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In vitro multiplication of wild *Manihot* species with different naphthaleneacetic acid and benzylaminopurine concentrations

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ABSTRACT. *In vitro* multiplication is an important tissue culture technique that is capable of efficiently producing seedlings at any scale. It is a propagation method based on the aseptic culture of small propagules in a suitable culture medium to enable plant regeneration. Multiplication experiments conducted *in vitro* to set protocols adapted to wild *Manihot* species have used modified mineral salts and MS vitamins as basic culture medium. Here, 25 treatments based on combinations of the regulators benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) at 0, 0.025, 0.05, 0.075, and 0.1 mg L^{-1} were used for *in vitro* multiplication of three genotypes of wild *Manihot* species (*M. violaceae* Pohl Müll. Arg., *M. pseudoglaziovii* Pax & Hoff., and *M. flabellifolia* Pohl). Plant height and the number of 1 cm minicuttings, number of roots, shoots, green leaves and senescent leaves were recorded 120 days after explant inoculation. *M. violaceae* Pohl. Müll. Arg. and *M. flabellifolia* Pohl. presented favorable results with 0.05 and 0.025 mg L^{-1} NAA, respectively. Culture medium lacking NAA and BAP favored the *in vitro* growth of *M. pseudoglaziovii* Pax & Hoff.

Keywords: auxin; cytokinin; cassava; micropropagation; phytohormones.

Introduction

In vitro micropropagation is one of the tissue culture techniques that can be an alternative to conventional vegetative propagation. The technique comprises a set of propagation methods based on the aseptic culture of small propagules (cells, tissues, and organs) in an appropriate growth medium to favor plant regeneration (Carvalho, Silva, & Medeiros, 2006). This process demands tight regulation of environmental conditions, such as luminosity, photoperiod, temperature, and relative humidity (Wendling, Dutra, & Grossi, 2006). There can also be other requirements particular to the species to be grown.

In vitro micropropagation has many advantages compared to conventional vegetative propagation methods. These advantages include the establishment of a large number of healthy plants that present genetic homogeneity in a short time, propagation of plants that are often not regenerated through traditional methods, the need for less space, seasonal independence, and the possibility of growing pathogen-free plants (Kozai, Kubota, & Byoung, 1997).

Several factors such as explant selection, culture medium composition, and culture conditions contribute to successful micropropagation. The culture medium is particularly important as it goes beyond the mere supply of essential nutrients for plant survival. It also functions in explant support (due to the presence of solidifying substances), growth control, and plant material development (Carvalho & Vidal, 2003). Plant hormones are crucial in plant growth control and differentiation. Plants grown *in vitro* often cannot sufficiently synthesize solidifying substances and so they must be added to the culture medium. Phytoregulators, which are synthetic substances with effects similar to those of endogenous hormones, also regulate growth and are often used in tissue culture processes (Pasqual, Hoffmann, & Ramos, 1997).

Auxins, cytokinins, gibberellins, ethylene, and abscisic acid are traditional growth regulator groups. They (especially cytokinins and auxins) contribute to successful micropropagation (Rogalski, Guerra, & Silva,

2003). The interaction between these two regulator classes helps control the formation and development of aerial parts, bud, and callus (Caldas, Haridasan, & Ferreira, 1999). Among the auxins, 1-naphthaleneacetic acid (NAA) plays a significant role in plant development, mainly in stimulating shoot growth (Seyyedyousefi, Kaviani, & Dehkaei, 2013) and the initial rooting of explants established *in vitro* (Azhar, Ghani, & Yusuf, 2018). Conversely, 6-benzylaminopurine (BAP) is one of the most responsive cytokinins. It regulates cell division in plant shoots and promotes lateral bud growth (Grattapaglia & Machado, 1999). These two growth regulators are widely used in combination, and their interaction often favors plant multiplication and development *in vitro*. Cytokinins in the culture medium play a key role in inducing shoots, whereas auxins favor rhizogenesis (Kerbauy, 2004).

