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Adventitious rooting induction of mulberry (Morus sp.) cuttings by sound frequencies and spermidine at different times

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Abstract. The use of sound frequencies has been reported to influence physiological and morphogenic responses in plants. Thus, the present work sought to study the effect of different sound frequencies on the adventitious rooting of mulberry cuttings (*Morus sp.*). Two experiments were carried out, the first consists treatment of cuttings by sound frequencies of 300 Hz, 1000 Hz or 0 Hz in two seasons - spring and autumn. For the second experiment, the frequency of 1000 Hz was used combined with inoculation of spermidine. After 60 days of inoculation, the parameters of the number and length of roots were evaluated, as well as the bud's development in the aerial part. The treatment of cuttings collected during the autumm with a sound frequency of 1000 Hz results in an increase in the number of roots in relation to the control. In the cuttings collected during the spring there was no increase, either in the exposure with frequency of 1000 Hz, or in the treatment with spermidine, alone or in combination with frequency. The treatment of cuttings with sound frequencies of 1000 Hz demonstrates the potential for an increase in the adventitious rooting response.
Keywords: biomechanics; mecanosensitivity; polyamines; propagation

Introduction

Cuttings are the main technique used for the vegetative propagation of tree species, allowing a genetic gain in a short period of time, with the implantation of orchards of selected individuals (Wendling et al. 2014). The use of vegetative propagation allows the maintenance of agronomic characteristics of the parent plant, the reduction of the juvenile phase and the obtaining of uniform production areas, in addition to being a cheap and simple technique (Wendling et al. 2014).

Cutting success depends mainly on the rooting ability to each species (Emer et al. 2016). Rhizogenesis is a complex anatomical and physiological process, which involves the entire expression of plant totipotentiality, leading the cells to de-differentiate and lead to the formation of adventitious roots (Da Costa et al. 2013). A number of factors can affect the rooting in cuttings, such as: age and physiological condition of the matrix plant; seasson of the year; sanity; light; temperature and type of substrate. (Steffens & Rasmussen 2016).

Mechanical frequencies, including sound frequencies, are one of the environmental components that have been ignored over time in their impact on plant development. Several authors claim that different mechanical frequencies can be felt by living organisms and influence their development (Leblanc-Fournier et al. 2014; Fernandes-Jaramillo et al. 2018; Jung et al. 2018; Geitmann et al. 2019; Gosselim 2019; Joshi et al. 2019; Kim et al. 2020). The use of mechanical frequencies that affect the development can be an interesting strategy in inducing the cuttings rooting.

Analyzing the factors responsible for the expression of totipotence and cellular fate, it is observed that the combination of chemical and physical factors play essential roles (Mirabet et al. 2011; Moreno et al. 2017; Marsollier and Ingram 2018). Sound frequencies can alter transport dynamics, affecting the concentration of phithormones, in addition to impacting the activation and inactivation proteins membrane (Da Costa *et al*. 2013; Basu & Haswell 2017; Toyota et al. 2018). Phytohormones have a classic role in chemical signaling, and some compounds have been reported recently as to their importance, with spermidine being one of those compounds.

Based on these, this work aimed to study the effect of different sound frequencies alone and in combination with spermidine in adventitious rooting. The species used in the experiment was mulberry (*Morus sp.*) since the species has a good expression of totipotence, with the formation of roots in cuttings without the need for previous treatment.

Methods

Use of sound frequencies in mulberry (Morus sp.) cuttings at two times of the year

Mulberry semi-woody cuttings (*Morus sp.*), with approximately 20 cm, with a pencil thickness and about 3 to 5 buds were collected from well-established tree from different portion of each branch, setting up experiments in two seasons - autumn 05/19/2017 and spring 10/03/2017.

The cuttings were previously subjected to different sound frequencies, by Function Signal Generator - VC2002 - Victor®, which are: 0 (T1) control; 300 Hertz (T2), and 1000 Hertz (T3), with an exposure time of 60 minutes, with 12 repetitions, totaling 36 experimental units. The frequencies exposure occurred in an acoustic isolation box containing an emitter coupled to a frequency generator.

