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Study of pulsed laser beams and magnetic field on radish seeds

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Abstract: In this work we report a study on the influence of innovative pulsed stresses utilizing an UV laser and a homemade generator of magnetic field on radish seeds (*Raphanus sativus L.*) growth. We analysed the seed germination and seedling growth. The UV pulsed laser was an excimer KrF operating at 248 nm, 23 ns of pulse duration, with a laser fluence of about 40 mJ/cm^2. The generator of pulsed magnetic field was realized by the electric discharge on a coil of a high voltage capacitor of 150 μF, 60 kV. The magnetic field pulse waveform exhibited damped oscillations at 215 kHz with a maximum intensity of 400 mT. Groups of uniform radish seeds were exposed to laser pulses at five different doses: 30000 shots (KrF/1), 80000 shots (KrF/2), 145000 shots (KrF/3), 225000 shots (KrF/4) and 275000 shots (KrF/5). Other groups were exposed to magnetic field at eight different doses: 3600 shots (MF/1), 7200 shots (MF/2), 10800 shots (MF/3), 14400 shots (MF/4), 18000 shots (MF/5), 21600 shots (MF/6), 32400 kshots (MF/7) and 36000 shots (MF/8). Simultaneously, untreated seeds were used as control. All treatments were performed at room temperature. Both untreated and treated seeds were transferred in Petri dishes and followed for their germination and seedling growth up to 96 h. The results showed that the stress induced by UV laser photons brought a significant stimulation on root growth which may contribute to improve the performance and the productivity of the plants. On the contrary, all physical stresses induced by magnetic fields did not have effect on seed germination, as well as on cell elongation growth and on hypocotyls in comparison to control seeds.

Keywords: Interaction of radiation with matter; Lasers

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2020 JINST 15 C03022

Contents

1 Introduction

The natural light we are exposed is due to incoherent sources. Laser instead offer coherent and monochromatic light. Therefore, laser beams allow to interact with the matter more selectively and strictly. In the laser-matter interaction, the laser energy is absorbed and/or reflected from the matter. The absorbed part is due to the electrons which change the energetic state. So that, the temperature increases and diffuses inside the target. In many applications, the laser beam is focused and for fluences >1 J/cm^2 one observes the ablation processes of the target [1] with the consequence of the formation of craters and generation of plasma. At low fluence values, the surface sample is not modified but just heated and/or photochemical reactions can be observed [2]. For UV photons, it is also possible to observe photoelectric processes [3] and the electrons can escape from the sample. In any case the application of laser beams operating in the UV region does not influence the bulk of the target but rather only the superficial part. The penetration depth of photons in biological matter is difficult to determine, but it is likely to image that the distribution of energy decreases with the penetration. However, the surplus of heat due to deposited energy does not remain limited in the interaction region but it diffuses inside. The penetration depth is governed by [4] $l_{th} \approx \sqrt{\pi D \tau_L}$ where τ_L is the laser pulse duration and D the thermal diffusivity of the sample which represents its reluctance to transmit heat. It is $D = k/\rho c_V$, where k is thermal conductivity, ρ is the mass density and c_V the specific heat for constant volume. Considering again the biological matter similar to water and a laser pulse of τ_L=23 ns, the penetration depth of the temperature is about 100 nm. This value is sufficient to stimulate the biological cell membrane which can react activating defense processes.

Instead, the influence of magnetic field acts inside the target without any limitation on its dimensions. The application of magnetic fields interacts with the magnetic moment of the matter which can change its orientation. Namely, free ions can move and their movement can activate the

2020 JINST 15 C03022

metabolic pathways by enhancing biochemical and physiologic feedback. The mechanical moment is $\vec{N} = \vec{M} \times \vec{B}$, where $\vec{M}$ represents the magnetic moment of matter and $\vec{B}$ the magnetic field. With the application of magnetic fields, the energy of molecules changes: $E_B = -\vec{M} \cdot \vec{B}$.

