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Rooting of acerola cuttings (*Malpighia emarginata* Sessé & Moc. Ex DC.)

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Abstract

This research was carried out with the purpose of establishing a vegetative propagation methodology to multiply selections of *Malpighia emarginata*. The objectives were: 1) to evaluate the rooting of apical cuttings under the following rooting media: sugarcane rum + organic manure from river bank (S1); worm humus + organic matter from river bank (S2); coconut shell + organic manure from river bank (S3); top soil + organic manure from river bank (S4); and the application or not of indole butyric acid (AIB) at 5000 mg kg^{-1}, and 2) to determine the primordial rooting on cuttings treated or non treated with AIB. It was used a completely randomized design using five replications $treatment^{-1}$. The experimental unit was five stakes for the first objective and 10 stakes for the second objective. The following variables were measured 60 days after the treatments of rooting: percentage (%) of alive stakes (PV), percentage (%) of rooting (PE), percentage (%) of primordial root (PP), root number (NR) and root length (LR). Microscope observations were done weekly to follow the primordial growth on five stakes. The results showed significant differences between the substrates (P<0.01), S1 induced the best rooting considering PE and LR (47.5 and 5.02 cm, respectively); AIB affected PE, NR, and LR when treated with AIB were of 37.5%, 1.91 roots $stakes^{-1}$ and 4.22 cm respectively in comparison with no treatment of AIB that were of 23.75%, 0.59 roots $cutting^{-1}$ and 2.06 cm (P<0.01). The primordium roots were favored by the AIB treatment at 5000 mg kg^{-1}, shortening their development in two weeks; a mean of 1.5 primordium roots $stakes^{-1}$ was obtained. Other rooting substrates and levels of AIB need to be evaluated for these plant specie.
Key words: *Malpighia emarginata,* cuttings, substrates, indolbutiric acid, primordial roots.

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Introduction

Between the fruits with the possibility of a wide commercialization in the world is acerola (*Malpighia emarginata* Sessé & Moc. ex DC), belonging the Malpighiaceae family. This fruit is world-wide known with different names: cherry orchard, semeruco, acerola, Barbados-cherry, cereja de para, among others. The fruits of this plant are eaten fresh, besides are widely used in the food industry as a nutritive additive of several sub-products obtained from other fruits due to its high percentage of vitamin C (ascorbic acid), it is also used as an ornamental tree, by its leafy foliage and the nice red color of its fruits that contrasts to the shiny green of leaves (6). The elevated nutritive value of fruits of this plant would allow supplementing nutritional requirements of the population, offering them on the national and international market.

In spite of, for some authors the botanical classification is not precise (12), others confirm that in Venezuela are two species of acerola, having very similar morphological characteristics though, these are *Malpighia glabra* and *M. emarginata* (synonym of *M. punicifolia*). *Malpighia emarginata* has obtuse, rounded and emarginated leaves in the apex, gathered in short nods and commonly with smaller peduncles of 5 mm long, and fruits with 4 to 9 lobules (15).

The relatively fast growth of the plant, the production of some crops in the year, and the high Vitamin C content, have turned the acerola crops as an alternative of economical importance, with commercial plantations in Puerto Rico, Florida, Hawaii and Brazil (19).

In Venezuela, this is a wild fruit and grows in arid and semiarid areas; there is only an experimental extensive plantation in Lara state (18), and research about the selection of materials with agronomical purpose are not known (6). On the north area of Zulia state, acerola is considered a fruit specie as cocoplum, plum, custard apple, cashew and pomegranate (2).

The dissemination of this plant can be done by seed and by a vegetative way (15), however, when propagating them by seeds has been observed a huge diversity in characteristics such as: precocity, growth's habit, flowering, productivity, rooting capacity and quality of the fruit, therefore, it is important to detect and select plants which genotype has characteristics of high horticultural value, in order to establish commercial clones (5).

In the national market, help is required for the clone propagation of selected acerola plants (16), in this sense the stakes might be the most convenient technique to prolong the characteristics of its variety, however, rooting percentages are not always too elevated (6).

The stake technique is considered a method which offer higher benefits to multiply acerola in big quantities; therefore it is advisable that the rooting mean be very porous in order to guarantee a good ventilation and enough drainage (vermiculite, sand, or mixtures with sawdust, zeolite, perlite or granite dust) (12). The acerola substrate must have three essential functions: the first one would be to nourish the stake in the rooting period; the second to proportion humidity, and finally to allow a good oxygenation in the base of it (3).

