Analysis of the Radish Seed Germination and Growth by Radiofrequency Stresses

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Abstract

In this work we report a study on the behavior of radish seed (*Raphanus sativum* L.) germination and growth under radiofrequency stresses. Groups of uniform seeds were irradiated at five duration time values of 60, 210, 375, 470 and $830\,h$ at medium frequency of $1\,MHz$, $(MF/1)$, $(MF/2)$, $(MF/3)$, $(MF/4)$ and $(MF/5)$, respectively; at very high frequency of $100\,MHz$, $(VHF/1)$, $(VHF/2)$ f$(VHF/3)$, $(VHF/4)$ and $(VHF/5)$, respectively; at ultra high frequency of $900 MHz$, $(UHF/1)$, $(UHF/2)$, $(UHF/3)$, $(UHF/4)$ and $(UHF/5)$, respectively. The exhibited magnetic field for the three frequencies was of about $240\,nT$ and the associate electric field inside the samples was less than $71\ V/m$, owing to the electric permittivity exposed sample. Another group of uniform radish seeds, irradiated by static magnetic (SM) field of $80\,mT$ for the same time duration, was used as comparison, whereas untreated ones were used as control. The results showed that all physical stresses induced by magnetic fields did not have effect on seed germination as well as on cell elongation growth of the radish hypocotyls. On the contrary, a stimulating effect was observed on root growth.

I. INTRODUCTION

It is known that the human population is surrounded by electric and electronic devices. All this involves electric currents that generate magnetic fields. It is also known that variable magnetic fields generate electric fields, called inducted fields different from the electrostatic ones, and they are regularized by the Faraday law. The knowledge of magnetic effects on the live organism has a twofold purpose: the first is to discover the role of magnetism on the behavior of living beings; the second is to understand the result of the biological effects, both positive and negative [1-3].

The importance of the magnetic field in biological matter consists in the fact that it can interact with the moving electric charges, on the contrary the electric field interacts with the electric charges, stopped or in moving by the Coulomb force.

Regarding the biological matter, the charges are very closely bound to atoms and they are responsible of the magnetic moment of the matter. The exhibited magnetic moment of matter is regulated by quantum mechanics and it is responsible of the ferromagnetism and paramagnetism phenomena of molecules, behaviors that are very evident. Minor evident is the diamagnetism, a characteristic of all molecules, and it is sensitive to the derivative of the magnetic flux due to the Lentz law. Therefore, only fields of high frequency could deliver to significant results to diamagnetism matter.

Generally, the biological matter can be considerate paramagnetic, and the interaction of the magnetic field with the matter applies a mechanical moment $\vec{N}$ corresponding to:

$$\vec{N} = \vec{M} \times \vec{B} \tag{1}$$

where $\vec{M}$ represents the magnetic moment of matter and $\vec{B}$ the magnetic field. Inside the matter the mechanical moment causes a variation of the orientation and as consequence of the energy of the same molecule, value expressed by:

$$E_B = -\vec{M} \cdot \vec{B} \tag{2}$$

The study of living matter exposed to fields is very difficult due to the complexity of the cell membrane structure. In these cases, it is reasonable to suppose that the mechanical moment induced by the magnetic field can influence the charge transport through the membrane and the energetic state. The magnetic field could interact directly with the DNA, but this is to be discovered. Instead, considering the only magnetic moment due to the electron whose spin is ± ½, the energy variation according to the following Eq. 3 can be positive or negative, that is:

$$E_B = \pm g \mu_B B m \tag{3}$$

where g is the factor of Landé which is close to 2.00 for free electrons and as well as for most organic radicals [4], μ_B is the magnet of Bohr, B is the applied magnetic field and m is the quantum constant.

It is known that on the earth a magnetic field is present and it is very relevant for many living organisms even if its intensity is very low, about $50\ \mu T$, as well as its variation. In general, more intense fields are used in laboratory experiments and are expressed by the term of moderate field and range from a few $100\ 10^{-6}$ to 1 Tesla.

By the above theory, it is easily to image that magnetic and electric fields have a great impact on growth and development of higher plants [5]. Nowadays, physical stimulations are considered an ecological and economical method, used in alternative to chemical ones, for increasing the performance and productivity of plants. Physical methods of plant growth stimulation are reported to be: i) static and variable magnetic and electrical fields, ii) microwave fields, iii) ionizing and laser radiations. These methods are considered not only more profitable, but they also improve the productivity of plants without harming the environment [6, 7].

