



## Minituber production in yam for alternative vegetative propagation: types of cuttings, substrates, and anatomy

Crislaine Alves dos Santos<sup>1\*</sup>  Maria de Fátima Arrigoni-Blank<sup>1</sup>  Itamara Bomfim Gois<sup>1</sup>   
Andréa Santos da Costa<sup>1</sup>  Sara Dayan da Silva Oliveira<sup>1</sup>  Arie Fitzgerald Blank<sup>1</sup> 

<sup>1</sup>Departamento de Engenharia Agrônômica, Universidade Federal de Sergipe (UFS), 49100-000, São Cristóvão, SE, Brasil. E-mail: [cris.al.sa@hotmail.com](mailto:cris.al.sa@hotmail.com).

\*Corresponding author.

**ABSTRACT:** Yam is a tuber vegetable crop with vegetative propagation. The crop is low yielding due to the difficulty of obtaining quality material for propagation and the high cost of acquisition. The cutting technique is a new alternative for vegetative propagation of yam. The present study evaluated the production of minitubers of yam through cuttings, and follow the formational growth of the minitubers. Different types of cuttings, obtained from three positions on the shoots, and three substrates were tested. The types of cuttings and substrates did not significantly affect the number of minitubers per cutting, this number ranging from 1.18 to 1.75. The best results were obtained using Tropstrato Florestal<sup>®</sup> and median-position cuttings for the following variables: minituber length (17.47 mm), minituber diameter (12.63 mm), minituber fresh weight (2.12 g), and percentage of cuttings with two minitubers (75%). Anatomical analysis showed cell divisions and starch accumulation in the nodal region at seven days after planting the cutting. Emergence of the minituber could be observed at 21 days. The production of yam minitubers through stem cuttings is enhanced using cuttings obtained from the median position of the shoot and using the commercial substrate Tropstrato Florestal<sup>®</sup>.

**Key words:** *Dioscorea* sp., commercial substrate, morphological changes, starch, tuberization.

## Produção de minitubérculos de inhame para propagação vegetativa alternativa: tipo de estacas, substratos e anatomia

**RESUMO:** O inhame é uma hortaliça tuberosa de propagação vegetativa. A cultura apresenta baixa produtividade devido à dificuldade de obtenção de material propagativo de qualidade e ao seu alto custo de aquisição. A técnica de estaquia se apresenta como nova alternativa para a propagação vegetativa do inhame. O objetivo do presente estudo foi avaliar a produção de minitubérculos de inhame por meio de estacas e acompanhar a formação dos minitubérculos. Para isso, foram testadas estacas obtidas de três posições na rama e três substratos. Os tipos de estacas e substratos não afetaram significativamente o número de minitubérculos por estaca, variando de 1,18 a 1,75. Os melhores resultados foram obtidos com a utilização do Tropstrato Florestal<sup>®</sup> e de estacas da posição mediana para as variáveis: comprimento de minitubérculos (17,47 mm), diâmetro de minitubérculos (12,63 mm), massa fresca do minitubérculo (2,12 g) e porcentagem de estacas com dois minitubérculos (75%). A partir da análise anatômica observou-se divisão celular e acúmulo de amido na região nodal aos sete dias após o plantio da estaca. A formação de minitubérculo pôde ser observado aos 21 dias. A produção de minitubérculo de inhame por meio de estacas caulinares é melhorada com estacas obtidas da posição mediana do caule utilizando o substrato comercial Tropstrato Florestal<sup>®</sup>.

**Palavras-chave:** *Dioscorea* sp., substrato comercial, alterações morfológicas, amido, tuberização.

## INTRODUCTION

The genus *Dioscorea* (Dioscoreacea) includes more than 600 species that have different purposes in the pharmaceutical and food

Yam is the fourth most important tuber vegetable crop in the world, after potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz), and sweet potato (*Ipomoea batatas* L.) (FAO, 2019). In 2019, planted area worldwide was 8,910,485 ha. Production is particularly strong in African countries;

Nigeria is the largest producer in the world, with production of 50,052,977 metric tons (FAO, 2019).