After exposure to the frequencies, the cuttings were inoculated in polystyrene trays containing an alternative substrate (cellulose pulp and vermiculite 1: 1) and kept in a nursery with 50% shade, with daily irrigation.

The cuttings were evaluated after 60 days, for the formation of buds developed (BD), swollen buds (SB), number of roots(NR) and size roots (SR).

Use of sound frequencies and spermidine in the mulberry (Morus sp.) cuttings rooting

As previously described, semi-woody cuttings of mulberry (*Morus sp*), with approximately 20 cm, with the thickness of a pencil and about 3 to 5 buds, with absence of leaves and fruits, were collected on 10/17/2019

Ten cuttings were used for each treatment, which were: absence of frequency and spermidine (Merck®), control (T1), only frequency exposure (1000 Hz) (T2), only inoculation with spermidine (T3), inoculation with spermidine and later use of frequency (Spd > Hz) (T4), use of frequency and later inoculation with spermidine (Hz> Spd) (T5) and use of frequency plus inoculation of spermidine simultaneously (Hz + Spd) (T6).

The exposure of the frequencies occurred in an acoustic isolation box containing an emitter coupled to a frequency generator for 60 minutes, and/or immersion in spermidine was for 30 minutes, at a concentration of 500 μM.

After treatments, the cuttings were inoculated in polystyrene trays containing alternative substrate (cellulose pulp and vermiculite 1: 1) and kept in a nursery with 50% shade, with daily irrigation.

The cuttings were evaluated after 30 days, for the formation of buds developed (BD), swollen buds (SB), number of roots (NR) and size roots (SR).

Statistical analysis and design used

For both experiments, a completely randomized design (DIC) was used, the data were subjected to analysis of variance and Tukey's test, both with a significance level of 5% (α = 0.05). For this, the statistical program Sisvar was used.

Results and discussion

Use of sound frequencies in mulberry cuttings (Morus sp.) in two seasons of the year

The cuttings submitted to the sound frequencies presented different responses for the analyzed parameters, varying the response according to the season of the year.

In the experiment initiated in autumn (May), when analyzed number of roots (NR) was observed by analysis of variance significant effect. According to the Tukey test (α = 0.05), the use of a frequency of 1000 Hz resulted in a higher average in terms of the number of roots (9.08 roots), which is statistically different when compared to the use frequency of 0 Hz (control), which presented an average of 2.08 roots (Figure 1). As for the experiment carried out in the spring (October), when the parameters related to root formation were analyzed, it was observed that there was no significant effect between treatments.

In work carried out by Alcantara et al. (2007), it was observed that a reduction in the rooting of cuttings of *Pinus taeda* L. during the spring period. On the other hand, Pizzato et al. (2016) and Souza et al. (2019), it was observed that the cuttings collected during the spring were more successful in rooting *Hibiscus rosa-sinensis* and *Campomanesia adamantium*, respectively.

Adventitious rooting is a complex process coordinated by several factors. The concentration of auxins in the tissue is one of the triggering factors to induce the formation of the root primordium, and subsequently the concentrations of auxin must reduce in order to have root elongation (Husen et al. 2017). The concentration and carbohydrates composition plays an important role not only in energy but also in signaling, acting in the regulation of gene expression (da Costa et al. 2013). Fukuda et al (2018) observed that the carbohydrate contents of cuttings are a more important factor in the rooting induction.

An important factor to be considered is that the mulberries bloom in the spring period, with fruiting during the summer (California Rare Fruit Growers 2020). When we compare the number of roots formed in the cuttings of the control group in the different seasons of the year, we observe that the spring cuttings have greater rooting, and the cuttings treated with frequencies do not differ from the control. The increase in rooting in the control treatment is possibly related to the alteration in the partition pattern of photoassimilates and to the breakdown of sugars (Janecek & Klimesova 2014; Jung et al. 2018). This result highlights the role of sound frequencies as a stimulator in the formation of roots in periods when it is naturally not conducive.

