


## Article

# The Influence of Physical Treatments on Seed Germination and Seedling Development of Spruce (*Picea abies* [L.] Karst.)

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**Abstract:** The aim of this research was to investigate the influence of an electric field and gamma radiation upon the germination of spruce seeds. In order to carry out the research, spruce seeds from different provenances have been subjected to different treatments: electric field (EF) with 10 V, 30 V, and 50 V voltages and intensity of  $E = 266\text{V/m}$ , exposure time of 15 and 35 min, and gamma (G) radiation with several treatments (1 Gy-31 min, 1.5 Gy-46 min, 2 Gy-62 min, and 6 Gy-186 min). Under the influence of EF, the best results upon seed germination (80.83%) were recorded when seeds were treated with 30 V for 15 min, for all provenances investigated. Regarding gamma radiation, the highest germination percentage (87.50%) was achieved in T5<sub>G</sub> when seeds were subjected to 6 Gy for 186 min. It was also considered the interaction between seeds origin and the different EF and G treatments applied to the seeds to induce germination and further seedlings' development. The results obtained after seeds were exposed to gamma radiation came out on top compared to electric field treatments, both for the germination and seedlings' height.

**Keywords:** electric field; gamma radiation; germination; plant development; spruce; treatment



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## 1. Introduction

Coniferous forests contribute to global forestry, therefore, natural forests and coniferous forest plantations are widely distributed across diverse climate zones on different continents [1]. Due to their carbon storage capacity, they play a key role in the global carbon cycle [2,3], helping to conserve and protect soil resources, and last but not least, enhancing the beauty of the landscape [4]. *Picea abies* L., known as Norwegian spruce, is one of the most important species of conifers but also one of the dominant species in the forests of the Carpathian Mountains, which is one of the largest forest-covered regions of the continent [5,6]. In addition, it is considered one of the most economically important species in Central and Northern Europe [7], extending to more than 30 million hectares [8]. It is one of the most commonly used wood species for construction, but also for the paper industry [9,10]; a special quality is its resonant wood, which, compared to pine or larch wood, has a lower density, a higher tensile strength, and a higher modulus of elasticity along the fiber [11], so that the species is suitable for the production of musical instruments [12]. Spruce forests also have an important role in soil protection and erosion control while having a great recreational value [13].

Spruce prefers a cold and humid climate, and fertile soils, being a shade-tolerant species that grows well in the companionship of other trees [14]. It is the main species

widespread in the boreal and temperate regions of Europe [15], whereas in Romania, it can be usually found at altitudes between 1200 and 1800 m [16], while it also grows at lower altitudes, in mixt forests of fir and beech [17].

Conifer seeds generally have a high degree of dormancy, even if they undergo favorable environmental conditions for germination [18]. Regarding spruce, the germination capacity of the seeds is unsatisfactory in many stands (less than 60%) [19], which increases the interest and preoccupation of any forestry department and research institute to find solutions to stimulate spruce seed germination and thus help reforestation movements. Several procedures, such as exposure to various forms of energy (radiation, light, ultrasound) or treatments with chemicals, have already been used to accelerate seed germination and produce high-quality planting material [20].

The optimum temperature for spruce seed germination is 20–22 °C [21–23], while the optimum pH is between 5–6, which indicates the need for acid soils [23,24]. Previous findings suggest that the germination capacity of trees can be highly influenced by species, age, seed traits, climatic conditions during the pollination process, and biotic factors such as fungal or insect infestation, which can decrease germination capacity [25]. Due to frequent periods of drought, found within many regions lately, the vitality of the seeds is lower, being affected by climatic factors at the time of ripening [20,26].

Currently, electric and magnetic fields are used as an alternative in non-chemical methods to induce germination [27], having fewer pollutant effects on the environment [28,29], so that the methods can also be applied to ecological agriculture [30]. It was demonstrated [31,32] that a magnetic field was beneficial for bean culture, also being used against pathogens and diseases. Chemical products can be harmful to the ecosystem and can increase the overall cost of a crop [30,33]. Since the use of chemical germination and plant growth regulators and nonorganic inputs has been disputed, even though being efficient for controlling pathogens, their ecological impact is not constructive [32]; therefore, the use of low-cost alternatives and ecological treatments such as gamma radiation, laser lights, microwaves, radio frequency energies, and magnetic field, became more feasible for seed bio-stimulation, thus increasing germination efficiency and accelerating initial growth and development of plants [32,34,35]. One of the most common practices for inducing genetic variation in plant species is gamma irradiation ( $\gamma$ -rays) [36], the method being proper to be also used for trees [37]. Gamma rays influence the growth and development of plants by inducing cytological, genetic, biochemical, physiological, and morphogenetic changes in tissues and cells [38]. According to previous research, irradiation of seeds with low doses of gamma  $\gamma$  rays stimulates seed germination, plant growth, and synthesis of photosynthetic pigments [39].

