



Germination and stress tolerance of oats treated with pulsed electric field at different phases of seedling growth

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ABSTRACT

This study explores the impact of pulsed electric field (PEF) application on oat seedling growth and stress tolerance. PEF treatment (99 monopolar, rectangular pulses lasting 10 μ s each, with a frequency of 13 Hz and a nominal electric field strength of 2250 V/cm) was applied at two growth stages: (i) when the seedlings had 0.2 cm roots emerging from the kernel, and (ii) when they had a 0.4 cm shoot emerging from the kernel. Post-treatment, the seedlings were hydroponically grown for 8 days. To induce stress, the hydroponic medium was augmented with PEG (15 %) to induce drought stress and NaCl (150 mM) to induce salinity stress. Results demonstrate that applying PEF improved the growth of the root and shoot of oat seedlings. This effect was more pronounced when applied to more developed seedlings. When PEF was applied during the later stage of germination, seedlings exposed to salinity stress showed enhanced shoot growth compared to the control. Under the studied conditions, the application of PEF had no impact on the growth of seedlings under drought stress.

1. Introduction

Seed germination is one of the most critical events affecting crop yield and quality in crop production. It encompasses processes beginning with the absorption of water by dried seeds and concluding with the emergence of the radicle (a part of the embryonic axis) [1,2]. Water uptake by dry seeds typically follows a triphasic pattern, with distinct physical and metabolic processes characterizing each phase. Phase 1 primarily involves the physical uptake of water, though physiological activities may commence within minutes of a cell becoming hydrated. Phase 2 is characterized by minimal or no water uptake, a significant increase in seed metabolic activity, and the transcription of new genes. The completion of germination is marked by the emergence of the radicle through the surrounding structures at the end of this phase. In Phase 3, there is further water uptake as the young seedling utilizes its major stored reserves [3]. The chemical composition and metabolic activity of oat seeds depend on the germination phase.

Abiotic stresses, including low or high temperatures, high salinity, and drought, have significantly reduced agricultural production and crop yields in recent years [4]. Drought and salinity are two of the main

abiotic stresses that limit the growth, development, and productivity of plants, thus posing a threat to food security [5]. Estimates indicate that 32–69 % of oat grain yield is lost due to drought stress [6], and more than 20 % of cultivated land was affected by salinity stress in 2014 [7].

Various chemicals, microbes, and electrostimulation technologies have been identified to enhance agricultural production by improving crop tolerance to environmental stresses. Zhang et al. [8] applied melatonin to alleviate drought damage in seedlings of naked oats (hullless oats). Melatonin has also been used to reduce salt stress damage in cotton seeds by regulating abscisic acid (ABA) and gibberellic acid (GA3)-related genes, promoting seed germination [4]. Several microorganisms are employed in agricultural production to enhance the production of secondary metabolites and induce the expression of plant-specific genes, improving crop plant tolerance. The application of Plant Growth-Promoting Rhizobacteria (PGPRs) is one such example, proving to be an effective way to alleviate drought stress [9]. However, with the increasing demand for chemical-free and sustainable technologies, electrostimulation technologies are emerging as potential alternatives in agricultural production [10]. Various forms of electrostimulation technologies can be applied, including magnetic fields and electric fields,

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which encompass alternating current (AC) electrical fields and PEF [11,12].

Among the different electrotechnologies tested on seeds and seedlings, PEF shows the most potential for industrial-scale applications. The effects of PEF on seed germination depend on factors such as field strength, pulse duration, seed type, and polarity. While PEF may enhance germination and seedling vigor in some cases, it can also inhibit or have negligible effects on germination under different conditions (for a comprehensive review, see [12]). In a few studies, PEF treatment of seeds has shown promise for improving seedling resistance to abiotic stresses such as drought, salinity, and temperature extremes. PEF treatment increased germination, seedling growth, and tolerance to cold and salt stress in wheat seeds [13]. He et al. [14] found that exposing maize seeds to extremely low-frequency PEF increased root growth and antioxidant activity in seedlings under drought stress. The PEF treatment reduced damage from free radicals and helped seedlings better withstand drought. However, these studies have not considered the possible influence of the germination phase on the effects of the PEF treatment, which is important as metabolic processes in the seeds and how electrical treatment may affect them, depend on the germination phase.