Brazil is the second largest producer in South America, with production of approximately 250,000 t (FAO, 2019). There are around 130 species in Brazil (SOUZA & LORENZI, 2012), and among them, *D. alata*, *D. cayennensis*, *D. rotundata*, *D. esculenta*, and *D. trifida* are the main species grown, all of them for food purposes (SIQUEIRA, 2011).

Yam is multiplied in a vegetative manner, and the method most adopted by farmers

is propagation through whole or fractioned seed tubers (GARCÍA et al., 2015). Nevertheless, this method has limitations, such as a low multiplication rate, which compromises planting uniformity (BORGES-GARCÍA et al., 2018); fungus and nematode contamination (ANDRADE et al., 2010); and high acquisition cost, which can represent 55% of the costs of yam production (SANTOS et al., 2021).

Studies have shown that minituber production from yam shoot cuttings may be an alternative for use as propagative material in commercial growing (AGELE et al., 2010; BEHERA et al., 2009; UYOH et al., 2016). In a study on two genotypes of *Dioscorea rotundata* Poir (TDr 335, TDr 97/00940), cuttings allowed minitubers to be obtained with mean weight of 1.30 g (TDr 335) and 1.44 g (TDr 97/00940) at 21 days after planting (AGELE et al., 2010).

When successful, this method provides a high multiplication rate without the use of seed tubers, which can contribute to reducing the costs of material for planting (ANDRES et al., 2016). In addition, it is a viable technology for producing seed yams with quality health when using a pathogen-free substrate (AIGHEWI et al., 2015).

Studies are necessary to understand and improve the tuberization process of stem cuttings of yam. Among the factors that affect rooting, we can mention the position of the cuttings on the branches and the substrate used. Apical cuttings have higher auxin rates compared to basal cuttings, but the latter have a greater capacity to provide reserves for the formation and growth of roots and shoots (CUNHA et al., 2015). With regard to the substrate, it must have chemical and physical characteristics suitable for the rooting process, absence of pathogens and be economically viable (MENDES et al., 2014).

The process of formation of the reserve organ from stem cuttings was described, through anatomical studies, for cassava (*Manihot esculenta* Crantz) (CHAWEEVAN & TAYLOR, 2015) and potato (*Solanum tuberosum* L.) (XU et al., 1998); however, for yam there are no reports on how this process occurs. Thus, this study evaluated the production of yam minitubers through cuttings obtained from three positions on the shoot and in three commercial substrates, and of following the morphological and anatomical growth of the minitubers.

## MATERIALS AND METHODS

### *Plant material*

The plant material used was obtained from growing the crop in the Agroecological Lived

Experience Area (Espaço de Vivência Agroecológica) of the Federal University of Sergipe. The seedlings were produced using sectioned yam tubers purchased at CEASA (Sergipe Supply Center). The cuttings were taken from plants of 120 days of age.

### *Production of minitubers from cuttings*

A randomized block experimental design was used in a 3 × 3 factorial arrangement, with four replications. Each replication was composed of four cuttings. The treatments consisted of three positions on the shoot to obtain the cutting (apical, median, and basal) and three commercial substrates (Tropstrato Florestal® – TF; Tropstrato Hortaliça® – TH; and mixture of the two substrates – TF:TH, in a 1:1 proportion). The chemical characterization of the substrates is shown in table 1.

Cuttings with two nodes and one pair of leaves were used; the lower pair of leaves from the second node was removed. The region from which the leaves were removed remained in contact with the substrate for rooting and forming minitubers. The cuttings were placed in 280 cm<sup>3</sup> plug pots and kept in an agricultural greenhouse with an intermittent water mist system.

At 60 days after setting up the experiment, the plants were fertilized with 42 g.L<sup>-1</sup> of nitrogen, using the 6-24-12 NPK formulation as a source. Each plug pot received 15 mL of the solution.

The following variables were evaluated at 100 days from planting: number of minitubers per cutting, minituber length and diameter (mm), minituber mean fresh weight (g), and cuttings that formed two minitubers (%). The minitubers formed were photographed through the Ground Eye S800 device for observation of the morphological changes over time.

Analysis of variance was performed by the F test on the data obtained and, when significant, the means were compared by Tukey's test ( $P < 0.05$ ) using the SISVAR statistical program (FERREIRA, 2011). The data on minituber mean fresh weight (g) were transformed into square root of ( $x+0.5$ ), and the data regarding percentage were transformed into arc sine of the square root of ( $X/100$ ).

