DOI: 10.53660/CLM-1383-23K63

Culture media in the in vitro cultivation of *Dioscorea* spp.

Meios de cultura no cultivo in vitro de *Dioscorea* spp.

Received: 2023-15-04 | Accepted: 2023-20-05 | Published: 2023-31-05

Denise dos Santos Vila Verde
ORCID: https://orcid.org/0000-0001-7773-5097
State University of Santa Cruz, Brazil
E-mail: denisevilaverde@hotmail.com
Maria Inês de Souza Mendes
ORCID: https://orcid.org/0000-0001-7129-2467
State University of Santa Cruz, Brazil
E-mail: inessm.123@gmail.com
Antônio da Silva Souza
ORCID: https://orcid.org/0000-0003-4535-7807
Embrapa Cassava and Fruticulture, Brazil
E-mail: antonio.silva-souza@embrapa.br
Camila Rodrigues Pinto
ORCID: https://orcid.org/0000-0002-7275-3699
Federal University of Bahia, Brazil
E-mail: camilarodrigues80@hotmail.com
Leila Vasconcelos Costa Nobre
ORCID: https://orcid.org/0000-0002-5132-3235
Federal University of Recôncavo da Bahia, Brazil
E-mail: leilacosta11@hotmail.com
Karen Cristina Fialho dos Santos
ORCID: https://orcid.org/0000-0002-3241-5302
Embrapa Cassava and Fruticulture, Brazil
E-mail: karen.santos@embrapa.br
Carlos Alberto da Silva Ledo
ORCID: https://orcid.org/0000-0001-9578-4167
Embrapa Cassava and Fruticulture, Brazil
E-mail: carlos.ledo@embrapa.br

ABSTRACT

The composition and physical state of the culture medium, as well as the effect of supplementation in different subcultures, are determining factors for the regeneration and good development of the in vitro explant. Therefore, this study aimed to study the effect of the culture medium on micropropagation during four subcultures, depending on the growth variables of the genotypes *Dioscorea alata* L., *D. alata* var. *purpurea* (Roxb.) A. Pouchet, and *D. rotundata*. Nodal segments of 1 cm length of plants previously cultivated in vitro were introduced into 10 mL of MS culture media supplemented with 100 mg L^{-1} of inositol, 20 mg L^{-1} of cysteine, 0.20 mg L^{-1} of ANA, 0.08 mg L^{-1} of AG_3 and 0.05 mg L^{-1} of BAP and 2GGC basic, both in solid and liquid states plus 3 g L^{-1} of activated charcoal and 30 g L^{-1} of sucrose and the pH adjusted by 5.8 before autoclaving. At every 30 days of in vitro culture, development variables, percentage of responsive explants, and the number of calluses were evaluated. The MS and solid 2GGC media are the most suitable for the multiplication of the genotypes *D. alata* and *D. alata* var. *purpurea*. For the *D. rotundata* genotype, the solid MS medium is the most suitable for its in vitro multiplication.

Keywords: Yam; Tissue culture; Nutritive media; Micropropagation; Subculture.

RESUMO

A composição e o estado físico do meio de cultivo, bem como o efeito da suplementação em diferentes subcultivos, são fatores determinantes para a regeneração e bom desenvolvimento do explante in vitro. Portanto, este trabalho teve como objetivo estudar o efeito do meio de cultura na micropropagação durante quatro subcultivos, em função das variáveis de crescimento dos genótipos *Dioscorea alata* L., *D. alata* var. *purpurea* (Roxb.) A. Pouchet e *D. rotundata*. Segmentos nodais de 1 cm de comprimento de plantas previamente cultivadas in vitro foram introduzidos em 10 mL de meio de cultura MS suplementado com 100 mg L^{-1} de inositol, 20 mg L^{-1} de cisteína, 0,20 mg L^{-1} de ANA, 0,08 mg $^{L\ -1}$ de AG_3 e 0,05 mg L^{-1} de BAP e 2GGC básico, tanto no estado sólido quanto no líquido mais 3 g L^{-1} de carvão ativado e 30 g L^{-1} de sacarose e o pH ajustado em 5,8 antes da autoclavagem. A cada 30 dias de cultivo in vitro, foram avaliadas as variáveis de desenvolvimento, porcentagem de explantes responsivos e número de calos. Os meios MS e sólido 2GGC são os mais indicados para a multiplicação dos genótipos *D. alata* e *D. alata* var. *purpurea*. Para o genótipo *D. rotundata*, o meio sólido MS é o mais adequado para sua multiplicação in vitro.

Palavras-chave: Inhame; Cultura de tecidos; Meio nutritivo; Micropropagação; Subcultivo.

INTRODUÇÃO

Yam is a monocotyledon belonging to the family *Dioscoreaceae* and the genus *Dioscorea*. Among more than 600 species of the genus, *D. alata* L. and D. *rotundata* Poir. are the most consumed by humans and are widely distributed worldwide, thus standing out as the main cultivated species of economic importance (Arnau et al., 2009; Salcedo-Mendoza et al., 2018).

Yam cultivation is an important source of income for farmers (Sêdami et al., 2017). It is a healthy, nutritious, and low-fat food, being an excellent source of carbohydrates and vitamins such as thiamine, riboflavin, niacin, ascorbic acid, and carotenoids. It has most essential amino acids (Cabrera Jova, 2009), is rich in minerals, and some of its species can be used for their medicinal properties (García et al., 2009).

The largest yam producers are the tropical countries of West Africa, mainly Nigeria and Côte d'Ivoire, where it occupies an important place in food security, concentrating 95% of the total produced in the world (Brito et al., 2011; Sêdami et al., 2017). In South America, Brazil is the second-largest producer, behind Colombia (FAO, 2018). The Northeast region has most of the culture production, playing an important socioeconomic role (Brito et al., 2011; Santos et al., 2007).

Despite its importance, the cultivation of yam in the field encounters some obstacles, which end up affecting production, such as the scarcity of seeds free of pests and diseases, the high cost of quality seed tubers, and their planting in low fertility soils, aspects that cause a loss of up to 90% in crop yield (Balogun et al., 2014). Damage caused by viruses, fungi of tuber rot, and bacterial infections are the main contributors to poor seed quality and low yields (Aighewi et al., 2015).

Plant tissue culture techniques have the potential to overcome the limitations of conventional yam propagation methods (Bömer et al., 2019). With in vitro cultivation, it is possible to obtain a clean clonal plant material of high phytosanitary quality, which would represent a sustainable solution for rapid production of pathogen-free planting material (Aighewi et al., 2015; Balogun et al., 2017).

However, aspects such as the adequacy of the culture medium, with the adjustment of macronutrients, micronutrients, and vitamins, in addition to the physical state of the medium that favors a better absorption of nutrients, depending on the species used, is a determining factor for the explant regeneration and a good in vitro development. In the in vitro cultivation of yam, the MS medium (Murashige and Skoog, 1962), with the addition of growth regulators, is the most used in the different species of Dioscorea (Chen et al., 2003; García et al., 2011; Carmona Wilches et al., 2013; Díaz Narváez et al., 2015; Taha and Abdelaziz, 2017). Some studies use other culture media, such as modified D-571 (García et al., 2004), Galzy glutamine (Sêdami et al., 2017), and modified WPM (Shukla And Shukla, 2014).

Therefore, this work aims to study the effect of culture medium during four subcultures, depending on the growth variables of the genotypes *D. alata*, *D. alata* var. *purpurea*, and *D. rotundata* on in vitro multiplication.