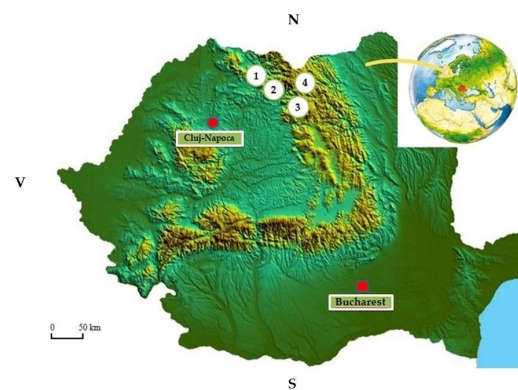
Beside gamma irradiation, another physical method used in the process of seed germination is the electric field (EF). Several studies have revealed the positive effects of electric fields on plants, even though these effects may vary depending on plant species [40]. Researchers [41,42] showed that EF increased the germination rate of several vegetables. Other findings indicate that cotton seeds had a higher growth rate when exposed to EF [43], as well as lettuce [41]. In rice, the electric field had no effect on the germination rate but positively influenced the development of seedlings [44]. An electric field exerts certain effects also on the soil, as reported in tomatoes, when the soil was treated, and the growth rate was accelerated [43,45]. The use of the electric field can be efficient under other specific conditions [46] as it induces electroosmosis, electrophoresis, and electrolysis in the soil [47].

On these bases, the main aim of the present study was to test the germination of spruce seeds under the influence of electric field and gamma radiation treatments and evaluate their effect considering different aspects (variation within treatments based on specialty literature, seed origin influence, and further seedlings development).

## 2. Materials and Methods

### 2.1. Biological Material

Spruce seeds (*Picea abies*) have been collected from different locations from Romania and were used as biological material for this research. The location (provenances) from where the spruce seeds were collected are: (1) Măria Mică (Bistrița-Năsăud County, Șanț City Hall, O.S. Izvorul Someșului, UB.VIII), coordinates (47°25' N/24°50' E); (2) Făina (Maramureș County, RNP-Romsilva, O.S. Vișeu, UP. III), coordinates (47°50' N/24°38' E); (3) Aluniș (Harghita County, RNP-Romsilva, O.S. Borsec, UP. VI), coordinates (46°45' N/25°20' E); (4) Putnișoara (Suceava County, RNP-Romsilva, O.S. Pojorâta, UP.III), registered in the National Catalog of basic materials for the production of forest reproductive materials [48] (Figure 1). Thus, the provenances (P) were noted as follows: P1 (Măria Mică), P2 (Făina), P3 (Aluniș), and P4 (Putnișoara) and were investigated within the perspective of different origin of seeds in the current study.



**Figure 1.** Romanian provenances of *Picea abies* seeds: 1—P1 (Măria Mică), 2—P2 (Făina), 3—P3 (Aluniș), 4—P4 (Putnișoara).

Spruce cones were harvested in October and stored at room temperature (20–25 °C) to dry and spill out the seeds. Further, the seeds were collected for each provenance, visually sorted so that only seeds with no visible damage were kept, and then evaluated based on morphological traits. All the incomplete and degraded seeds were eliminated. The selected seeds were cleaned, using 50 seeds/provenance/treatment, in 3 repetitions and further subjected to electric fields and gamma radiation treatments, then germinated along with control (non-treated seeds) in Linhardt pots. After germination, the seedlings were transferred to seedling trays using a peat and perlite mixture (Figure 2).



**Figure 2.** (a) Cones and spruce seeds; (b) sprouts; (c) the Gamma Chamber apparatus; (d) electric current generator; (e) spruce seedlings.

## 2.2. Exposure of the Seeds to Electric field (EF) Treatments

The seeds were subjected to electric field (EF) treatments in the Biophysics and Afforestation laboratory of the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca. The electric field was generated with an electric field generator (with an armature of 26 cm and  $d = 0.073$  m). The applied voltages were as follows: 10 V, 30 V, and 50 V, with an exposure time of 15 and 35 min. The combination of the above-mentioned treatments were named as follows: T1<sub>EF</sub> (Control), T2<sub>EF</sub> (10 V-15 min), T3<sub>EF</sub> (10 V-35 min), T4<sub>EF</sub> (30 V-15 min), T5<sub>EF</sub> (30 V-35 min), T6<sub>EF</sub> (50 V-15 min), and T7<sub>EF</sub> (50 V-35 min).

## 2.3. Exposure of Seeds to Gamma Irradiation (G) Treatments

The irradiation of the biological material was carried out at the Babeş-Bolyai University from Cluj-Napoca, in the laboratory of Anatomical and Nuclear Physics, Isotopes. The Gamma Chamber apparatus was used to irradiate the seeds using the 60Co Isotope. This unit consists of an annular source, which has an activity of 900 Curries. For the irradiation of spruce seeds, the experimental treatments were set as follows: a non-treated variant was used as control T1<sub>G</sub> (Control), T2<sub>G</sub> (1 Gy-31 min), T3<sub>G</sub> (1.5 Gy-46 min), T4<sub>G</sub> (2 Gy-62 min) and T5<sub>G</sub> (6 Gy-186 min). Irradiation and exposure intervals were selected based on speciality literature.

## 2.4. Seeds and Seedlings Characteristics Measurements

Seed characteristics were measured with the help of existing tools in forestry laboratories. The length (mm) and diameter (mm) of the seeds were measured with an electronic caliper. For determining the weight (mg) of the seeds, an analytical balance was used. Regarding seedlings' development, their heights were determined with electronic callipers, since it was considered the most relevant quantifiable parameter at this stage.

