The effect of Salicylic Acid on the growth of seedling roots of *Brosimum alicastrum*, a perennial tree from the Mexican tropics which produces recalcitrant seeds.

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Abstract

The effect of salicylic acid (SA) on the length of roots and shoots of *Brosimum alicastrum* seedlings was studied. Twelve-day-old seedlings developed in 20 cm long; PVC tubes filled with perlite were sprayed either with water or 1µM SA. When roots protruded from the base of the tube, the seedlings were harvested and measured. Results showed that SA treatment significantly increases the length of roots and shoots by 22 % and 21%, respectively, in comparison with those of the control. These significant differences were also present in the fresh weight of the roots. This is the first report to show that SA affects the growth of tree seedlings from the Mexican tropics that produce recalcitrant seeds.

Key words: *Brosimum alicastrum*, perennial, recalcitrant seeds, root, salicylic acid, seedling, steam.

Introduction

Salicylic acid (SA) is a plant growth regulator which has been reported to mediate in different physiological processes in plants and also to be involved in responses to pathogens (Raskin 1992). More recently, its effect on processes relating to the bioproductivity of cultivated plants has been considered (Hayat and Ahamad 2007, 2012)

One of the most interesting reports describes the effect of spraying seedlings with

low concentrations of salicylic acid to stimulate significant root growth; this has been extensively investigated in studies conducted in soybean, Habanero pepper, Madagascar rosy periwinkle (old maid), etc. (Gutiérrez-Coronado, et al 1998; Echeverría-Machado et al. 2007).

Studies have indicated that the transformed roots of *Catharanthus roseus* are sensitive to the application of SA to the culture media, and respond to concentrations at femtomole levels by increasing length and differentiation of secondary roots (Echeverría-Machado et al. 2007).

Brosimum alicastrum, locally known as ramon, is an arboreal specie of the Mexican tropics which has attracted much attention due to the environmental services it offers, most importantly, its high potential as a food source (National Academy of Sciences. 1975; Pardo-Tejeda et al. 1976). This tree produces recalcitrant seeds, that is, they are seeds which rapidly lose their viability as they lose humidity; thus, they cannot be stored in germplasm banks in the same way as orthodox seeds. If we consider the seeds production of this tree as food source we need to ensure that the seedlings will be resistant in natural conditions and provide them hormonal stimulation using salicylic acid that will ensure the increase of the root, fundamental part which contributes in the survival and development of the plant. The aim of the present study was to determine if the spraying of salicylic acid would have an effect on the root elongation in seedlings of *Brosimum alicastrum*.

Materials and methods

To measure the effect of SA on the root length in *Brosimum alicastrum*, a specific

biotest was carried out. Seedbeds consisting of PVC tubes 23 cm high and 4 cm in diameter were filled with perlite substrate and 1 mm mesh was placed at the base to avoid substrate loss. One seed was planted in each PVC tube. A daily irrigation of 10ml of distilled water per tube was applied. The seedbeds were placed in a randomized experimental block, designed with 6 repetitions per treatment and were kept in a growth room at a temperature range between 28°C and 30° with 12 hours of light and 12 hours of darkness.

The SA solution applied in the biotest was prepared using the methodology published by Gutiérrez-Coronado et al (1998). The treatments were a) salicylic acid 10^{-6} M equivalent to 1.0µM and b) Control of distilled water.

The SA solution was sprayed on the shoots of 13-day-old Ramon seedlings with an approximate height of 4.70 cm, three times a week in the mornings in 10 occasions.

When the seedling roots from the different treatments were seen to protrude from the base of the PVC tube, they were harvested. In order to measure the length of the root and stem of the seedlings, the mesh at the base of the tube was removed and the substrate was carefully separated from the root. The root was then washed with distilled water to eliminate perlite residues, and it was finally measured with a ruler.

For the measurement of the biomass weight, after the roots were harvested and measured, they were dried in an oven at 40°C, for a 12-hour period.

Statistical analysis was performed using the Statgraphics, V.16.1.15 package by Fisher's Least Significant Difference (LSD) of multiple comparisons, by means of the ANOVA technique with 95% confidence interval.

Results

Based on the results obtained from previous experiments carried out in our laboratories, in this study, only the concentration of 1.0 µM of SA was tested to evaluate its effect on root elongation. Among the species in which this concentration has shown to have a positive effect on root elongation, the most significant results have been obtained in *Capsicum annum, Lycopersicum esculentun, Pinus patula, Cucumis sativum,* among others (Larqué-Saavedra et al. 2012).