Wild *Manihot* species are important in breeding programs focused on genetically enhancing cultivated species. However, these wild species are often resistant to propagation processes based on any system. Unlike for the wild species, a protocol for *in vitro* multiplication has already been established for *Manihot esculenta* Crantz. The protocol has been adopted in crops for several purposes including increasing multiplication rates and maintaining the quality and uniformity of plant development (Souza et al., 2013).

The MS culture medium developed by Murashige and Skoog (1962) is the most popular medium for the *in vitro* propagation of several species, including *M. esculenta* Crantz. However, the composition of the medium developed for this species does not enable the effective *in vitro* multiplication of most wild *Manihot* species. Therefore, different growth regulator concentrations need to be assessed to improve explant development (Morais, Asmar, & Luz, 2014).

The basic medium used for *in vitro* multiplication often requires adjustments, such as changing its composition to meet the specificities of the target crop. This study investigated the use of MS medium supplemented with different concentrations of NAA and BAP to establish *in vitro* multiplication protocols suitable for three wild *Manihot* species.

Material and methods

The experiments were carried out at the Tissue Culture Laboratory (*Laboratório de Cultura de Tecidos*, LCT) of the Center for Advanced Biology (*Núcleo de Biologia Avançada*, NBA), Embrapa Cassava and Fruits, Cruz das Almas County, in the Brazilian state of Bahia.

Three experiments were conducted to individually investigate three wild *Manihot* species: *M. violaceae* (Pohl) Müll. Arg., *M. pseudoglaziovii* Pax & Hoff., and *M. flabellifolia* Pohl. The three species had been previously established at LCT.

Each experiment used a basic culture medium comprising minerals and MS vitamins (Murashige & Skoog, 1962). Different combinations of BAP and NAA were added to the media at the following concentrations: 0.025, 0.05, 0.075, and 0.1 mg L^{-1}. The pH was adjusted and maintained at 5.8 with NaOH or HCl (0.1 N) when necessary. Phytagel® (2.4 g L^{-1}) was used as the gelling agent. Ten milliliter aliquots of each type of medium were added to 20 test tubes (25 mm diameter and 150 mm height), which were subsequently capped, packed, and autoclaved at 121°C for 20 min.

The experiments were performed in a laminar flow chamber. The cassava plants were sectioned into minicuttings, approximately 1 cm in size, each containing one axillary bud. Each explant was placed in a test tube filled with culture medium, sealed with stretchable PVC film, and labeled. Cultures were placed in a growth room operating at 27 ± 1°C and a 16 hours photoperiod with 30 µmol m^{-2} s^{-1} light intensity. The conditions were favorable for rapid plant development.

At 120 days after explant inoculation, plant height (PH; cm), number of 1 cm minicuttings (NM), number of roots (NR), number of shoots (NS), number of green leaves (NGL), and number of senescent leaves (NSL) were recorded.

The study utilized a completely randomized experimental design, with 5 × 5 factorial arrangement and 20 repetitions. Each repetition was represented by a plant grown in a test tube. Each combination of BAP and NAA in multiplication experiments conducted *in vitro* was individually applied to the three selected genotypes. This process resulted in the three experiments. Data collected during the evaluations were subjected to analysis of variance based on the F test. Polynomial regression models were adjusted for phytoregulatory means. Statistical analyses were carried out using SISVAR statistical software (Ferreira, 2011). The NM, NR, NS, NGL, and NSL values were transformed into $\sqrt{x} + 0.5$ to meet assumptions based on the variance analysis.

Results and discussion

Given the pronounced genetic variability in the genus *Manihot*, and the lack of information about the behavior of wild cassava species *in vitro*, three experiments were performed as described above. Results have been presented and discussed below.

Experiment I: Effect of different BAP and NAA concentrations on the micropropagation of *M. violaceae* (Pohl) Müll. Arg.

Table 1 shows the highly significant effect ($p < 0.01$) of the BAP × NAA interaction on PH, NM, NS, and NSL. BAP alone had a significant effect ($p < 0.05$) only on NGL. Variable NR values were not significantly affected by the concentrations of BAP and NAA.

