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IN VITRO EVOLUTION OF SOME SWEET POTATO (*IPOMOEA BATATAS* [L.] LAM.) GENOTYPES UNDER THE INFLUENCE OF DIFFERENT PLANT GROWTH REGULATORS (AUXINS AND CYTOKININS)

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Abstract

A study was initiated with the aim of identifying an effective nutrient medium formulation capable of stimulating the growth and development of microplants. The in vitro development of five sweet potato genotypes (DK 19/1, DK 19/2, DCh 19/3, DK 19/4, and DK 19/5) was monitored. One month after culture initiation, the sweet potato microplants were evaluated for shoot length, number of leaves, and root length. Six nutrient media were formulated and compared with the control (Murashige-Skoog - without phytohormones). Regarding shoot length, number of leaves, and root length, the media variants V1 (MS+BAP 0.5 mg/L + IAA 0.5 mg/L), V4 (MS+KIN 0.5 mg/L+IAA 0.5 mg/L), and V0 (MS without phytohormones) showed the most notable effects. Media variants V6 (MS+KIN 0.5 mg/L+NAA 0.5 mg/L+IBA 1 mg/L) and V3 (MS+BAP 0.5 mg/L+NAA 0.5 mg/L+IBA 1 mg/L) had a positive influence on the number of roots. The study highlighted BAP 0.5 mg/L and IAA 0.5 mg/L, positively influencing the in vitro development of microplants across all five sweet potato genotypes.

Key words: *auxin, cytokinin, genotype, plant tissue culture, sweet potato.*

INTRODUCTION

Ipomoea batatas (L.) Lam., commonly known as sweet potato, is a resilient, nutritious, and versatile crop traditionally propagated using conventional methods. However, these methods have limitations, which has led to the adoption of micropropagation as an efficient alternative for producing healthy, cost-effective plantlets in a shorter time (Adu Donyina et al., 2025; Neja and Tileye, 2020).

Plant tissue culture has emerged as a powerful technique, offering the potential not only for rapid, clonal, and large-scale propagation of plant species needed in plantation programs, but also for the conservation of important and rare species. This technique has been widely applied across horticulture, plantation, and forestry sectors, significantly contributing to the enhanced production of high-quality planting material for the market (Siddique et al., 2015).

Plant growth regulators are chemical substances that play a vital role in the growth and development of plants (Singh, 2021; Zhang et al., 2022; Woodward and Bartel, 2005). They act as chemical messengers that facilitate cell to cell communication to induce the growth and differentiation of plant cells, tissues, and organs (Feraru and Friml, 2008; Singh, 2021). Under *in vitro* conditions, plants can't synthesize hormones for their growth and development, and that's why exogenous hormones are required (Singh, 2021; Gerdakaneh et al., 2020; Bernula et al., 2020).

Auxins are considered the most important hormones because they play a role in all physiological processes of plants. They induce the formation of root buds, contribute to cell division, and are involved in tropic responses. Cytokinins influence cell division, thereby impacting plant growth, and they also promote the development of lateral buds while delaying the aging of organs and plant tissues (Sosnowski et al., 2023). Auxins are a growth-promoting substance that is involved in the shoot and root tips and promotes cell division, stem and root growth. In tissue culture conditions, auxins are used to induce root development (Falasca and

Altamura, 2003; Singh, 2021; El-Sherif, 2018). Sometimes, a high ratio of auxin to cytokinin is required for proper organ development in some species (Singh, 2021). The auxin response is concentration-dependent in most tissues, with different tissues exhibiting distinct responses to varying amounts of exogenous auxin (Thimann, 1937 cited by Zhang et al., 2022). Cytokinins play a role in the transport and accumulation of photosynthetic products and in regulating the activity of other enzymes, and they also have a significant impact on physiological and biochemical processes (Wang et al., 2022 cited by Sosnowski et al., 2023).

Auxins are produced by plants in the apical meristems of shoots, as well as in young leaves, seeds, and fruits. They promote the elongation of shoots and initiate the formation of adventitious and lateral roots. Cytokinins, on the other hand, are synthesized in root tips and in unripe fruits and seeds. These hormones stimulate the growth of lateral shoots and also promote cell division (Sosnowski et al., 2023).

