SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 21, NUM. 01 2025
doi: 10.14808/sci.plena.2025.010201

In vitro tuberization of yam (*Dioscorea* sp.): influence of sucrose, light spectra, and plant growth regulators

Tuberização *in vitro* de inhame (*Dioscorea* sp.): influência da sacarose, espectros de luz e reguladores de crescimento vegetal

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(Recebido em 14 de dezembro de 2024, aceito em 27 de janeiro de 2025)

The effects of different sucrose concentrations, light spectra, and plant growth regulators on *in vitro* tuberization of yam (*Dioscorea* sp.) were determined. A completely randomized experimental design was used in a factorial arrangement, with five replications. In the first experiment, three concentrations of sucrose (30, 60, and 90 $g.L^{-1}$) and four concentrations of naphthalene acetic acid (NAA) (0.0, 1.0, 2.0, and 4.0 $mg.L^{-1}$) were tested. The highest percentage of tuberization was observed with the use of 90 $g.L^{-1}$ of sucrose, regardless of the NAA concentrations used. Greater diameter (7.88 mm) and fresh weight (166.13 mg) were obtained by increasing the sucrose concentration to 90 $g.L^{-1}$. In the second experiment, three LED light spectra (white, blue. and red), two concentrations of NAA (0.0 and 1.0 $mg.L^{-1}$), and three concentrations of kinetin (KIN) (0.0, 1.0, and 2.0 $mg.L^{-1}$) were tested. Tuberization (%) showed higher averages with the use of blue (90.5 %) or red (95.5 %) light spectra or with 1.0 $mg.L^{-1}$ KIN (91.1%). The culture medium without NAA led to a larger number of microtubers (1.67), as did the use of blue (1.65) or red (1.73) lights, and KIN at concentrations of 1.0 $mg.L^{-1}$ (1.58) or 2.0 $mg.L^{-1}$ (1.68). Higher averages of fresh microtuber weight were observed with the use of 0.0 $mg.L^{-1}$ KIN, 1.0 $mg.L^{-1}$ NAA, and the blue (276.9 mg) or white (320.4 mg) light spectra. The use of 90 $g.L^{-1}$ of sucrose, 1 $mg.L^{-1}$ KIN and 1 $mg.L^{-1}$ NAA, and the blue-light spectrum increase production of microtubers.
Keywords: micropropagation, LED lamps, carbohydrate.

Foram determinados os efeitos de diferentes concentrações de sacarose, espectros de luz e reguladores de crescimento vegetal na tuberização *in vitro* de inhame (*Dioscorea* sp.). Adotou-se o delineamento experimental inteiramente casualizado, em esquema fatorial, com cinco repetições. No primeiro experimento, foram testadas três concentrações de sacarose (30, 60, 90 $g.L^{-1}$) e quatro de ácido 1-naftaleno-acético (ANA) (0,0; 1,0; 2,0 e 4,0 $mg.L^{-1}$). Maior porcentagem de tuberização foi observada com a utilização de 90 g de sacarose independente da concentração de ANA. Maior diâmetro (7,88 mm) e massa fresca (166,13 mg) foram obtidos ao aumentar a concentração de sacarose para 90 $g.L^{-1}$. No segundo experimento, foram avaliados três espectros de luz LED (Branco, Azul e Vermelho), duas concentrações de ANA (0,0 e 1,0 $mg.L^{-1}$) e três de cinetina (CIN) (0,0; 1,0; 2,0 $mg.L^{-1}$). A tuberização (%) apresentou maiores médias com a utilização das luzes azul (90,5%) ou vermelha (95,5%) ou com 1,0 $mg.L^{-1}$ de CIN (91,1%). O meio de cultivo sem ANA proporcionou maior número de microtubérculos (1,67), assim como a utilização das luzes azul (1,65) ou vermelha (1,73), e de CIN nas concentrações 1,0 $mg.L^{-1}$ (1,58) ou 2,0 $mg.L^{-1}$ (1,68). Maiores médias de massa fresca de microtubérculo foram observadas com a utilização de 0,0 $mg.L^{-1}$ de CIN, 1,0 $mg.L^{-1}$ de ANA e os espectros de luz azul (276,9 mg) ou branco (320,4 mg). A utilização de 90 $g.L^{-1}$ de sacarose, 1 $mg.L^{-1}$ de CIN e 1 $mg.L^{-1}$ de ANA, e o espectro de luz azul proporcionam maior produção de microtubérculos.
Palavras-chave: micropropagação, lâmpadas LED, carboidrato.

