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Conference Paper · August 2016

DOI: 10.1061/9780784480120.005

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Enhanced Germination and Growth of *Arabidopsis thaliana* Using IrO_2-Ta_2O_5|Ti as a Dimensional Stable Anode in the Electro-Culture Technique

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Abstract: Plants are sensitive to many different forms of stimuli, and they respond to many well-known environmental conditions such as temperature, light quality, moisture, and gravity, among others. Electro-culture or electro-farming consists of stimulation by electric fields applied in the soil in order to improve the germination rate, growth rate, yields and crop quality. It has been demonstrated that the treatment also protects plants from diseases; even so, this technology remains poorly studied. Here, we study the electro-culture process for *Arabidopsis thaliana* using IrO_2-Ta_2O_5|Ti as a dimensional stable anode (DSA) with a titanium cathode. We expect to accelerate the germination and growth rate of this plant's cotyledons, leaves, and roots in the early stages of growth. This process is beneficial due to the fact that the ions undergo electro-migration and permeate the nutrients across the seeds. Furthermore, a decrease in pH occurs nearby the DSA via electrolysis of the water during the electro-culture process (pH = 1), and we observe the generation of OH radicals during this electrolysis when the seed is exposed to low electric fields in a short time with direct currents (less than 5V in 1 hour)..

INTRODUCTION

The environmental conditions of our life has been affected negatively due to the rapid growth of the world population in recent years, which has resulted in increasing consumption of food and energy (Aladjadjiyan 2012). It is important to increase food and energy production but the tendency for satisfying growing needs has led to intensive development of plant production through the use of chemical additives, which in turn has caused more and more pollution of soil, water and air.

A fundamental property of all living organisms is their capacity to collect information from the environment and to express physiological processes that are aimed to optimize their performance under the new environmental conditions and by these means maintain homeostasis (Gurovich 2012). The application of electricity, magnetism, monochrome light, and sound can stimulate the growth of plants to a great extent. The technology of applying electric fields to the soil in order to improve the efficiency of germination and growth of plants is called electro-culture or electro-farming which are defined as any and all applications of electricity to agriculture (Matthews 1922). This technology has been used for different purposes including seed treatment, seedling growth, plant growth, insect control, among others (Gui et al. 2013).

Electric current is broadly defined as charged particles in motion or as a flow of charge. It can be direct current (DC) or alternating current (AC), with direct current being characterized by a uniform direction of flow and amount (voltage) of electricity.

Brief exposure of seeds to electric current ends their dormancy, accelerates development throughout the period of vegetation, ultimately increases yields, and metabolism of the seedlings is stimulated. Respiration and hydrolytic enzyme activity are intensified for many types of plants (Matthews 1922; Wang et al. 2009; Artem and Albertovna 2012).

There are four main soil transportation mechanisms induced by the application of electric fields: electro-migration refers to the transportation of ions in solution in the interstitial fluid in the soil matrix towards the electrode of the opposite charge; electro-osmosis is the net flux of water or interstitial fluid induced by the electric field; electrophoresis refers to the transport of charged particles of colloidal size; and diffusion refers to the mass transport due to a concentration gradient (Cameselle and Gouveia 2013).

DSAs are currently being used in industry for the treatment of wastewater with reduced operational costs. These electrodes consist of different metal bases (titanium, zirconium, and tantalum) which are coated with different oxides: IrO_2, RuO_2, or SnO_2, including $RuO_2|Ti$ and $IrO_2|Ti$ (Papastefanakis, Mantzavinos, and Katsaounis 2010; J. Souza, Martínez-Huitle, and Ribeiro da Silva 2011). The use of a modified anode (IrO_2-$Ta_2O_5|Ti$) and cathode (Ti) has been reported previously by our research group, when they were used in the treatment of polluted soils in the electro-remediation technique of hydrocarbons and mercury (Pérez-Corona et al. 2013). These kinds of electrodes are resistant to extreme experimental conditions (3 A cm^{-2} at 353 K in concentrate H_3ClO_4 or ± 1V for 90 s in 0.5 M NaCl at 297 K) with a life span of around of 40 cycles of use under extreme conditions (Méndez et al. 2012).