Table 1. Analysis of variance applied to plant height (PH, cm), number of minicuttings (NM), number of roots (NR), number of shoot (NS), number of green leaves (NGL), and number of senescent leaves (NSL) in *Manihot violaceae* (Pohl) Müll. Arg. using different BAP and NAA concentrations.

SV	DF	MS					
		PH	NM	NR	NS	NGL	NSL
BAP	4	60.27**	5.07**	1.10ns	0.28**	0.74*	4.78**
NAA	4	82.95**	0.83**	0.73ns	0.31**	0.46ns	0.33ns
BAP × NAA	16	98.08**	0.71**	0.59ns	0.42**	0.25ns	0.88**
Error	315	13.10	0.19	1.10	0.08	0.27	0.34
CV (%)		25.40	12.70	30.06	18.63	21.96	18.55
Mean		14.25	11.53	6.66	1.86	5.39	9.73

ns = non-significant; ** and * significant at 1 and 5%, respectively, based on the F test.

These results were similar to those reported by Rodrigues, Santos, Takane, & Carvalho (2017). The authors found no evidence of a significant effect of the interaction between BAP and NAA on *Cattleya labiata* roots *in vitro*.

The *in vitro* development of *M. violaceae* (Pohl) Müll. Arg varied markedly, as evidenced by coefficient of variation (CVs) recorded for NM and NR, which ranged from 12.70 to 30.06%, respectively (Table 1). These CV values were low as compared to the values ranging from 18.9 to 82.10% for *Physalis peruviana* L. *in vitro* (Rezende, Pasqual, Carvalho, Pereira, & Villa, 2018).

Werner, Motta, Martins, Lima and Schmildt (2012) used a general analysis of different variables in an investigation of plant tissue culture. The obtained mean CVs indicated satisfactory experimental accuracy. It is likely that the CVs resulted from genetic variability in the investigated wild species and from the difficulty in establishing the same conditions for each minicutting that was used, mainly from a physiological perspective.

Polynomial regression models are presented in Table 2. Adjustments were made in the 1st and 2nd degree models, whose coefficients of determination (R^2) ranged from 52.16 to 98.95%.

Comparison between NAA and BAP concentrations revealed that the interaction of 0.1 mg L^{-1} of both growth regulators increased shoot formation and led to the highest mean value of 2.05 (Table 2). This result was similar to that found by Kaviani, Sedaghathoor, Safari Motlagh and Rouhi (2019), who obtained the best NS values for the interaction between BAP and NAA for *Aglaonema widuri*.

BAP at 0.04 mg L^{-1} increased NGL (2.45). The use of 0.05 mg L^{-1} NAA and 0.1 mg L^{-1} BAP produced the highest NSL value (12.51). The findings highlighted the important influence of plant aging, which restricts *in vitro* micropropagation and conservation of many plant species.

According to values estimated from the regression equations (Table 2), the highest mean PH (17.38 cm) resulted from the combination of 0.025 mg L^{-1} BAP and 0.04 mg L^{-1} NAA.

The highest mean NM (17.57) was observed using 0.075 mg L^{-1} BAP and the absence of NAA. Oliveira-Cauduro, Lopes, Bona, Alcantara and Biasi (2016) found that the use of BAP in the culture medium was beneficial for the efficient *in vitro* multiplication of pineapples. Silva et al. (2017) recorded the highest shoot values at 1.0 and 1.5 mg L^{-1} BAP for *Rosa* sp. Porfírio, Titon, Castro, Pereira and Knegt (2019) reported that cytokinins concentration-dependently promoted the development of the aerial part of plants, with the effects varying with plant species.

Table 2. Polynomial regression equations, coefficients of determination (R^2), optimal dose, and estimated values for plant height, number of minicuttings, number of shoots, number of green leaves, and number of senescent leaves in *Manihot violaceae* (Pohl) Müll. Arg. using different BAP and NAA concentrations.