1. INTRODUCTION

Tuberization is a physiological process that can be influenced by various factors, such as carbohydrate source and concentration, plant growth regulators, and light intensity [1]. In species with storage organs, this process can be induced during *in vitro* cultivation from nodal and apical explants, resulting in the production of microtubers [2]. These microtubers can be used as

propagative material and for distribution of pathogen-free plant material in germplasm exchange programs [3].

The carbohydrate source in the culture medium contributes to induction of the *in vitro* tuberization process, and sucrose has been widely used as the main source [4]. Studies on species within the *Dioscorea* genus have shown that increasing the concentration of sucrose in the culture medium influenced the induction and growth of microtubers [5, 6, 3].

Besides the carbohydrate source, plant growth regulators are also known to induce microtuber formation. Auxins and cytokinins have been recognized as playing a crucial role in tuberization [1]. In different potato (*Solanum tuberosum* L.) cultivars, auxin increased the size of microtubers, whereas cytokinin increased the number of microtubers produced [7]. In *Dioscorea nipponica*, the induction of microtubers was enhanced when combining 1-naphthaleneacetic acid (auxin) and N6-benzyladenine (cytokinin) [5]. The effects of 6-benzylaminopurine and kinetin on tuberization in *Dioscorea deltoidea* have also been reported, where the use of 0.9 $mg.L^{-1}$ of 6-benzylaminopurine with a 6% sucrose concentration was considered ideal for tuberization, while kinetin only promoted tuberization when combined with 6-benzylaminopurine and 1-naphthaleneacetic acid [8].

Light is another crucial factor that can interfere with tuberization in various species [9]. Artificial illumination through LED (light emitting diode) lamps can provide the necessary quantity and quality of light for plant development, offering specific spectra and lower energy demand [10]. Different light spectra are known to induce the formation and growth of microtubers in potato (*Solanum tuberosum* L.) [11]. Red LED light is recognized for accelerating tuber induction, whereas blue light might delay the onset of tuberization in potatoes [12]. The combination of red, blue, and white LED light (70:20:10%, respectively) increased the number of potato tubers produced; however these tubers had lower fresh weight [10].

Studying these factors is of great importance to facilitate the propagation process for tuber-producing species, such as yam. Belonging to the *Dioscorea* genus, yam (*Dioscorea* sp.) is considered the fourth most important tuberous vegetable globally [13]. Its edible tubers are significant sources of nutrients, such as vitamins, calcium, and minerals [14], and they display potential industrial use in flour and chip production [15]. It is a relevant crop in the Northeast region of Brazil, which accounts for approximately 90% of Brazilian domestic production [16], providing income, employment, and sustenance for the region's farmers [17].

Yam propagation is mainly vegetative, which is conducted through whole seed tubers or their fragments [18]. However, this method has limitations, such as dormancy and a low sprouting index, the risk of contamination of growing areas by pathogens in the tuber [17], and high acquisition costs [19]. In this context, there is a need to explore alternatives to improve production of yam seedlings in the Northeast region, which is affected by a lack of high phytosanitary quality seed tubers and uniform sprouting. The induction of *in vitro* tuberization could serve as a source of microtubers for this crop propagation. Therefore, this current study was conducted to evaluate the effect of sucrose, light spectra, and plant growth regulators on *in vitro* tuberization of yam, aiming to support the development of microtuber production protocols to be used in planting yam by local yam producers.