The histological analysis might be a very valuable tool by being fast and simple in the visualization of root primordium to determine the efficiency of an applied treatment to accelerate and improve rooting, without waiting the completed period that according to the potentiality employs the specie about to root.

The initiation of root primordium, the organization and the development of roots generally occurs before the appearance through the cortex in the stake. Root primordiums are considered meristematic regions separated by rows of elongated cells (13). In acerola, primordiums are observed as areas of cellular proliferation, product of the meristematic activity, which produce an increment on the radial parenchyma tissue, pushing the phloem and the nodules of the phloem fibre toward the cortex (16).

The objectives of this research are: 1) to evaluate the effect of the type of substrate and of the indole butyric acid (AIB) at 5000 mg kg^{-1} on the rooting of apical stakes and 2) to determine the formation of root primordium through the histological analysis in treated and non treated stakes with AIB.

Material and methods

I Experiment. Rooting of acerola stakes: effect of the type of substrate and of the butyric indole acid (AIB)

The vegetal matter had apex stakes of green wood, with three pair of leaves and 20 cm long, selected from mother plants which were approximately ten years old, located on the Fruit Center of Zulia State (CORPOZULIA). Stakes were transported on containers, immersed in water until arrive at the University Nursery (University of Zulia) where were established. This nursery is geographically located at 10º41’12” north latitude and 71º38’05” of west longitude, and in an altitude of 25 msnm. The region is classified as a life area with very dry tropical forests with mean annual temperature of 29ºC, precipitation with 500 mm $year^{-1}$, 76% of relative humidity and solar radiation of 1101, 72 mmol m^2 s^{-1} (9).

On the stakes treated with AIB 5000 mg kg^{-1}, it was applied in powder soaking approximately 2 cm of the base of the previously wet stakes. Subsequently, treated and non treated stakes with AIB were established in four types of substrates, which had the following components: vegetal surface + river manure in 2:1 proportion, coconut shell + river manure in 1:1 proportion, cane rum + river manure in 1:1 proportion and worm humus + river manure in 1:3 proportion; which were distributed in plastic cups of 80 cc of capacity. Finally, stakes were put in wet individual chambers in order to avoid their dehydration.

From the combination of all factors that are being studied, eight treatments in a completely randomized design were produced, with five replications. Every replication had five stakes as an experimental unit, for a total of twenty five stakes by treatment.

Eight weeks after established the essay were measured the variables: percentage of alive fence, percentage of rooting stakes, percentage of stakes with primordium, roots number by stake and average longitude of the longest root.

The analysis of the information was done using the GLM procedure of the Statistical Analysis System software (22), and the Duncan multiple comparison test was employed.

II Experiment. Determination of root primordium in treated and non treated stakes with AIB.

Stakes were established in the substrate by cane rum + river manure, because it presented the best results when was evaluated in the experiment I. Likewise, AIB was applied in a concentration of 5000 mg kg^{-1} which was compared with a witness

With the aim of analyzing this phase of the evaluation, material with the described characteristics in the experiment I was selected. A total of one hundred stakes were distributed in five replications by treatment, each replication formed by ten stakes, using a completely randomized design.

With the purpose of determining the appearance time of root primordium in treated and non treated stakes with AIB, five stakes by replication were taken out in the following interval of time: 7, 1 and 21 days of established the essay; besides stakes with intact stems were histological analyzed in order to compare the histological changes occurred in the tissues of these.

Stakes were fixed in a mixture of formaldehyde (5%), glacial acetic acid (5%), and ethylic acid at 90% (FAA). Transversal cuts with a thickness of 15 ìm were done in the base of each stake, exactly at 3 mm under the last node with a hand microtome; these were tinged with phloroglucinol (20 $g.L^{-1}$ of ethanol at 70%) and concentrated hydrochloric acid in an exposure time of 1 min.

The observation and the exam of cuts was done with a Olympus KC® microscope, and the microphotography were done with a Leitz Standard Phot 2 photomicroscope.

Results were expressed with the average number of root primordium, these were identified as group of just formed cells with radial direction from the vascular cambium region to the periphery of the transversal cut.

Results and discussion

I Experiment. Stakes rooting: effect of the type of substrate and of AIB

The variance analysis showed significant effects (P<0.01) of the rooting substrate (SE) and of the AIB application on the variables that are being studied; while the interaction between these factors did not present statistical differences. SE influenced on the percentage of rooting stakes and the longitude of roots, and AIB had a significant effect on these variables and on the roots number.