MATERIAL AND METHODS

The experiment was conducted at the Tissue Culture Laboratory of Embrapa Cassava and Fruticulture in Cruz das Almas, Bahia. Nodal segments of approximately 1 cm in length of plants from *D. rotundata*, *D. alata*, and *D. alata* var. *purpurea*, previously grown *in vitro*, were introduced into test tubes containing 10 mL of MS media (Murashige and Skoog, 1962) supplemented with 100 mg L^{-1} of inositol, 20 mg L^{-1} of cysteine, 0.20 mg L^{-1} of 1-naphthaleneacetic acid (ANA), 0.08 mg L^{-1} of gibberellic acid (AG_3) and 0.05 mg L^{-1} of 6-benzylaminopurine (BAP), and 2GGC basic (Doukoure et al., 2000), both in solid and liquid states. In all culture media, 3 g L^{-1} of activated charcoal and 30 g L^{-1} of sucrose were added. In the solidified media, 2.4 g L^{-1} of Phytagel® was used, and in the liquid media, to support the explants, filter paper bridges were used; all media had their pH adjusted to 5.8 before autoclaving.

An experiment was installed in a completely randomized design for each genotype, in a factorial 4 x 4 [4 subcultures (every 30 days) x 4 culture media], containing 20 repetitions per treatment. Each experimental plot consisted of a test tube containing a nodal segment.

After introduction into the culture medium, the explants were kept in a growth room under temperature conditions of 27 ± 1 ºC, photon flux density 30 μmol m^{-2} s^{-1}, and 16-hour photoperiod.

At every 30 days of *in vitro* culture, the following variables were evaluated: aerial part height (APH), in centimeters (cm), number of green leaves (NGL), number of senescent leaves (NSL), number of nodal segments (NNS), number of roots (NR), and number of calluses. After each evaluation, the plants were subcultured in the same culture medium until 4 growing periods were completed.

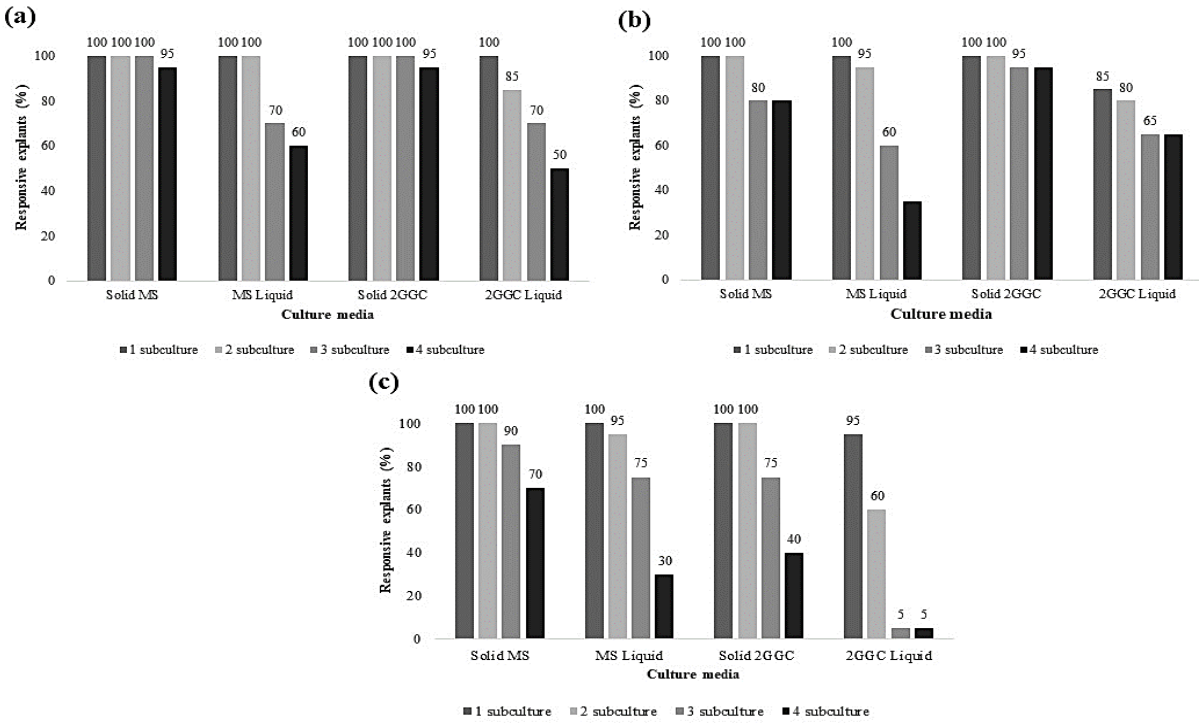
To calculate the percentage of responsive explants, the number of regenerated nodal segments in each treatment was considered, from which the overall mean of the experiment was obtained. The same process was used to calculate the callus percentage.

The other data obtained were submitted for analysis of variance with the aid of the statistical program R, version 3.4 (R CORE TEAM, 2017), using the ExpDes.pt package (Ferreira et al., 2018). The means of subcultures, culture media, and genotypes were compared at 5% probability by the Tukey test. The count values, number of green leaves, number of senescent leaves, number of nodal segments, and number of roots were transformed into $\sqrt{(x+0.5)}$ aiming to meet the assumptions of the analysis of variance.

RESULTS

For the *D. alata* genotype, in MS and solid 2GGC culture media, there was 100% regeneration in the first three subcultures, reducing to 95% in the fourth subculture (Figure 1). The liquid media presented 100% regeneration in the first subculture. In the second subculture, the MS medium maintained this percentage, while the 2GGC medium had a reduction to 85%. In the third subculture, the two media presented 70% of responsive explants, and in the fourth subculture, the MS medium had a reduction to 60% and the 2GGC medium to 50%.

Figure 1 - Percentages of responsive explants of the genotypes *D. alata* (a), *D. alata var. purpurea* (b), and *D. rotundata* (c), in supplemented DM and 2GGC media, in solid and liquid physical states, during four subcultures.



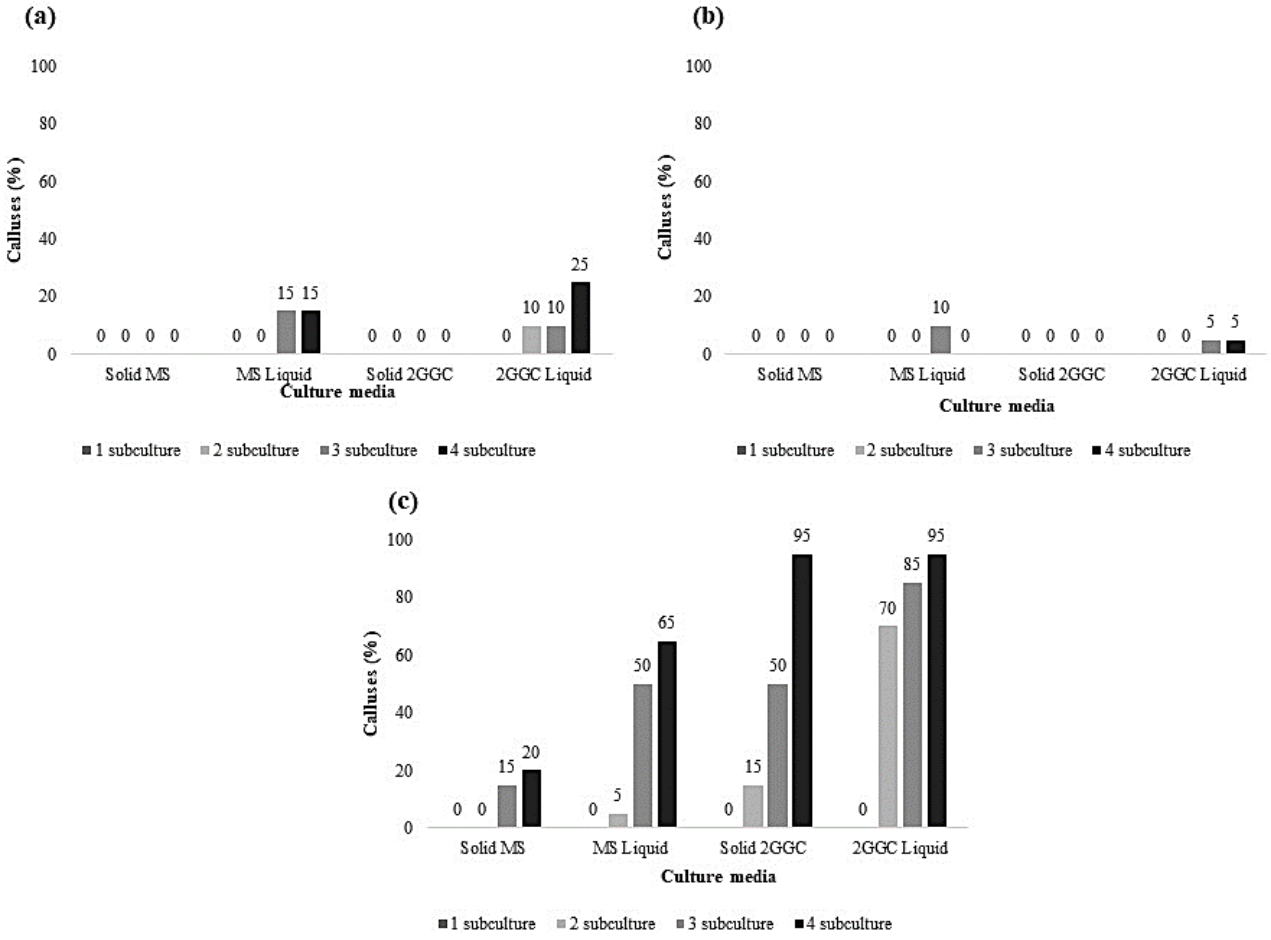
Source: Prepared by the authors (2023).