2. MATERIALS AND METHODS

2.1 Plantlet production

Yam tuber genotypes were acquired from the "*Central de Abastecimento de Sergipe*" (CEASA/SE) for plantlet production. They were kept in a dry and airy location until they sprouted. They were segmented and planted in 10-L pots containing soil and manure (3:1). The resulting plants were maintained in a greenhouse and used as a source of explants for conducting the experiments. These explants were taken from the secondary branches of 120-day-old plants.

All experiments were conducted in the Tissue Culture and Plant Breeding Laboratory of the Department of Agronomic Engineering at the Federal University of Sergipe, São Cristóvão, Sergipe, Brazil (10°55'45.8" S, 37°06'14.3" W).

2.2 In vitro cultivation

2.2.1 Disinfection of explants

Nodal segments of approximately 10 cm were used as explants (Figure 1A). They were disinfected using a recipient with water and a neutral detergent, which was kept under an open water tap to have running water for 30 minutes. Then, within a laminar flow chamber, they were immersed in 70% ethanol for 1 minute, followed by immersion in a 0.1% mercury chloride solution for 3 minutes, and subsequently rinsed three times with autoclaved distilled water.

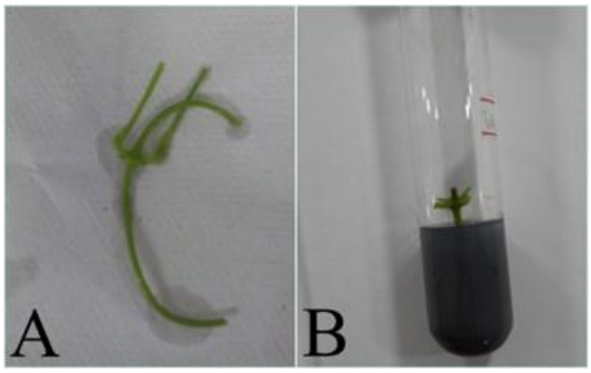

Figure 1: A – nodal segment of approximately 10 cm; B – nodal segment of approximately 2 cm inoculated in a test tube.

2.2.2 In vitro establishment

After disinfection, the explants were cut into sections of approximately 2 cm and inoculated into tubes (Figure 1B) containing 15 mL of MS medium [20], supplemented with 3.0 $g.L^{-1}$ of activated charcoal, 30 $g.L^{-1}$ of sucrose, and 7 $g.L^{-1}$ of agar, adjusted to a pH of 5.8 ± 0.1. The culture medium was autoclaved at 121 ± 1 °C and 1.05 atm for 15 minutes. The inoculated explants were maintained in a growth room at a controlled temperature of 25 ± 2 °C, with a 12-hour photoperiod and a photosynthetic photon flux density of 40 $\mu mol.m^{-2}.s^{-1}$.

2.3 *In vitro* tuberization experiments

2.3.1 Experiment 1: Tuberization under different concentrations of sucrose and 1-naphthaleneacetic acid (NAA)

Plants established *in vitro*, at 60 days of age, were transferred to flasks containing 30 mL of MS medium supplemented with 1.5 $g.L^{-1}$ of activated charcoal and 7 $g.L^{-1}$ of agar, and they were subjected to the treatments (concentrations of sucrose and NAA).

A completely randomized experimental design was adopted, following a 3×4 factorial arrangement consisting of three sucrose concentrations (30, 60, and 90 $g.L^{-1}$) and four NAA concentrations (0.0, 1.0, 2.0, and 4.0 $mg.L^{-1}$). For each treatment, five replicates were used, each consisting of four flasks containing one plant each. The control treatment was the combination of 30 $g.L^{-1}$ of sucrose and 0.0 $mg.L^{-1}$ NAA.