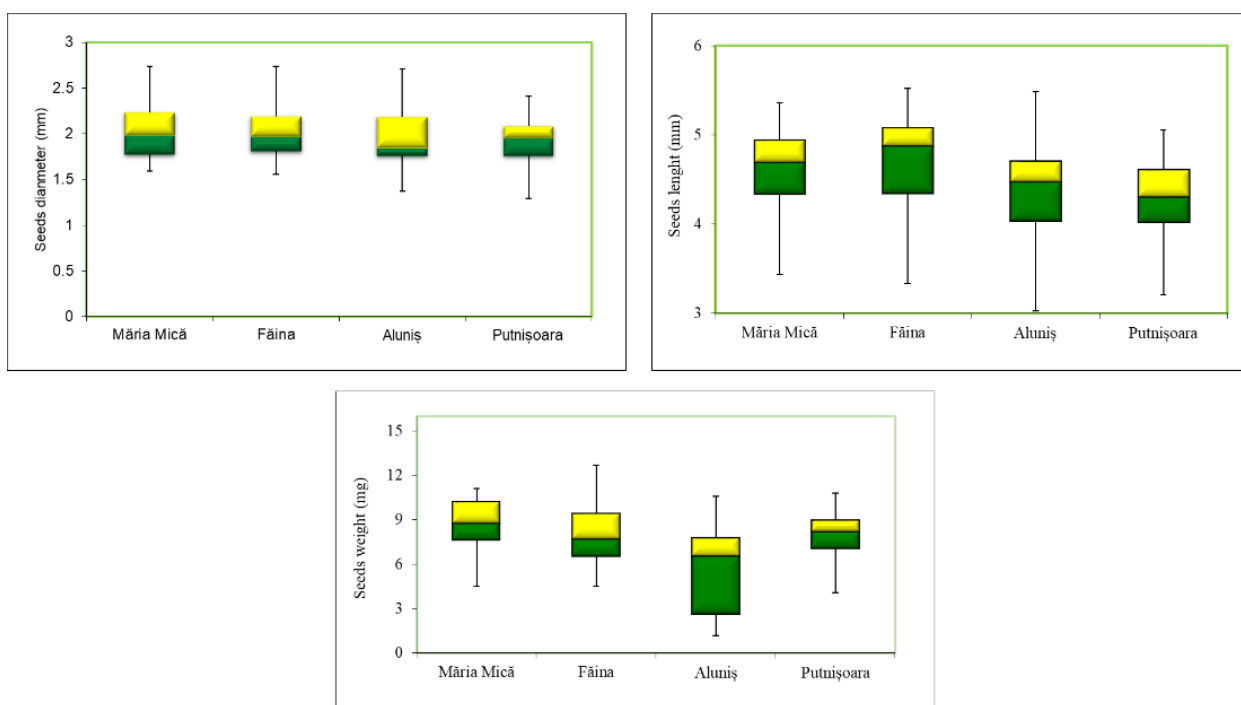
## 2.5. Experimental Design and Data Analysis

The experimental design was a factorial combination of a physical factor (EF, G; fixed factor) and seeds' provenance (four sites; random factor). Data were analyzed by a mixed model 2-Way ANOVA to test for the significance of the mean of the main effects of the factors and their interaction. When the null hypothesis was rejected, Duncan's multiple range test at  $p < 0.05$  was applied to determine statistically significant differences between the means. In total, 4,050 spruce seeds were used for the experiment (50 seeds/provenance/treatment, in 3 repetitions). Different lowercase letters above the bars indicate significant differences between the means, according to Duncan's Multiple Range Test (the data presented are means  $\pm$  standard error) [49].

# 3. Results

## 3.1. Morphological Traits of Spruce Seeds

Seed characteristics such as length, diameter, and weight were measured and are presented as a box-plot diagram, which consists of the minimum and maximum values of the range noted within the experiment, the upper and lower quartiles, as well as the median, to be able to summarize the distribution of the recorded data set, as shown in Figure 3. The minimum recorded seed length was 2.11 mm from P3 (Aluniş) provenance, while the maximum length recorded was 5.52 mm from P2 (Făina). The diameter of the seeds ranged between 1.06 mm at P4 (Putnişoara) to 2.74 mm within seeds from P1 (Măria Mică) and P2 (Făina) provenances. The highest seed weight (12.7 mg) was recorded in P2 (Făina) seeds. It will be further investigated how the variability of seeds' characteristics, with regard to the influence of their origin investigated hereby, can influence germination.



**Figure 3.** Chart diagrams of spruce seed length, diameter, and weight. Each box plot represents the minimum, median, and maximum values (yellow represents the maximum values and green represents the minimum values).

### 3.2. Seed Germination after EF Treatments

The results of the interaction between seeds origin (four provenances) and the different EF treatments applied to the seeds to induce germination are presented in Table 1. It was intended to analyze the impact of these two distinct factors so that all aspects can be discussed and objective recommendations can be formulated. It was noted that the T4<sub>EF</sub> (30 V-15 min) treatment gave the best results for seed germination within all provenances investigated, closely followed by T3<sub>EF</sub> (10 V-35 min) with significant differences only for P2 seedlings, which was also the provenance with the highest seed weight.

