Scientific Paper

Effect of efficient microorganism ME-50 on rooting and survival of agricultural seed of *Thitonia diversifolia* (Hemsl.) A. Gray[1]

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Abstract

Objective: To evaluate the effect of the efficient microorganism ME-50 on the rooting and survival of the agricultural seed of *Tithonia diversifolia* (Hemsl.) A. Gray.

Materials and Methods: The effect of three conditioning times with ME-50 was evaluated: 0 hours (control), 24 and 48 hours. During the study, sprouting was carefully monitored during the first 14 days, while morphological quality of shoots and roots was systematically evaluated at 14, 28 and 42 days after planting.

Results: The conditioning of cuttings with ME-50 had a significant positive effect on the vegetative development of the species. Cuttings treated with the biostimulant showed a faster and more abundant shoot emission compared to the control group, with the 48-hour conditioning treatment standing out as the most effective. As for the root system, a particular development pattern was observed. Although initially there was a delay in root emission in the treated cuttings, after 42 days they significantly outperformed the control in both root length and root density, especially in the 48-hour treatment.

Conclusions: The use of ME-50 represents an effective strategy for the improvement of vegetative propagation of *T. diversifolia* and 48-hour conditioning was the most recommended protocol. The initial delay in root development did not compromise the final results, as it was compensated by a more vigorous growth in the medium term.

Key words: sprouting, growth, plant propagation

Introduction

Studies on the performance during establishment, as well as the characteristics that the propagation material must meet to achieve a fast and effective development have been carried out in an ad hoc manner (Medina *et al*., 2009). In this sense, Arias *et al*. (2015) stated that due to the few works that exist on this subject it is not possible to establish an accurate and broad discussion about most of the agrotechnical indicators of Tithonia diversifolia (Hemsl.) Gray. It is necessary to keep in mind the wide interest that this plant is acquiring for animal feed and also its genetic variability, as indicated by Ruiz *et al*. (2010) when evaluating 29 ecotypes collected in Cuba. However, it is important to continue research to corroborate the growth dynamics and biomass production of the species.

Ruiz *et al*. (2009) stated that despite the use of *T. diversifolia* in animal feeding, especially by farmers, little research has been conducted on this subject and suggested that scientific evaluation of the productive response of its forage as animal feedstuff was necessary, in addition to studying further aspects related to plant management, specifically to the rooting and efficient establishment of this plant in the field.

One of the fundamental limitations for the extension of this species is that propagation is done almost 100% through the use of cuttings, which are easy to acquire, but it should be noted that this type of propagation presents some difficulties such as the cost of transportation, limitations of mineral fertilizers that guarantee good rooting and storage that can only be done for short periods, without affecting the quality of the cuttings (Sánchez *et al*. 2014).

Solving the previous problematic situation constitutes a challenge in this research that involves developing comprehensive research that optimizes the processes of emission and conservation of agamic seed.

Hence, the objective of the work was to evaluate the effect of the efficient microorganism ME-50 on the rooting and survival of the agricultural seed of *Tithonia diversifolia* (Hemsl.) A. Gray.

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Materials and Methods

Plant material. Cuttings of *T. diversifolia* obtained from adult plants of one year of cultivation at the Juan Tomás Roig Experimental Station, belonging to the Bioplants Center of Ciego de Avila, Cuba, were used.

Characterization of ME-50. It consists of a mixed culture of beneficial microorganisms containing yeasts, lactic acid bacteria, photosynthetic bacteria, actinomycetes and fungi with a concentration of > 10^6 CFU/mL of beneficial microorganisms in 2 % and 98 % water. This microorganism is produced and commercialized by LABIOFAM company, Ciego de Avila.

Experimental procedure. To determine the effect of the efficient microorganism as pre-germinative treatment on agricultural seeds of *T. diversifolia* through morphological traits in seedlings up to 42 days of culture, the conditioning of agamic seeds was carried out.