2.3.2 Experiment 2: Tuberization under different light spectra and plant growth regulators

Explants of approximately 2 cm were used for the experiment. They were inoculated into flasks containing 30 mL of MS medium [20], supplemented with 3.0 $g.L^{-1}$ of activated charcoal,

90 g.L^{-1} of sucrose, and 7 g.L^{-1} of agar, adjusted to a pH of 5.8 ± 0.1, and they were subjected to the treatments.

A completely randomized experimental design was adopted in a 3×3×2 factorial arrangement, consisting of three light spectra (white, blue, red), three concentrations of kinetin (KIN) (0.0, 1.0, and 2.0 mg.L^{-1}), and two concentrations of NAA (0.0 and 1.0 mg.L^{-1}). Five replications were used, with four flasks per replication, each containing one explant. The control treatment was the combination of white light, 0.0 mg.L^{-1} of KIN, and 0.0 mg.L^{-1} NAA.

LED (Light-Emitting Diode) lamps (9W, G-light brand) were used for the different light spectra. The photosynthetic photon flux density on the shelf was measured at 19.7 µmol.m^{-2}.s^{-1}, 11.56 µmol.m^{-2}.s^{-1}, and 9.22 µmol.m^{-2}.s^{-1} for the white, blue, and red LED lamps, respectively.

2.4 Variables analyzed

The following factors were evaluated at 60 days after starting the experiments: the percentage of tuberization (%), the number of microtubers per plant, and the diameter (mm) and fresh weight (mg) of the microtubers. The percentage of tuberization was calculated based on the presence or absence of microtubers per plant. The diameter was measured using a digital calipers, and the fresh weight was obtained using an analytical balance with a precision of 0.1 mg.

Analysis of variance was performed on the data, and when significant, the means of the sucrose, light spectra, NAA, and KIN factors were compared using Tukey's test at a 5% probability level. The means of the NAA factor (Experiment 1) were fit using polynomial regression models. Analysis was conducted using the SISVAR statistical software [21].

3. RESULTS AND DISCUSSION

Tuberization under different concentrations of sucrose and 1-naphthaleneacetic acid (NAA)

There was a significant interaction between the sucrose concentration and NAA factors for tuberization percentage (Figure 2). A quadratic model was fitted to the data when using 30 g.L^{-1} of sucrose, with the lowest tuberization percentage (78.92%) obtained at a concentration of 2.2 mg.L^{-1} NAA. A decreasing linear model was fitted for the culture medium with 60 g.L^{-1} of sucrose, where the tuberization percentage decreased with an increase in NAA concentration (Figure 2). Polynomial regression models were not significant when using a concentration of 90 g.L^{-1} of sucrose (Figure 2).

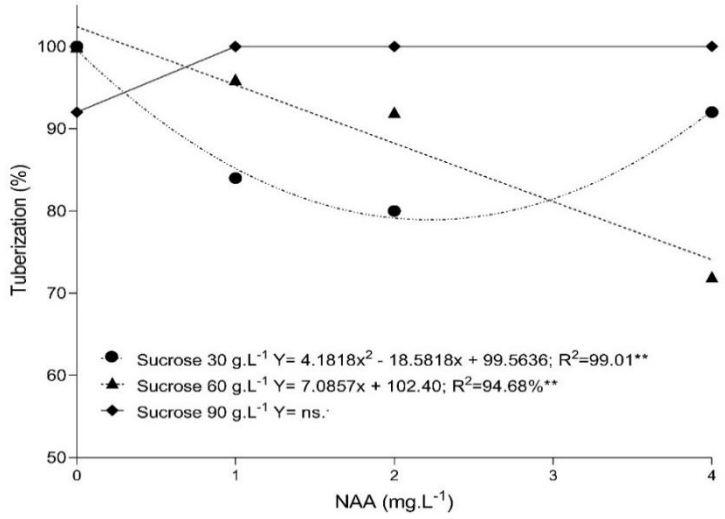

Figure 2: Percentage of in vitro tuberization of Dioscorea sp. as a function of NAA and sucrose concentrations.