Other studies, however, have reported negative effects in relation to seed germination time. It is reported that radish seeds exposed to a variety of magnetic field intensity conditions (alternating current and direct current) showed a reduction of germination time when treated under highest magnetic field ($110\ mT$ exposure for $10\ min$) [8].

Referring to the reported data, we have studied the effect of two different physical stresses on radish seeds and followed their germination and seedling growth. In particular, we investigated the behavior of the seeds under the effect of radiofrequency and under the effect of a static magnetic field. Three different radiofrequency we utilized 1, 100 and $900\ MHz$ which generated a field of $240\ \mu T$, while the static magnetic field was of $80\ mT$. Five exposure times were applied for all stresses 60, 210, 375, 470 and $830\ h$. The results were compared to the untreated samples ones.

II. EXPERIMENTAL APPARATUS

To perform experiments by electromagnetic fields it is necessary to make transmission lines [$9, 10$] connected to radiofrequency (RF) generators of different frequency. All generators have got an output impedance of $50\ \Omega$ and as a consequence all lines have to have a characteristic impedance of the same value, $50\ \Omega$. On the contrary, the static magnetic field set up, used as compare, was much simpler.

Medium frequency (MF) set up

The medium frequency we used corresponds to the one used to radio communication in amply modulation. We chose $1\ MHz$ e the corresponding wavelength in air is $300\ m$. The line was designed with a height $h = 1.4\ cm$, width $a = 9\ cm$ and with a lengthy of $20\ cm$, see *Fig. 1*.

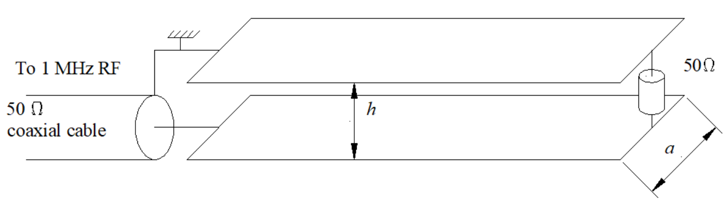

Fig. 1: $50\ \Omega$ transmission line operating at MF, $1\ MHz$.

The length of the line does not affect the characteristic impedance R_0 but allows to have a large operating area and to treat simultaneously more samples. The expression of the characteristic impedance of the flat line, excluding the external radiation, is looked at the following formula:

$$R_o = \sqrt{\frac{L}{C}} = \sqrt{\frac{\mu_o}{\varepsilon_o}\frac{h}{a}} \qquad (4)$$

where L and C represent the inductance and capacitance for unit length, instead ε_o and μ_o are the electrical permittivity and magnetic permeability, respectively. From $Eq. 4$ it is deduced that our line has an impedance of about $50\ \Omega$. As a consequence, the line input was connected to a $50\ \Omega$ generator via a high frequency $50\ \Omega$ coaxial cable and the output of the line was connected to a $50\ \Omega$ resistors, *Fig. 1*.

The RF generator was a $RHODE$ & $SCHWARZ\ SM$ 300. Its maximum output power is $20\ mW$ and the voltage utilized was $1\ V$.

Very low frequency (VHF) set up

The second radiofrequency value we chosen was $100\ MHz$ which corresponds to the frequency modulation of radio communication. Its wavelength in air is $3\ m$. The line we made had the geometric characteristic similar to the one of the $1\ MHz$, height $h = 1.4\ cm$ and width $a = 9\ cm$. In this case, to improve the matching of the line at $100\ MHz$, we closed the output of the line by 4, $200\ \Omega$ resistors in parallel, *Fig. 2*.

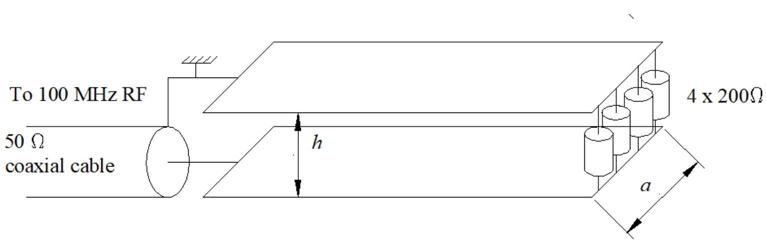

Fig. 2: $50\ \Omega$ transmission line configuration operating at VHF, $100\ MHz$.

The RF generator was again a $RHODE$ & $SCHWARZ\ SM$ 300 and the voltage utilized was again $1\ V$.