This study aimed to identify an auxin-cytokinin combination that promotes the growth and development of sweet potato microplants, supporting the optimization of *in vitro* cultivation methods.

MATERIALS AND METHODS

Biological Material

Five sweet potato genotypes were chosen for this study: DK 19/1 (white flesh); DK 19/2 (yellowish-white flesh); DCh 19/3 (purple flesh); DK 19/4 (yellow flesh); DK 19/5 (pale yellowish flesh with pink streaks). These sweet potato genotypes belong to the Research and Development Station for Plant Culture on Sands Dăbuleni, Dolj County, Romania.



Figure 1. Source of explants for initiating *in vitro* cultures

To initiate *in vitro* cultures, sweet potato shoots obtained in the greenhouse were used as a source of nodal explants (Figure 1).

The *in vitro* regenerated microshoots (1.5-2 cm long) from these explants constituted the biological material for the experiment, which aimed to identify an optimal nutrient medium recipe to stimulate the *in vitro* growth and development of sweet potato microplants.

The experiment was conducted at the National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania, Research Laboratory for Plant Tissue Culture.

Media and culture conditions

The aim was to identify an optimal culture medium formulation that would produce well-developed sweet potato plantlets, characterized by larger leaves and a vigorous root system.

For this experiment, six artificial media formulations were designed as substrates for the *in vitro* cultivation of sweet potato explants and were compared with a control variant (V0), represented by the Murashige-Skoog Murashige & Skoog, 1962) medium without added phytohormones (Table 1).

The six medium variants were based on the MS formulation supplemented with a cytokinin (6-benzylaminopurine or kinetin) and auxins (either indole-3-butyric acid at different concentrations, or a combination of two other auxins - namely α-naphthalene acetic acid and indole-3- acetic acid).

Table 1. Composition of medium variants for *in vitro* cultivation of sweet potato

V0 (Control)	MS hormone-free
V1	MS + BAP 0.5 mg/l + IAA 0.5 mg/l
V2	MS + BAP 0.5 mg/l + IAA 1 mg/l
V3	MS + BAP 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l
V4	MS + KIN 0.5 mg/l + IAA 0.5 mg/l
V5	MS + KIN 0.5 mg/l + IAA 1 mg/l
V6	MS + KIN 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l

MS - Murashige-Skoog; BAP - 6-benzylaminopurine; KIN - kinetin; IAA - indole-3-acetic acid; NAA - α-naphthalene acetic acid; IBA - indole-3-butyric acid.

Sucrose 20 g/l as a carbon source and agar 9 g/l were added to all medium variants. Prior to autoclaving, the pH of the medium was adjusted to 5.7. The nutrient medium was dispensed into glass test tubes (150 mm in height; 20 mm in diameter), previously sterilized in a drying oven for 2 hours at 180°C. After sealing the tubes with aluminum foil, the culture medium was sterilized in an autoclave at 120°C for 20 minutes.

The cultures were incubated in a growth room under artificial lighting conditions provided by neutral-white LED tubes (luminous flux of 2300 lm; 2000 lux light intensity) with a controlled temperature (21 ± 2°C) and photoperiod (16 hours light/8 hours dark). After one month from the initiation of the *in vitro* cultures, the sweet potato microplants were evaluated for shoot length (cm), number of leaves, number of roots, and root length (cm), depending on the genotype and the culture medium variant.

RESULTS AND DISCUSSIONS

Influence of growth regulators on the length of sweet potato microshoots

Regarding this parameter, the addition of 0.5 mg/l BAP to the nutrient medium along with 0.5 mg/l IAA (V1) led to the best result (1.98 cm), a favorable aspect in the micropropagation process, being the only variant that exceeded the control (Figure 2). On the other five medium variants, insignificant differences were recorded compared with the control (V0).

Comparing the evolution of the genotypes regarding microshoot length (Table 2), genotypes DCh 19/3 (2.56 cm) and DK 19/1 (1.78 cm) are distinguished, which recorded very significant differences (1.44 cm and 0.65 cm, respectively) compared to the control.

