Effects of physical stresses on radish seed germination and growth

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Abstract
In this work we explore the consequences on variable magnetic fields applied to radish seeds (*Raphanus sativum* L.) on germination and seedling growth. Three, different alternate magnetic fields have been tested having a high intensity, $40\ mT$, at very low frequency, $0.2\ Hz$ (B0.2), a low intensity, $577\ \mu T$, at low frequency, $370\ kHz$ (B370), and a very low intensity, about $250\ nT$, at ultra high frequency, $900\ MHz$ (RF900). These last generate electromagnetic waves which are nowadays very used for mobile communications. Groups of uniform radish seeds were exposed to alternate magnetic fields at three duration time values: i) $120\ h$ (BO.2/1), $260\ h$ (B0.2/2) and $360\ h$ (B0.2/3) at high intensity and very low frequency, 0.2 Hz; ii) $120\ h$ (B370/1), $260\ h$ (B370/2), $360\ h$ (B370/3) at low intensity and low frequency, 370 kHz; iii) $120\ h$ (RF/1), $260\ h$ (RF/2), $360\ h$ (RF/3) at very low intensity and ultra high frequency, 900 MHz. Furthermore, another group of seeds was treated by laser beams of 248 nm wavelength whose magnetic field is coupled to an electric field. These two fields, by the Planck theory, origin the quantum energy, expresses by photons. Therefore, in the laser-matter interaction photons are absorbed dependent on laser shots. Again, we performed experiments at three duration time values that applied: $30\ kshots$ (KrF/1), $80\ kshots$ (KrF/2) and $135\ kshots$ (KrF/3) at $40\ \mathrm{mJ/cm^2}$ fluence. The magnetic field and electric field presented by the laser beam are approximatively $250\ kV/m$ and $850\ \mu T$, respectively.
All treatments were performed at room temperature. Both untreated seeds and each group of seeds exposed to the physical stresses were transferred in Petri dishes and followed for their germination and seedling growth up to $96\ h$. The results showed that all physical stresses induced by alternate magnetic fields, radiofrequency and laser radiations did not have effect on seed germination as well as on cell elongation growth of the radish hypocotyls in comparison to control seeds. On the contrary, all physical stresses showed a significate stimulation on root growth.

1. INTRODUCTION
During the 8th century BC, Magnes found the magnetism. Nowadays, the magnetism is continually increasing. This consequence is due to the presence of electronic instruments which are feed by electric currents. It is also known 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, while the electric field also interacts with the electric charges, stopped or in moving by applying the Coulomb force:

$$\vec{F} = q\vec{E} \tag{1}$$

Regarding the biological matter, the charges are closely bound to atoms but 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 of molecules, behaviors 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 can deliver to significant results to diamagnetism matter.

Generally, the biological matter can be considerate paramagnetic, but generally the interaction of the magnetic field with the matter applies a mechanical moment corresponding to:

$$\vec{N} = \vec{M} \times \vec{B} \tag{2}$$

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 of the energy of the same molecule, value expressed by:

$$E_B = -\vec{M} \cdot \vec{B} \tag{3}$$

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 Eq. 4 can be positive or negative, that is:

$$E_B = \pm g\mu_B Bm \tag{4}$$

where g is the factor of Landé which is close to 2.00 for free electrons and for most organic radicals [4], μ_B is the magnet of Bohr, B is the 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 frequency. 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 [6]. 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) fixed and variable magnetic and electrical fields, ii) microwave, iii) ionizing and laser radiation. These methods are considered not only more profitable, but they also improve the productivity of plants without harming the environment [7, 8].

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 $10\ min$) [9].

Referring to the reported data, we have studied the effect of four different physical stresses on radish seeds and followed their germination and seedling growth. In particular, we investigated the effects on seeds of two different alternative magnetic fields named B0.2 and B370 and the effects of irradiation with radiofrequency (RF) on seedling growth for 120 h, 260 h and 360 h. Likewise, the seed germination and growth were also analyzed after laser radiation (KrF).

2. EXPERIMENTAL APPARATUS AND SEE TREATMENTS

Different suitable experimental set ups have been made.