Effect of the type of substrate

Percentage of alive fences and stake percentage with primordium

Results did not determine statistical differences between the substrates for these two variables (table 1). The percentage of alive fences was generally centered in a rank from 80 to 97.5%, proving that the conditions where took place the experiment (mother plants, environmental conditions and used techniques), were very approximated to the adequate ones. According to the percentage of stakes with primordium, it must be mentioned that it was between 0-10%, this allows to infer that the employed time during the essay (8 weeks) was enough to obtain the highest number of rooting stakes; after this period and with commercial purpose it turns to be unpractical and expensive for the maintenance of plants.

In other fruit species, the type of substrate has had an effect on the percentage of alive stakes, for example in *Prunus* sp., six types of substrates were evaluated where the proportion of the soil and organic matter (cow dung) varied. The best rooting mean (80% of stakes survival) was the one done with 50% of sand + 50% dung, followed with 50% soil + 50% sand and 33% of each components previously mentioned (10).

Percentage of rooting stakes

For this variable, significant differences (P<0.01) were obtained

Table 1. Means and meaning for the variables percentage of alive stakes (PV), rotted stakes (PE), with primordium (PP), roots number (NR) and root longitude (LR), by the effect of the rooting substrate (SE) in acerola.

SE	PV	PE	PP	NR	LR (cm)
Cane rum + river manure	97.5^{a}	47.5^{a}	7.5^{a}	1.65^{a}	5.02^{a}
Worm humus + river manure	87.5^{a}	40^{ab}	10^{a}	1.67^{a}	4.44^{ab}
Vegetal layer + river manure	80^{a}	20^{bc}	5^{a}	1.22^{a}	1.85^{ab}
Coconut shell + river manure	85^{a}	15^{c}	0^{a}	0.45^{a}	1.26^{b}

Means with different letters in the same column differ statistically (P<0.01).

between the evaluated substrates, the best was the mix of cane rum with river manure to which was obtained a rooting percentage of 47.5%; this substrate did not differ from the one of humus compound and river manure, which reached a value of 40%. The lowest percentage (15%) was presented by the mix of coconut shell and river manure (table 1). The obtained results with the mix of cane rum and river manure were attributed by the properties that cane rum has, characterized by being a spongy, formless, with a dark to black color material, which absorbs huge quantities of water and has a high content of phosphorous, calcium and nitrogen (24).

Regarding Humus, it has been determined that most of the direct effects are not related to the mineral nutrition of plants, but instead to their growth and development. Thereby, for example, there are papers which describe the effect of humic substances on the generation of roots and their growth, seeds germination, among others, which are related effects to their hormones properties (17). Humic substances in low concentrations act on the rhyzogenesis because increase the permeability of the cellular membrane, elevate the activity of the synthesizing ferments as well as the chlorophyll content and the ventilation intensity (7).

Particularly, worm humus is rich in phytostimulines: gibberellins from 2.05 at 2.75 µg kg^{-1} of dry matter (MS), citoquinines 1.05 to 1.08 µg g^{-1} of MS and auxins 3.07 to 3.8 µg g^{-1} of MS. Humic acids are considered phytostimulants because these have a similar effect to the inducted by phytohormones favoring rooting, the development of the radical system and growth of the stems (8).

The low rooting percentages obtained with the mix of coconut shell and river manure, did not agree with other authors, who, when comparing in annatto (*Bixa orellana* L.) washed sand of river with coconut sawdust for rooting, it resulted to be the best treatment (21). In the properties of the coconut sawdust, is noticeable the fact of the lack of pathogens of soil by being a product with aerial origin, the physical properties that it has, allows to sow in thin layers and the radical development is more intense with a higher number of absorbent rootlets than the one obtained with other substrates (20).

Roots number (NR) and roots longitude (LR) by stake

For the NR variable, there were not differences between the evaluated substrates; instead, the type of substrate did affect significantly ($P<0.01$) at LR being the mixture of rum + river manure, and humus + river manure, the best with 5.02 and 4.44 cm respectively. These positives effects were conferred to the excellent physical and chemical properties of both substrates previously mentioned (2, 8). In a research done in *B. orellana*, the effect of the rooting substrate produced differences for the longitude of the longest root, and the interaction size of the stake with the rooting substrate was significant for the number of roots (21).

