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High-Intensity Nanosecond Pulsed Electric Field effects on Early Physiological Development in *Arabidopsis thaliana*

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Abstract**—**The influences of pulsed electric fields on early physiological development in *Arabidopsis thaliana* were studied. Inside a 4-mm electroporation cuvette, pre-germination seeds were subjected to high-intensity, nanosecond electrical pulses generated using laboratory-assembled pulsed electric field system. The field strength was varied from 5 to 20 kV.cm^{-1} and the pulse width and the pulse number were maintained at 10 ns and 100, respectively, corresponding to the specific treatment energy from 300 J.kg^{-1} to 4.5 kJ.kg^{-1}. Statistical analyses on the average leaf area 5 and 15 days following pulsed electric field treatment showed that the effects appear significant the second week after treatments with a maximum increase of 80% compared to the control ($P < 0.01$).

Keywords**—***Arabidopsis thaliana*, full-wave analysis, leaf area, high-intensity nanosecond pulsed electric fields

I. INTRODUCTION

THE use of to pulsed electric fields (PEFs) of high intensity and short duration to expose a living cell can result in disruption of cell membranes. This is because the transmembrane potential induced by the applied field causes the cell membrane to lose its impermeability, which, beyond a critical value, leads to the formation of pores on the membrane surface resulting in an disintegration of the cell itself [1]-[3]. Relatively low electric field strength of about 0.1–1.0 kV.cm^{-1} and long pulse durations of 0.1 ms–10 μs exhibit the reversible effect if pores are electrically small relative to the critical membrane surface [2]. Such an effect, also known as electroporation [3], is routinely used in a number of applications, e.g. gene and drug delivery into cells [4],[5], bacterial decontamination [6],[7] and food hygiene [8],[9].

Currently, techniques to control the pulse width in a nanosecond regime have received great attention in practical applications of medicine and biology. The manipulation of inner structures of biological cells can be achieved while maintaining considerably low thermal absorption through a delivery of pulses with relatively large electric field intensities in several kilovolts per centimeter. Applications of a high-intensity nanosecond pulsed electric field (nsPEF) have been occupied to study for the response of mammalian cells [10],[11].

Treatments of pre-germination seeds with electromagnetic and magnetic fields, varying from low to high frequency have shown to produce positive effects. Celestino et al. [12] reported the application of extremely low-frequency (ELF) electromagnetic fields in enhancing early growth of cork oak. Similarly, Vashisth and Nagarajan [13] successfully increased germination rates and seedling growths of chickpeas with the use of uniform ELF magnetic fields. Another study on the germination potential of seeds was reported by Amyan and Ayrapetyan [14], in which ELF electromagnetic fields and vibrations modified the water structure, affecting the processes of barley seed growth. For high-frequency treatments, Azharonok et al. [15] observed effects of using radio wave electromagnetic field at 5.28 MHz to enhance the arable germination and to increase the crop yield of legume seeds. Lynikiene et al. [16] have also shown the potential of applying 2.4-GHz electrical fields for a short duration to maximize carrot, radish, beet, beetroot and barley seed germinations and viabilities. Huang and Wang [17], and Jinapang et al. [18] reported an enhancing effect on the early growth of mung beans with 20 and 60 Hz magnetic fields, and 425-MHz electromagnetic fields, respectively.

Thus far, little has been reported on investigation of effects of nanosecond electrical pulses, which is another form of time-varying electromagnetic waves, on plant seeds. *Arabidopsis thaliana*, whose genome was the first to be sequenced, is one of popular tools providing insight into the molecular biology [19]. Due to its rapid life cycle and small size, *Arabidopsis thaliana* therefore offers great advantages for research on the growth characteristics of plants. Previous attempt to examine nsPEF exposure effects on *Arabidopsis thaliana* was done with seven day old seedlings [20]. It was shown that nsPEF treatment with a field strength of 5 kV.cm^{-1} and a specific treatment energy of 100 J.kg^{-1} revealed a growth effect. Treating the pre-germination seeds with nsPEF, however, would expose key information of other essential growth mechanisms such as seed imbibing ability and seed survival rate. Thus, the objective of this study is to investigate

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high-intensity nsPEF effects on early physiological development in pre-sowing seeds of *Arabidopsis thaliana*. Following the introduction, Section II reports the configurations of the high-intensity nsPEF system. The remaining of this section details treatment materials, methods, and preparation. The results are analyzed and discussed in Section III and concluded in Section IV.