Sucrose and the auxin NAA are known to favor induction of *in vitro* tuberization [5]. In this study, their combination had an effect on tuberization percentage. The lowest tuberization (78.92%) was observed in the culture medium with 30 $g.L^{-1}$ of sucrose and 2.2 $mg.L^{-1}$ NAA. Similar results were obtained with *Dioscorea hirtiflora*, where the use of an NAA concentration of 2.5 $mg.L^{-1}$ led to 80% tuberization in a culture medium with 3% sucrose [22]. The different NAA concentrations evaluated did not influence the tuberization percentage when increasing the sucrose concentration to 90 $g.L^{-1}$. A higher sucrose concentration in the culture medium seems to provide a signal to induce tuberization [4]. It has been reported that increasing the sucrose concentration to 80 $g.L^{-1}$ brought about a higher tuberization percentage in potato plants cultivated *in vitro* [6].

The sucrose concentrations tested showed significant differences for the number of microtubers per plant and microtuber diameter and fresh weight variables. However, no significant effect was observed for these variables across the NAA concentrations tested (Table 1). At least one microtuber per plant formed at all sucrose concentrations tested. However, the highest number of microtubers per plant was observed for sucrose concentrations of 60 and 90 $g.L^{-1}$, with means of 1.60 and 1.73, respectively (Table 1). Furthermore, increasing the culture medium supplementation to 90 $g.L^{-1}$ of sucrose resulted in microtubers with larger diameter (7.88 mm) and fresh weight (166.13 mg) (Table 1).

Table 1: Number of microtubers per plant and diameter and fresh weight of microtubers of Dioscorea sp. under different concentrations of sucrose and 1-naphthaleneacetic acid (NAA).

Sucrose ($g.L^{-1}$)	Number of microtubers per plant	Diameter of microtubers (mm)	Fresh weight of microtubers (mg)
30	1.34 b	6.15 c	64.88 c
60	1.60 a	7.04 b	124.75 b
90	1.73 a	7.88 a	166.13 a
NAA ($mg.L^{-1}$)			
0.0	1.5	6.8	119.2
1.0	1.6	6.9	119.8
2.0	1.6	7.2	119.7
4.0	1.5	7.0	115.7
Equation (Y)	ns	ns	ns
CV (%)	16.8	9.69	23.7

Means followed by the same lowercase letter in the column do not differ significantly according to Tukey's test at a 5% probability level. ns - not significant; CV - coefficient of variation.

Higher sucrose supply in the culture medium appears to favor signals for the induction and formation of yam microtubers [4], increasing their number, as well as their weight and diameter. For *Dioscorea alata*, increasing the concentration to 6% and 8% sucrose in the culture medium favored the production of a larger number of microtubers per explant [3]. Presumably, sucrose provides the necessary carbon and energy to initiate and promote the growth of microtubers in the culture medium [5]. Additionally, the increase in sucrose concentration enhances the osmolarity of the medium, leading to development of storage organs due to induced stress [23].

In trials with *Dioscorea nipponica*, increasing the sucrose concentration in the culture medium to 7% enhanced the production of microtubers with higher fresh weight [5]. Similar results were obtained with *Dioscorea deltoidea*, where increased sucrose supplementation resulted in increased number, fresh weight, and length of microtubers [8]. Therefore, sucrose appears to have a dual effect on *in vitro* tuberization: it provides a signal to initiate this process and a nutritive substrate for the development of microtubers [7].

Tuberization under different light spectra and plant growth regulators

The results of the experiment with different light spectra and the plant growth regulators NAA and KIN are presented in Tables 2 and 3. The different light spectra and KIN concentrations showed significant differences for the tuberization percentage variable. For the number of microtubers per plant variable, all three factors evaluated (light spectra and concentrations of KIN and NAA) showed a significant effect. For the microtuber diameter variable, only the light spectra factor was significant.