Arabidopsis thaliana is a small plant in the mustard family (Brassicaceae) that has become the model system of choice for research in plant biology (Meyerowitz and Sommerville 1994) and it has a natural distribution throughout Europe, Asia and North America. This plant has different accessions, or ecotypes that have been collected from natural populations, and others which have been developed in laboratories. *Arabidopsis thaliana* has several accepted standards for genetic studies and plant biology, among which is Columbia and Landsberg ecotypes, because they can be traced for their entire life cycle including seed germination, formation of rosette plant, bolting of the main stem, flowering, and maturation of the first seeds, all in six weeks and in laboratory conditions (Meinke et al. 1998).

The aim of this work was to prove the efficiency of the electro-culture technique for a model plant using a IrO_2-Ta_2O_5│Ti anode and a Ti cathode in low DC electric fields (0.1, 0.2, 0.4 and 0.8 V/cm) applied for 4 hours in a Vertisol-type soil and *Arabidopsis thaliana* Col-0 seeds to evaluate seed germination rate and early seedling development. The results obtained in this set of experiments shows that IrO_2-Ta_2O_5│Ti modified electrodes used as anode and titanium cathode when a DCEF up to 0.2 V/cm is applied for 4 hours improves seed germination rate and seedling development, and that higher DCEF treatments with the same electrodes negatively affect *Arabidopsis thaliana* development.

METHODOLOGY

Vegetal material. *Arabidopsis thaliana* seeds Col-0 ecotype was obtained as a donation from Stefan de Folter, PhD from Plant Development Functional Genomics in the National Laboratory of Genomics for Biodiversity (LANGEBIO) in the National Polytechnic Institute in Mexico.

Soil sampling. Clean agricultural-purpose Pelic Vertisol-type soil was collected near a farm in Sanfandila, Queretaro, Mexico and dried in the dark according to Mexican norm NMX-12/1-1987b and United States norm from the series SW82-EPA Chapter 9. Soil samples were collected from the superficial layer of the site (between 0 and 0.2 m with respect to the natural ground level). These were transported in sterilized glass containers and kept at 277 K until used. Before experimentation, collected samples were allowed to dry for two weeks at room temperature in dark conditions; this step allowed soil moisture elimination and after that, soil samples were sieved through a 2 mm mesh for the removal of roots, gravel and non-soil components. All analytical determinations were done in triplicate in order to assure that experimental error was less than 5 % (Perez Corona et al. 2013).

Electro-culture conditions. Electro-culture was conducted as follows: low DCEF was used in all experiments (0.1; 0.2; 0.4 and 0.8 V/cm) (Bi et al. 2011; Eing et al. 2009), using a power source; 500 gr of dry and sieved soil was placed in a plastic container of 10 x 12 cm and wetted with tap water at pH 7.3 and electric conductivity 451 µS/cm; the electrodes were placed 10 cm apart in a 1D face to face arrangement (Perez Corona et al. 2013) (the anode placed in front of the cathode) using a IrO_2-Ta_2O_5│Ti electrode as anode and a Ti electrode as a cathode (Méndez et al. 2012); and finally *Arabidopsis thaliana* Col-0 seeds were sown in 5 lines of 5 seeds each with a distance between the seeds of 2 cm inside the space between electrodes. Once sown, seeds were allowed a period of 24 hours of vernalization at 4º C. After the vernalization period, the electrodes were connected to a DC Power Supply GP-4303DU, which acted as a power source. The treatment was for 4 hours in each of the electric fields described above. A control without DCEF was left in every set of experiments. After electro-culture treatments, the cells were placed in a growing chamber with artificial light in a room without windows applying a photoperiod of 8 hours of light and 16 hours of darkness; the temperature of the chamber was 26 ± 1 ºC and was maintained until the end of experiment.