For the conditioning of the cuttings, ME-50 at 5% (5 mL L^{-1}) and three conditioning times (0, 24 and 48 hours) were used. The cuttings were placed in plastic containers (0,018 m^3), complying with the previously established imbibition time for each treatment. After the time period dedicated to conditioning, the cuttings were planted in polyethylene bags (0,0015 m^3) at the rate of one cutting per bag for a total of 150 bags per treatment, divided into three replicates of 50 bags. The bags were filled with a substrate based on compacted Ferrallitic Red soil and earthworm humus (50/50). Irrigation was performed daily to maintain humidity during the 42 days of the experiment.

Determinations made

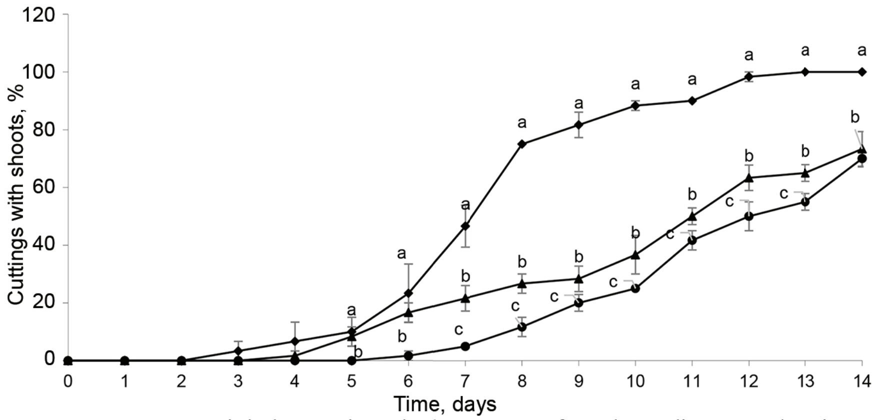
- *Percentage of emitted shoots (%).* During 14, 28 and 42 days after planting, the total number of cuttings with shoots was counted (n = 30), a daily reading was made and those measuring more than one centimeter were considered shoots.
- *Number of shoots per cutting.* The total number of shoots per cutting (n = 30) was counted during 14, 28 and 42 days after planting.
- *Percentage of shoots per cutting.* The total number of shoots per cutting (n = 30) was counted and the percentage was calculated in reference to the total number of buds on the cutting at 14, 28 and 42 days after planting.
- *Number of roots per cutting.* The total number of roots per cutting (n = 30) was counted 14, 28 and 42 days after planting.
- *Number of leaves per shoot*. The number of leaves on the largest shoot of each cutting (n = 30) was counted during 14, 28 and 42 days after planting.
- *Shoot thickness*. The thickness of the largest shoot of each cutting (n = 30) was measured with a digital caliper during 14, 28 and 42 days after planting.

Statistical analysis. Simple analysis of variance was performed. The means of the treatments were compared with Tukey's multiple range test ($p \leq 0,05$), after testing the assumptions of normality (Kolmogorov-Smirnov) and homogeneity of variances (Levene). In some cases it was necessary to transform the data to achieve the assumptions of the performed parametric tests. SPSS® (Version 11.5 for Windows, SPSS Inc.) was used for data processing.

Results and Discussion

Figure 1 shows the percentage of cuttings with shoots during the first 14 days after planting. Significant statistical differences between treatments, could be appreciated at each evaluation time from the fifth day onwards. The first shoots were observed on the third day in the 48-hour ME-50 immersion treatment, while in the 24-hour treatment the emission started on the fourth day and in the control on the sixth day. The percentage of cuttings with sprouts increased rapidly during the first eight days in the 48-hour ME-50 immersion treatment, differing significantly from the other treatments. At 13 days, 100 % of cuttings with shoots were reached in this treatment (48 hours), while in the remaining treatments (24 hours and control) only 73 and 70 % were reached at 14 days, respectively.

