Master Degree Project in Food Engineering, Nutrition and Food Chemistry

Exploring the effect of pulsed electric field on the growth and stress tolerance of oat seedlings

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Popular Science Abstract

Applying a non-thermal, non-chemical, and environmental friendly technology to promote growth and stress tolerance in germinated oat seeds.

Oats are nutritious crops that can be added to gluten-free diets and provide numerous health benefits. However, environmental stresses like drought and salinity are affecting grain productivity, which includes oat production. Especially drought stress, as oats require large amounts of water during growth period. In this thesis work, Pulsed Electric Field (PEF) was applied to oat seeds to promote the growth and stress tolerance of the oat plant. PEF is a non-thermal technology that was used to apply short, high voltage electrical pulses to oat seeds at different stages of germination that were placed between two electrodes. The effect of PEF on the oat seeds can either be reversible or irreversible. If the cells are destroyed, it is an irreversible permeabilization. And if the cells recover after PEF treatment, it is a reversible permeabilization. In this thesis work, the desired outcome was the reversible permeabilization. Starting with 15 different PEF protocols and narrowing it down to the "best" PEF protocol was possible as the selected PEF protocol did significantly promote the growth of germinated oat seeds. The selected PEF protocol was applied to oat seeds at three different stages of germination for growth experiments and additionally to expose the PEF treated germinated seeds to salinity and drought stress. Different activities on molecular, biochemical and physiological levels occur at different germination and growth stages. For example, during the germination phase (phase 1 of growth), the dry oat seed tries to absorb as much water as possible so that the roots and shoots can grow and break the seed coat. Therefore, the main research question was: does PEF treatment at different levels of oat seed germination have different impacts on the development of the oat seeds? The results from this thesis work showed that treating oat seeds at different stages of germination with the same PEF protocol had different outcomes and it depended on the germination stage. For growth experiments in water and nutrients, it was observed that PEF decreased the growth of oat seeds at early stages of germination, whereas treating seeds at later stages of development did promote the growth. As for growth experiments with stress, there was also a difference in the results, which depended on the growth stage of the seeds when treated with PEF. Therefore, PEF is a promising non-chemical technology to promote oat growth and resistance towards stresses; however, PEF parameters adjustment and germination stage are two main factors affecting the effect of PEF.

Abstract

Plants are exposed to harsh environments due to climate changes, which lead to reduction in productivity and yield of the crops. In order to promote stress tolerance in plants, several chemical, microbial and pulsed power methods are applied in agriculture. Pulsed Electric Field (PEF) is an alternative non-chemical and environmentally friendly technology that can be applied to promote the growth of crops. This study is a systematic study investigating the effect of 15 different PEF protocols on the development of germinated oat seeds. To the best of our knowledge, this is the first systematic study on the effect of PEF on oat seeds. One PEF protocol was selected among the 15 PEF protocols which showed a significant promotion in oat seed germination and seedling development ($p<0.05$). The PEF parameters for the selected PEF protocol were E = 2.25 kV/cm, t = 10 µs and n = 99 pulses. The selected PEF protocol was applied to oat seeds at three different stages of germination to investigate the effect of this PEF protocol on oat seeds at different germination stages. Seeds at stage-1 of germination had no roots or shoots, seeds at stage-2 of germination had only one root and seeds at stage-3 of germination had both roots and shoots when treated with PEF. Three different growth experiments were performed: growth without stress, growth with salinity stress and growth with drought stress. The results from the growth experiments without stress showed a significant decrease in root growth for stage-1, no significant difference for stage-2, and a significant increase in root and shoot growth for stage-3 of germination ($p<0.05$). As for germinated oat seeds exposed to drought stress, a significant decrease in growth of seeds at stage-1 was observed, and no significant differences appeared at stage-2 and stage-3 of germination ($p<0.05$). And for growth in salinity stress, the results showed no significant differences at stage-1, a significant increase in shoot weight at stage-2 and a significant promotion of shoot growth for stage-3 ($p<0.05$). The results from this thesis work shows that the PEF protocol applied and the germination stage of the seeds have to be investigated to find the right PEF parameters and germination stage.

Acknowledgments

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Contents

List of Figures

List of Tables

List of Abbreviations

ABA Abscisic acid

AC Alternating Current

ATP Adenosine triphosphate

CO2 Carbon dioxide

DC Direct Electric Current

DRW Dry root weight

DSW Dry shoot weight

E Electrical field strength

ETR Electron transfer rate

FRW Fresh root weight

FSW Fresh shoot weight

GA3 Gibberellic acid

MDA Malondialdehyde

NaCl Sodium chloride

NADPH Nicotinamide adenine dinucleotide phosphate

nsPEF Nanosecond PEF

N No. of pulses

PEF Pulsed Electric Field

t Pulse width

PGPRs Plant-growth-promoting rhizobacteria

PS II Photosystem II

ROS Reactive oxygen species

RH Relative humidity

RL Root length

SL Shoot length

SOD Superoxide dismutase

SLU Swedish University of Agricultural Sciences

1 Introduction

Climate change is posing a severe threat to the ecosystem and agriculture which has resulted in land plants growing in a harsh environment. This includes different environmental factors (abiotic stresses) like high salinity, ultraviolet radiation, heavy metals, drought and high or low temperatures (He *et al.*, 2018). World food production is dependent on cereals, but the agricultural production of cereals is greatly affected by stresses, especially salinity and drought stress (Daryanto *et al.*, 2016). More than 20% of cultivated land was affected by salinity stress in 2014 (Gupta & Huang, 2014), and it is one of the primary threats to agricultural production. Most plants cannot survive if the Sodium chloride (NaCl) concentration exceeds 200 mM (He *et al.*, 2018). However, the yield loss of crops varies depending on the cultivar. Data from field work experiments showed that a yield loss of 21% in wheat and 39% in maize occurred when water was reduced by approximately 40% (Daryanto *et al.*, 2016). By 2100 it is expected that drought intensity will increase by 1% to 30% (Xie *et al.*, 2021). Despite the ongoing effort by breeders to develop stress-tolerant crops, these are still not meeting the demand of food security (Hu & Xiong, 2014).

Oat (*Avena sativa* L.) is an important global cereal crop that is high in protein and oil. It ranks sixth in world grain production (Kamal *et al.*, 2022). It is expected that the global oat market will continue to grow and by 2027 reach $9.46 billion (Oats global market report 2023, 2023). Oat has a low carbon footprint, it can potentially replace animal-based food products and it can be added safely to gluten-free diets (Kamal *et al.*, 2022). Due to a growing awareness of the importance of consumption of functional foods, an interest in new food products containing oats is increasing (Strychar, 2011). However, despite being well adapted to a wide range of soil types (Sanchez-martin *et al.*, 2015), the production of oats is affected by climate change conditions like drought and salinity (Strychar, 2011). For example, oats have a high water requirement compared to other cereals (other than rice), which makes lack of water one of the main abiotic stresses affecting the production of oats (Valentine, 2011).

Different methods are used to improve growth and stress tolerance of crops, which includes the use of chemicals, microbes and pulsed power technologies. A study by Zhang *et al.*, (2022a) exposed naked oat seedlings to drought stress and observed that melatonin application did promote growth. Microorganisms, like the Plant-growth-promoting rhizobacteria (PGPRs), are also applied to improve crop growth and tolerance, for example to alleviate drought stress (Mathur & Roy, 2021). However, electrotechnologies can also find useful implementations to promote plant growth or enhance plant

tolerance towards abiotic stresses (Takaki *et al.*, 2021). These technologies are non-chemical, non-thermal and environmentally friendly. It includes the application of pulsed electric field (PEF) (Demir *et al.*, 2023). Ahmed *et al.* (2020) applied PEF to wheat (*Tritium aestivum* L.) seeds before water imbibition (electric field strength: 2-6 kV/cm and the number of pulses: 25 and 50) and observed that water absorption was enhanced which resulted in a faster germination and an increase in the content of antioxidant compounds in the resultant seedlings (Ahmed *et al.*, 2020). PEF treatments have also shown to promote germination and seedling rates, improve vigor and induce cold and salt stress tolerance of wheat seeds (Akdemir Evrendilek *et al.*, 2021).

The application of PEF has a high potential to induce seed germination, seedling growth and improve stress tolerance of crops. When applying PEF, processes on biochemical, physiological, cellular and molecular levels are regulated (Dannehl, 2018). For example, the application of PEF induces stress which increases endogenous production of secondary metabolites by the tissues, such as polyphenols and carotenoids (Demir *et al.*, 2023). Among the different electrotechnologies that have been tested on seeds and seedlings, PEF is the one that has more potential on an industrial scale.

To the best of our knowledge, no systematic study has been done to test the effect of PEF on oat seeds at different stages of germination. This study examines different PEF protocols with increasing field strength on oat seeds, which is the most common practice to determine the boundaries for a specific system (Demir *et al.*, 2023).

2 Aim of the study

The overall aim of this study is to assess the impact of PEF on growth, drought and salinity stress tolerance of oat seeds at different stages of germination. The specific aims of this study are:

(I) Evaluate the growth of oat seeds treated with 15 different PEF protocols.

(II) Determine the best PEF protocol to be used for treatment of oat seeds at three different stages of germination for growth experiments.

(III) Expose PEF treated oat seeds at three different stages of germination to drought and salinity stress to examine whether the PEF treatments will improve stress tolerance in oat seeds.

3 Theoretical foundation

3.1 Oats

Oat (*Avena sativa* L.) is a global annual crop that ranks sixth in world grain production (Kamal *et al.*, 2022). It is a member of the economically important grass family Gramineae, that includes barley, rice, wheat, maize, sugarcane, sorghum and common millet. Oat is mainly grown for livestock feed, however, it is also used in the production of human food products like oatmeal, oat bran, oat flakes and oat flour (Paudel *et al.*, 2021).

3.1.1 Nutrient composition and health benefits

Oat is a good and well-balanced source of carbohydrates, lipids, minerals, vitamins, phytochemicals and quality proteins. It is the cereal-grain crop with the highest protein content. Oats contain four times more fat, one-third more protein and less starch, compared to wheat (Strychar, 2011). Table 1 shows the nutrient composition of oats (Rasane *et al.*, 2013). Beta-glucan and the powerful antioxidants in oats are especially attracting interest (Klose & Arendt, 2012; Tian *et al.*, 2010). Studies in animals and humans have revealed that oats and other grains that have a high content of soluble fibers are effective in lowering blood cholesterol levels. Other epidemiological and observational studies have reported that whole-grain diets may protect against type 2 diabetes, cardiovascular disease, and hypertension (Behall, 2011). Furthermore, after revealing the mosaic oat genome, it was confirmed that oats can be added to gluten-free diets (Kamal *et al.*, 2022).

Table 1 **Nutrient Composition of Oats (from Rasane *et al.*, 2013).**

Component	Availability in oats
Starch	60 %
Protein	11-15%
Lipid	5-9 %
Dietary fibers	2.3-8.5 %
Phenolic compounds	5.7 %
Trace minerals	0.54 %
Vitamins	Riboflavin 0.001 % Niacin 0.032 %

3.1.2 Oat grain structure

Oats are monocotyledons that have only one cotyledon or seed-leaf. The root systems of oats are fibrous, not relying on a dominant taproot (shown on figure 1). Oats (and grasses in general) produce single seed fruits, called caryopsis or grain, and each one of the fruits is formed from a single carpel (White, 1995).

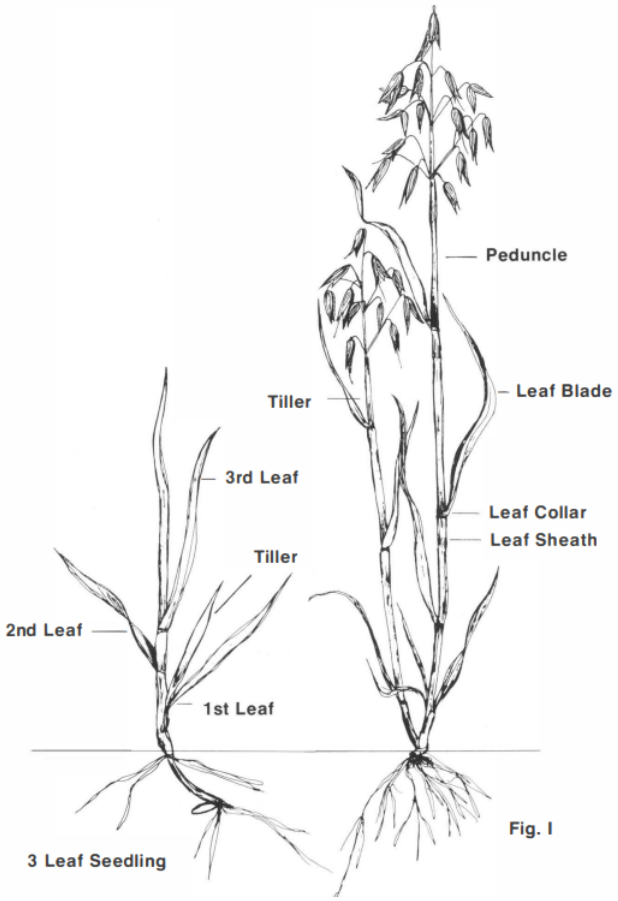

Figure 1 **Young oat seedling and mature oat plant.** A representative illustration of a young oat seedling to the left and a mature oat plant to the right showing the development of an oat plant. The illustration is from Revess *et al.*, 1976.