Ultra high frequency (UHF) set up

The third wavelength value, we chosen, was $900\ MHz$. This frequency is used for mobile communication and its wavelength in vacuum is about $0.33\ m$. For this reason, the $50\ \Omega$ transmission line, we designed, was very similar to the previous ones but modified in order to limit the external irradiation. It had the conductor lateral outlines bended and the final line width was of about $10\ cm$, while the height again $h = 1.4\ cm$, and the length shorter than the previous ones. It was $12\ cm$. To matching the line at $900\ MHz$, we closed its output by 4, $200\ \Omega$ resistors in parallel, *Fig. 3*.

The RF generator was a RHODE & SCHWARZ SMF 100A having the maximum output power of $20\ W$ and the voltage utilized was again $1\ V$.

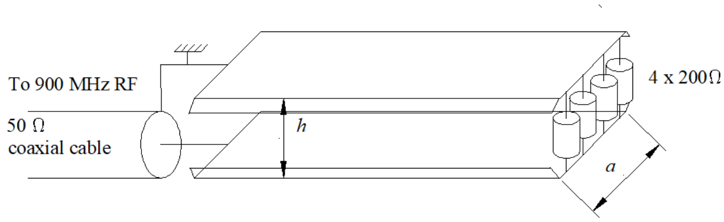

Fig. 3: 50 Ω transmission line configuration operating at UHF, 900 MHz.

Static magnetic field (SM) set up

The static magnetic field was realized by a magnetic circuit with two 40 mT magnets and a ferromagnetic core. As shows in *Fig. 4*. The magnetic field in the gap was of 80 mT.

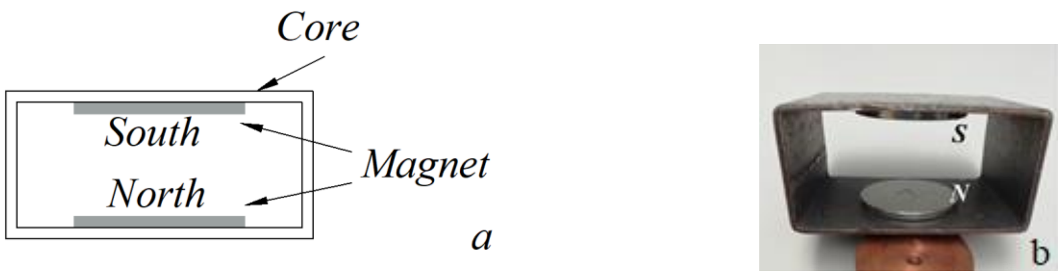

Fig. 4: Set up of static magnetic SM field. a) sketch; b) photo.

III. SAMPLES AND PREPARATION

Plant Material

Radish seeds (*Raphanus sativus*, L.) used for all experiments were obtained from Riccardo Larosa (Andria, BT, Italy). They were not treated with any chemicals and showed uniform germination rates within the same batch of seeds. Seeds were selected on the basis of uniform size and without visible morphological defects or deformities.

Physical Treatment and growth conditions

Radish seeds were placed in Petri dish and then inserted under the various magnetic field generators, while a sample was take far from the generators. Each experimental treatment was replicated three times.

Untreated and exposed to physical stress seeds, rapidly sterilized with NaClO 2%, were placed in Petri dishes (10 seeds for dish) on water-wetted Whatman n° 1 paper for germination and growth under sterile conditions. The Petri dishes were transferred into a growth chamber setted with a photoperiod of $16/8$ h day/night, at a temperature of 22 $°C$, with light intensity of 25 μE, up to 96 h. At the end of 24, 48, 72 hours of incubation the Petri dishes were taken out from the growth chamber and photographed, whereas at the end of 96 h the seedlings were removed from Petri dishes and the hypocotyl and root of each sample were photographed and then their dimensions were measured.

IV. RESULTS

The exposition of the samples was performed storing the choice seeds in Petri cell make of polystirene. The output voltage of all RF generators was fixed to $1V$. The currents inside the lines at the voltage peak resulted of $0.02\,A$ and the generated magnetic field for the three frequencies was the same, about $240\,nT$. The electric field inside the lines resulted of $71\ V/m$, but inside the samples can decrease owing to the electric permittivity of the exposed sample. Instead, when samples are inserted into the line the system becomes considerate like stratified dielectric medium composed of more layers: air, polystyrene and sample. Pointing out the electric field in area without sample, $71\ V/m$, the ones in correspondence of the samples are regularized by the following law $[9, 10]$:

$$E_i = E_0 \frac{h_0}{\varepsilon_i \sum_{n=1}^{3} \frac{h_n}{\varepsilon_{rn}}} \tag{5}$$