2.1 Very low frequency (VLF) magnetic field irradiation

The low frequency magnetic field is an alternation field by $0.2\ Hz$ frequency, $40\ mT$ peak. It is constructed with four static magnets of disc of $4\ cm$ in diameter. The magnets are placed along a circumference supported by two ferromagnetic strips, see *Fig. 1*. The support was moved by a low frequency motor which frequency was of about $0.1\ Hz$

Fig. 1: Photo of VLF magnetic field set up.

2.2 Low frequency (LF) magnetic field irradiation

The realization of variable and intense magnetic fields is rather difficult due to the fact that for our purposes we need rather high currents, about $10\ A$, variable. The common RF generators are of low current and for our purposes it was necessary to create an ad hoc one. Therefore, we have made a solenoid with an internal diameter of about $5\ cm$ and a height of $11\ cm$ with a number of turns of 18 in order to have a low inductance and a low resistance. The value of the inductance is about $4\ \mu\ H$. The inductor is coupled in parallel with a capacitor. The coupling of a capacitor with an inductor forms an oscillating LC circuit. To have a frequency $\omega = 2\pi f$ it is necessary to utilize a capacitor of about $C = 1/(L\omega^2)$. To reach a frequency of about $370\ kHz$ we used a capacitor of about $50\ \mu F$. The photo of the coil is in *Fig. 2*.

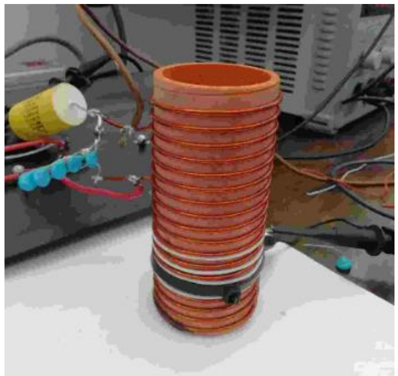

Fig. 2: Photo of LF magnetic field set up.

All real LC circuits are dispersive, that is they are unable to maintain continuum oscillations. Therefore, it is necessary to restore the energy lost per cycle. To get this goal the LC circuit was feed by a continuum voltage and connected to ground by a switch circuit formed by four transistors in collector common configuration, *Fig. 3*.

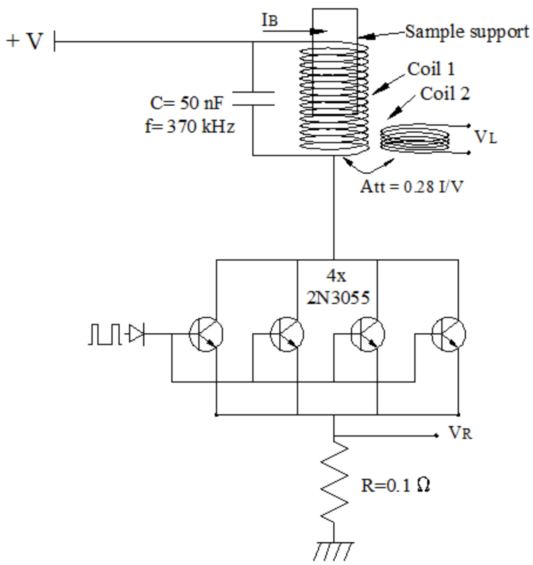

Fig. 3: Electronic scheme for oscillation circuit.

All transistor gates were connected to a RF generator which signal was in resonance with current of the LC circuit. This signal was also used to trigger the oscilloscope.

Moreover, in order to control the magnetic field, since there are no gaussometers for magnetic fields variable to hundreds of kHz, we opt to measuring the voltage value in the inductor of a secondary inductor concatenated with the principal and closed on an infinite impedance. The latter consists of 4 turns and the attenuation coefficient depends on frequency. At $370\,kHz$ the attenuation coefficient was $A_{tt} = I_B\,/\,V_{out}\ \approx\ 0.28\ A/V$. By Ampère law, the magnetic field inside the inductor is controlled by the relation $B = I \cdot 206\,x\,10^{-6}$ T. Therefore, with $10\,V$ of output voltage the maximum magnetic field resulted $577\ \mu T$.