Figure 2 shows the composition of an oat grain (Zwer, 2010). The hull contains different tissues (photosynthetic and vascular tissues) during early development of the seed, where it contributes to the nutrition of the grain. The bran is the outer layer of the grain that contains minerals, vitamins, phytate and the antioxidant activities of the kernel (Zwer, 2010; Miller & Fulcher, 2011).

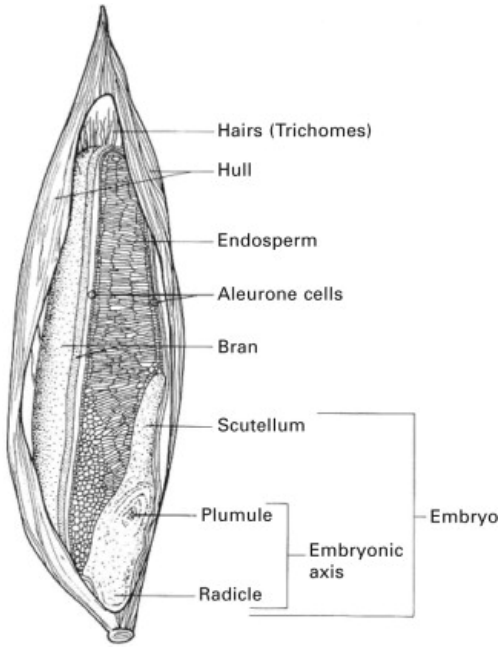

Figure 2 **Composition of an oat kernel.** A representative illustration of an oat kernel. The illustration is from Zwer, 2010.

As for a viable seed to develop and become a new plant, it is important that the seed kernel contains (Zwer, 2010; Miller & Fulcher, 2011):

(I) Quiescent vegetative tissue, which is the embryo, that generates new roots and shoots during the germination processes. It is made up of the embryonic axis and the scutellum and is capable of metabolic activity. The embryonic axis is made up of coleorhiza and coleoptile that contains rudimentary leaves and roots. The embryonic axis is attached to the scutellum that is composed of two distinct tissues: parenchyma (function as a nutrient storehouse) and epithelium.

(II) Nutrients that play a role during the first days of growth when the young leaves and roots establish absorptive and photosynthetic functions. The starchy endosperm is the largest tissue in oats and constitutes up to 70% of the weight of a mature oat grain. It is the major food reserve for the emerging radical during germination and contains starch, protein, lipid and beta-glucan, which are hydrolysed during germination.

(III) Mechanisms to release and transport these nutrients from their storage in a soluble form to reach the germinating embryo. Aleurone cells make up a layer with a thickness ranging from 50 to 150 µm. Enzymes derived from the aleurone layer are responsible for hydrolyzing macromolecules found in the starchy endosperm during oat seed germination.

3.1.3 Germination of oat seeds

The growth and development of oat plants is divided into 9 stages, starting with germination and ending with ripening (Revess *et al.*, 1976). Only seed germination, which is an important stage in the life of plants, will be considered in this thesis work (Demir *et al.*, 2023). Figure 3 shows some important changes that are associated with germination of seeds (Bewley *et al.*, 2013).

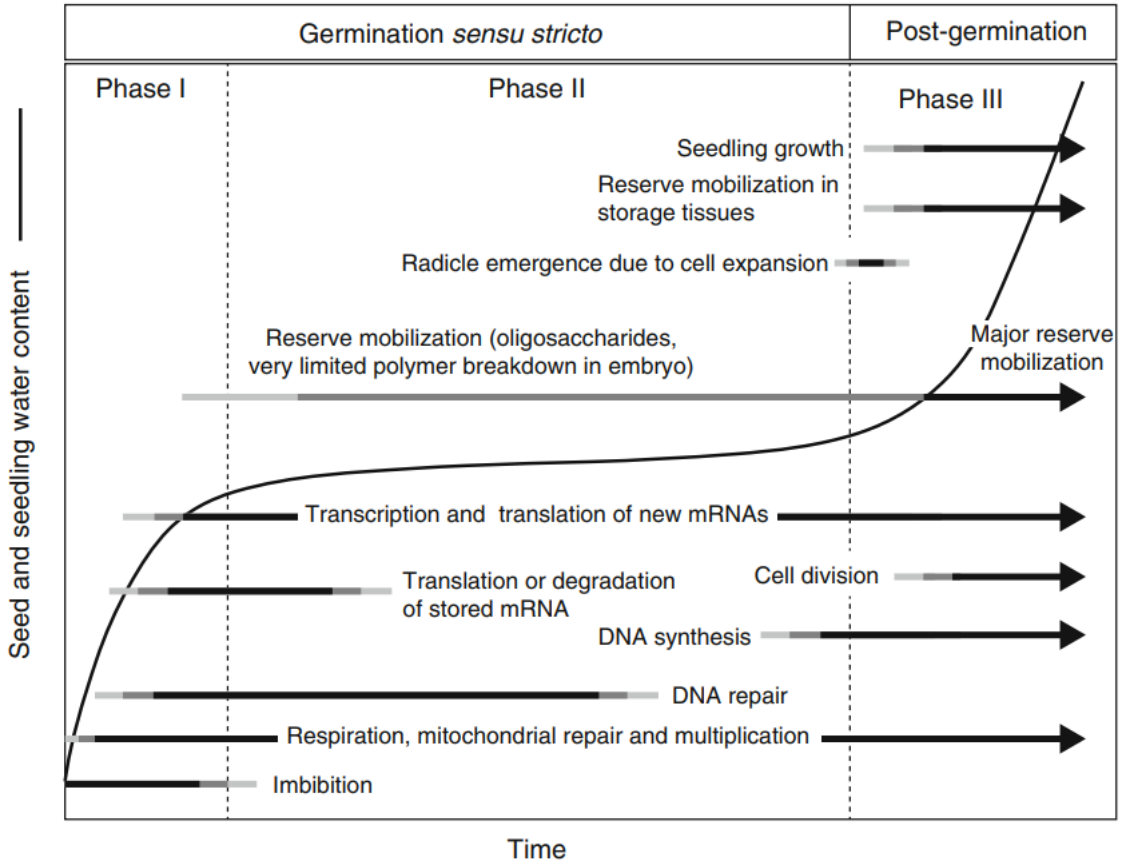

Figure 3 **Seed Germination.** Some important changes that appear during germination of seeds which are divided into three phases. The illustration is from Bewley *et al.*, 2013.

The first phase of germination is water imbibition by the quiescent oat seed, which is the resting seed with low moisture content (5-15%). It needs to uptake water to induce metabolic activities in the seed that are at standstill before water imbibition. When water is absorbed into the seed and the cell wall and cellular components become hydrated, the rate of water uptake will slowly decrease and the seed will enter the second phase, known as the lag phase (Bewley *et al.*, 2013). During the lag phase, major metabolic activities take place, which was also shown in a study by Tian *et al.* (2009) that investigated the physicochemical changes of oat seeds during germination. Some

of the complex cellular activities that appear are storage material breakdown, mitochondrial repair, DNA repair, cellular integrity restoration, respiration initiation, and synthesis of mRNA or proteins that are associated with germination. However, some of these events, like reestablishment of cellular activities, appear as the cell needs to recover from the damage caused by drying and oxidation. It can be difficult to distinguish between events that are directly linked to germination completion and activities for cellular repair. The third phase of germination is the completion phase, where the growth of the embryo into a seedling is initiated. Thus, the chemical composition and metabolic activity of oat seeds depends on the stage of germination.

3.2 Agricultural production

3.2.1 Environmental factors affecting agricultural production

Abiotic stresses (environmental stresses) including low or high temperatures, high salinity, ultraviolet radiation, heavy metals and drought have in recent years significantly reduced agricultural production and crop yields (Chen *et al.*, 2020). Drought and salinity are two of the main abiotic stresses that limit growth, development and productivity of plants and thereby threatening food security (Gupta & Huang, 2014; Jia, 2002). Estimates indicate that 32-69% of oat grain yield is lost due to drought stress (Tian *et al.*, 2022) and more than 20% of cultivated land was affected by salinity stress in 2014 (Gupta & Huang, 2014). Plants must be able to sense these environmental stresses and respond to them rapidly in order to cope with the climate change conditions.

Compared to other cereal crops (other than rice), oats have a higher water requirement for growth (Behall, 2011). Studies have revealed that the shortage of water damages physiological metabolism and photosynthesis in oats by decreasing intercellular Carbon dioxide (CO2) content, photosynthetic rate and stomatal conductance. Under water shortage, the efficiency of light use and capacity of photosynthesis by the leaves decrease, which decreases the overall performance of Photosystem II (PS II). As leaves are the primary photosynthetic organ and PS II is the most sensitive protein complex to leaf damage, water stress also results in a decrease in the accumulation of dry matter (Zhang *et al.*, 2022b). Also, the production of Reactive oxygen species (ROS) is increased, which causes oxidative damage to DNA, proteins and lipids and can results in cell death (Konieczna *et al.*, 2023).

The main form of salt that is present in soil is NaCl. During salinity stress, the capacity of the roots to

absorb water decreases and the leaves lose water due to osmotic stress caused by high salt accumulation in the plant and soil. Salt stress can cause cell dehydration, decrease stomatal conductance, and reduce carbon assimilation effectiveness due to the absorption of toxic ions by the roots (Bai *et al.*, 2018). Often, high salt concentrations induce oxidative stress and ion imbalance in plants which is associated with overproduction of ROS. To achieve salt tolerance in plants, growth control activities, homeostasis and detoxification are required (Akdemir *et al.*, 2021).

3.2.2 Improving agricultural production

Different chemicals, microbes and electrostimulation technologies have been identified to improve agricultural production. A study by Zhang *et al.*, (2022a) did apply melatonin to alleviate drought damage to naked oat (hulless oats) seedlings, due to drought being critical during the seedling stage. Melatonin, a phytohormone, is naturally found in plants and is involved in plant growth regulation and reduces environmental stress. By spraying melatonin on seedlings, an alleviation of the decline of growth caused by drought stress was observed. Melatonin has also been used to reduce salt stress damage in cotton seeds by regulating Abscisic acid (ABA) and Gibberellic acid (GA3) related genes and promoting seed germination (Chen *et al.*, 2021). Several microorganisms are used in agricultural production to produce food crops. PGPRs is one example that enhance production of secondary metabolites and induce the expression of plant-specific genes, which improve the tolerance of the crop plants and make the application of PGPRs an effective way to alleviate for example drought stress (Mathur & Roy, 2021). However, as the demand for chemical-free and more sustainable technologies are increasing, electrostimulation technologies may pose a potential to be an alternative technology in agricultural production (Takaki *et al.*, 2021). Different types of electrostimulation technologies can be applied, like magnetic fields and electric fields including Alternating Current (AC) electrical field, Direct Electric Current (DC) electric field and PEF (Dannehl, 2018).

Li *et al.* (2019) did examine the effect of exogenous electrical stimulation on seed germination, seedling growth, and the thermotolerance of maize seeds imbibed in water for 12h. The water imbibed maize seeds were sown in trays with filter papers, where the ends of two wires were linked to an electrophoresis apparatus and the other ends were inserted into the trays to touch the filter paper. They observed that electrical stimulation did increase the germination rate of maize seeds and seedling growth (the significant level was 22 V for 10 minutes). However, an intensity of electrical

stimulation performed at 22 V for 15 minutes did reduce germination rate and decreased seedling growth. Therefore, it is important to strictly control electric signaling parameters to reach the desired improvement in seed germination, plant growth or tolerance towards stresses. Further experiments did confirm the interaction between electrical signaling and calcium and ROS signaling. Electrical stimulation did activate ion channels which triggered calcium and ROS signaling and increased catalase, peroxidase, and carbon metabolism enzymes. It did also increase the uptake of nutrients like magnesium, calcium, and potassium. This could explain the boost in seed germination and seedling growth (Li *et al.*, 2019). Additionally, studies have shown that magnetic fields do also impact photosynthesis in plants. Anand *et al.* (2012) did observe that pre-treating corn seeds with magnetic flux density of 100 mT for 2h did increase photosynthesis and stomatal conductance (Anand *et al.*, 2012).

However, among the different electrotechnologies that have been tested on seeds and seedlings, PEF is the one that has more potential on an industrial scale. A study by Eing *et al.* (2009) did apply Nanosecond PEF (nsPEF) to seven days old seedlings of Arabidopsis thaliana. They reported growth stimulation, which they assume was a stress response towards nsPEF exposure. Akdemir *et al.* (2021) investigated the effect of PEF with the energy range of 1.07-17.28 J on wheat grains and observed vigor improvement, cold and salt tolerance promotion and inactivation of surface microflora. For more studies on the effect of PEF on seed germination, read the review by Edmir *et al.* (2023).

