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Association between Cuttings Maturity and Alternative Substrates in the Rooting of Acerola Cherry

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HIGHLIGHTS

- Herbaceous cutting of acerola cherry in vermiculite substrate are recommended to higher rooting rate.
- The use of soil and tanned bovine manure as substrate is promising to produce seedlings of acerola cherry

Abstract: Vegetative propagation methods of acerola cherry are important in standardizing orchards and fruit quality, and the cutting process has been investigated as a promising alternative. The present work aimed to propose the best combination of substrate and cutting type (herbaceous and semi-woody) to provide a greater root rate and seedling development. The trial was conducted in a greenhouse at the State University of Maranhão, in São Luís - MA under intermittent nebulization conditions. The cuttings with dimensions of 10 cm were treated with indole butyric acid (IBA) at a concentration of 2,000 mg L^{-1}, and as substrate were used vermiculite (MV), Plantmax ® (PL) (commercial substrate based on decomposed pine bark), agricultural soil + tanned and sieved bovine manure (S + M) (50% + 50%, v / v); and vegetable soil (VS) composed of fresh soil with the remains of decomposed plants (leaves, stems, bark, and tree fern). The experiment was laid out in a randomized complete design in a 2 x 4 factorial arrangement (2 types of cuttings x 4 substrates) with eight replications with five cuttings per plot. At 60 days after the establishment of the trial, it was concluded that the best combination for rooting cuttings was vermiculite with herbaceous cuttings because they favor a higher rooting rate (95.0%). The alternative substrate composed of soil and tanned bovine manure provided promising results in the rate of rooting (72.5%), root formation, and vegetative development of the aerial part.

Keywords: *Malpighia emarginata* DC.; propagation, herbaceous cuttings, organic substrates.

INTRODUCTION

The acerola cherry (*Malpighia emarginata* Sessé & Moc. Ex DC.), also known as Cherry of the Antilles, is a fruit tree native to the Caribbean, Central America, and northern South America, which found in Brazil favorable conditions for its commercial cultivation [1, 2]. Brazil is the largest producer, consumer, and exporter of acerola cherry in the world, and northeast Brazil is the largest producing region [3].

The growing consumption of acerola cherry is basically due to its high content of ascorbic acid (vitamin C) that can reach, in some cultivars, up to 5,000 mg 100 g^{-1} of pulp, that is, up to 100 times more than the orange [4]. However, there is a wide variation in the vitamin C content, from 779 to 3,094.43 mg 100 g^{-1} of pulp [5], which means that the vitamin C content, next to the red color of the peel and pulp, is an important criterion for the selection and multiplication of cultivars.

Almost all orchards have a marked mixture of acerola cherry plant types and forms due to the obtaining of seedlings by seeds [6]. This has caused serious problems for acerola cherry producers, because plants' unevenness causes management difficulties, such as the need for pruning, and losses in orchard productivity and fruit quality [7]. Orchards must be from well-defined acerola cherry varieties, with agronomic and technological characteristics, suitable for their intended purpose [8].

Vegetative propagation is based on the capacity of part of the plant to regenerate from somatic cells [9] such as the rooting of cuttings. This faculty depends on two basic characteristics: totipotence and differentiation [10, 11], and implies the mitotic division of cells, in which there is a duplication in the chromosome and cytoplasm system [12]. In addition to generating a high number of individuals, the process maintains the essential characteristics of the genotype, contrary to what occurs when heterozygous plants come from seeds [13]. This allows the multiplication of selected plants through cuttings and establishing uniform clonal plantations [14].

The technique of vegetative multiplication most used for cloning woody and herbaceous plants on a large scale has been cutting [15]. By this method, new plants are produced by rooting parts of the branches, which can be herbaceous, semi-woody, woody, or mini cuttings [13, 2, 16]. In this method, a regenerative process of sprouts and adventitious roots occurs in the nodal regions of the cutting [2].

Woody cuttings are obtained from lignified branches or stems, where they are preferably collected during the period of vegetative rest, and herbaceous cuttings are collected during the period of vegetative growth when the tissues present high meristematic activity and low degree of lignification [11]. the term “semi-woody” refers to intermediate cuttings between herbaceous and woody. The stake preparation should be 10 to 20 cm long [23].

Lima and coauthors (2006) [23] confirmed the viability of the acerola cherry asexual propagation by cutting rooting. This method ensures greater precocity in production as well as the transmissibility of the genetic characteristics of the propagated plant [8]. In a study [17], they obtained 87% of rooted cuttings and a high survival rate, evidenced by these characteristics in acerola cherry cuttings.