2.3 Very high frequency (UHF) magnetic field irradiation

To perform treatments at $900\ MHz$ we utilize a short transmission line. It was designed with a height $h = 1.4\ cm$, width $a = 9\ cm$ and with a lengthy of $20\ cm$. The length of the line does not affect the characteristic impedance but allows to treat more samples. The RF generator was a RHODE & SCHWARZ SM 300. Its output power is $20\ mW$. The expression of the characteristic impedance of the flat line, excluding the external radiation, is looked at the following formula:

$$R_o = \sqrt{\frac{L}{C}} = \sqrt{\frac{\mu_o}{\varepsilon_o}\frac{h}{a}} \quad = \qquad (5)$$

where ε_o and μ_o are the electrical permittivity and magnetic permeability, respectively. From Eq. 5 it is deduced that our line has an impedance of about $50\,\Omega$ like the one of the generator. As a consequence, the line input was connected to the generator via a high frequency $50\,\Omega$, while the line output was connected to a $50\,\Omega$ utilizing 4 $200\,\Omega$ resistors *Fig. 4*.

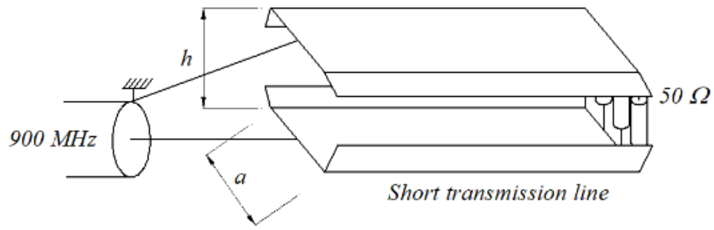

Fig. 4: Short $50\,\Omega$ transmission line.

The wavelength at $900\ MHz$ in vacuum is about $0.33\ m$, while the line width is $0.10\ m$. So, to limit the irradiation, it is necessary to modify the conductors bending the lateral outline. The applied signal was $1\ V$ at $900\ MHz$ and the correspondent magnetic field peak was $250\ nT$. All measurements were made with a Le Croy Wavepro 7100 fast oscilloscope, $20 GS/s$ with $1\ GHz$ band limitation.

2.4 Excimer laser (KrF) irradiation

The experiment was carried out by a KrF excimer laser (photon energy of 5 eV) with pulse time duration τ=23 ns at full-width at half-maximum (FWHM). The laser beam was not focused and its spot dimension was of $3\ cm^2$. Utilising an output energy of $120\ mJ$, the fluence value resulted of $40\ mJ/cm^2$. Considering an average value of the radish diameter of $2.5\ mm$, the incident energy for each seed was the radish seed of $2\ mJ$. In this case, the magnetic field presented by the laser beam was about $850\ \mu T$ and the associate electric field was $250\ kV/m$.

2.5 Plant material

Epigeal 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.

2.6 Physical treatment of radish seeds

Each group of radish seeds was placed in Petri dish and then inserted ~~into~~ under the various magnetic field generators, while a group of seeds was used as control. Each experimental treatment was replicated three times.

2.7 Growth conditions

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 $25\ \mu E$, 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 were photographed, *Fig. 5*, and then their dimensions were measured.