II. EXPERIMENTAL SETUP

A. nsPEF Treatment System

The configuration of the high-intensity nsPEF system is shown in Fig. 1 where the main components include a high-voltage DC supply V_1, a pulse forming line (PFL) consisting of a set of three 1-m long coaxial cables connected in parallel (T_1 – T_3), a self-breakdown switch U_1, and a load R_2. A LT60P33 high-voltage DC source (Glassman High Voltage, USA) supplies the energy to the PFN through the decoupling resistor R_1 of 100 MΩ. The coaxial cable of PFL is RG8/U (10.29-mm ø, 96.79 pF/m) with the maximum rating of 10 kV. Each cable has a characteristic impedance of 52 Ω with solid polyethylene as an insulator material. This setup gives the PFL input and output impedances of approximately 52 Ω and 17 Ω, respectively. The value of the load R_2 is estimated from the conductivity σ of the buffer solution and the size of the electroporation cuvette (VWR Scientific Products, West Chester, PA), i.e., $R_2 = \sigma^{-1} \cdot L \cdot A^{-1}$ where L and A are the electrode gap and area, respectively. Under a matching condition, i.e., the value of R_2 is identical to that of the PFL's characteristic impedance Z_0, the output voltage peak across the load can be estimated by a half of the input voltage peak supplied to the network. The output voltage's pulse duration τ is 10 ns, which is equivalent to the double transit time of 1-m long cable. The temporal behavior of generated pulses was monitored using a P6015A high-voltage probe (Tektronix, Inc., Beaverton, OR, USA), reading through a TD53O52C digital oscilloscope (Tektronix, Inc., Beaverton, OR, USA). The self-breakdown switch is a spark gap with a few nanosecond rise time.

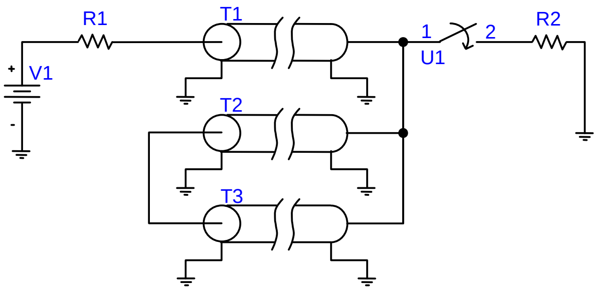


Fig. 1 Schematic diagram of high-intensity nsPEF system for a generation of 10-ns electrical pulses with the field strength varying from 5 to 20 kV.cm^{-1}.

B. Materials

Arabidopsis seeds (Columbia-0 ecotype, Col-0) were surface-sterilized in order to remove putative contaminants. The seeds were soaked in purified water in a 1.5 ml microcentrifuge tube and incubated at room temperature for 15 minutes. The seeds were then sterilized with 70% ethanol for 5 minutes and in 20% sodium hypochlorite solution with a small amount of Tween-20 for 10 minutes. Supernatants were removed under axenic conditions. Seeds were then washed five times with sterile water and then stored in 1 ml sterile agar solution (0.1% w/v) overnight at 4 °C for stratification.

C. Methods

Prior to experiments, three-dimensional full-wave analyses were carried out to observe the electric field distributions inside the medium-filled electroporation cuvette under nanosecond electrical pulse stimulation. Simulations were done by the commercial electromagnetic solver (CST Microwave Studio, Darmstadt, Germany) based on the finite integration technique (FIT) [21]. The materials for cuvette electrodes and walls were, respectively, perfect electric conductor (PEC) and the clear polystyrene ($\varepsilon_r = 2.6$, $\sigma \approx 0$).