These results proved that the species *T. diversifolia* can be adequately propagated by agamic way in an adequate way, and if a conditioning treatment to the cuttings with the use of efficient microorganisms is used, sprouting can be increased in a shorter period of time. The number of shoots per cutting (figure 2, A), the percentage of shoots per cutting (figure 2, B) and the number of roots per cutting (figure 2, C) were also evaluated 14 days after planting the *T. diversifolia* cuttings. The number of shoots per cutting was significantly higher at all times of evaluation in those conditioned with ME-50 for 48 hours. Meanwhile, there were no statistical differences between those conditioned for 24 hours and the control treatment. The same result was seen in the percentage of shoots per cuttings (figure 2, B). On the other hand, the number of roots per cutting was significantly higher in the cutting of the control treatment 14 days after planting (figure 2, C).



p ≤ 0,05; n = 30. For statistical processing only, data were transformed according to y'= 2*arcsin ((y/100)^{0,5}).

Figure 1. Percentage of *T. diversifolia* cuttings with shoots during the first 14 days after planting. Results with unequal letters, on each evaluation day, are statistically different

Figure 3 shows *T. diversifolia* shoot development, number of leaves per shoot (figure 3, A) and shoot thickness (figure 3, B) 14 days after planting. The 48-hour conditioned seed treatment showed the highest number of leaves per shoot and shoot thickness, which differed significantly from the 24-hour conditioned cuttings and the control treatment. This corroborates what Martinez *et al*. (2014) stated, showing that the reserve substances present in the cuttings, along with the new shoots and leaves that are formed, increase photosynthetic activity, as well as the production of endogenous auxins that stimulate the formation and differentiation of shoots.