because of the integral of the electric fields for the three stratified dielectric $E_1 h_1 + E_2 h_2 + E_3 h_3$ must be to $E_0 h$, whose value corresponds to the applied voltage. In the Eq. (5) h_n and ε_{rn} are the thickness and the relative dielectric constant of the nth layer, respectively. Note that $\sum_1^3 h_n = h_0$. From the Eq. (5) the value of the electric field inside the samples becomes $1.2\ V/m$ having fixed for the seeds $h =\cong 2.5\,mm$, $\varepsilon_r \cong 80$, and for the Petri cells $h =\cong 2\,mm$, $\varepsilon_r \cong 2.6$.

The measurements were performed by Le Croy Wavepro 7100 fast oscilloscope, $20GS/s$ with $1\,GHz$ band limitation. They also were addressed to the length of the roots and of the hypocotyls comparing to the control ones.

The measurements on the control seeds –exhibited a mean length of the roots of $24.00\,mm$ with a statistic error of $2.14\,mm$, and the related value of the hypocotyls was $10.75\,mm$ with a statistic error of $0.7\,mm$. On the contrary, all the analysis by new stresses on exposition time were very variable.

The results of the length of the roots on different doses are reported in $Fig. 5$, while the ones of the hypocotyls are reported in *Fig. 6*. In all figures the error values of the statistic distribution are reported. They are pointed out by the vertical bars. It is possible to observe that the length of the roots changes along the dose (*Fig. 5*), instead the values of the hypocotyls do not suffer of strong variation (*Fig. 6*).

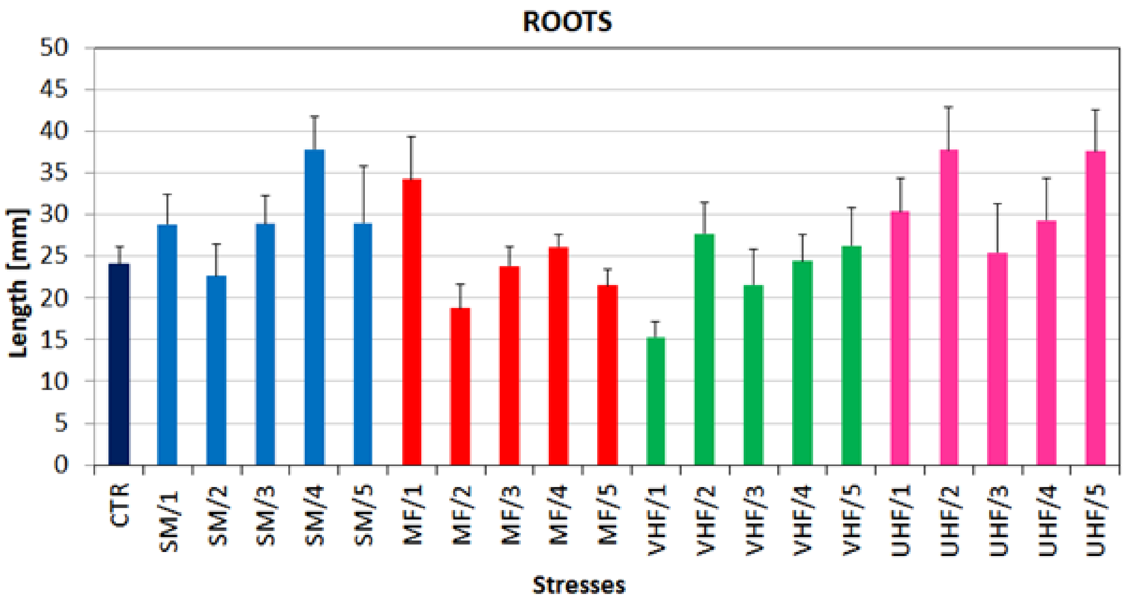

Fig. 5: Experimental results of the root length on different stresses and doses.