All prepared seeds were divided into 10 groups of 20 seeds in accordance with the study plan, which included change in electric field strength, i.e., 0 (for non-treated control), 5, 10, 15, and 20 kV.cm^{-1} and maintaining the number of pulses, the pulse width, and the pulse repetition rate at 100, 10 ns, and 5 Hz, respectively. At every parameter setting, two treatments with 20 seeds each were made. Two mock treatments were performed identically to the pulsed electric field treatment procedure but without applying electrical pulses to the seeds. Immediately before nsPEF treatment, each group were removed from the agar solution and suspended into 800 μl of 1% sodium chloride solution prepared inside an electroporation cuvette. For each experiment, 70% ethanol was used as an antimicrobial disinfectant applied to the treatment area. Following the treatments, the seeds were re-surface sterilized and sown with a pipette approximately 1-cm apart on 90 mm petri dishes containing solid MS medium (3% sucrose and 0.8% agar, pH 5.8). The seeds were then cultivated in a growth room for fourteen days under 12 h light/ 8 h dark cycle at 25 °C.

The evaluation of nsPEF effects on the development activity of the plant was determined by the average leaf area where ImageJ software (version 1.42q, National Institutes of Health, USA) was used for image analysis and processing. For statistical significance tests, the P values were determined with all replication results together through one-tailed paired sample t-test using a Microsoft Excel 2003 spreadsheet running on Windows XP. Conventionally, if $P < 0.05$, the result is statistically significant, and if $P < 0.01$, the result is highly significant with more confidence of true effect. The error bar for each replication was determined from the standard error (SE).

III. RESULTS AND DISCUSSIONS

A. Electric Field Distribution

The field distributions on the cuvette's *xz* and *yz* planes are shown in Fig. 2 with the presence of the buffer solution between electrodes. Once the electrical pulse was delivered into the treatment volume, the field uniformity can be observed across the gap between electrodes, thus ensuring the uniform treatment environment under nsPEFs.

B. Inactivation Effects of nsPEF

The photo of 15-day old *Arabidopsis thaliana* seedlings is shown in Fig. 3(a) after the treatment of 10-ns electrical pulses with the electric field strength of 5 kV.cm^{-1}. Fig. 3(b) shows associated image following the threshold adjustment process for all 20 seedlings. For quantitative evaluations, Fig. 4(a) compares the average leaf area (mm^2) of both treated and control groups 5 days after treatments. Only a significant increase in the leaf area of 43.7% (determined by the difference between the average leaf area of the control and treated samples) was observed for the electric field strength of 10 kV.cm^{-1} ($P < 0.05$, one-tailed paired sample t-test). The rest show no significant difference between the two groups.

In the case of 15 days after treatments, as shown in Fig. 4(b), all treated groups of different field strengths show significant increases in the leaf area compared to the control group. Significant increases of 80%, 60%, 70%, are observed, respectively, for the field strengths of 10, 15, and 20 kV.cm^{-1} ($P < 0.01$, one-tailed paired sample t-test) where an increase in the average leaf area with less confidence is observed at 65% for the field strength of 5 kV.cm^{-1} ($P < 0.05$, one-tailed paired sample t-test).The observed enhancements in the growth of *Arabidopsis thaliana* upon exposure to high-intensity nsPEFs at pre-sowing stage have been reported for the first time. A few similar studies have previously reported the enhancing effects of electromagnetic fields, in general, on the germination and growth of seeds [12]-[17]. In this work, the effects on the growth development in terms of the leaf area were somewhat visible when compared with the untreated seeds and became more pronounced two weeks after treatments. The results seem to be similar with those reported on the effects of nsPEF on the growth development in seven-day old seedlings by Eing et al. [19] where the optimum growth appeared with a few hundreds kJ.kg^{-1} up to 4 kJ.kg^{-1} of the specific treatment energy level. Possible explanation for an increase in growth development under nsPEF treatments is based on the fact that the disturbance due to the time-varying fields on the tissue structures leads to the transport of essential