### *Histological analysis of the formation of minitubers on stem cuttings*

Histological analysis was performed for the purpose of following the development of minitubers on stem cuttings. For the histological study, cuttings obtained from yam shoots were planted in plug pots containing commercial substrate

Table 1 - Chemical characterization of the substrates.

Test	Unit	Tropstrato Florestal® (TF)	Tropstrato Hortaliça® (TH)	TF:TH (1:1) mixture
pH		6.07	5.75	5.98
Calcium+magnesium	cmolc.dm <sup>-3</sup>	12.60	12.70	13.10
Calcium	cmolc.dm <sup>-3</sup>	9.44	8.75	9.21
Aluminium	cmolc.dm <sup>-3</sup>	< 0.08	< 0.08	< 0.08
Sodium	mg.dm <sup>-3</sup>	29.50	62.30	47.00
Potassium	mg.dm <sup>-3</sup>	433.00	400.00	333.00
Phosphorus	mg.dm <sup>-3</sup>	218.00	288.00	281.00
Sum of Bases	cmolc.dm <sup>-3</sup>	13.80	14.00	14.20
CEC	cmolc.dm <sup>-3</sup>	15.60	16.00	16.00
PES	%	0.82	1.69	1.28
Base saturation	%	88.50	87.50	88.80
Organic matter	g.dm <sup>-3</sup>	131.00	122.00	136.00

CEC – Cation Exchange Capacity; PES – Percentage of Exchangeable Sodium.

(Tropstrato Hortaliça®) and kept in a greenhouse with an intermittent water mist system.

Every seven days after planting, for 56 days, six cuttings were removed from the substrate for analysis. They were washed in running water to remove the substrate. Before making the histological sections, the cuttings were photographed with the assistance of the Ground Eye S800 device and LEICA EZ4D magnifying glass for observation of the morphological changes over time.

Sectioned parts of the nodal region that was in contact with the substrate were fixed in 70% FAA (70% ethanol, 5% glacial acetic acid, and 5% formaldehyde) for 72 hours and then fixed in 70% alcohol (JOHANSEN, 1940).

The material was dehydrated by passing through a series of ethanol solutions of increasing concentration (70%, 80%, 90%, and 100%) for two hours each. The fragments were transferred to a pre-infiltration solution containing 100% ethanol and 100% methacrylate (Historessin-Leica) for 12 hours (TITON et al., 2007). They were then placed in an infiltration solution (100% methacrylate) for 24 hours. The material was formed into tissue blocks in 250 µL silicon cuvettes.

After being formed into blocks, the material was sectioned at a thickness of 10 µm using a Leica RM 2235 semi-automatic microtome. The material was stained with toluidine blue for 20 seconds and with 2% iodine for 30 seconds before mounting on permanent slides with microscope slide sealer. The sections were photographed in an optical microscope equipped with a LEICA DM500 camera

and visualized on the computer with the assistance of the LAS EZ® program.

## RESULTS AND DISCUSSION

### *Minituber production*

The position of the cutting exhibited a significant statistical difference for the variables of minituber diameter (mm) and minituber fresh weight (g). The interaction between the substrate and position factors was significant for the variables of minituber length (mm) and of cuttings that formed two minitubers (%) (Table 2).

In all the treatments there was formation of at least one minituber per cutting, and no significant difference was observed among the treatments (Table 2). Results indicated that tubers can grow from the axillary buds present in yam cuttings, regardless of the cutting position and the substrate. This may be related to the mechanisms of induction, initiation, and development of the storage organ in the cuttings (MA et al., 2015).

For minituber length, the median-position cutting had better results than the others when the Tropstrato Florestal® substrate was used, and had results equal to the basal-position cutting when the Tropstrato Hortaliça® and the TF:TH mixture substrates were used (Table 2). There was a significant difference among the substrates only for the median-position cutting, in which Tropstrato Hortaliça® was inferior to the others, with a mean of 14.26 mm.