The study of living matter exposed to magnetic and electromagnetic fields is very difficult due to the complexity of the biological cell membrane structure. In these cases, it is reasonable to suppose that the mechanical moment induced by the magnetic field could influence the charge transport through the membrane. 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 $\pm\frac{1}{2}$, the energy variation can be positive or negative [5]: $E_B = \pm g\mu_B Bm$ where g is the factor of Landé which is close to 2.00 for free electrons (as well as for most organic radicals [5]), μ_B is the magnet of Bohr, B is the applied magnetic field and m is the quantum constant. Such difference of energy can make easier chemical reactions.

In this work, we intend to study the effects of new stresses that can have a great impact on seed growth and development of higher plants [6]. These stresses could be considered an ecological and economical method, used in alternative to chemical ones, for increasing the performance and productivity of plants. In the proposed experiment, the choice sample was the radish seeds (*Raphanus sativus L.*). They belong to the family of cruciferae [7] and contains important nutrient such as protein, sugar and vitamin C. Some authors have studied the germination, the seedling growth and the yield of radish seeds and other seeds under the influence of moderate magnetic fields [8–10]. We report the effect of two innovative physical stresses, both pulsed, on radish seeds and we follow their germination and seedling growth. In particular, we investigated the effects on seeds under the irradiation of UV laser pulses at five different doses maintaining constant the laser fluence at about 40 mJ, and under magnetic field pulses (400 mT at the peak) at eight different doses.

2 Materials and methods

2.1 Set-up for UV laser irradiation

The UV source was a KrF excimer laser (Lambda Physics, Compex), operating at 248 nm wavelength (5 eV photon energy) and 23 ns of pulse duration. Figure 1 shows the experimental set-up. The laser fluence was fixed at about 40 mJ/cm^2 and the corresponding electric and magnetic fields associated to the beam were estimated to have peak values of 3.6 MV/m and 12 mT, respectively.

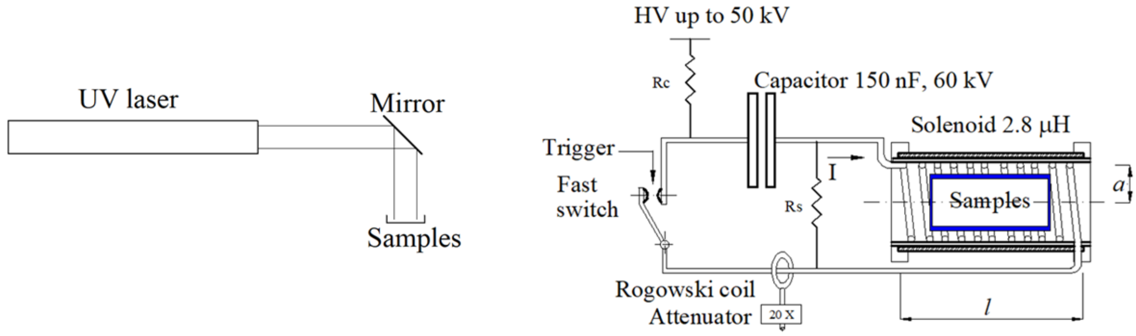
2.2 Magnetic field (MF) set-up

The magnetic field was generated by a homemade LC circuit with a main pulse lasting of about 20 µs. To realized it, we connected a solenoid to a pulser composed by a capacitor of 150 nF, 60 kV (Maxwell) and a homemade spark gap as fast switch. The capacitor was charged by a high-voltage power supply up to 50 kV. Supposing the internal non-zero resistance of the circuit, the discharge occurring in the pulser presents a time dependence for the current $i(t)$:

$$i\,(t) = \frac{V}{\omega L} e^{-\frac{R}{2L}t} \cdot \sin\,(\omega t) \tag{1}$$

where $\omega = \sqrt{\frac{1}{LC} - \frac{R^2}{4L^2}}$ is the frequency, V is the applied voltage on capacitor and R the resistance value (circuit and spark gap). By the Ampère law, the waveform of the magnetic field was similar to

2020 JINST 15 C03022



(a) **(b)**

Figure 1. Set-ups for (a) UV laser irradiation and (b) magnetic field (high voltage pulser: l = 12 cm and a = 3 cm).