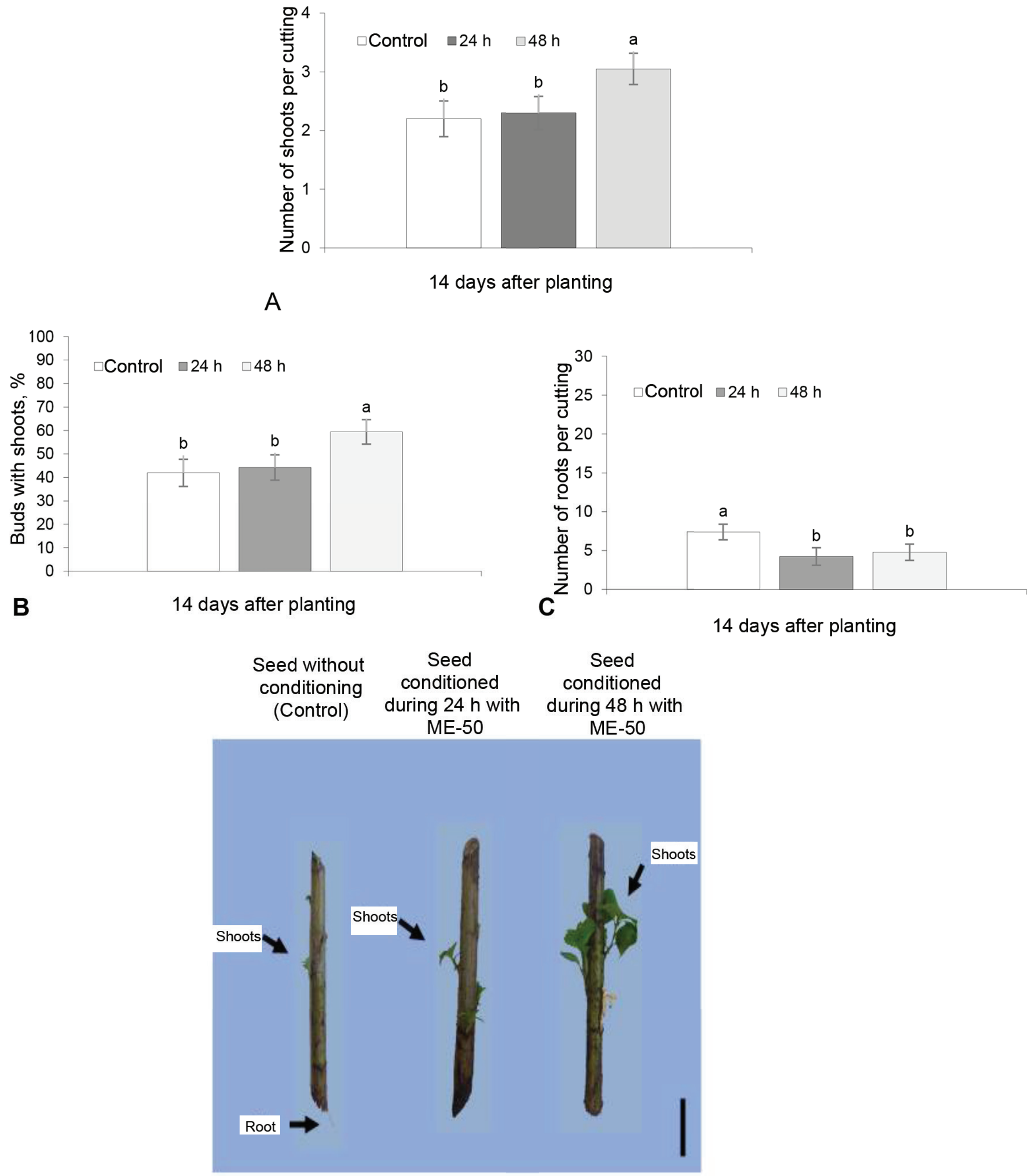
Twenty-eight days after planting *T. diversifolia* cuttings, the number of shoots per cutting (figure 4, A), the percentage of shoots per cutting (figure 4, B) and the number of roots per cutting (figure 4, C) were evaluated. The number of shoots per cutting and the percentage of buds per cutting were significantly higher in those conditioned with ME-50 for 48 hours. In the number of roots per cutting (figure 4, C), no differences were observed among the evaluated treatments. The morphological quality of the *T. diversifolia* cuttings showed that the cuttings conditioned for 48 hours with ME-50 had greater vigor, which could be associated with the phytohormonal effect of the microorganism that stimulates shoot growth. However, this whole process is not only mediated by the interaction of the microorganisms, but also by the environmental conditions that make it possible for the benefits of the product used in conditioning to be expressed in the plant species.

At 42 days after planting *T. diversifolia* cuttings, the number of shoots per cutting (figure 5, A), the percentage of shoots per cutting (figure 5, B) and the number of roots per cutting (figure 5, C) were evaluated. The number of shoots per cutting, the percentage of buds per shoot and the number of roots per cutting were significantly higher at all times of evaluation in those conditioned with ME-50 for 48 hours

During rooting and sprouting of the cuttings after planting, the number of leaves per shoot (figure 6, A) and the thickness of each shoot (figure 6, B) were evaluated. Both indicators were significantly greater, at all evaluation times, in the cuttings that were conditioned with ME-50 for 48 hours.

Conditioning activates several processes that stimulate seed germination, rooting and bud emission (Gupta *et al*., 2022; Pagano *et al*., 2023). At the time of planting agamic seeds (cuttings), the conditioned ones have absorbed a considerable amount of hormones, amino acids, proteins and metabolites that can favor metabolism, and achieve a higher emission and rooting rate, followed by improved seedling development (Wright *et al*., 2003; Gupta *et al*., 2022).