The longer minitubers observed for the median-position cutting with the use of

Table 2 - Mean values of number of minitubers per cutting, minituber length (mm), diameter (mm), and fresh weight (g), and percentage of cuttings with two minitubers of yam (*Dioscorea* sp.) at 100 days after planting, in accordance with type of cutting and substrate.

Cutting type	Substrate		
	Tropstrato Florestal® (TF)	Tropstrato Hortaliça® (TH)	TF:TH (1:1) mixture
----- Number of minitubers per cutting <sup>n.s.</sup> -----			
Apical	1.25 a A	1.25 a A	1.50 a A
Median	1.75 a A	1.43 a A	1.68 a A
Basal	1.43 a A	1.31 a A	1.18 a A
----- Tuber length <sup>++</sup> (mm) -----			
Apical	9.89 b A	9.10 b A	8.98 b A
Median	17.47 a A	14.26 a B	16.17 a AB
Basal	8.05 b A	12.55 ab A	14.59 a A
----- Tuber diameter <sup>+</sup> (mm) -----			
Apical	8.46 b A	7.37 b A	7.67 b A
Median	12.63 a A	11.75 a A	11.93 a A
Basal	5.97 b A	10.03 ab A	9.96 ab A
----- Tuber fresh weight <sup>+</sup> (g) -----			
Apical	0.33 b A	0.48 a A	0.37 b A
Median	2.12 a A	1.50 a A	1.90 a A
Basal	0.26 b A	0.90 a A	1.28 ab A
----- Percentage of cuttings with two minitubers <sup>++</sup> (%) -----			
Apical	32.77 b A	29.16 a A	66.66 a A
Median	75.00 a A	58.33 a A	68.75 a A
Basal	58.33 ab A	41.66 a AB	18.75 b B

Mean values followed by different lowercase letters in the column and uppercase letters in the row differ from each other by Tukey's test ( $P < 0.05$ ).

n.s. not significant.

<sup>+</sup> Position of the cutting significant.

<sup>++</sup> Interaction between the substrate and position of cutting significant.

the Tropstrato Florestal® and the TF:TH mixture substrates may be related to the greater organic matter content, 131 g.dm<sup>3</sup> and 136 g.dm<sup>3</sup>, respectively, compared to Tropstrato Hortaliça®. Organic matter raises water-holding capacity and provides better structuring, aeration, aggregate stability, and nutrient availability (MAGGIONI et al., 2014), characteristics that provide a better environment for minituber development (DANTAS et al., 2013).

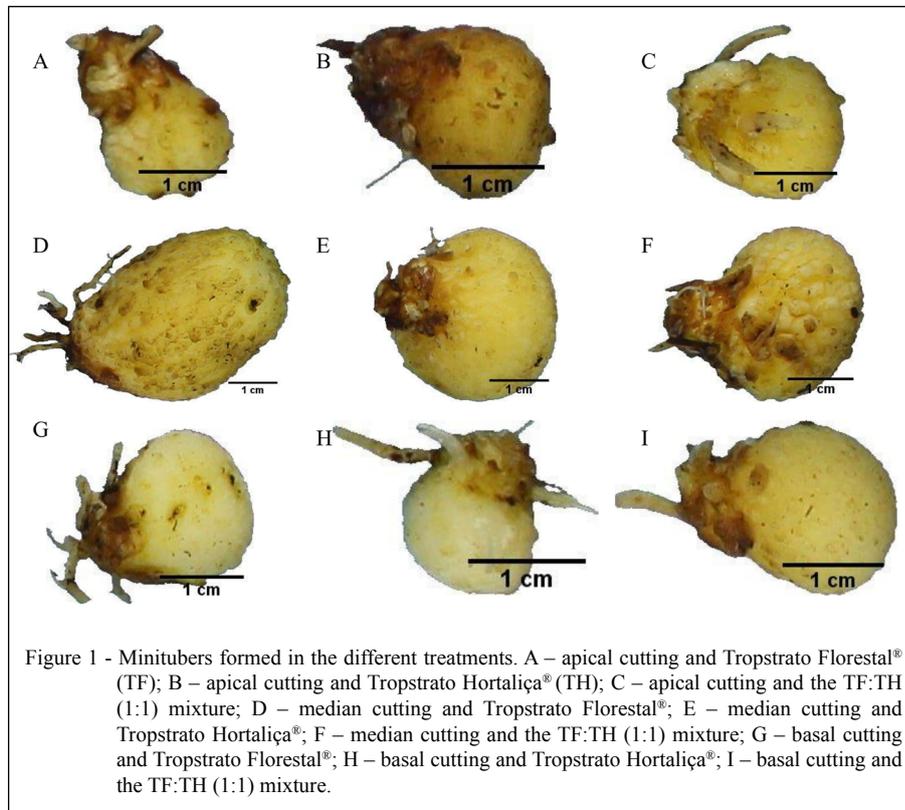
For yam, the second most absorbed mineral element is potassium (DIBY et al., 2011) and, in that regard, Tropstrato Florestal® had the largest amount of that element (433 mg.dm<sup>3</sup>); that may have contributed to the higher values observed for the fresh weight and minituber diameter variables. Regarding the other chemical characteristics, the different substrates showed little variation in their composition.