The importance of the rooting medium (substrates) in the cutting rooting process is related to its ability to provide sufficient porosity to allow good aeration and to ensure adequate oxygen availability for the root system’s growth and development. Regarding low-cost or alternative substrates, one should choose simple materials or mixtures of common and easily available types. There are several materials on the market used as plant substrates, such as vermiculite, carbonized rice husks, sawdust, and sand, among others [18, 19, 20, 21, 22].

Acerola cherry was introduced in the state of Maranhão in the late 1980s and its expansion in the state occurred with ungrafted seedlings, being cultivated in small orchards that exhibit high variability of plants and fruits. There is no offer of cloned seedlings obtained by vegetative propagation by either grafting or cutting.

Acerola seedlings produced through vegetative propagation (cuttings) in combination with a good substrate can result in greater uniformity, reduce seedling production time in nurseries, and increase vigor and productivity. From this perspective, the present work aimed to propose the best combination of substrate and cutting type (herbaceous and semi-woody) to provide a greater root rate and seedling development.

MATERIAL AND METHODS

Collection of cuttings and substrate preparation

We collected cuttings at two maturity stages (herbaceous and semi-woody) from healthy and vigorous plants, in a domestic orchard of the acerola cherry 'Andiroba' selection, aged five years, which produces red-colored fruits (bark and pulp). We removed the branches from the median and upper part of the plant canopy [23] and kept them with the base immersed in clean water and protected in the shade.

In laboratory conditions and on the same day we standardized the cuttings with 10 cm length with five pairs of leaves and with dormant buds. We made a bevel cut at the base of the stake at 1.0 cm from the node and a straight cut at the apex. We reduced the leaves to half of their size and kept each cutting with three pairs of half leaves [24]. The cutting portions buried in the substrate correspond to two pairs of buds, without leaves.

We grouped the cuttings in bundles and treated them with indole butyric acid (IBA) at a concentration of 2,000 mg L^{-1} [24]. The IBA hydroalcoholic solution was prepared [24]: with 0.2 g of IBA weighed on a semi-analytical scale and dissolved in 50 mL of ethyl alcohol in a Becker. After the IBA complete dissolution, we transferred it to a flask and completed the volume up to 100 ml with distilled water, obtaining the desired concentration (2,000 mg L^{-1}). We immersed the cuttings to half their length in the said solution for a period of 10 seconds.

We used expanded vermiculite (MV), Plantmax ® (PL) (commercial substrate based on decomposed pine bark), agricultural soil + tanned and sieved bovine manure (S + M) (50% + 50%, v / v); and vegetable soil (VS) composed of fresh soil with the remains of decomposed plants (leaves, stems, bark, and tree fern), as substrates. Chemical analyses of the substrates were carried out and the contents of organic matter, pH, P, K, Ca, Mg, Na, Al, H, and C were determined, adopting the methodology of Raij and Quaggio [26] (Table 1).

Table 1. Chemical analysis results of the substrates used for rooting of the acerola cherry (*Malpighia emarginata* Sessé & Moc. ex DC.) cuttings. São Luís, MA, 2017.

Substrate	OM	pH	P[1]	K^+	Ca^{2+}	Mg^{2+}	H^++Al^{3+}	Al^+	H^+	C
	g dm^{-3}	$CaCl_2$	mg dm^{-3}	------------------------$mmol_c$ dm^{-3}					----------------------------	g dm^{-3}
Vermiculite	4	7.9	6	5.9	7	122	8.0	0.0	8	2.5
Plantmax	66	8.2	129	25.6	210	119	6.1	0.10	6	38.0
Soil + Manure	61	7.2	334	48.2	30	89	12.1	0.10	12	35.4
Vegetable Soil	39	8.0	137	11.9	46	73	8.0	0.0	8	22.8

[1]Extractor Mehlich.

Conducting the experiment

The experiment was installed at the São Luís School Farm from the Center for Agricultural Sciences, State University of Maranhão, located on the Paulo VI campus, in São Luís, Maranhão, Brazil (02°31'51" S and 44°18'24" W)). The average temperature is around 27° C, with a maximum of 37°C and a minimum of 23°C.

The polystyrene trays with 128 cells (53 x 27 cm) (dimension of each cell: 5 x 3 cm), containing the different substrates, were used for the cutting rooting. The trays with the cuttings were kept under programmed intermittent nebulization conditions with a 60-second nebulization shift and operation every two minutes, due to the high local temperature.

The experiment was laid out in a randomized complete design in a 2 x 4 factorial arrangement (2 types of cuttings x 4 substrates), with eight replications, with each experimental unit composed of 5 cuttings. Two types of cuttings were used (herbaceous and semi-hardwood) which, in combination with different substrates, resulted in eight treatments.

