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Biological effects of low frequency electromagnetic field in *Curcubita pepo*

Mihaela Racuciu^a, Dorina-Emilia Creanga^{b,*}

^a "Lucian Blaga" University, Faculty of Science, 10 Blvd Victoriei, Sibiu, 550012, Romania ^b "Al.I.Cuza" University, Faculty of Physics, 11A Blvd. Copou, Iasi, Romania

Abstract

Plants of agrotechnical interest, during their early ontogenetic stages have been chronically exposed to low frequency electromagnetic field (50Hz, 10 mT) generated by adequately Helmholtz coils system. Spectrophotometric assay was carried out to evidence the putative changes at the level of the assimilatory pigments involved directly or indirectly in the photosynthesis mechanisms. Chlorophylls ratio (chlorophyll a / chlorophyll b) was found enhanced for 1-2-4 hours exposure times. The total carotene pigment level was found correlated with the chlorophyll a level. © MISM2005. All rights reserved

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1. Introduction

Plants became more and more an attractive model system for the study of the biological effects of the magnetic fields. In the last decades the number of bioelectromagnetism research reports focused upon the investigation of living organisms magnetosensitivity, by using various types of magnetic field exposure has significantly increased. The need for such research has been enlarged following the huge industrial development, the explosive extension of wireless communications and the army production diversification at the end of the XXth century. Nevertheless the larger and larger utilization of electric devices within family and office activities had a significant contribution to the people interest in occupational exposure effects to electromagnetic sources. High level preoccupation and even medical concern have been supplied following the common acceptance of EMF harmful effects in the conditions of the rapid increase of the EMF sources spreading. The putative indirect effects of plant exposed to EMF upon the human being health led to the re-consideration of experimental projects based on vegetal material. So, one can mention the interest in seeds exposure to magnetic field -that revealed the acceleration of plant growth, the protein synthesis stimulation and the plant root development [1-6]. As well known, photosynthesis is a very complex phenomenon resulting in the transformation of solar electromagnetic energy into chemical energy, stored in organic compounds, synthesized from inorganic carbon and water. The study of assimilatory pigments from plant green tissue is able to provide information upon the capacity of solar energy conversion at the level of chloroplast membranes, during photosynthesis. In this paper the authors present some quantitative observations of electromagnetic exposure influence on the growth of Cucurbita pepo (pumpkin)

* Corresponding author. Tel.: +40232201180; E-mail address: mracuciu@yahoo.com

agrotechnical plants.

2. Experimental

Biological material. Caryopsides of *Cucurbita pepo* (pumpkin) harvested from a single plant, (in order to minimize the gene pool variation) have been chosen to compose the samples (30 seeds let to germinate at 24 ^oC in darkness and 98% humidity on watered paper support in a Petri dish constituted a sample).

Magnetic field generator. To generate the low frequency magnetic field (50 Hz, 10 mT), a pair of Helmholtz coils, was employed (Figure 1) which creates a uniform magnetic field into a relatively large space volume allowing the plant uniform exposure between and in the center of the coils.



Figure 1. Magnetic exposure arrangement

Magnetic induction was measured using a HTS type teslameter with Hall probe with an accuracy of 10^{-4} T.

Plant exposure to magnetic field. After germination, the vegetal organisms in early ontogenetic stages were let to grow within the static magnetic field, which was aligned parallel to their stems. Chronic magnetic exposure has been arranged by repeated exposures of 1-2-4-12 hours, daily, of already germinated plantlets, 9 during days. After germination the growth was conducted in constant conditions of temperature (24 °C), illumination (10 h: 14 h light: dark) (magnetic exposure system being introduced within the acclimatization room). Only distilled water in adequate volumes has been supplied to the samples.

Spectral investigation. JASCO V spectrophotometer (Japan) provided with glass cells 1 cm width was used to assay the content of chlorophyll a, chlorophyll b and carotenoid pigments (Meyer-Bertenrath's method [7]).

Statistic analysis. Five repetitions for every measurement in the test and control samples have been accomplished. Average values and standard deviations have been calculated for graphical plots.

The statistical signification of the differences between the control and the magnetically exposed samples was carried out by means of t-test pair type, two tailed.

Box-plot representation method was applied to compare the distributions of plant lengths measured after 9 days [8].



Figure 2. Chlorophylls and carotene contents in the plants exposed to electromagnetic field

3. Results and discussions

The assimilatory pigment levels have been comparatively studied on the basis of graphical representations of chlorophyll \mathbf{a} , chlorophyll \mathbf{b} and total carotene (average values and standard deviations).

In figure 2 the stimulatory influence of EMF chronic exposures on the biosynthesis of chlorophyll a and carotenes (except the 12 hours exposure in the case of chlorophyll \mathbf{a}); up to 21% increase in chlorophyll \mathbf{a} level was recorded in the 1 hour exposed plantlets. On the contrary, slight inhibitory influence (up to 19% diminution in the 4 hours exposed plantlets) on the chlorophyll b biosynthesis



Figure 3. Total assimilatory pigments in plants exposed to electromagnetic field

was noticed (except the 12 hours exposure time).

