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Stimulation of *Pinus tropicalis* M. seeds by magnetically treated water

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A b s t r a c t. Magnetically treated water (MTW) was used to stimulate the germination process of *Pinus tropicalis* M. seeds. This species of *Pinus* is an endemic of the western part of Cuba and at present is threatened due to a visible decrease that has been detected in its populations. The main cause of this decrease is the low seedling production at nurseries, since the germination percentage of this species rarely exceeds 50%. To carry out our work, normal water (water + ions) was treated using 1200 G isotropic strontium magnets. This was done in two forms: statically and dynamically. The MTW was applied to the samples as irrigation water and as water for imbibition during 24 h. The results showed an increase in the germination percentage up to 70-81% with respect to the control samples (43%) as well as greater seedling growth after germination. The best results were obtained while the treated water was applied dynamically and statically with initial inbibition. In this last case the germination percentages reached 81%, which represents an increase of 88 % compared to the control samples (43%).

K e y w o r d s: pines, seeds, germination, magnetic treatment, irrigation water

INTRODUCTION

The use of magnetically treated water (MTW) for irrigation in agriculture takes an important place in the list of environmentally clean methods *eg* Bugatin *et al*. (1999), Radeva and Mamarova (1988), Rokhinson *et al*. (1994), Sala (1999), and Tian *et al*. (1989). It consists in exposing water to magnetic fields so that the intensity and configuration of the field can vary depending on its final use. Effects of magnetic fields on biological material have been studied extensively (Chou, 2007; Tenuzzo *et al*., 2006; and Trebbi *et al*., 2007). Specifically, MTW has been used for crop irrigation, the results reported showing better germination percentages, larger growth and better yields (Alexander and Ganeshan, 1990; Chauhan and Agarwal, 1977; Dayal and Singh, 1986; Hilal and Hilal, 2000; Kavi, 1989; Namba *et al*., 1995; and Nargis and Thiagarajan, 1996). Results show that MTW is more easily absorbed by the seed tissue and in this way it can stimulate internal metabolic processes which are conducive to germination. On the other hand, according to experiments realized by Belov *et al*. (1988), Henkenius and Retseck (1992), and Yakovlev and Kolobenkov (1976), MTW also stimulates the growth of seedlings, reported as an increase of height, size and number of leaves.

The *Pinus tropicalis* M. is an endemic of the western part of Cuba and has an enormous importance for the repopulation plans in the region, due to its ecological characteristics. In recent years a visible decrease in the populations of this species has been detected, provoking its declaration as a threatened species by the International Union for Conservation of Nature (SSC/UICN, 1995). The main cause of the decrease in its population is the low seedling production, since the germination percentage of this species rarely exceeds 50%, according to Matos (1963) and Samek (1967).

In the present work we applied MTW obtained statically and dynamically as an alternative seed treatment to stimulate the germination process of *Pinus tropicalis* M. seeds in order to increase the present results for the germination percentage at nurseries (under 50%).

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MATERIALS AND METHODS

Water treatment

In spite of many devices that have been created for the treatment of irrigation water, we preferred to build up our own magnetic equipment in laboratory conditions. Figure 1 shows the experimental arrangement for statically treated water. A cylindrical plastic container (8.7 cm in diameter) with normal water was exposed to four isotropic permanent magnets of strontium, arranged in bipolar configuration (facing the magnetic poles). The distances between the magnets were 7 cm. On the right side of Fig. 1 we have shown the shape and dimensions of the magnets used. The intensity of the magnets was 1200 G at the surface. For the magnetic treatment the water remained in the container for a period of 48 h. We have called the aforementioned treated water as magnetically treated water (statically).

Figure 2 shows the experimental arrangement for dynamically treated water. A normal water flow was created, of 1.5 m s^{-1}, through a cylindrical plastic hose (1 cm in diameter). Four magnets of the same type as in Fig. 1 were arranged in bipolar configuration (Fig. 2). They were placed at 20 cm distance from each other as shown in the figure. The water passed through the hose only one time. We have called the afore mentioned treated water as magnetically treated water (dynamically).

