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ADAPTATION OF *Acrocomia aculeata* VITROPLANTS, WITH THE APLICATION OF AMF AND BIOBRAS-16

Adaptación de vitroplantas de *Acrocomia aculeata*, con la aplicación de HMA y Biobras-16

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ABSTRACT. An experiment was carried out at the Federal University of Paraná, Brazil, which was established and developed under controlled conditions of greenhouse. The aim of this research was to obtain a methodology to improve the adaptation process of seedlings of the oil palm *Acrocomia aculeata* (Jacq.) Lodd. ex mart., obtained from the *in vitro* culture of zygotic embryos. A substrate composed of soil and river sand washed in proportion (3:1) was used and the following products were applied to the seedlings: Arbuscular Mycorrhizal Fungi (AMF) and a Spirostic Analogue of Brassinosteroid (Biobras-16), with four treatments, which were: 1-Control, 2-Inoculation of the Plants with AMF and one application of Biobras-16 to the transplant, 3- Inoculation of the plants with AMF and 4- Inoculation of the plants with AMF and two applications of Biobras-16, one at the transplant time and another 30 days later. The results showed that the best treatment was the number 2, in which the vitroplants were inoculated with AMF and a foliar spray was performed with the Biobras-16 at the time of transplantation, which produced an increase in the different growth and development rates of the plants with respect to the other treatments.

Key words: acclimatization, brassinosteroids, fungi, inoculation, palm oils

RESUMEN. Se llevó a cabo un experimento en la Universidad Federal de Paraná, Brasil, el cual se estableció y desarrolló bajo condiciones controladas de invernadero. El objetivo de la investigación fue obtener una metodología para mejorar el proceso de adaptación de plántulas de la palmera oleaginosa *Acrocomia aculeata* (Jacq.) Lodd. ex mart., obtenidas a partir del cultivo *in vitro* de embriones cigóticos. Se utilizó un sustrato compuesto por suelo y arena de rio lavada en proporción (3:1) y se aplicó a las plántulas los productos: Hongos Micorrízico Arbusculares (HMA) y un Análogo Espirostánico de Brasinoesteroide (Biobras-16), con un total de cuatro tratamientos, los cuales fueron: 1-Testigo, 2-Inoculación de las plantas con HMA y una aplicación de Biobras-16 al trasplante, 3- Inoculación de las plantas con HMA y 4- Inoculación de las plantas con HMA y dos aplicaciones de Biobras-16, una al momento del trasplante y otra 30 días después. Los resultados obtenidos, mostraron que el mejor tratamiento fue el número 2, en el cual las vitroplantas fueron inoculadas con HMA y se les realizó una aspersión foliar con Biobras-16 al momento del trasplante, lo cual produjo un incremento en los diferentes índices de crecimiento y desarrollo de las plantas con respecto a los demás tratamientos.

Palabras clave: aclimatación, brasinoesteroides, hongos, inoculación, palmas oleaginosas

INTRODUCTION

The production of vegetable oils from new sources such as palms has been gradually increasing since the 90s to the present day due to the increase in demand for their use globally, because they have a wide use, which It goes from satisfying the growing food needs of the population, to the industrial production of soaps, cosmetics and the use of lubricants and biodiesel as a source of renewable energy (1,2). The palm oil industry, *Elaeis guineensis*, is responsible for supplying almost 40 % of the world's oil and fat needs (3); However, *Acrocomia aculeata*, second in productive volume by surface, has been receiving in the last decades an important development, because it adapts better to lower quality soils and is less demanding in water (1).

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However, in countries where oils are produced from *A. aculeata*, mainly in Paraguay and Brazil, the harvest of the fruits is mostly done through family extractivism from naturally grown plants (4).

During the last five years work has been done to domesticate the species in order to organize the harvest with lower costs, selecting the planting material, since it has a lot of genetic variability and thus achieve plants that have the most homogeneous characters, which will allow greater efficiency productive (5).