Interactions	Doses (mg L^{-1})	Equation	R^2 (%)	Optimal dose	Estimated values
		Plant height (cm)			
BAP (NAA)	0	$\hat{y}^{**} = 1818.3x^2 - 165.2x + 17.265$	52.16	0.05	13.51
BAP (NAA)	0.075	$\hat{y}^{**} = -2157.4x^2 + 232.62x + 10.309$	98.95	0.05	16.58
NAA (BAP)	0	$\hat{y}^{**} = 2524.4x^2 - 274.33x + 16.513$	83.84	0.05	9.06
NAA (BAP)	0.025	$\hat{y}^{**} = -1680.3x^2 + 118.69x + 15.289$	98.12	0.04	17.38
NAA (BAP)	0.05	$\hat{y}^{**} = 52.967x + 10.713$	70.58	0.1	16.01
NAA (BAP)	0.1	$\hat{y}^{**} = 1260x^2 - 172.1x + 18.205$	96.66	0.07	12.33
		Number of minicuttings			
BAP (NAA)	0.025	$\hat{y}^{**} = -1344.3x^2 + 198.22x + 6.6475$	62.93	0.07	13.95
BAP (NAA)	0.05	$\hat{y}^{**} = -1390.5x^2 + 180.38x + 6.9619$	81.94	0.06	12.81
BAP (NAA)	0.075	$\hat{y}^{**} = -1100.2x^2 + 149.29x + 7.3485$	90.57	0.07	12.41
NAA (BAP)	0	$\hat{y}^{**} = 1684.5x^2 - 174.38x + 10.826$	93.81	0.05	6.31
NAA (BAP)	0.075	$\hat{y}^{**} = -59.576x + 17.574$	78.85	0	17.57
NAA (BAP)	0.1	$\hat{y}^{*} = -19.143x + 12.554$	57.49	0	12.55
		Number of shoots			
BAP (NAA)	0	$\hat{y}^{**} = -617.3x^2 + 64.036x + 0.9567$	82.14	0.05	2.62
BAP (NAA)	0.05	$\hat{y}^{*} = 215.09x^2 - 18.114x + 1.736$	53.57	0.04	1.35
NAA (BAP)	0	$\hat{y}^{**} = 8.3197x + 1.3292$	63.81	0.1	2.16
NAA (BAP)	0.025	$\hat{y}^{*} = 235.54x^2 - 12.926x + 1.8202$	76.58	0.03	1.64
NAA (BAP)	0.05	$\hat{y}^{**} = 655.24x^2 - 74.19x + 2.999$	96.46	0.06	0.90
NAA (BAP)	0.1	$\hat{y}^{*} = 6.5357x + 1.3958$	64.11	0.1	2.05
		Number of green leaves			
BAP		$\hat{y}^{*} = -59.809x^2 + 5.0861x + 2.3407$	61.13	0.04	2.45
		Number of senescent leaves			
BAP (NAA)	0.025	$\hat{y}^{**} = -1666.7x^2 + 227.09x + 4.5448$	90.57	0.07	12.28
BAP (NAA)	0.05	$\hat{y}^{*} = 50.325x + 7.5064$	81.07	0.1	12.51
NAA (BAP)	0	$\hat{y}^{**} = 1036.6x^2 - 86.048x + 7.3471$	81.63	0.04	5.56

** and * significant at 1 and 5% probability level, respectively, based on ANOVA carried out in the F test.

Experiment II: Effect of different BAP and NAA concentrations on the micropropagation of *M. pseudoglaziovii* Pax & Hoff.

The NR, NS, and NSL values significantly increased ($p < 0.01$) in the presence of BAP alone (Table 3).

Table 3. Summary of the analysis of variance applied to plant height (PH, cm), number of minicuttings (NM), number of roots (NR), number of shoot (NS), number of green leaves (NGL), and number of senescent leaves (NSL) in *Manihot pseudoglaziovii* Pax & Hoff. using different BAP and NAA concentrations.