This study aims to evaluate the effect of PEF on the germination and stress tolerance of oats at two stages of seedling growth. Tolerance to drought and salinity was assessed for seedlings growing in a hydroponic system.

2. Materials and methods

2.1. Raw material handling

Dry oat seeds, cultivar Galant, used 6 months after harvest, with a germination rate of 90 %, were provided by the company Lantmännen, Svalöv, Sweden. The seeds were stored at room temperature in a dark, dry place before the experiments.

2.2. Sample preparation

For the upcoming experiments, seedlings in two different stages of germination (as shown in Fig. 1) were prepared. Five hundred undamaged, dry oat seeds were sorted and spread on wet tissue papers in boxes. The boxes were sealed with parafilm to prevent dehydration and were then kept in a climate chamber (MLR-352 PE, Panasonic) for germination for 3 days at 10 °C and 60 % RH in the darkness.

To prepare seedlings at two different stages of germination, the seeds

were treated as follows:

- (i) Removed from the chamber.
- (ii) Transferred to another climate chamber (MLR-352 PE, Panasonic) and stored at 20 °C and 60 % RH with a 16-h photoperiod for one to two days (stage 1).
- (iii) Stored for three to four days (stage 2).

At the end of stage 1, 40 seedlings with a root length of 0.2 ± 0.1 cm (Fig. 1A) were manually selected for further experiments. At the end of stage 2, 40 seedlings with a shoot length of 0.4 ± 0.2 cm (Fig. 1B) were manually selected for further experiments.

2.3. Electrical treatment

Twenty seeds from each described germination stage were subjected to PEF treatment, while the other 20 were in the control group. A pulse generator (Thor, OptiCept Technologies AB, Lund, Sweden) was connected to the treatment chamber, which contained two square stainless steel electrodes of 20 cm width x 20 cm length, placed in parallel with a 1.6 cm gap between them. Tap water with an electrical conductivity of 200 $\mu\text{S}/\text{cm}$ was positioned in the gap between the electrodes. In the treatment chamber, 20 seeds were exposed to the following PEF protocol: 99 monopolar, rectangular pulses lasting 10 μs each, with a frequency of 13 Hz and a nominal electric field strength of 2250 V/cm (equivalent to a treatment energy of 1.6 kJ/kg of wet seeds).

The electroporation conditions were established in preliminary experiments, where 15 different PEF protocols, with field strengths ranging from 1.25 to 5 kV/cm, pulse widths ranging from 10 to 100 μs , and pulse numbers ranging from 10 to 100, were tested on seedlings in stage 1 of germination. This range of parameters either failed to elicit any visible effect on rooting, facilitated growth, or significantly hindered the rooting process. These conditions were also tested on *Scutellaria baicalensis* Georgi. by [15].

The entire procedure was repeated, starting with 500 seeds for the preparation of seeds in the two stages of germination and concluding with the electrical treatment. Therefore, all reported data result from two replications, with a total of 80 seeds for each growth phase, 40 PEF-treated and 40 untreated.

2.4. Seedlings growth

Once the seedlings in stage 1 of germination had roots measuring 0.2 ± 0.1 cm in length, they were placed in the bottom of plastic pipette tips



Fig. 1. Oat seeds at two different stages of germination. (A) stage 1, where seedlings with 0.2 cm root length were selected (radicle indicated with arrows); (B) stage 2, where seedlings with 0.4 cm shoot length were chosen (plumule indicated with arrows). The background, brightness and contrast of the original pictures were adjusted for clarity.

with a capacity of 200 μL , which were cut at the bottom using scissors. Seedlings in stage 2 were also placed in similarly cut pipette tips.