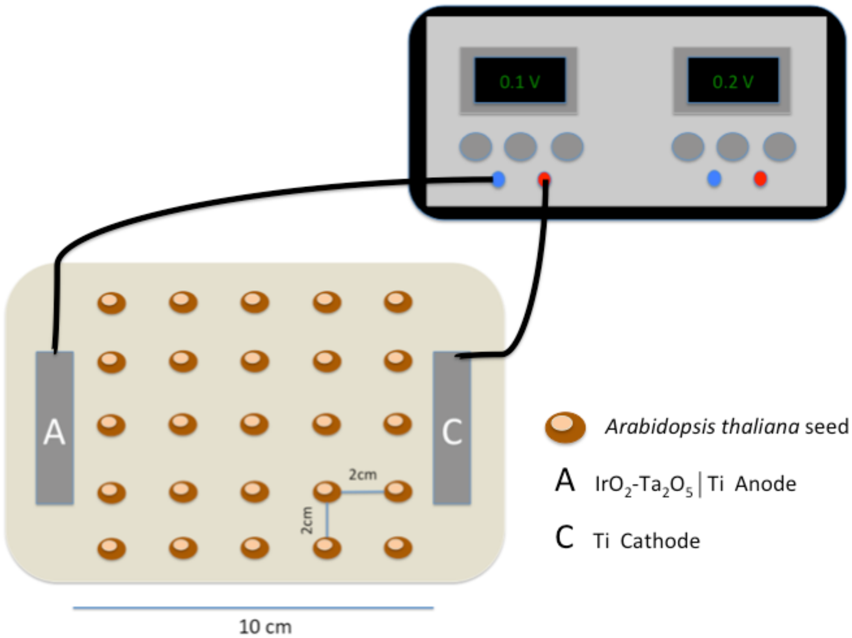

Figure 1. Arrangement of Arabidopsis thaliana Col 0 seeds in on the electro-culture cell. Seeds are arranged in five rows of five seeds located at 2 cm from each another in the space between anode and cathode. After a vernalization period, the electro-culture cell is connected to a power source to implement an electric field.

Germination rate. Germination rate was calculated by counting the total number of seeds that broke dormancy, that is when the epicotyl emerged. Germinated seeds were counted from the first week and every seven days until the majority of seeds had germinated, that is four weeks after treatment (Bewley 1997; A. De Souza et al. 2014; Patwardhan and Gandhare 2013).

Plantlet Development. To evaluate plantlet development after electro-culture treatment, an analogic Vernier was used to assess the diameter of the rosette of the germinated plants from tip to tip of the longest leaves for every plant in the cell. All determinations were done at least in triplicate to assure that statistical error was less than 5 % (Yang and Shen 2011; Costanzo, Catania, and Doria 2008).

RESULTS AND DISCUSSION

Germination rate. Germination rate in Arabidopsis thaliana was increased when using IrO_2-Ta_2O_5|Ti electrode as anode and Ti as cathode when compared with the Control with no DCEF applied as shown in Figure 2. As previously reported, IrO_2-Ta_2O_5|Ti electrodes permit the mobilization of ions in the soil due to the effect of change in pH in the anode, compared with this value in the cathode thus allowing the mobilization of ions that can contribute to the break of the dormancy of the seeds.