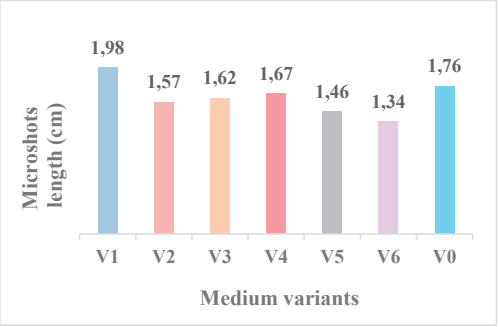

Figure 2. Effect of MS and plant growth regulators on sweet potato microshoot length after 1 month of culture

Table 2. Behavior of sweet potato genotypes regarding microshoots length

Genotype	Microshoots length (cm)		
	Mean	Diff.	Sign.
DK 19/1	1.78	0.65	***
DK 19/2	1.33	0.20	ns
DCh 19/3	2.56	1.44	***
DK 19/5	1.34	0.21	ns
DK 19/4 (Control)	1.13	-	-

LSD 5%: 0.33; LSD 1%: 0.43; LSD 0.1%: 0.56

After 1 month from the initiation of the cultures, the highest value of microshoot length was recorded in the DCh 19/3 genotype (3.23 cm) using the hormonal combination BAP (0.5 mg/l) + IAA (0.5 mg/l) in the culture medium (Table 3). In fact, this genotype also obtained the best results on all medium variants, except for the control variant (V0), where it was surpassed by the DK 19/1 genotype. Regarding the microshoot length parameter, the DCh 19/3 and DK 19/1 genotypes recorded distinctly significant and very significant differences compared to the DK 19/4 (control).

Table 3. Microshoot length of sweet potato genotypes, depending on the composition of the culture medium (after 1 month from culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.
DK 19/1	1.45	-0.43	1.55	0.08	1.60	0.58	2.15	1.23**
DK 19/2	1.63	-0.25	1.60	0.13	1.30	0.28	0.63	-0.30
DCh 19/3	3.23	1.35**	2.20	0.73	2.68	1.65***	3.13	2.20***
DK 19/5	1.73	-0.15	1.00	-0.48	1.48	0.45	1.53	0.60
DK 19/4 (Control)	1.88	-	1.48	-	1.03	-	0.93	-
Genotype	Medium variants							
	V5		V6		V0			
	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.		
DK 19/1	1.80	1.20**	1.53	0.83	2.35	1.08*		
DK 19/2	1.45	0.85	1.33	0.63	1.38	0.10		
DCh 19/3	2.60	2.00***	2.13	1.43**	1.98	0.70		
DK 19/5	0.83	0.23	1.03	0.33	1.80	0.53		
DK 19/4 (Control)	0.60	-	0.70	-	1.28	-		

LSD 5%: 0.86; LSD 1%: 1.14; LSD 0.1%: 1.47

The minimum value of microshoot length was determined in the DK 19/4 genotype (0.60 cm) by using the hormonaL combination KIN (0.5 mg/l) + IAA (1 mg/l). In the DK 19/4, DK 19/5 and DK 19/2 genotypes the microshoots have the Lowest length in most medium variants, except for those that had the hormonal combination BAP (0.5 mg/l) + IAA (0.5 mg/l and 1 mg/l).

Influence of growth regulators on the leaves number

The average values recorded for this parameter varied between 5.50 on V4 and 4.00 on V2 (Figure 3).

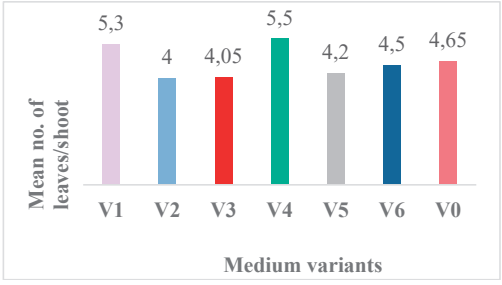

Figure 3. Effect of MS and plant growth regulators on the number of leaves/shoot after 1 month of culture

It can be observed that, irrespective of the cytokinin used (BAP or KIN), when the auxin concentration was higher (IAA 1 mg/L) or when a combination of two auxins was applied (NAA 0.5 mg/L + IBA 1 mg/L), the number of leaves per plantlet was lower.