In the first subculture, the species *D. alata* var. *purpurea* presented 100% regeneration in solid and liquid MS and solid 2GGC media and 85% in liquid 2GGC media. In the second subculture, the solid media presented 100% of responsive explants, while in the liquid media, there was a reduction to 95% in the MS medium and 80% in the 2GGC medium. Solid MS medium showed 80% of explants regenerated in the third and fourth subcultures, while solid 2GGC medium showed 95%. In the liquid media with the other subcultures, there was a reduction in the regeneration rate as the subcultures were performed, reaching 65% in the 2GGC medium and 35% in the MS in the third and fourth subcultures, respectively (Figure 1b).

As shown in Figure 1c, the *D. rotundata* genotype showed 100% regeneration in the first two subcultures in the solid media, a rate that decreased in the third and fourth subcultures to 90% and 70% in the MS and 75% and 40%, respectively in the 2GGC. The liquid media showed a reduction along with the subcultures when, in the MS medium, there were indices of 100%, 95%, 75%, and 30% of responsive explants in the first, second, third, and fourth subcultures, respectively, while in the 2GGC there was an even more pronounced reduction, with 95% regeneration in the first subculture, 60% in the second and 5% in the third and fourth subcultures.

There was callus formation in the liquid media, which prevented the development of the explants from the second subculture of the *D. alata* genotype in the 2GGC medium, where there were callus percentages in 10%, 10%, and 25% of the explants in the second, third, and fourth subcultures, respectively. In the MS medium, there was 15% callogenesis in the explants in the third and fourth subcultures (Figure 2a).

Figure 2 - Percentages of calluses of the genotype *D. alata* (a), *D. alata* var. *purpurea* (b), and *D. rotundata* (c), in supplemented DM media and 2GGC, both in solid and liquid physical states, during four subcultures.



Source: Prepared by the authors (2023).

In the genotype *D. alata* var. *purpurea*, 10% of explants with callus formation in the third subculture occurred in the liquid MS medium, and 5% in the liquid 2GGC medium in the third and fourth subculture (Figure 2b).

In the species *D. rotundata*, callus formation in the explants was more pronounced, starting from the second subculture in the liquid MS and solid and liquid 2GGC media. In all culture media, there was callogenesis in the third subculture, with the highest percentage in the liquid 2GGC (85%). In the fourth subculture there were 95% of calluses formed in the 2GCC media in both physical states and in the solid and liquid MS media, in 20% and 65%, respectively (Figure 2c).

In the analysis of variance, for the genotype *D. alata*, the height of the aerial part, number of green leaves, number of nodal segments, and number of roots showed a highly significant effect on the isolated factors, subculture and culture medium. However, in the interaction between the factors (subculture x culture medium) there was a significant effect only on the variables height of the aerial part and number of green leaves. For all genotypes, the number of senescent leaves was not significant for the factors due to the low rate of senescence found between treatments. For this variable, in the genotypes *D. alata*, *D. alata* var. *purpurea*, and *D. rotundata*, means of 0.04, 0.01, and 0.00 senescent leaves were obtained, respectively.

As in *D. alata*, for the genotypes *D. alata* var. *purpurea* and *D. rotundata*, there was a highly significant effect on the variables height of the aerial part, number of green leaves, and number of nodal segments. For *D. rotundata*, there was also a highly significant effect on the number of roots. Besides, for *D. alata* var. *purpurea* and *D. rotundata*, there was a significant effect on the interaction of factors (subculture x culture medium), height of the aerial part, number of green leaves, number of nodal segments, and number of roots.

The height of the aerial part, for the genotype *D. alata*, in the first three subcultures presented the highest means in the solid and liquid MS and solid 2GGC media, differing statistically from the fourth subculture. The liquid 2GGC medium did not present statistical differences between the subcultures (Table 1). The means did not present statistical differences in the first and third subcultures. In the second subculture, the solid MS and 2GGC media provided the highest means of 5.79 cm and 4.42 cm, respectively. However, the solid 2GGC media did not differ statistically from the liquid media. In the fourth subculture, the solid MS and solid and liquid 2GGC media have the highest means; the solid MS and liquid 2GGC media did not differ from the liquid MS media, which produced the lowest mean for the height of the aerial part (Table 1).

Table 1 - Mean values of height of the aerial part (cm) of the genotypes *D. alata*, *D. alata* var. *purpurea*, and *D. rotundata* grown in supplemented DM and 2GGC media in solid and liquid physical states.

Height of the aerial part (cm)				
	Culture medium			
Subculture	Solid DM	Liquid DM	Solid 2GGC	Liquid 2GGC
	Dioscorea alata			
1	4.88 aA	4.84 aA	5.03 aA	3.96 aA
2	5.79 aA	4.06 aB	4.42 abAB	3.68 aB
3	4.68 aA	4.40 aA	5.20 aA	4.26 aA
4	3.25 bAB	2.17 bB	3.48 bA	3.27 aAB
CV (%)				16.52
Dioscorea alata* var. *purpurae				
1	6.15 aA	4.66 aAB	6.01 aA	4.10 aB
2	3.94 bA	4.66 aA	4.79 aA	4.21 aA
3	3.66 bB	2.85 bB	6.49 aA	5.57 aA
4	2.30 cB	2.31 bB	6.67 aA	4.65 aA
CV (%)				18.35
Dioscorea rotundata				
1	3.79 Aa	2.78 aB	3.72 aA	3.03 aAB
2	3.27 abA	2.22 aB	2.85 abAB	2.28 aB
3	2.84 bcA	2.76 aA	2.37 bcA	4.20 aA
4	2.27 cA	1.93 aA	1.83 cA	1.80 aA
CV (%)				13.43

Means followed by the same lowercase letter in each column and uppercase in each row do not differ statistically from each other at 5% probability by the Tukey test. Source: Prepared by the authors (2023).

In the genotype *D. alata* var. *purpurea*, 2GGC media showed no statistical differences between subcultures in both physical states. The solid MS medium had the highest mean height of the aerial part (6.15 cm) in the first subculture, differing statistically from the others, while the last subculture had the lowest mean (2.30 cm). As for the liquid MS medium, the first two subcultures presented the same mean (4.66 cm) and were statistically superior to the third and fourth subcultures (Table 1).

According to Table 1, the solid 2GGC, solid and liquid MS media for genotype *D. alata* var. *purpurea*, do not present statistical differences in the first subculture. The highest mean height

(6.15 cm) was obtained in the solid MS medium. Still, in this subculture, the liquid media did not differ from each other. In the second subculture, there was no statistical difference between the culture media. In contrast, in the third and fourth subcultures, the 2GGC media in both states presented the highest means and differed statistically from the MS media.