The tips containing the seedlings were arranged in a rack that allowed the roots of the seedlings to be submerged in different nutrient solutions:

- (i) 1.1 g/L of Murashige and Skoog (MS) basal medium with vitamins pH 5.8 [16]
- (ii) To assess the seedlings' tolerance to drought stress, they were immersed in a 15 % polyethylene glycol 6000 solution, pH 5.7 (PEG-6000, Sigma–Aldrich, St. Louis, MO, USA). This solution was prepared by adding 15 g of PEG-6000 to 1 L MS media ($\frac{1}{2}$ strength) and mixing it with a magnetic mixer for 30 min [17]. Control samples were also immersed in the same solution.

- (iii) To assess the seedlings' tolerance to salinity stress, the seedlings were submerged in a 150 mM NaCl solution. Control samples were also immersed in the same solution.

After PEF treatment, seedlings in this simple hydroponic system were placed in a growth chamber at 20 °C and 60 % RH with a 16-h photoperiod for 8 days before analysis.

2.5. Analysis

2.5.1. Root and shoot lengths

Root length was measured on the longest root. For the shoot measurement, the primary shoot was separated from the seed, and tillers were not included in the measurement.

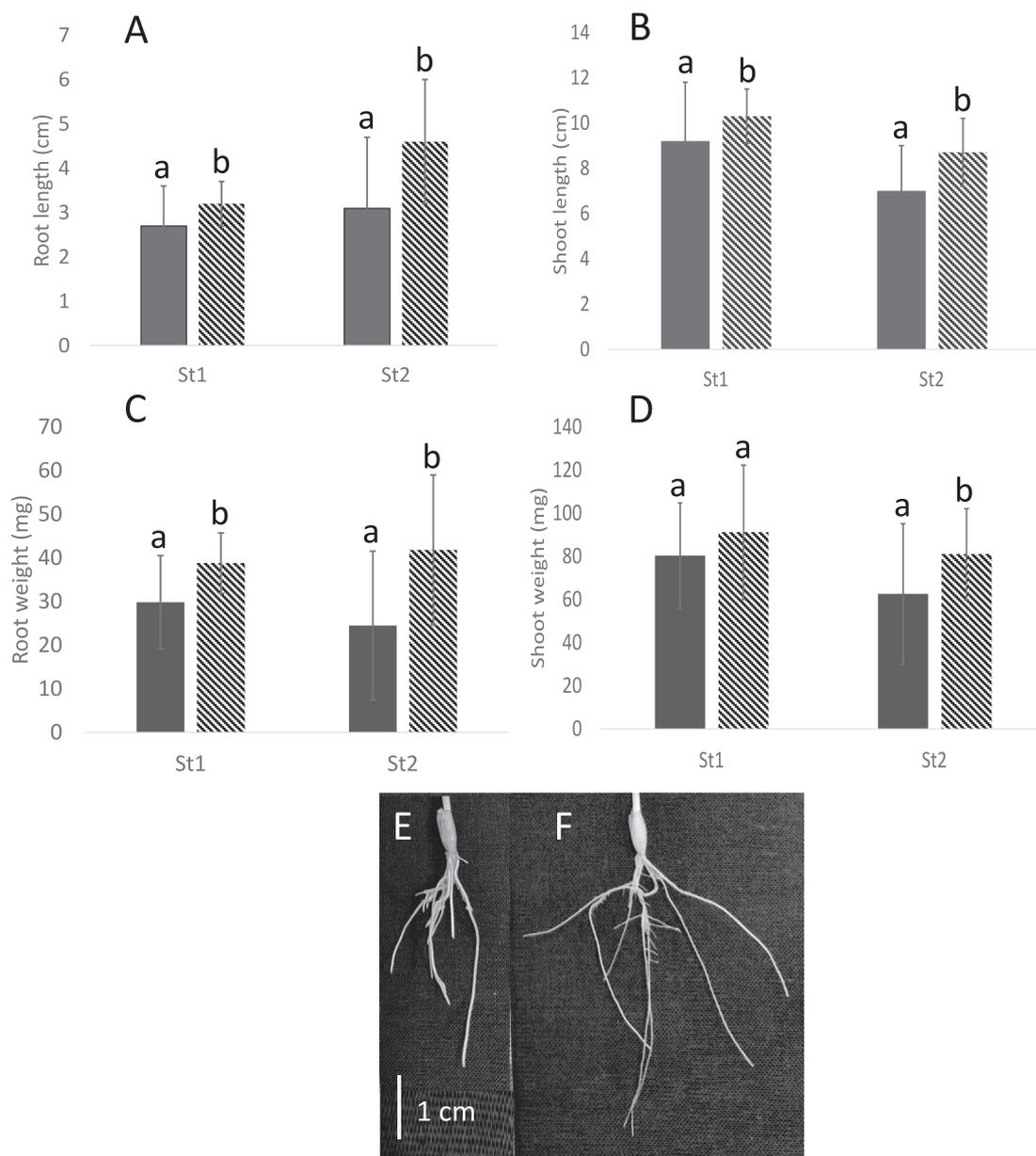


Fig. 2. Response of oat seedlings treated with PEF at two stages of germination (St1 and St2) grown under non stress conditions. (A and C) Comparison of root length and weight of seedlings between the control (black bars) and PEF-treated (striped bars); (B and D) Comparison of shoot length and weight of seedlings between the control (black bars) and PEF-treated (striped bars). Error bars represent the standard deviation of measurements taken from 40 seeds. Different letters above the error bars indicate statistical significance ($p < 0.05$). (E-F) Representative pictures show a root length comparison between the control (E) and the PEF-treated seedlings (F) treated in stage 2. For further details, see Materials and methods.

2.5.2. Fresh, dry weights and visual observations

The roots and shoots were separated from each of the seeds by opening the seed kernel. Excess water was removed by carefully blotting it with tissue paper before weighing. A group of 40 roots and shoots, with 20 from treated seedlings and 20 from the control group, were collected and weighed. Dry weights were determined after drying the samples in an oven (Termaks, Norway) at 65 °C until a constant weight was achieved.

Results were used to calculate the root/shoot ratio (R/S) according to [18]:

$$R/S = \frac{\text{dry root weight}}{\text{dry shoot weight}} \quad (1)$$

Photographs were taken with the camera of a Samsung Galaxy S21 mobile phone to compare shoot length and root structure, including the formation of secondary roots.

2.5.3. Statistical analysis

The statistical significance ($p < 0.05$) of the treatments was tested by performing a one-way ANOVA using MINITAB 21.4.1 (Minitab Inc., State College, PA, USA). The Tukey–Kramer multiple comparison test was used to evaluate differences between treatments.

3. Results

3.1. Growth without drought or salinity stress

A significant difference in root and shoot growth was observed under normal growth conditions in oat seedlings treated with PEF (Fig. 2A-F). The effect of the applied electric field demonstrates a clear dependence on the seed germination phase. PEF treatment stimulated root growth and fresh weight compared to the control when applied to seedlings in stage 2 (as depicted in Fig. 2A and C and the comparison between Fig. 2E and F). This rooting stimulation effect was less pronounced when PEF

was applied to seedlings in stage 1, although it was still statistically significant (Fig. 2A and C). The application of PEF resulted in a statistically significant increase in shoot length in both tested germination stages. However, this increase only led to a significant fresh weight increase in the shoot when PEF was applied to seedlings in stage 2 of germination (Fig. 2D).

3.2. Growth under drought stress

Fig. 3A-D show the results of the measurements conducted on roots and shoots when the seedlings were grown under drought stress conditions. Drought mostly affected the development of the shoot, as shown with the comparison between Fig. 2B and D with Fig. 3B and D. Notably, no significant differences on root or shoot development were observed when PEF was applied to the seedlings growing under drought stress for both germination stages.

3.3. Growth under salinity stress

Fig. 4A-F present the results of measurements conducted on roots and shoots when the seedlings were grown under salinity stress. As expected, salinity was found to be detrimental to the growth of seedlings in both germination phases. However, a pronounced effect on both shoot growth and weight was observed when PEF was applied to seedlings in stage 2. In this stage, a statistically significant impact of PEF was also noted on root weight, although no significant effect was observed on the length of the longest root.