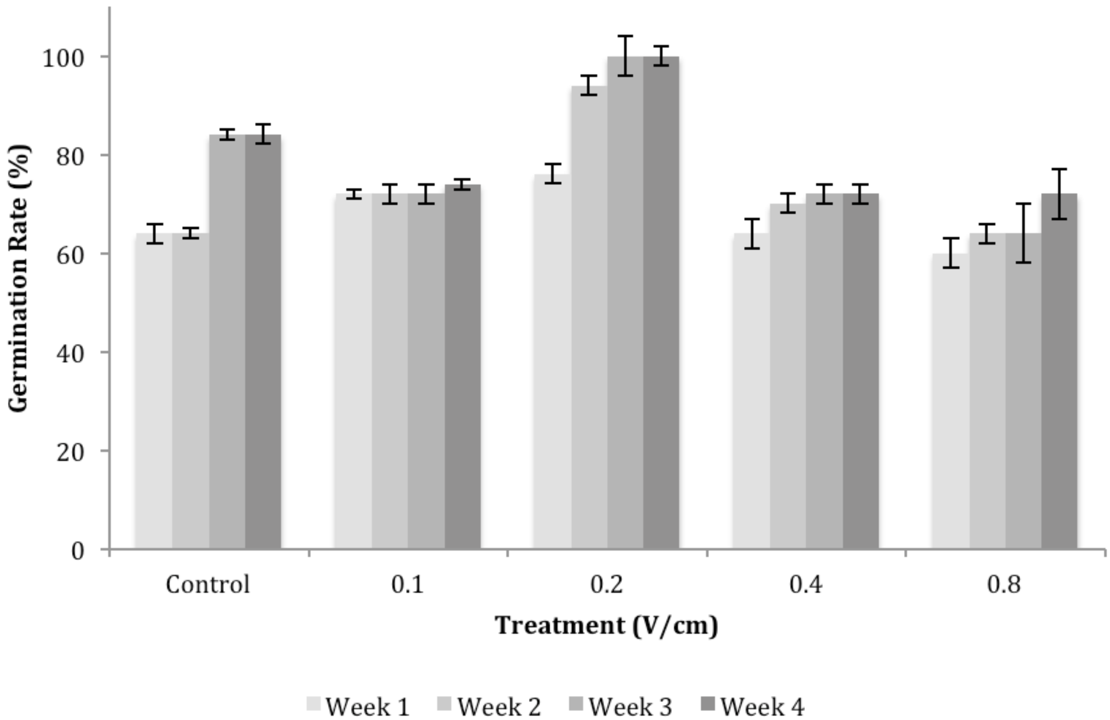

Figure 2. Germination Rate of *Arabidopsis thaliana* in four weeks with different low intensity DCEF using IrO2-Ta_2O_5│Ti electrodes as anode and a Ti electrode as a cathode.

Plantlet Development.

Plantlets were evaluated for rosette diameter 6 weeks after treatment with the electro-culture technique. Results showed that this electro-culture condition (IrO2-Ta_2O_5│Ti anode and Ti cathode) also improves plantlet development. The plants were analyzed for the rosette diameter six weeks after germination and the results showed that when we applied 0.2 V/cm direct current treatment plantlets showed a tendency to develop faster.

When plantlet development of Arabidopsis thaliana was assessed, we found that using IrO_2-Ta_2O_5│Ti and Ti electrodes increased the diameter of the rosette as shown in Figure 3.

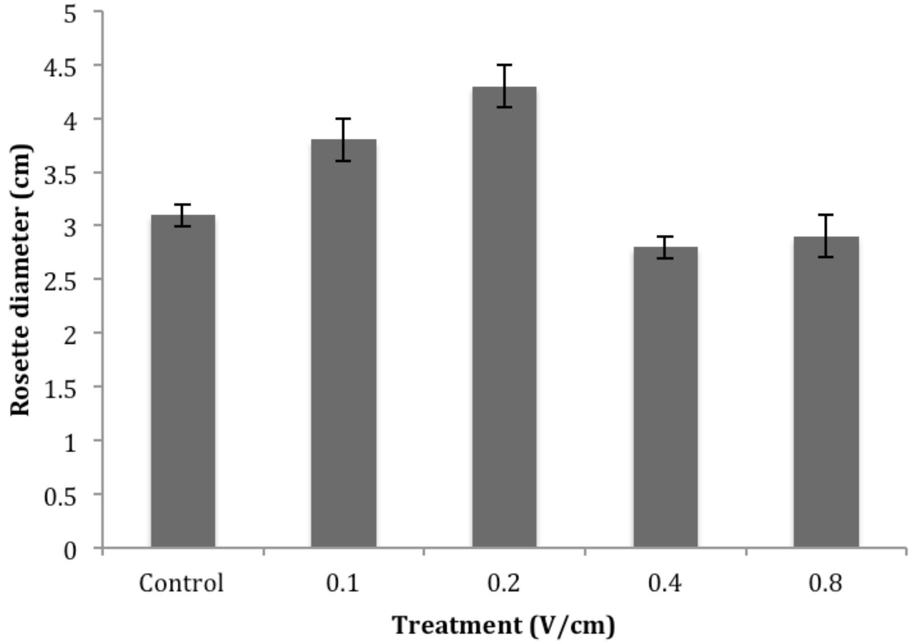

Figure 3. Rosette diameter of plants of *Arabidopsis thaliana* measured six weeks after treatment with low intensity DCEF.

After this step, we decided to evaluate the behavior of the development in three different areas of the electro-culture cell, so we analyzed the area close to the IrO2-Ta_2O_5 | Ti anode (CA), the middle of the cell (MA) and in the area close to the Ti cathode. As previously reported (Oszust et al. 2013), when this set of electrodes is used, the middle area of the cell is the most efficient for the development of living organisms, followed by the area close to the IrO2-Ta_2O_5 | Ti cathode. This might be due to the increase of organic carbon sources in these areas and in the mobilization of ions in the electro-culture cell as well as the increase of living bacteria and fungi in these areas after the application of the DCEF.