The results showed that 1 µM SA treatment significantly increased the length of the roots of *Brosimum*, at the end of the test (Fig 1). This value was, on average, 4.3 cm longer than the one of the control treatment. Fresh weight of the root also increased significantly in comparison with the control. Dry weight of the roots, however, was not significant, although the pattern does indicate that the treatment with SA showed a higher value.

SA treatment also favored stem height of *Brosimum* with 4.2 cm higher than that of the control (Fig. 2). From these results, we can infer that spraying SA on the leaves of Brosimum will stimulate root growth while increasing the capacity to absorb water and nutrients which will facilitate a more successful development, particularly in adverse conditions such as drought.

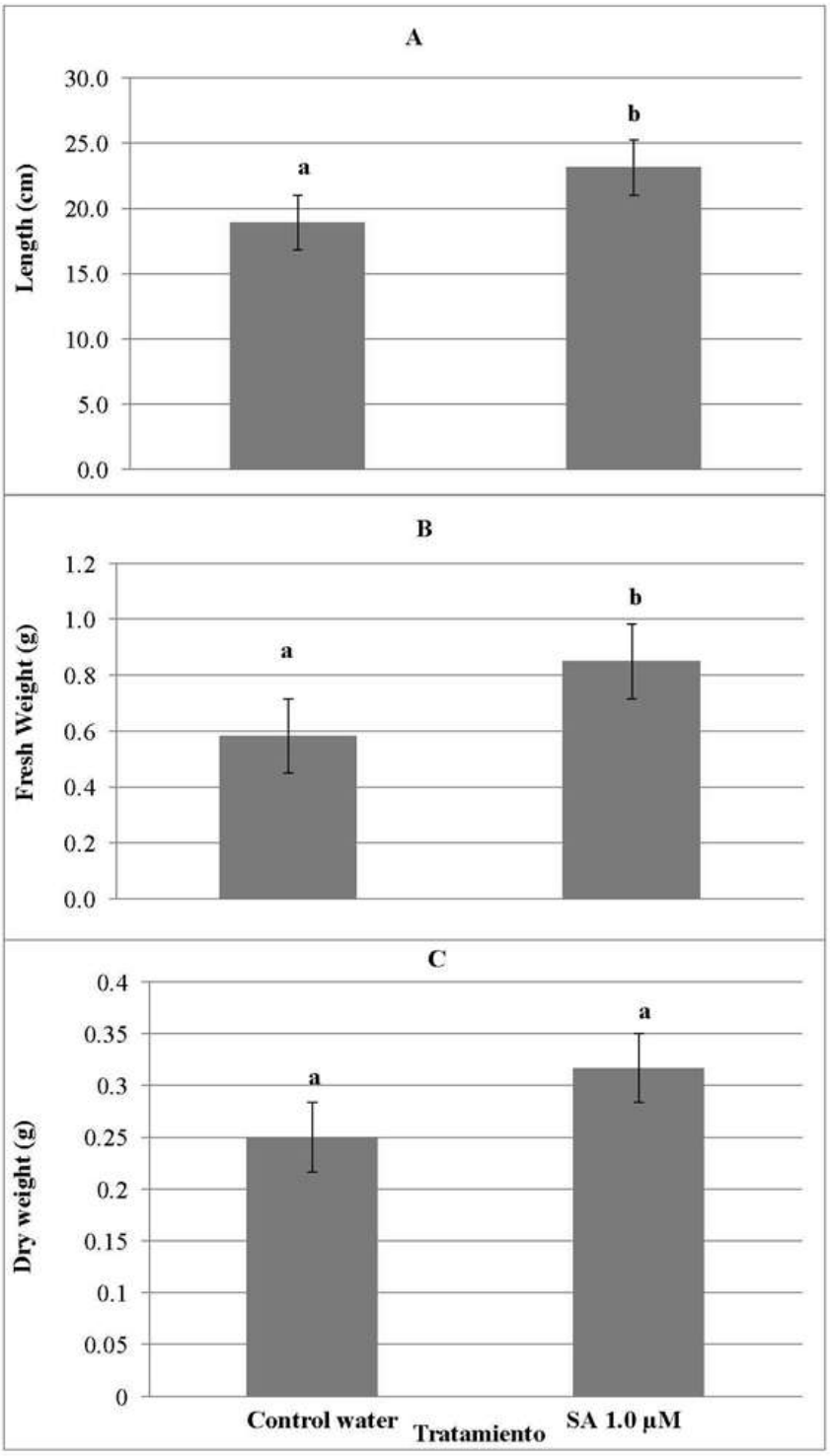

Figure 1. Effect of the spray application of 1µM of Salicylic Acid on the length (A) fresh weight (B) and dry weight (C) of *Brosimum alicastrum* seedling roots. The average of 6 repetitions ± standard error is shown. Similar letters indicate no significant difference (Fisher, $p \leq 0.05$).