3.3 Pulsed Electric Field

PEF is a non-thermal technology that applies short (µs-ms), high voltage electrical pulses to materials placed in a chamber between two rektangular shaped electrodes. The intense PEF is produced when short-duration pulses are applied (Dannehl, 2018). When applying an external electric field to a cell, it induces the transmembrane potential which disrupts the cell membrane if it reaches a critical value of approximately 1 V for a bimolecular lipid membrane (Aronsson *et al.*, 2001). The electromechanical model introduced by Zimmerman *et al.* (1974) is the most accepted theory explaining what happens at cellular level when an external electric field causes the cell membrane to break down (Soliva-Fortuny *et al.*, 2009; Zimmermann *et al.*, 1974). The cell membrane is considered as a capacitor that has a low dielectric constant with free charges of opposite polarities present internally and externally in the cell. This results in a naturally occurring transmembrane potential. The increase

of transmembrane potential is induced by accumulation of internal and external charges across the membrane. Membrane thinning is also induced due to attraction between the opposite charges on both sides of the membrane which raises compression pressure on the cell membrane. Due to membrane thinning, electrostatic attraction between the opposite charges is further increased which results in membrane permeabilization and hydrophilic pore formation. The structural changes in the plasma membrane cause temporary disturbance of cellular homeostasis which lead to molecular transport across the membrane (Demir *et al.*, 2023; Soliva-Fortuny *et al.*, 2009).

The application of PEF leads either to reversible or irreversible electroporation. If the cell recovers and survives the PEF treatment by resealing the created pores in the membrane and restoring homeostatic disturbance, it is termed reversible electroporation. This is reached when the applied electric field is below a critical threshold. However, if it is above the electrical threshold, the cell cannot recover the homeostatic disturbance and leads to cell death. This is termed irreversible electroporation. What determines the reversibility of the PEF treatment is two things: the PEF protocol applied (which include the parameters such as pulse shape, pulse width, number of pulses, burst numbers, duration between bursts and electric field strength) and the cell characteristics (shape, size and cytoplasm conductivity) (Demir *et al.*, 2023; Miklavcic & Davalos, 2015). The common way to determine the critical electric field is to perform a systematic study with a series of experiments with increasing electric field strengths to find the boundaries of PEF protocols. Figure 4 shows how PEF treatment can either lead to reversible or irreversible electroporation (Demir *et al.*, 2023).

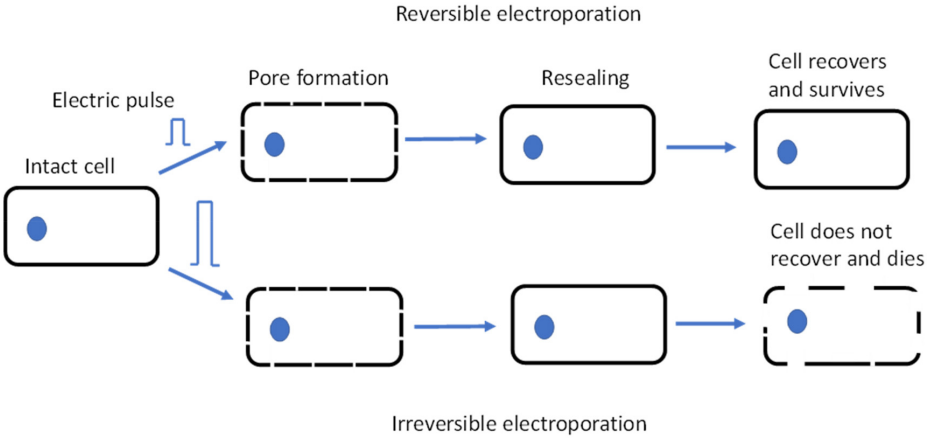

Figure 4 **Reversibility of PEF treatment.** A Schematic representation of the mechanism of PEF that leads to either reversible or irreversible electroporation. The illustration is from Demir *et al.*, 2023.

4 Experimental Overview

This study is divided into three parts. First part is the preliminary experiments. The aim of preliminary germination experiments was to establish the most proper conditions for germination and to determine if pre-treatments promote germination. Preliminary salinity experiment was performed to determine the concentration of NaCl that will be used in the third part of the experimental work. The second part is growth experiments without stress, which includes the systematic study that evaluates 15 different PEF protocols on oat seed germination and seedling growth. The third part is treating germinated oat seeds at three different stages of germination with the selected PEF protocol to determine the tolerance of PEF treated oat seeds towards salinity and drought stress. The experimental overview is shown on figure 5.

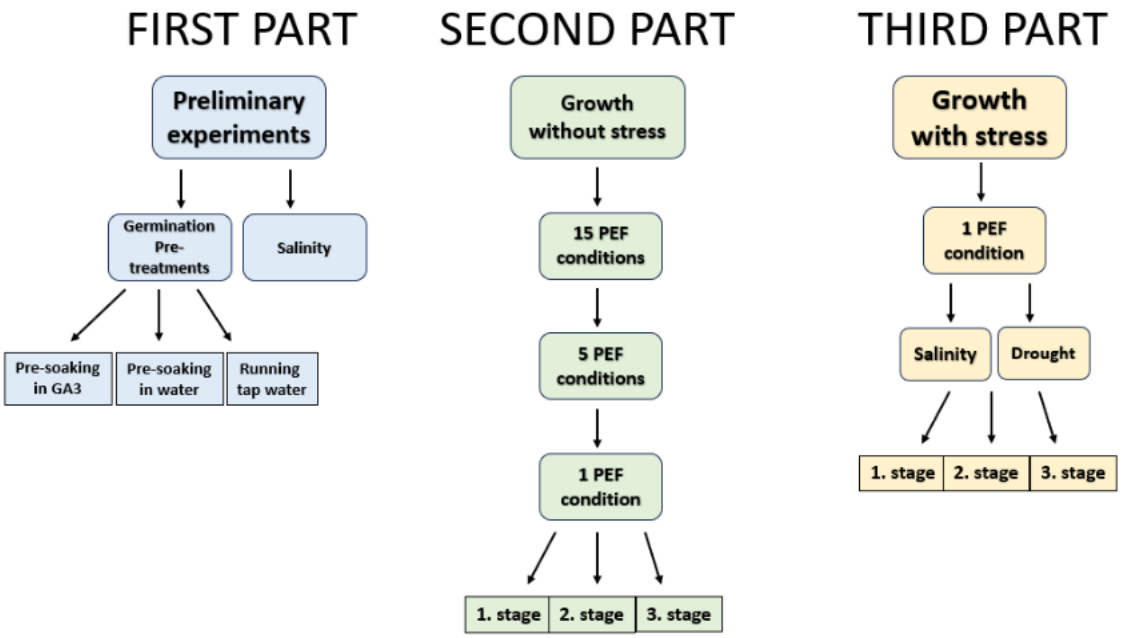

Figure 5 **Experimental Overview.** The experimental work is divided into three parts: preliminary experiments and growth experiments with and without stress.

5 Materials and Methods

5.1 Source of oat seeds

Dry oat seeds were provided by Lantmännen, Svalöv, Sweden. Galant is the name of the cultivar used in this study. They reported that the germination rate was 90% (provided by Lantmännen). Dry oat seeds were provided in plastic bags and kept dry in the dark at room temperature.

5.2 First part: Preliminary experiments

5.2.1 Germination experiments

Several germination experiments were performed to determine if pre-treatment of oat seeds promotes an even germination. Germination conditions were tested according to a protocol provided by the company Lantmännen and the use of petri dishes with double-layer filter paper was implemented as described by several studies (Ghizlane, 2023; Bai et al., 2018). For all preliminary germination experiments, oat seeds (n=15) were spread on wetted filter papers in a petri dish (diameter = 8.5 cm), sealed with parafilm to prevent dehydration (figure 6). Petri dishes were placed in a growth chamber (Panasonic MLR-352 PE Climate Chamber) and pre-chilled at 10 °C, no light, and 60% Relative humidity (RH) for three days and then moved to another growth chamber with a 16h photoperiod, 20 °C and 60% RH. Water was added to keep the filter paper wet. Each germination experiment was performed in duplicates (n=30 for each treatment). The germination experiments were as following:

(I) No pre-treatments. Dry oat seeds were spread directly on wetted filter paper.

(II) Dry oat seeds were pre-soaked in water for six hours and spread on wetted filter paper.

(III) Dry oat seeds were exposed to running tap water for six hours and spread on wetted filter paper.

(IIII) Dry oat seeds were pre-soaked in different concentrations of GA3 (100, 250, 500 and 750 ppm) for 24h and spread on wetted filter paper.

Measurements were performed after six days of germination (Root length (RL), Shoot length (SL), number of roots and germination rate (%)).

Figure 6 **Germination of oat seeds in petri dishes.** Oat seeds were spread on wetted filter paper for germination.

5.2.2 Salinity experiment

To determine the tolerance of PEF treated germinated oat seeds towards salinity, the concentration of NaCl by which oat seeds leaves and roots were affected was determined. The experiment was performed on MS (½ strength; Murashige & Skoog Medium, including vitamins, Duchefa Biochemie, BH Haarlem, Nederlandene) agar plates. MS medium was prepared by adding 275 mg to 250 mL Milli-Q water, mixed and poured into five 50 mL bottles. The plant agar (0.8%; Duchefa Biochemie, BH Haarlem, Nederlandene) equaling 0.4 g was added to each bottle and boiled in the microwave. Four saline concentrations (100, 150, 200, and 250 mM) were created from a stock solution of 5 M NaCl, by adding the required volume of 5 M NaCl to each bottle, using the dilution formula for calculation:

$$c1 * v1 = c2 * v2$$

Bottles were mixed and poured into petri dishes. When the agar medium was solidified, 10 handpicked germinated seeds (uniform germination, having only roots) were inoculated in a row on the agar medium. The agar plates were sealed with parafilm and placed in a growth chamber with a 16h photoperiod, 20 °C and 60% RH. Each treatment condition was performed in duplicates (n=20 for each concentration). After six days of growth, measurements were performed (RL, SL, and number of roots).

5.3 Second part: Growth experiments without stress

After performing the preliminary growth experiments, dry oat seeds without pretreatment and the protocol provided by Lantmännen was chosen for all the coming growth experiments; with and without stress. Each treatment condition was performed in duplicates. Starting from scratch for each duplicate, where different batches of oat seeds were used. For each duplicate, 20 oat seeds were used.

5.3.1 Testing PEF protocols

The study by Grzelka *et al.*, (2023) that examined the effect of electroporation on the growth of the plant *Scutellaria baicalensis* Georgi did treat the plant with 15 different PEF protocols. These 15 PEF protocols are shown on figure 7. In this study, the same PEF protocols were applied on oat seeds. The germination was performed on petri-plates as explained in section 5.2.1. The 15 PEF protocols were applied on:

(I) Completely dry oat seeds.

(II) Oat seeds pre-chilled at 10°C, no light and 60% RH for three days and moved to 16h photoperiod, 20°C and 60% RH for one day. In total four days of water absorption.

Parameter	Group 1			Group 2			Group 3			Group 4			Group 5			Control
E [kV/cm]	1.25			1.75			2.25			3			5			–
t [µs]	10	25	100	10	25	100	10	25	100	10	25	100	10	25	100	–
N	100	40	10	100	40	10	100	40	10	100	40	10	100	40	10	–

E - electric field strength, t - duration of impulse, N - number of impulses delivered, - means no applied electric field (control plants).

Figure 7 **15 PEF protocols applied to oat seeds.** This table is from Grzelka *et al.* (2023) and was used in this study to treat oat seeds with the same 15 PEF protocols.

Dry oat seeds were placed in a dry tube to be transported to the company OptiCept in Lund, where the PEF treatments were performed. Water imbibed oat seeds were removed from the growth chamber at the Swedish University of Agricultural Sciences (SLU) and ensured that no dehydration occurred during transport, by adding more water to the petri dish to keep the filter paper wet. Untreated seeds were also brought to the company. At the company OptiCept, an electroporation chamber was filled with tap water (conductivity of 200 µs/cm) and the dry and water imbibed seeds were placed in the chamber, respectively. The controls were also placed into the chamber, but without applying electric pulse field. When closing the lid of the chamber, bubble formation was prevented. The width and

length of the chamber was 20 cm, respectively. The gap between the electrodes was 1.6 cm. The chamber was connected to an OEM generator (Arc Aroma Pure AB, Lund, Sweden) that delivered the pulses. This was done for each PEF condition shown on figure 7 for dry and water imbibed oat seeds, respectively. After each treatment, the seeds were directly transferred to a petri plate with wetted filter paper. After finishing all treatments, the seeds were transported back to SLU and placed in the growth chamber for germination with a 16h photoperiod, 20°C and 60% RH. In total 480 oat seeds were spread for germination in this experiment. After six days of germination, measurements were performed (RL, SL, number of roots and germination rate (%)).

5.3.2 First method development

For the coming experiments, 500 dry oat seeds were sorted and spread on wetted tissue papers in boxes, sealed with parafilm to prevent dehydration and placed in growth chambers for germination (Kaliniewicz & Tylek, 2019). Only healthy oat seeds were chosen when sorting and the choice was made on the size and color of the seed. This is due to the weight of oat seeds that varies a lot within one sample (Revess *et al.*, 1976). The definition of germinated oat seeds was changed to oat seeds being in stage-2 of germination. This is the stage where the roots just protrude the structure (figure 8). The germination duration was changed to eight days. Seeds at three different germination stages were handpicked from the 500 seeds to ensure an even germination under PEF treatments. The three germination stages were described as following and shown on figure 8:

(I) Seeds at stage-1 of germination were treated with PEF after three days of pre-chilling at 10 °C, no light and 60% RH and before moving to 16h photoperiod, 20 °C and 60% RH. The seeds had absorbed water. After PEF treatment, the seeds were placed in boxes again to continue germination at 16h photoperiod, 20 °C and 60% RH. When the roots emerged with a root length of 0.2 cm $\pm$ 0.1 cm, the germinated seeds were handpicked.