Parameters evaluated

After 60 days of experimenting, the following variables were evaluated: the number of rooted cuttings from which the rooting percentage was calculated; the number of new leaves; the length of the aerial part, and the root system. Rooted cutting was defined when it presented at least one root with a minimum of 5 mm, with or without the presence of a callus. The number of leaves was obtained by counting new leaves fully expanded in the emitted shoots. The length of the aerial part was measured from the sprout emitted from the cutting to the apical bud, while the length of the root system was measured in centimeters from the base of the cutting to the apex of the largest root, both in centimeters using a measuring tape.

Statistical analysis

The data obtained were subjected to tests of normality, homogeneity, and analysis of variance, with the treatment, and means compared by the Tukey test at the level of 5% probability. Non-normal data even with transformations were analyzed using the Kruskal-Wallis non-parametric test (root length and number of rooted cuttings). For percentage data, the √x / 100 arc sine transformation was adopted. The data were analyzed by the software ASSISTAT version 7.7 beta [27].

RESULTS

A statistically significant difference was observed when considering the main effects of the treatments, that is, the type of cuttings about the substrate and between the types of cuttings.

The type of cutting affects rooting. Herbaceous cuttings obtained the highest rooting rates (71.2%), while semi-hardwood cuttings reached 50.6% rooting percentage (Table 2). The rooting is related to species and mainly, to their age, in which younger plants rooted better than older plants [15].

Table 2. Rooted cuttings (%)* of the acerola cherry (*Malpighia emarginata* Sessé & Moc. ex DC.) 'Andiroba' selection under the influence of as affected by different substrates and two maturity stages, at 60 days after installation of the experiment. São Luís, MA, 2017.

Cutting type	**Substrates**				**Cutting mean**
	MV	PL	S+M	VS	
Herbaceous	95.0	47.5	72.5	70.0	71,25 a
Semi - woody	77.5	7.5	65.0	52.5	50,62 b
Substrate mean	86.2 A	27.5 C	68.7 B	61.2 B	

CV(%) = 30.19. Means followed by the same lower case letters in the column and upper case letters in the row, do not differ by Tukey's test ($p < 0.05$). * data in %: transformed into arc sine of √x / 100. MV: vermiculite, PL: Plantmax, S + M: soil + manure, and VS: vegetable soil.

Other authors have reinforced that those tissues with a lower degree of lignification, such as branches harvested from the apical position, have adequate physiological conditions for the emission of new structures such as adventitious roots [16, 23].

It was found that the vermiculite commercial substrate provided a higher percentage of rooted cuttings (86.2%) compared to the other treatments (Table 2), confirming its good characteristics as a rooting material [18, 19]. The results for this substrate were superior to those reported in another study [17] that found a rooting rate of 76.25%. Observed that the use of vermiculite resulted in greater rooting of acerola cuttings [28].

To mint seedlings (*Mentha arvensis* L.) formation, and asexual propagation of common horsetail (*Equisetum arvense* L.) Plantmax® is the recommended substrate [29,30]. In this work, Plantmax® presented the lower rooting rate (27.5%) probably due to the association of limited space in the polystyrene trails and the humidity caused by the irrigation system, also induced by the occurrence of root fungi.

Considering the combination of herbaceous cuttings and substrates, the greatest rooting was ensured by vermiculite (95.0%) while the lowest rate was due to Plantmax (47.5%). For semi-hardwood cuttings, the rooting rate in the different substrates was lower than the apical cuttings (Table 2). The strategy of treatment of cuttings with indole butyric acid proved to be adequate in this study. Similar results were observed with peach [25] and acerola [16].

The so-called alternative materials, the substrates soil + manure (S+M) and vegetable soil (VS) did not differ from each other and were surpassed only by vermiculite. The results of the rooting rate of S + M and VS, on average, were considered relevant. This may be related to the good characteristics of these substrates such as porosity, neutral pH, and high levels of mineral nutrients, as shown in Table 2.

Regarding the root length, a significant difference was also observed between the types of cuttings, with an emphasis on herbaceous cuttings (Table 3). Herbaceous acerola cherry cuttings produced longer root lengths, showing the speed of the use of tissue reserves in the promotion of roots for other functions [31].

Table 3. Root length (cm) of the acerola cherry (*Malpighia emarginata* Sessé & Moc. ex DC.) 'Andiroba' selection under the influence of different substrates and two maturity stages, at 60 days after installation of the experiment. São Luís, MA, 2017.

