Effect of Negative Electrostatic Field Treatment on Germination of Seeds Soaked GA3

Antonio Piras, Zhibin Gui, Limin Qiao, Kai Gui, Yongxiang Fan

Abstract— Tree seed germination improvement of tree species with shallow dormancy may represent an effective process to enhance restocking of forests and woodlands that have been depleted. Although sometimes conflicting results have been obtained, some studies showed the beneficial effects of applied static electric field (electrostatic field) on seed germination and seedling growth. We think that the different treatment effects reported maybe depended on the dosage, exposition time, process and vigor index of the seeds. We previously showed improved tree seed germination of pine seeds by positive electrostatic field treatment using our apparatus and procedure, and the purpose of the present study was to investigate the effects of the negative electrostatic field treatment on germination, using the same experimental procedure. The results indicated that the negative electrostatic field treatment on pine seeds soaked with 100 ppm of gibberellic acid (GA3) is not so effective as the positive one, with slightly improved germination percentage at the electrical voltage of -500 kV/m 10 min, and increasing the intensity of the negative electrostatic gradient resulted in a reduction of seedling growth.

Index Terms—Electrostatic Field, GA3, Germination, Seedling Growth, Soaked Seed.

I. INTRODUCTION

Over the last six decades the effects produced by the direct interaction of electrostatic fields on biological systems have represented a great topic of debate, especially since the application of electricity in agro-forestry science has been considered in order to evaluate the possibility to improve germination of seeds, seedling growth, plant growth, quality of seedlings, and quality and yields from cultivation through insect control [1-3]. Murr [2,4] showed that high intensity electric fields appear to inhibit development in grass seedlings. Conversely, Jorgenson and Priestley [5], and Shibusawa and Shibata [6], observed that electric field increased plant growth. Krueger *et al*. [7] reported reduced time of germination under exposure to positively or negatively ionized air in seeds of *Avena sativa*. Yang and Shen [8] reported that electrostatic field treatment of dry seeds of *Sorbus pohuashanensis* resulted in fast germination at room temperature under light conditions, possibly through an increased water absorption ability of seeds. Also, combined treatment of electrostatic field with cold stratification could enhance percentage of seed germination (to 42.2%), promote seedling height growth, and could raise contents of total chlorophyll, soluble protein, and total soluble sugar in leaves.

Seed soaking time had a significant effect on seed relative electroconductivity, seed germination under light, soluble protein and total soluble sugar content of seedling leaves. Electrostatic field intensity exerted a moderate effect on these indexes, and the exposition time only had no evident effect except for the total chlorophyll contents.

Sidaway [1] observed inhibition of plant development by positive electrostatic field, and the nature of the plant response to the applied field depended on the sign and not merely by the presence of the field itself. Furthermore, light reduced the effect of positive polarity on plant development, compared to dark condition. A source of electrons provided by an electrical conductor with negative charge, was able to extend the viability of seeds during artificial aging [9]. Such anti-aging effect may be due to the reduction of the free radical attack species to the subcellular structures and biological macromolecules, counteracted by the electron donor negatively charged conductor.

On the basis of this assumption, and considering that many investigators often reported contradictory results as showed by Lund [10], we have studied the application of electrostatic field in forestry science for many years in order to improve germination of tree seeds, using different electrical methods, single and multiple factor treatments. Through the experience gained in this field of research by our group, we think that in many of the experiments conducted over the past years, the electrical field intensity was too weak and its distance from biological matter was too far; moreover the experimental designs were not standardized, because many studies did not solve insulation, dosage, experimental condition, and so on. Based on the above problems, it is difficult to get ideal results and conclusions. Therefore, we decided to investigate how the electrostatic treatment determines changes in seed vigor index through our apparatus and methods [11,12], also, analyzing separately the effects generated by different electrostatic charges.

Pine seeds are widely used for reforestation in mountain areas by aerial seeding. Sowing by aerial seeding, a broadcast method of seeding, allows an efficient coverage of a large area in the least amount of time to a relatively low cost. After sowing seeds, 30-100 mm of rainwater over 3 to 5 days with a suitable temperature, 30 to 40% of seeds can germinate and become young tree seedlings. Otherwise, 50 to 70% of the seeds on the surface were quickly eaten by birds, insects, and rodents within 2-3 weeks. In order to improve germination percentage, time of germination and development of young seedlings, soaking of seeds is an effective practice to accomplish these targets.