For the *D. rotundata* genotype, the MS and 2GGC media in the liquid state did not differ over the four subcultures performed. In the solid state, the media presented the highest means in the first two subcultures, which did not differ from each other, noting, however, that there was a decrease in height within the subcultures (Table 1). Solid MS and solid and liquid 2GGC media showed the highest means in the first subculture. Nevertheless, the liquid media did not differ from each other. In the second subculture, the highest means of 3.27 cm, and 2.85 cm were observed in the solid MS and 2GGC media, respectively, however, the solid 2GGC media did not differ statistically from the liquid media. There were no differences between the culture media in the third and fourth subcultures.

Concerning the variable number of green leaves in the genotype *D. alata*, the 2GGC media did not present statistical differences for the subcultures, while in the solid MS medium, the means of the second, third, and fourth subcultures were the highest. In this last subculture, there was the highest number of green leaves (6.00). In the liquid MS medium, the highest mean (5.21) was obtained in the third subculture, being statistically higher than the values achieved in the others. The means did not present statistical differences between each other in the first and third subcultures, while in the second subcultures, the solid MS and 2GGC media presented the highest means, 4.68 and 3.37, respectively. In the fourth subculture, the solid MS medium presented the highest number of green leaves (6.00), differing from the other media, which, in turn, did not differ from each other (Table 2).

Table 2 - Mean values of the number of green leaves of the genotypes *D. alata*, *D. alata* var. *purpurea*, and *D. rotundata* grown in supplemented DM and 2GGC media, in solid and liquid physical states.

Number of green leaves				
	Culture medium			
	Solid DM	Liquid DM	Solid 2GGC	Liquid 2GGC
Subculture	***Dioscorea alata***			
1	3.55 bA	3.21 bA	3.80 aA	4.10 aA
2	4.68 abA	2.68 bB	3.37 aAB	2.75 aB
3	5.10 aA	5.21 aA	3.63 aA	3.86 aA
4	6.00 aA	2.58 bB	3.12 aB	2.50 aB
CV (%)				20.38
	Dioscorea alata* var. *purpurae			
1	5.50 aA	4.10 aA	5.30 aA	4.59 aA
2	3.80 bA	4.10 aA	3.85 abA	3.38 aA
3	6.00 aA	3.33 aB	3.63 bB	3.58 aB
4	4.63 abA	2.86 aB	3.42 bAB	3.54 aAB
CV (%)				17.50
	Dioscorea rotundata			
1	3.20 bA	3.55 aA	4.30 aA	3.37 aA
2	3.20 bA	2.22 abAB	3.25 aA	1.75 aB
3	3.89 abA	3.48 aA	3.13 aA	3.00 aA
4	5.21 aA	1.67 bAB	1.50 bB	5.00 aA
CV (%)				22.68

Means followed by the same lowercase letter in each column and uppercase in each row do not differ statistically from each other at 5% probability by the Tukey test. Source: Prepared by the authors (2023).

For *D. alata* var. *purpurea*, there were no statistical differences in the four subcultures performed in the liquid media. In the solid MS medium, the highest means were those of the first and third subcultures, with 5.50 and 6.00 green leaves, respectively, statistically higher values than those found in the second subculture (3.80). In the solid 2GGC medium, the highest mean occurred in the first subculture, with 5.30 green leaves, which differed from those observed in the third and fourth subcultures. Among the culture media, there were no differences in the first and second subcultures. In contrast, in the third subculture, the solid MS medium presented the highest mean (6.0), differing statistically from the others. In the fourth subculture, the solid MS medium

was responsible for the largest number of green leaves (4.63), differing statistically from the liquid MS medium (Table 2).

Also in Table 2, it can be observed that the number of green leaves for the genotype *D. rotundata* did not differ along the subcultures carried out in the liquid 2GGC medium. In solid MS, the highest means were of the third and fourth subcultures, 3.89 and 5.21 green leaves, respectively. In the liquid MS and solid 2GGC media, the highest means were from the first three subcultures, which statistically did not differ from each other but were higher than the mean observed in the fourth subculture, except for the mean obtained in the second subculture for the liquid MS medium, which was statistically equal to that presented in the fourth cultivation period. In the first and third subcultures, there were no differences between the culture media, while in the second subculture, the highest means were in the solid 2GGC, solid and liquid MS media, 3.25, 3.20, and 2.22, respectively, with the means obtained in the solid media being statistically higher than in the liquid 2GGC media. In the fourth subculture, the solid MS and liquid 2GGC media presented the highest means, respectively, 5.21 and 5.00, and were statistically superior to those of the solid 2GGC.

The number of nodal segments, for *D. alata*, in the first and third subcultures presented the highest means, of 2.50 and 2.66, respectively (Table 3).

Table 3 - Mean values of the number of nodal segments (NNS) of the genotype *D. alata* in different subcultures and the supplemented DM media and 2GGC in the solid and liquid physical states.

1 Subculture	**2 Subculture**	**3 Subculture**	**4 Subculture**
2.50 a	1.94 b	2.66 a	1.98 b
Solid DM	**Liquid DM**	**Solid 2GGC**	**Liquid 2GGC**
2.58 a	2.06 b	2.28 ab	2.13 b
CV (%)			19.30

Means followed by the same letter in each row do not differ statistically from each other by the Tukey test at 5% probability. Source: Prepared by the authors (2023).

For variable number of nodal segments, the solid media provided the highest means, and the solid 2GGC medium did not differ statistically from the liquid media (Table 3).

In the first subculture, *D. alata* var. *purpurea* presents the highest means of nodal segments for the solid MS (4.50) and 2GGC (3.90) media, with the means being statistically higher than those obtained in the other subcultures. The first two subcultures had the highest means in the liquid MS medium. However, the value achieved in the second subculture did not differ from the others. In the liquid 2GGC medium, the highest means are from the first and third subcultures, and the third subculture did not differ from the second and fourth subcultures (Table

4). Solid media have the highest means in the first subculture, but solid 2GGC media did not differ statistically from liquid MS media. In the second subculture, the solid 2GGC and solid and liquid MS media have the highest means, 2.75, 2.20, and 2.32, respectively, and only the solid 2GGC media was statistically superior to the liquid 2GGC media. Even with a statistically mean for the solid MS medium, there was no difference between the solid and liquid 2GGC media in the third subculture. No statistical differences were found in the fourth subculture between the culture media (Table 4).

Table 4 - Mean values of the number of nodal segments of the genotypes *D. alata* var. *purpurea* and *D. rotundata* grown in supplemented DM and 2GGC media in solid and liquid physical states.

Number of nodal segments				
	Culture medium			
Subculture	Solid DM	Liquid DM	Solid 2GGC	Liquid 2GGC
	Dioscorea alata* var. *purpurea			
1	4.50 aA	2.95 aBC	3.90 aAB	2.71 aC
2	2.20 bcAB	2.32 abAB	2.75 bA	1.69 bB
3	2.94 bA	1.83 bB	2.26 bAB	2.25 abAB
4	1.94 cA	1.86 bA	2.05 bA	1.77 bA
CV(%)				16.77
	Dioscorea rotundata			
1	1.85 aB	1.70 abB	2.95 aA	1.95 aB
2	1.65 aA	1.22 bA	1.90 bA	1.25 aA
3	2.17 aA	2.27 aA	1.73 bA	2.00 aA
4	2.43 aAB	1.17 bB	1.12 bB	3.00 aA
CV(%)				17.56

Means followed by the same lowercase letter in each column and uppercase in each row do not differ statistically from each other at 5% probability by the Tukey test. Source: Prepared by the authors (2023).

For *D. rotundata*, the solid MS and liquid 2GGC media showed no difference in the number of nodal segments in the subcultures. The liquid MS medium presented the highest means in the first and third subcultures. For the solid 2GGC medium, the highest mean of nodal segments (2.95) was observed in the first subculture, which differed statistically from the others (Table 4). In the second and third subcultures, there was no statistical difference between the culture media; in the fourth subculture, the liquid 2GGC and solid MS media presented the highest means, 3.0 and 2.43 nodal segments, respectively, with the liquid 2GGC media differing statistically from the liquid MS and solid 2GGC media (Table 4).