More than 90% of the plants tuberized when exposed to blue- and red-light spectra, while under white light, an average of 60% exhibited tuberization (Table 2). A larger number of microtubers per plant was also achieved using blue- and red-light spectra (1.65 and 1.73, respectively), with the lowest mean observed under white light (1.33). The largest microtuber diameter (5.23 mm) was obtained under white light, not differing statistically from the diameter under blue light (5.02 mm) (Table 2).

Table 2: Tuberization percentage and number and diameter of microtubers of Dioscorea sp. as a function of the light spectrum, 1-naphthaleneacetic acid (NAA), and kinetin (KIN).

	Tuberization (%)	**Number of microtubers per plant**	**Diameter of microtubers (mm)**
Light spectrum			
White	63.3 b	1.33 b	5.23 a
Blue	90.5 a	1.65 a	5.02 ab
Red	95.5 a	1.73 a	4.51 b
Kinetin (mg.L^{-1})			
0.0	81.6 ab	1.43 b	5.06 a
1.0	91.1 a	1.58 ab	4.91 a
2.0	76.6 b	1.68 a	4.79 a
NAA (mg.L^{-1})			
0.0	86.2 a	1.67 a	5.02 a
1.0	80.0 a	1.46 b	4.82 a
CV (%)	25.18	24.35	22.09

Means followed by the same lowercase letter in the column do not differ significantly according to Tukey's test at a 5% probability level. CV - coefficient of variation.

Blue- and red-light spectra have been studied due to their easy absorption by plants and have shown different responses, depending on the species [24]. One of these responses is the induction of tuberization, corroborating what was observed in this study, where blue- and red-light spectra promoted greater tuberization. The effect of these light spectra was assessed for potatoes (*Solanum tuberosum* L.), revealing that blue light promoted early formation and an increase in the number of microtubers per plant [25], while extreme red light led to accelerated tuberization initiation [12]. Additionally, in potatoes, the combination of red and blue LED lights in a 3:1 ratio favored *in vitro* tuberization, increasing the number of microtubers produced [26].

A white-light source resulted in a lower tuberization percentage and fewer microtubers per plant. However, they exhibited a larger diameter (5.23 mm). The use of blue light provided a mean microtuber diameter statistically equal to that of white light, while also promoting a higher tuberization percentage and larger number of microtubers formed. Meanwhile, red light, which showed the highest tuberization and largest number of microtubers, exhibited a smaller mean diameter (4.51 mm) (Table 2). Producing larger microtubers can be crucial when using them as propagative material, as their increased reserves lead to more vigorous plant growth [1]. On the other hand, red light may result in a greater total quantity of microtubers produced, increasing the overall yield.

This can be explained by the fact that red light is associated with stem elongation, potentially causing photosynthetic products to accumulate primarily in the above-ground part of the plant

[27]. In potatoes (*Solanum tuberosum* L.), extreme red light brought about an increase in the number of tubers, favoring overall yield per plant [9].

The concentration of 1.0 mg.L^{-1} KIN resulted in a higher tuberization percentage (91.1%), which was statistically similar to the treatment without KIN (81.6%) (Table 2). Supplementing the culture medium with 2.0 mg.L^{-1} KIN resulted in a larger number of microtubers per plant (1.68), which was statistically similar to the result observed for the 1.0 mg.L^{-1} concentration (1.58). The KIN concentrations evaluated did not show statistical differences in microtuber diameter (Table 2).

The use of KIN in the culture medium resulted in an increase in the number of microtubers formed. A study on factors affecting *in vitro* potato tuberization showed that KIN acted on tuber initiation when using a concentration of 2.5 mg.L^{-1} in a medium with a high sucrose content (90 g.L^{-1}) [4]. It was also shown that KIN primarily leads to an increase in the number of tubers due to its effect on cell elongation [7].