The assimilatory pigment sum appeared increased for the 1 hour electromagnetic exposure (about 22%) while for longer exposure times non distinguishable changes could be noticed (Figure 3).

The chlorophyll ratio is also providing important indirect data on the photosynthesis since it is an indicator on the activity of Light Harvesting Complex II (LHC II) from the vegetal cell tylakoidal structures (that is known as rather sensitive to the action of external factors). In figure 4 the chlorophyll ratio is presented. This ratio being considered an important physiological parameter regarding the photosynthesis efficiency, the results displayed in figure 4 might be taken as a premise upon the slight stimulation of the photosynthesis (about 23% increasing) in the case of low electromagnetic exposure times (1- 2- 4 hours); these results provide also a suggestive picture for the slight inhibitory effect (about 16%) of EMF in the case of 12 hours magnetic exposure sample. This is concordant with the effect observed in the case of chlorophyll a (Figure 2), which is the most important assimilatory pigment involved directly in the conversion of solar energy into chemical energy at the molecular level in the frame of photosynthesis complex phenomena.

In figure 5 the positive linear correlation between the chlorophyll a and carotene contents is given. These results can be taken as an indication upon the correlated influences of EMF in both chlorophyll a and the secondary carotene like pigments (with a very consistent role in the sustaining of photosynthesis efficiency, by means of the absorbed energy transfer



Figure 4. The effects of EMF exposure on chlorophyll ratio



Figure 5. Linear correlation between the chlorophyll a and the carotene contents in samples exposed to electromagnetic field $(R^2$ - correlation coefficient).

to chlorophyll a molecules). Consequently the assimilatory pigment sum increasing in the sample exposed for 1 hour daily is given by the stimulation of biosynthesis of chlorophyll a as well as of carotenes.

The statistical analysis accomplished for the chlorophyll ratio (by applying the t-test to compare control and test sample data) revealed statistic significance (p<0.05) for all exposed samples.

For the comparative study of stem lengths we have been applied box-plot graphical method. In Figure 6 is presented the box-plot graphic for control and electromagnetic exposure samples. In all boxes the median (corresponding to 50% cumulated frequency of data appearance) represented as a line parallel to the box edges) is close to the average value (represented as an asterisk), which is reflecting the relative high symmetry of the distribution curves. The



Figure 6. Box-plot representation for the EMF exposed plants length

same think is reflected by the equal length of the box tails.

The stimulatory effect of EMF exposure is obvious only for the longest exposure time (where the whole distribution curve, i.e. the correspondent box is shifted to higher values than the control sample distribution) apparently in contrast with the assimilatory pigment situation. It is notable the rather uniform plant lengths was recorded in the sample corresponding to 2 hours EMF exposure where the box length is considerable smaller than in the other samples (about twice smaller than in the control box).

In fact the complexity of plant growth phenomena can not be evaluated only by means of the chlorophylls and carotenes behavior but also by considering the enzymes activity, the nucleic acid levels, and the capacity of water accumulation and so on.

It is designed that our further experimental projects to be focused on the diversification of the investigation methods able to provide data regarding the plant magnetosensitivity in the range of EMF exposure parameters tested inhere.

Based on the fact that such EMF exposure can often occur in natural plant populations (because of both natural and artificial sources of low frequency EMF) but no phenotypic changes are reported regarding the perturbation of the main biological patterns, the presumption that the recover mechanisms are also activated following the EMF exposure is a challenging issue. Young plantlets aged of no more than ten days seems to characterized not only by their magnetosensitivity but also by their ability to balance the electromagnetic stress.

In the same time, in the case of the present experiments the needs of controlling the plant growth conditions (and aiming to assure the result reproducibility) have imposed their supply with only distilled water – fact that is obviously not correlated with the natural situation where many other chemical, physical, biological and ecological factors have their own contributions to the plant development.

4. Conclusions

The low frequency magnetic field (50 Hz and 10 mT) treatment has revealed that Cucurbita pepo young plantlet development may be slightly affected during their first ten days of growth. When the exposure time is of about 1 hour stimulatory effect could be noticed at the level of assimilatory pigment biosynthesis (except the chlorophyll b). The chlorophyll ratio was simultaneously enhanced for the samples corresponding to 1-2-4 hours, which is reflecting the significant EMF effect on the energy specific conversion processes (located in the tylakoidal membranes). Appreciable negative influence on these energy mechanisms was emphasized for 12 hours magnetic exposure while the plant lengths are enhanced.

The interest for the continuation of such bioelectromagnetism experiments is sustained by the recognized high level of electromagnetic pollution of the biosphere and by the persistence of some biological effects of longer electromagnetic exposure at the biochemical level of foods prepared from exposed plants.

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