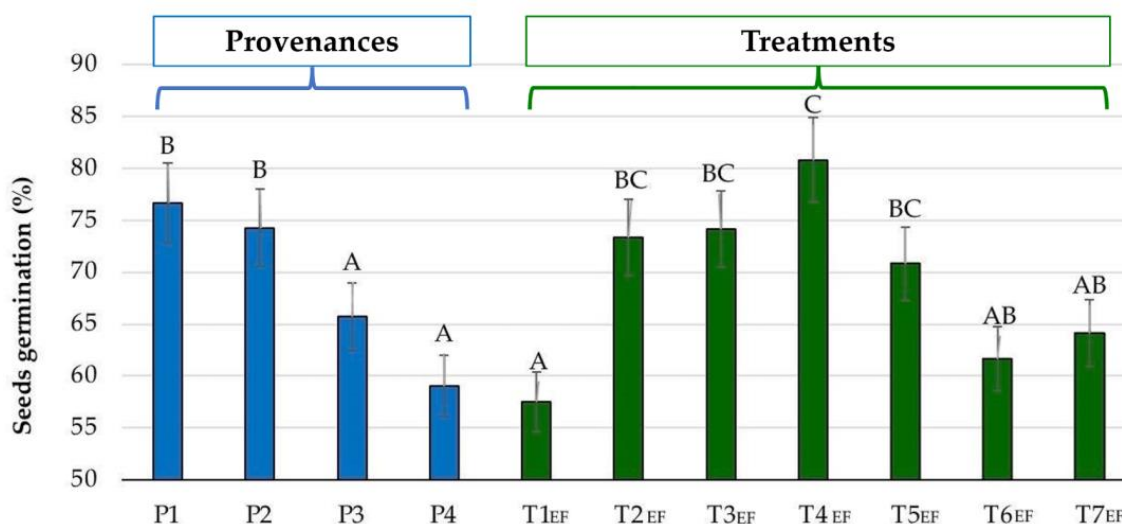
**Table 1.** Seed germination percentage (%) depending on the interaction between EF treatments and provenances.

Treatment	Provenance			
	Măria Mică (P1)	Făina (P2)	Aluniș (P3)	Putnișoara (P4)
T1 <sub>EF</sub> (Control)	60.00 a-c	63.33 a-d	56.67 ab	50.00 a
T2 <sub>EF</sub> (10 V-15 min)	80.00 bd	76.67 a-d	73.33 a-d	63.33 a-d
T3 <sub>EF</sub> (10 V-35 min)	86.67 cd	83.33 b-d	63.33 a-d	63.33 a-d
T4 <sub>EF</sub> (30 V-15 min)	86.67 cd	90.00 d	76.67 a-d	70.00 a-d
T5 <sub>EF</sub> (30 V-35 min)	76.67 a-d	76.67 a-d	70.00 a-d	60.00 a-c
T6 <sub>EF</sub> (50 V-15 min)	73.33 a-d	60.00 a-c	63.33 a-d	50.00 a
T7 <sub>EF</sub> (50 V-35 min)	73.33 a-d	70.00 a-d	56.67 ab	56.67 ab

The means on the columns inside the table, followed by different small letters (a–d), are significantly different according to Duncan's MRT test ( $p < 0.05$ ) regarding the interaction between provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, P4—Putnișoara), and EF treatments: T1<sub>EF</sub> (Control), T2<sub>EF</sub> (10 V-15 min), T3<sub>EF</sub> (10 V-35 min), T4<sub>EF</sub> (30 V-15 min), T5<sub>EF</sub> (30 V-35 min), T6<sub>EF</sub> (50 V-15 min), and T7<sub>EF</sub> (50 V-35 min).

Regarding the origin of the seeds and the EF treatments applied, based on F calculated ( $F_{\text{provenance}(P)} = 15.68$ ,  $F_{\text{treatment}(T)} = 3.71$ ,  $F_{P \times T} = 0.23$ ), our results showed that out of the four provenances, seeds from P1 (Măria Mică) (76.67%) and P2 (Făina) (74.29%) had significantly higher germination percentages as compared to other provenances (Fig-

ure 4). In the context of the EF treatments applied to the seeds, it was observed that T4<sub>EF</sub> (30 V-15 min) induced the highest germination percentage (80.83%), followed by T3<sub>EF</sub> (10 V-35 min) (74.17%), while the lowest recorded percentage was in T1<sub>EF</sub> (Control) with 57.50% germination rate (Figure 4). These results show that both provenance and treatment, as well as their interaction (Table 1), can influence the success of seed germination, with significant differences among variants; the best results were recorded at the provenance P2 (Făina) seeds, along with T4<sub>EF</sub> (30 V-15 min) treatment.



**Figure 4.** The percentage of seed germination for provenances and EF treatments (mean  $\pm$  SE; n = 50). The means represented with blue color reflect the influence of the provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, and P4—Putnișoara), regardless of the treatment applied; the means represented with green color reflect the influence of the EF treatment (T1<sub>EF</sub> (Control), T2<sub>EF</sub> (10 V-15 min), T3<sub>EF</sub> (10 V-35 min), T4<sub>EF</sub> (30 V-15 min), T5<sub>EF</sub> (30 V-35 min), T6<sub>EF</sub> (50 V-15 min), and T7<sub>EF</sub> (50 V-35 min)), regardless of the provenance. Different (upper case) letters at the top of the bars indicate statistically significant differences between provenances, and respectively treatments, according to Duncan's MRT test ( $p < 0.05$ ).

