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Research Article

Non-chemical treatments to improve the seeds germination and plantlets growth of sessile oak

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Abstract

The current study aimed to investigate the effect of different treatments applied to sessile oak seeds in order to improve the germination features, development and growth of seedlings. The seeds were subjected to electric field, electromagnetic field and γ rays' treatments. For the electric field study three distinct voltages (10V, 30V and 50V) and three values of exposure time (15 min., 35 min. and 60 min.) were considered. Displaying the seeds in an electric field for 60 minutes increased the germination rate with 58.89%. The highest value for the seedling's height was obtained when seeds were exposed for 15 minutes at an intensity of 30V. The interaction between the intensity and the timing showed that treating seeds with 30V for 35 minutes improved significantly the seedlings peculiarities. In regard to the electromagnetic field, the seeds were subjected to a single value of electromagnetic field (B=0,4mT; i=0,680 A) and three values of exposure time: 10 min., 20 min. and 30 min. The sessile oak seeds subjected to the electromagnetic field for 20 minutes induced the highest value of germination percentage (90.00%) and germination index (1.81) and those seeds produced plantlets with high diameter (2.69 mm) and with significant differences towards non-treated plants. For γ rays treatments a dosage of 2 Gy induced the highest value of germination percentage (86.67%), of germination index (2.02); of germination energy (7.7); and of germination speed (7.24). A dosage of 6 Gy decreased the values of the same parameters. Our results showed that a dosage of 2 Gy improved also the growth parameters such as plantlets diameter (2.65 mm) and height (11.25 cm). In short, the physical treatments represent an ecological substitute approach towards the chemical substances presently used to improve seeds germination. Using physical treatments for seeds germination and growth of seedlings might be a harmless alternative for environment.

Keywords: electric field; electromagnetic field; γ rays' treatments; seed germination; seedlings

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Introduction

Quercus petraea (Matt.) Liebl., commonly known as sessile oak is taxonomically framed in *Quercus* genus, *Fagaceae* family along with other 500 species (Oldfield and Eastwood, 2007; Aldrich and Cavender-Bares, 2011) defining a systematic group with a great biological richness in forest ecosystems (Manos *et al.*, 1999; Reyes and Casal, 2006).

The sessile oak is a species of tree native to Central and Western Europe, spread from Scandinavia to the Iberian Peninsula (Doody and O'Reilly, 2008; Pukacka *et al.*, 2011). The sessile oak is the national tree of Ireland (Alan, 1974) and it is located mainly in hilly regions. In Romania the sessile oak occupies around 670.000 ha, being the most spread species of oaks, representing more than half (56%) of the total amount of oak species (Nicolescu, 2010). *Quercus petraea* is a deciduous tree of great height, upon 40 m tall, with acorn fruits of 1.5-2.5 cm length, arranged in a sessile cup or very shortly pedunculated, acorn which matures in about 6 months (Rushforth, 1999). From ecological point of view, sessile oak is less demanding upon summer heath and more drought-tolerant, that allows it to raise more in altitude than other tree species (Mijnsbrugge *et al.*, 2016). *Quercus petraea* is drought tolerant and prefers to grow in more Atlantic climates on light and well-drained, often rocky, soils (hence the specific Latin name *petraea* = of rocky places), generally occurring on slopes and hill tops, and preferring a more acid soil pH (Eaton *et al.*, 2016; Aas, 2000). Sessile oaks start producing acorns from the age of 40-60 years (Pukacka *et al.*, 2011), fruiting is irregular and occurs in 4–6-year intervals (Suszka *et al.*, 1996; Giertych and Suszka, 2011). Most deciduous tree seeds have "orthodox" characteristics which means that seeds can be dried to a moisture content of <10% for long-term storage (Özbingol and O'Reilly, 2005). However, sessile oak seeds are recalcitrant, which means that they do not tolerate moisture loss without adversely affecting viability (Gosling, 1989; Poulsen and Eriksen, 1992; Bonner, 1996; Xia *et al.*, 2012), making it difficult to store them for a useful period of time (Suszka and Tylkowski, 1980; Muller, 1990; Gordon, 1992b). The acorns of some species are best stored at mild freezing temperatures around -2 or -3 °C (Suszka *et al.*, 1996), whereas others are best stored at normal refrigerator temperatures around 2-4 °C (Connor and Bonner, 2001; Connor and Sowa, 2002, 2003). The sessile oak acorns appear to be well stored over the full range of these temperatures, from -2 °C to 4 °C (Gordon, 1992b).