Effect of the application of AIB

Percentage of alive fences and percentage of stakes with primordium

The variance analysis did not determine significant effects of the application of AIB on the variables: percentage of alive fences (PV) and percentage of stakes with primordium (PP), values were 88.75 and 7.50 with AIB and 86.25 and 3.75 without AIB, for PV and PP respectively (table 2).

Percentage of rooted stakes

Significant differences (P<0.01) were found, when comparing stakes with and without application of AIB (table 2). The highest value in relation to this variable was presented by the first with 37.5%, compare to 23.75% of the witness. These results agree with those cited by other researchers, who said that when using this same growth regulator in the same acerola concentration, the best results were obtained (6).

Though of considering 5000 mg g^{-1} as a high solution, in other fruit species of difficult rooting the best results have been presented. In guava (*Psidium guajava* L.) was evaluated the AIB effect in stakes, comparing doses of 4000, 5000, 6000 and 7000 5000 mg kg^{-1}; it was deduced that at the same time that increased the concentration of the growth regulator, increased the rooting percentage (23).

The most adequate doses of AIB depend on the genetic characteristics of the plant besides other factors, for example, in peach (*Prunus persica* (L.) Batsch) was evaluated its effect on stakes of different cultivars. The used concentrations were: 0, 1000, 2000, 3000 and 4000 mg L^{-1}. The highest percentage of rooted stakes was obtained with the concentration of 2318 mg L^{-1} with 36.65% (10).

Number and longitude of roots

With the application of AIB, the best results for both variables were obtained (1.91 roots $stake^{-1}$ and 4.22 cm), significantly distinguishing (P<0.01) this effect with the produced by the witness, which obtained 0.59 roots $stakes^{-1}$ and 2.06 cm (table 2). Auxins, besides improving the percentage of stakes with roots, accelerate the radical initiation, and increased the number and quality of produced roots. Endogens or artificially applied auxins are a requirement for the initiation of

Table 2. Means and meaning for the percentage of alive stakes (PV), rooted stakes (PE), with primordium (PP), roots number (NR) and longitude of the longest root (LR) by application effect of AIB in acerola.

AIB	EV	EE	EP	NR	LR (cm)
Treated stakes with 5000 mg kg^{-1} of AIB	88.75^a	37.50^a	7.50^a	1.91^a	4.22^a
Non-treated stakes (witness)	86.25^a	23.75^b	3.75^a	0.59^b	2.06^b

Means with different letters in the same column differ statistically (P<0.01).

adventitious roots in the stem, and in fact, it has been proved that the division of first cells initiators of the roots depends on the auxin (14).

Experiment II. Determination of root primordium through the histological analysis in treated and non treated stakes with AIB.

Histological study in intact stakes

The acerola stem studied on intact stakes presented peridermis with pluristratified cork, external cortex with parenchyma cells of a big size, besides of isolated vascular axes, elements of the primary phloem, where were found rows groups of fibers in their external part, rounded secondary xylem in the external part by the vascular cambium and on the internal area by the parenchyma cells of the medulla (figure 1).

Seven days after had been put the stakes on the rooted mean were not evidenced histological changes guided to the formation of root primordium in any of the cases (figure 2a). After had passed 14 days of established, stakes with hormonal treatment showed completely organized radical primordium and a well defined procambium passing through the cortex and emerging by the peridermis (figure 2b). The microscope observations allowed to detect that adventitious roots were originated from radial parenchyma cells associated to the vascular cambium (figures 2b and 2c), agreeing with the reported determinations in other research (16). The number average of primordium by stake was of 1.5. This low quantity was justified by the limited time to make the observations.

In stakes without AIB, were not observed formation of primordium for this period of time of the essay.

Roots were visible 21 days of established the stakes. The period of time, where passed the emergency of roots, did not agree with the one of the other research done in the same

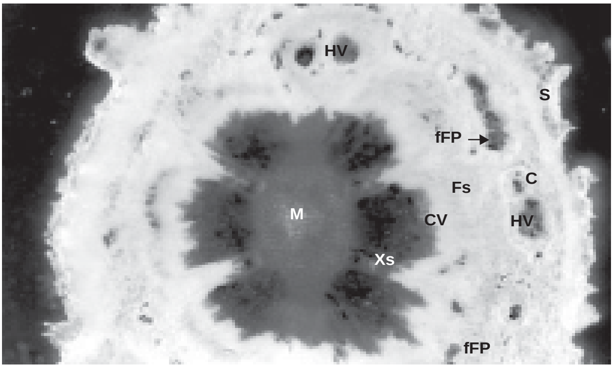

Figure 1. Transversal cut of intact stake of acerola (*Malpighia emarginata*). (S= Cork; C= cortex; HV= vescular bundle; fFP= Fibre of the primary phloem; FS= Secondary Phloem; XS= Secondary xylem; M= Medulla) (100x).