### 3.3. Seedlings Development after EF Treatments Applied to the Seeds

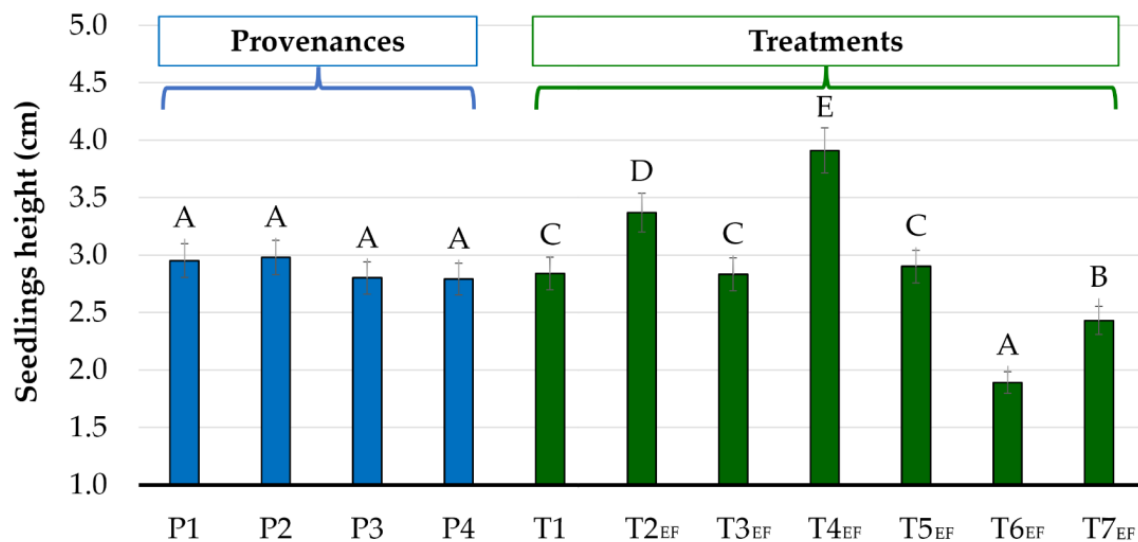
The effect of EF treatments in the interaction between provenance and different voltages and duration revealed obvious differences between the mean values of seedlings' height (Table 2). It was noted that T4<sub>EF</sub> (30 V-15 min) induced a height between 3.54 and 4.18 cm, within all four provenances analyzed, with superior and significant differences. At the same time, it was observed that the interaction between the two factors (provenance and treatment) weakened seedlings' development in T6<sub>EF</sub> (50 V-15 min) and the seedlings' height ranging between 1.66–2.17 cm (Table 2).

Seedlings obtained from seeds subjected to various EF treatments showed statistically significant differences in terms of length (Figure 5), even though the provenance was not directly influencing their growth. It can be noted that T4<sub>EF</sub> (30 V-15 min) induced a height of 3.91 cm, with superior and significant differences, whereas no differences have been recorded when the impact of EF upon seeds provenances were analyzed separately. On the contrary, the shortest seedlings were those obtained from seeds that had undertaken T6<sub>EF</sub> (50 V-15 min).

**Table 2.** Seedlings' height (cm) depending on the interaction between treatment and provenance after seeds were exposed to EF treatments.

Treatment	Provenance			
	Măria Mică (P1)	Făina (P2)	Aluniș (P3)	Putnișoara (P4)
T1 <sub>EF</sub> (Control)	2.89 <sup>d-f</sup>	3.08 <sup>e-g</sup>	2.59 <sup>b-e</sup>	2.79 <sup>b-e</sup>
T2 <sub>EF</sub> (10 V-15 min)	3.66 <sup>gh</sup>	3.57 <sup>f-h</sup>	3.11 <sup>e-g</sup>	3.13 <sup>e-g</sup>
T3 <sub>EF</sub> (10 V-35 min)	2.70 <sup>b-e</sup>	2.78 <sup>b-e</sup>	3.00 <sup>e-g</sup>	2.83 <sup>c-e</sup>
T4 <sub>EF</sub> (30 V-15 min)	4.18 <sup>h</sup>	4.00 <sup>h</sup>	3.54 <sup>f-h</sup>	3.91 <sup>h</sup>
T5 <sub>EF</sub> (30 V-35 min)	2.99 <sup>d-g</sup>	2.63 <sup>b-e</sup>	3.09 <sup>e-g</sup>	2.90 <sup>d-f</sup>
T6 <sub>EF</sub> (50 V-15 min)	1.93 <sup>a</sup>	2.17 <sup>a-c</sup>	1.66 <sup>a</sup>	1.82 <sup>a</sup>
T7 <sub>EF</sub> (50 V-35 min)	2.3 <sup>a-d</sup>	2.66 <sup>b-e</sup>	2.62 <sup>b-e</sup>	2.14 <sup>ab</sup>

The means on the columns inside the table, followed by different small letters (a–h), are significantly different according to Duncan's MRT test ( $p < 0.05$ ) regarding the interaction between provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, P4—Putnișoara), and EF treatments (T1<sub>EF</sub> (Control), T2<sub>EF</sub> (10 V-15 min), T3<sub>EF</sub> (10 V-35 min), T4<sub>EF</sub> (30 V-15 min), T5<sub>EF</sub> (30 V-35 min), T6<sub>EF</sub> (50 V-15 min), T7<sub>EF</sub> (50 V-35 min)).



**Figure 5.** Seedlings' height (cm), after EF treatments, with regard to seed provenances and treatments (mean  $\pm$  SE;  $n = 50$ ). The means represented with blue color reflect the influence of the provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, and P4—Putnișoara), regardless of the treatment applied; the means represented with green color reflect the influence of the EF treatment (T1<sub>EF</sub> (Control), T2<sub>EF</sub> (10 V-15 min), T3<sub>EF</sub> (10 V-35 min), T4<sub>EF</sub> (30 V-15 min), T5<sub>EF</sub> (30 V-35 min), T6<sub>EF</sub> (50 V-15 min), and T7<sub>EF</sub> (50 V-35 min)), regardless of the provenance. Different (upper case) letters at the top of the bars indicate statistically significant differences between provenances, and respectively treatments, according to Duncan's MRT test ( $p < 0.05$ ).