Gamma irradiation (γ rays) is one of the most frequent practices of genetic variation induction at many species of plants (De Micco *et al.*, 2011; Moussa, 2006) including trees (Iglesias-Andreu *et al.*, 2012) and morphological parts of plants. It has a considerable influence on growth and development of plantlets through genetical, cytological, biochemical, physiological inductions (Kiong *et al.*, 2008) and morphogenetical modifications in cells and tissues closely related to irradiation levels (Ikram *et al.*, 2010). The material and the necessary energy for initial growth are already available in seed, but some stimulants are necessary in order to activate these substances already deposited in cotyledons. The small dosages of γ rays can activate the enzymes, and the young embryo, stimulating in the same time the cellular division and thus affect not only germination but also vegetative growth (Sjödin, 1962; Piri *et al.*, 2011). The biological effect of γ rays is due principally to formation of free radicals through water hydrolysis, that can lead to modulation of an anti-oxidation system, accumulation of phenolic compounds and chlorophyl pigments (Wi *et al.*, 2006; Ashraf, 2009).

Studies regarding γ rays were performed in order to achieve a delay in fruits maturation (World Organisation of Health and Nutrition in Agriculture, Organisation of United Nations, 1988) or to reduce bacterial populations, mushrooms, insects and other germinative pathogen agents (Gruner *et al.*, 1992) or to optimize seeds storability. Low doses of γ rays treatments in the seeds stimulate germination and increase fruit number and total production up to 86% (Wiendl *et al.*, 1995).

Despite the utility of γ rays to improve the germinative potential there are not many references in literature regarding the benefits of gamma radiation on tree species (Iglesias-Andreu *et al.*, 2012). Most studies on seeds germination in forestry species were performed with classic methods, using physical/chemical pre-seeding treatments. Low doses of γ rays treatments were used mainly to generate variation in aims of reproduction through mutation. It is well known that plants' response to γ rays treatments is depending on many factors as: irradiation dosage, species or plant genotype, or which morphological part of plant is analysed (Esnault *et al.*, 2010; De Micco *et al.*, 2011).

Regarding the lack of references on γ rays treatments in the seeds, the present study aimed to assess the effects of low gamma irradiation dosage on seeds' germination and growth of sessile oak plantlets.

In addition, another aim of the current study was to achieve the positive effects of the electromagnetic field (EMF) on germination and growth of plantlets. An electromagnetic field (EMF) is a natural environmental factor which has an influence to all the plants (Maffei, 2014). Numerous studies showed that along evolution process, the EMF of Earth was a natural component of the environment for living organisms (Belyavskaya *et al.*, 2004). The phototropism and the gravitropism have been intensely studied, but there is a lack of researches regarding the impact of EMF on germination and plant growth and development. Different studies indicate positive effects of treating seeds with an EMF in some cereals and vegetable species such as bean (Huang and Wang, 2008), onion (Alexander and Doijode, 1995), tomato (De Souza *et al.*, 2006), chickpea (Grewal and Maheshwari, 2011) and pea (Es'kov and Rodionov, 2010). Based on these studies, different species respond differently to the EMF. There is a lack of researches on the impact of EMF on sessile oak seeds, their germination and the growth of plants.

Another physical factor which may be used in the process of seed preparation and seed germination is the electric field (EF). The EF and the EMF are both non-chemical und harmless physical factors with less damaging effects on the environment (Molamofrad *et al.*, 2013).

Several studies reported positive effects of high voltage on the yield and yield components of potatoes (Smigel *et al.*, 1968), of cotton cultivars (Mustafayev, 1974), or of rice (Rotcharoen *et al.*, 2002). Lynikiene *et al.* (2006) reported that the EF not only increased the rate of germination but also increased the germination percentage of carrot, garden radish, beet, and barely. Vasilevski (2003) showed that the use of EF and EMF in agriculture has lot of the advantages such as low toxicity and pollution of surface and underground waters which means the decrease of costs of agricultural products. This article aimed to analyse the positive effects of electric, electromagnetic field and γ rays treatments on seeds' germination and growth of sessile oak plantlets in an experimental design.

Materials and Methods

The biological material

The sessile oak acorns were harvested manually, with the help of harvesting bags, during October 2019 from seeds' reservation Transylvania, Romania. After harvesting, seeds were deposited in well ventilated rooms (inside temperature 20 °C) for two months. Before sowing, seeds were immersed in water for 24 hours. The seeds that arose on surface of water were removed, being considered not viable, and those from the bottom of the pot were subsequently sorted for sowing (flotation process). A visual sorting of acorns was performed using the exterior condition as criterion, and only the seeds without visible damage were kept for study. When the relative humidity content of acorns reached the 40-50% value, the seeds were subjected to three treatments: EF, EMF and γ rays treatments. In order to test the germination capacity, the seeds were sown in pots type Hiko, using as edaphic substrate peat (70%) + humus (15%) + perlite (15%). Four repetitions each of 50 seeds were used and arranged in a completely randomized design.