Once the planting material has been selected, plants should be produced in an accelerated manner with homogeneous characteristics, one of the methods to be used *in vitro* multiplication from zygotic embryos. With the aim of obtaining a methodology to improve the process of adaptation of the vitro plants to *ex vitro* conditions, a research work was developed under controlled conditions, with the application of two bioproducts, the Arbuscular Mycorrhizal Fungi (AMF), which are of great help in the solubilization and absorption of nutrients for plants (3,6) and a spirostanic analogue of Brassinosteroid, of which some of their formulations have been shown to stimulate growth when plants are subjected to stress. There are good results in treatments carried out in adaptation of vitroplants and in the treatment of plants of other crops subjected to stress, such as the production of plants in nurseries, and other types of vegetable production (7,8).

MATERIALS AND METHODS

The experimental work was carried out under controlled conditions in a Van der Hoeven greenhouse with automatic temperature and relative humidity programming system, which maintained the average temperature between 25 and 26 ºC and the relative humidity at 70 %, belonging to the Department of Plant Sciences and Phytosanitarism, Sector of Agricultural Sciences of the Federal University of Paraná, Curitiba, Brazil. To carry out the experiment, vitroplants obtained from the *in vitro* culture of zygotic embryos of *A. aculeata* were used in the tissue culture laboratory of said university, obtained in Woody Plant Medium (WPM) culture medium (Lloyd and McCrown 1980). The embryos used were obtained from seeds of selected plants in the "Campanario" farm, municipality Bodoquena, state of Mato Grosso do Sur.

Four experimental treatments were established, which had three replicates of ten plants each (Table 1). The substrate used was composed of soil + washed river sand, in proportion (3: 1 v/v), its chemical composition being shown in Table 2 and the granulometric composition in Table 3.

Table 1. Description of treatments

No.	Composition of treatments
1	Control
2	Plants inoculated with AMF + application of Biobras-16 at the time of transplant
3	Plants inoculated with AMF at the time of transplant
4	Plants inoculated with AMF + 2 applications of BioBras-16, the 1st. at the time of transplant and the 2nd. 30 days after transplant

Table 2. Chemical characterization of the substrate used in the adaptation of vitroplants

pH		Al^{+3}	$H^{+}+Al^{3}$	Ca^{+2}	Mg^{+2}	K^{+}	P	C	Ca/Mg
$CaCl_2$	SMP			$cmol_c\ kg^{-1}$			mg kg^{-1}	g kg^{-1}	
4,5	4,8	2,60	14,4	3,90	2,90	0,10	4,60	54,5	1,3

Table 3. Granulometric composition of the substrate used in the adaptation of vitroplants, expressed in g kg^{-1}

Gross sand	Fine sand	Silt	Clay
85	95,5	369,5	450,0

Composition of bioproducts and mineral fertilizer used. Form of preparation and application

The Arbuscular Mycorrhizal Fungi (AMF) used for inoculation, corresponding to the EcoMic® series, a commercial product patented and produced in Cuba by the National Institute of Agricultural Sciences (INCA), using the species Glomus cubense (9), with a content of 40 spores per gram. It was applied by placing two grams of powder product inside the hole made in the substrate of the bag that would receive the plant to adapt.

The Brassosteroid Spirostannic analogue used for the sprinkled application corresponded to "Biobras-16", which is produced by the Natural Products Laboratory of the Faculty of Chemistry of the University of Havana, Cuba, which has a concentration of 1 g L^{-1}. To be applied, it was prepared at a concentration of 0.05 mg L^{-1}, mixing it in water, agitated and sprinkled in the form of a spray to the point of drip to the plants after transplanting or at the time corresponding to the treatment (Table 1). To apply the bioproduct a hand sprinkler was used.

The fertilization of the seedlings in adaptation was done with the commercial product "Nutriverde" liquid formula (6-6-8 + micronutrients), at a rate of 2 ml of the liquid fertilizer in three liters of water. It was applied evenly to the substrate of the bags at a rate of 25 ml in each and then lightly irrigated; This fertilization was carried out in two moments, when the plants had 180 days of transplanted and 210 days.

Studied variables

The final evaluation of the plants was made when they were 399 days after the transplant (DAT).