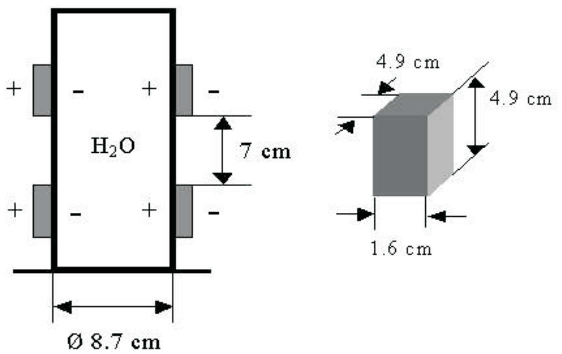

Fig. 1. Experimental arrangement for statically treated water. Blocks in grey represent the isotropic strontium magnets.

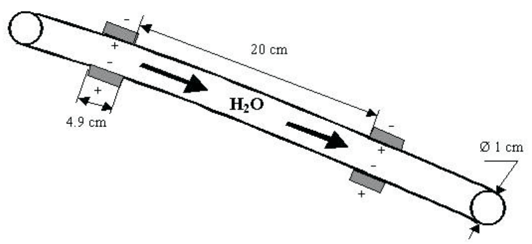

Fig. 2. Experimental arrangement for dynamically treated water. Blocks in grey represent the isotropic strontium magnets.

The water used in the experiments was not pure water but a dissolution (water + ions, pH=8.6 and electric conductivity 8 μs cm^{-1}). Magnetically treated water properties, such as vibration modes and electrolytic potential, are changed in the presence of dissolved oxygen which presents weak paramagnetic properties (Otsuka and Ozeki, 2006). This fact makes possible a change in the solubility and surface tension of a dissolution of water plus ions (Golovleva *et al*., 2000; Srebrenik *et al*., 1993), which leads to changes favouring the increase of the germination percentage and growth of seedlings (De Souza *et al*., 2006). In this work, we focus on the use of normal water (water + ions), also due to practical reasons, since this is the type of water commonly used for imbibition and irrigation at *Pinus* nurseries (Samek, 1967).

Selection of samples

We established four seed treatments in the experiments, as well as a control sample as a comparison pattern. The treatments were the following:

a) Initial imbibition of 24 h in normal water and irrigation with normal water at the moment of sowing. We will refer to this treatment in the figures as Control samples.

b) Initial imbibition of 24 h in normal water and irrigation with MTW, statically, at the moment of sowing. We will refer to this treatment in the figures as MTW (statically).

c) Initial imbibition of 24 h in MTW, statically, and irrigation with MTW, statically, at the moment of sowing. We will refer to this treatment in the figures as MTW (statically with imbibition).

d) Initial imbibition of 24 h in normal water and irrigation with MTW, dynamically, at the moment of sowing. We will refer to this treatment in the figures as MTW (dynamically).

e) Initial imbibition of 24 h in MTW, dynamically, and irrigation with MTW, dynamically, at the moment of sowing. We will refer to this treatment in the figures as MTW (dynamically with imbibition).

A number of 50 samples of 100 seeds for each seed treatment were randomly drawn from the seed lot. Seeds were germinated in the laboratory according to test prescriptions of the Association of Official Seed Analysts (1981). Each sample was irrigated one time with 30 ml of water (the kind of water depended on the seed treatment) at the moment of sowing. Seeds were placed in 100 g of sterilized silica sand.

RESULTS AND DISCUSSION

The germination percentages for a period of 45 days of germination are shown in Fig. 3, along with the standard deviation error boxes. It can be noticed that all seed treatments yielded a higher germination percentage compared with the control sample. The change in the germination

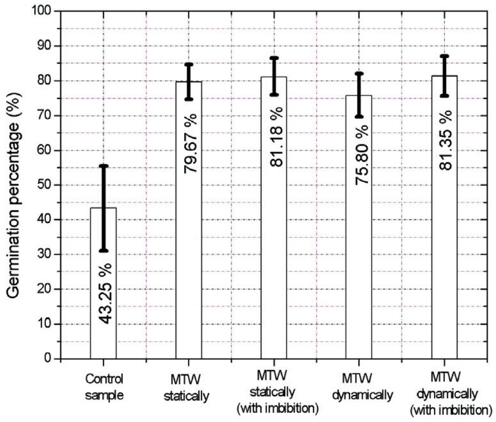

Fig. 3. Germination percentage for each seed treatment.

percentage is very well seen from the figure, since the confidence intervals do not overlap. In this sense we also performed an ANOVA test which yielded significant differences among the treatments for a significance level of 0.05%. In order to locate the differences between the mean va lues in the treatments we realized a Duncan test (Table 1). Results for this test show that there are significant differences between the control sample and the rest of the treatments, and also between the MTW, dynamically, and the rest of the treatments. As statistical software, we used the professional package SPSS version 10.0 for Windows (1999). The highest values of the germination percentages were reported for MTW, statically, (81%) and dynamically, (81%) with initial imbibition. In general, the relative increase of the germination percentage ranged from 75 to 81% with respect to the control samples.