The effect of electric field (EF) on seed germination and seedlings

The effect of EF on seed germination and seedlings was designed in the Biophysical Laboratory of the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania.

The EF generator consisted of two fittings, with an adjustable distance between them, powered by a variable voltage source, between which the seed lots were placed. The EF intensity was adapted by the distance between the armature and by the voltage. The distance between the condenser armatures was 7.3 cm and the armature diameter was 26 cm (Figure 1). Three voltage values: 10V, 30V and 50V and three distinct values of exposure timing: 15 min., 35 min. respectively 60 min, were considered in this study and also a control variant.

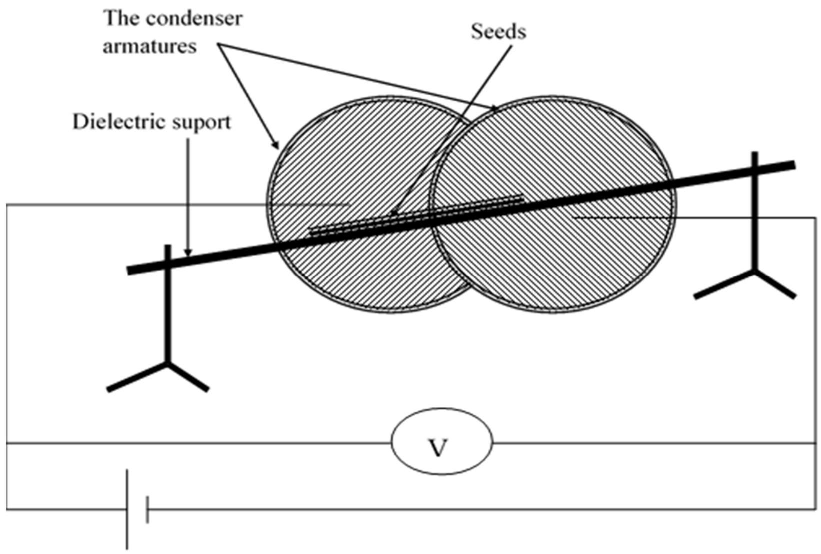

Figure 1. The diagram of seeds exposure in the electric field

The effect of electromagnetic field (EMF) on seed germination and seedlings

The effect of EMF on seed germination and seedlings was considered for a single value of EMF B=0.4mT and i=0.680A (B - electromagnetic field, 1 Tesla (T)=1000 millit (mT) and i - electromagnetic field intensity) and three values of exposure time: 10 min., 20 min. and 30 min. The EMF was performed in a Helmholtz system, where the electromagnetic induction is constant inside the coil. By modifying the supply current, is obtained the modification of the internal electromagnetic current. The seeds were exposed inside the coil, according to Figure 2.

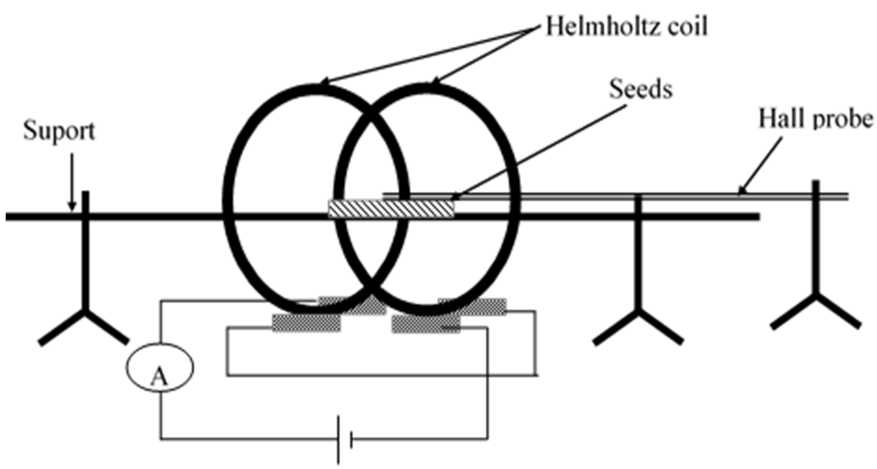

Figure 2. The diagram of seeds exposure in the electromagnetic field

The effect of γ rays treatments on seed germination and seedlings

The irradiation treatments of the biological material were designed within Atomic and Nuclear Physics Department of Babeş-Bolyai University, Cluj-Napoca. For this study, a Gamma Chamber 900 type device was used, using as source isotope 60 C. This device contains a ringly source, with an activity of 900 Currie.