- Height of the plants: it was measured with a tape measure from the neck of the plant to the apex.
- Diameter of the stems: it was measured with a Vernier at a height of one centimeter above the neck of the plant.
- Number of leaves issued: the number of leaves issued was counted.
- Average length of the leaves: the length in cm was measured with a tape measure of each of the leaves per plant and the average of them was taken out.
- Dry mass of the different parts of the plants and total in the final sampling: once the selected plants were extracted, they were separated by sections: roots, corm, stem, leaves and rachis. The green weight was taken by weighing the different parts in the technical balance with 0.01 g of precision. Once weighed, they were put in a stove until constant dry weight, and weighed again.
- To determine the root colonization by AMF, 200 mg of roots were taken from each sample that were stained with trypan blue (10), then the intercept method was used to quantify mycorrhizal colonization (11). The visual density was determined by means of the evaluation of the fungal occupation of the fungus at the root of each intercept, assigning a level to the occupation percentages (12).
- Determination of foliar area of the plants: the disc method was used for this, according to (Watson & Watson 1953) cited by Vidal (13), taking an average of 10 discs of 1 cm^{-2} per plant in leaves of different stage of development and a total of nine plants sampled per treatment. Subsequently, they were dried in a forced circulation oven at a temperature of 70 ºC, up to a constant weight. Both the leaves and the discs extracted to each plant were weighed on an analytical scale with error (± 1 mg), thus being able to calculate the leaf area of the plants based on the disks of area and known average weight.

Extraction of nutrients by Plants

In order to determine the extraction of nutrients made by the plants in the evaluated period, dried samples of complete plants were sent to the laboratory for each treatment for the extraction of the contained nutrients, using the Dumas method for the determination of N and the Nitroper method for determinations of P, K, Ca, Mg and S expressed in mg/gr. Then, based on the average dry matter per plant for each treatment, the amount of extracted nutrients was calculated.

Method used for tube-to-bag transplantation

During the process of passing from tube to bag with substrate, great care was taken to put into operation in the interior of the greenhouse the system of irrigation by micro-spraying before performing the transplant of the vitroplants, in order to increase the humidity relative within the premises and minimize the stress of the seedlings when performing the transplant.

The vitroplants were transplanted from the tubes into small polyethylene bags (3x7 cm) containing a soil/sand substrate previously described. It was removed with sterile water and the help of a washing bottle, the remains of culture medium from the roots of the vitroplants. The small bags where the vitroplants were placed were in turn deposited inside transparent plastic boxes with a lid, which contained 30 bags each.

In the case of the plants that were inoculated with AMF, the bioproduct was applied in powder form at a rate of two grams per bag, just before the transplant, depositing in the hole that was made in the bag that had been previously moistened to receive the seedling.

The application of Biobras-16 was performed as appropriate by treatment and time (Table 1); once the transplant was finished, the plants were sprayed and the plastic boxes closed, placed on a table. These transparent plastic boxes with lid, and dimension of 35 cm long x 20 cm wide x 10 cm deep and lid with 10 cm high on the axis of the closure, act as a humid chamber for the adaptation of the vitroplants. The plants were kept in the wet chamber stage for the first 30 days and they were opened little by little until 10 days later the lids were finally removed.

Vitroplants already adapted in the small bags were transplanted at three months of age to larger black polyethylene bags (18x30 cm) containing a substrate with the same composition as used in the small bags. They were placed on a wooden table covered with nylon, distributed in a random block design of four treatments and three repetitions, each treatment having 10 plants.

The irrigation of the plants was carried out every seven days in the adaptation boxes, after these were extracted from them and transplanted; the irrigation was carried out every three days.

Statistical analysis

The data corresponding to percentage were transformed by the formula arc sin $\sqrt{x}$ and the averages of the counts by the formula $\sqrt{(x + 1)}$ to make the statistical analyzes.

The data obtained were analyzed by means of a simple classification Variance Analysis. In the cases in which a significant difference was found between the treatments, the comparison of means was carried out using Duncan's multiple range tests, and Statgraphics plus, version 5, was used for statistical processing.