Regarding minituber diameter, the median-position cutting was superior to the others when the Tropstrato Florestal® substrate was used,

and equal to the basal-position cutting when the Tropstrato Hortaliça® and the TF:TH mixture substrates were used (Table 2).

For minituber fresh weight, the median-position cutting was superior to the others upon using the TropstratoFlorestal® substrate, and equal to the basal-position cutting when the TF:TH mixture was used. The cuttings obtained from the three positions were statistically equal in the TropstratoHortaliça® substrate (Table 2). The minitubers obtained from the median-position cuttings had a mean of 12.1 mm for diameter and of 1.8 g for fresh weight. These minitubers are visually larger than those of the other treatments (Figure 1).

The greater diameter and fresh weight observed for the median-position cuttings may be related to the tendency of better rooting in physiologically juvenile yam cuttings that already have more lignified tissues and, possibly, greater carbohydrate reserves (SILVA et al., 2014). Thus,



the age of stem tissue is related to the beginning of root primordia, and more lignified cuttings can create larger storage organs (GREGORY & WOJCIECHOWSKI, 2020).

The importance of obtaining minitubers with greater fresh weight comes from the relation between the amount of reserves with the emergence, vigor, and survival of the plantlets obtained. Thus, small seed tubers produce smaller tuber roots and might not sprout (MORSE, 2018).

For the variable of cuttings that formed two minitubers (%) (Table 2), 75% of the median-position cuttings produced two minitubers when the Tropstrato Florestal® substrate was used, and no significant difference was observed with the basal-position cutting. The TF:TH mixture substrate had a lower percentage of production of two minitubers (18.75%) when the basal-position cutting was used.

The advantage arising from production of two minitubers per cutting is the increase in the total number of seed tubers obtained. However, depending in the position of the cutting, minitubers with lower fresh weight may be produced, which may compromise the sprouting and development of the plants obtained.