3.4. Root/shoot ratio

Fig. 5 reports the results obtained for the root/shoot (R/S) ratio at each of the studied germination stages and the three growing conditions. Growing under stress increased the R/S ratio of seedlings. The R/S ratio was higher in seedlings in stage 2 of germination, and the PEF treatment

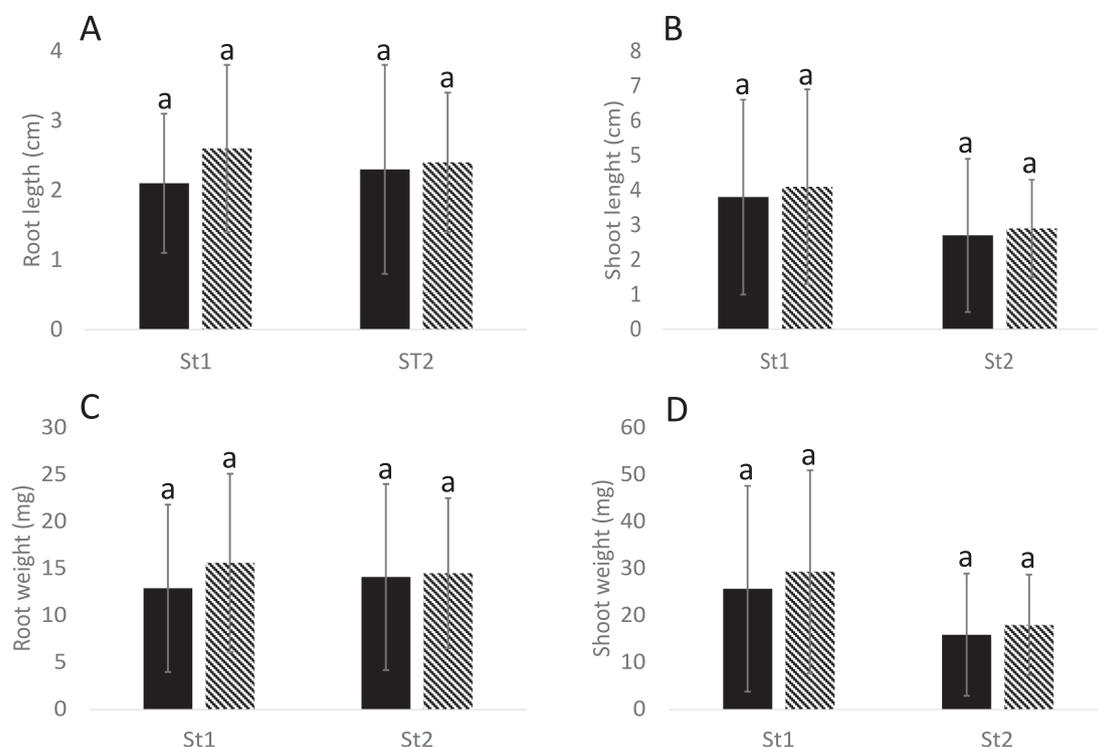


Fig. 3. Response of oat seedlings treated with PEF at two stages of germination (St1 and St2) grown under induced drought stress. (A and C) Comparison of the root length and weight of seedlings between control (black bars) and PEF-treated (striped bars); (B and D) Comparison of the shoot length and weight of seedlings between control (black bars) and PEF-treated (striped bars). Error bars represent the standard deviation of measurements taken from 40 seeds. Different letters above the error bars indicate statistical significance ($p < 0.05$). For further details, see Materials and methods.