RESULTS AND DISCUSSION

Table 4 shows the results of the effect of the different treatments applied to the vitroplants in the adaptation phase.

As can be seen in the Table, the best results were obtained when the root system of the plant was inoculated with AMF, and Biobras-16 was applied at the time of transplantation (treatment 2).

The variables height of the plants, average length of the leaves and average number of roots per plant showed significant differences with the rest of the treatments, which shows the existence of synergism between the two applied bioproducts. Similar results regarding the application of Biobras-16 were obtained in the adaptation of banana vitroplants (14); however, it can be seen that when the radical system was inoculated with AMF, and two applications of Biobras-16 were made, one at the time of transplantation, and another 30 days after the transplant (Treatment 4), a lower growth of plants.

This answer does not coincide with the works of the aforementioned author, which obtains a greater growth of the plants with the immersion of the roots of the vitroplants in a Biobras-16 solution for 15 minutes before the transplant and a foliar application at the concentration of 0.02-0.2 μmol L^{-1}, 15 days after the transplant.

Table 4. Influence of treatments on the growth of plants at 399 days after transplantation

Treatments	Height of Plants (cm)	Number of leaves per plant	Average length of the leaves (cm)	Average diameter of stems (cm)	Roots	
					Average length in (cm)	Average number
1	18,12 b	3,64 b	30,63 b	2,52 b	70,89 b	5,11 ab
2	29,50 a	3,80 a	82,78 a	3,23 a	119,06 a	6,33 a
3	16,23 b	3,41 c	27,45 b	1,89 d	57,61 b	4,11 b
4	17,65 b	3,35 c	28,47 b	2,15 c	60,20 b	4,44 b
ES$\bar{x}$	1,100*	0,023*	3,89 *	0,018*	0,286*	0,363*
CV %	9,36	3,54	15,92	1,265	0,64	12,57

Means with common letters by columns, do not differ significantly according to Duncan's multiple range for $p < 0.05$

With respect to the dry matter index accumulated by the plants by organ and total, it can also be seen that treatment 2 has the highest amount of dry matter accumulated at the time of final sampling with significant differences with the rest of the treatments (Table 5), followed by treatment 4, where the radical system of the plants with AMF was inoculated and two applications of Biobras-16 were made.

Experiments carried out with the application of brassinosteroids in several agricultural crops, showed that with foliar sprays of this bioproduct, at concentrations of 0.1 mg L^{-1}, plant growth was markedly promoted in height, stem thickness, length of the main root, and dry mass per plant, foliar area and photosynthesis (15-17).

In terms of adaptation and growth stimulated by AMF, Schultz (2001) (3), it was reported that *E. guineensis* seedlings propagated *in vitro* and inoculated with 12 AMFF isolates (Arbuscular Mycorrhizal Formation Fungi), showed an increase in survival during the stage of hardening between 83 and 100 %, greater growth both radical and aerial part, and an increase in phosphorus intake, the most efficient species being *Glomus manihotis*, *Entrophospora colombiana*, *Acaulospora mellea* and *Acaulospora appendicula*. In works carried out with inoculations with AMF in oil palm, but propagated from sexual seed, important results have been obtained when performing the inoculation of the plants in the nursery phase, observing that after the transplant to the field, the inoculated plants surpass height and dry matter formation at least three times to the plants that were not inoculated, with highly significant differences between them (18-21).

On the other hand besides the already known benefits of symbiosis with mycorrhizae, such as the help not only to a more efficient nutrient intake by the plant, but also contributes to tolerance to water deficit, resistance to attack by pathogens, tolerance to high levels of heavy metals and the agility of growth in early stages of development, especially under nursery conditions (23). The case of the species *Coccotrinax readii* Quero (20) is also mentioned about the tolerance of the palm species to the hydric deficit when its radical system is performing symbiosis with AMF.

Regarding treatment 3 (plants inoculated with AMF at the transplantation time), the plants did not show an increase in growth as expected, due to having the substrate in which they are developing a fairly acid pH and a high aluminum content can be seen in Table 2, which is not suitable for the *Glomus cubense* species, since it adapts better to pH ranging from slightly acidic to neutral (21).

