Scientific Paper

Effect of auxin treatments on the rooting of two *Malpighia* species through the air layering technique

Maribel del Carmen Ramírez-Villalobos[1], Aly Segundo Urdaneta-Fernández[2], Verónica Chinquinquirá Urdaneta-Ramírez[2] and Danny Eugenio García-Marrero[3]

[1]*Departamento de Botánica, Facultad de Agronomía, Universidad del Zulia (LUZ), Apdo. 15205, ZU4005, Venezuela*
[2]*Unión de Ganaderos El Laberinto (UGALAB), Venezuela*
[3]*FMF-Freiburg Materials Research Center, Institute of Forest Utilization and Work Science, Germany*
Email: mcramire@fa.luz.edu.ve

Abstract

A study was conducted in order to determine the effect of auxin treatments on the rooting of two *Malpighia* species (semeruco), through the air layering technique. Air layers were made on branches of *M. glabra* and *M. emarginata*, and then eight auxin treatments were applied (AT): 0, 2 000, 4 000 and 6 000 mg kg^{-1} of naphthalene acetic acid (NAA) and of indole butyric acid (IBA). A completely randomized design was used, with factorial arrangement and six repetitions. After seven weeks the percentage of rooted layers (PRL), percentage of live layers (PLL), number of roots per layer (NR) and length of the longest root (RL), were evaluated. No interaction of the studied factors (species by AT) was found. There were no significant differences either between the species in the evaluated variables. With the doses of 4 000 and 6 000 mg kg^{-1} the highest PRL were found (85,2 and 87,5 %, respectively), as well as the highest NR and RL, without differing significantly between them. Good rooting was also obtained (62,5-64,0 %) with the AT of 0 mg kg^{-1} (control), which differed significantly from the other treatments. The propagation of the two species through the air layering technique was achieved by the application of 4 000 and 6 000 mg kg^{-1} of NAA and IBA, which produced the best rooting.

Keywords: auxin, concentration, vegetative propagation

Introduction

Cattle production systems in Venezuela have high socioeconomic importance; most of them are developed extensively, and grass monocrop and absence of tree cover in the grazing areas prevail. Nava (2010) stated that this model does not take into consideration the climate conditions of the different tropical ecosystems, in which variables such as temperature and relative humidity can limit the productive and reproductive efficiency of the animals. The presence or incorporation of trees and shrubs in livestock production systems, without affecting the forage production and quality, allows to decrease the heat stress of the animal, because under the canopy temperature is reduced between 2 and 9 ºC with regards to the open paddock areas.

In recent years, in Venezuela, an increase has been observed in the environmental temperature, as well as changes or deficit in rainfall (quantity and distribution), which has generated loss of large forest extensions, and of trees and shrubs in the paddocks. Such situation has been associated with El Niño phenomenon (INAMEH, 2015) and with the increase of deforestation (Ramírez *et al*., 2014a).

Agroforestry systems are one of the alternatives for livestock production (Delgado and Ramírez, 2008; Lamela *et al*., 2010; Petit *et al*., 2010; Ramírez *et al*., 2012, 2013, 2014b, 2014c), which includes trees and shrubs with multipurpose qualities. Additionally, the shade provided by them contributes to improve animal welfare (Navas, 2010), especially in the driest and warmest season of the year.

Malpighia spp. is a shrub of globe-shaped and dense crown which is native from northen South America and Central America (Avilan *et al*., 1992); it can reach 2-4 m of height, and grows naturally in several localities (Hoyos, 1994) and livestock production systems of Venezuela. This species, native from the above-mentioned regions, shows high adaptation to the dry and warm zones (Avilan *et al*., 1992). Cattle, sheep and goats browse its leaves and consume its fruits. This species is considered a promising multipurpose plant genetic resource for livestock production systems and urban green areas, because it can maintain its crown covered with leaves during long drought periods (up to nine months).