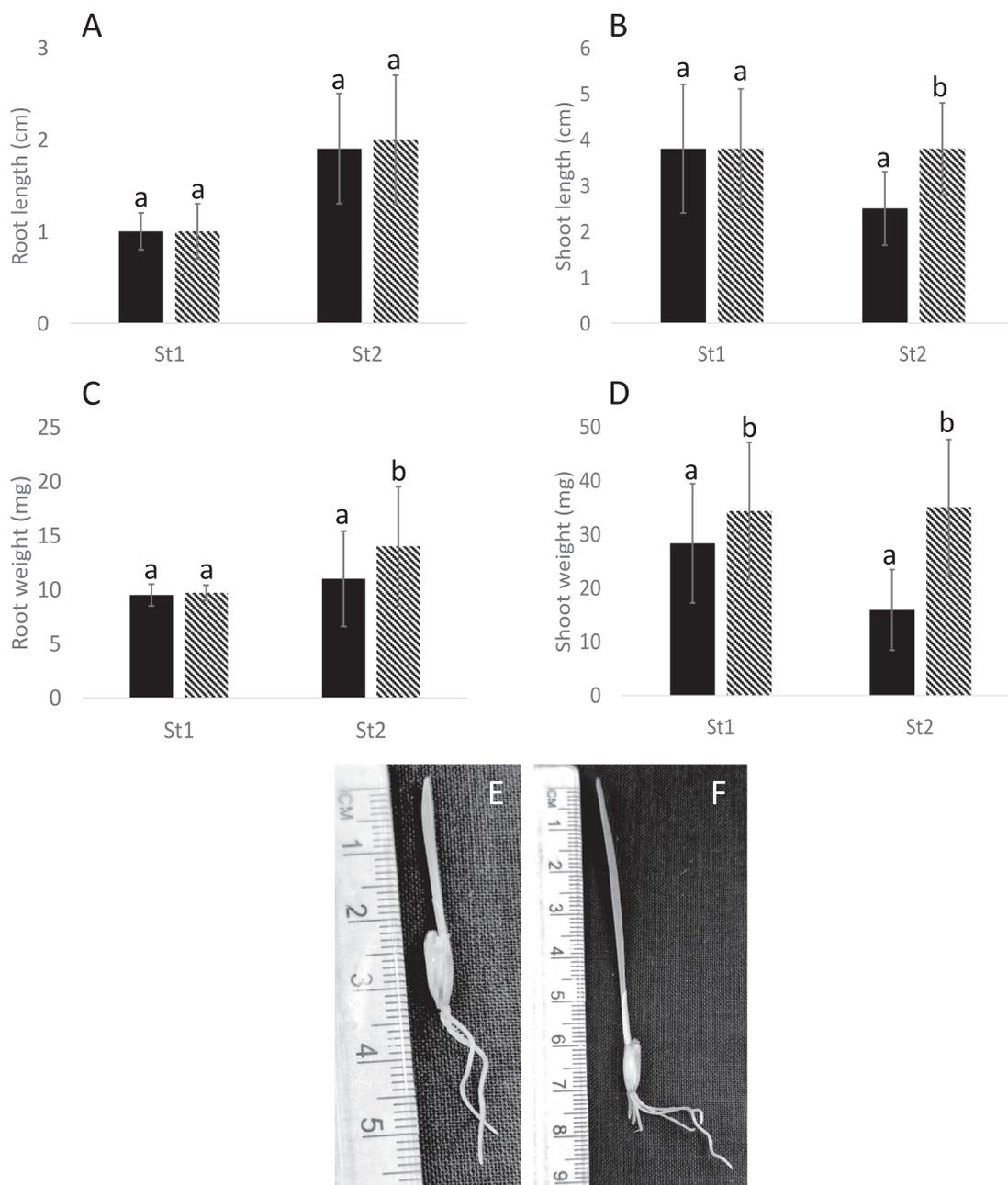


Fig. 4. Response of oat seedlings treated with PEF at two stages of germination (St1 and St2), grown under induced salinity stress. (A and C) Comparison of root length and weight of seedlings between the control (black bars) and PEF-treated (striped bars); (B and D) Comparison of shoot length and weight of seedlings between the control (black bars) and PEF-treated (striped bars). Error bars represent the standard deviation of measurements taken from 40 seeds. Different letters above the error bars represent statistical significance ($p < 0.05$). (E-F) Representative pictures show a root and shoot length comparison between the control (E) and the PEF-treated seedlings (F) treated in stage 2. For further details, see Materials and methods.

did not influence the ratio under any of the studied conditions.