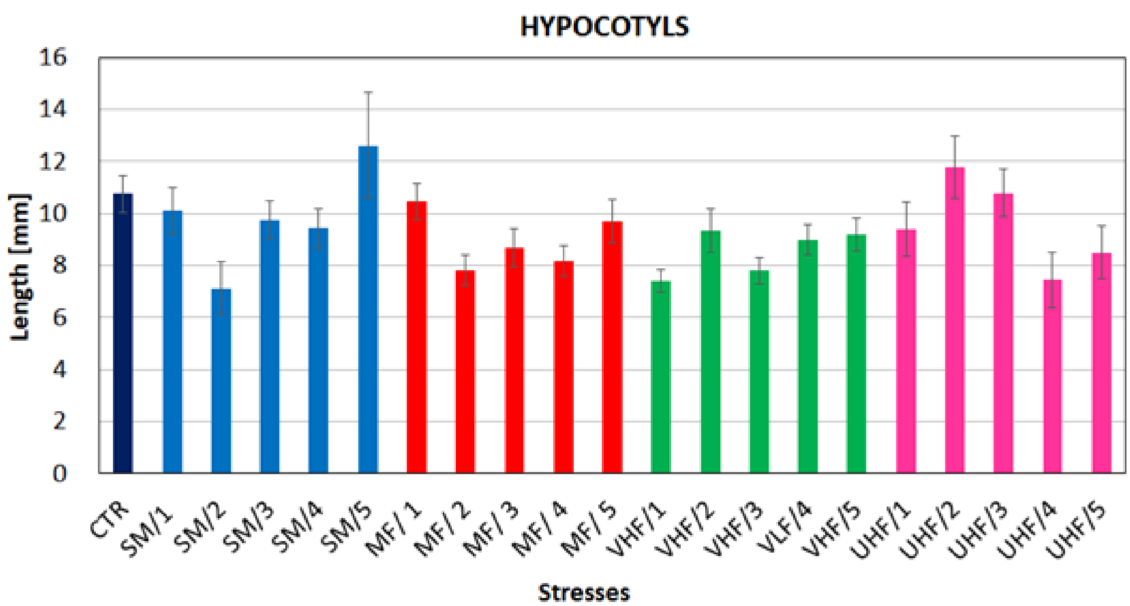

Fig. 6: Experimental results of the hypocotyls length on different stresses and doses.

Moreover, to have information on the modification of the applied stress, we utilized the *t*-student test whose *p* value determines the level of significance when it results ≤ 0.05.

In *Table 1* the value of parameter p for the length of the roots and of the hypocotyls for the five doses with SM stress are reported.

Table 1 *Value of p for the root and hypocotyl length for each dose treated by SM stress (*p<0.05).*

	Control	$SM/1$	$SM/2$	$SM/3$	$SM/4$	$SM/5$
p-value Roots	--	0.229	0.738	0.221	0.003*	0.355
p-value hypocotyls	--	0.561	0.006*	0.347	0.263	0.273

In this case only two events are significant.

In *Table 2* the value of parameter p for the length of the roots and of the hypocotyls for the five doses with MF stress are reported.

Table *2 Value of p for the root and hypocotyl length for each dose treated by MF stress (*p<0.05).*

	Control	$MF/1$	$MF/2$	$MF/3$	$MF/4$	$MF/5$
p-value Roots	--	0.115	0.055	0.974	0.459	0.432
p-value hypocotyls	--	0.771	0.012*	0.063	0.009*	0.344

In this case only two events of the hypocotyls are significant.

In *Table 3* the value of parameter p for the length of the roots and of the hypocotyls for the five doses with VHFstress are reported.

Table 3 *Value of p for the root and hypocotyl length for each dose treated by VHF stress (*p<0.05).*

	Control	$VHF/1$	$VHF/2$	$VHF/3$	$VHF/4$	$VHF/5$
p-value Roots	--	0.012*	0.141	0.582	0.797	0.607
p-value hypocotyls	--	0.001*	0.459	0.005*	0.173	0.135

In this case only three events are significant.

In *Table 4* the value of parameter p for the length of the roots and of the hypocotyls for the five doses with UHF stress are reported.

Table 4 *Value of p for the root and hypocotyl length for each dose treated by UHF stress (*p<0.05)*.

	Control	$UHF/1$	$UHF/2$	$UHF/3$	$UHF/4$	$UHF/5$
p-value Roots	--	0.133	0.008*	0.776	0.264	0.008*
p-value hypocotyls	--	0.266*	0.417	0.981	0.012*	0.067

In this case four events are significant.

From the above results it is possible to observe that no all stresses have induced strong variation. About the roots length only four cases have exhibited significance, whereas about the hypocotyl length nine cases have exhibited significance.

Analyzing the results of Figure 5 and 6 vs. the exposition time for all stresses, it possible to observe a specific behavior at $470\ h$, see *Fig. 7* and *8*. At this time the root length reaches the maximum value with the SM stress, and also the minimum one, but with the VLF stress. About the hypocotyl results, again at $470\ h$ a specificity is present: all stresses exhibited a minimum value with respect to the CTR value.