Regarding the number of roots, for *D. alata*, the second and third subcultures produced the highest means, which did not differ statistically from each other (Table 6). The solid MS and 2GGC media provided the highest means and were statistically superior to the liquid 2GGC medium. Liquid media have the lowest means and did not differ from each other (Table 5).

Table 5 - Mean values of the number of roots (NR) of the genotype *D. alata* in different subcultures and the supplemented DM media and 2GGC in the solid and liquid physical states.

1 subculture	**2 subculture**	**3 subculture**	**4 subculture**
2.51 b	3.94 a	3.94 a	2.10 b
Solid DM	**Liquid DM**	**Solid 2GGC**	**Liquid 2GGC**
3.51 a	3.23 ab	3.39 a	2.30 b
CV(%)			34.54

Means followed by the same letter in each row do not differ statistically from each other by the Tukey test at 5% probability. Source: Prepared by the authors (2023).

The liquid media and solid 2GGC do not present statistical differences in the number of roots throughout the four subcultures for *D. alata* var. *purpurea*. In the solid MS medium in the first three subcultures, the highest means occurred, and the highest of them (6.81), in the third subculture, differed statistically from that achieved in the fourth subculture. There were no differences between the culture media in the first and fourth subcultures. At the same time, the solid MS media had the highest mean in the second subculture, which corresponded to 5.35 roots and differed statistically from the 2GGC media, also solid. In the third subculture, the solid MS and solid and liquid 2GGC media had the highest means, differing statistically from the liquid MS media (Table 6).

Table 6 - Mean values of the number of roots of the genotypes *D. alata* var. *purpurea* and *D. rotundata* grown in supplemented DM media and 2GGC in solid and liquid physical states.

Number of roots				
	Culture medium			
Subculture	Solid DM	Liquid DM	Solid 2GGC	Liquid 2GGC
	Dioscorea alata* var. *purpurea			
1	4.15 abA	3.30 aA	4.35 aA	3.00 aA
2	5.35 abA	3.84 aAB	3.20 aB	3.94 aAB
3	6.81 aA	1.92 aB	4.00 aA	4.08 aA
4	4.12 bA	1.86 aA	3.58 aA	4.23 aA
CV(%)				26.41
	Dioscorea rotundata			
1	1.30 abA	1.60 aA	0.75 aA	1.16 aA
2	2.35 aA	1.56 aAB	0.85 aBC	0,17 aC
3	1.44 abAB	0.00 bB	0.00 aB	4.00 aA
4	0.79 bA	0.17 abA	0.50 aA	2.00 aA
CV(%)				47.62

Means followed by the same lowercase letter in each column and uppercase in each row do not differ statistically from each other at 5% probability by the Tukey test. Source: Prepared by the authors (2023).

For the *D. rotundata* genotype, the 2GGC media did not present differences between the four subcultures, the solid MS media presented the highest means in the first three subcultures, but the highest mean of them (2.35), observed in the second subculture, differed statistically from the mean of the fourth subculture. The first and second subcultures presented the highest means in the liquid MS medium and differed statistically from the value obtained in the third subculture. As observed in *D. alata*, the culture media showed no statistical differences in the first and fourth subcultures. In the second subculture, the MS media have the highest means, with the solid differing statistically from the 2GGC media that did not differ from each other. In the third subculture, the highest means, 4.00 and 1.44, corresponded to the liquid 2GGC and solid MS media, the first differing statistically from the liquid MS and solid 2GGC media, which did not present root formation (Table 6).

The genotypes presented different characteristics depending on the culture media and subcultures. The genotypes *D. alata* and *D. alata* var. *purpurea* developed better than the genotype *D. rotundata*. In addition, had a lower frequency of calluses and a higher percentage of responsive explants (Figures 3, 4, and 5).

Figure 3 - Plants of genotype *D. alata* in supplemented solid and liquid DM, and solid and liquid 2GGC media, respectively, from left to right in the first (a), second (b), third (c), and fourth (d) subcultures after 30 days. Source: Prepared by the authors (2023).

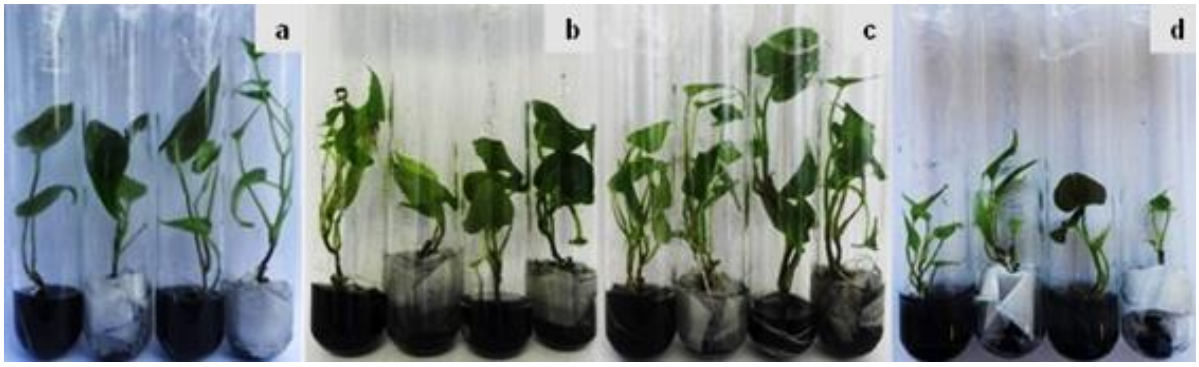


Figure 4 - Plants of the genotype *D. alata* var. *purpurea* in supplemented solid and liquid DM and solid and liquid 2GGC media, respectively, from left to right, in the first (a), second (b), third (c), and fourth (d) subcultures, after 30 days. Source: Prepared by the authors (2023).

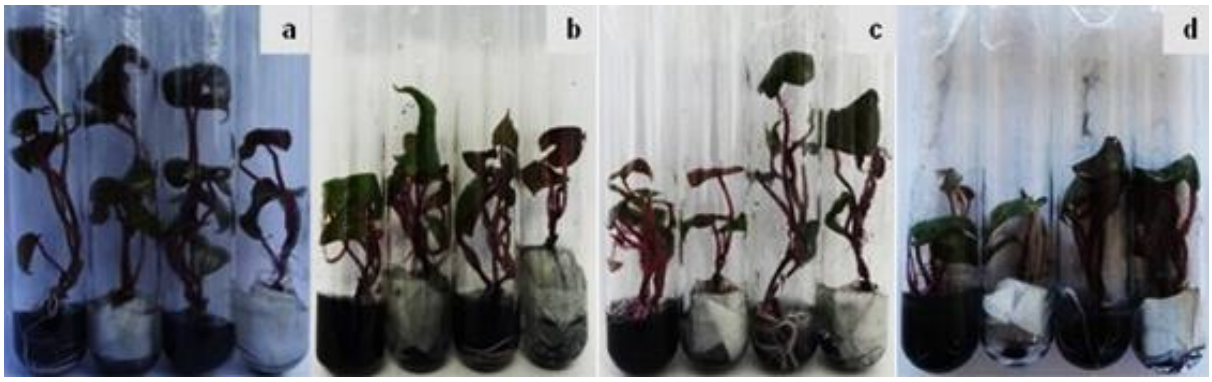
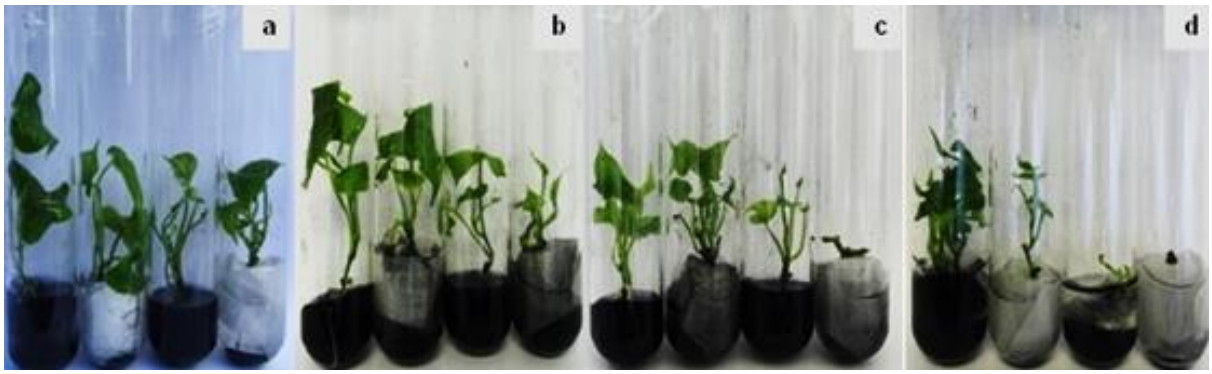


Figure 5 - Plants of the genotypes *D. rotundata* in supplemented solid and liquid DM, and solid and liquid 2GGC media, respectively, from left to right, in the first (a), second (b), third (c), and fourth (d) subcultures, after 30 days. Source: Prepared by the authors (2023).