ME-50 conditioning of *T. diversifolia* cuttings actually activates firstly the metabolism in charge of bud emission and then that of root formation, presumably influenced by hormonal balance (Paparella *et al*., 2015). As could be seen in this research,



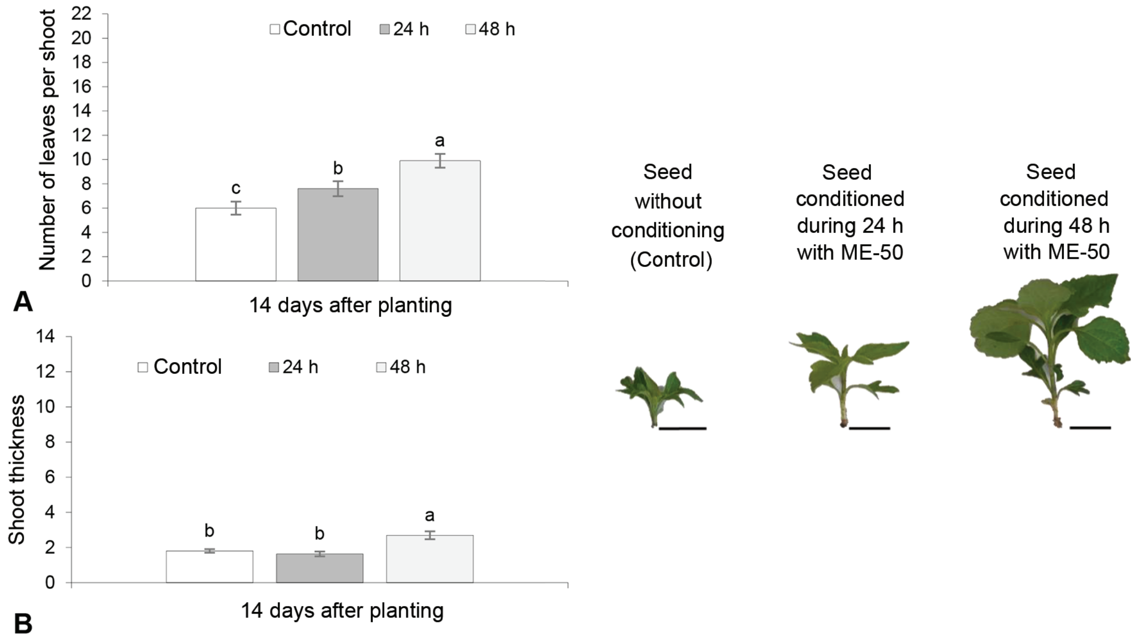
Results with unequal letters, at each evaluation time, are statistically different ($p \leq 0{,}05$; $n = 30$).

Figure 2. Morphological traits evaluated on *T. diversifolia* cuttings 14 days after planting. (A) Number of shoots per cutting, (B) Percentage of shoots per cutting and (C) Number of roots per cutting.

the conditioning of cuttings improved shoot size and the number of leaves per shoot, which may lead to improved responses of cuttings to adverse environmental conditions after planting, such as high temperatures, water deficit and pathogen attack.

Conclusions

ME-50 conditioning of *T. diversifolia* cuttings improved shoot and root emission, although conditioning for 48 hours was the conditioning time that showed the best results.



Results with unequal letters, at each evaluation time, are statistically different ($p \leq 0{,}05$; n = 30).

Figure 3. Morphological traits evaluated on shoots of *T. diversifolia* at 14 days after planting. (A) Number of leaves per shoot and (B) Shoot thickness.

Shoot emission was more accelerated in the conditioned cuttings, while root emission was delayed compared to the control treatment cuttings, although at 42 days of evaluation this indicator was significantly higher in this treatment.

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Conflict of interest

The authors declare that there is no conflict of interest among them.

Author contributions

- Dayami Fontes-Marrero. Conception of the research, collection, analysis and interpretation of the results and drafting of the manuscript.
- Yanier Acosta-Fernández. Photographs, collection, data collection, analysis of the results and compliance with the writing of the manuscript.
- Dayanis Hernández-Fontes. Establishment of the experimental area, data collection, interpretation of the results and conformity with the writing of the manuscript.
- Carlos Mazorra-Calero. Design, analysis of the results and conformity with the writing of the manuscript.
- Julio Ynchausti-Rodríguez. Harvest, data collection and conformity with the drafting of the manuscript.
- Jorge Martínez-Melo. Harvest, data collection and conformity with the drafting of the manuscript.

