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ON THE IMPACT OF EXPOSING MAIZE SEEDS TO A LOW FREQUENCY FIELD ON BIOLOGICAL FEATURES AND ON ASSIMILATING PIGMENT SYNTHESIS

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Abstract. *Magnetic field, particularly low-frequency magnetic field (LMF), induces in the biological tissues, currents that result in biological effects. They claim that biological effects induced by electromagnetic fields are adaptive or compensatory effects and that the body should adjust to this environmental factor. This paper presents the effect produced by pre-germination exposure of maize seeds to a magnetic field produced by low-frequency electromagnetic waves on the biological features and on the synthesis of assimilation pigments.*

Trials were carried out within the Laboratory for the testing of seed quality and of seeding material of the Department of Agricultural Technology of the Banat University of Agricultural Science and Veterinary Medicine in Timisoara (Romania). The trial aimed at assessing the effect of LMF on germinating energy, germinating ability, root length, fresh root volume, length of aerial part, fresh aerial volume, as well as a chlorophyll content, b chlorophyll content, carotenoids, lipids, proteins, and fibbers measured in maize leaves 30 days after treatment.

The plant pigments were determined with the spectrophotometer method, in accordance with the guidelines for analytical validation of the FDA, EMEA, FAO/WHO and the Laboratory Standard Operating Procedures "Benchmarking for design and development of innovative biotechnology, sustainable and strategic selection of genetic resources rich in bioactive compounds of plant origin" of the Department of Exact Sciences of the B.U.A.S.V.M. in Timisoara.

The study showed significant differences from the point of view of the parameters analysed compared to the control that was not exposed to the magnetic field produced by low-frequency electromagnetic radiations.

Keywords: low-frequency magnetic field, biological features, assimilating pigment,

INTRODUCTION

These last years, the study of the effect of electromagnetic radiations and their action on physiological mechanisms in living organisms lead to an interesting field of interdisciplinary research called, by many authors, "electro-magneto-biology". [15]

This new area of interdisciplinary research covers subjects specialised in the investigation of the impact of the magnetic field (EMF) on biological systems. [11,12]

Numerous studies carried out particularly these last years have shown that non-ionised electromagnetic radiations have on the living systems numerous effects depending on the wave length. [6,16]

The adepts of bio-electro-magnetism cited by Calugăreanu (1998) show that bio-electro-magnetism studies different electro-magneto-sensitive processes at the fundamental level of interaction of the fields with the substance. The bio-electro-magnetic effect is mainly the result of the different processes in a living body – from the (bio)physical molecular one to the complex adaptive biological ones. [1,2,9]

Synthesizing the basis of this approach from the data obtained from the different and numerous trials, we can claim the following: electromagnetic fields can alter the metabolism of any biological system, and the effects in each tissue or system are independent enough from the type of electromagnetic field; thus, on one hand, studies show that there are common ways of developing phenomena for the different spectral electromagnetic fields and that the major effect associated with the specificity of the electromagnetic field is that it determines the size and direction of the biological effect and, on the other hand, some spectral features (modulation, etc.) appear to be among the most important since they can change fundamentally the biological response. [6]

There are authors who claim that organisms' response to the action of the electromagnetic field is partially determined by their physiological history and partly by their genetic predisposition; individual organisms, even from a population apparently homogeneous, can have changes in different senses of a biological parameter depending on the action of the electromagnetic field. [1,8]

The Earth's electromagnetic field is $2x10^{-4}$T, and the human body is adapted to it. It is well-known that, in plants, lack or diminution of the electromagnetic field result in plant growth and, from certain levels, plant death. [9]

Most of the researchers are studying the influence of electromagnetic fields (EMF) and electromagnetic radiation (EMR) which could be easily generated for various purposes, including future agriculture applications of alternating low magnetic field (LMF). [11]

Wadas (1992) shows that there are biochemical, physical, and physiological changes under the impact of magnetic fields in the cells structure.

