

The chemical group characterization of coffee embryos was made by Near Infrared Spectroscopy (IR) with Fourier transformed (Bruker Vector 33). The half capacity is between 500-4000 nm, 5nm of interval. 0,1mg of embryos was triturated with the same amount of potassium bromide. The mixture was compressed in a mechanic press to make a translucent pill. The technique of Total Attenuated Reflectivity in a range of 400-4000 nm was used. The result was analyzed with OMNIC software for Windows 09.

Statistics

Data for control and treated samples were statistically compared by Student´s t-test ($P< 0.05$). SISVAR Software (License: 820459851, Lavras, Minas Gerais, Brazil) was used for statistical analyses.

Results

Magnetically treated plants in establishment phase shown great differences for calcium (55 %), aluminum (73 %), and magnesium (43,2 %) respect to control seedlings. Whereas, the control plants results for iron and zinc was superior respect treated plants (figure 2).

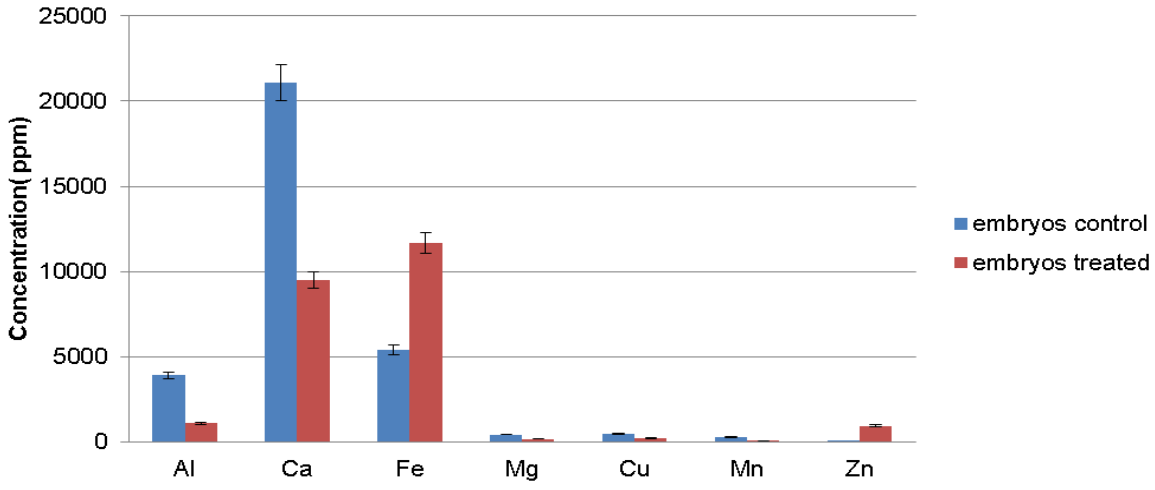

Figure 2. Effects on mineral content of ELF-MFs (2 mT of induction and 3 minutes of exposition) in *Coffea arabica* cv Catuai amarillo in establishment phase (t-Student, P≤0.05)

The germination, beginning for update of water in seed, and it finish with root emergence. In this moment, differential permeability temporal alteration is made accomplish with passive loss of different low molar mass metabolites (sugars, organic acid, ions, amino acid and peptides). This activity shows the transformation of

phospholipids component in cell membrane. An increase of ATP synthesis, respiratory activity and great numbers of enzymes are produced [18].

The coffee embryos are collocated in a culture medium during the establishment phase. The culture medium is formed by macronutrients and micronutrients. The macro and micronutrients are absorbed by the embryos and inside of tissue the mineral participate in many cellular processes like calcium. The calcium is a co-factor for many enzymes involved in ATP hydrolysis.

The results shown an increase of ion like calcium, aluminum and magnesium in treated plants that probably improve the growing in these seedlings, related with the increased of membrane permeability, a manner to agree with [17] and others authors. These authors reported during the expositions to ELF-MFs, the membrane cell channels stay open for more time and consequently an increase of nutrient absorption and cell metabolism [19-23].

The plasmatic membrane has not special structure to act in electromagnetic signal perception unlike electric signal, voltage-dependents channels. The behavior is assign to phospholipids anisotropic properties. The phospholipids is reoriented in membrane and cause the membrane channels deformation and conductivity [10]. Probably this phenomenon happened in coffee embryos treated with ELF-MFs respect to control embryos.

In multiplication phases an increase for calcium (17,9 %), iron (29,2 %) and zinc (26 %) with significant difference was observes in treated plants respect to control plants (figure 3).