Based of literature reports, the following treatments were considered: 1Gy-31 min, 1.5 Gy-46 min, 2 Gy-62 min. and 6 Gy-186 minutes and a control variant.

The studied seeds and seedlings parameters

The following seeds' parameters were considered in the present study: the length (cm), the diameter (cm) and the mass of the seeds (g). The length and the diameter of acorns were measured with a calliper. The diameter of acorn was determined as average of two made measurements. The mass of acorn was determined with the help of analytical balance. As for growth features of plantlets, height and diameter were also considered.

The studied germination parameters

For the EMF and γ rays treatments, seeds germination was analysed with help of the following indexes:

- GP (Germination percentage - %) where,
- $GP = \frac{Number\ of\ seeds\ germinated\ per\ day}{Total\ number\ of\ seeds\ placed\ to\ germination} \times\ 100$ (Fetouh and Hassan, 2014).
- Global method GI (Germination index) where,
- $GI = \frac{Number\ of\ germinated\ seeds}{Days\ from\ the\ first\ control} + \cdots + \frac{Number\ of\ germinated\ seeds}{Days\ from\ the\ last\ control}$ (AOSA, 1983).
- SE (Germination speed/Germinative energy) where,
- $SE = \frac{Number\ of\ germinated\ seeds\ in\ the\ first\ day\ of\ germination}{Number\ of\ germinated\ seeds\ in\ the\ last\ day\ of\ germination} \times 100$ (Islam *et al.*, 2003).
- CRG (Coefficient of germination speed) where,
- $CRG = \frac{n_1 + n_2 + \cdots + n_n}{n_1 \times T_1 + n_2 \times T_2 + n_3 \times T_3 + \cdots + n_n \times T_n} \times 100$
- n_1 = number of seeds germinated in day 1 (T1)
- n_2 = number of seeds germinated in day 2 (T2)
- n_n= number of seeds germinated in day n (Tn) (Bewley and Black, 1985; Chiapusio *et al.*, 1997).

Data analysis

To evaluate the differences between the obtained data of sessile oak seeds Boxplot was used. Two - way ANOVA was considered for the treatment of electric field, with intensity (I) and timing (T) as main factors, as well as interaction between these.

ANOVA single factor was considered for data regarding the height and the diameter of seeds both for electromagnetic field study and gamma ray study. If statistically significant values between averages were registered, the significance test of Tukey was applied (α = 0.05).

Results and Discussion

Results regarding seeds' characteristics

The seeds' parameters for the mass, diameter and length were represented as a boxplot diagram (Figure 3). The minimum seeds' length recorded was 2 mm, the maximum was 4.3 mm, and the average 2.7 mm. The seed diameter ranged between 1.3 mm and 2.9 mm, and the seeds weighed 6.1 g (Figure 3).

According to the literature, a higher germination is identified in larger seeds because they contain a greater number of resources that support the germination (Kheloufi *et al.*, 2018) and subsequently healthier seedlings (Pedrol *et al.*, 2018).

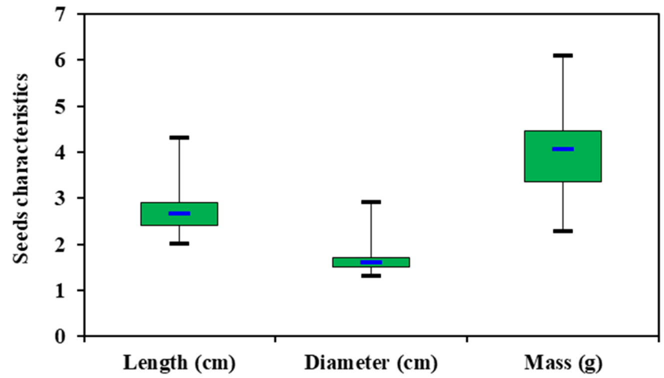

Figure 3. The chart diagram for the length, the diameter and the mass of sessile oak seeds

Seed germination and plantlets growth after exposing seeds in EF

The answer of sessile oak seeds exposed to three distinct voltages of EF (10V, 30V, 50V) and three values of exposure time (15 min., 35 min., 60 min.) was represented as germination capacity and some important parameters of plantlets (diameter and height).

The best germination rate was registered in the case of intensity I3 = 50V with 56.67%, upon intensity I2 = 30V with 47.78% and intensity I1 = 10V with 46.67%. Displaying the seeds in an EF for 60 minutes increases, the germination rate with 58.89%, meanwhile timing sequences of 15 minutes, or 45 minutes enhance the germination rate only with 45.56% and 46.67% respectively. The interaction between these two factors showed that I3 with 50 V and timing T3 with 60 minutes registered significantly superior differences (Figure 4).