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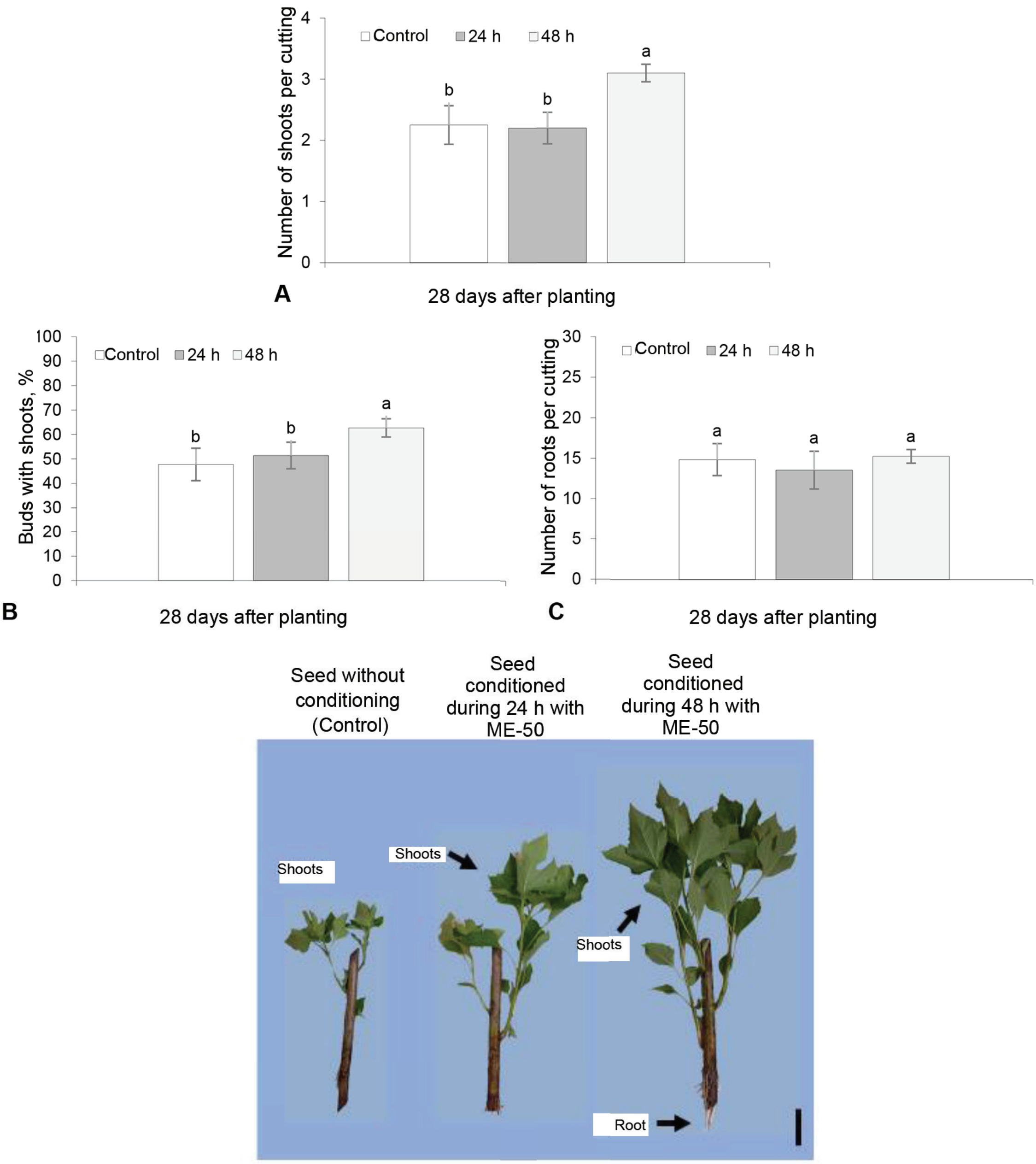

Results with unequal letters, at each assessment time, are statistically different ($p \leq 0,05$; n = 30).