In the country there are two species, *Malpighia glabra* L. and *Malpighia emarginata* Sessé & Moc.

ex DC.; the former is the most cultivated in America as a fruit tree, and both are mainly reproduced by seed (Hoyos, 1994). Nevertheless, papers have been found that report heterogeneity in the germination of *M. glabra* (García-Hoyos *et al*., 2011) and low germination percentage (12 %) in *M. emarginata* (Moratinos *et al*., 2008), , for which some authors recommend the layering and grafting techniques for an efficient propagation of the species (Avilan *et al*., 1992; Hoyos, 1994).

Fernández and Rivero (2004) found a high rooting percentage in *M. glabra* cuttings through the application of the auxin indole butyric acid (IBA) at a concentration of 4 000 mg kg^{-1}; while other studies have reported 48 % of rooting in *M. emarginata* and *M. glabra* cuttings treated with 5 000 mg kg^{-1} of IBA (Rivero *et al*., 2005a) and 750 mg kg^{-1} of IBA (Rivero *et al*., 2005b), respectively.

The application of auxins in species of difficult rooting is a common, viable and decisive practice, which allows to increase the number of cuttings with roots, advance rooting onset, increase root number and quality, besides providing higher uniformity in rooting. The most widely used auxins are indole butyric acid (IBA) and naphthalene acetic acid (NAA) (Hartmann and Kester, 2001).

In the case of air layering there are aspects that have not been yet studied in *Malpighia* spp., such as the species and treatments with auxins –concentration and type–, which could limit plant production in nursery or field. Hence the objective of this research was to determine the effect of auxin treatments on the rooting of two *Malpighia* species using the air layering technique.

Materials and Methods

Location of the study. The trial was conducted in the Fruit Tree and Beekeeping CESID- CORPOZULIA, Mara municipality, Zulia state (10° 49’ 15’’ NL, 71° 46’ 20’’ WL); which is framed in a zone classified as very dry tropical forest of intervened area, at 25 masl, with an annual rainfall average of 500 mm, temperature of 29 ºC, relative humidity of 79 % and evapotranspiration of 2 500 mm (Huber and Oliveira, 2010).

Plant material. Two *Malpighia* spp. species were used: *M. glabra* and *M. emarginata*, from the germplasm collection located in the Fruit Tree and Beekeeping CESID-CORPOZULIA.

Air layering technique. From each *Malpighia* species six plants with homogeneous size and crown were selected (Ramírez and Urdaneta, 2004); the latter was divided into four quadrants, considering the cardinal points North, South, East and West (Sánchez *et al*., 2009).

In each quadrant eight branches 60-70 cm long and 1,0-1,5 cm diameter were taken, without flowers or fruits, on which the air layering technique was applied, which consisted in withdrawing a bark ring of approximately 2 cm –with the aid of the back of a pocketknife tip– (Hartmann and Kester, 2001; Ramírez and Urdaneta, 2004) in an intermodal zone, at 10-15 cm over the base of the branch.

Afterwards the auxin was applied, which was prepared in paste with petrolatum at 100 % and dissolved with absolute isopropyl alcohol considering the suggestion made by Hartmann and Kester (2001). The auxin was impregnated in the basal part of the layered branch (Ramírez and Urdaneta, 2004), that is, in the bark zone that was above the ring (one centimeter approximately, with 0,5 mL of the auxin).

As substrate river sediment (decomposed plant material, swept along by the water currents and deposited on the shores) was used, recommended by Moratinos *et al*. (2008) for the *Malpighia* spp. cuttings. The well moist substrate was placed on a piece of transparent polyethylene (15 cm x 22 cm); afterwards, very carefully, it was taken to the ringed zone to cover or wrap it with the plastic, so that the auxin was not removed; the ends were firmly tied with plastic string (Ramírez and Urdaneta, 2004), 25 cm long; and, finally, the whole layer was wrapped with transparent plastic paper (Envoplast) to prevent moisture loss.