the solenoid current one. It exhibited damped oscillations at 215 kHz. The solenoid was composed by 11 rings made by a 0.8 cm diameter copper tube. It had a length of 12 cm, a radius of 3 cm and an inductance of 2.8 µH. Figure 1b shows the sketch of the high voltage pulser. The discharge current was recorded by a fast Rogowski coil, composed of 140 rings with a resulting inductance L = 11 µH. Closing the coil to a load resistor R_L=0.5 W, the detector system becomes an auto-integrator for pulse times approximatively up to 10 µs [11]. A typical output signal of the Rogowski is showed in figure 2 for a power supply of 23 kV. Being the first oscillation of the current contained in a time shorter than 2 µs, the maximum magnetic field generate can easily be estimated by the Rogowski coil signal. It was 400 mT.

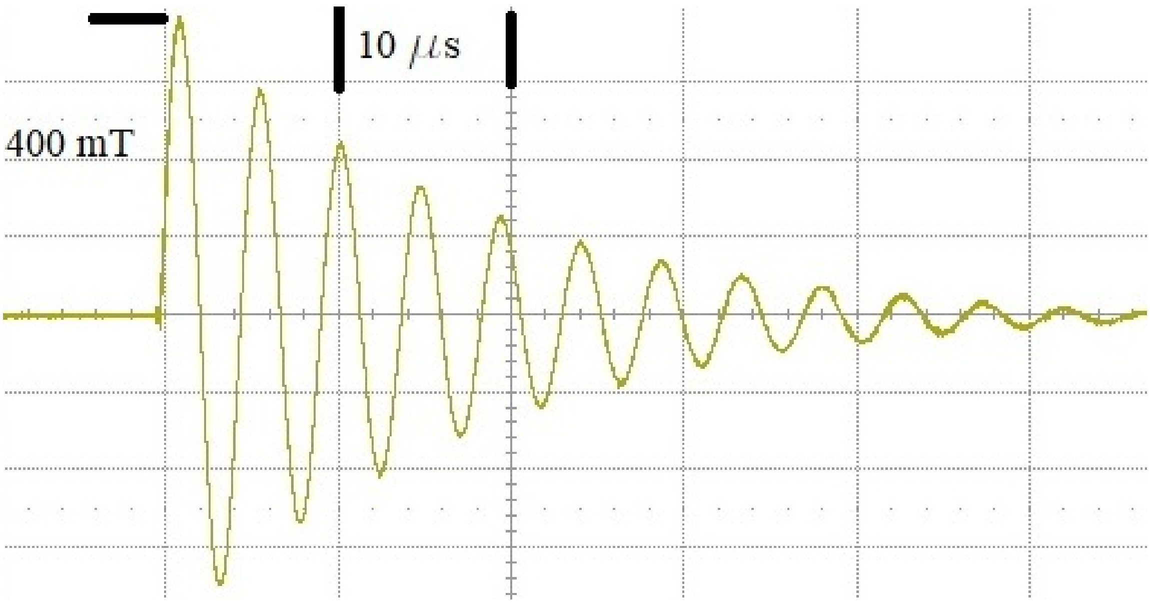


Figure 2. Waveform of magnetic field versus the time at 23 kV charging voltage.

2020 JINST 15 C03022

2.3 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.

2.4 Physical treatment of radish seeds

Each group of radish seeds was placed in Petri dish and then undergone to stresses: one to pulsed laser beams and other to pulsed magnetic fields, while a different group of seeds was used as control. Each experimental treatment was replicated three times. The exposition times for the two stresses were different. For laser irradiations we applied a maximum number of shots up to 275000, while for magnetic field one up to 36000 shots. This difference is justified by the fact that the pulse duration time of magnetic field (2 μs) is long if compared to the laser pulse (23 ns). In this way it is possible to get satisfying reaction conditions for both stresses and to have the important information on seed behaviour.

2.5 Growth conditions

Radish seeds, untreated and exposed to physical stress were rapidly sterilized (NaClO 2%) and placed in Petri dishes containing a water-wetted Whatman n° 1 paper (10 seeds for dish) for germination and growth. The Petri dishes were transferred into a growth chamber set 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. The length of hypocotyls and roots of the seedlings at 96 h of incubation were measured.