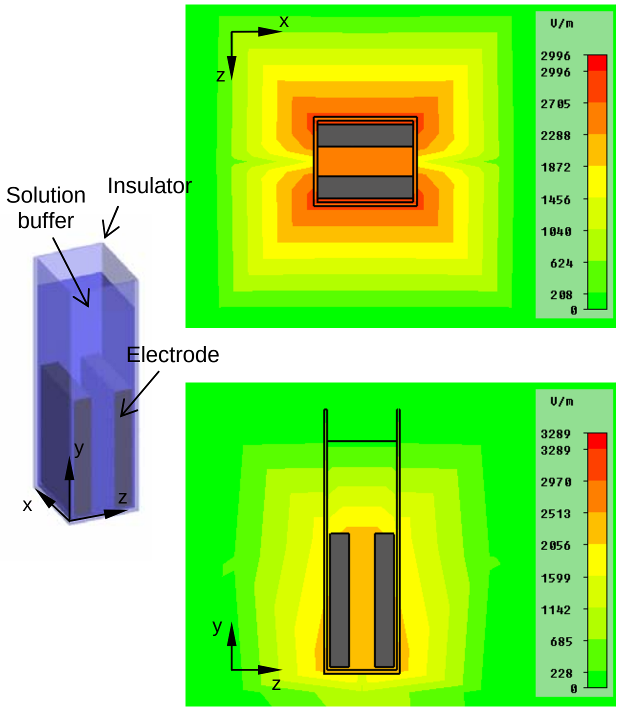
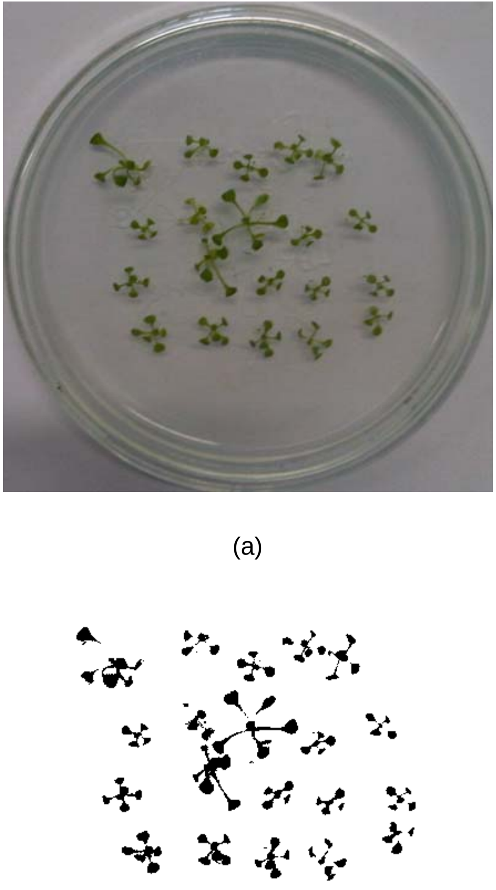


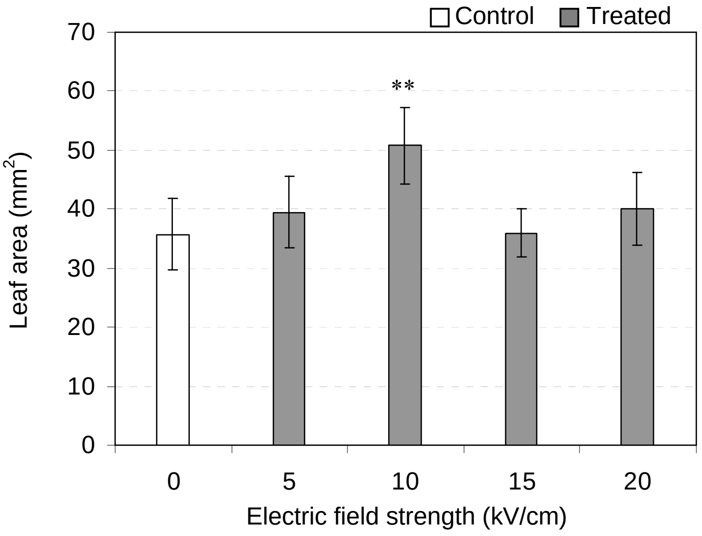
Fig. 2 Homogeneous field distributions inside the cuvette on the *xz* and *yz* planes under high-intensity nanosecond pulse stimulation.