#### *Histological analyses*

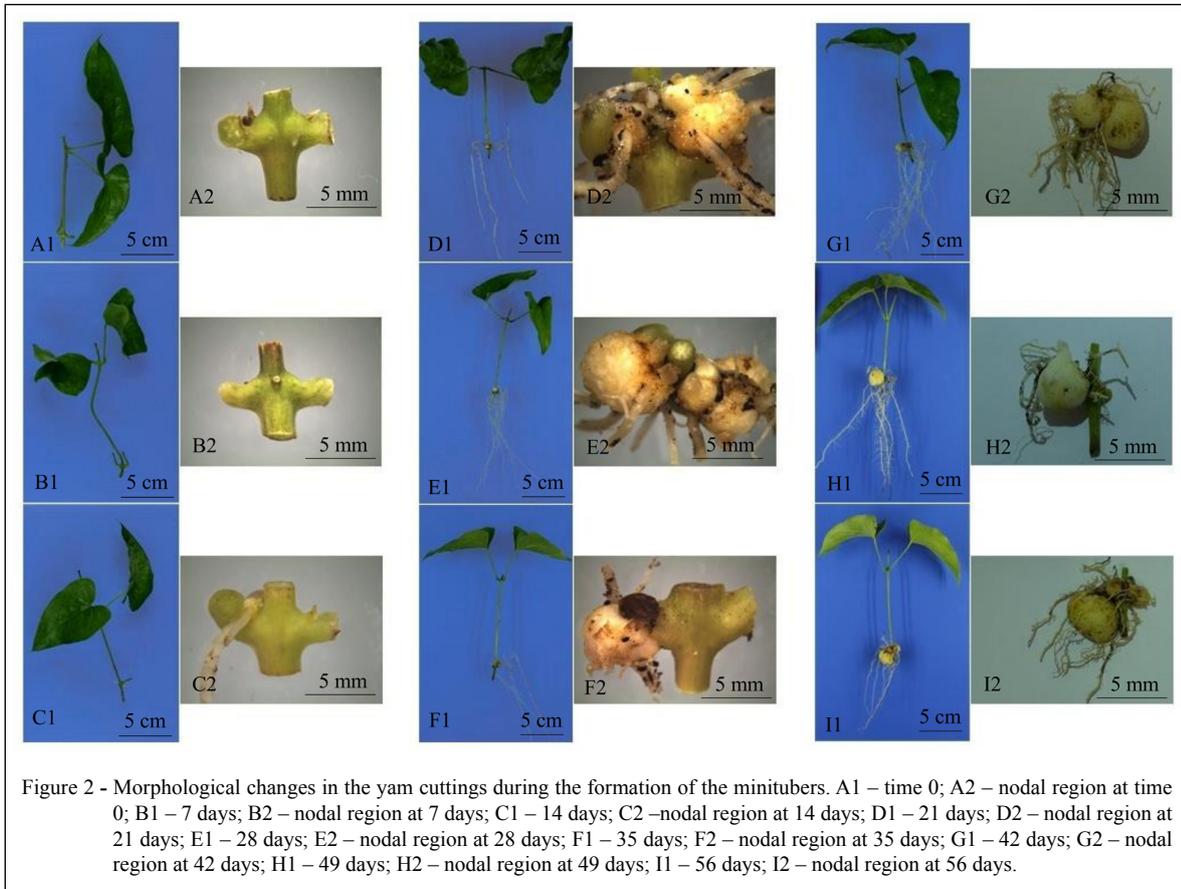
The first morphological changes were observed at 14 days after planting, with the emergence of small, white, fragile roots (Figure 2). They arose from swollen tissues near the nodal region of the stem.

The beginning of formation of minitubers was observed at 21 days after planting in the same region where the first roots formed. At this same time, it was also possible to observe a larger number of roots and greater root growth.

At 28 days, the minitubers already had a mean size of 2 mm. At 35, 42, 49, and 56 days after planting, minitubers were observed with weight of 0.8 g, 1.3 g, 1.8 g, and 2.5 g, respectively, showing that over time, starch accumulated to form the storage organ (Figure 2).

From histological analysis of the nodal region of the stem, the following structures could be observed: epidermis, cortical parenchyma, phloem, and xylem (Figure 3A). The absence of starch in the parenchyma of the cuttings at time 0 was confirmed through staining with 2% iodine.

The beginning of tuberization was characterized by the process of cell division for formation of the starchy parenchyma. At seven days



after placing the cutting in the substrate (Figure 3B), the process of cell division near the region of the cortical parenchyma and the presence of starch in the cortical region were observed. The tuberization process probably began with metabolic changes related to starch synthesis and from sucrose export from the leaves (FISCHER et al., 2008) which is under complex environmental and endogenous regulation. In the present work, we studied the regulatory mechanisms and the role of different morphogenetic factors in a newly isolated potato mutant, which exhibited spontaneous tuberization (ST). This is because the process of photoassimilate translocation from the green parts of the plant is important for formation of storage organs (SUNGTHONGWISES et al., 2016). Similar to what was observed in this study, in the taro (*Colocasia esculenta* L. Schott) tuberization process, the accumulation of starch grains in the proplastids and formation of amyloplasts was observed after day 8 (DU et al., 2013).