The hormonal combination consisting of KIN 0.5 mg/l and IAA 0.5 mg/l had a positive influence on the number of leaves, followed by the variants V1 and V0.

Regarding the influence of the genotype on the number of leaves/shoot (Table 4), the genotypes DK 19/1 (6.64), DK 19/5 (6.04) and DCh 19/3 (4.00) were noted. These were statistically different from the control, recording very significant positive differences (3.82 and 3.21), respectively significant (1.18).

Table 4. Behavior of sweet potato genotypes regarding number of leaves/shoot

Genotype	Number of leaves/shoot		
	Mean	Diff.	Sign.
DK 19/1	6.64	3.82	***
DK 19/2	3.50	0.68	ns
DCh 19/3	4.00	1.18	*
DK 19/5	6.04	3.21	***
DK 19/4 (Control)	2.82	-	-

LSD 5%: 0.93; LSD 1%: 1.24; LSD 0.1%:1.60

Under the combined influence of genotype and culture medium composition, in terms of leaf number (Table 5), genotypes DK 19/1 and DK 19/5 were prominent across all medium variant.

Table 5. Behavior of sweet potato genotypes regarding the number of leaves/shoots, depending on the composition of the culture medium (1 month after culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.
DK 19/1	6.00	1.25	6.25	4.25***	4.75	2.75*	8.75	5.50***
DK 19/2	4.25	-0.50	3.75	1.75	3.00	1.00	2.75	-0.50
DCh 19/3	4.25	-0.50	3.50	1.50	3.75	1.75	5.00	1.75
DK 19/5	7.25	2.50*	4.50	2.50*	6.75	4.75***	7.75	4.50***
DK 19/4 (Control)	4.75	-	2.00	-	2.00	-	3.25	-

Genotype	Medium variants					
	V5		V6		V0	
	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.
DK 19/1	4.75	1.50	8.50	7.25***	7.50	4.25***
DK 19/2	4.00	0.75	3.75	2.50*	3.00	-0.25
DCh 19/3	4.25	1.00	4.00	2.75*	3.25	0.00
DK 19/5	4.75	1.50	5.00	3.75**	6.25	3.00*
DK 19/4 (Control)	3.25	-	1.25	-	3.25	-

LSD 5%: 2.46; LSD 1%: 3.27; LSD 0.1%: 4.22

The highest number of leaves was observed in genotype DK 19/1 (8.75) on medium variant V4, while the lowest value was recorded for genotype DK 19/4 (1.25) on medium variant V6. For genotypes DK 19/1 and DK 19/5, the hormonal treatment consisting of KIN 0.5 mg/L + IAA 0.5 mg/L had a positive effect on leaf number, resulting in the best outcomes (8.75 and 7.75, respectively). In contrast, genotypes DK 19/4 and DK 19/2 produced fewer leaves across all tested medium variants.

Both the culture medium without added phytohormones (V0-control) and the medium variants V1 and V4, in which an auxin (IAA) at a lower concentration (0.5 mg/L) was added alongside a cytokinin (BAP or KIN), resulted in an average of more than 6 leaves per plantlet for genotypes DK 19/1 and DK 19/5.

Influence of growth regulators on the number of roots

Regarding the number of roots/shoots, the hormonal balance in the composition of the culture medium had a major influence. The results obtained in this study confirmed the role of auxins in inducing rhizogenesis (Fogaça and Fett-Neto, 2005; Kanungo et al., 2012; Singh, 2021; Shum and Dohm, 2003). The data presented in Table 6 highlight that medium variants containing a cytokinin (BAP or KIN) at 0.5 mg/L and two auxins (NAA 0.5 mg/L + IBA 1 mg/L) produced the best results in terms of root number.