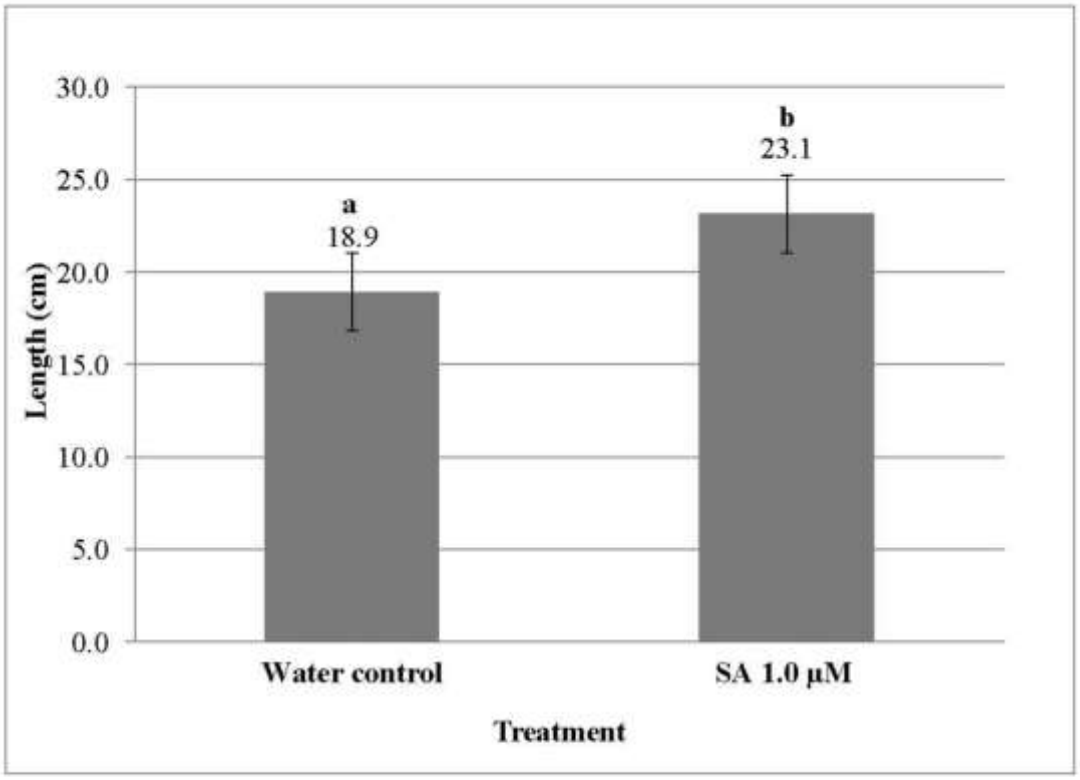

Figure 2. Effect of the spray application of 1µM of Salicylic Acid on the length of *Brosimum alicastrum* seedling stems. The average of 6 repetitions ± standard error is shown. Similar letters indicate no significant difference (Fisher, $p \leq 0.05$).

Discussion

The results obtained here are of particular importance as this is the first report on the effect of SA on a woody plant of the Mexican tropics which produces recalcitrant seeds. There is no other research in perennial trees to be compared with our investigation so far, but similar effects have already been reported by (Larqué-Saavedra et al. 2007,2012) in vegetables and they show the positive effect of salicylic acid in the root system through its elongation.

Further studies are still required to let us evaluate if the increase in length, dry weight and fresh weight detected in the present work could have an effect on nutrient

absorption, flowering, etc., as it has been reported in vegetables (Larqué-Saavedra et al. 2007, 2012). It is also important to consider that this is a perennial species of the Moraceae family which responds to a treatment of growth stimulators, and since the cultivation of these species in commercial plantations has been proposed, it is particularly important to strengthen the root system in order to achieve optimal establishment.

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