The culture medium without the addition of NAA provided the highest number of microtubers per plant (1.67), while for tuberization percentage and microtuber diameter, the different concentrations of NAA did not result in statistical differences (Table 2). NAA has been reported as an auxin that may stimulate cell division, leading to increased cell volume and a larger number of microtubers [28]. However, the response to tuberization varies depending on the hormonal concentration applied to the culture medium [29]. In the case of this experiment, the NAA concentrations used did not cause a response for tuberization induction.

There was a triple interaction among the factors of light spectra, NAA concentrations, and KIN concentrations for the fresh weight of microtubers (Table 3). Higher means were observed for white- and blue-light spectra (320.4 and 276.9 mg, respectively) without the addition of KIN (0.0 mg.L^{-1}) and with 1.0 mg.L^{-1} NAA. The means of fresh microtuber weight were statistically equal for the three light spectra without the addition of NAA (0.0 mg.L^{-1}) and KIN (0.0 mg.L^{-1}) in the culture medium. The use of blue light provided a higher mean compared to red light and was statistically similar to white light when using 1.0 mg.L^{-1} KIN and 0.0 mg.L^{-1} NAA (161.8 - white and 218.8 mg - blue) or 1.0 mg.L^{-1} NAA (150.4 - white and 276.9 mg - blue) were used. In the absence of NAA, red light was superior to blue and statistically equal to white light at a concentration of 2.0 mg.L^{-1} KIN (185.4 - red and 164.8 mg - white). Additionally, the use of 1.0 mg.L^{-1} NAA and 2.0 mg.L^{-1} KIN provided a higher mean when blue light was used (189.1 mg - blue).

The absence of NAA and KIN (0.0 mg.L^{-1}) resulted in higher mean fresh weight values with the use of red light (222.1 mg); and with 0.0 mg.L^{-1} NAA and 2.0 mg.L^{-1} KIN, higher mean fresh weight was observed with white (164.8 mg) and red light (185.4 mg) (Table 3). The NAA concentrations evaluated were statistically equal for the assessed light spectra when using 1.0 mg.L^{-1} KIN.

Considering the KIN concentrations, with the use of 0.0 mg.L^{-1} NAA and blue light, higher mean fresh weight values were observed for 0.0 mg.L^{-1} and 1.0 mg.L^{-1} KIN (192.2 and 218.8 mg, respectively). For the 0.0 mg.L^{-1} KIN concentration with 1.0 mg.L^{-1} NAA concentration, a higher mean was observed for white light (320.4 mg) (Table 3). The different KIN concentrations were statistically equal when using white light and 0.0 mg.L^{-1} NAA, as well as red light and 1.0 mg.L^{-1} NAA. The 0.0 mg.L^{-1} KIN concentration was superior to 2.0 mg.L^{-1} KIN and statistically equal to 1.0 mg.L^{-1} KIN when using blue light and 1.0 mg.L^{-1} NAA. The 0.0 mg.L^{-1} KIN concentration was superior to 1.0 mg.L^{-1} KIN and statistically equal to 2.0 mg.L^{-1} KIN when using red light and 0.0 mg.L^{-1} NAA (Table 3).

Table 3: Fresh weight of microtubers (mg) Dioscorea sp. as a function of light spectra and different concentrations of 1-naphthaleneacetic acid (NAA) and kinetin (KIN).

Spectrum light	NAA (mg.L^{-1})	
	0.0	**1.0**
	Kinetin (0.0 mg.L^{-1})	
White	158.1 a B α	320.4 a A α
Blue	192.2 a B α	276.9 a A α
Red	222.1 a A α	127.8 b B α
	Kinetin (1.0 mg.L^{-1})	
White	161.8 ab A α	150.4 ab A β
Blue	218.8 a A α	228.1 a A αβ
Red	110.1 b A β	147.5 b A α
	Kinetin (2.0 mg.L^{-1})	
White	164.8 ab A α	89.9 b B β
Blue	106.8 b B β	189.1 a A β
Red	185.4 a A αβ	102.7 b B α
CV (%)	29.52	

Means followed by the same lowercase letter within the column, uppercase letter within the row, and Greek letter among kinetin concentrations do not differ significantly according to Tukey's test at a 5% probability level. CV - coefficient of variation.