Cutting type	Substrates				Cutting mean
	MV	PL	S+M	VS	
Herbaceous	6.02	3.38	4.75	4.43	4.65 a
Semi - woody	4.58	0.11	3.87	3.55	3.03 b
Substrate mean	5.31 A	1.75 B	4.31 A	3.99 A	

CV(%) = 43.26. Means followed by the same lower case letters in the column and upper case letters in the row, do not differ by Tukey's test p <0.05). MV: vermiculite, PL: Plantmax, S + M: soil + manure, and VS: vegetable soil.

The substrates soil + manure and vegetable soil did not differ from vermiculite about the root lengths, proving to be suitable for this variable (Table 3). These results are relevant since they investigate lower-cost alternatives for seedling formation. In contrast [16] the use of substrates added with sugarcane bagasse and chicken manure did not prove to be effective on mulberry (*Morus nigra* L.) cuttings. The good porosity of vermiculite and other physical characteristics were offset by the high levels of organic matter and nutrients in the alternative substrates S + M and VS [28, 19].

Four characteristics must be considered when choosing the substrate for cutting rooting: the ability to sustain cuttings and provide aeration, good moisture retention, and a dark environment at the base of the cutting [10].

The substrate vermiculite associated with herbaceous cuttings favored greater development of adventitious roots of acerola cherry cuttings. For rooting of fig (*Ficus carica* L.) observed a similar effect in quince (*Cydonia oblonga* M.) [18, 32]. This is probably due to the low apparent particle density, high porosity, neutral pH, and high moisture retention capacity found in this substrate.

Regarding the shoot length, the substrate vermiculite and S + M favored the development of acerola cherry cuttings, with average values of 6.46 cm and 6.01 cm, respectively. In contrast, the substrate plantmax had a shoot length lowest value (1.97 cm). It was observed that the herbaceous cuttings systematically showed a significantly higher average than the semi-hardwood ones. Therefore, they are the most suitable material for acerola cherry cuttings (Table 4).

Table 4. Shoot length (cm) of the acerola cherry (*Malpighia emarginata* Sessé & Moc. ex DC.) 'Andiroba' selection under the influence of different substrates and two maturity stages, at 60 days after installation of the experiment. São Luís, MA, 2017.

Cutting type	Substrates				Cutting mean
	MV	PL	S+M	VS	
Herbaceous	7.12	3.65	6.40	5.45	5.65 a
Semi - woody	5.81	0.30	5.63	3.96	3.92 b
Substrate mean	6.46 A	1.97 C	6.01 AB	4.70 B	

CV(%) = 37.49. Means followed by the same lower case letters in the column and upper case letters in the row, do not differ by Tukey's test p <0.05). MV: vermiculite, PL: Plantmax, S + M: soil + manure, and VS: vegetable soil.

The development of the aerial part of cuttings of fruit seedlings may be related to the higher levels of carbohydrates present in the branches that served as an energy source for the development of the seedlings [10]. Parallel to the rooting, the cuttings emitted generalized shoots of branches with new leaves, a variable that did not differ statistically between the types of cuttings, as shown in Table 5. The largest number of leaves, regardless of the type of cutting, was observed in the soil + manure treatments and vegetable soil. The plantmax substrate provided fewer leaves for semi-hardwood cuttings and intermediate values for vermiculite.

Table 5. Number of leaves of the acerola cherry (*Malpighia emarginata* Sessé & Moc. ex DC.) 'Andiroba' selection under the influence of different substrates and two maturity stages, at 60 days after installation of the experiment. São Luís, MA, 2017.

Cutting type	**Substrates**				**Cuttin mean**
	MV	PL	S+M	VS	
Herbaceous	2.50	1.95	5.70	3.75	3.47 a
Semi - woody	4.00	0.07	5.97	4.10	3.53 a
Substrate mean	3.25 B	1.01 C	5.83 A	3.92 AB	

CV(%) = 60.24. Means followed by the same lower case letters in the column and upper case letters in the row, do not differ by Tukey's test p <0.05). MV: vermiculite, PL: Plantmax, S + M: soil + manure, and VS: vegetable soil.

Almeida and coauthors (2011) obtained similar results with castor seedlings (*Ricinus communis* L.) grown in substrates with the presence of goat manure [21]. Sousa and coauthors (2000) reported that enriched manure substrate promotes better development of banana seedlings (*Musa paradisiaca* L.) due to the presence of nitrogen, which is essential for vegetative growth [33].

CONCLUSION

The combination of vermiculite with herbaceous cuttings provided a higher rooting rate of the acerola cherry ‘Andiroba’ selection.

The alternative substrate composed of soil and tanned bovine manure showed promising results in the rooting rate, root formation, and vegetative development of the aerial part.

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