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Micropropagation of Arrowroot (*Maranta arundinacea*). Micropropagação de Araruta (*Maranta arundinacea*).

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Abstract
The study aimed at establishing a protocol to produce arrowroot plants quickly. Seedlings were grown from stem nodes on modified MS medium in controlled growth chamber, green house and filed and again aclimatized in green house on washed sands and soil. Chlorophyll content both of a and b in seedlings were higher in the plants grown in field (32.97 and 9.80 respectively) and likewise greenhouse grown seedlings had greater contents than in plants grown *in vitro* (16.66 and 3.76 respectively). Plants acclimatized in green house soil for 120 days achieved an average rhizome height up to 32.7 cm along with having higher number of leaves (7.0) and dry weight (100 g) compared to those acclimatized in washed sands (15.00 cm, 7.0 and 50 g respectively). So, the results showed that is possible to obtain rhizomes of arrowroot in less time.
Keywords: Micropropagation, *in vitro* culture, greenhouse, chlorophyll

Resumo
O estudo teve como objetivo estabelecer um protocolo para produzir plantas de araruta rapidamente. As plântulas foram cultivadas a partir de nós do caule em meio MS modificado em sala de crescimento controlada, casa de vegetação e novamente aclimatizadas em casa de vegetação em substrato de areia lavada e solo. Os teores de clorofila tanto de a quanto de b nas plântulas foram maiores no tratamento em campo (32,97 e 9,80, respectivamente) e as plantas cultivadas em casa de vegetação tiveram maior conteúdo do que plantas cultivadas *in vitro* (16,66 e 3,76, respectivamente). Plantas aclimatizadas em solo de casa de vegetação por 120 dias atingiram altura média acima de 32,7 cm e maior número de folhas (7,0) e massa seca de rizoma (100 g) em relação às aclimatizadas em areia lavada (15,00 cm, 7,0 e 50 g respectivamente). Assim, os resultados mostraram que é possível obter rizomas de araruta em menos tempo.
Palavras-chave: Micropropagação, cultivo *in vitro*, casa de vegetação, clorofila

Introduction

The arrowroots originated in South American continent, are also found in native form in the Venezuelan forests. Size of the arrowroot (*Maranta arundinacea* L.) rhizomes vary depending on age of the plants which contains more than 20% starch (PEREIRA et al., 1999).

Common arrowroots plants are characterized by plant height up to 60 cm with clear conical or spindle shaped fleshes-covered by scales reaching up to 30 cm in length, depending on the quality of the soil, although the normal size varies from 10 to 25 cm. Some are of flowering type in tropical conditions (LEONEL; CEREDA, 2002; MONTEIRO; PERESSIN, 2002).

ROSSI et al. (2011) detected that araruta rhizome has 45.83% of inulin on dry basis, together with an oligofructose (fructose polymers which have been found to be important in relation to their health benefits and technological properties in the food industry. In arrowroot, inulin is re-mobilized from the rhizome for the formation of new shoots and a high content of inulina is found during the formation of rhizome in adult plant. The arrowroot starch has characteristics and qualities that are unparalleled.

Arrowroot gives lightness and high digestibility to the confectionery. Another important feature of arrowroot foods is the absence of gluten (protein wheat, oats, rye and barley and derivatives), which makes them recommendable to people who have food intolerance to this protein (NEVES et al., 2005).

MONTEIRO e PERESSIN (2002) report that the world production of arrowroot is not plenty but commercial crop data are available in Barbados and Saint Vincent in the Caribbean. They referring IBGE (1997) reported that Brazilian production of arrowroot in 1996 was 1,141 tons, with an estimated value of R $ 283,565.15, in São Paulo contributing 54 tons. The current importance of arrowroot is a is considered relation to the peculiar culinary characteristics of its starch.

As the price of its starch in the international market is reached higher the interest of the industry in its production is has been great (MONTEIRO; PERESSIN, 2002).

However, arrowroot culture is almost very insignificant in Brazil, being limited in some municipalities of Bahia. So, for increasing its micro production in the culture media has been necessary.

The objective of this work was to establish the micropropagation of this species *in vitro* and in greenhouse conditions to produce plants with rhizomes in a shorter time for increased starch production on pick demanding period.

Materials and methods

The seedlings for micropropagation *in vitro* were obtained by Stem nodes, which were cut with an approximate size of 4 cm. The plants were grown in farm land (S 20° 26' W 54° 38' to 592m of altitude). After cutting, the nodes were taken to a laminar flow chamber where they were washed with sterile distilled water and immersed in 2% bleach solution for about 5 minutes for decontamination. The nodes were then dried with autoclaved paper and inserted into Hoagland nutrient solution. For each glass bottle 5 ml of nutrient solution was used. The vials were sealed with film paper. Filter paper bridges were also used to support plants in the early stages of development. In total, 108 glass vials each containing 03 stem nodes and as such 124 explants in total were used.