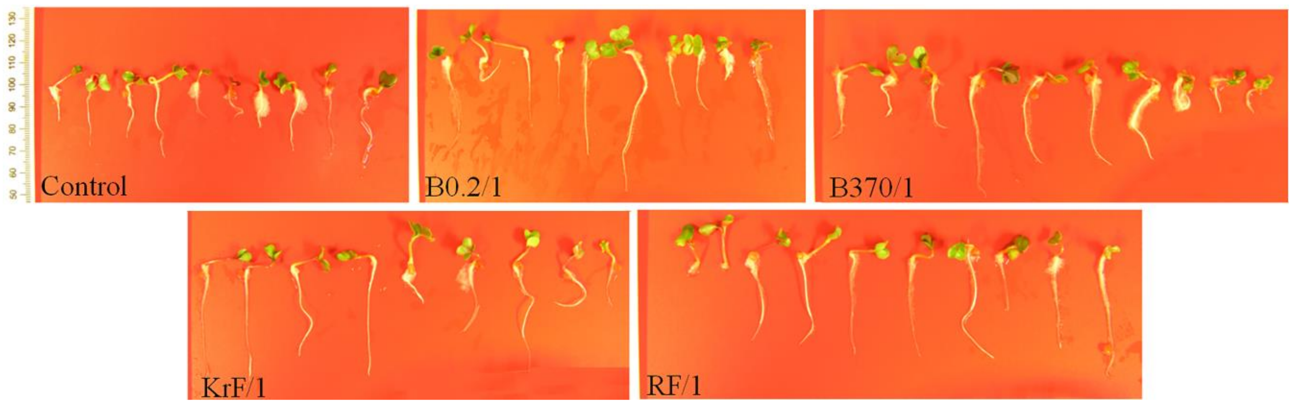

Fig. 5. Seedlings from seeds untreated (control); seedlings from seeds exposed to magnetic field at 0.2 Hz (B0.2/1), to magnetic field at 370 kHz (B370/1), to radiofrequency at 900 MHz (RF/1) for 120 h and to laser (excimer laser) KrF/1 at 248 nm for 30 *kshots*.

3. RESULTS

The data obtained by the measurement of the root and hypocotyl lengths, in three independent experiments, were statistically analyzed using a One-Way ANOVA test in SigmaStat software (Systat Software Inc., http://www.systat.com/). The Holm-Sidak post-hoc method was used to establish significant differences between means with a confidence level of at least 95% [10].

All radish seeds germinated after $24\ h$, even though, the emergence of the primary root was more prominent for all physical treatments than to control. This characteristic was better evaluated during the time course of growth at 48, 72 e 96 h. Thus, at the end of $96\ h$ of incubation, root and hypocotyl lengths were recorded for each seedling and the data are reported in *Fig. 6* and *Fig. 7*.

The B0.2 treatments caused an increase of root length correlated to the time of exposure at the alternate magnetic field (high intensity at low frequency). In fact, a gradual increase was observed in the samples treated for 120 h (B0.2/1), 260 h (B0.2/2) and 360 h (B0.2/3), from 37% up to 75.5% (*Fig. 6*). The radish seeds exposed with the alternate magnetic field B370 (low intensity at high frequency) showed a significative increase (67%) of root length only after 360 h of treatment (B370/3) (*Fig. 6*). The laser radiations (KrF) showed the highest stimulation effect on root length (61%) after 30 *kshots* (KrF/1). Increasing the number of *shots* (80*k*, 120 *kshots*) the stimulation effect of KrF treatment slightly decreased (KrF/2, KrF/3) (*Fig. 6*). The radish seeds exposed to radiofrequency (RF) presented a significative increase of 64% only after 260 h of treatment (RF/2) (*Fig. 6*). All four physical stresses had no effect on the hypocotyl growth in comparison to control seedlings (*Fig. 7*).