4. Discussion

The results presented in this study provide evidence that PEF treatment influences oat seedling development (Fig. 2). This result aligns with other reports demonstrating the growth stimulation effects of various plant species upon the application of an electric field [12,19–21]. The stimulation depends on the intensity and time of exposure to the electric field [20]. The literature shows a vast range of PEF conditions that have been applied to seeds and seedlings to improve germination and/or growth stimulation. However, applied voltages above 1.4 kV/cm, as in the case of the present study, have proven

successful in various types of seeds when authors work with pulse widths in the order of μs [12].

In this investigation, we demonstrate that growth stimulation is also dependent on the developmental stage of the seedling prior to the application of PEF. The reported effect was more pronounced when applied to more developed seedlings (Fig. 2), where the shoot has emerged approximately half a centimeter from the kernel (Fig. 1). A significant effect on root growth was also observed when PEF was applied during early germination (Fig. 2), and the root breaking through the kernel was 0.2 cm long (Fig. 1). In this stage of germination, an opposite result was found in barley (husks not removed), where the application of the electric field had a detrimental effect on the growth of the seedlings [2], an effect attributed to the decrease in α -amylase

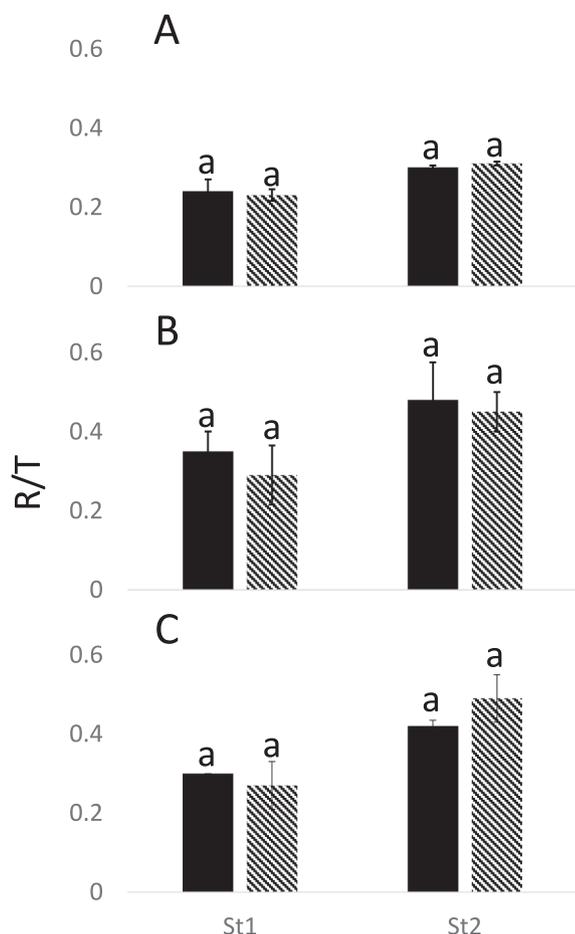


Fig. 5. Comparison of the root to shoot ratio (R/S) of oat seedlings between the control (black bars) and PEF-treated (striped bars). (A) The seedlings grew without drought or salinity stress. (B) The seedlings grew under drought stress, and (C) The seedlings grew under salinity stress. Error bars represent the standard error of two replications. Different letters above the error bars represent statistical significance ($p < 0.05$). St1 and St2 denotes Stage1 and Stage2, respectively. For further details, see Materials and methods.

concentration.

The mechanism behind the stimulation of seedling growth by applying an electric field is not well defined. However, it has been reported that external electric stimulation can influence various critical physiological processes of plants, including root hair formation, water uptake, activation of ion channels, active ion transport, changes in gene expression, antioxidant accumulation, respiration, photosynthesis, and growth [22,23].