In view of the semiarid conditions of the zone and considering that the experiment was conducted during the dry season, it was necessary to apply irrigation of 60 mL per layer every two weeks, with a disposable plastic syringe or injector without needle, recommended by Albany et al. (2004).

Experimental design and treatments. A completely randomized design with 2 x 8 factorial arrangement was used, for a total of 16 treatments. The studied factors were the two plant species and eight auxin concentrations (AT). The latter consisted in the application of 0, 2 000, 4 000 and 6 000 mg kg^{-1} of NAA (Ramírez and Urdaneta, 2004) and IBA (Moratinos *et al*., 2008), which were replicated six times. The experimental unit was formed by four layers.

Variables. Seven weeks later the percentage of rooted layers (PRL), percentage of live layers (PLL), number of roots per layer (NR) and length of the longest root (RL) (Ramírez and Urdaneta, 2004), were evaluated. The PRL was calculated through the ratio of the number of rooted layers between the total number of live layers, multiplied by 100.

Statistical analysis. For the data processing a variance analysis (ANOVA) was used through the Statistical Analysis System program, after verifying that the assumptions fulfilled the variance homogeneity and normal distribution adjustment. For the variable PRL the data were transformed through the equation arcsin $(x + 1)^{1/2}$. The means were compared through Tukey's test for 5 % of significance.

Results and Discussion

No interaction was found of the studied factors (species by auxin treatment). There were no significant differences either for the species in the variables PRL, PLL, NR, RL (table 1). *M. glabra* and *M. emarginata* recorded statistically equal PRL values (79,8 and 71,0 %, respectively). Moratinos *et al.*, 2008) stated that these *Malpighia* spp. species did not influence the layer rooting –expressed in terms of percentage of rooted layers, number of roots per layer and root length–; this is similar to the result obtained in this study through the air-layering technique.

Regarding the other variables, *M. glabra* showed 79,8 % of PLL, 4,1 roots per layer and 5,8 cm of RL, values statistically similar to those of *M. emarginata* (table 1). It is important to state that dead layers could have occurred due to the presence of a high population of ants, which caused substrate loss.

For the variables PRL, NR and RL significant differences were found when applying the different ATs (table 2). After seven weeks, it was observed that the IBA and NAA treatments at 4 000 and 6 000 mg kg^{-1} showed the highest values of PRL, NR and RL, which did not significantly differ among them, but they did show differences ($p < 0,05$) with regards to the others. Of these four treatments, it is convenient to use the ones with lower concentration in both auxins, from which NAA has the lowest cost, because the price of auxins is in the range from moderately high to very high.

According to reports by Ramírez and Urdaneta (2004), Azcón-Bieto and Talón (2008) and Taiz and Zeiger (2013), the responses produced due to the application of auxins to the plants depend on the concentration of the phytohormone or auxin, as well as the type of organ treated. In that sense, the result obtained with 4 000 mg kg^{-1} of NAA and IBA is likely to be due to the property of auxins of being capable to participate in several processes of plant growth. Likewise, it is possible that they affect division, growth and differentiation of the cells that promote the

Table 1. Effect of species on the PRL, PLL, NR and RL, in air layers of *Malpighia* spp.

Species	PRL (%)	PLL (%)	NR	RL (cm)
M. glabra	79,8	79,8	4,1	5,8
M. emarginata	71,0	82,6	4,6	6,2
Mean ± SE	75,4 ± 4,4	81,2 ± 1,4	4,4 ± 0,3	6,0 ± 0,2

Table 2. Effect of auxin treatments on the PRL, NR, RL and PLL, in air layers of *Malpighia* spp.