Correct explanation of the interaction of MTW and biological systems is a very complex issue nowadays and most experiences are only in practice, anyway some experimental results have pointed to some hypothesis. Some studies have shown that when normal water (water + ions) is exposed to magnetic fields some of its physical and chemical properties are changed, such as: surface tension, conductivity, solubility of salts, refractive index and pH, which has been shown by Aidárov *et al*. (1985), Smikhina (1981), and Srebrenik *et al*. (1993), Yakovlev and Kolobenkov (1976). Figure 3 shows the great increase in the germination percentage, which can be attributed to the hypothesis that as MTW changes its properties, like solubility and surface tension, it can penetrate much easier to the inner parts of the seed structure, making possible a better absorption which starts up the metabolic processes that lead to germination.

T a b l e 1. Results for the Duncan test in the germination percentage (number of samples = 40)

Treatments	Mean (%)	Homogeneous groups
Control sample	43.3	A*
MTW (statically)	75.8	B
MTW (statically with imbibition)	79.9	C
MTW (dynamically)	81.2	C
MTW (dynamically with imbibition)	81.4	C

*Capital letters indicate the homogeneous groups between which there are significant differences for a significance level of 0.05%.

In spite of slight differences observed among seed treatments, the cases of MTW treated dynamically and statically (with imbibition) presented the best values of the germination percentage. This behaviour has to do with the great importance of water absorption prior to germination. As it was explained in the paragraph above, the MTW can be absorbed more easily by the tissues than normal water, breaking the dormancy and shortening the latency period.

The daily germination was registered for a period of 45 days. In this respect, Fig. 4 shows the daily accumulative seed counts. Results illustrate a visible increase in the number of germinated seeds. The seeds under the influence of MTW germinated faster in comparison to the control samples. The MTW, statically and dynamically with initial imbibition, showed the best behaviours. All seed treatments yielded a better germination response in time and in number of germinated seeds than the control samples. It is also seen that MTW, dynamically and statically, with imbibition, presented the best behaviour. It is also visible that on day 17 there is a change in the germination rate when the control sample curve separates from the rest. These last keep the initial germination rate up to day 34. This shows that treatments maintain the germination conditions longer with respect to the control samples. This result also reaffirms the idea that MTW can be absorbed more easily by the seed tissues than normal water (not treated).

In order to test the influence of the MTW on the seedling growth after germination, we have represented, in Fig. 5, histograms of seedling lengths for three periods of time, from: 1 to 15, 16 to 30 and 31 to 45 days. Seedlings counts per interval of length were taken at the end of each period and over all germinated seeds. The seedlings were taken out of the samples after the measurements. For the first period there can noticed a shift of the counts bands to the higher lengths in all seed treatments with respect to the control sample. The same behaviour is observed in the second period, but less pronounced, and for the third period almost no difference was noticed.

These histograms revealed the influence of the MTW on the growing of seedlings for all treatments in comparison to the control samples (Fig. 5). This could be explained by the great absorption of MTW by the seeds in the first and second period after germination, when MTW is rapidly used in the seedling growth process.

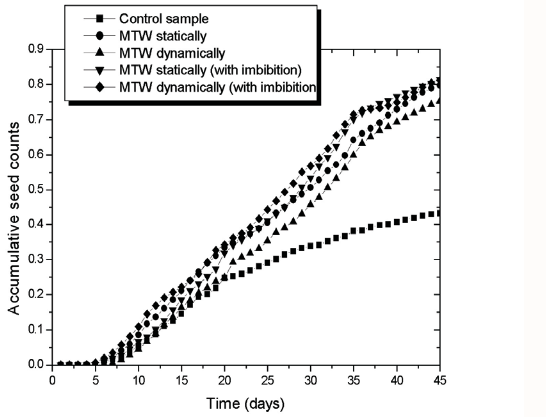

Fig. 4. Curves of the accumulative seed counts for each seed treatment.