(II) Seeds at stage-2 of germination were treated with PEF after three days of pre-chilling and one to two days in 16h photoperiod, 20 °C and 60% RH. Germinated seeds were chosen based on roots with a root length of 0.2 cm $\pm$ 0.1 cm.

(III) Seeds at stage-3 of germination were handpicked after three days of pre-chilling and three to four days in 16h photoperiod, 20 °C and 60% RH. Germinated seeds were chosen based on shoot length with a protruding radicle of 0.4 cm $\pm$ 0.2 cm.

Figure 8 **Three different germination stages of oat seeds.** a) Stage-1 of germination, b) Stage-2 of germination, and c) Stage-3 of germination.

5.3.3 Narrowing down PEF protocols

Based on the results of the different measurements, from the 15 PEF protocols described in section 5.3.1, five PEF protocols were chosen (shown on table 2). Only seeds at stage-3 of germination were handpicked from the 500 sorted oat seeds. Seeds were transported to the company OptiCept and followed the same procedure explained in section 5.3.1, but only for the five PEF treatments shown on table 2. After PEF treatments, seeds were returned to petri dishes with wetted filter paper and continued germination in the growth chamber. In this experiment, 180 seeds were handpicked for PEF treatment (no PEF treatments applied to controls). After six days of germination, the measurements were performed (RL, SL, number of roots and germination rate (%)).

Table 2 **Five selected PEF protocols.**

-	**Unit**	**Gr 1**	**Gr 2**	**Gr 3**	**Gr 4**	**Gr 5**
Treatment Energy	kJ/kg	0.05	1.00	1.60	2.90	5.00
E-field (monopolar)	V/cm	1250	1750	2250	3000	5000
Pulse width	µs	25	25	10	10	25
Pulse per volume	Pulses	40	41	99	101	40
Frequency	Hz	5	5	13	13	5

5.3.4 Second method development

The method was developed further by using a simple hydroponic system instead of petri dishes. The same procedure for sorting and spreading 500 seeds on wetted tissue papers in boxed and handpicking of germinated seeds were followed, but after PEF treatment at the company OptiCept, the germinated seeds were placed in a small hydroponic system (except for seeds at stage-1 of germination, as these

seeds had to continue germination before handpicking and placing in hydroponics). The hydroponic system consisted of a plastic box, racks, and tips (figure 9). The plastic box was filled with a nutrient solution (1.1 g/L MS media), each seed was added to a plastic tip and placed in the rack in the box.

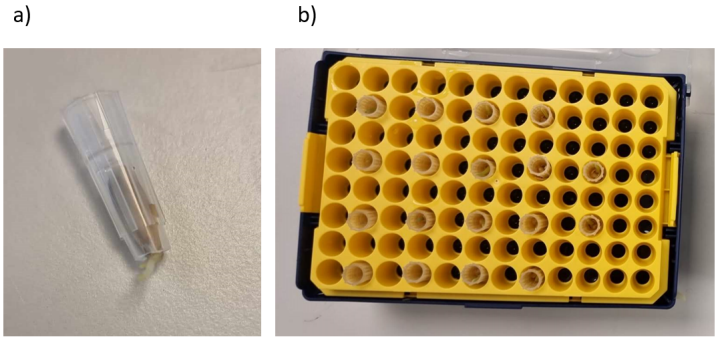

Figure 9 **A simple hydroponic system.** a) plastic tip that the germinated oat seeds were placed in after PEF treatments. In this photo, an oat seed at stage-2 of germination is shown. b) plastic box, plastic racks, plastic tips and germinated seeds.

5.3.5 Selected PEF protocol

Based on the results from the five PEF protocols described in section 5.3.3, one PEF protocol was selected, which is shown on table 3.

Table 3 **The selected PEF protocol.**

	Unit	**Gr 3**
Treatment Energy	kJ/kg	1.60
E-field (monopolar)	V/cm	2250
Pulse width	µs	10
Pulse per volume	Pulses	99
Frequency	Hz	13

Seeds were at stage-1, stage-2 and stage-3 of germination when treated with this PEF protocol. The hydroponic systems were placed in a growth chamber at 16h photoperiod, 20 °C and 60% RH. In this experiment, 240 seeds were handpicked for PEF treatment (controls were not treated with PEF) and placed in the hydroponic system. Eight days after PEF treatment, roots and shoots were separated for measurements. Fresh root weight (FRW), Fresh shoot weight (FSW), Dry root weight (DRW), Dry shoot weight (DSW), RL, SL and number of roots were measured. After fresh biomass measurements, roots and shoots were dried for 48h at 70°C for dry weight measurements.

5.4 Third part: Growth experiments with stress

The third part of the experiments was performed to determine oat tolerance towards drought and salinity. Seeds at the three different germination stages were treated with the selected PEF protocol (table 3) in the simple hydroponic system. In these experiments 480 oat seeds were handpicked, treated with PEF (except the controls) and placed in the hydroponic system. Eight days after PEF treatment, roots and shoots were separated for measurements. FRW, FSW, DRW, DSW, RL, SL and number of roots were measured. After fresh biomass measurements, the roots and shoots were dried for 48h at 70 °C for dry weight measurements.

5.4.1 Drought stress experiment

Polyethylene glycol 6000 (PEG-6000; 15%; Sigma–Aldrich, St. Louis, MO, United States) was prepared by adding 15 grams PEG-6000 to 1 L MS media (½ strength) and mixing on a magnetic mixer for 30 minutes (according to Gong *et al.*, 2022).

5.4.2 Salinity stress experiment

NaCl (150 mM) was prepared as explained in section 5.2.2, despite that 150 mM NaCl solution was poured into the hydroponic system instead of agar media.

5.5 Analysis

5.5.1 Measurements

Germination rate

Germination rate was calculated as following:

$$\text{Germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{No. of total seeds}} * 100$$

Fresh and dry weights

FSW and FRW were weighted after ensuring that no excess of liquid was attached to the roots (by using paper tissues). The roots were cut from the seed and the shoot was separated from the seed by

opening the seed kernel and removing the kernel and hull from the shoot. As for the DSW and DRW, the roots and shoots were removed from the dryer after ensuring that a constant weight was reached.

Root/shoot ratio

Root to shoot ratio was calculated from the DSW and DRW according to (Rogers *et al.*, 1995) as following:

$$\text{Root/shoot ratio} = \frac{\text{DRW}}{\text{DSW}}$$

Root and shoot lengths

RL was measured for the longest root. SL was measured for the primary shoot and not eventual tillers (see figure 10). However, as for the shoot length, the shoot was separated from the seed before the measurement.

5.5.2 Visual observations

Visual observations were performed to compare shoot length and width, root structure including the formation of secondary roots and the roots branches.

5.5.3 Statistical analysis

Data are presented as means ± standard deviations for replicates. Statistical analysis between the data from treated and non-treated germinated oat seeds was carried out using MS Excel by means of one-way ANOVA (significance level of $p<0.05$).

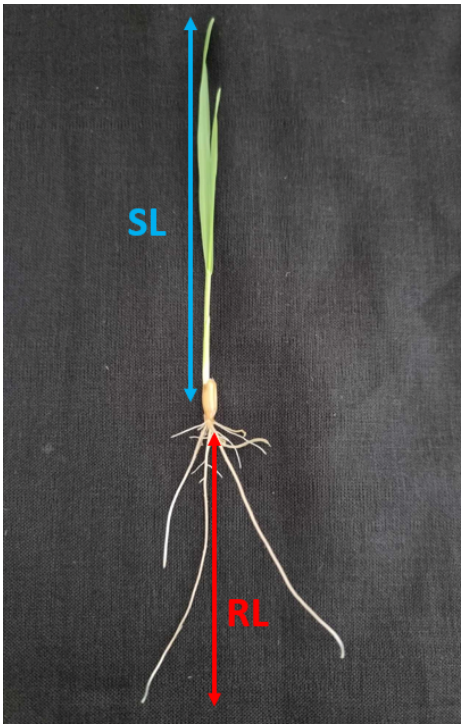

Figure 10 **Root and shoot length measurements.** An example of how the root and shoot lengths were measured.

6 Results

6.1 First part: Preliminary experiments

6.1.1 Germination experiments

Figure 11 shows the results from the germination experiments. Germination was reached when roots started penetrating the structure. A germination rate of 80% was observed for seeds pre-soaked in 100 ppm GA3 and 75% for dry oat seeds. Despite a significant difference between germination rates of dry seeds and seeds treated with 100 ppm GA3, it was decided not to apply any pretreatment for the next experiments, as GA3 did not promote a more evenly germination.

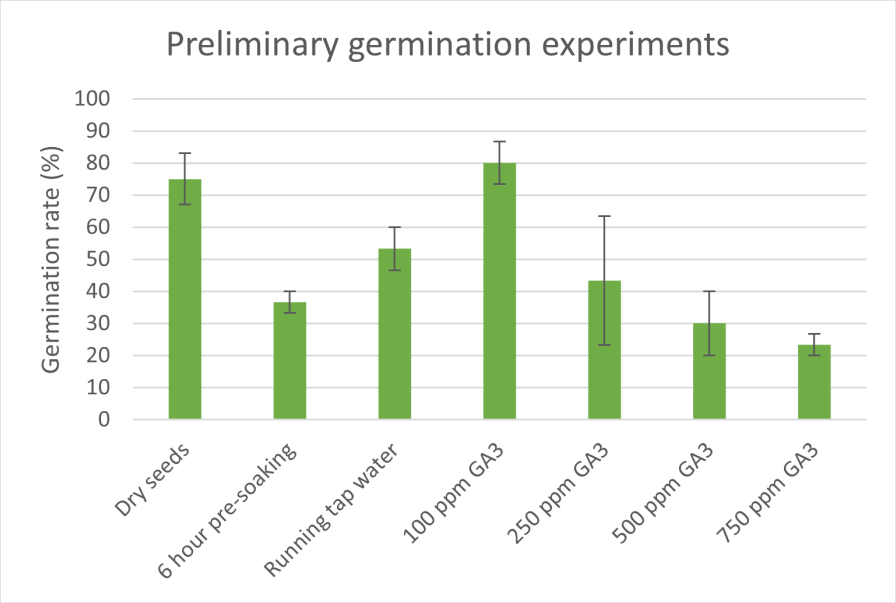

Figure 11 **Preliminary germination experiments results.** Results from the preliminary germination experiments showing the germination rate in percentage.

6.1.2 Salinity experiment

After exposure to NaCl for six days, the biomass of oat leaves and roots were affected by 50% at 150 mM. The growth started to reduce extensively by 200 mM (figure 12). Therefor, 150 mM NaCl was used for the growth experiment with salinity stress.

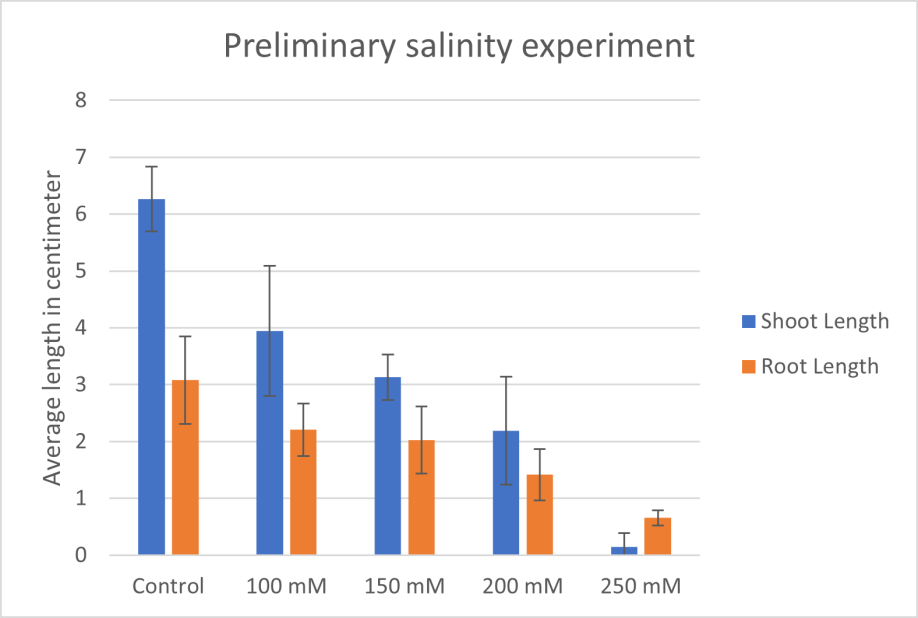

Figure 12 **Preliminary salinity experiment results.** Results from growing germinated oat seeds in four different NaCl concentrations.

6.2 Second part: Growth experiments without stress

6.2.1 Testing PEF protocols

In this experiment, completely dry oat seeds and water imbibed oat seeds were treated with 15 different PEF protocols. Figure 13 is a representative photo of dry oat seeds treated with PEF and figure 14 shows the results after six days of germination. An uneven germination was observed, which affected the results (see figure 13).

Figure 13 **Dry oat seeds treated with PEF.** A representative photo of the uneven germination of dry oat seeds treated with the following PEF protocol:E= 5 kV/cm, t=15 µs and N= 50 Pulses.