The effect of EF treatments was also analyzed, considering the interaction between provenance and different voltages and duration (Table 2). It was noted that T4<sub>EF</sub> (30 V-15 min) induced a height between 3.54 and 4.18 cm with all four provenances investigated, with superior and significant differences. At the same time, it was observed that the interaction between the two factors (provenance and treatment) weakened seedlings' development in T6<sub>EF</sub> (50 V-15 min), with seedlings' height ranging between 1.66–2.17 cm (Table 2).

### 3.4. Seed Germination after Gamma (G) Irradiation

Regarding the interaction between the two factors, provenance and gamma irradiation of the seeds (Table 3), it was observed that T5<sub>G</sub> treatment (6 Gy-186 min) upon seeds from P1 (Măria Mică) recorded the highest germination rate (96.67%), followed by P2 (Făina) seeds exposed to the same treatment (93.33%). These two provenances are also the ones

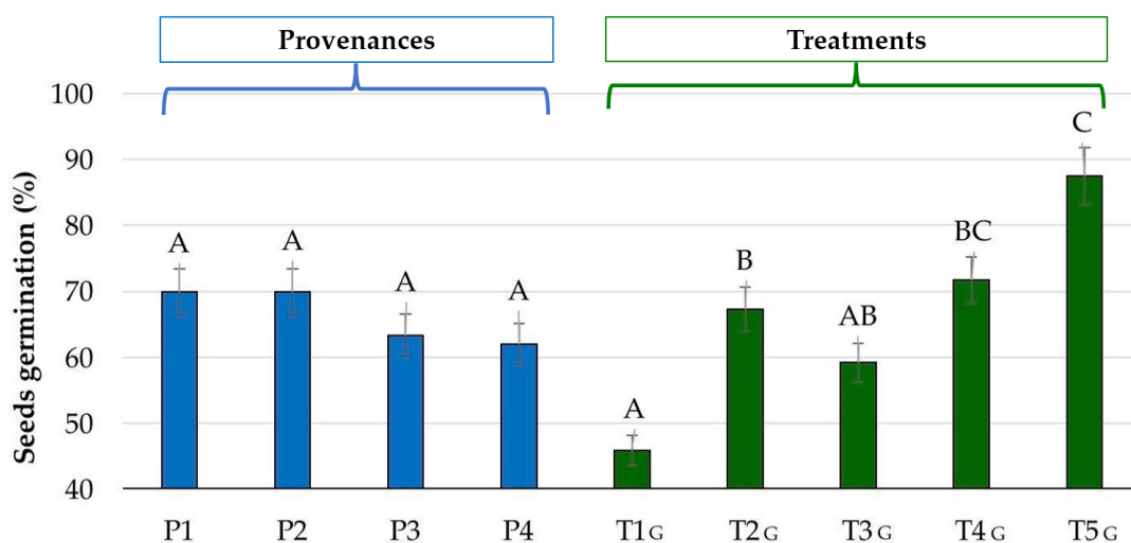
that had the largest diameter of seeds, as noted for seeds characteristics analysis, whereas P2 had seeds with high values for length and weight also.

**Table 3.** Seeds' germination (%) depending on the interaction between G treatments and provenance.

Treatment	Provenance			
	Măria Mică (P1)	Făina (P2)	Aluniș (P3)	Putnișoara (P4)
T1 <sub>G</sub> (Control)	43.33 <sup>a</sup>	50.00 <sup>a,b</sup>	43.33 <sup>a</sup>	46.67 <sup>ab</sup>
T2 <sub>G</sub> (1 Gy-31 min)	66.00 <sup>a-d</sup>	66.33 <sup>a-d</sup>	70.00 <sup>a-d</sup>	66.67 <sup>a-d</sup>
T3 <sub>G</sub> (1.5 Gy-46 min)	63.33 <sup>a-d</sup>	63.33 <sup>a-d</sup>	53.33 <sup>ab</sup>	56.67 <sup>a-c</sup>
T4 <sub>G</sub> (2 Gy-62 min)	80.00 <sup>a-d</sup>	76.67 <sup>a-d</sup>	66.67 <sup>a-d</sup>	63.33 <sup>a-d</sup>
T5 <sub>G</sub> (6 Gy-186 min)	96.67 <sup>d</sup>	93.33 <sup>c-d</sup>	83.33 <sup>b-d</sup>	76.67 <sup>a-d</sup>

The means on the columns inside the table, followed by different small letters (a–d), are significantly different according to Duncan's MRT test ( $p < 0.05$ ), regarding the interaction between provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, and P4—Putnișoara) and G treatment (T1<sub>G</sub> (Control), T2<sub>G</sub> (1 Gy-31 min), T3<sub>G</sub> (1.5 Gy-46 min), T4<sub>G</sub> (2 Gy-62 min), and T5<sub>G</sub> (6 Gy-186 min)).

Our results suggest that due to gamma irradiation, the germination rate of spruce seeds was considerably influenced (Figure 6). Germination percentages varied from 45.83% within T1<sub>G</sub> (Control) to 87.50% for T5<sub>G</sub> (6 Gy-186 min). Even though the provenance itself did not differ statistically within this stage of the experiment, whereas the F calculated had small values ( $F_{\text{provenance (P)}} = 0.75$ ,  $F_{\text{treatment (T)}} = 7.61$ ,  $F_{\text{P} \times \text{T}} = 0.19$ ), the results for the G treatments were significantly different among the specific gamma irradiation applied seeds.