According to some authors, electromagnetic impulse act on the water structure in the cell, which has a beneficial impact on plants. [5]

Other authors consider that exposing seeds to electromagnetic radiations results in a change of the enzymatic activity. Pietruszewski (1996), citing Marino and Becker (1977) and Blank (1995) show that *in vitro* studies pointed to enzyme activity as well as a stimulation of biosynthetic processes involving polymerase and free radical ribonucleic acid (RNA).

Pietruszewski (1996) claims that changes in cell synthesis, especially in protein synthesis, activated by LMF, are similar to those induced by cellular stress called 'heat shock'. But energy needed to start protein transcription form magnetic field is many orders of magnitude lower than energy needed for thermal stimulation.

The results presented in this study refer to the RIES (Radiation Impulse Electromagnetic Stimulation) method in seeds.

MATERIALS AND METHODS

The terminology used in scientific papers concerning low frequency electromagnetic waves is the following:

- very low frequency electromagnetic waves or (**VLF)** with frequencies between 30 kHz and 3 kHz
- ultra low frequency electromagnetic waves or (**ULF**) with frequencies between 3 kHz and 300 Hz
- super low frequency electromagnetic waves or (**SLF**) with frequencies between 300 Hz and 30 Hz
- extreme low frequency electromagnetic waves or (**ELF**) with frequencies below 30 Hz.

In this study concerning the treatment of maize seeds we have used extremely low frequency electromagnetic waves.

In treating the seeds with the electromagnetic wave generator of the Faculty of Agriculture in Novi Sad (Serbia) we used 5 wave lengths in our trial.

Frequency codification under study was: V_1- 22 Hz; V_2- 22,5 Hz; V_3- 23 Hz; V_4- 23,5 Hz; V_5- 24 Hz; MT- control (not treated).

The treatment was applied 1.5 km far from the seed storage place to avoid interferences during seed treatment with different experimental wave lengths. Thus, we treated the first variant for 10 minutes, after which we carried it to the storage place and we treated the other sample, for each trial wave length.

We used the maize hybrid PR37Y12, a maize hybrid developed by the Pioneer Hi Bred Seed Company that is being cultivated at present on the largest areas of the Western Plain.

After treatment, the seeds were put to germinate in boxes 30/20 cm. In a box, we sowed 35 treated seeds, each variant being sowed in three replications. The support on which we sowed the seeds was quartz sand, and watering the seeds was done with distilled water. The laboratory temperature was maintained at 20-21^{0}C.

We monitored the effect of 5 frequencies compared to the control variant (not treated) and we made the following measurements:

- germinating energy (%), 4 days after treatment;
- germinating ability (%), 7 days after treatment.

To monitor the impact of electromagnetic stimulation on the growing rate and on the synthesis of assimilation pigments, we transplanted the plantlets to nutrient solutions after we calculated the plants' germinating ability, using boxes 10/10 cm in size. To render as exact as possible the soil conditions, the nutrient solution we used was the R. Jork method:

KH_2PO_4	27,2 g/l		
KCl	74,54 g/l		
$CaCl_2x6H_2O$	218,53 g/l	$\longrightarrow$	
$MgSO_4x7H_2O$	98,58 g/l		120 ml/20 l H_2O
NH_4NO_3	120,18 g/l		
$ZnSO_4$ x$7H_2O$	0,15 g/l		
H_3BO_3	1,5 g/l		
$CuSO_4$ x$5H_2O$	0,06 g/l	$\longrightarrow$	5 ml/20 l H_2O
MnCl x$4H_2O$	1,5 g/l		
Na_2MoO4 xH_2O	0,0,3 g/l		

Measurements aimed at the effect produced by 5 frequencies compared to the control (not treated) variant, monitoring the following:

- root length (cm) 30 days after treatment;
- length of aerial part (cm) 30 days after treatment;
- fresh root volume (g) 30 days after treatment;
- fresh aerial part volume (g) 30 days after treatment.