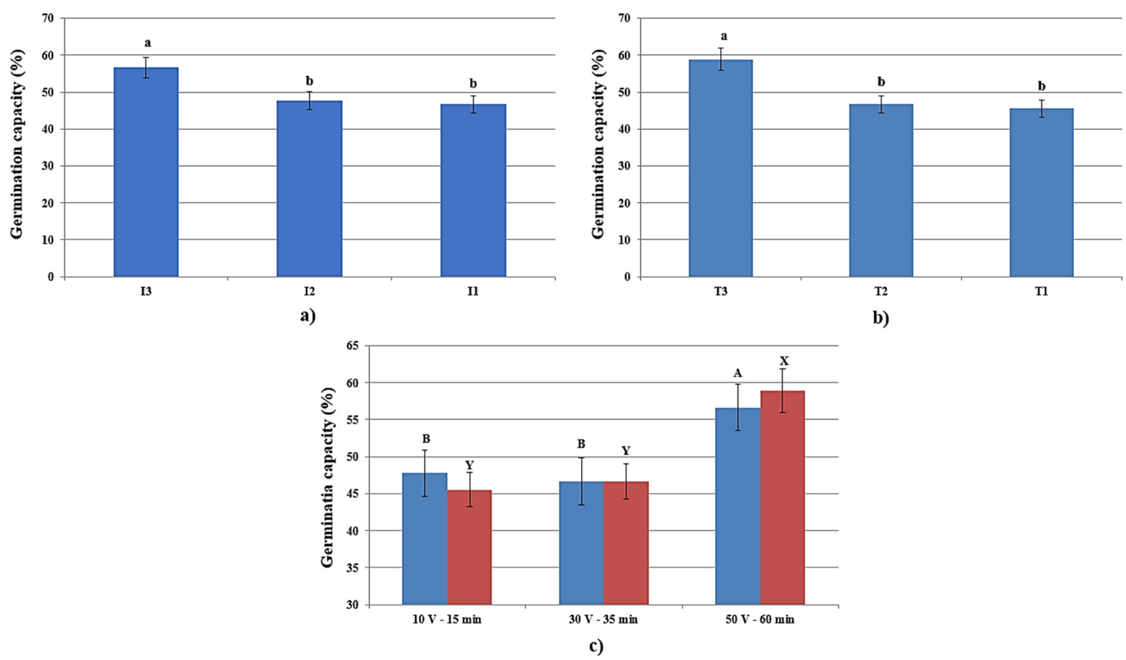

Figure 4. The effects of electric field intensity (a) and timing intervals (b) as well as the interaction between these factors (c) on seeds germination of sessile oak

The bars represent the average ± SE (n = 30). For each factor, different letters from top of bars indicate significant differences between treatments, according to Tukey's test (α=0.05). I3 = 50V, I2 = 30V, I1 = 10V; T1 = 15 min, T2 = 35 min, T3 = 60 min.

Regarding the two studied factors, EF intensity and time interval of seeds' exposure, our results highlighted that the diameter of plantlets was significantly different for 50V and timing 15 min respectively. The intensity of 50V, registered significant differences towards the two other values (10V, 30V) for plantlets' diameter (Figure 5 a). Exposure timing of 15 minutes registered significantly inferior differences towards the two other applied timing intervals, respectively T2=35 minutes and T3=60 minutes (Figure 5 b).