SV	DF	SM					
		PH	NM	NR	NS	NGL	NSL
BAP	4	91.98*	0.22ns	6.63**	0.87**	0.56ns	1.54**
NAA	4	10.11ns	0.24ns	2.03*	0.17ns	0.45ns	0.28ns
BAP × NAA	16	38.25*	0.60ns	1.04ns	0.18ns	0.33ns	0.75ns
Error	266	21.11	0.38	0.79	0.12	0.31	0.47
CV (%)		29.24	29.24	27.08	22.14	23.08	26.00
Mean		15.71	10.13	11.18	2.00	5.67	6.94

ns = non-significant; ** and * significant at 1 and 5%, respectively, according to the F test.

The interaction between BAP and NAA had a significant effect ($p < 0.05$) only on PH. This outcome differed from the report by Imtiaz et al. (2019), where increased NS was observed in *Chrysanthemum morifolium* treated with NAA and BAP.

CVs for NS and PH varied from 22.14 to 29.24%, respectively. These values were less than those reported by Sá et al. (2018). The latter authors reported CVs varying between 12.88 and 72.46% in the micropropagation of wild species of *Manihot*.

Table 4 summarizes the findings for the polynomial regression models. The R^2 values for PH and NR ranged from 55.77 to 96.58%, respectively.

The highest NS value (1.67) was observed in the presence of BAP, with 0.05 mg L^{-1} as the most suitable dose (Table 5). A slightly higher dose (0.06 mg L^{-1}) resulted in an increased NSL value (7.89). However, micropropagation of *M. pseudoglaziovii* Pax & Hoff. occurred in the absence of NAA and BAP, considering that the mean PH (19.09) and NR (13.68) were higher in the culture medium devoid of NAA and BAP.

Table 4. Polynomial regression equations, coefficients of determination (R^2), optimal dose, and estimated values for plant height, number of roots, number of shoots, and number of senescent leaves in *Manihot pseudoglaziovii* Pax & Hoff. grown in the presence of different BAP and NAA concentrations.

Interactions	Doses (mg L^{-1})	Equation	R^2 (%)	Optimal dose	Estimated values
		Plant height (cm)			
BAP (NAA)	0	$\hat{y}^{**} = 1531.8x^2 - 209.79x + 19.493$	55.77	0.07	12.31
NAA (BAP)	0	$\hat{y}^{**} = -36.093x + 19.095$	55.77	0	19.09
		Number of roots			
BAP		$\hat{y}^{**} = -56.782x + 13.68$	96.58	0	13.68
NAA		$\hat{y}^{ns} = 11.30281$	-	0	11.30
		Number of shoots			
BAP		$\hat{y}^{**} = -102.4x^2 + 10.501x + 1.4$	90.95	0.05	1.67
		Number of senescent leaves			
BAP		$\hat{y}^{**} = -664.88x^2 + 76.481x + 5.6861$	74.34	0.06	7.89

** and * significant at 1 and 5 % probability level, respectively, based on ANOVA carried out in the F test.

Table 5. Summary of the analysis of variance applied to plant height (PH, cm), number of minicuttings (NM), number of roots (NR), number of shoots (NS), number of green leaves (NGL), and number of senescent leaves (NSL) in *Manihot flabellifolia* Pohl. grown in different concentrations of BAP and NAA.

SV	DF	MS					
		PH	NM	NR	NS	NGL	NSL
BAP	4	47.19^{ns}	5.54**	3.30**	2.90**	1.23**	3.38**
NAA	4	69.38^{ns}	1.67**	2.86**	1.82**	1.83**	2.30**
BAP x NAA	16	14.87^{ns}	0.43**	0.99**	0.90**	0.39*	0.48^{ns}
Error	118	39.99	0.20	0.17	0.35	0.22	0.44
CV (%)		109.52	25.58	19.44	26.37	24.19	26.67
Mean		5.77	2.94	4.43	5.11	3.62	6.30

ns = non-significant; ** and * significant at 1 and 5%, respectively, based on the F test.