The dry volume of the root and of the aerial part was determined within the Laboratory for the testing of seed quality and of seeding material of the Department of

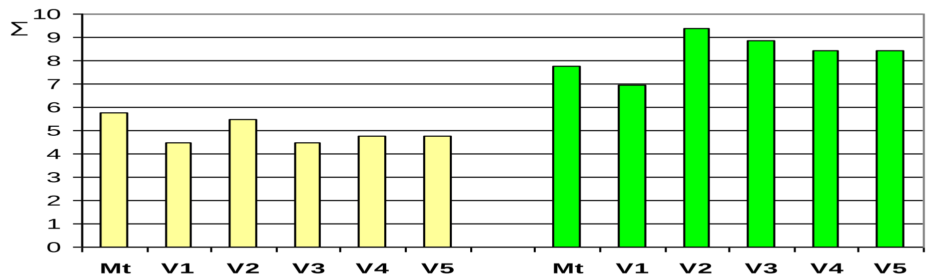
Agricultural Technology of the Banat University of Agricultural Science and Veterinary Medicine in Timisoara (Romania) with a "Thermobalance".

We also measured, from the leaves, lipid content (%), crude protein content (%), crude fibber content (%), carbohydrate content (%), a chlorophyll content (mg/100 g), b chlorophyll content (mg/100g), and carotenoid content (mg/100g).

The plant pigments were determined with the spectrophotometer method, in accordance with the guidelines for analytical validation of the FDA, EMEA, FAO/WHO and the Laboratory Standard Operating Procedures "Benchmarking for design and development of innovative biotechnology, sustainable and strategic selection of genetic resources rich in bioactive compounds of plant origin" of the Department of Exact Sciences of the B.U.A.S.V.M. in Timisoara.

RESULTS AND DISCUSSION

Figure 1 presents results concerning the measurement of germinating energy (calculated 4 days after treatment) and of germinating ability (calculated 4 days after treatment) depending on 5 wave lengths compared to the control (not treated).



Variant	Mt	V1	V2	V3	V4	V5		Mt	V1	V2	V3	V4	V5
∑	5,75	4,5	5,5	4,5	4,75	4,75		7,78	6,967	9,4	8,877	8,417	8,417
Difference		-1,25	-0,25	-1,25	-1	-1			-0,813	1,62	1,097	0,637	0,637
Sum of the germinating energy (4 days after)								Sum of the germinating ability (7 days after)					

Fig. 1. Calculus of the sum of the germinating energy index and of germinating ability depending on 5 wave lengths compared to the control (not treated)

If, from the point of view of the value of the germinating energy index, the value of the control was above the trial variant values, in germinating ability the value of the control variant was below trial variant values.

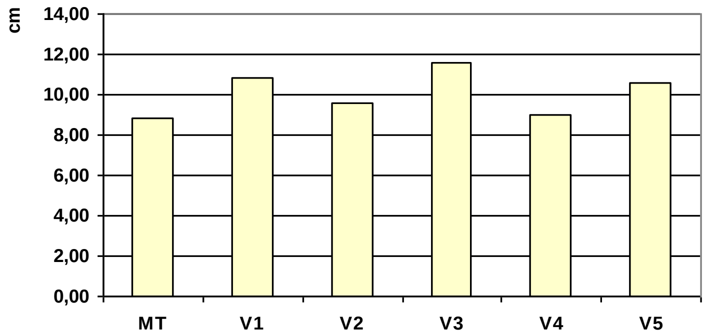
These values prove the stimulation effect of germination positively, particularly favouring explosive sprouting.

The measurements concerning root length depending on the trial wave length compared to the control (not treated) are shown in Figure 2.

Data show that, depending on the trial wave length, root growth compared to the control (not treated) ranged between 0.2 cm in the variant V_4 (23.5 Hz) and 2.8 cm in the variant V_3 (23.0 Hz). The variants V_1 (22.0 Hz) and V_5 (24.0 Hz), with about 2 cm, and the variant V_2 (22.5 Hz), with 0.8 cm ranged on intermediary positions.