**Figure 6.** Percentage of seed germination for provenances and G treatments (mean ± SE; n = 50). The means represented with blue color reflect the influence of the provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, and P4—Putnișoara), regardless of the treatment applied; the means represented with green color reflect the influence of the G treatment (T1<sub>G</sub> (Control), T2<sub>G</sub> (1 Gy-31 min), T3<sub>G</sub> (1.5 Gy-46 min), T4<sub>G</sub> (2 Gy-62 min), and T5<sub>G</sub> (6 Gy-186 min)), regardless of the provenance. Different (upper case) letters at the top of the bars indicate statistically significant differences between provenances, and respectively treatments, according to Duncan's MRT test ( $p < 0.05$ ).

### 3.5. Seedlings Development after G Treatments Applied to the Seeds

Analyzing the interaction between the two factors, namely the different origin of seeds and the G treatments, it can be seen that the T5<sub>G</sub> treatment (6 Gy) retained its positive influence in the development of seedlings in the interaction with the four sources analyzed (Table 4), so it turns out that P1 (Măria Mică) showed higher values (4.13 cm) compared to the other sources analyzed. On the other hand, it can be seen that the interaction between P4 (Putnișoara) and T3<sub>G</sub> (1.5 Gy) showed the lowest value for the seedlings' height (1.92 cm).

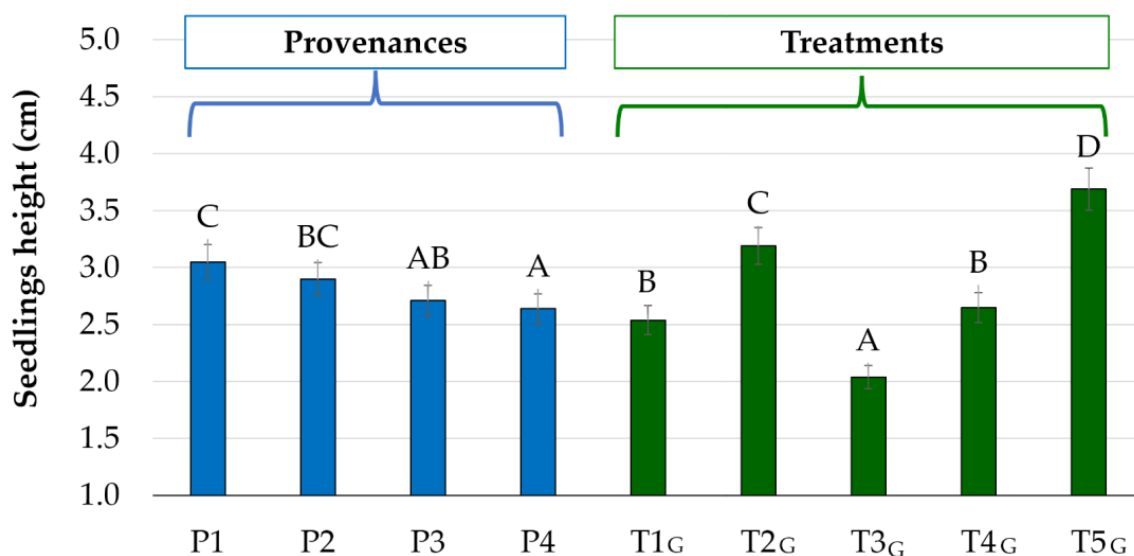


**Table 4.** The seedling's height (cm) depending on the interaction between G treatment and provenance.

Treatment	Provenance			
	Măria Mică (P1)	Făina (P2)	Aluniș (P3)	Putnișoara (P4)
T1 <sub>G</sub> (Control)	2.55 <sup>a-c</sup>	2.47 <sup>a-c</sup>	2.52 <sup>a-c</sup>	2.61 <sup>b-d</sup>
T2 <sub>G</sub> (1 Gy-31 min)	3.63 <sup>f-h</sup>	3.21 <sup>d-f</sup>	2.96 <sup>c-e</sup>	2.97 <sup>c-e</sup>
T3 <sub>G</sub> (1.5 Gy-46 min)	2.10 <sup>ab</sup>	2.03 <sup>ab</sup>	2.12 <sup>ab</sup>	1.92 <sup>a</sup>
T4 <sub>G</sub> (2 Gy-62 min)	2.83 <sup>c-e</sup>	2.87 <sup>c-e</sup>	2.49 <sup>a-c</sup>	2.41 <sup>a-c</sup>
T5 <sub>G</sub> (6 Gy-186 min)	4.13 <sup>h</sup>	3.91 <sup>gh</sup>	3.45 <sup>e-g</sup>	3.28 <sup>ef</sup>

The means on the columns inside the table, followed by different small letters (a–h), are significantly different according to Duncan's MRT test ( $p < 0.05$ ) regarding the interaction between provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, and P4—Putnișoara) and G treatments (T1<sub>G</sub> (Control), T2<sub>G</sub> (1 Gy-31 min), T3<sub>G</sub> (1.5 Gy-46 min), T4<sub>G</sub> (2 Gy-62 min), and T5<sub>G</sub> (6 Gy-186 min)).

Regarding the seedlings' development, all gamma irradiation treatments differently affected their growth (Figure 7). For example, seedlings obtained from seeds originating from P1 (Măria Mică) were the only ones that exceeded 3 cm, so that the seedlings reached a height of 3.05 cm, followed by P2 (Făina) seedlings which had 2.90 cm. Among the G treatments applied to seeds, T5<sub>G</sub> (6 Gy-186 min) induced the obtained seedlings to the highest value for height (3.69 cm), followed by T2<sub>G</sub> (1 Gy-31 min), whereas seedlings reached 3.19 cm, with significant differences.