Similar results were observed for the micropropagation of *Plathymenia reticulata* Benth. (Moura, Titon, Miranda, Moreira, & Oliveira, 2012) and *Hancornia speciosa* Gomes (Oliveira, Freire, & Aloufa, 2016), whose explants were more responsive in MS culture medium devoid of BAP and NAA.

Vidal, Diniz and Silva (2013) found that BAP, despite being widely used in the *in vitro* multiplication phase, may not exert a satisfactory effect, depending on the species studied. Mallón, Rodríguez-Oubina and González (2011) attributed this behavior to the concentrations of endogenous auxins and cytokinins being sufficient to induce plant development *in vitro*.

The present study differed from those of Kaviani et al. (2019) and Silva et al. (2017). The former group observed the highest NR values in plants of *Aglaonema widuri* grown with NAA and BAP, whereas the latter group obtained higher rates of responsive explants at the highest concentrations of BAP.

Experiment III: Effect of different BAP and NAA concentrations on the *in vitro* micropropagation of *M. flabellifolia* Pohl.

An analysis of variance revealed the significant effect of the BAP and NAA interactions on most variables. With the exceptions of PH and NSL, variables were significantly affected when plants were grown in the presence of either BAP or NAA (Table 5).

These findings differed from those reported by Marín, Albarrán, Fuenmayor and Perdomo (2009), who recorded the highest PH values in plants grown in the presence of combined doses of regulators, such as NAA and GA$_3$.

The highest NR value (7.54) was observed in plants treated with 0.025 mg L^{-1} NAA, in the absence of BAP (Table 6). A similar response was observed during the *in vitro* multiplication of *Satureja hortensis* L. stem segments (Navroski et al., 2014) in cultivation medium containing a low concentration of NAA and no BAP. The effect on NR provided evidence of the role of auxin in root development.

Taiz, Zeiger, Møller and Murphy (2017) reported that lower concentrations of auxins may assist normal root growth, while conversely higher concentrations may have an inhibitory effect. NAA (0.025 mg L^{-1}) alone significantly increased the NGL value, with the mean NGL reaching approximately five leaves per plant.

Table 6. Polynomial regression equations, coefficients of determination (R^2), optimal dose and estimated values for the number of minicuttings, number of roots, number of shoots, number of green leaves, and number of senescent leaves in *Manihot flabellifolia* Pohl. grown using different BAP and NAA concentrations.

Interactions	Doses (mg L^{-1})	Equation	R^2 (%)	Optimal dose	Estimated values
		Number of minicuttings			
BAP (NAA)	0	$\hat{y}^{**} = 941.71x^2 - 146.65x + 6.8222$	84.3	0.08	1.11
BAP (NAA)	0.025	$\hat{y}^{**} = 1190.5x^2 - 192.18x + 8.7664$	92.7	0.08	1.01
		Number of roots			
BAP (NAA)	0	$\hat{y}^{**} = -1004.3x^2 + 74.715x + 5.0186$	96.69	0.04	6.41
BAP (NAA)	0.025	$\hat{y}^{**} = -54.689x + 7.5439$	94.99	0.00	7.54
BAP (NAA)	0.1	$\hat{y}^{**} = -1243.8x^2 + 95.248x + 4.8386$	73.04	0.04	6.66
NAA (BAP)	0.05	$\hat{y}^{**} = 1633.4x^2 - 171.71x + 6.8095$	60.09	0.05	2.30
NAA (BAP)	0.075	$\hat{y}^{**} = 1081.9x^2 - 106.06x + 5.5124$	90.53	0.05	2.91
		Number of shoots			
BAP (NAA)	0	$\hat{y}^{**} = -1824.4x^2 + 193.87x + 2.5254$	97.78	0.05	7.68
BAP (NAA)	0.025	$\hat{y}^{**} = -2337.8x^2 + 213.56x + 3.49$	91.57	0.05	8.37
NAA (BAP)	0.075	$\hat{y}^{*} = 1447.6x^2 - 115.43x + 6.5095$	97.77	0.04	4.21
		Number of green leaves			
BAP (NAA)	0	$\hat{y}^{**} = -953.51x^2 + 80.665x + 3.6372$	92.39	0.04	5.34
BAP (NAA)	0.025	$\hat{y}^{**} = -29.667x + 5.0917$	65.80	0	5.09
NAA (BAP)	0.025	$\hat{y}^{**} = 931.97x^2 - 103.44x + 4.9566$	75.35	0.06	2.09
NAA (BAP)	0.05	$\hat{y}^{**} = 1204.4x^2 - 142.59x + 6.367$	76.78	0.06	2.15
NAA (BAP)	0.075	$\hat{y}^{**} = 1649.5x^2 - 120.29x + 4.5019$	91.47	0.04	2.31
		Number of senescent leaves			
BAP		$\hat{y}^{**} = -44.663x + 8.1471$	81.40	0	8.14
NAA		$\hat{y}^{**} = -27.185x + 7.4335$	56.13	0	7.43