Figure 4. Morphological traits evaluated on *T. diversifolia* cuttings at 28 days after planting. (A) Number of shoots per cutting, (B) Percentage of shoots per cutting, and (C) Number of roots per cutting.

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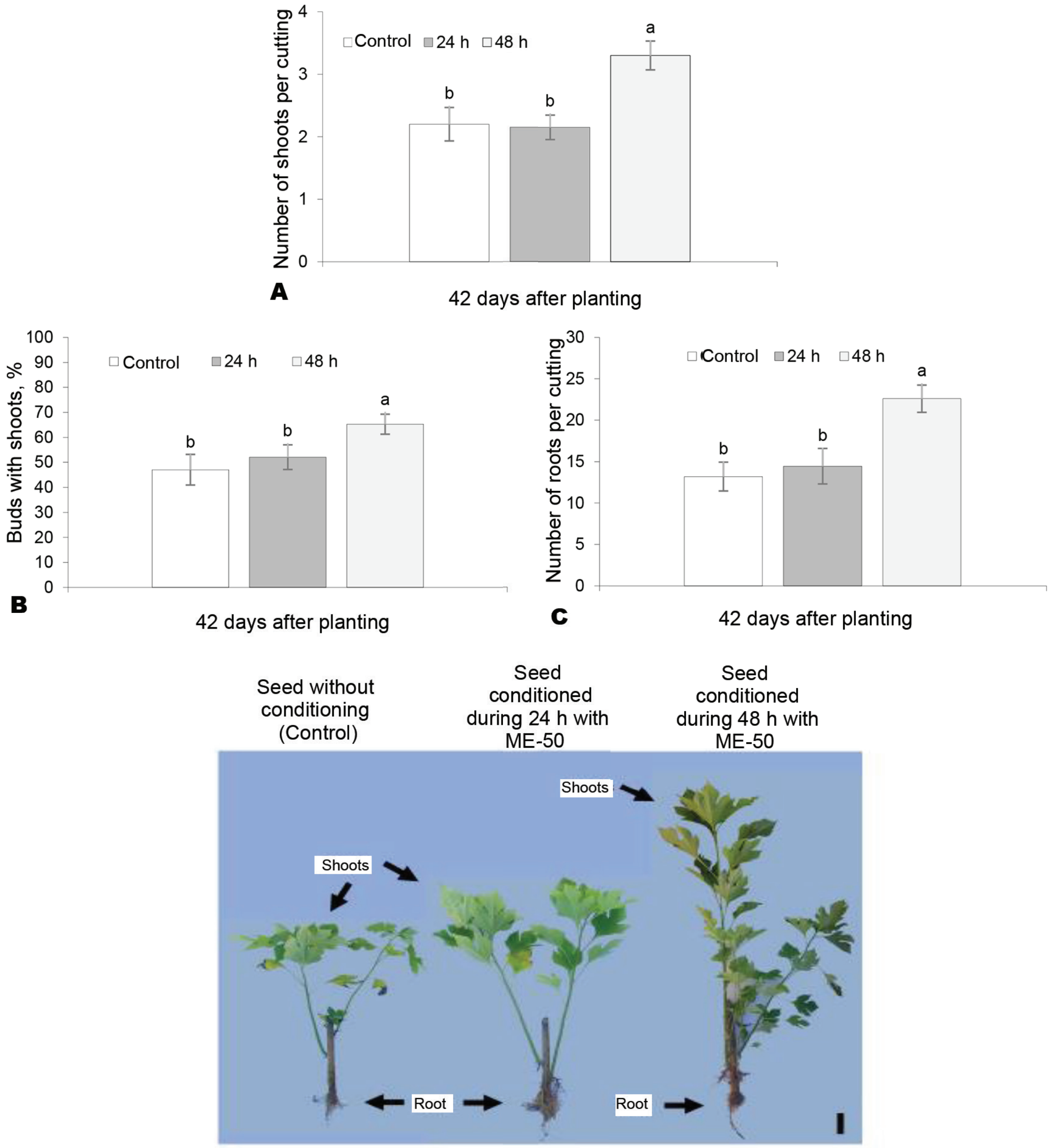