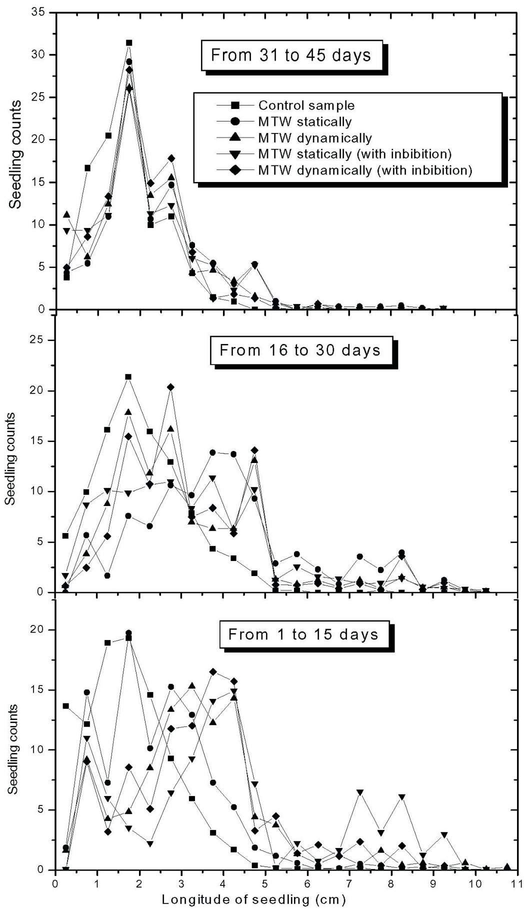

Fig. 5. Counts of seedling lengths for three periods of time after germination, from 1 to 15 days, from 16 to 30 days, and from 31 to 45 days. The behaviour for each seed treatment is shown.

From the accumulative daily germination (Fig. 4) and the histograms of seedling lengths (Fig. 5) we have built up Fig. 6 which illustrates the mean length of the seedlings as an average over three periods (from 1st to 15th, 16th to 30th and from 31st to 45th) versus their age in days. All seed treatments presented a greater growth rate per day than the control samples. The best results correspond to the MTW, dynamically (with imbibition), followed by MTW, statically (with imbibition), MTW, dynamically and MTW, statically. It is now better noticed that the best results for the seedling growth were revealed for the cases of MTW, statically and dynamically (with imbibition), being much better in the last case.

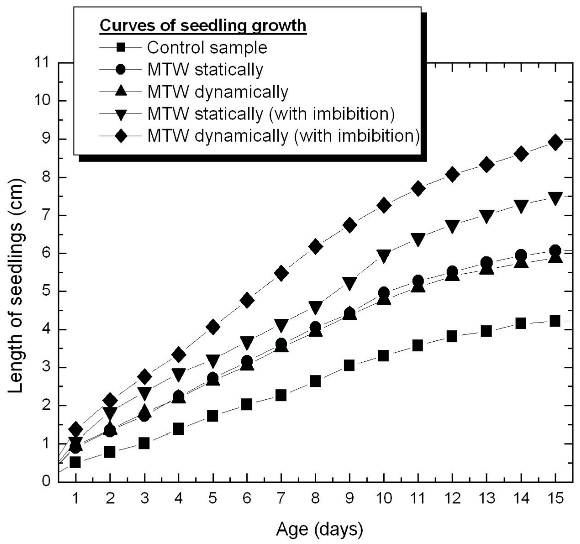

Fig. 6. Average length of the seedlings versus the age. The behaviour for each seed treatment is shown.

CONCLUSIONS

1. The germination percentage of *Pinus tropicalis* M. seeds was increased from 43% (control samples) to 75-81% (seed treatments).

2. Magnetically treated water largely influences the seedling growth. In general the best results were found for the cases of MTW, statically and dynamically, with initial imbibition.

3. The basic mechanism consisted in the change in the physical and chemical properties of water (plus ions) under the influence of magnetic field, such as: surface tension, solubility of salts, which allowed the MTW water to be better incorporated to the inner parts of the seeds, thus increasing the probability of germination.

4. Those simple and basic experiments could lead to a better understanding of the influence of MTW on the germination process and in this respect create scientific bases of upcoming technologies.

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