DISCUSSION

The survival and regeneration of explants are related to several factors, among which one can highlight the adequate nutrition to its *in vitro* response provided by the culture medium. The maximum percentage of survival for *D. alata* achieved in all culture media, as well as in the genotypes *D. alata* var. *purpurea* and *D. rotundata*, except for the liquid 2GGC medium in the first subculture is similar to those observed in other studies such as Pereira and Fortes (2003a). The authors obtained 100% survival and regeneration of the explants in MS culture medium, in solid and liquid consistencies, with the addition of different concentrations of ampicillin, after 21 days, in the cultivar of Baronesa potato. Kadota; Niimi (2004), testing the BM medium (Linsmaier and Skoog, 1965), in liquid and solid physical states, with different solidifiers (agar and gellan gum), also obtained survival rates between 97% and 100%. In the other subcultures, there was a decrease in the regeneration of the explants in the yam genotypes studied, with a more relevant reduction in the media of liquid consistency, especially in the 2GGC medium.

Doukoure et al. (2000) developed a study with five culture media (2GH1, CL82-1, MHW78, 2GGC, and M50) in *D. alata* (23 cultivars), D. esculenta (7 cultivars) and D. caynensis – *D. rotundata* (72 cultivars and 13 hybrids), and obtained, in 2GGC and M50, more than 50% of regenerated nodal segments, while in 2GH1, CL82-1, MHW78 media, there was favoring for callus production.

The callus formation observed in the explants is related to the physical state of the culture medium. In the liquid consistency, a greater number of calluses was obtained, something unwanted according to the objectives of this study since its occurrence, especially in the species *D. rotundata*, made the development of the explant impossible, even after new subcultures in new culture media. Also, as Vega (2012) mentioned, the callogenesis process depends on the morphology and physiology of each species. The effect of subcultures on the origin of calluses is evidenced by the realization of more than one subculture of the same plant in the same culture medium. In the species *D. alata* and *D. rotundata*, the first occurrences of calluses were verified from the second subculture, with an increasing increase in the others. In the species *D. alata* var. *purpurea*, this process was evidenced by the third subculture.

Moreno et al. (2016), studying creole clones of the species *D. alata*, obtained callus formations from the second week on in solid and liquid media, being the most evident formation in the solid, which differs from the results of this work. These same authors emphasize that callus formation can obtain a greater number of shoots, especially when the objective is the formation of microtuberization.

In liquid culture media, the tissue remains constantly submerged in the medium, which can cause oxygen deprivation and aeration (Chen and Ziv, 2001), probably being one of the reasons for the higher proportions of calluses that occur in the liquid media. Pereira also observed

this behavior; Fortes (2003b), when they cultivated meristems in MS medium in different physical states, when there was 99.6% of callus formation in the Baronesa potato cultivar in the liquid medium, while in the solid consistency, this value represented only 0.4% after 30 days. According to these authors, callus formation is somewhat unfavorable, as it can result in undesirable genetic variations.

In the height of the aerial part, it is observed that in the solid media, the highest means occurred. Differing from these results, Moreno et al. (2016) obtained, in liquid MS medium under constant agitation, in clones of the species *D. alata* L., sprout lengths of 17.30 cm, in plants of the Colombian Creole clone under 12 hours of light, and 12.10 cm, for the Venezuelan Creole clone under continuous light, in 2 months. This divergence in the results may infer that constant agitation is an important factor to consider. Nevertheless, it adds a higher cost to the use of the liquid medium.

Pereira and Fortes (2004), even with the formation of calluses in the development of meristems, obtained the best responses for the height of the aerial part with the culture medium containing MS salts and supplemented with 1.0 mg L^{-1} of thiamine, 5.0 mg L^{-1} of pantothenic acid, 0.25 mg L^{-1} of AG_3, in the liquid consistency in the potato multiplication step.

For the study over the four subcultures, the achievement of the highest means in the solid MS medium for the variable number of green leaves in the genotypes *D. alata*, *D. alata* var. *purpurea*, and *D. rotundata*, are in line with the work carried out by Rodríguez et al. (2016), who, when studying the physical state of the culture medium, obtained in yam cv. Belep (*D. alata*) grown in MS medium, positive results in static liquid culture medium with filter paper support, for the height of the aerial part (2.37 cm), number of internodes (2.82), number of leaves (3.62), without the presence of buds and calluses. They demonstrated, therefore, that the static liquid medium with filter paper support reduced the callus produced at the base of the explants, differently from what occurred in this study. It is observed that the number of leaves found by these authors (3.62), with 30 days of cultivation, is lower than the means found in the solid MS medium, except for the *D. rotundata* genotype (Table 2). However, it is necessary to emphasize that these authors did not consider the subculture factor, which was relevant in this study for the formation of calluses from the second cultivation period, especially in the liquid medium.

Solid media showed the highest number of nodal segments in the genotype *D. alata* and *D. alata* var. *purpurea* (Tables 3 and 4). In the case of the *D. rotundata* genotype, the solid MS medium is the most indicated for allowing greater stability throughout the subcultures, as the liquid media resulted in callus formations that impaired the development of the explants (Table 4). Differing from this result, Pereira; Fortes (2003b) found that the use of liquid culture medium in ‘Eliza‘ potato provided a significant increase in the multiplication rate in the M2 medium [formed by MS salts, 30 g L^{-1} of sucrose, 2 mg L^{-1} of pantothenic acid, 0.4 mg L^{-1} of thiamine, and the growth regulators ANA (0.01 mg L^{-1}) and AG_3 (0.25 mg L^{-1})].

Dos Santos et al. (2022), studying the physical state of three culture media in *D. rotundata*, did not obtain statistical differences between the variables studied in a solid medium. In the liquid consistency, the MS and 2GGC media presented the highest means for the variables plant height, fresh mass of the aerial part, and fresh and dry root masses. The liquid 2GGC medium resulted in a higher mean for the variable number of nodal segments (5.19), a value higher than that found for this genotype in the four subcultures performed in the culture media of this work (Table 4). Probably, the difference found between the two studies may have been due to the evaluation time, which was approximately 60 days less than the work compared.

Kadota and Niimi (2004), in *Dioscorea japonica* Thunb, obtained better results for fresh weight in the gelated medium with 0.1% gellan gum. These authors observed that there was no significant effect on the proliferation of sprouts among the physical states. However, the sprouts in the liquid medium were much heavier and had three times more nodes than the sprouts in the solid medium. In turn, although there was a higher frequency of rooting in the solid medium, the highest number of roots was found in the liquid medium (9.80); for the number of leaves, there were no differences between the gelling agents and the consistencies of the media.

According to the variables analyzed in the three genotypes in four subcultures, the solid media showed the best results and more stability; this can be concluded mainly from the realization of two or more subcultures under the same experimental conditions. In accordance with these results, Chacón et al. (2000) state that Phytagel® allows a greater accumulation of water in the tissues of plants grown in solidified medium, which can favor the absorption of nutrients present in the culture medium, thus allowing a greater growth of plants in solid media.