The uneven germination appeared for both dry and water imbibed oat seeds and PEF treatment did not promote an even germination. Therefore, based on the data (figure 14) and due to an uneven germination, one PEF protocol from each group (referred to as G1 to G5 in figure 14) was chosen for further investigation. The five selected PEF protocols (shown in section 5.3.3) had the "best" results within the respective group (regarding root and shoot lengths and germination rate). PEF protocols in one group share the same Electrical field strength (E) and treatment energy. What differs is No. of pulses (N) and Pulse width (t).

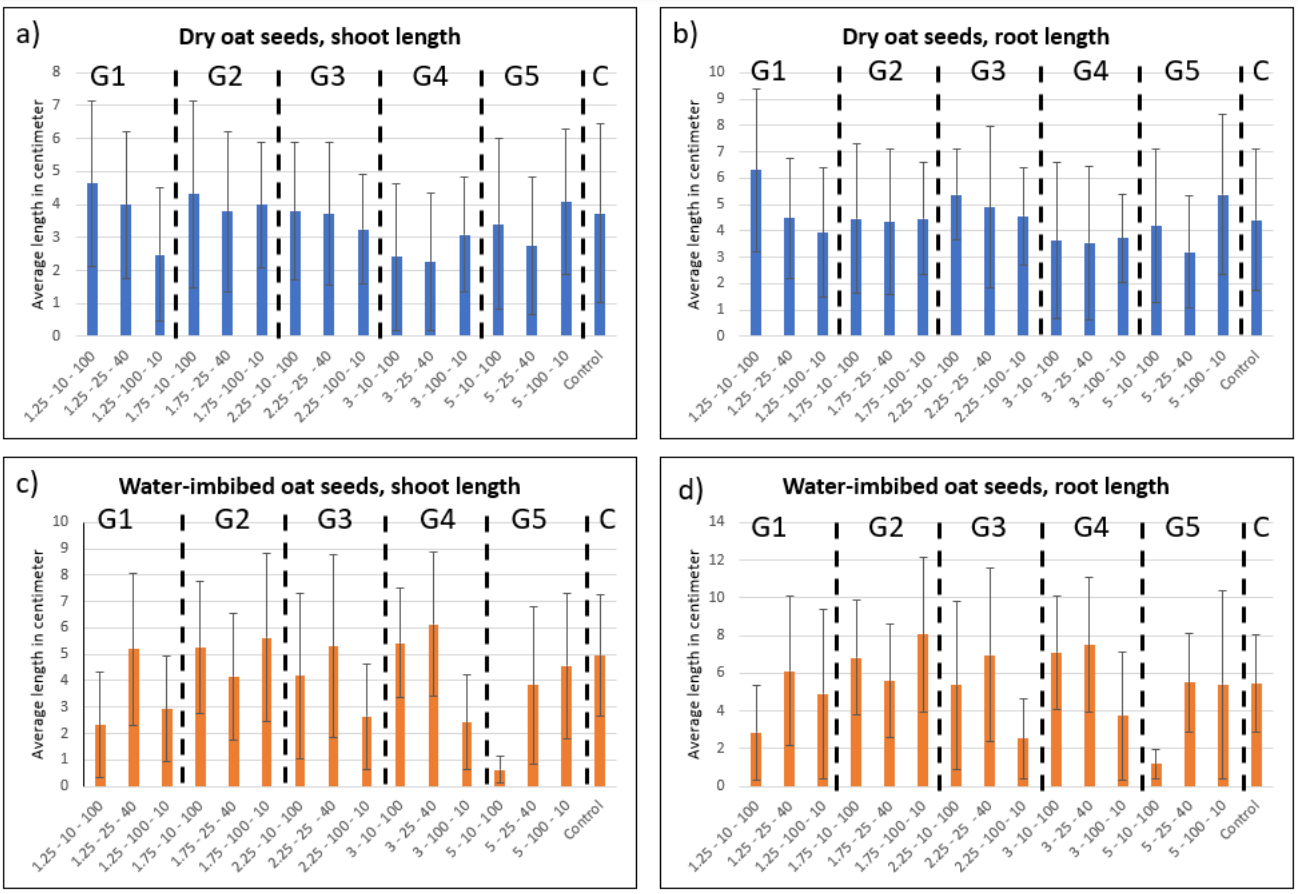

Figure 14 **Results from testing 15 PEF protocols.** The PEF protocols are defined as: Electric field (kV/cm) - Pulse width (µs) - Pulses per volume. a-b) RL and SL of dry oat seeds treated with 15 different PEF protocols after six days of germination. c-d) RL and SL of germinated oat seeds treated with 15 different PEF protocols after six days of germination. The 15 PEF protocols were divided into 5 groups, which are written as G1, G2, G3, G4 and G5 in the bar plots.

6.2.2 Narrowing down PEF protocols

For this experiment the seeds were at the stage-3 of germination when treated with PEF (selection was based on the shoot length). A more even germination and growth was obtained this time (figures 15

and 16). According to one-way ANOVA, there was a significant increase in SL and RL of germinated oat seeds treated with the following PEF protocol: E =2.25 V/cm, t =10 µs and N = 100 pulses ($p<0.05$). This PEF protocol was therefore chosen for the next experiments.

Figure 15 **PEF treated germinated oat seeds.** Oat seeds at stage-3 of germination treated with the following PEF protocol: E=2.250 kV/cm, t=25 µs and N=99 Pulses.

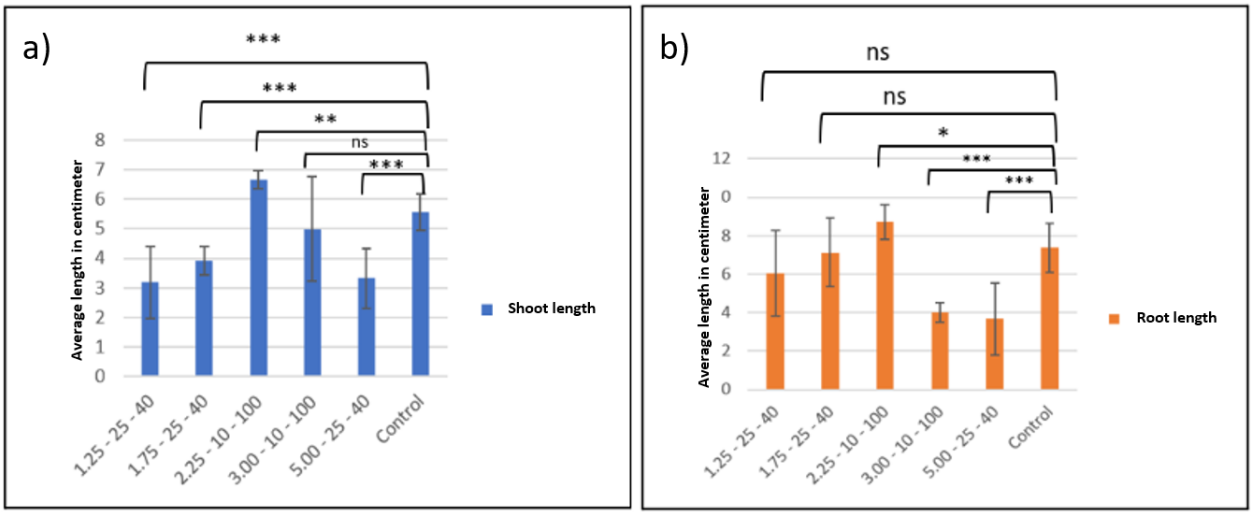

Figure 16 **Results from testing five PEF protocols.** Bar plots of a) shoot lengths and b) root lengths. The parameters of the PEF protocols are defined as: Electric field (kV/cm) - Pulse width (µs) - Pulses. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

6.2.3 Selected PEF protocol

The selected PEF protocol applied in the next experiments is mentioned in section 5.3.5. The results in this section are for germinated oat seeds at stage-1, stage-2 and stage-3 of germination after eight days of growth in hydroponics post PEF treatment. The visual observations show the shoot, roots and seedling development.

Stage-1 of germination According to one-way ANOVA, there was a significant reduction in RL and FRW of PEF treated seeds compared to the controls ($p<0.05$), which is shown on figures 17 and 18. The roots of the resultant seedlings from the seeds treated with PEF had shorter secondary roots compared with the controls and the controls had longer roots. Root/shoot ratio was calculated to 0.22 for the control and 0.20 for the PEF treated resultant seedlings from the germinated seeds.

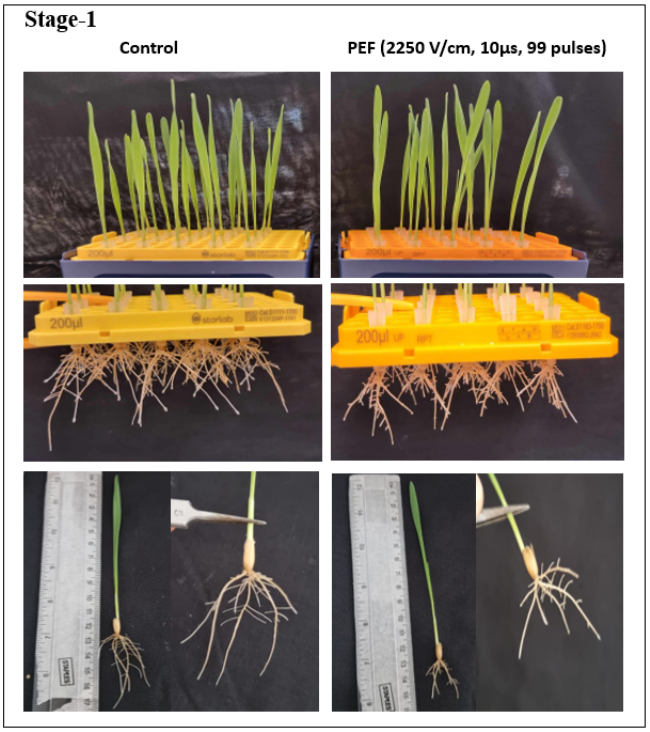

Figure 17 **Visual observations: Growth experiment without stress, stage-1 of germination.** Resultant seedlings from the controls and seeds at stage-1 of germination treated with the selected PEF protocol.

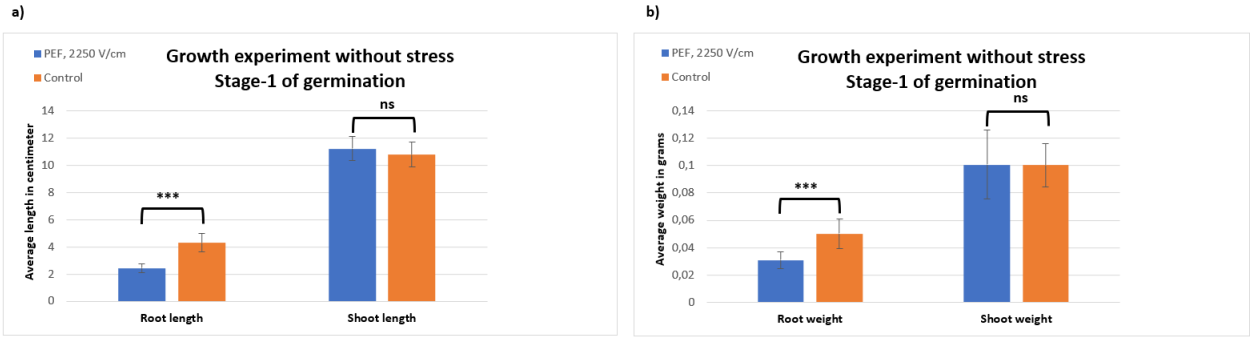

Figure 18 **Bar plots: Growth experiment without stress, stage-1 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-1 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

Stage-2 of germination There were no significant differences in RL, SL, FSW and FRW between the control and the resultant seedlings from the PEF treated seeds ($p<0.05$), which was also observed visually (figures 19 and 20). Both the control and the PEF treated seedlings had long secondary roots. Root/shoot ratio was calculated to 0.24 for the control and 0.22 for the resultant seedlings of the PEF treated germinated seeds.

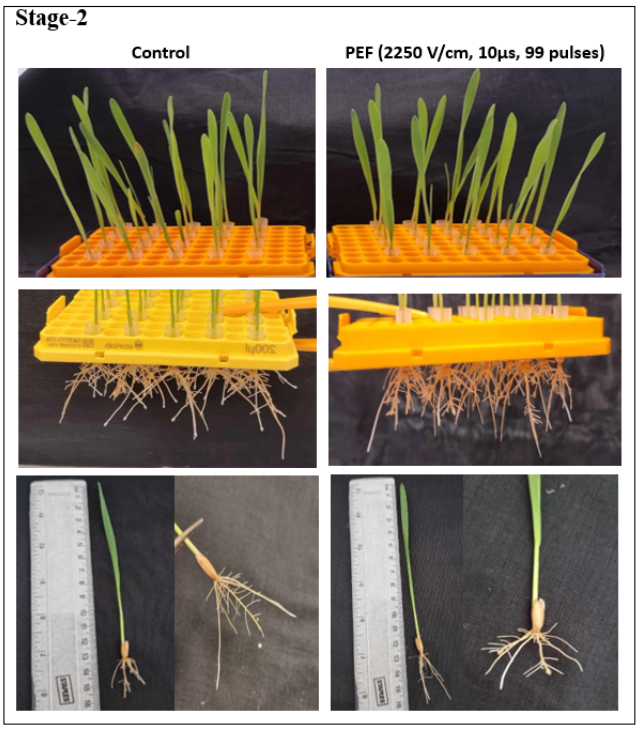

Figure 19 **Visual observations: Growth experiment without stress, stage-2 of germination.** Resultant seedlings from the controls and seeds at stage-2 of germination treated with the selected PEF protocol.