The process of cell division and of starch accumulation in the region of the starchy parenchyma

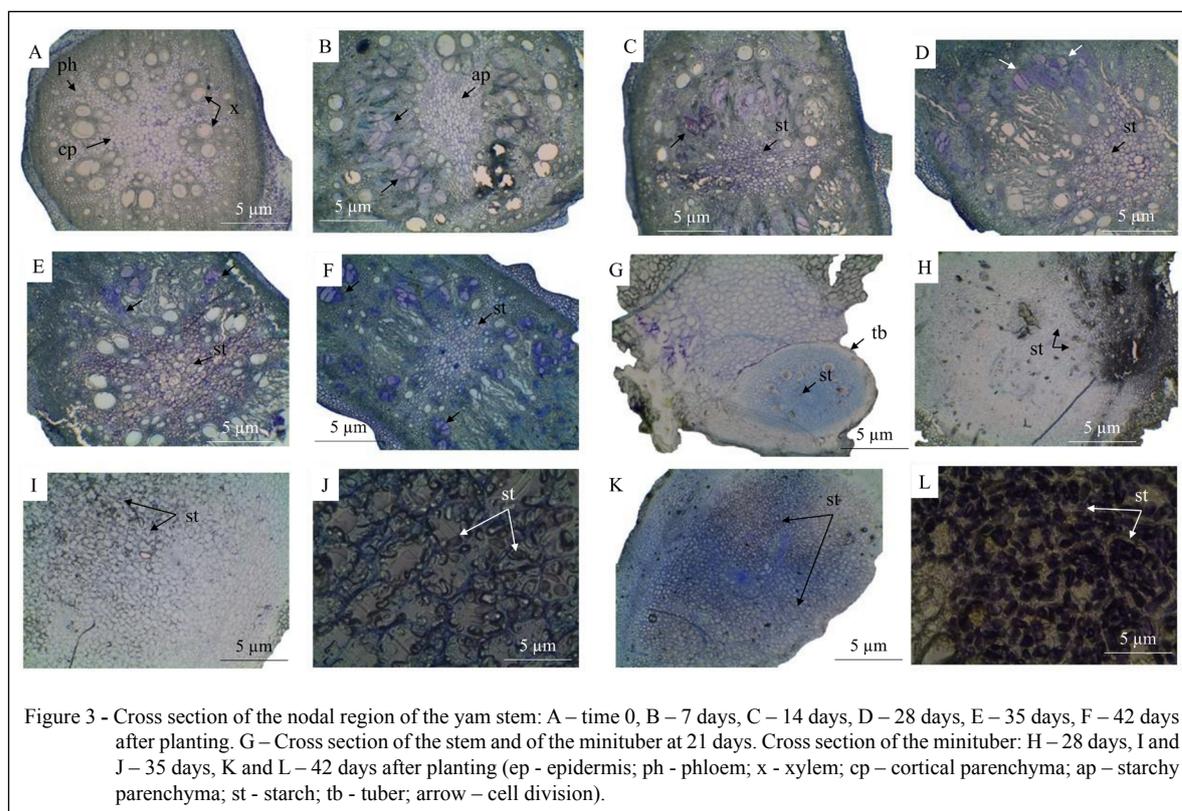
can be observed in Figures 3C, 3D, 3E, and 3F. Up to day 35, the cell divisions observed in the cortical parenchyma region were longitudinal; however, at 42 days after planting, random divisions were observed.

The accumulation of starch in the parenchyma of the minituber can be seen beginning on day 28 after planting (Figures 3G, 3H, 3I, 3J, 3K, 3L).

The tuberization process in yam stem cuttings was similar to the process described for formation of potato tubers *in vitro*. The cells pass through a process of longitudinal cell division, and when they reach 2 to 4 mm, longitudinal division ceases and is replaced by random divisions and cell enlargement (XU et al., 1998).

## CONCLUSION

Yam minituber production can be improved through the use of stem cuttings obtained from the median position of the shoot and the Tropstrato Florestal® substrate.



Anatomical analysis makes it possible to monitor the formation of minitubers in yam stem cuttings, which begins through the process of cell division in the stem and the change of the cortical parenchyma to a starchy parenchyma, with translocation of assimilates for formation of the storage organ.

#### ACKNOWLEDGMENTS

The authors thank the Brazilian Federal Agencies: Fundação de Apoio a Pesquisa e Inovação Tecnológica do Estado de Sergipe (Fapitec/SE), Brazil; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Financecode 001), Brazil; and the Financiadora de Estudos e Projetos (FINEP), Brazil, for financial support.

#### DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

#### AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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