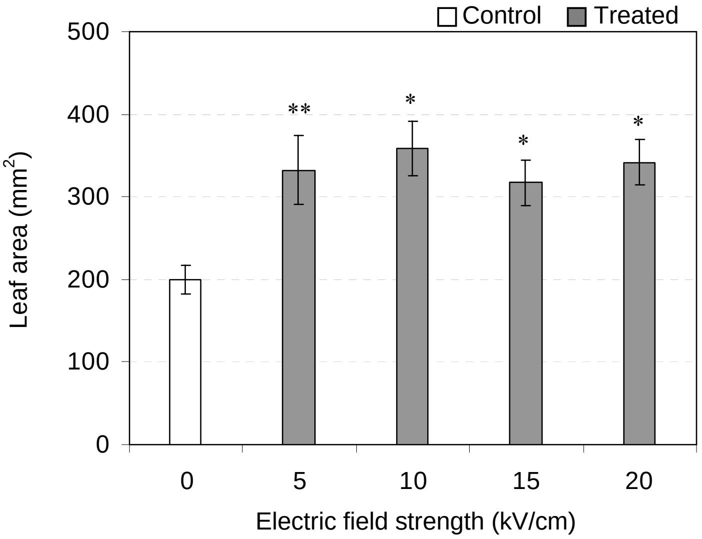
(a)

(b)

Fig. 3 (a) 15-days old *Arabidopsis thaliana* seedlings after treatment of 10-ns electrical pulses with the electric field strength of 5 kV.cm^{-1} for 100 pulses at 5 Hz. (b) Associated image after threshold adjustment process



(a)



(b)

Fig. 4 (a) and (b) Average leaf area in mm^2 of 5-days old and 15-days old *Arabidopsis thaliana* seedlings, respectively, of both treated and control groups from five sets of electric field strengths. The asterisks * and ** indicate significant growth improvements for $P < 0.01$ and $P < 0.05$, respectively.

substances through the channels induced on the cell membranes [3]. Besides, a potential difference across the membrane caused by an alternating action of pulsed electric fields possibly leads to permeable cell wall. An appropriate level of induced membrane pressure can then create a number of pores, and weakening of tissues surrounding the embryo, which is required for radical growth to occur [22]. Once carrying out with observations on physiological characteristics, the study of influencing factors on the germination of the seed using molecular techniques through related gene expressions to identify the morphological mechanisms is expected.

IV. CONCLUSION

In this work, we have exposed the seeds of *Arabidopsis* *thaliana* to high-intensity nanosecond pulsed electric fields. The objective was to determine the effects of nsPEFs on early growth development in the seeds. Preliminary simulations ensured the homogeneity of the field distribution inside the treatment volume for the uniform treatment environment under nsPEF exposures. The experiments were conducted using the custom-built nsPEF system, delivering nanosecond pulses to the seeds suspended inside the electroporation cuvette. There appeared growth improvement when treated pre-germination seeds with all given sets of exposure parameters, corresponding to the specific energy from 300 $J.kg^{-1}$ to 4.5 $kJ.kg^{-1}$. Statistical analyses showed pronounced effects with a maximum increase in the average leaf area of 80% ($P < 0.01$) 15 days following pulsed electric field treatments.

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