3 Results and discussion

The laser irradiation was performed with 30000, 80000, 145000, 225000 and 275000 shots utilizing the set-up of figure 1a at 40 mJ/cm^2 of fluence and 2 Hz of repetition rate. During the irradiation, the samples were moved in order to change the exposition surface. Figure 3 shows the photos of the seedlings grown up to 96 h in control condition (Control) and those grown from seeds laser irradiated at different doses (Laser KrF pt. 1, pt. 2, pt. 3, pt. 4, pt. 5).

Instead, stress induced by magnetic fields (400 mT) was performed with 3600 (pt. 1), 7200 (pt. 2), 10800 (pt. 3), 14400 (pt. 4), 18000 (pt. 5), 21600 (pt. 6), 28800 (pt. 7) and 36000, (pt. 8) shots utilizing the pulser schematized in figure 1b at 23 kV of power supply and 1 Hz of repetition rate. Figure 4 shows some photos (as examples) of the seedlings grown from seeds treated with the magnetic field for 96 h.

The laser experiments were performed in December/18 as well as the measurements of the relative control, while the ones of magnetic field were performed in March/19 as well as the control. The performed measurements were addressed to the length of the roots and of the hypocotyls.

The analyses on the control seedlings in December/18 showed a mean length of the roots of 24.0 mm with a standard deviation of 10.1 mm, while the related values of the hypocotyls was 10.8 mm with a standard deviation of 2.8 mm. The control seeds analysed in March/19 exhibited a

2020 JINST 15 C03022

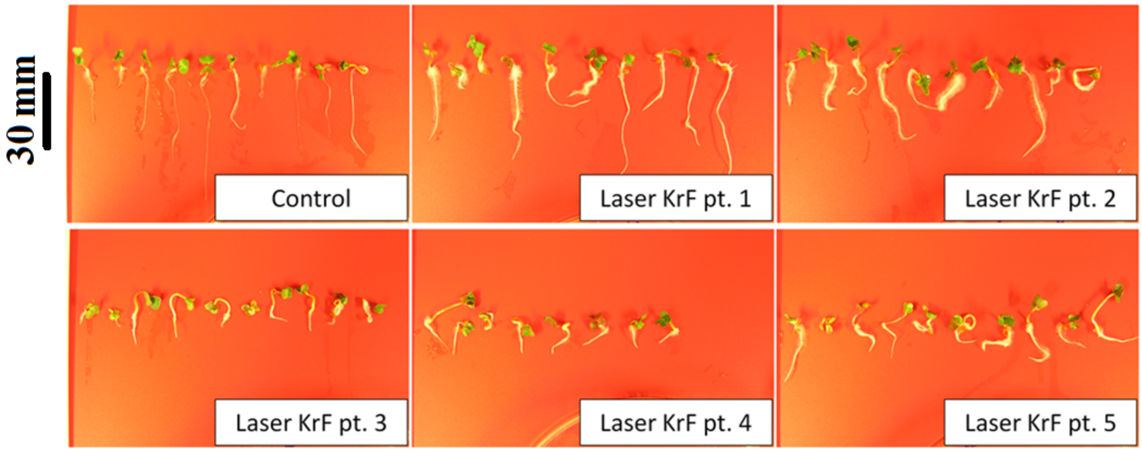


Figure 3. Photos of seedlings grown from stressed seeds by KrF laser after 96 h at different doses with 30000, 80000, 145000, 225000 and 275000 shots, identified by pt.1, pt.2, pt.3, pt.4 and pt.5, respectively.