Díaz and Sánchez (2007) state that liquid media offer more homogeneous conditions, making nutrients more accessible to tissues and, moreover, the gelling agent is one of the most expensive inputs used in culture media. However, in this work, statically using the liquid medium, without movement, it was possible to observe that the activated charcoal accumulated at the bottom of the test tube, preventing the explant from coming into direct contact with the antioxidant. Furthermore, other effects may result from the use of a static culture medium, such as hypoxia (low oxygen availability) or hyperhydricity (high water content inside cells and tissues with a translucent aspect), causing a depressive effect on the growth of plant tissues, as cited by Polzin et al. (2014). These factors may have resulted in the formation of calluses, a low percentage of responsive explants and lower results in the growth variables analyzed in this study for the liquid media.

Chacón et al. (2000), studying the effect of the liquid medium and different types of gelling agents, obtained, with Phytagel®, higher values of height of the aerial part and fresh and dry weight in the species *D. alata* and *D. trifida*, with the highest means obtained with 1.3 g L^{-1} and 1.8 g L^{-1} of this gelling agent. Even with a high percentage of micro-cuttings cultivated in the liquid medium, they did not develop in both species. Using the concentration of Phytagel ® of

2.3 g L^{-1}, close to that used in this study (2.4 g L^{-1}), in 7 weeks of cultivation in *D. alata*, the authors obtained 3.28 leaves and the height of the aerial part of 3.79 cm.

In the micropropagation of other yam species, the solid MS medium is used, as in the studies by Chu and Ribeiro (2002), which employed 0.7% (w/v) of agar in the species *D. bulbifera* L., *D. delicata* R. Knuth, and *D. olfersiana* Kl.; Jasik and Mantell (2000) used 2 g L^{-1} of Phytagel® in the species *D. alata*, *D. caynensis*, and *D. rotundata*; Chen et al. (2003) used 8 g L^{-1} of agar in *D. zingiberensis* E.; the studies by Mahesh et al. (2010) in *D. wughtii* Hook.f. and Souza et al. (2011) in *D. multiflora* Grised, that did not specify the solidifying agent used.

Most studies use the MS medium, often supplemented with growth regulators, such as the supplemented MS medium, which was developed by the International Institute of Tropical Agriculture [IITA] (1985) and modified in relation to the BAP dose based on the study by Simões et al. (2016).

The 2GGC medium, developed by Duokoure et al. (2000), was used by Rabêlo (2019) with *D. rotundata*, providing satisfactory results in liquid consistency. The two basic nutrient media used in this study have the same macros and micronutrients. They differ mainly in the constitution of vitamins. In the 2GGC medium, there is biotin, calcium pantothenate, and ascorbic acid, absent in the MS medium and presenting higher amounts of thiamine-HCL (B1), pyridoxine-HCL (B6), and glycine. The MS medium, in turn, has nicotinic acid (B3), absent in the 2GGC, and a greater amount of inositol, in addition to growth regulators ANA, BAP, and AG3, and cysteine. Doukoure et al. (2000), using the 2GGC medium, attributed the reduction of oxidation in yam explants to the presence of ascorbic acid and activated charcoal. It is worth mentioning that the cysteine present in the MS medium also acts as an antioxidant, and the amounts used of activated charcoal did not differ between the culture media.

Throughout the subcultures, the liquid 2GGC medium was one of the ones that differed the least. In the other media, the subcultures reduced the mean values of the variables in some cases, which may have occurred due to the reduction of the responsive explants or the effect of the culture medium on the explants. There are no studies with *Dioscorea* in the literature that have tested the effect of culture medium after several subcultures, a fundamental factor for the proper determination of an efficient *in vitro* multiplication protocol.

CONCLUSION

The solid MS and 2GGC media are the most indicated for multiplication of the genotypes *D. alata* and *D. alata* var. *purpurea* during four subcultures. For the *D. rotundata* genotype, the solid MS medium is the most suitable for use by four subcultures.

ACKNOWLEDGMENTS

To the Coordination for the Improvement of Higher Education Personnel (CAPES) and Foundation for Research Support of the State of Bahia (FAPESB), for the financial support to the scholarships of the students involved in the study. To UFRB (Federal University of Recôncavo da Bahia), specifically to the Graduate Program in Agrarian Sciences, for supporting the development of the dissertation from which this article was extracted.

REFERENCES

Aighewi, B. A., Asiedu, R., Maroya, N., Balogun, M. 2015. Improved propagation methods to raise the productivity of yam (*Dioscorea rotundata* Poir.). *Food Security*, 7 (4): 823-834. Doi: 10.1007/s12571-015-0481-6.

Arnau, G., Némorin, A., Maledon, E., Abraham, K. 2009. Revision of ploidy status of *Dioscorea alata* L. (*Dioscoreaceae*) by cytogenetic and microsatellite segregation analysis. *Theoretical and Applied Genetics*, 118 (7): 1239-1249. Doi: 10.1007/s00122-009-0977-6.

Balogun, M. O., Maroya, N. 2014. Status and prospects for improving yam seed systems using temporary immersion bioreactors. *African Journal of Biotechnology*, *13*(15): 1614 - 1622. Doi: 10.5897/AJBX2013.13522.

Balogun, M., Maroya, N., Augusto, J., Ajayi, A., Kumar, P. L., Aighewi, B., Asiedu, R. 2017. Relative efficiency of positive selection and tissue culture for generating pathogen-free planting materials of yam (*Dioscorea* spp.). *Czech Journal of Genetics Plant Breeding*, 53 (1): 9-16. Doi: 10.17221/117/2016-cjgpb.

Bömer, M., Rathnayake, A. I., Visendi, P., Sewe, S. O., Sicat, J. P. A., Silva, G., Seal, S. E. 2019. Tissue culture and next-generation sequencing: A combined approach for detecting yam (*Dioscorea* spp.) viruses. *Physiological and molecular plant pathology*, *105*: 54-66. Doi:10.1016/j.pmpp.2018.06.003.

Brito, T., T., Soares, L. S., Furtado, M. C., Castro, A. A., Carnelossi, M. A. 2011. Composição centesimal de inhame (*Dioscorea* sp.) in natura e minimamente processado. *Scientia Plena*, *7*(6): 1-7.

Cabrera Jova, M. A. 2009. Empleo de métodos biotecnológicos para la propagación de ñame. *Biotecnología Vegetal*, *9*(4): 195-209.

Carmona Wilches, O. E., Díaz Narváez, L. C., Beltrán Herrera, J. D. 2013. Efecto de los osmolitos sacarosa, manitol y sorbitol en la conservacion *in vitro* de *Dioscorea alata*, *D. bulbifera*, *D. rotundata* y *D. trifida* por el método de crecimiento mínimo. *Magazine of the Colombian Association of Biological Sciences (ACCB)*, 25: 41-51.

Chacón, A. G., Saborío, F., Gómez, L., Torres, S., Valverde, R. 2000. El tipo de gelificante en el desarrollo in vitro y la aclimatización de plantas de yampi (*Dioscorea trifida*) y ñame (*Dioscorea alata*). *Agronomía costarricense, 24* (2): 57-64.

Chen, J., Ziv, M. 2001. The effect of ancymidol on hyperhydricity, regeneration, starch and antioxidant enzymatic activities in liquid-cultured Narcissus. *Plant Cell Reports*, *20* (1): 22-27. Doi: 10.1007/s002990000283.

Chen, Y., Fan, J., Yi, F., Luo, Z., Fu, Y. 2003. Rapid clonal propagation of *Dioscorea zingiberensis*. *Plant cell, tissue and organ culture, 73*(1): 75-80. Doi:10.1023/A:1022683824635.

Chu, E. P., Ribeiro, R. D. C. L. F. 2002. Growth and carbohydrate changes in shoot cultures of *Dioscorea* species as influenced by photoperiod, exogenous sucrose and cytokinin concentrations. *Plant cell, tissue and organ culture*, *70*(3): 241-249. Doi:10.1023/A:1016565500201.