Probably the application of Biobras-16 acts as an anti-stress before the effect of acidity on the symbiosis that takes place in the inoculated plants, which can be corroborated in Table 6 where it is also observed that treatment 3 (plants inoculated with AMF at the time of transplantation), radical colonization is almost nil with respect to treatment 1 (control), where there is obviously a native AMF strain of the soil, which is more abundant and colonizes with greater intensity but is inefficient , because the plants have little growth and development which can be seen in Table 7.

Table 7 shows the foliar area of the plants by treatment, where the treatment 2, also shows a greater leaf area than the rest of the treatments, product of the synergism between AMF and Brassinosteroid since the solubilization and absorption of nutrients by that treatment is greater than in the rest, which is observed in Table 8.

The macro and micro elements extracted by the plants are found in a greater quantity in treatment 2, demonstrating the absorption of elements made by the plant product of synergism (Table 8).

Table 5. Influence of the treatments on the average dry matter index per organ of the plants and total

Treatments	Dry mass of the leaves	Dry mass of rachis	Dry stem mass + corm	Dry mass of the roots	Total dry mass
1	0,20 b	0,047 c	0,42 b	0,24 bc	0,907 c
2	1,30 a	0,307 a	3,25 a	0,99 a	5,847a
3	0,15 c	0,043 c	0,26 c	0,20 c	0,653d
4	0,21 b	0,058 b	0,45 b	0,27 b	0,988 b
ES$\bar{x}$	0,008*	0,001*	0,029*	0,019*	0,001*
CV %	2,845	1,949	4,536	7,774	0,106

Means with common letters by columns, do not differ significantly according to Duncan's multiple range for p <0.05

Table 6. Radical colonization, visual density and endophyte weight by treatments at 399 days after transplantation

Treatments	Root colonization %	Visual density %	Endophyte weight
1	3 b	0,06 b	0,09 b
2	6 a	0,22 a	0,33 a
3	1 d	0,01 c	0,015 c
4	2 c	0,02 c	0,028 c
ES $\bar{x}$	0,005*	0,006*	0,008*
CV %	0,289	12,87	11,922

Means with common letters by columns, do not differ significantly according to Duncan's multiple range for p <0.05

Table 7. Average leaf area of plants by treatment

Treatments	Average dry weight of leaves in grams per plant	Average dry mass in grams of a disk of 1 cm²	Foliar area calculated in cm² per plant
1	0,20 b	0,00364 c	54,94 b
2	1,30 a	0,00375 b	346,66 a
3	0,15 c	0,00357 d	42,01 b
4	0,21 b	0,00457 a	45,95 b
ES $\bar{x}$	0,008*	0,001*	16,128 *
CV %	2,845	0,386	22,24

Means with common letters by columns, do not differ significantly according to Duncan's multiple range for p <0.05

Table 8. Extraction of nutrients in (mg plant^{-1}) per treatment

Treatments	N	P	K	Ca	Mg	S
1	10,16 b	0,65 b	10,86 b	8,89 b	4,13 b	2,15 b
2	54,37 a	5,03 a	38,24 a	37,19 a	27,9 a	8,36 a
3	6,07 d	0,37 c	5,67 d	4,03 d	2,47 d	0,86 d
4	8,99 c	0,48 c	10,32 c	6,27 c	3,12 c	1,39 c
ES $\bar{x}$	0,035*	0,037*	0,034*	0,033*	0,019*	0,042*
CV %	0,305	3,962	0,361	0,403	0,351	2,286

Means with common letters by columns, do not differ significantly according to Duncan's multiple range for p<0.05

CONCLUSIONS

The obtained results showed that applying the treatment where the inoculation of the root system of transplanted plants with AMF, *Glomus cubense* species, is combined, applying 2 g of the product in the hole of the bag that received the seedling, and making the application of the Spirostannic analog of Brassinosteroid (Biobrás- 16) at the time of transplantation and before the closure of the humid chamber in the adaptation phase, the best results can be obtained in the growth and development of the plants obtained from the *in vitro* culture of zygotic embryos of *Acrocomia aculeata*.

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