Data show that, compared to the control (not treated) variant when stimulated with wave measuring 23.5 Hz, the aerial part grew, compared to the control, with 3.8 cm; in the variant treated with 22.5 Hz, length increased with 3.0 cm, and in the variant treated with 23.0 Hz, the length of the aerial part increased with 2.8 cm. In the stimulation variants 22.0 Hz and 24.0 Hz, the growth of the plants was positively impacted.

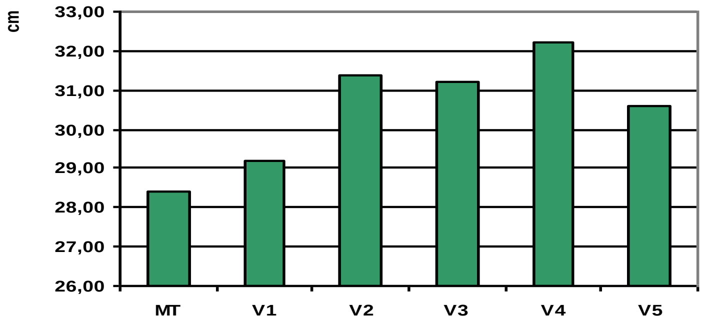
Results concerning the effect of exposing seeds to low-frequency electromagnetic waves on maize root weight are shown in Figure 4. In the variants with the highest values of the root length we obtained the highest values of the fresh root weight, with differences between 0.15 g, in the variant V_4 (23.5 Hz) and 0.44 g, in the variant V_3 (23.0 Hz).



Root legth (cm)	8,80	10,80	9,60	11,60	9,00	10,60
%	100	123	109	132	102	120
Difference		2,00	0,80	2,80	0,20	1,80
DL5%=1,1; DL 1%=1,7; Dl 0,1%= 2,38						

Figure 2. Root length (cm)

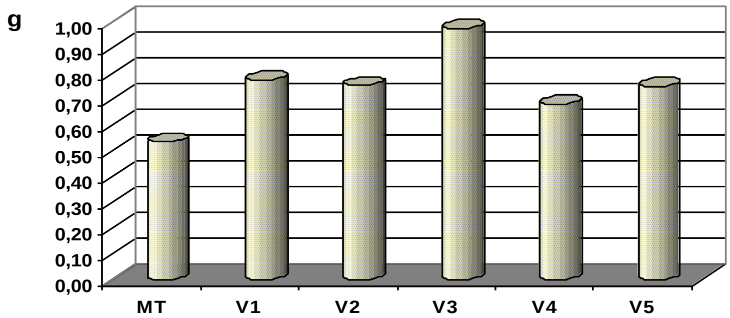
The effect produced by exposing the seeds before sowing for 10 minutes at 5 low-frequency wave lengths on the growth of the aerial part is shown in Figure 3.



Legth of aerial part (cm)	28,40	29,20	31,40	31,20	32,20	30,60
%	100	103	111	110	113	108
Difference		0,80	3,00	2,80	3,80	2,20
DL5%=1,6; DL 1%=2,22; Dl 0,1%= 3,38						

Figure 3. Length of aerial part (cm)

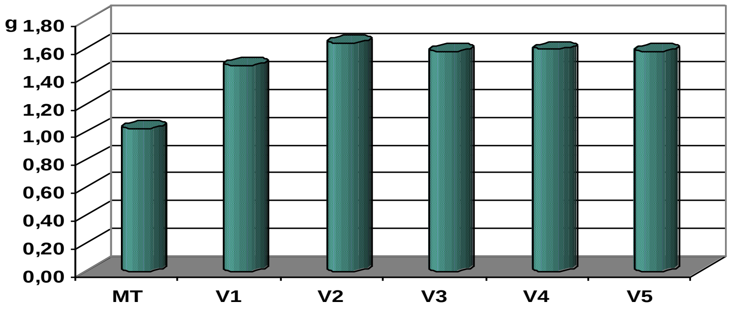
Figure 5 present the results concerning the effect of exposing seeds to low-frequency electromagnetic waves on the fresh maize aerial part weight. To note that the highest values of the aerial part weight were obtained in the stimulation variants V_2 and V_4, variants in which we obtained the lowest values of root length and weight.