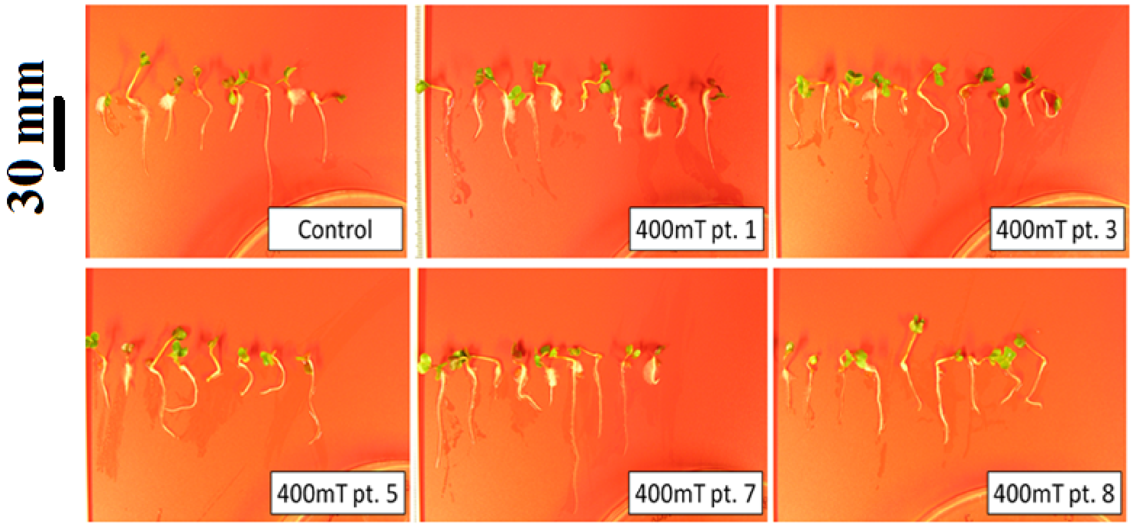


Figure 4. Photos of seedlings grown from stressed seeds by 400 mT magnetic field at different doses after 96 h. The reported doses refer to 3600, 10800, 18000, 28800 and 36000 shots, identified by pt.1, pt.3, pt.5, pt.7 and pt.8, respectively.

mean length of the roots of 26.6 mm with a standard deviation of 12.2 mm, and the related value of the hypocotyls was 9 mm with a standard deviation of 2.9 mm.

The results of laser experiments at different doses are reported in figure 5, while the ones of magnetic field are reported in figure 6. The error values of the statistic distribution are also reported (they are pointed out by the vertical bars). From figure 5 it is possible to observe that the root length changes on laser dose, instead the value of the hypocotyls does not suffer of strong variation. Instead, from figure 6 it is possible to see that either the root or the hypocotyl lengths do not exhibit variations (lack of statistical significance).

2020 JINST 15 C03022

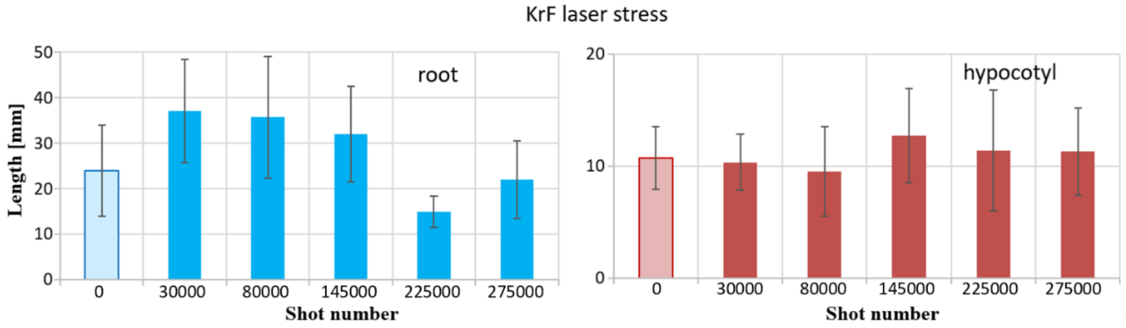


Figure 5. Results due to the laser stress: root length versus the dose (left) and hypocotyl length versus the dose (right). Zero represents the control.