II. EXPERIMENTAL PROCEDURE

Gibberellins (GAs) are important growth regulator factors that induce seed germination, promotion of radical elongation,

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and mobilization of endosperm reserves during early embryo growth. Moreover this family of plant hormones influences dormancy, flowering and various other processes [13,14]. Presoaked seeds with GAs are widely used for agro-forestry production, they cause rapid germination of many highly dormant seeds, improve quality and increase fruit production.

In our study, we used 100 ppm of gibberellic acid (GA3), 3 Kg solution, and 0.5 kg of soaked seeds of *Pinus tabuliformis* Carr. Seeds of pine were placed into cold water containing 100 ppm GA3 for 24h, then removed from the water and drained for 5 minutes, and subsequently divided into four sublots. One sublot was retained as control and the other three were treated under a negative electrostatic field of -300 kV/m, -500 kV/m, and -700 kV/m, respectively. Treatment time was 10 minutes. The electric field gradient E was calculated as same as Murr [2], and the circuit for high voltage generator is described in Fig.1. Treatment electrodes were two horizontal, circular metal plates of 300 mm in diameter and 4 mm thick, separated and maintained in parallel orientation by three insulating posts, 3 cm in length and 2 cm in diameter. The top one was the negative electrode; and the bottom one was the positive electrode (Fig. 1).

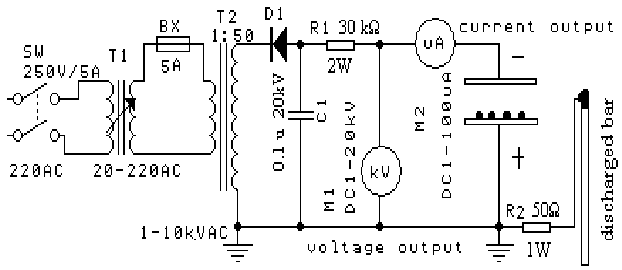

Fig. 1. Circuit for high voltage generator

Seeds of pine were placed on the top surface of the bottom plate. In the high voltage circuit, positive and negative electrodes were connected to the positive and negative output terminals with insulating high-voltage line, respectively, of 1 to 10 kV high-voltage generator and adjusted output voltage with a knob. The positive electrode must ground when use a negative electrostatic treatment. To check and regulate the voltage between electrodes a kilovolt meter was used, and the extremely small electric currents passed through seeds were measured by a microammeter. For the treatment, seeds were placed in a single or more layers on the top of the positive plate with 50 to 100 g containing about 1000 to 2000 pine seeds at a time. The distance between the surface of the seeds and the negative electrode is decided by electrical field intensity. After treatment was over, before removing the treated seeds, plates were discharged with an insulated conducting rod for safety. Presoaking GA3 seeds control sample was not treated and kept at least 3-4 meters away from any electrical source. After treatment was finished, four replicates of 100 seeds each of treated and control sublots were randomly selected and placed into glass culture containers with sandy medium. They were watered in timely fashion and germinated at 25 ºC constant temperature with full photoperiod. The temperature of the seeds was measured through a temperature sensor and was not changed during and after the seed treatment. To monitor temperature with a more accurate method, sensor networks can be used. Research about sensor networks can be found in [15,16]. When the length of the radical was equal to the length of the seed, seeds were considered to have germinated. Germination percentage was calculated according to the rules for seed testing (ISTA, 1996) [17]. For data analysis, a standard deviation was used as to express the degree of variation, and the F-test was used as to examine differences among tested samples.

III. RESULTS AND DISCUSSION

Table I summarizes the germination percentage at 10 days of the wet lot of pine seeds soaked 100 ppm GA3 under negative electrostatic field treatment, compared to control seeds (not electrically treated). The results show that treatments of -300 kV/m, -500 kV/m, and -700 kV/m for 10 min., slightly improved germination percentage from 45.0% to 51.7%, 52.6%, and 49.0%, compared with control. Although the percentage differences between treatments and control are not statistically significant, the negative electrostatic field treatment of soaked 100 ppm GA3 pine seeds improves germination percentage, but the experimental results are not good as those previously obtained from positive electrostatic field treatment [12]. The exposition time used in the negative electrostatic field treatment was the same (10 min.), but different gradients were tested. The negative electrical field appears to be not so fast and effective as the positive one, the more effective electrical gradient was -500 kV/m 10 min. The range of the temperature variation in the seeds before and after the treatment was little.