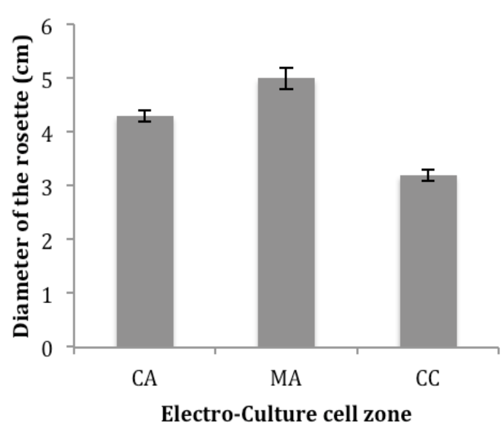

Figure 4. Diameter of the rosette of *Arabidopsis thaliana* plants after the exposure to low intensity DCEF electro-culture.

In Figure 5, we can observe the development of Arabidopsis thaliana plants after treatment with electro culture, and it shows that the plants treated with a 0.2 V/cm using this electrode arrangement presents better development than the plants with no treatment (control) and the plants treated with other DCEF as described in the measurements presented above.

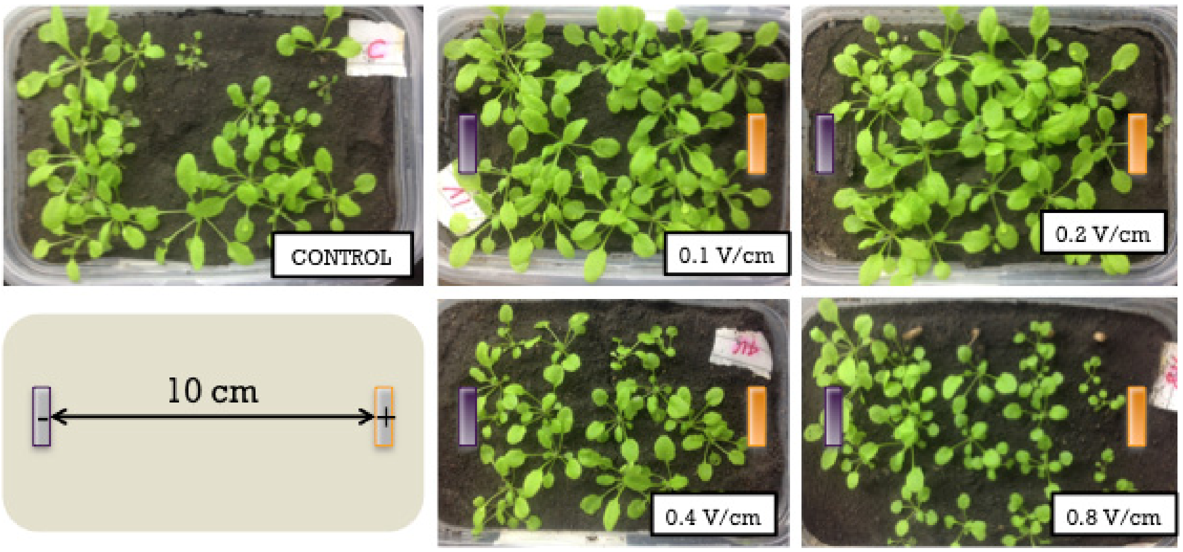

Figure 5. *Arabidopsis thaliana* plants treated with different low intensity DCEF using IrO2-Ta_2O_5│Ti and Ti electrodes as an electro-culture treatment.

CONCLUSIONS

The use of a dimensionally stable anode (IrO_2-Ta_2O_5│Ti) and a Ti cathode to generate a low intensity direct current electric field increases the germination rate and germination velocity of *Arabidopsis thaliana* seeds as well as the growth of plantlets in early stages of development. We suspect that this effect is due to the movement of ions in soil in the process called electro migration, which can lead to bioavailability of these ions, first for the germination of seeds by helping them to break dormancy, and in early stages of development helping the plantlets to synthesize organic molecules necessary to increase growth. It is imperative to complete experiments that can help us understand this effect.

ACKNOWLEDGMENTS

The authors want to thank Consejo Nacional de Ciencia y Tecnología de los Estados Unidos Mexicanos (CONACyT) for their financial support granted for the development of this investigation and Centro de Investigación y Desarrollo Tecnológico en Electroquímica, S.C. We also want to thank Dr. Stefan De Folter at LANGEBIO, Mexico for the kind donation of the vegetal material used in this project, and to Curtiss Palin, Peace Corps volunteer at CIDETEQ for the English revision of this manuscript.

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