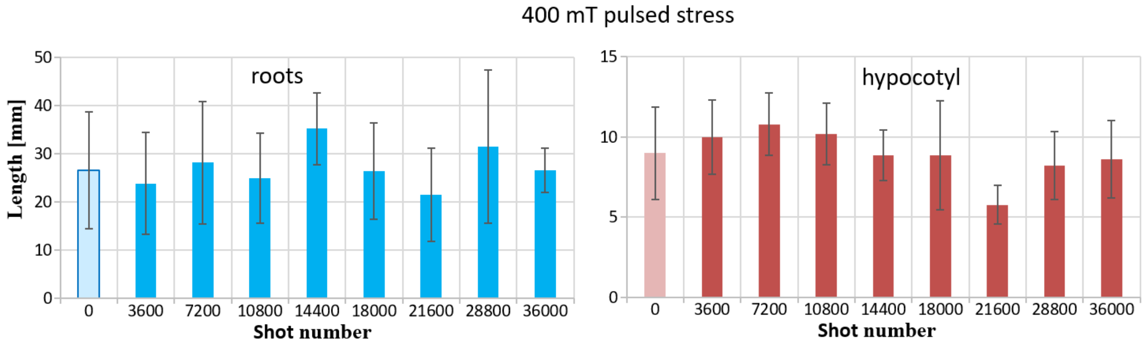


Figure 6. Results due to the magnetic field stress: root length versus the dose (left) and hypocotyl length versus the dose (right). Zero represents the control.

To understand the significance of the treatment we applied the statistic of t-Student whose p value must be ≤ 0.05. Table 1 reports the value of parameter p for the length of the roots and of the hypocotyls under the laser stress for the five doses.

Table 1. Value of p for the root and hypocotyl length for each dose treated by laser (*$p \leq 0.05$).

	Control	pt. 1	pt. 2	pt. 3	pt. 4	pt. 5
***p*-value roots**	—	0.0058*	0.0160*	0.0500*	0.0088*	0.5693
***p*-value hypocotyls**	—	0.7089	0.3532	0.1615	0.7074	0.6745

Almost all treatments exhibited a significance value for the root length, while never significance was exhibit for the hypocotyl length. We can suppose that the photon act is very specific. The p-values related to the results delivered by the magnetic field stress are reported in table 2.

Table 2. Value of p for the root and hypocotyl length for each dose treated by magnetic field (*$p \leq 0.05$).

	Control	pt. 1	pt. 2	pt. 3	pt. 4	pt. 5	pt. 6	pt. 7	pt. 8
***p*-value roots**	—	0.606	0.793	0.752	0.127	0.964	0.345	0.489	0.995
***p*-value hypocotyls**	—	0.425	0.122	0.306	0.909	0.938	0.007*	0.531	0.075

2020 JINST 15 C03022

In this case all treatments did not exhibit a significance for the root length, while only one significance was exhibited for the hypocotyl length corresponding at pt. 6. We can suppose that the act of the magnetic field is different from the laser one. The motivation of such result must be find. In any way, by physical considerations on the different characteristics of two stresses, we can affirm that the laser stress acts with electric and magnetic fields (whose peak values were 3.6 MV/m and 12 mT, respectively) on the superficial part of the samples and it increases the root length which depends primarily from cell division followed by cell elongation. On the contrary, the magnetic field acts inside the sample (with a field of peak intensity of 400 mT) and it is responsible of eventual orientation of the magnetic moment and of the internal energy. It is clear that the relation between stress signaling and control of cell division and cell elongation requires to be better understood.

4 Conclusions

Cell division and cell elongation are cellular processes driving plant growth and differentiation, and their regulation is a highly dynamic process that changes during development, as well as the adaptation to variations of the external stimuli. In this work, we report preliminary results on the effect on germination and growth of the hypogean radish seeds treated by pulsed laser beam operating in the UV region (248 nm) and pulsed magnetic field oscillating at 215 kHz for about 20 µs. The obtained results are interesting but required better understanding. In particular, we observed that the laser stress induces significative changes in the root lengths but not in the value of the hypocotyls, whereas the magnetic stress does not induce variations on either the root or the hypocotyl lengths. We can suppose that the laser stress acts with electric and magnetic fields on the superficial part of the samples and it increases the root length which depends primarily from cell division followed by cell elongation. On the contrary, the magnetic field acts inside the sample and it is responsible of eventual orientation of the magnetic moment and of the internal energy.

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2020 JINST 15 C03022