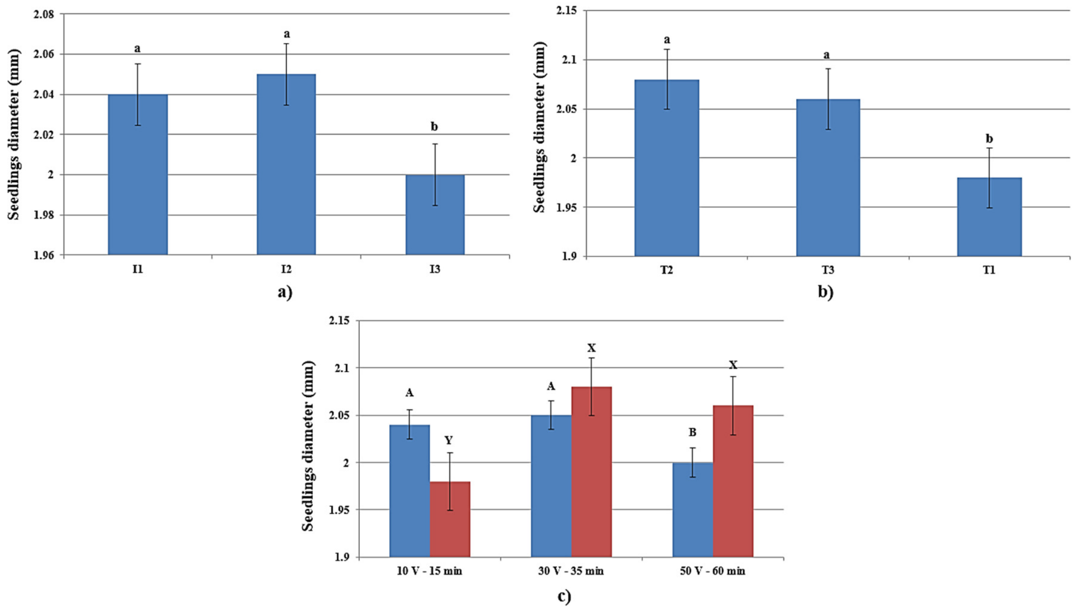

Figure 5. The effects of electric field intensity (a) and timing intervals (b) as well as the interaction between these factors (c) on diameter of sessile oak seedlings

The bars represent the average ± SE (n = 30). For each factor, different letters from top of the bars indicate significant differences between treatments, according Tukey's test (α=0.05). I3 = 50V, I2 = 30V, I1 = 10V; T1 = 15 min, T2 = 35 min, T3 = 60 min.

The highest value for the plantlet's height was obtained when seeds were exposed for 15 minutes at an intensity of EF of 30V registering significant superior differences upon the other values (Figure 6 a, b). Regarding the interaction between the analysed factors may be noted that the interaction between I2 (30V) and T2 (35 minutes) registered significantly differences upon the two other interactions, respectively I1 (10V) with T1 (15 minutes) and I3 (50V) with T3 (60 minutes) (Figure 6 c).

The use of EF and EMF in agriculture has had various advantages, such as low toxicity and pollution of surface and groundwater (Vasilevski, 2003). Various studies reported positive effects of high voltage on the yield and yield components of potatoes (Smigel *et al.*, 1968). Similarly, it has been reported for certain varieties of cotton (Mustafayev, 1974) and rice (Rotcharoen *et al.*, 2002).

Lynikeine *et al.* (2006) reported that the EF not only increased the germination rate, but also increased the germination rate of carrot, garden radish, beet and barely. In our study, we noticed that displaying the seeds in an EF for 60 minutes increase the germination rate with 58.89%, meanwhile timing sequences of 15 minutes, or 45 minutes enhance the germination rate with 45.56% and 46.67% respectively.

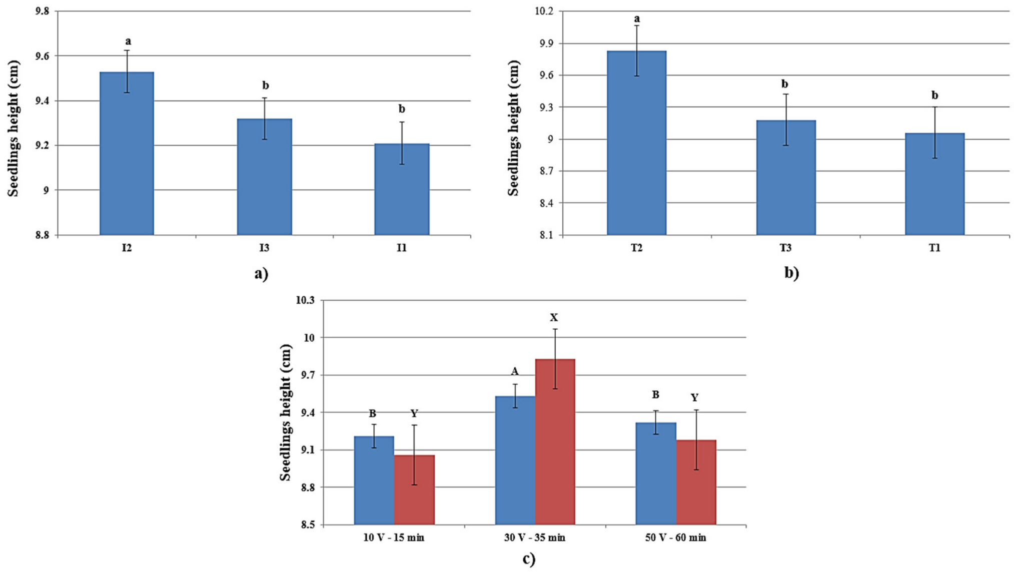

Figure 6. The effects of electric field intensity (a) and timing intervals (b) as well as the interaction between these factors (c) on height of sessile oak seedlings

The bars represent the average ± SE (n = 30). For each factor, different letters from top of bars indicate significant differences between treatments, according Tukey's test (α=0.05). I3 = 50V, I2 = 30V, I1 = 10V; T1 = 15 min, T2 = 35 min, T3 = 60 min.