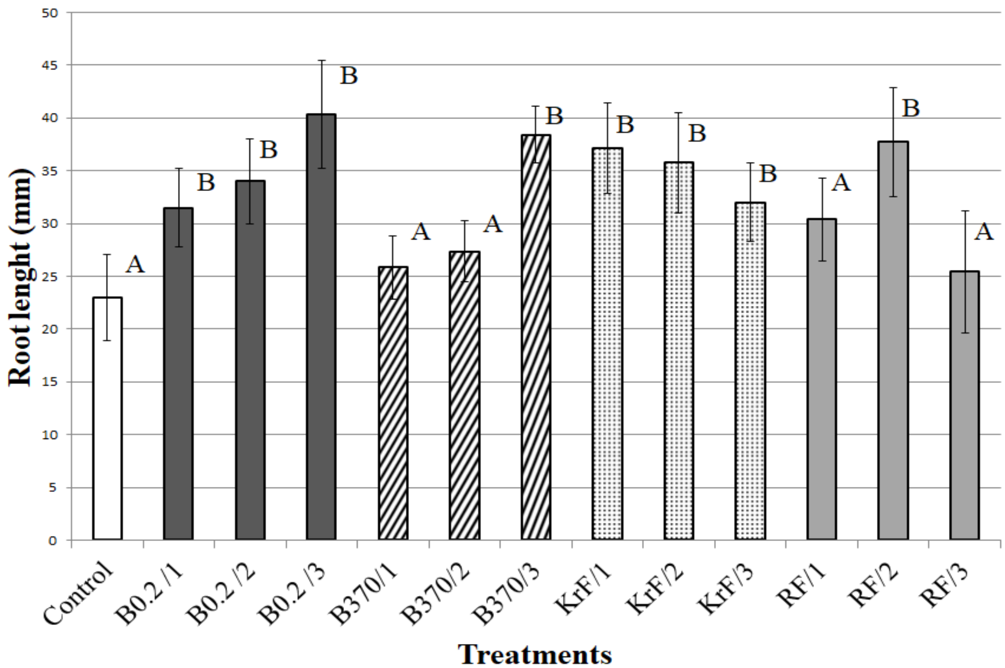

Fig. 6. Average value of root length. Data are means ± standard deviation of three independent experiments. Different letters indicate values that differed between treatments within each treated seed group (Holm-Sidak test, P< 0.05).

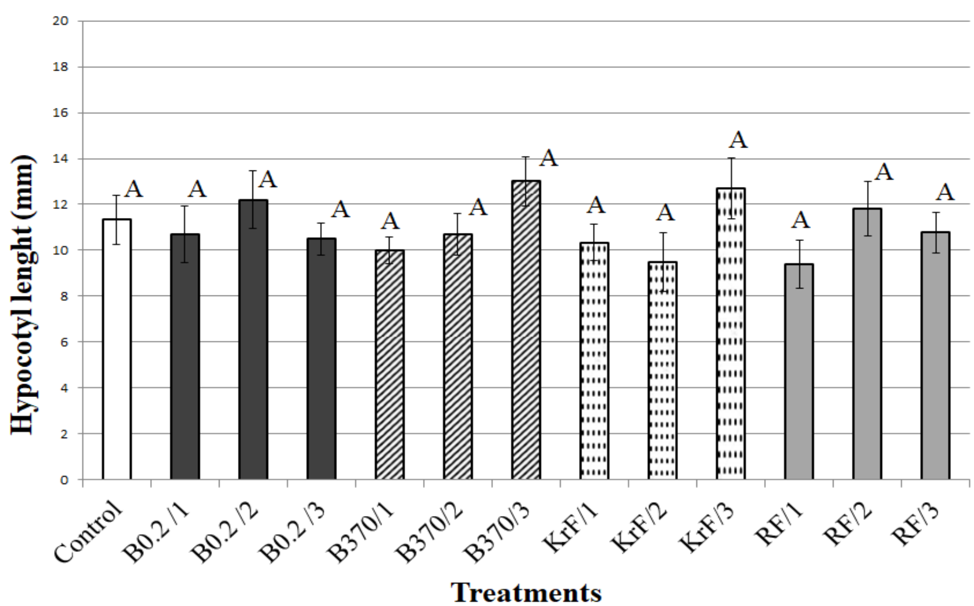

Fig. 7. Average value of hypocotyl length. Data are means ± standard deviation of three independent experiments. Different letters indicate values that differed between treatments within each treated seed group (Holm-Sidak test, P< 0.05).

4. DISCUSSION

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 report the effect on germination and growth of the epigeal radish seeds treated with two different alternate magnetic fields (B0.2 and B370), radiofrequency (RF) and laser irradiation (KrF). Our results demonstrate that exposure of radish seeds to all physical treatments used in our experimental conditions significantly stimulated root growth.

It is important to underline that when a significative elongation of roots occurred during the different physical treatments, the increase of percentage of root length was between 61 % (KrF/1) and 75.5 % (B0.2/3) (*Fig. 6*). It is clear that the stimulation of root growth due to the physical stresses involves some unknown physiological mechanisms. On the contrary, all physical stresses did not have effect on radish hypocotyls. The hypocotyl growth in epigeal radish seeds is strictly related to cell elongation events, whereas the increase of the root length depends primarily from cell division followed by cell elongation. These two processes are tightly coupled. The present data indicate that all physical stresses mainly affect both cell division and elongation processes.

A modulation of the cell division and cell elongation as response to abiotic stresses is controlled by hormones as well as by transcriptional and/or post-transcriptional regulation of other cellular events. The relation between stress signaling and control of cell division and cell elongation requires to be better understood.

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