Adventitious organ formation involves biophysical aspects, since the formation of a new organ generates a new balance of forces (Marsollier

& Ingram 2018). It has been shown that sound frequencies can cause significant changes in cell number and wall flexibility, causing a considerable effect on cell wall turgidity and improving the relative water content (Sassi & Traas 2015). Sound frequencies can act on the membrane leading to changes in the signaling, modifying the transport flow and dynamics of auxins, as well as acting on microtubule dynamics, influencing the root formation (Druege et al. 2019; Haas et al. 2020).

Apparently, specific sound frequencies trigger specific responses. Gosh et al (2016) observed different physiological responses in different plants under different frequencies, changing patterns of expression in transcriptome, proteomics and in the phytohormone profile.

In the parameters related to the aerial part - swollen buds (SB) and developed buds (DB) - there was no significant effect of the treatments, indicating that there is no difference between the frequencies used, both in the first evaluation period and in the second period.

Use of sound frequency and inoculation of spermidine in mulberry cuttings (Morus sp.)

Taking into account the first experiments, in which the frequency of 1000 Hz led to an increase in the number of roots, but that this response was not repeated in the period of october, we tried to use the sound frequency most effective associated with chemical treatment with spermidine.

Polyamines may play an essential role in adventitious rooting (Tsafouros & Roussos 2020). The isolated application of spermidine increased the formation of sprouts and roots of cuttings in Christmas pines (Tang et al. 2004). Wang et al (2020) demonstrated that spermidine affects rooting by changing the pattern of phytohormones and regulating gene expression.

For the autumn, cuttings from the control group should have a low concentration of sugars or a low hydrolysis capacity, corroborating with data that suggest that the low allocation of carbohydrates for root formation may be one of the limiting factors for rooting (Da Costa et al. 2013; Husen et al. 2017).

When analyzing the variables of the rooting and the aerial part, it was observed that there was no significant effect between treatments, according to the analysis of variance.

Despite reports about the role of spermidine in adventitious rooting, its application did not result in an increase.

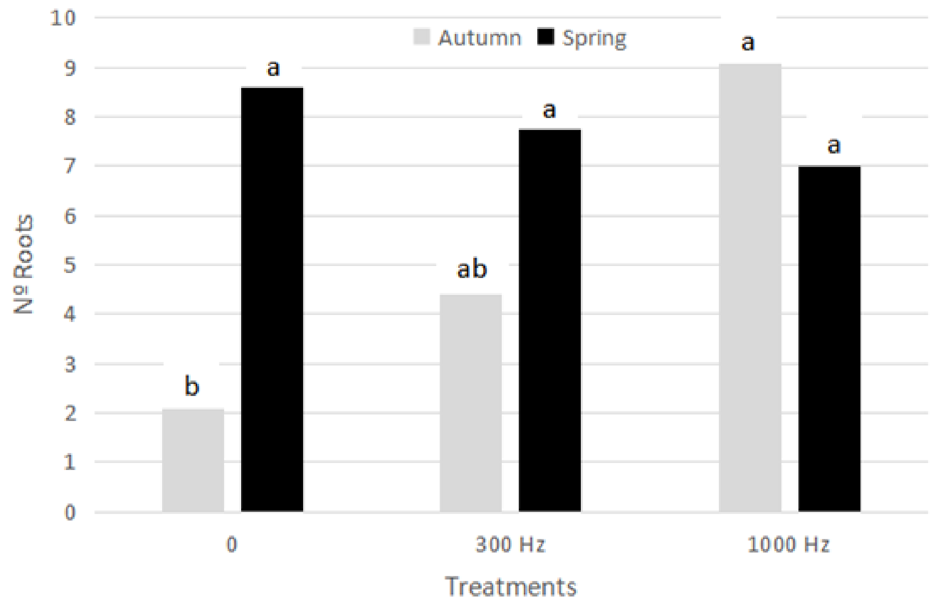

Figure 1. The number of roots of mulberry cuttings submitted to differents sounds frequencies (0, 300 and 1000 Hertz) and different seasons (autumn or spring). Averages followed by the same lower case letter do not differ at the 5% probability level on the Tukey test.

Conclusion

Sound frequencies increased the number of roots formed on cuttings harvested in the autumn, especially in the treatment with 1000 Hz.

Higher concentrations of spermidine should be tested, especially in the autumn, when rooting responses are lower in cuttings with no frequency exposure.

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