** and * significant at 1and 5% probability level, respectively, based on ANOVA carried out in the F test.

The CVs ranged from 19.44 to 109.52%, similar to the values of 12.25 to 99.53% reported by Miranda et al. (2016) during *in vitro* multiplication of *Eremanthus incanus* (Less.) Less. According to Werner et al. (2012), the genotype can influence coefficients of variation in plant tissue cultures.

The regression equations in Table 6 indicated that the best NM results were obtained in plants grown in the presence of 0.025 mg L^{-1} NAA and 0.08 mg L^{-1} BAP. NM is an essential variable in micropropagation processes, since it defines the number of explants necessary to perform new subcultures. Thus, NM determines the multiplication rate. Afonso, Paranhos, Tabaldi and Soriani (2018) emphasized the importance of using growth regulators to produce the most vigorous *Tabernaemontana catharinensis* A. DC. seedlings derived from minicuttings.

Among their actions, cytokinins stimulate shoot formation, mainly in association with auxins. However, the nature and concentration of these regulators can change from species to species (Gana, 2010). In this context, Goelzer, Déo, Lopes and Damiani (2019) found that the explants of *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae) grown in a medium supplemented with BAP exhibited a significant increase in NS values.

The interaction between 0.025 mg L^{-1} NAA and 0.05 mg L^{-1} BAP led to the largest mean NS value (8.37) in *M. flabellifolia* Pohl.. The highest mean NSL values (8.14 and 7.43) were observed in the absence of these regulators. These findings were different from those of Deswiniyanti & Lestari (2020), who recorded the highest NS in *Lilium longiflorum* treated with 1.0 mg L^{-1} NAA and 1.0 mg L^{-1} BAP.

Concerning wild cassava species, only a few studies have examined the multiplication of germplasm under controlled conditions *in vitro*. Thus, the present results are essential for understanding the behavior of the studied genotypes *in vitro*. The data concerning the three species, and the BAP and NAA concentrations have not been reported before. Xavier, Wendling, & Silva (2009) affirmed that the required concentrations of phytoregulators vary with plant species and the type of explant used. Thus, the use of culture media supplemented with growth regulators is important in determining their role in the growth and control of the *in vitro* development pattern of explants.

Another point that further emphasizes the significance of the work is that the species we used are wild. Seiler, Qi, & Marek (2017) have suggested that these genotypes provide valuable attributes for traditional genetic improvement. Dempewolf et al. (2017) also emphasized the importance of these species in the development of genotypes adapted to factors such as diseases and adverse climatic conditions. Thus, *in vitro* micropropagation of these plants has a key role in supporting genetic improvement programs.

Conclusion

BAP and NAA supported the *in vitro* multiplication of *M. violaceae* (Pohl) Müll. Arg. and *M. flabellifolia* Pohl. Addition of NAA or BAP to the culture medium was not necessary for the *in vitro* multiplication of *M. pseudoglaziovii* Pax & Hoff.

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