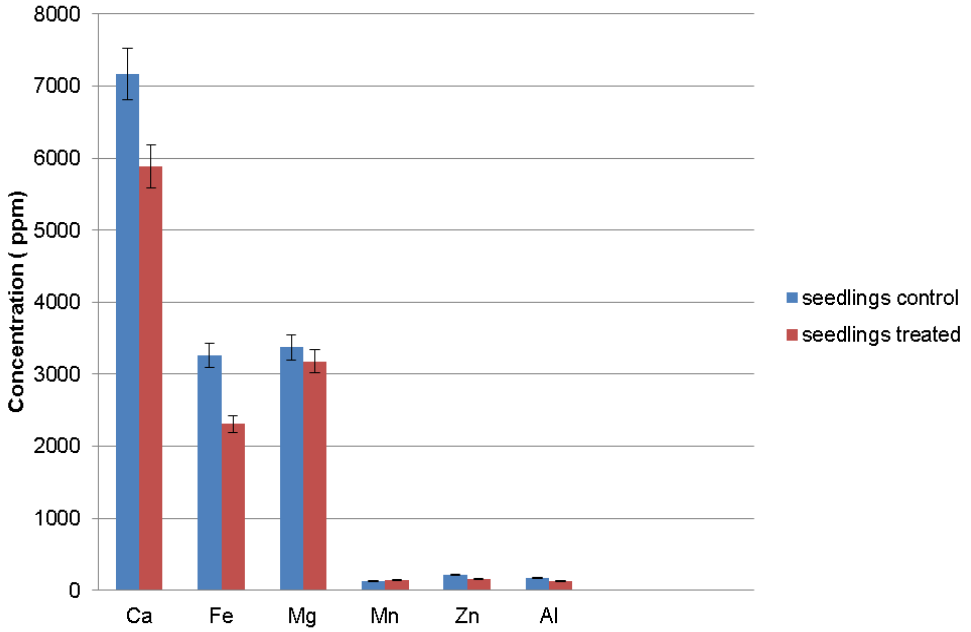

Figure 3. Effects on mineral content of ELF-MFs (2 mT of induction and 3 minutes of exposition) in *Coffea arabica* cv Catuai amarillo in multiplication phase (t-Student, P≤0.05)

Like was said before, electromagnetic fields act on membrane permeability process and it have a positive influence on physiologic process like photosynthesis, hormonal transport, and others. The iron is considering a constituent of cytochromes and non-heme iron proteins involved in photosynthesis, nitrogen fixation, and respiration. The other hand, the zinc is constituent of process glycolytic enzymes and calcium is required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids and acts as a second messenger in metabolic regulation [18].The results indicate that 60 Hz- MF (2 mT and 3 min of exposure) induced a positive influence on the increase of this three elements improve the physiological process in coffee plants to guarantee de quality of seedlings.

Infrared spectroscopy results are shown in figure 4. We can to identify the increase of some components in treated embryos respect to control embryos.

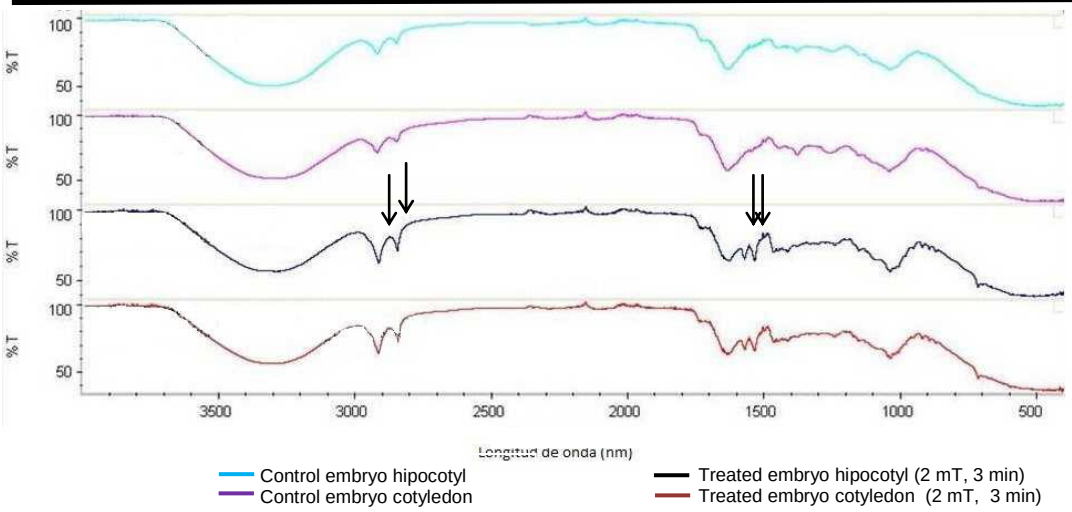

Figure 4. Infrared Spectroscopyresults of EMF-MFs effects (2 mT of induction and 3 minutes of exposition) on *Coffea arabica* L. cv Catuai amarillo embryos. ***X axiswave length (nm); Y axis first absorbance derived.***

The interpretation of four sensible points in the figure 3, that changed for treated embryos respect to control, agree with NO_2 (1 500 nm); double chemical bound C=C (1 600 nm) and aldehydes (2 800 nm).

This result probably is related with a superior metabolic activity in treated embryos, associated with the compound structural formation. In the germination process activation the metabolism of proteins takes place. It well know that double chemical bound (C=C) is a component of nitrogen bases, and the same time this nitrogen bases take place in nucleotide biosynthesis, who are direct precursor of DNA and RNA.

Besides, NO_2 point in treated embryos could be related with the reduction for nitrite reductase to ammonium. Finally the amide form is linked to glutamine to be transformed in others amino acid for produce others proteins and nucleic acid [18].

The results allows to consider the presence of this reaction of nucleotide metabolism in coffee embryos studied, agree with a positive influence on production rates of DNA, RNA, and mRNA, tRNA and rRNA transcripts [10, 19, 24].

Conclusion

The data presented here on EMF- MFS induced changes in mineral content and chemical group during establishment and multiplication phases of embryos coffee is a reference to show a probably increase of membrane permeability in plant cell.

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