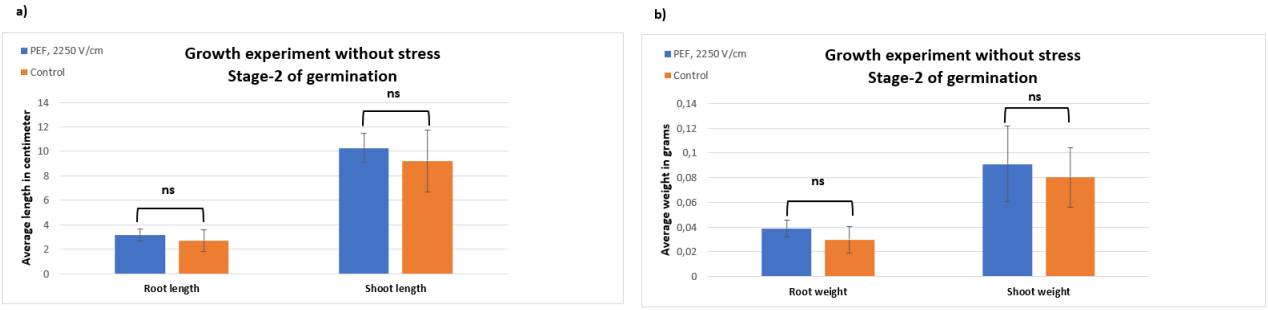

Figure 20 **Bar plots: Growth experiment without stress, stage-2 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-2 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

Stage-3 of germination There was a significant increase in RL, SL, FSW and FRW ($p<0.05$). The differences in SL was also observed visually (figures 21 and 22). The root/shoot ratios were calculated to 0.31 for the PEF treated germinated seeds and 0.30 for the control.

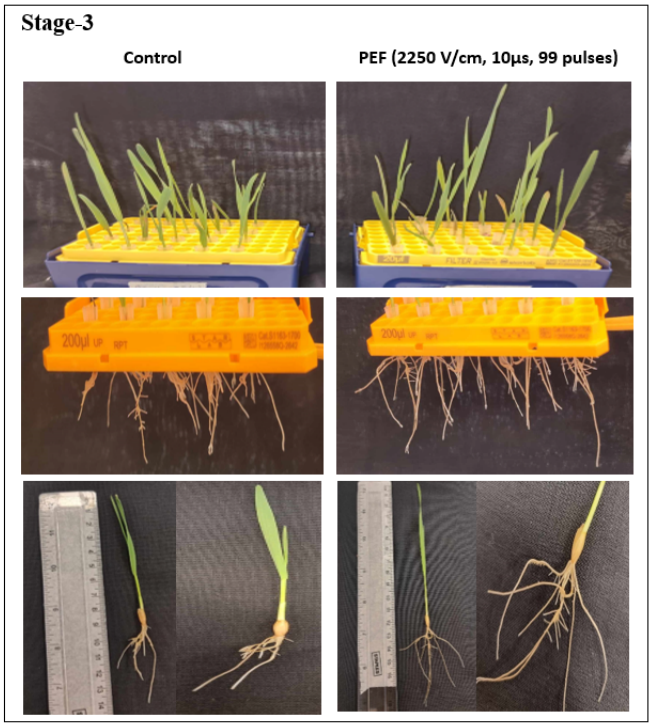

Figure 21 **Visual observations: Growth experiment without stress, stage-3 of germination.** Resultant seedlings from the controls and seeds at stage-3 of germination treated with the selected PEF protocol.

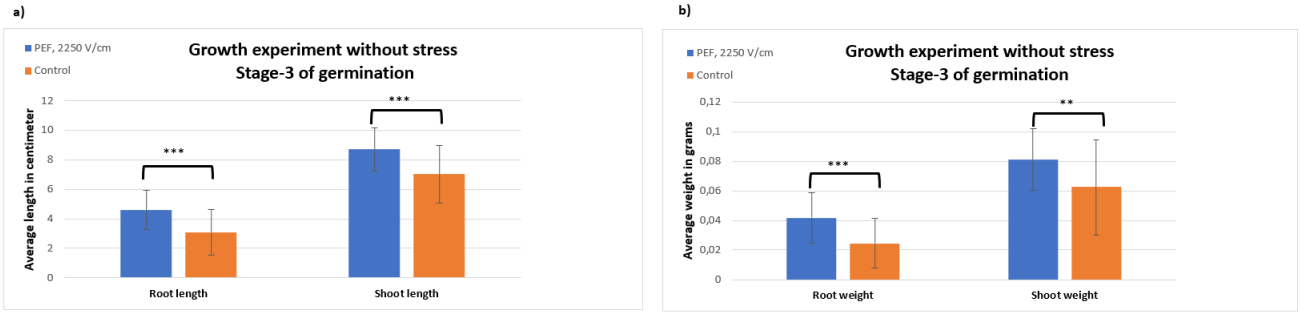

Figure 22 **Bar plots: Growth experiment without stress, stage-3 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-3 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

6.3 Third part: Growth experiments with stress

6.3.1 Drought stress experiments

Stage-1 of germination There were a significant reduction in the growth of PEF treated germinated oat seeds compared to the control ($p<0.05$). The roots and shoots of the PEF treated seedlings were thinner and shorter than the control (figures 23 and 24). The root/shoot ratio were calculated to 0.42 for control and 0.40 for PEF treated seedlings.

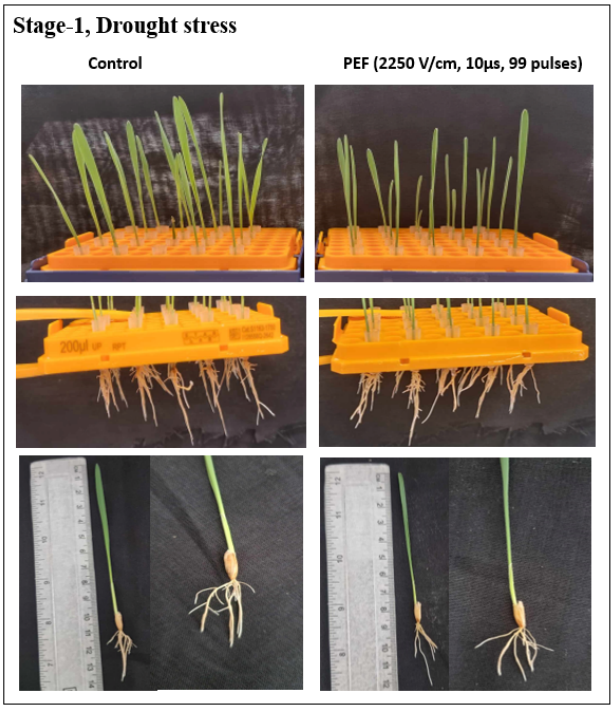

Figure 23 **Visual observations: Growth experiment with drought stress, stage-1 of germination.** Resultant seedlings from the controls and seeds at stage-1 of germination treated with the selected PEF protocol and exposed to 15% PEG-6000.

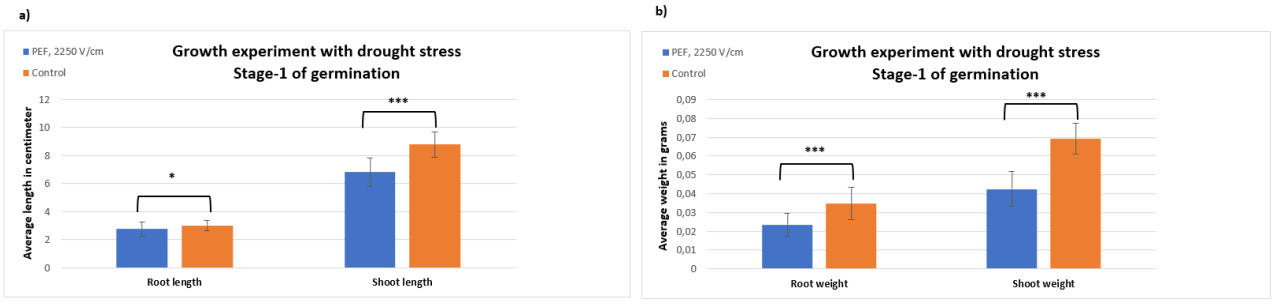

Figure 24 **Bar plots: Growth experiment with drought stress, stage-1 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-1 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses and exposed to 15% PEG-6000. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

Stage-2 of germination There were no significant differences in RL, SL, FSW and FRW of control and PEF treated seedlings ($p<0.05$). This is shown on figures 25 and 26. There was also a big variation between the RL and SL of the samples. The root/shoot ratio was calculated to 0.37 for the control and 0.39 for the resultant seedlings of the PEF treated germinated seeds.

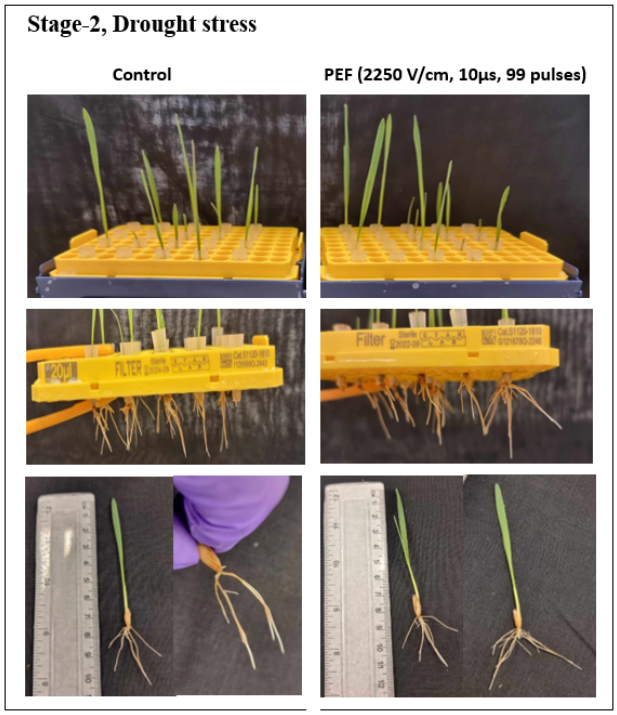

Figure 25 **Visual observations: Growth experiment with drought stress, stage-2 of germination.** Resultant seedlings from the controls and seeds at stage-2 of germination treated with the selected PEF protocol and exposed to 15% PEG-6000.

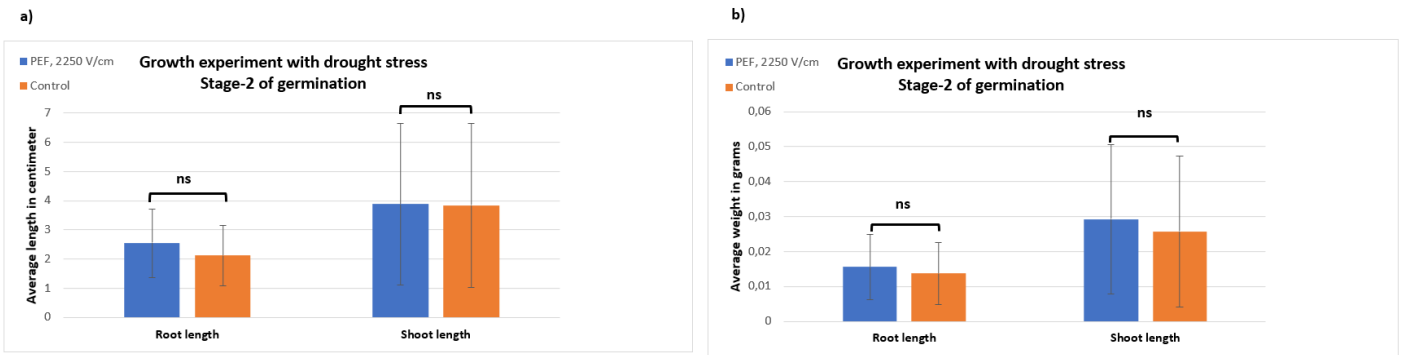

Figure 26 **Bar plots: Growth experiment with drought stress, stage-2 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-2 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses and exposed to 15% PEG-6000. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

Stage-3 of germination There were no significant differences between the biomass of the controls and PEF treated seedlings ($p<0.05$). This was also observed visually (see figures 27 and 28). There was also a big variation in RL and SL within one sample. The root/shoot ratio was calculated to 0.47 for the control and 0.45 for the PEF treated germinated seeds.