Díaz Narváez, L. C., Carmona Wilches, O. E., Beltrán Herrera, J. D. 2015. Optimización de la conservación *in vitro* de germoplasma de *Dioscorea* spp por crecimiento mínimo. *Revista Colombiana de Biotecnología*, *17*(1): 32-39. Doi: 10.15446/rev.colomb.biote.v17n1.50842.

Salazar Díaz, R., Hoyos Sánche, R. A. 2007. Multiplication and in vitro tuberization of yam (*Dioscorea alata* L.) in temporary immersion system. *Revista Facultad Nacional de Agronomía Medellín, 60* (2): 3907-3921.

Doukoure, S., Ahoussou, N., Zoundjihekpon, J., Tio-Toure, B. E. 2000. Culture *in vitro* chez l'igname (*Dioscorea* sp.): Influence du milieu de culture sur la regeneration des microboutures.: *In vitro* culture of yam (*Dioscorea*): Influence of the culture medium on the regeneration of microcuttings. *Agronomie Africaine, 12* (3): 105-114.

FAO. Food And Agriculture Organization Of The United Nations/Data, Crop. Available in: < http://www.fao.org/faostat/en/#data/QC >. Access in: 05 maio 2019.

Ferreira, E. B., Cavalcanti, P. P., Nogueira, D. A. 2018. Pacote Experimental Designs (Portuguese). R package version 1.2.0. Available in: https://CRAN.R-project.org/package=ExpDes.pt.

García, M. B., Batista, D., Rodríguez, M., Kosky, G., Malaurie, B., Hamon, P., Charles Demenorval, L. 2011. Optimización de un medio de cultivo para plantas micropropagadas de *Dioscorea alata* L. *Revista Colombiana de Biotecnología*, *13* (2): 221-228.

García, M. B., Sánchez, Y. A., Malaurie, B., Jerez, Y. H., & Pupo, J. J. S. 2009. Conservación *in vitro* de *Dioscorea alata* L. clon caraqueño (*Dioscoreaceae*). *Revista Peruana de biología*, *16* (2): 203-208.

García, M. B., Ceiro, W., Meneses, S., Aguilera, N., Vázquez, J., Infante, Z., Fonseca, M. 2004. Regeneration and multiplication of *Dioscorea alata* germplasm maintained *in vitro*. *Plant Cell, Tissue and Organ Culture*, *76*(1), 87-90. Doi: 10.1023/A:1025804516226.

IITA. International Institute of Tropical Agriculture. 1985. Root and tuber improvement program. Research Highlights, Nigeria, p. 1981-1984.

Jasik, J., Mantell, S. H. 2000. Effects of jasmonic acid and its methylester on *in vitro* microtuberisation of three food yam (*Dioscorea*) species. *Plant Cell Reports*, *19* (9): 863-867. Doi: 10.1007/s002990000207.

Kadota, M., Niimi, Y. 2004. Improvement of micropropagation of Japanese yam using liquid and gelled medium culture. *Scientia horticulturae*, 102 (4): 461-466. Doi:10.1016/j.scienta.2004.06.004.

Linsmaier, E. M., Skoog, F. 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiologia Plantarum*, 18: 100-127. Doi:10.1111/j.1399-3054.1965.tb06874.x.

Mahesh, R., Muthuchelian, K., Maridass, M., Raju, G. 2010. *In vitro* propagation of wild yam, *Dioscorea wightii* through nodal cultures. *Int J Biol Technol*, 1 (1): 111-113.

Moreno, M. E., Durango, R., Cabrita, Y., Oropeza, M. (2016). Establecimiento y caracterización del banco de germoplasma *in vitro* de ñame (*Dioscorea* sp.). Memorias del Instituto de Biología Experimental, 8: 129-132.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, 15 (3): 473-497.

Pereira, J. E. S., Fortes, G. R. D. L. 2003a. Toxicidade de antibióticos no cultivo in vitro da batata em meios semi-sólido e líquido. *Pesquisa Agropecuária Brasileira*, 38 (11): 1273-1279. Doi: 10.1590/S0100-204X2003001100004.

Pereira, J. E. S., Fortes, G. R. D. L. 2003b. Protocolo para produção de material propagativo de batata em meio líquido. *Pesquisa Agropecuária Brasileira*, 38 (9): 1035-1043. Doi: 10.1590/S0100-204X2003000900003.

Pereira, J. E. S., Fortes, G. R. D. L. 2004. Organogênese de ápices meristemáticos de batata em meios de isolamento e multiplicação in vitro. *Horticultura Brasileira*, 22 (2): 197-201.Doi: 10.1590/S0102-05362004000200007.

Polzin, F., Sylvestre, I., Déchamp, E., Ilbert, P., Etienne, H., Engelmann, F. 2014. Effect of activated charcoal on multiplication of African yam (*Dioscorea cayenensis-rotundata*) nodal segments using a temporary immersion bioreactor (RITA®). *In Vitro Cellular & Developmental Biology-Plant*, *50* (2): 210-216. Doi: 10.1007/s11627-013-9552-6.

R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2016. Available in: http://www.R-project.org/. Access in: 27. Dez. 2017.

Rodríguez, D., López, J., Torres, Y., Basail, M., Rayas, A., Santos, A., Gutiérrez, Y., Medero, V., Beovides, Y., Bauta, M. 2016. Influencia del medio de cultivo líquido estático con soporte en la micropropagación de *Dioscorea alata* L. cultivar 'Belep'. *Agricultura Tropical*, *2* (2): 79-82.

Salcedo-Mendoza, J., García-Mogollón, C, Salcedo-Hernández, D. 2018. Propiedades funcionales de almidones de ñame (*Dioscorea alata*). Biotecnología en el sector agropecuario y agroindustrial, 16 (2): 99-107. Doi: 10.18684/bsaa.16n2.1170.

Dos Santos, K. C. F., Rabelo, J. S. S., Sampaio, L. F. S., da Silva Ledo, C. A., & da Silva Souza, A. (2022). Meios de cultura na multiplicação in vitro do 'Inhame da Costa' (*Dioscorea rotundada* Poir.). *Revista Brasileira de Ciências Agrárias*, *17*(1), 1-6. Doi: 10.5039/agraria.v17i1a128.

Santos, E. D., Cazé Filho, J., Lacerda, J. D., & Carvalho, R. A. (2007). Inhame (*Dioscorea* sp.) tecnologia de produção e preservação ambiental. *Tecnologia & Ciência Agropecuária*, *1* (1): 31-36.

Sêdami, A. B., Todjro, C. G. H., Justine, D. S., Arnaud, A., Clément, A., Corneille. 2017. Effects of activated charcoal on medium-term conservation of yam (*Dioscorea* spp.) cultivated in Benin. *African Journal of Biotechnology*, *16*(15), 819-825. Doi:10.5897/AJB2016.15504.

Shukla, S., Shukla, S. K. 2014. *In vitro* regeneration of *Dioscorea hispida* through nodal explants-A rich source of starch. *GSTFJ BioSciences*, *3* (1): 30-35.

Simões, K. da S., Lino, L. S. M., Souza, A. da S., Silva, S. de O. E, Ledo, C. A. da S. 2016. Micropropagação de Inhame da Costa sob concentrações de BAP em distintos meios de cultura. *Magistra*, *28* (3/4): 342-351.

Souza, A. V. D., Bertoni, B. W., França, S. D. C., Pereira, A. M. S. 2011. Micropropagação de *Dioscorea multiflora* Grised. *Ciência e Agrotecnologia*, *35*, 92-98. Doi: 10.1590/S1413-70542011000100011.

Taha, S. S., Abdelaziz, M. E. 2017. *In vitro* propagation of yam via nodal segment culture. *Bioscience Research*, *14* (4): 1217-1222.

Vega, M. E. G. 2012. El Ñame (*Dioscorea* spp.). Características, usos y valor medicinal. Aspectos de importancia en el desarrollo de su cultivo. *Cultivos tropicales*, 33(4): 05-15.