All biological material disinfested and conditioned in glass bottles with nutritive solution was taken to a growth room, where they were exposed to two fluorescent lamps branded Grow 2000 μmol.

$m^{-2}s^{-1}$ at 27 °C (+ -2 °C) with photoperiod of 16 hours of light and 8 hours of dark. After 40 days, the in vitro culture plants were transferred to acclimatization under greenhouse conditions. Therein the explants were planted in 2 treatments: a substrate consisting of ¾ of soil, ½ part of roasted straw. The second treatment consisted of sand washed 5 times in distilled and autoclaved water. The plants were measured at zero time (planting time) until the determination of the fresh and dry mass (end of the experiment). 60 plants were transferred to the plastic prothynetic trays (34x36x16 cm) of the treatment with substrate and 60 plants for plastic trays of the treatment with washed sand. The volume of substrate or sand used for each tray was 16 cm^3. In each tray 4 plants were transferred, 15 trays in the substrate treatment and 15 trays in the treatment with washed sand.

The height of the plants was obtained with the use of a ruler millimeter-from the base of the soil to the tip of the petiole at the end. Regarding the leaf count, the leaves that entered the inclusion criterion were only those that were mature and fully expanded. For the determination of the dry mass, a drying oven with forced air circulation at 54ºC was used for 7 days. For analysis of chlorophyll, 2 leaves per plant, were harvested fresh, weighed in analytical balance, the measures of width and height were measured and later crushed in porcelain crucible. Two fully expanded mature green leaves were collected from 3 plants of each treatment (*in vitro* culture, greenhouse and field), in a total of 9 plants. After maceration, 1 gram of leaf material from each treatment was weighed for evaluation of chlorophyll a and b, made after extraction in 2 ml of distilled water and 8 ml of ethyl alcohol PA, and incubated at 60 °C. After extraction, compound's absorbance was read at 663 nm and 645 nm in spectrophotometer. The means of the results of each experiment were analyzed by the Anova One Way (Tukey 5%, p = 0.05) and student test.

Results and discussion

The use of nodes in propagation *in vitro* culture was positive, because it produced new 5 cm plants in 15 days (figure 1).

Figure 1. *In vitro* Propagation of arrowroot stem nodes.

This result shows that it is not necessary to use rhizomes to for to multiplication *in vitro*. Thus, the rhizome could be used in the large-scale planting in farm level.

LAURA et al. (2000) evaluated the shoot propagation using two classes of rhizomes (class I = rhizomes with mass less than 5.0 g and class II = rhizomes with mass between 5.1 and 15.0 g) under immersion in indolebutiric acid (doses of 0, 150 and 300 mg L^{-1}). They concluded that the rhizome mass was extremely important for the growth of the aerial part, because the roots and the new rhizomes,

being recommended to propagation the rhizomes with weight than 5.0 g and without the use of phytoregulators.

However, the information of those authors does not corroborate with the results found for the arrowroot produced from the stem nodes, which was much faster and less costly and did not require the use of phytohormones for multiplication.

There was no significant difference in chlorophyll a content in plants grown in full sun (field) and those kept in greenhouse (table 1).

Table 1. Chlorophyll a and b in leaves of *Marantha arundinacea* grown in field (10 months), growth chamber (6 months) and greenhouse (4 months). Figures with dissimilar letter differ significantly at 5% level of significance in Tukey's test.

Treatments	chrorophyll a	chrorophyll b
Field (Full sun)	32.97 a	9.80 c
Greenhouse	26.84 a	8.41 c
In vitro cultivation	16.66 b	3.76 d

Chlorophyll *a* had always been higher in comparison to the chlorophyll *b* content of arrowroot plants grown in full sun, greenhouse and *in vitro* culture (Table 1).

Leaf length was higher in mature and senescent leaves of field plants when compared to plants kept in greenhouse (Table 2).

Table 2. Length, width and leaf weight of arrowroot plants grown under greenhouse and field grown plants. Figures with dissimilar letter differ significantly at 5% level of significance in Tukey's test.

Treatment	Leaf	length (cm)	width (cm)
Greenhouse	Fully expanded leaf	12.3B	4.3B
	Senescence	7.5C	2.5C
Field	Fully expanded leaf	26.5ª	6.8 B
	Senescence	23.0 A	11.0A

However, in regards to leaf width, it was observed that there was no difference between leaves of the field grown plants and of the greenhouse grown ones. Although the appearance of new leaves in adult arrowroot plants occurs in the lateral part when there are new branches issuing, the senescent leaves are not positioned in the basal part, but distributed among the mature leaves.

And again, the information of this study does not agree well with the results found from the arrowroot produced from the stem nodes, which was much faster and less costly and does not require the use of phytohormones for multiplication.