Seed germination and plantlets growth after γ rays treatments

Gamma rays treatments have a positive impact of seeds germination. Qi *et al.* (2015) investigated the impact of treatments with γ rays on seeds of *Arabidopsis thaliana* and noted that seeds irradiation with lower dosages of 100 Gy stimulated the germination index. Maity *et al.* (2005) described the effects induced by γ rays (50-350 Gy) on seeds of *Oryza sativa* and *Phaseolus mungo.* While irradiation at small dosages improved morphological features, exposure at higher dosages had a negative impact on the same parameters. Araújo *et al.* (2016) noted that stimulating effects of γ rays were observed at small dosages (2-30 Gy), while large dosages (70 Gy) proved to be harmful to plants. Gamma rays proved to be, also, an efficient approach to improve seeds germination performance and seedlings stability for forestry species.

In this study a dosage of 2 Gy induced the highest value of the following germination indexes: (GP (%) = 86.67; GI = 2.02; SE = 7.7; and CRG = 7.24), and at 6 Gy there was observed a decrease of values of the same parameters (Table 1). The similar behaviour was reported by Iglesias-Andreu *et al.* (2012) in *Abies religiosa*, the germination percentage grew with the growing frequency of mutation until 10 Gy and then lowered gradually with growth of γ rays dosage. Habba (1992) for seeds of *Hyoscyamus muticus* observed that the germination percentage grew gradually until 100 Gy. Marcu *et al.* (2013) and Bodele (2013) reported, also, the growth of germination parameters in small dosages of γ rays.

Table 1. The γ ray treatments of sessile oak seeds (*Quercus petraea*)

Germination parameter	Symbol	Control	Irradiation dosage			
			Gy	1,5 Gy	2 Gy	6 Gy
Germination percentage	GP (%)	76.67	56.67	73.33	86.67	63.33
Germination index	GI	1.42	1.02	1.30	2.02	1.06
Speed of emergence	SE	4.3	5.9	4.5	7.7	5.3
Coefficient of germination speed	CRG	5.79	5.52	5.71	7.24	5.43

Our results showed that a dosage of irradiation of 2 Gy improved the growth parameters (plantlets diameter and height) in comparison with untreated plants (Figure 7).

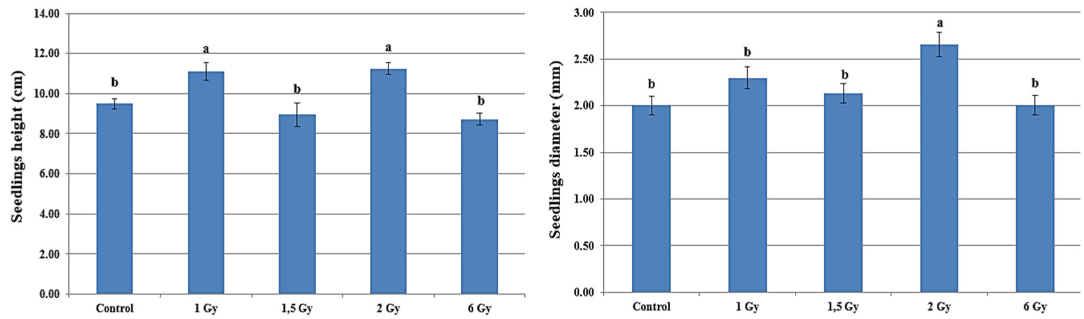

Figure 7. The effect of gamma rays treatments on diameter and height of sessile oak seedlings
The bars represent the average ± SE (n = 30). For each character, different letters from top of bars indicate significant differences between treatments, according to Tukey's test (α=0.05).

At the maximum dosage considered in this study (6 Gy) there was noted a decrease of growth parameters (diameter and height of plantlets) in comparison with control and more with those subjected to some smaller irradiation dosages (Figure 7). Similar to previous study and with the present study, γ rays applied in small dosages (until 5 Gy) grew the germination percentage in comparison with non-irradiated or irradiated samples with larger dosages (Minisi *et al.*, 2013). Gamma rays with smaller dosage stimulated, also, seeds' germination, vigour and seedlings growth at savage oat (Maherchandani, 1975), garden cherry (Majeed *et al.*, 2010), nightshade (Abdel-Hady *et al.*, 2008), okra (Dubey *et al.*, 2007) and arugula (Moussa, 2006).