In Table II are shown the effects of the negative electrical treatment on root length of young seedling after germinated at 10 days. Pine seeds were treated with -300 kV/m, -500 kV/m, and -700 kV/m for 10 min. Within 10 days germination period, the root length of treated young seedling decreased, compared to control sample. The difference of seedling heights ranging from 9.4 cm to 8.93 cm, 6.39 cm and 6.88 cm, comparing with the control one. The general trend indicates that increasing the intensity of the negative electrostatic gradient results in a reduction of seedling growth. Also, electrostatic field force altered vigor index of seeds. Seed treatment includes many methods, such as low and high frequency electrical fields, magnetic field, X-ray, microwave field, infrared ray, and electrostatic field. According to our recent observations [11,12] compared to other methods, electrostatic field represents a fast, effective and economic method for the pretreatment of thin coat seeds with shallow dormancy.

Table I. Effect of negative electrostatic field treatment on germination percentage at 10 days of *Pinus tab.* Carr. seeds soaked 100 ppm GA3.

Test samples	Germination percentage (%)	Percentage difference
-300 kV/m 10’	51.7	6.7
-500 kV/m 10’	52.6	7.6
-700 kV/m 10’	49.0	5.0
Control	45.0	0

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Table II. Effect of negative electrostatic field treatment on root length at 10 days of *Pinus tab.* Carr. seeds soaked 100 ppm GA3.

Test samples	Mean root length (cm)	Root length difference
-300 kV/m 10'	8.93	-0.47
-500 kV/m 10'	6.39	-3.01
-700 kV/m 10'	6.88	-2.52
Control	9.4	0

The direction and magnitude of electrostatic field did not change during the exposure. Treated seeds were in contact with the electrode. The temperature of the seeds was kept changeless during and after seed treatment. Using sensor networks it is possible to measure temperature from different points [18,19]. Exposure to high intensity electrostatic field to promote germination could be an effective substitute for the chemical treatments, wich are quite expensive and potentially harmful to soil. In all tissue cells of the seed there are small electric potential differences. During electrostatic field exposure, positive ions in the seed tissue would move and be concentrated on the surfaces of each seed. Positive ions would transfer from original positions toward the negatively polarized electrode. The external electric field induces an inner electric field in the seed, with a opposite direction respect to the external electric field. Therefore the magnitude of the inner electric field in the seed tissue will depend on the magnitude of the external electric field, according to conducting electrical theory of physics.

Comparing the methods of Edwards [3], Jorgensen and Priestley [5], Sidaway [1], Murr [2,4,20], Krueger et al. [7], we think that they used weaker electric fields and longer times for the stimulation period. They placed samples into a small space that was formed by plate electrodes in a higher moisture condition. Pammenter et al. [9] used a lower electrostatic gradient, as that of 140 V existing in the earth's surface. Provision of a -500 kV/m static electric charge for 10 min. on *Pinus tab.* Carr. seeds soaked 100 ppm GA3, was the suitable dosage of negative charge that has shown to increase weakly the percentage of germination, but at the same time, dramatically reduced seedling growth, compared to other dosages and control.

These and our previous results suggest to consider a method that takes into account different variables such as electric charge, dosage, pretreatment, etc., when using electrostatic field treatment for forestry production.

IV. CONCLUSION

This study indicates that negative electrostatic field treatment practically has no effect in improving percentage germination of seeds of pine, *Pinus tabuliformis* Carr. soaked with 100 ppm of GA3, moreover increasing the negative gradient voltage reduced seedling height and root length during seedling development. Probably, enhancing the intensity of the negatively charged conductor may determine electrical and biochemical changes that reduce the ability of seed development at tissue, cellular, and subcellular levels. The application of a specific procedure that takes into account the standardization of parameters such as electrostatic charging, dosage, exposure time and any pretreatment of the seeds, results decisive for improvement tree seed germination by electrostatic field treatment.

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