Fresh root weight (g)	0,53	0,77	0,75	0,98	0,68	0,75
%	100	145	141	183	127	140
Difference		0,24	0,22	0,44	0,15	0,22
DL5%=0,3; DL 1%=0,68; Dl 0,1%= 1,02						

Figure 4. Fresh root weight (g)

This aspect is very important since through electromagnetic stimulation of the maize seeds we can induce a root growth that results in better fixation with positive effects on drought resistance, a phenomenon that is steadily increasing these years.



Fresh aerial part weight (g)	1,02	1,47	1,63	1,58	1,59	1,58
%	100	144	161	155	156	155
Difference		0,45	0,62	0,56	0,57	0,56
DL5%=0,41; DL 1%=0,69; Dl 0,1%= 1,06						

Figure 5. Fresh maize aerial part weight (g)

Data concerning assimilation pigment synthesis are shown in Tab. 1.

Table.1.

Proximate analysis of the hybrid component (% dry weight)

Samples		Lipid (%)	Crude protein (%)	Crude fibre (%)	Carbohydrate (%)	*Chl a* (mg/100g)	*Chl b* (mg/100g)	Carotene (mg/100g)
PR37Y12	Standard	1,50±0,02	36,28±0,05	18,29±0,11	45,38±0,03	49.0±0.25	23.9±0.72	19.4±1.05
	V_1	1,05±0,10	36,73±0,03	18,99±1,47	49,01±0,10	53.2±1.65	20.1±1.67	19.1±0.05
	V_2	1,17±0,02	37,79±0,06	17,60±1,88	51,28± 0,04	53.1±0.25	20.4±1.45	19.7±2.45
	V_3	1,31±0,14	38,11±0,00	18,29±0,11	51,82±1,22	54.7±1.40	24.1±0.30	21.1±0.65
	V_4	1,57±0,10	30,86±0,09	18,99±1,47	55,62±2,08	57.0±2.67	21.3±0.95	21.5±0.65
	V_5	1,82±0,02	38,88±0,01	19,25±0,07	59,01±0,05	61.4±1.05	24.2±0.30	21.7±0.95

Lipid content (%) in maize leaves, depending on the type of maize hybrid experimented and wave length used resulted in the highest values in the variant V_5 – 1.82%, followed by the variant V_4 – 1.57%. In the variants V_1, V_2, and V_3, lipid content was below that of the control (not treated) variant.

Crude protein content (%) depending on the maize hybrid and on the wave length experimented recorded, in 4 of the 5 trial variants, values above that of the control (not treated) variant. The highest crude protein content was in the variant V_5 – 38.88%, followed by the variants V_3 – 38.11%, V_2 – 37.79%, and V_1 – 36.73%.

The impact of the wave length on fibber content in maize leaves also recorded, in 4 of the 5 trial variants, values above that of the control (not treated) variant.

Carbohydrate content depending on maize hybrid and trial wave length was above the values of the control (not treated) variant in all 5 trial variants.

The highest value was recorded in the variant V_5 – 51.02%, compared to only 45.38% in the control variant (not treated). The values of a chlorophyll content depending on the maize hybrid and on trial wave length ranged between 49.00% in the control (not treated) variant and 61.40% in the variant V_5. To mention that, in all 5 trial variants, the values of the a chlorophyll content were above that of the control (not treated) variant.

The content of b chlorophyll in 2 of the 5 trial variants (V_3, and V_5) was above the value of the control (not treated) variant (23.90%). In the variants V_1, V_2, and V_4, the values were below that of the control (not treated) variant.

The values of the carotenoid content depending on maize hybrid and on trial wave length recorded values almost equal to those of the control variant in 2 variants (V_1 and V_2), while in 3 of the trial variants were above the control value with 2.00%.