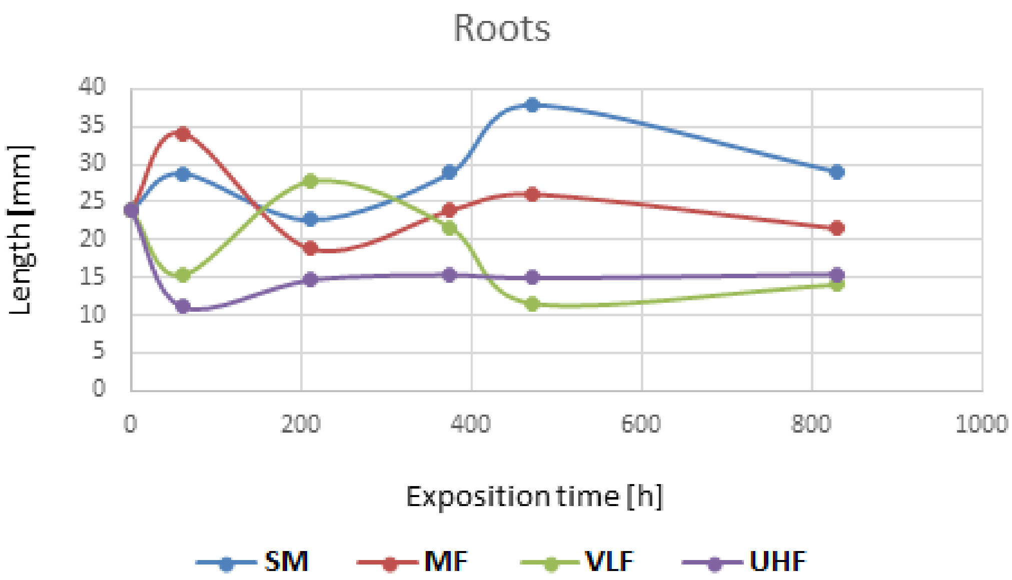

Fig. 7: Experimental results of the root length vs. exposition time.

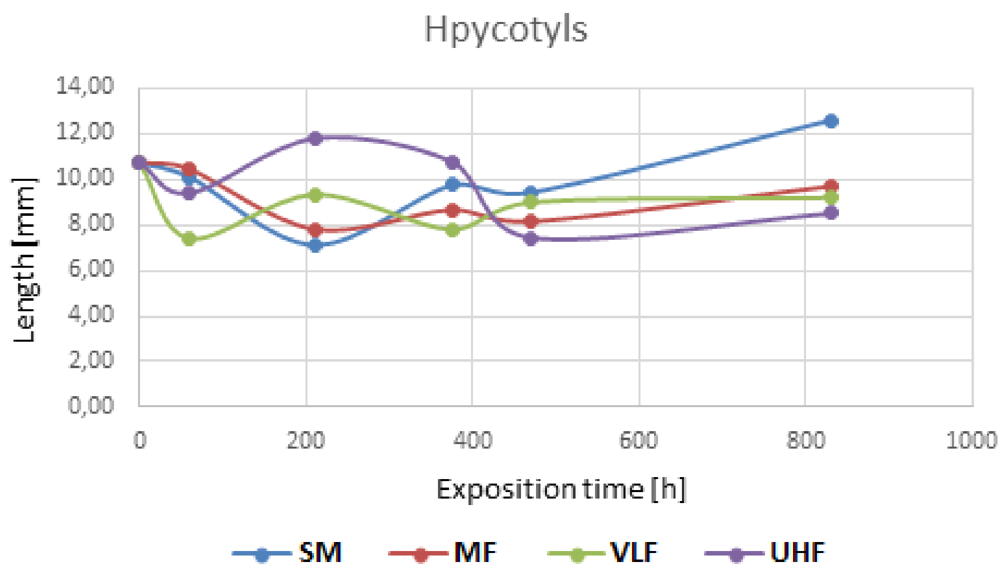

Fig. 8: Experimental results of the hypocotil length vs. exposition time.

V. CONCLUSIONS

Cell division and cell elongation are cellular processes driving plant growth and differentation, and their regulation is a highly dynamic process that changes during development as well as the adaptation to variations of the external stimuli. We have reported the effects on germination and growth of the hypogean radish seeds treated by a different radiofrequency fields.

Our results demonstrated that exposure of radish seeds to all radiofrequency treatments used in our experimental conditions did not stimulated either the root growth or the hypocotyls length.

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