Table 6. Effect of culture medium composition on the number of roots/shoots

Medium variants		Number of roots		
		Mean	Diff.	Sign.
V1	MS + BAP 0.5 mg/l + IAA 0.5 mg/l	5.65	2.05	ns
V2	MS + BAP 0.5 mg/l + IAA 1 mg/l	5.60	2.00	ns
V3	MS + BAP 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	7.05	3.45	**
V4	MS + KIN 0.5 mg/l + IAA 0.5 mg/l	3.75	0.15	ns
V5	MS + KIN 0.5 mg/l + IAA 1 mg/l	4.60	1.00	ns
V6	MS + KIN 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	8.25	4.65	***
V0	MS hormone-free (Control)	3.60	-	-

LSD 5%: 2.07; LSD 1%: 2.84; LSD 0.1%: 3.86

Sweet potato plantlets produced the highest number of roots on medium variants V6 (8.25) and V3 (7.05), showing highly significant (4.65) and significant (3.45) differences, respectively, compared to the control (medium without added phytohormones). On the other medium variants, where a hormonal combination consisting of a cytokinin (0.5 mg/L) and an auxin (0.5 mg/L or

1 mg/L IAA) was applied, differences relative to the hormone-free medium were positive but not statistically significant.

Regarding the response of genotypes in terms of the number of roots/shoot, the most affected was genotype DK 19/4, which recorded a value of 2.50. All other genotypes showed highly significant differences compared with the control, with an average of more than 6 roots per shoot (Table 7).

Table 7. Behavior of sweet potato genotypes regarding the number of roots/shoot

Genotype	Number of roots/shoot		
	Mean	Diff.	Sign.
DK 19/1	6.21	3.71	***
DK 19/2	6.18	3.68	***
DCh 19/3	6.36	3.86	***
DK 19/5	6.25	3.75	***
DK 19/4 (Control)	2.50	-	-

LSD 5%: 1.74; LSD 1%: 2.31; LSD 0.1%: 2.98

Under the combined influence of genotype and the culture medium composition, the best results were recorded for genotype DK 19/1 (13.00) on the medium supplemented with KIN 0.5 mg/L + NAA 0.5 mg/L + IBA 1 mg/L, and for genotype DK 19/3 (12.50) on the medium supplemented with BAP 0.5 mg/L + NAA 0.5 mg/L + IBA 1 mg/L (Table 8).

As regards the number of roots per shoot, the medium variant containing KIN 0.5 mg/L + NAA 0.5 mg/L + IBA 1 mg/L promoted root formation in all sweet potato genotypes, resulting in an average of at least 6 roots per plantlet. On this medium, even genotype DK 19/4 had an average root number greater than 6, whereas on the other medium variants this parameter did not exceed 3 roots per shoot (Table 8).

Table 8. Behavior of sweet potato genotypes regarding the number of roots/shoot, depending on the composition of the culture medium (1 month after culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.
DK 19/1	6.75	4.50	6.25	5.25*	4.75	1.75	4.75	3.25
DK 19/2	7.25	5.00*	8.50	7.50**	5.50	2.50	3.00	1.50
DCh 19/3	5.00	2.75	7.00	6.00*	12.50	9.50***	2.75	1.25
DK 19/5	7.00	4.75*	5.25	4.25	9.50	6.50**	6.75	5.25*
DK 19/4 (Control)	2.25	-	1.00	-	3.00	-	1.50	-

Genotype	Medium variants					
	V5		V6		V0	
	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.	"""Number of roots	Diff./ Sign.
DK 19/1	3.25	1.00	13.00	6.75**	4.75	3.50
DK 19/2	9.50	7.25**	6.00	-0.25	3.50	2.25
DCh 19/3	4.00	1.75	9.50	3.25	3.75	2.50
DK 19/5	4.00	1.75	6.50	0.25	4.75	3.50
DK 19/4 (Control)	2.25	-	6.25	-	1.25	-

LSD 5%: 4.60; LSD 1%: 6.11; LSD 0.1%: 7.89

Influence of growth regulators on root length

According to the results obtained (Table 9), regarding the composition of the culture medium, the variant in which no growth regulators were added (the control) determined the best results in terms of root length (6.51 cm). This was followed by the variants in which, along with cytokinin (BAP or KIN at a concentration of 0.5 mg/l), a lower concentration of auxin (0.5 mg/l IAA) was used. And on these two variants, the average root length exceeded 6 cm (6.10 cm on variant V1, respectively 6.03 cm on variant V4), but without statistically significant differences compared to the control.