Factors such as light spectrum, cytokinins, and auxins are known to influence the tuberization process [1]. In this study, these factors had a combined effect on the fresh weight of the microtubers variable. There was no significant difference in fresh weight among light spectra without the addition of NAA and KIN. In this case, the use of plant growth regulators seems to have been important for increasing the weight of the microtubers, similar to findings in *Dioscorea alata*. For this species, the use of plant growth regulators like NAA in the culture medium stimulated microtuber growth, while cytokinins such as KIN, BA, and zeatin produced microtubers with lower fresh weights [30]. This might be due to the roles of these regulators, where KIN allows cell division, while NAA regulates organ growth [8]. In the *in vitro* cultivation of cassava, the synergism between BA and NAA was favorable for the growth of tuberous roots when concentrations of 0.5 mg.L^{-1} of BA and 0.5 mg.L^{-1} NAA were used together in the culture medium [31]. The fresh weight of microtubers in *Dioscorea deltoidea* increased with high concentrations up to 2.0 mg.L^{-1} NAA and with 1.0 mg.L^{-1} of the cytokinin BA [5].

Higher mean fresh weight values of microtubers were observed in white or blue light with the addition of 1.0 mg.L^{-1} NAA and without the presence of KIN. Both blue- and white-light spectra influence the growth and increase the fresh weight of potato microtubers [11, 26]. Blue light, in turn, increases sugar and starch contents during the tuberization process, favoring biomass accumulation in microtubers [32].

The use of 90 g.L^{-1} of sucrose, 1 mg.L^{-1} KIN, 1 mg.L^{-1} NAA, and the blue-light spectrum appears to favor the production of yam microtubers. Hormonal changes are observed during the induction and initiation of tuberization. Auxin contributes to tuber enlargement, while cytokinin influences the number of tubers produced, with the response to these hormones being dependent on the sucrose concentration in the culture medium. After induction, an increase in photosynthesis and carbohydrate transport to the developing tubers is noted. Therefore, increasing the supply of sucrose is necessary for starch biosynthesis and subsequent tuber growth [1]. Blue light is used by stomata as a signal to open, a sensory response that allows CO_2 to enter the leaf. It is believed that after the perception of stimuli by the phytochrome present in the leaves, the signal is sent through plant hormones. This signal initiates cell division, cell expansion, and orientation of cell growth, leading to the formation of a new organ [33].

Conventional production of yam seedlings is hindered by a lack of seed tubers with adequate phytosanitary quality and uniform sprouting. In this context, the aim of *in vitro* tuberization of species from the *Dioscorea* genus is to produce disease-free planting material with uniform sprouting, intended to replace conventionally used materials. Our results provide a foundation for

further research on the production of yam microtubers, highlighting the need for additional studies to optimize the protocol, increase the number and fresh weight of microtubers, and assess their sprouting capacity and viability for use by farmers in commercial yam cultivation.

4. CONCLUSION

Increasing the sucrose concentration to 90 $g.L^{-1}$ in the culture medium enhances *in vitro* tuberization of yam.

The blue-light spectrum and varying concentrations of kinetin and 1-naphthaleneacetic acid influence the induction and yield of microtubers.

The MS culture medium supplemented with 90 $g.L^{-1}$ of sucrose, 1 $mg.L^{-1}$ of kinetin, and 1 $mg.L^{-1}$ of 1-naphthaleneacetic acid, combined with cultivation under a blue-light spectrum, promotes greater yam microtuber production.

5. ACKNOWLEDGMENTS

The authors thank the Brazilian Federal Agencies: *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), Brazil; *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES – Finance code 001), Brazil; and *Financiadora de Estudos e Projetos* (FINEP), Brazil, for financial support.

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