Seed germination and plantlets growth after exposing seeds in EMF

It was assessed that an EMF applied to latent seeds grows the development rate of barley, corn, beans, wheat and of some species of trees (Maffei, 2014). Moreover, an EMF with reduced frequency (16 Hz) can be used as method of improving seeds of sensitive species that germinate at low temperatures (Rochalska and Orzeszko-Rywka, 2005).

The germination study showed that sessile oak seeds subjected to EMF for 20 minutes induced the highest value of germination indexes (GP = 90.00% and GI = 1.81) while for SE no higher values were registered. The seeds subjected to EMF for 30 minutes registered the highest value for CRG = 7.07 (Table 2).

Table 2. The effect of electromagnetic field on seeds' germination of sessile oak (*Quercus petraea*)

Germination parameter	Symbol	Control	10 min	20 min	30 min
Germination percentage	GP (%)	63.33	73.33	90.00	70.00
Germination index	GI	1.09	1.28	1.81	1.61
Speed of emergence	SE	10.5	4.5	3.7	9.5
Coefficient of germination speed	CRG	5.92	6.15	6.24	7.07

Our results showed that seeds exposed in EMF for 20 minutes produced plantlets with high diameter and with significant differences towards non-treated plants. In addition, an exposure of seeds in EMF for 20 minutes, or 30 minutes registered significantly superior differences for plantlets height (Figure 8).

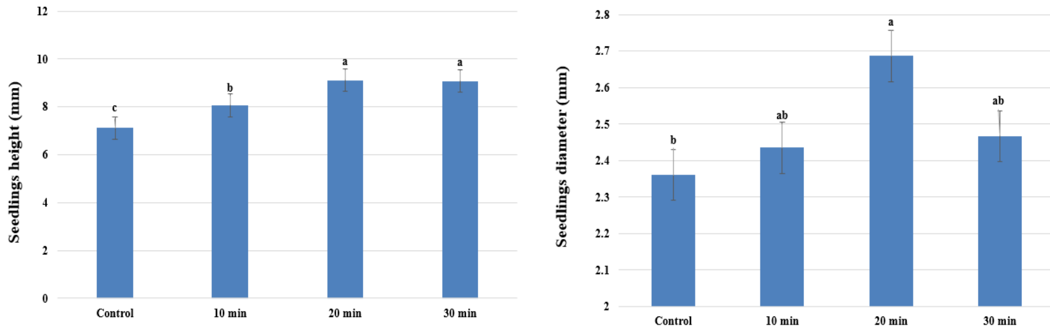

Figure 8. The seedlings diameter (a) and seedlings height (b) under electromagnetic field B=0,4mT and i=0,680 A

The bars represent the average ± SE (n = 30). For each of the character, different letters from top of bars indicate significant differences between treatments, according to Tukey's test (α=0.05).

Application of EMF promoted also the germination of beans seeds (Sakhnini, 2007). For beans seeds that were exposed to EMF of 87 until 226 mT intensity, for 100 minutes, Sakhnini (2007) assessed a linear growth of germinative traits with the growing intensity of EMF. Mahajan and Pandey (2014) noted that an applied EMF improved germination of beans seeds even out of season

Iqbal *et al.* (2012) followed a study with pea seeds exposed to three different values of EMF and three different time intervals before sowing. The results of Iqbal *et al.* (2012) study showed an increase of vigour index with 86, respectively, 205%, and underlined that the treatment could be used in practice for accelerating germination.

At chickpea the application of EMF with a resistance from 0 to 250 mT improved significantly the germination speed, length of seedlings and dried weight of seedlings. It was assessed that chickpea seeds treated electromagnetically can have better results under pluvial conditions (non-irrigated) in which a restrictive regime of soil humidity existed (Vashisth and Nagarajan, 2008).

Conclusions

The current results suggest that treatments of seeds with non-chemical factors might improve the seed germination and the seedlings features. The physical methods might be an alternative approach towards the chemical substances used nowadays and offers also ecological advantages as well as the possibility to be used at a scale with high efficiency.

Authors' Contributions

RH, OV and AMT designed the manuscript, wrote and authored most of the manuscript; IMM, SS, CS, HDV and PT collected the data; RH, IMM, HC, LH and AMT contributed data or analysis tools; RH, OV and AMT performed the analysis; HDV, HC and LH reviewed drafts of the paper and corrected the manuscript. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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