Auxin treatment (mg kg^{-1})	PRL (%)	NR	RL (cm)	PLL (%)
0	62,5^{b}	3,2^{b}	4,4^{b}	75
2 000 NAA	65,5^{b}	3^{b}	4,9^{b}	80
4 000 NAA	85,2^{a}	5,6^{a}	7,3^{a}	82
6 000 NAA	87,5^{a}	5,2^{a}	7,2^{a}	83,3
0	64^{b}	2,9^{b}	4,6^{b}	82
2 000 IBA	61,7^{b}	3,6^{b}	4,5^{b}	84,2
4 000 IBA	88,6^{a}	6^{a}	7,5^{a}	75
6 000 IBA	87,5^{a}	5,1^{a}	7,1^{a}	88
Mean ± SE	75,3 ± 12	4,3 ± 1,2	5,9 ± 1,3	81,2 ± 4,2

Means with different letters significantly differ ($p < 0,05$).

onset of rooting, increase root number and quality, increase rooting uniformity and reduce the time required for the process (Hartmann and Kester, 2001).

On the other hand, the AT of 0 mg kg^{-1} or control showed that without the application of the auxins rooting of the air layers in *Malpighia* spp. could be achieved, which was associated to the stimulation received by the branches once the bark ring was removed; this technique caused wounds and the interruption of the translocation downwards, through the phloem, of organic materials, carbohydrates, auxins and other growth factors, condition that allows the accumulation of such compounds in the basal part of the layer and favors the rooting of the branch still attached to the plant. Among other aspects that could have promoted this process are the absence of light in the zone where the roots will be formed, continuous moisture and good aeration of the substrate, as well as the moderate temperature (Hartmann and Kester, 2001; Ramírez and Urdaneta, 2004). Moratinos *et al*. (2008) found that the rooting of the *Malpighia* spp. layers was determined by the formation of new growths on the layer, for which the application of IBA was not necessary.

The results in the PRL were similar to the ones reported for the cuttings by Fernández and Rivero (2004), who obtained at eight weeks high rooting in *M. glabra*, specifically when they applied 4 000 mg kg^{-1} of IBA; although they exceeded the values found by Rivero *et al*. (2005a, 2005b) and Moratinos *et al*. (2008), because layer rooting was achieved in higher time (60 and 63 days, respectively).

On the other hand, in *M. emarginata* 47,5 % of rooting has been reported in green wood cuttings with three pairs of leaves, treated with 5 000 mg kg^{-1} if IBA (Rivero *et al*., 2005a); and in *M. glabra*, 48 % in cuttings with 750 mg kg^{-1} of IBA (Rivero *et al*., 2005b).

Nevertheless, Moratinos *et al*. (2008) achieved 45 and 52 % of rooting in cuttings of *M. emarginata* and *M. glabra*, respectively, and concluded that the rooting of the species was determined by the formation of new growths and not by the application of the IBA-type auxin, which was not necessary, or whose requirement was very low. In the air layers of *Malpighia* spp. the auxins were found to favor the process.

The NR in *Malpighia* spp. (table 2) was above the report for *M. marginata* and *M. glabra* cuttings (Rivero *et al*., 2005a, 2005b; Moratinos *et al*., 2008). These differences were associated with the rooting techniques used (cuttings, concentration, substrate, experimental conditions, among others) RL (7,1-7,5 cm) was similar to the ones obtained in *Malpighia* spp. cuttings by Rivero *et al*. (2005a, 2005b) and Moratinos *et al*. (2008).

The results in the two *Malpighia* spp species with the air layering technique and rooting are considered pioneer and represent a great contribution for these crops present in Venezuela, because there is little information referred to multiplication by cuttings (Fernández and Rivero, 2004; Rivero *et al*., 2005a, 2005b; Moratinos *et al*., 2008).

It is concluded that the propagation of the two *Malpighia* spp. species through the air layering technique was achieved through the application of 4 000 and 6 000 mg kg^{-1} of NAA and IBA, treatments which showed the highest rooting percentage, as well as the highest quantity of roots and the highest root length, in seven weeks. The layered branches of both species could also root, in lower proportion, without applying auxins.

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