In this context, the application of PEF has emerged as a promising technology for metabolism modulation. It has been suggested that PEF could potentially modulate the effects of plant hormones, particularly the synthesis of gibberellin (GA), on the root growth of plants like eucalyptus [24] and *Scutellaria baicalensis* [25]. Additionally, electromagnetic field applications have been shown to increase the content of gibberellin (GA) in germinating pea seedlings, provoking faster germination [26] as well as the decline of abscisic acid (ABA) in winter wheat seedlings [27]. This technology has even been proposed as a substitute for traditional hormone applications to enhance rooting in oregano cuttings [28].

The influence of gibberellin (GA) on the growth of both roots and stems is well-documented, with its effects depending on its concentration. Notably, stem elongation demands a substantially higher GA concentration compared to what is required for regulating root growth [29]. A change in gene expression was also reported upon the

application of 10 ns PEF on *Arabidopsis thaliana* seeds. Increased gene expression of *RetOx*, *PAD3*, *PR1*, *81F2*, and *GST6* genes in the treated seeds was associated with the faster growth of the seedlings. These genes are known to be upregulated by reactive oxygen species (ROS) [30], which is a well-known effect of PEF application [31].

For the most effective application of electric field stimulation in seedlings, it is essential to consider their physiological state. This effectiveness may depend on the balance between different hormones, especially the GA/ABA (gibberellic acid/abscisic acid) ratio, which tends to increase during germination [32]. Monitoring the levels of these hormones in the seedlings may serve as markers to determine the optimal timing for the treatment, ensuring that the electric field's impact is maximized.

Gibberellins have been demonstrated to enhance plants' resistance to various environmental stresses, among them salinity [33,34] and drought [35]. The response to salinity is further influenced by the growth stage of the plant [36]. For instance, rice exhibits varying sensitivity to salinity stress during germination, early seedling stage, active tillering and initiation of flowering [37]. Interestingly, in our study, the effect of PEF on seedlings growing under salinity stress was influenced by the growth stage (Fig. 4), indicating that the growth stimulation effect under stress is more effective when PEF is applied as a post-germination treatment.

Remarkably, our results show a clear effect of the electric treatment when seedlings grew under salinity stress but not under drought stress (Figs. 3 and 4). The early responses to water and salt stress are similar, except for the ionic component [38]. Exposure to drought or salinity stress elicits a variety of common reactions in plants. Both stressors induce cellular dehydration, resulting in osmotic stress. However, the oat seedlings in our experiments have been exposed to two stressors in sequence: PEF + drought and PEF + salinity. The combination of these stressors may have provoked a distinctive response with specificity for salt tolerance. According to Choudhury et al. [39], different ROS signatures induced by different abiotic stresses determine the specificity of the acclimation response and help the plant to encounter the exact stress.

Further studies should focus on the cross-talk between ROS and hormones to better understand the mechanism of PEF-induced growth and stress tolerance in seedlings.

5. Concluding remarks

This study, which reports the effects of the application of pulsed electric field on the growth and stress tolerance of oat seedlings, has produced remarkable results as well as raised interesting questions. The following remarks underline important findings:

- (1) The application of PEF, as a post-germination treatment, increases the growth of both roots and shoot of oat seedlings. This effect was more pronounced when more developed seedlings (with shoot grown 0.4 cm outside the kernel) were treated.
- (2) When PEF was applied to seedlings in the later stage of germination used in this study, seedlings grown under salinity stress exhibited a drastic improvement in shoot growth compared to that of the control. Interestingly, the application of PEF did not influence the drought tolerance of the seedlings.

Our exploration study has laid the foundation for further investigations into the mechanisms for PEF-induced growth and stress tolerance in seedlings, including the search for biological markers to determine the most appropriate stage of seedling development for PEF application.

CRediT authorship contribution statement

Alia Hussain Al-Khafaji: Formal analysis, Investigation,

Methodology. Stephen Kwao: Investigation, Resources. **Federico Gómez Galindo:** Conceptualization, Project administration, Writing – original draft. **Radha Sivarajan Sajeevan:** Conceptualization, Funding acquisition, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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