Table 9. Effect of culture medium composition on root length/shoot

Medium variants		Root length (cm)		
		Mean	Diff.	Sign.
V1	MS + BAP 0.5 mg/l + IAA 0.5 mg/l	6.10	-0.40	ns
V2	MS + BAP 0.5 mg/l + IAA 1 mg/l	3.47	-3.04	oo
V3	MS + BAP 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	2.72	-3.79	oo
V4	MS + KIN 0.5 mg/l + IAA 0.5 mg/l	6.03	-0.47	ns
V5	MS + KIN 0.5 mg/l + IAA 1 mg/l	4.22	-2.29	o
V6	MS + KIN 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	2.19	-4.32	ooo
V0	MS hormone-free (Control)	6.51	-	-

LSD 5%: 2.16; LSD 1%: 2.96; LSD 0.1%: 4.03

Table 10. Behavior of sweet potato genotypes regarding root length, depending on the composition of the culture medium (1 month after culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.
DK 19/1	8.90	0.73	7.10	6.80**	4.30	3.20	8.18	3.78
DK 19/2	5.83	-2.35	4.73	4.43	2.70	1.60	4.93	0.53
DCh 19/3	1.40	-6.78^{oo}	2.83	2.53	2.20	1.10	5.53	1.13
DK 19/5	6.20	-1.98	2.38	2.08	3.30	2.20	7.13	2.73
DK 19/4 (Control)	8.18	-	0.30	-	1.10	-	4.40	-

Genotype	Medium variants					
	V5		V6		V0	
	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.
DK 19/1	6.85	3.85	4.30	3.60	12.33	8.63***
DK 19/2	4.30	1.30	2.50	1.80	5.10	1.40
DCh 19/3	3.33	0.33	0.75	0.05	3.63	-0.07
DK 19/5	3.60	0.60	2.68	1.98	7.78	4.08
DK 19/4 (Control)	3.00	-	0.70	-	3.70	-

LSD 5%: 4.73; LSD 1%: 6.27; LSD 0.1%: 8.10

Analyzing the data presented in Table 9, it can also be observed that the addition to the culture medium of the auxin combination NAA 0.5 mg/L + IBA 1 mg/L together with kinetin (0.5 mg/L) had a negative influence on root length, with the minimum value (2.19 cm) recorded for this medium variant and a very significant negative difference (-4.32 cm) compared with the control (V0). Although, on this medium variant (V6) the sweet potato genotypes obtained the best results in terms of the number of roots, the root length was strongly affected.

From the analysis of sweet potato genotype performance, genotype DK 19/1 stands out, having recorded the greatest root length (7.42 cm) and showing a highly significant difference (4.37 cm) compared with the control (Table 11). It was followed by genotypes DK 19/5 (4.72 cm) and DK 19/2 (4.30 cm), although the differences between them were not statistically significant.

Table 11. Behavior of sweet potato genotypes regarding root length

Genotype	Root length (cm)		
	Mean	Diff.	Sign.
DK 19/1	7.42	4.37	***
DK 19/2	4.30	1.24	ns
DCh 19/3	2.81	-0.25	ns
DK 19/5	4.72	1.67	ns
DK 19/4 (Control)	3.05	-	-

LSD 5%: 1.79; LSD 1%: 2.37; LSD 0.1%: 3.06

The combined influence of genotype and culture-medium composition on root length (Table 10) highlights the V1 medium variant, in which the hormonal treatment consisting of 0.5 mg/l BAP + 0.5 mg/l IAA exerted a positive effect on root elongation in most genotypes, with the exception of genotype DCh 19/3 (1.40 cm), which showed a highly significant negative difference (–6.78 cm) compared with the control.