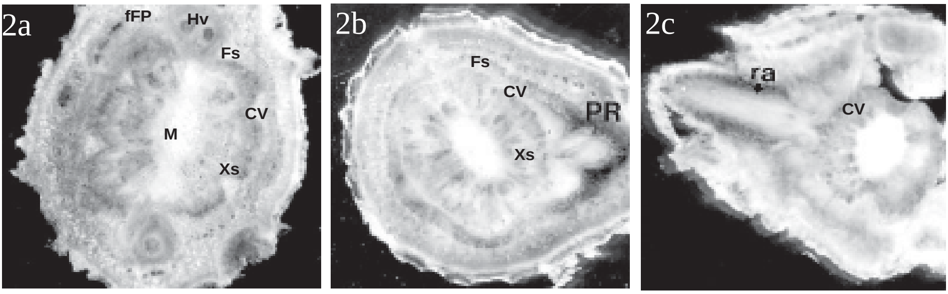

Figure 2. Transversal cuts of acerola stakes (*Malpighia emarginata*). 2a) Stake once passed 7 days (fFP= Fibre of the Primary Phloem, Hv= Vascular bundle, Fs= Secondary Phloem, CV= Vacular cambium, Xs= Secondary xylem, M= Medulla); 2b) Stakes once passed 14 days (Fs Secondary phloem, CV= Vascular cambium, PR= Radical Primordium, Xs= Secondary xylem); 2c) ra= Stakes with adventitious root, CV= Vascular cambium(40x).

specie and with the same AIB concentration (5000 mg kg^{-1}), where was reported that these did not emerged but 35 days later (16). However, these were similar when were compared treated and non treated stakes, determining a delay in relation to the initiation and sequence of histological events that originated the formation of adventitious roots from 7 to 10 days for the last, due to in this research in 28 days non treated stakes had roots a week later of the treatment with AIB (table 3).

Anatomical research conducted *in vitro* on the *Malus domestica* specie determined that in micro-stakes, the initiation of roots depended on the rooting inductor components of the mean, these show the beginning of the cellular division previous to the formation of the root primordium in areas where did not previously existed. Treated micro-stakes with water during the induction of roots, presented a scarce or invalid meristem activity or formation of meristemoids. Stakes treated with AIB were similar to those put in

Table 3. Appearance sequence of histological events in treated and non-treated acerola stakes with AIB.

	Days			
	7	14	21	28
Stakes without AIB	Without primordium	Without primordium	With primordium	With roots
Stakes with AIB	Without primordium	With primordium	With roots	With roots

water, showing little meristem activity. Treatments with Saccharose + AIB formed meristemoids of roots in all micro-stakes (13).

In the previous research, was also determined that the origin of the adventitious roots was associated to cells of the phloematic parenchyma. The same as in *M. domestica* in stakes of the *Hedera helix* specie of difficult rooting, roots were originated from the radial parenchyma of the phloem (11).

The determination of radical primordium constitutes a very important aspect because besides of histologically studying the rhyzogenesis it allows to corroborate in a fast and trustable way, the efficiency of the hormonal effect in terms of acceleration of the rooting process, considering that the reported period for acerola was from eight to ten weeks (6). With these results, was determined that the AIB application in a concentration of 5000 mg kg^{-1} improved the rooting process accelerating the formation phase of roots in acerola stakes.

Conclusions and recommendations

On the vegetative dissemination through apical stakes of green wood of acerola (*M. emarginata*), the compound substrate by cane rum and river manure in 1:1 proportion resulted favorable for rooting, by registering the highest values of the variables percentage of rooted stakes and longitude of the root.

The application of AIB in a concentration of 5000 mg kg^{-1}, improved the rooting process of the specie, considering that presented a higher percentage of rooted stakes, higher number of roots by stake and higher root longitude, compare to the witness without application.

The rooting stakes of this acerola specie, was favored with the application of indole butyric acid at 5000 mg kg^{-1}, due to it shortened the formation of radical primordium 14 days later of established the stakes.

It is recommended the evaluation of other substrates with similar characteristics to the one of cane rum, as well as testing other doses of indole butyric acids with the purpose of determining the adequate doses.

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