The dependence of the maize hybrid components on low frequency electromagnetic wave evaluated by the simple regression and correlation analysis are shown in Fig. 6.

Dependence of the maize hybrid component on the low frequency electromagnetic wave (variant PR37Y12) is a simple regression:

- linear model *Chl b* (mg/100g) equation is $y = 2.1429x + 47.233$ and R squared value $R^2 = 0.9186$; *Chl b* (mg/100g): $y = 0.2089Ln(x) + 22.104$; and R squared value $R^2 = 0.9051$;
- logarithmic model from Carbohydrate (%) equation is $y = 6.8968Ln(x) + 44.457$, and R squared value $R^2 = 0.9037$;
- polynomial model from protein (%) $y = 0.0823x^2 – 0.6988x + 37.639$; and R squared value $R^2 = 0.9123$, and carotene (mg/100g): y = 0.0304x2 + 0.3618x + 18.69; and R squared value $R^2 = 0.8839$;
- exponential model from: Lipid (%) equation is $y = 1.0977e^{0.0653x}$ and R (regression type) squared value $R^2 = 0.8647$
- exponential model from crude fibber (%) y = 18.196 x 0.018; and R squared value $R^2 = 0.9268$.

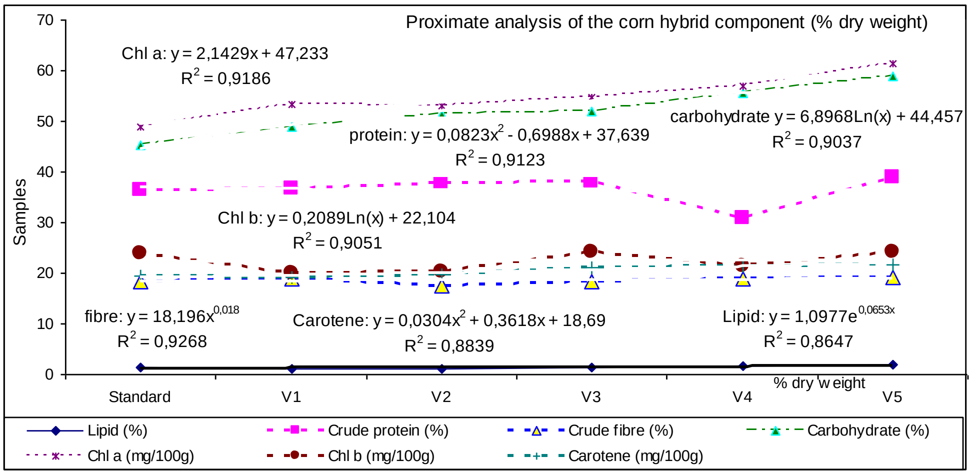


Fig. 6. Dependence of the maize hybrid components on low frequency electromagnetic wave

CONCLUSIONS

Results of the present study concerning the impact of the magnetic field developed by low frequency electromagnetic waves on the biological features and on the synthesis of assimilation pigments in maize allow the following conclusions beneficial for the adoption of the cultivation technology.

As a result of exposing the seeds for 10 minutes before sowing, we could see there is an increase of the sum of the germinating ability index in all trial variants compared to the control (not treated) variant.

Positive impacts were also from the point of view of root and stem length as well as of the fresh root and stem weight; the stimulation effect was different in the different variants, which shows that it is determined by the wave length. To note that in the variants with the highest values of the root length we obtained the lowest values of the stem length, which is beneficial for maize, since in the first vegetation phases there is deep rooting, plants develop a vigorous stem, and drought resistance increases, a phenomenon more and more common these last years in Romania’s Western Plain.

As for the synthesis of assimilation pigment, we could notice increases of the values of the analysed parameters depending on trial wave length but not constantly in the parameters analysed and in trial wave length.

In the conditions in which, in most springs, there are frequent problems caused by germination particularly because of lack of water, results are valuable; if we take into account the price of the treatment (about 5-10 Euros per ha), the value of the results is higher and with real chance to be applied in agriculture.

Acknowledgements

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