The hormone-free medium variant (V0) is also noteworthy, where the minimum root length recorded was 3.63 cm (DCh 19/3) and the maximum was 12.33 cm (DK 19/1). Genotype DK 19/1 stood out with respect to root length, achieving the highest values regardless of the culture-medium composition.

A negative effect on root elongation was observed in the V6 medium variant, where the minimum value was recorded in genotype DK 19/4 (0.70 cm), while the highest value, 4.30 cm, was obtained for genotype DK 19/1.

The use of BAP was more effective compared with KIN in terms of microshoot development, an aspect also observed in other studies (Tsemah et al., 2022; Koleva et al., 2012; Prabhakaran and Abraham, 2025).

In this study, the hormonal combination of a cytokinin (BAP or KIN) and IAA, in lower concentrations (0.5 mg/l), had a positive influence on leaf number. This aspect has also been observed in other research (Tsemah et al., 2022; Ali et al., 2015; Mayada and Seliem, 2025).

Regarding the length of microshoot roots, in our study the best results were obtained on the medium variant without the addition of phytohormones, followed by those with a low concentration of cytokinins or auxin. Inhibition of *in vitro* rooting due to the presence of cytokinins in the environment has also been observed by other researchers (Dobránszki et al., 2010; Dewir et al., 2016; Tahir et al., 2024; Luna et al., 2014). Also, as stated by Van Staden et al. (2008), the presence of cytokinins in high concentrations in the culture medium inhibits *in vitro* rooting, root growth, or delays the formation of roots. These results were previously demonstrated by Huetteman and Preece, who stated that cytokinins inhibit root formation, especially when used in shoot proliferation medium at high concentrations or for long periods of time.

CONCLUSIONS

In this study, seven medium variants and five sweet potato genotypes were tested with the aim of identifying a medium formulation that would promote the growth and development of microplants under controlled conditions. The *in vitro* performance of the five genotypes was assessed by measuring growth parameters such as shoot length, number of leaves/shoot, number of roots/shoot, and root length.

Regarding the influence of culture-medium composition on shoot length, the addition of 0.5 mg/l BAP together with 0.5 mg/l IAA (V1) led to the highest value observed (1.98 cm).

Concerning the average number of leaves per shoot, the hormonal combination of 0.5 mg/l KIN and 0.5 mg/l IAA had a positive effect on this parameter, followed by variants V1 and V0. When a higher auxin concentration was used (1 mg/l IAA) or a combination of two auxins (0.5 mg/l NAA + 1 mg/l IBA) was applied, the number of leaves per shoot was lower, regardless of the cytokinin used (BAP or KIN).

Regarding root length, the hormone-free medium variant produced the best results (6.51 cm). This was followed by variants in which a cytokinin (BAP or KIN at 0.5 mg/l) was combined with a lower concentration of auxin (0.5 mg/l IAA). Thus, the use of a cytokinin in combination with a lower concentration of an auxin in the culture medium, as well as the use of a hormone-free medium, led to the best outcomes in terms of shoot length, leaf number, and root length.

The hormonal balance in the culture medium had a major influence on the average number of roots per shoot. The results highlighted the medium variants containing a cytokinin (BAP or KIN at 0.5 mg/L) combined with two auxins (0.5 mg/L NAA + 1 mg/L IBA). Sweet potato microshoots produced the highest number of roots in medium variants V6 (8.25) and V3 (7.05), showing highly significant (4.65) and distinct significantly (3.45) differences compared with the control (medium without added phytohormones).

Among the seven culture-medium variants tested in this study, the hormonal combination of 0.5 mg/L BAP and 0.5 mg/L IAA (medium V1) was particularly effective, producing a positive response in all five sweet potato genotypes for every growth parameter measured. This medium consistently supported optimal *in vitro* development of the microplants. Research on culture-medium composition, particularly the presence of phytohormones, their combinations and the concentrations used, is of great importance for establishing an

effective *in vitro* cultivation protocol for sweet potato.

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