Plants grown on substrate had height up to 32.7 cm over a period of four months (Table 3).

Table 3. Average height, number of leaves and dry weight of plants arrowroot grown in soil (composting) and washed sands for 120 days in a greenhouse. Figures with dissimilar letter differ significantly at 5% level of significance in Tukey's test.

Treatment	height (cm)	Leaves number/plant	Dry weight/plant (g)
Washed sand	15.0 B	3.5 B	50.0 B
Soil	32.7 A	7.0 A	100.0 A

In respect of leaf production, the treatment 'soil' provided higher number of leaves, compared to plants grown on washed sands.

Mean number of leaves/plant was significantly higher on manipulated soil than on washed sands at 120 days of acclimatization (table 3). Greater productivity in terms of dry mass observed in arrowroot plants grown on manipulated soil substrates (Table 3).

MINHONI and AULER (2003) found that the addition of increasing doses of phosphorus alone exerted significant positive effects on the height, number of leaves and stem diameter of papaya plants. As a result of the manipulation of the substrate used to propagate arrowroot, this may have been a contributing factor to a higher degree of development of the plants compared to the other type of substrate (washed sand). Arrowroot does not require large amounts of abiotic or biotic resources for rhizomatic formation, but the only factor that the plant does not tolerate is water stress. Probably, with genetic improvement, new varieties resistant to drought may be one the options to grow crops under drought stress in future.

The ideal climatic conditions for arrowroot culture are found in the climatic type Cfa, humid mesothermic climate, without drought. As for the soil, it is in those that present the porous superficial layer, loose soils, where the best productions are obtained, so that this culture prefers the sandy alluvial rich in organic matter and the soils of the taxonomic units, Podzolic red-yellow ortho Podzolised and Gravel (MONTEIRO; PERESSIN, 2002).

The use of soil in this experiment did not have a porous surface layer which probably promoted a good growth and tuberization of the arrowroot plants, indicating that arrowroot could be planted in compacted soils. The availability of phosphorus in Cerrado areas under natural conditions is very low. In this way, phosphate fertilization is an essential practice because it is a very important nutrient in the first days of plant life, being determinant for success in the establishment and no other nutrients can replace it. The plant needs the phosphorus to complete its normal production cycle (LOPES, 1989).

The use of the substrate had a positive effect due to the high water retention capacity in arrowroot planting for a long period of time. SILVEIRA et al. (2002) evaluated the use of a consortium between the humus toasted coconut shell and the use of only the toasted coconut shell in the germination of the tomato seeds.

In another trial it was observed that due to the high water retention capacity, the use of a suitable substrate (humus) provided an ideal condition for the germination of tomato seeds. This indicates that to be efficient as a substrate, this material should be used in a mixture enriched in nutrients (SILVEIRA et al., 2002). In the field, the harvesting of the rhizomes can be done from 9 to 12 months after planting when the leaves are wilted with a brown coloration which lateral becomes straw-yellow and whitish (MONTEIRO; PERESSIN, 2002). The plantlets grown in *in vitro* culture and then acclimatized in greenhouse had a mean growth of up to 32.7 cm in 120 days, and rhizomes between 10 to 15 cm, depending on the substrate. However, the greenhouse experiment showed that the harvest could be made in **4** four months' time. Plants obtained from arrowroot shoot nodes promoted early tubering in

a greenhouse with significant starch accumulation. With stem propagation it is possible to have rhizomes in less time.

In another work, the use of the substrate was found to have a positive effect on rhizome production due to the higher water retention capacity in arrowroot planting for a long time period. SILVEIRA et al. (2002) evaluated the use of a consortium between the humus toasted coconut shell and the use of only the toasted coconut shell in the germination of the tomato seeds. Due to the high water retention capacity, the use of a suitable substrate (humus) provided an ideal condition for the germination of tomato seeds. Such evidences indicate that to be efficient as a substrate, this material should be used in a mixture enriched in nutrients (SILVEIRA et al., 2002).

In the field study of MONTEIRO AND PERESSIN (2002), the harvesting of the rhizomes could be done from 9 to 12 months after planting, when the leaves were wilted, with a brown coloration, becames straw-yellow and whitish.

Arrowroot seedlings obtained from cauline nodes promoted early tuberization in a greenhouse with starch accumulation. Utilizing Stem propagation it was possible to have rhizomes in less time.

SILVA (1996) argues that the industrialization of arrowroot and sweet potato, for starch production, could rationalize the cassava industries during their milling periods, avoiding the idleness of the off season. In the same way, arrowroot could be used in the off-season of the starch industries.

Conclusion

Arrowroot seedling production *in vitro* and then transferred to greenhouse for acclimatization produced rhizomes with starch in only 120 days. It is recommended that stem nodes might be used instead of rhizomes for arrowroot production in less time in the field.

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