**Figure 7.** Seedlings' height (cm), after G treatments, with regard to seed provenances and G treatments (mean  $\pm$  SE;  $n = 50$ ). The means represented with blue color reflect the influence of the provenance (P1—Măria Mică, P2—Făina, P3—Aluniș, and P4—Putnișoara), regardless of the treatment applied; the means represented with green color reflect the influence of the G treatment (T1<sub>G</sub> (Control), T2<sub>G</sub> (1 Gy-31 min), T3<sub>G</sub> (1.5 Gy-46 min), T4<sub>G</sub> (2 Gy-62 min), and T5<sub>G</sub> (6 Gy-186 min)), regardless of the provenance. Different (case upper case) letters at the top of the bars indicate statistically significant differences between provenances, and respectively treatments, according to Duncan's MRT test ( $p < 0.05$ ).

#### 4. Discussions

Efficient management and conservation of planting material for the production of forest reproductive material and the genetic resources of forest species suffer from variations in their characteristics and seed germination [50,51]. The differences between the analyzed origins were statistically assured, while the germination of seeds and seedlings' development was certainly influenced both by the ecological parameters in the area of origin and by the EF and G treatments applied. Seed size has been considered an important trait affecting the reproductive outcome of several plant species [52], so that can directly

influence germination interval [53], germination percentage [54], and seedling vigor [55,56]. Seed weight and size are directly related to the amount of energetic (nutritional) reserves that will be allocated for seedlings' growth [56]. According to the literature, larger seeds often have a higher germination rate because they are supposed to contain more resources to support the intense, energy-intensive biological process [57] and also result in healthier seedlings [50,57], as was noted in the results obtained hereby just as well, especially for P1 and P2 provenances. Even so, although germination efficiency has been shown to be largely correlated with seed mass, larger seeds do not always germinate faster than smaller ones [58,59]. In our study, the provenance P1 (Măria Mică) and P2 (Făina) had the largest seeds, whereas the two provenances had the best germination percentages for all applied treatments.

Regarding the action of the electric field (EF) on the germination of spruce seeds, the best germination percentage was registered in the case of the T4<sub>EF</sub> treatment (30 V-15 min) with a value of 80.83%, compared to the variant T1<sub>EF</sub> (Control) with a value of 57.50%. In this case, it was observed that the provenance also positively influenced the germination of the seeds, significant differences being registered at P1 (Măria Mică) and P2 (Făina). Further investigating, significantly higher values in the case of seedlings' development were recorded for seedlings obtained from seeds exposed to T4<sub>EF</sub> treatment (30 V-15 min), with a height of 3.91 cm. The interaction between EF treatment and the provenance gave the best results within P1 (Măria Mică). Most studies show us the positive effects of electric fields on plants [36,42,60]; researchers show that EF significantly increased the germination rate of sessile oak, carrot, garden radish, and beet. It has also been shown that cotton seeds have a higher growth rate when exposed to EF [43], as well as lettuce [41]. Positive results have also been reported for acacia and corn [61], beets and barley [43,45], and even more, in the case of oak [62].

Gamma treatments have a positive impact on seed germination, being used nowadays in several breeding strategies [63]. Small doses of irradiation improved vigor and plant development [64], while 30–50 Gy led to a significant decrease in *Nicotiana tabacum* plants' growth, whereas 70 Gy killed the plants [65]. Even so, the impact of  $\gamma$ -ray treatments was investigated on various seeds [66], and it was observed that irradiation with doses lower than 100 Gy stimulated the germination rate. Researchers [67] described the effects induced by  $\gamma$ -rays (50–350 Gy) on the seeds of *Oryza sativa* and *Phaseolus mungo*; while low-dose irradiation improved morphological characteristics, exposure to higher doses had a negative impact on the same parameters. It was also observed [68] that  $\gamma$ -ray had stimulant effects at low doses (2–30 Gy), while high doses (approx. 70 Gy) were found to be harmful to plants. It can be said that the overall pattern of germination treatments takes an approximately similar shape across the provenances but with varying degrees of strength between the provenances (as shown by the variable results of Duncan's test of the provenance means down the columns in the Tables).

In our study, the most influential irradiation was the treatment T5<sub>G</sub> (6 Gy) with a germination rate of 87.50%; it is important to mention that this treatment has presented the same beneficial influence on the seedlings' development. Thus, gamma rays have also been shown to be an effective approach for improving seed germination performance and seedlings' stability for forest species.

## 5. Conclusions

It can be concluded that spruce seeds' germination was significantly stimulated by an electric field and gamma irradiation. Based on the results obtained in the present investigation, it may be concluded that lower doses of radiation may facilitate better germination, growth, and development by overcoming all the barriers, including dormancy specific to spruce seeds. Treatment T4<sub>EF</sub>, meaning 30 V-15 min, gave the best results among the tested EF variants (almost 81%), whereas the better result of the experiment in regard to germination was obtained after gamma irradiation of seeds, 87.50% for T5<sub>G</sub> (6 Gy-186 min) respectively. The same treatments further induced a proper growth, so

that the corresponding seedlings also had the best results for height. The results of the study illustrate that non-chemical treatments could significantly improve spruce seeds' germination and seedlings' characteristics. Consequently, physical methods (EF and G) could be considered to be valuable alternatives for stimulating germination and plant development in the first stages, while the hereby methods have even more advantages—being both ecological and economic—offering the possibility of being used on a large scale.

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