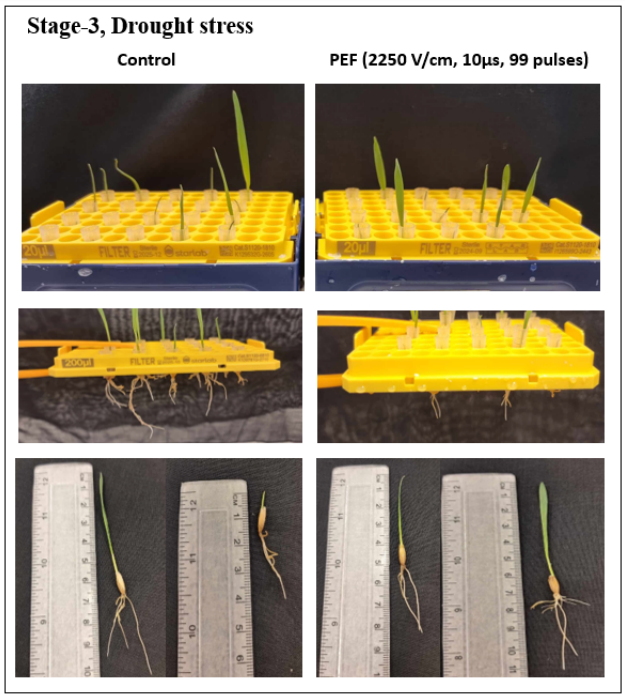

Figure 27 **Visual observations: Growth experiment with drought stress, stage-3 of germination.** Resultant seedlings from the controls and seeds at stage-3 of germination treated with the selected PEF protocol and exposed to 15% PEG-6000.

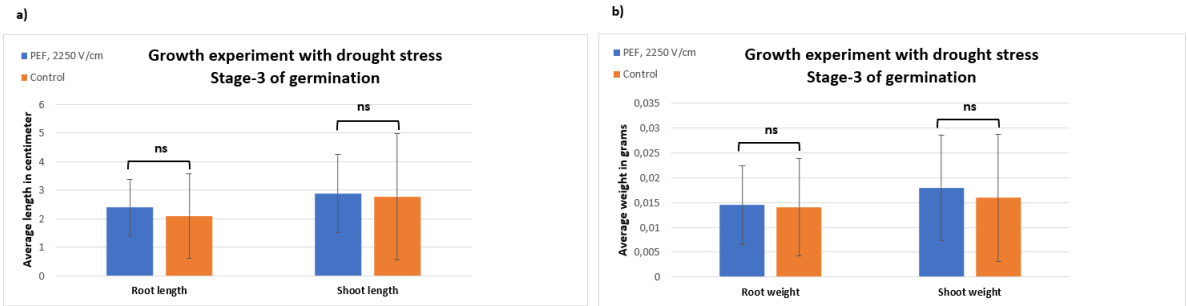

Figure 28 **Bar plots: Growth experiment with drought stress, stage-3 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-3 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses and exposed to 15% PEG-6000. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

6.3.2 Salinity stress experiments

Stage-1 of germination There were no significant differences in the growth of the controls and PEF treated seedlings, as shown on figures 29 and 30 ($p<0.05$). The roots of the seedlings (both PEF treated and the controls) were small and rigid. The root/shoot ratio was calculated to 0.14 for the control and 0.22 for the PEF treated germinated seeds.

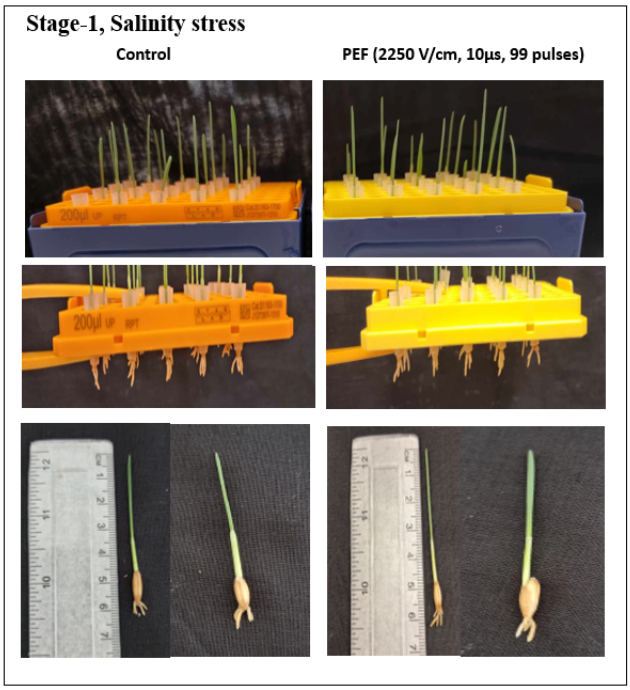

Figure 29 **Visual observations: Growth experiment with salinity stress, stage-1 of germination.** Resultant seedlings from the controls and seeds at stage-1 of germination treated with the selected PEF protocol and exposed to 150 mM NaCl.

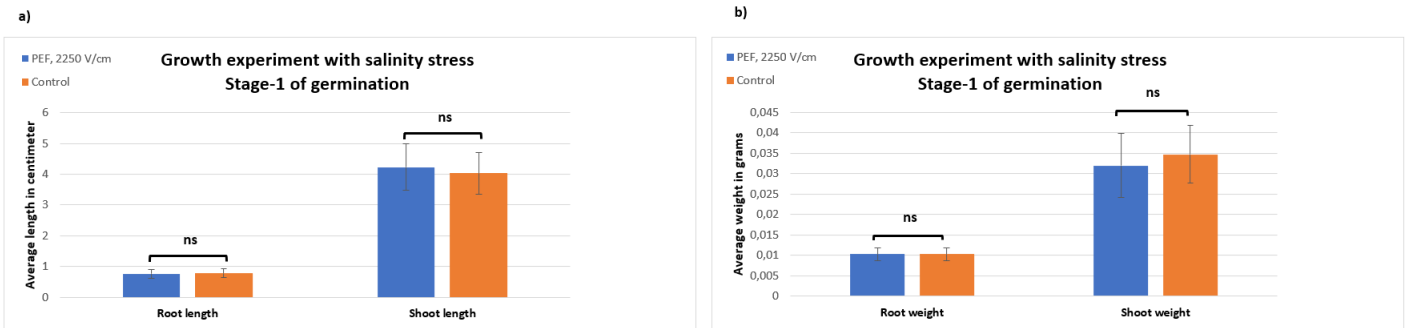

Figure 30 **Bar plots: Growth experiment with salinity stress, stage-1 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-1 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses and exposed to 150 mM NaCl. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

Stage-2 of germination There were no significant differences in RL, SL and FRW of controls and PEF treated seedlings ($p<0.05$). This was also observed visually (see figures 31 and 32). The roots of the seedlings (both PEF treated and the controls) were short and rigid. As for the FSW, there was a significant increase in the PEF treated seedlings. The shoots of the controls were a bit thinner than the shoots of the PEF treated seedlings. The root/shoot ratio was calculated to 0.30 for the control and 0.37 for the PEF treated germinated seeds.

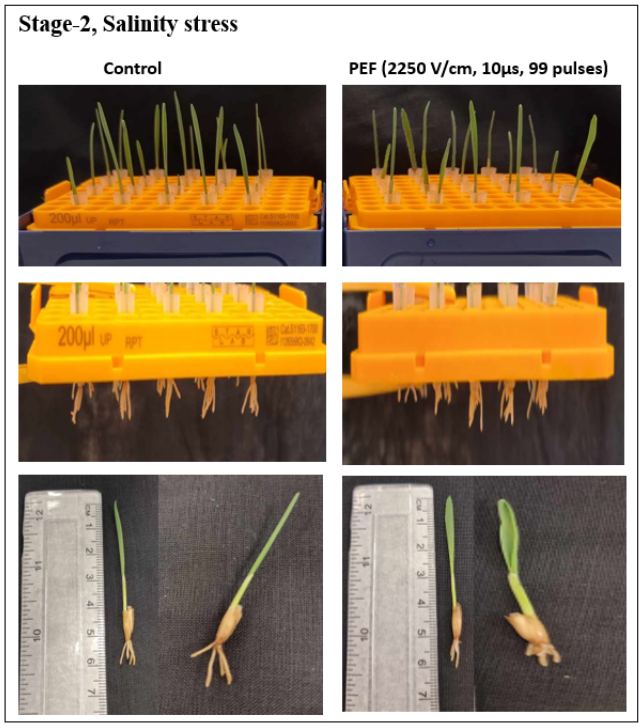

Figure 31 **Visual observations: Growth experiment with salinity stress, stage-2 of germination.** Resultant seedlings from the controls and seeds at stage-2 of germination treated with the selected PEF protocol and exposed to 150 mM NaCl.

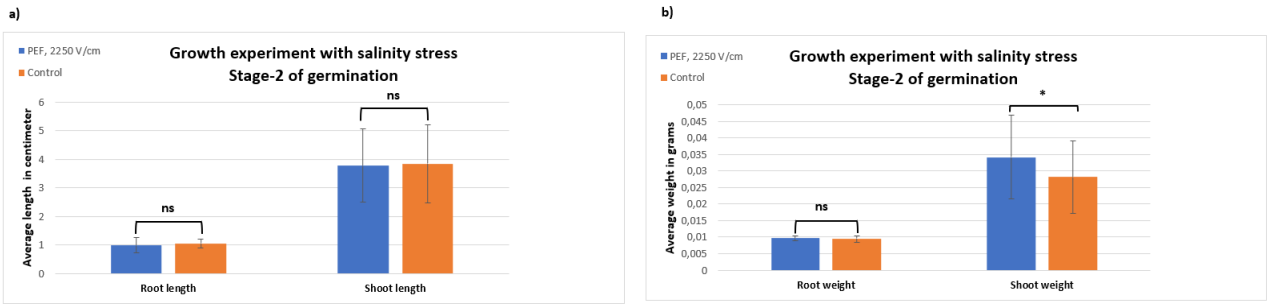

Figure 32 **Bar plots: Growth experiment with salinity stress, stage-2 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-2 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses and exposed to 150 mM NaCl. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

Stage-3 of germination There were no significant differences in RL and FRW of controls and PEF treated seedlings ($p<0.05$). This was also observed visually (see figures 33 and 34). Many small and rigid roots were growing for both samples. However, there was a significant difference in SL and FSW of PEF treated seedlings and the controls. The root/shoot ratio was calculated to 0.42 for the control and 0.48 for the PEF treated germinated seeds.

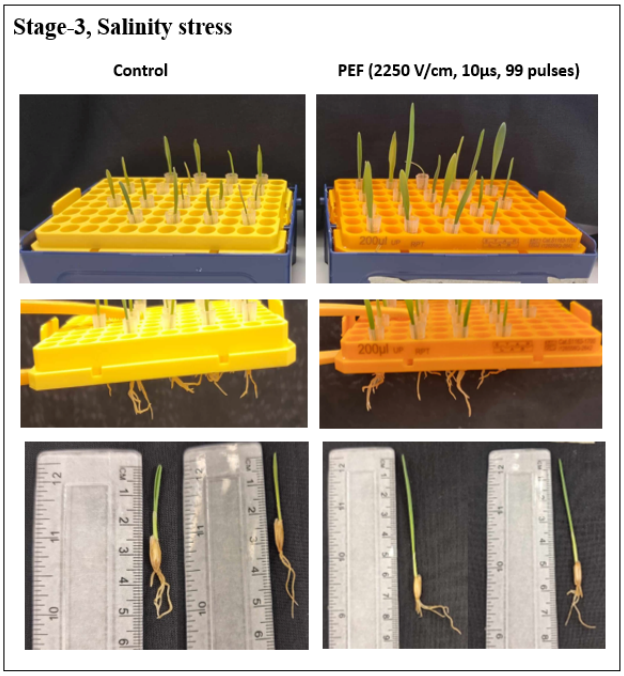

Figure 33 **Visual observations: Growth experiment with salinity stress, stage-3 of germination.** Resultant seedlings from the controls and seeds at stage-3 of germination treated with the selected PEF protocol and exposed to 150 mM NaCl.

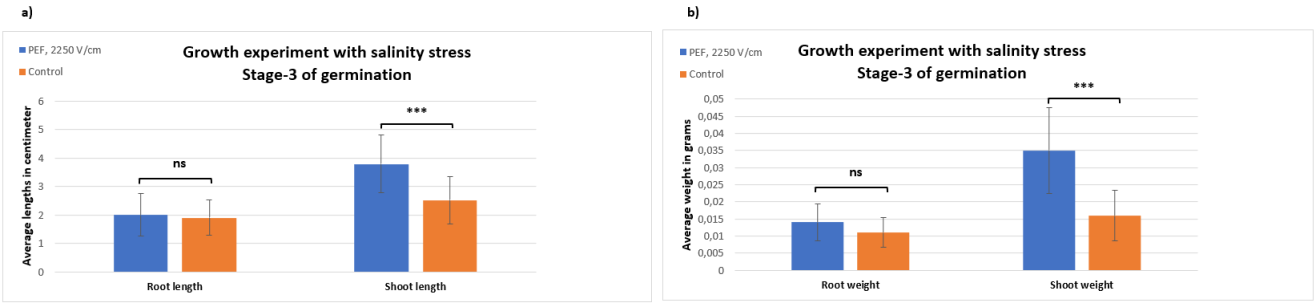

Figure 34 **Bar plots: Growth experiment with salinity stress, stage-3 of germination.** Bar plots of a) RL and SL and b) FSW and FRW of germinated oat seeds at stage-3 of germination treated with the following PEF protocol: E = 2250 V/cm, t = 10 µs and N = 99 Pulses and exposed to 150 mM NaCl. The meaning of the small letters and stars: ns = no significant difference, * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$.