Results with unequal letters, at each evaluation time, are statistically different ($p \leq 0.05$; $n = 30$).

Figure 5. Morphological traits evaluated on *T. diversifolia* cuttings 42 days after planting. (A) Number of shoots per cutting, (B) Percentage of shoots per cutting and (C) Number of roots per cutting.

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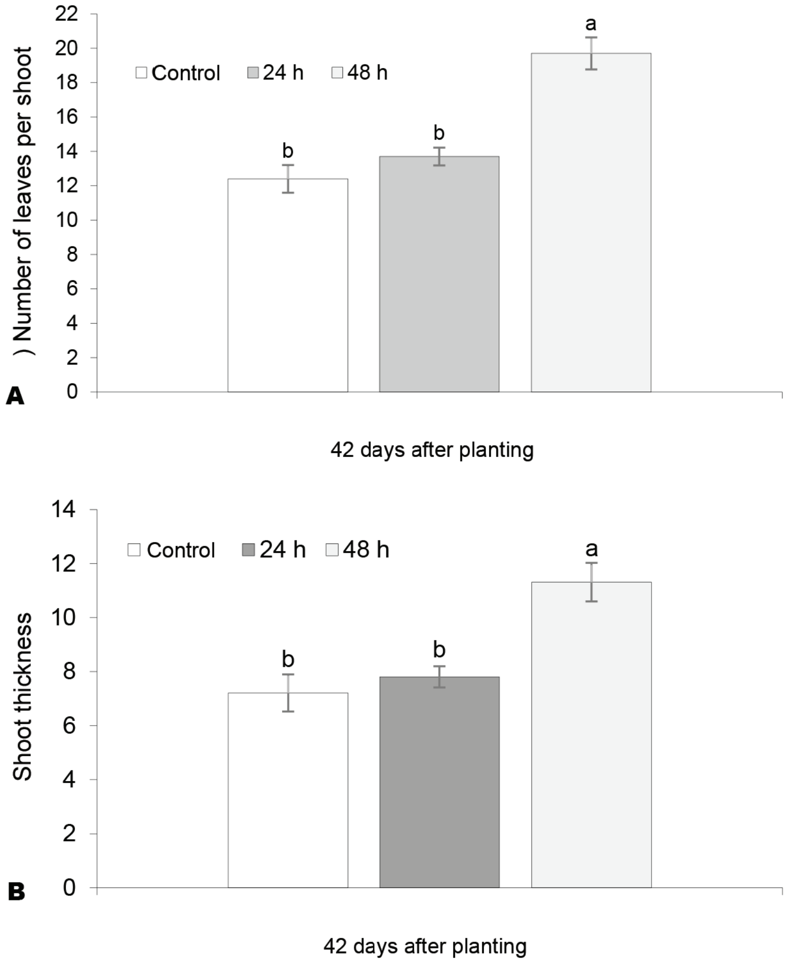

Results with unequal letters, at each evaluation time, are statistically different $p \leq 0,05$; n = 30).

Figure 6. Morphological traits evaluated on shoots of *T. diversifolia* at 42 days after planting. (A) Number of leaves per shoot and (B) Shoot thickness .

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