6.4 Summary of results

The results from sections 6.2.3 and 6.3 are summarized here. Figure 35 presents the root/shoot ratios. Table 4 shows the statistical results. The significant difference is at the 0.05 probability level.

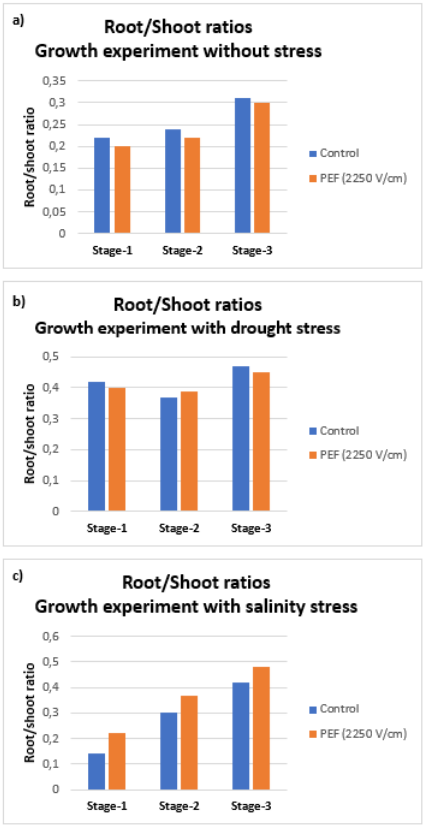

Figure 35 **Root to shoot ratios.** a) growth experiments without stress, b) growth experiments with drought stress and c) growth experiments with salinity stress.

Table 4 **Statistical analysis results.**

	Growth without stress	**Growth with drought stress**	**Growth with salinity stress**
Stage-1	Decrease in root length and weight.	Decrease in root and shoot length and weight.	No differences.
Stage-2	No differences.	No differences.	Increase in shoot weight.
Stage-3	Increase in root and shoot length and weight.	No differences.	Increase in shoot length and weight

7 Discussion

7.1 First part: Preliminary experiments

Several studies have applied different methods to promote germination by pre-treating seeds. Pre-soaking in different GA3 concentrations (GE, 2019; Yildirim *et al.*, 2022), pre-soaking in water and washing in running tap water (Farooq *et al.*, 2021) were the methods applied in this study. As the pre-treatments did not promote an even germination, we decided not to apply any pretreatment. As for the salinity preliminary experiment, Muscolo *et al.* (2023) did observe that 150 mM of NaCl did reduce the growth of kikuyu grass by 50%, which was also observed in this study with germinated oat seeds. No preliminary experiment was performed for drought experiment as growth experiment with drought stress did rely on a study by Gong *et al.* (2022) did use 15% PEG-6000 when they investigated the underlying molecular mechanism of oats' response to drought stress (Gong *et al.*, 2022). By having these results, the primary experiments with PEF were initiated.

7.2 Second part: Growth experiments without stress

Starting with 15 PEF protocols (according to Grzelka *et al.* (2023)) in this systematic study and narrowing it down to one PEF protocol was achieved by developing the method used throughout this study. Petri dishes were replaced with a simple hydroponic system as roots did penetrate through the filter papers from the first days of germination, and it was complicated to remove the fast growing seedlings from the filter papers especially if germinating for more than six days. Monitoring root health and growth was also easily obtained by applying the hydroponic system, which made it a great research tool (Langenfeld, 2022).

Several studies have investigated the effect of PEF protocols with increasing electric field strengths on seed germination and seedling development and they have concluded that the results depend on the PEF protocol used. Leong *et al.* (2016) observed that treating wheatgrass (*Triticum aestivum*) with field strengths $\leq$ 1.4 kV/cm did not affect seed germination but did influence the size of the seedlings produced from the PEF treated seeds. However, a PEF treatment with a field strength of 2.0 kV/cm did reduce coleoptile and primary leaf growth. Therefore, the boundaries for a specific PEF system can be determined by systematic studies, like the one applied in this thesis (Demir *et al.*, 2023). The selected PEF protocol had the following PEF parameters: E = 2.25 kV/cm, t = 10 µs and N = 99

Pulses. The aim of the upcoming experiments was to evaluate the effect of the selected PEF protocol on oat seeds at three different stages of germination. A study by Dymek *et al.*, (2012) did investigate the effect of PEF on germinating barley seeds (investigating only one stage of germination) and they did also suggest further investigations using different PEF protocols on different stages of development of barley seeds. The results from this thesis study showed that the effect of PEF on oat seeds at three different stages of germination differed. As explained in section 3.1.3 seed germination is divided into different phases with different molecular, biochemical and physiological activities in each phase, which will most probably respond to the PEF treatment in different ways. This was also highlighted by Demir *et al.*, 2023, as they explained that the cell characteristics and the PEF protocol determines the reversibility of the PEF treatment.

7.2.1 Selected PEF protocol

A significant reduction in root growth in oat seeds at stage-1 of germination treated with PEF was observed. The study by Dymek *et al.* (2012) observed that metabolic processes of germinating barley seeds were not impaired by PEF treatment (1.2 kV/cm), however, a reduction in shoot length and impairment of root growth appeared. The impairment of root growth in barley seedlings has been associated with oxidative stress, which is induced during the resealing process (after pulse power application) (Dymek *et al.*, 2012; Ktitorova *et al.*, 2006). On the other hand, a study by Gómez Galindo *et al.* (2008) showed that oxidative stress (after pulse application) did affect gross metabolic activities of potatoes (Gómez Galindo *et al.*, 2008). A possible explanation for no effect on the metabolic activity of germinating barley seeds is the tight redox control system found in germinating barley. This did probably confer metabolic resistance to the stress induced by PEF (Dymek *et al.*, 2012). A possible explanation for root growth reduction in oat seeds at stage-1 of germination could be oxidative stress as oat seeds were treated with PEF when the oat seeds were in a crucial germination stage, where major biochemical and physiological changes are involved, including root and shoot protrusion (Han & Yang, 2015).

Another explanation could be that as the seeds had absorbed water before PEF treatment, it could have caused the seed coat to swell and be more susceptible to rupture. When applying PEF to seeds at this stage of germination, the seed coat is not thick enough to protect the embryo. The PEF treatment could have damaged the embryo which results in decreasing seedling emergence. This has been shown

in other studies, whereas treating completely dry seeds with PEF at the same intensity did damage the seed coat and thereby allowed the seed to absorb more water (Foshee *et al.*, 2007). Another interesting observation was that PEF treatment did influence oat seedling development and not oat seed germination. This was observed during germination of the oat seeds post PEF treatment, as root and shoot emergence followed the same pattern as for the control despite that PEF was applied before root or shoot emergence. Some studies have shown that changes in biochemical properties (especially when applying PEF protocols with high electric field strengths) has no correlation with seed germination, but the changes in protein levels and other biochemical properties has an impact on plant growth and yield (Attri *et al.*, 2022). This was also confirmed by the study by Leong *et al.* (2016), however, with a growth induction, as explained earlier.

A possible explanation for increased shoot elongation and bigger shoot size for seeds at stage-3 of germination could be that PEF affected the photosynthetic mechanisms. This was observed by Wang *et al.* (2022) that investigated the effect of different PEF protocols on photosynthesis in lettuce. They found optimal values for photosynthetic activation after PEF treatment. PEF caused an increase in Electron transfer rate (ETR) which in turn increased Adenosine triphosphate (ATP) and Nicotinamide adenine dinucleotide phosphate (NADPH) (chemical energy, products of light reactions of photosynthesis). Additionally, they observed an increase in stomatal aperture, which meant that CO2 did increase and the photosynthetic carbon reduction (calvin) cycle did progress. It utilizes ATP and NADPH to utilize atmospheric CO2 in the biosynthesis of starch and sucrose (Wang *et al.*, 2022). The enhanced sucrose production from this process also promotes primary root growth (Li *et al.*, 2021). Despite the fact that the root lengths differed under PEF treatments for seeds at stage-3 of germination, it could be interesting to investigate the effect of PEF on germinated seeds with the same length of protruding roots and shoots. As seeds at stage-2 of germination did not have protruding shoots when treated with PEF, this may have been the reason why no effect was observed on the growth of the germinated seeds after PEF treatment.

As for the root/shoot ratios (figure 35) of seeds at stage-1, stage-2 and stage-3 of germination, it was not affected by the PEF treatment.

7.3 Third part: Growth experiments with stress

7.3.1 Drought stress experiment

Seeds at stage-1 of germination were much more sensitive towards PEF treatment followed by drought stress than seeds at stage-2 and stage-3 of germination. As shown in section 6.2, PEF treatment did reduce root development in seeds at stage-1 of germination. Roots absorb water and nutrients from soil, which is essential for plant growth and yield. Studies have shown that roots are closely related to drought resistance as roots were affected first when applying drought stress (He *et al.*, 2017). This could explain the impairment of seedling development for seeds at stage-1 of germination when exposed to drought stress. As the root development of seedlings at stage-1 of germination were reduced by PEF (without exposure to stress), this may have affected the root development further when the germinated seeds were exposed to drought.

Results from a study on maize seedlings (after two days of germination) where extremely low frequency PEF (ELS-PEF) was applied (E = 200 Kv/m, t = 80 ms and frequency = 1 Hz), showed dry weight increase of PEF treated maize seedling roots, enhancement of Superoxide dismutase (SOD) activity of root cells and reduction in Malondialdehyde (MDA) content, which indicate a boost in root cell growth and drought stress tolerance (He *et al.*, 2017). However, this was not observed in this study as the PEF treatment with the selected PEF protocol did not have a significant effect on seeds at stage-2 and stage-3 of germination. As for the root/shoot ratios (figure 35) of seeds at stage-1, stage-2 and stage-3 of germination, it was not affected by the PEF treatment.

7.3.2 Salinity stress experiment

If the concentration of NaCl exceeds 200 mM, most of the plants will not survive (Gupta & Huang, 2014). Salinity stress is known to affect root length more than shoot length in plants (Muscolo *et al.*, 2003). During the initial phase of salinity stress the capacity of root systems to absorb water decreases and the loss of water from the shoots will be accelerated. This was observed in all three stages of germinated seeds; small and rigid roots for PEF treated germinated seeds and the controls with no significant differences in fresh root growth. For seeds at stage-2 of germination, there was a lower significant increase in shoot weight of PEF treated germinated seeds (p-value of 0.028) compared to stage-3 of germination (with a p-value<0.001). PEF treatment may have triggered (in seeds at stage-3 of germination) one of the mechanisms responsible for salinity stress tolerance to make the germinated

seeds more resistant to salinity concentration of 150 mM NaCl. This was shown by Akdemir *et al.*, 2021 in wheat grains, where PEF did induce salt tolerance in the seedlings. Another explanation could be the increase in root/shoot ratio for PEF treated seeds at stage-1, stage-2 and stage-3 of germination. Despite that no significant difference was found in fresh root growth of PEF treated and control seeds at the three stages of germination, the dry weights (figure 35) showed that all the PEF treated germinated oat seed had a higher proportion of roots, which can potentially increase water uptake (Zarebanadkouki *et al.*, 2019). However, as the effect on root growth is not visible (by observations and from the fresh root weights), the germinated oat seeds have to be exposed to salinity stress for more than eight days to evaluate the development of the roots.

8 Conclusion and future work

To the best of our knowledge, this is the first systematic study on the effect of PEF on oat seeds at different stages of germination. The results showed that PEF is a promising, chemical-free method to promote growth of germinated oat seeds during normal and harsh conditions. Oat seeds at three different stages of germination were treated with one PEF protocol (E = 2.25 kV/cm, t = 10 µs and N = 99 pulses) that was selected from 15 PEF protocols. The method used for germination was developed throughout this work, starting with spreading seeds on wetted filter paper to growing the germinated seeds in a simple hydroponic system. Three different growth experiments were performed; with stress (salinity and drought) and without stress. For the growth experiment without stress, a significant increase in the biomass of oat seeds at stage-3 of germination was observed ($p<0.05$), however, a significant decrease in root growth appeared for seeds at stage-1 of germination ($p<0.05$). This indicates that the germination stage at which the PEF treatment is applied is important for the results of the PEF treatment. As for drought stress, no significant increases were observed for seeds at stage-2 and stage-3 of germination, but a significant decrease in growth was observed for seeds at stage-1 of germination ($p<0.05$). As for salinity stress, a significant increase in shoot growth was observed for seeds at stage-3 of germination when treated with PEF ($p<0.05$), with an increase in root/shoot ratios of oat seeds at all three stages of germination.

These results from this systematic study are promising for the use of PEF as an alternative technology to chemical applications in promoting oat seed germination and seedling growth, both in environments with stress and without stress. This study with its promising results is a starting point to investigate the effect and potential application of PEF on germinated seeds. The selected PEF protocol can potentially be applied to other crops and other oat genotypes, however, most probably, the PEF parameters need to be adjusted for different plants. Additionally, the effect of PEF on germinated oat seeds has to be investigated on the fully grown oat plant and on the yield and quality of the oats. This will require a shift from the simple hydroponics system to real field conditions in soil. Also, to understand the mechanism of PEF on oat seed metabolism, further studies are required at the biochemical and molecular levels to investigate the seed responses to PEF treatment.

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