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Micriopropagation Of Two Cultivavars of Taro [*Colocasia esculenta* (L.) Schott] Produced In Benin by Direct Organogenesis

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Abstract

Taro [*Colocasia esculenta* (L.) Schott] is a very nutritious plant. It is traditionally multiplied. This production strategy is a route for the dissemination of microorganisms. It is essential to foresee the path of in vitro micropropagation. This study aims to determine the effect of hormonal combinations of naphthalene acetic acid (0.5 mg / L; 1 mg / L) and benzylamino purine (2 mg / L; 4 mg / L) on micropropagation and in vitro organogenesis from apical explants of pink-fleshed and white-fleshed taro cultivars produced in Benin. A logistic regression was performed to understand the effect of different doses of naphthalene acetic acid, benzylamino purine and their combination on the apical buds of taro. The results showed that the combination of 0.5 mg / L of naphthalene acetic acid with 4 mg / L of benzylamino purine promoted the formation of the highest average number of roots in both cultivars. In relation to the number of leaves, the combination MS + 4 mg / L BAP + 0.5 mg / L ANA was more suitable for leaf formation in the white-fleshed cultivar while the combination MS + 2 mgl- 1 BAP + 1 mgl-1 ANA was more suitable for leaf formation in the pink-fleshed cultivar. On the other hand, the combination of 0.5 mg / L of naphthalene acetic acid and 2 mg / L of benzylamino purine favored the highest average number of suckers (3) in the pink-fleshed cultivar whereas in the cultivar with white flesh it was the combination of 0.5 mg / L of naphthalene acetic acid and 4 mg / L of benzylamino purine that favored the formation of the highest average number of rejections (5). The present study, realizing the success of healthy seed production, offers the opportunity of in vitro conservation in order to limit the risks of contamination and increase the production of taro in Benin.

Keywords: Growth Regulators; (*Colocasia esculenta* (L.) Schott); Apical Buds; Micropropagation

Introduction

Taro [*Colocasia esculenta* (L.) Schott] was an important food crop with a world production of around 11 million tonnes per year over an area of around 2 million hectares [1]. The main producers in West Africa were Ghana (3,303,118 tonnes), Nigeria (1,460,938 tonnes), Togo (16,620 tonnes) and Benin (1,069 tonnes) [1]. Taro is a very nutritious plant and is considered one of the healthiest food products [2]. It was particularly useful for people with allergies to cereals and may be recommended for children sensitive to milk. The bulb was an excellent source of carbohydrates, mainly starch [3]. It contains higher amounts of vitamin B complex than whole milk. It was low in fat and protein, but its protein content was higher than that of other root and tuber crops such as yam (*Dioscorea alata* L.), cassava (Manihot esculenta Crantz) and sweet potato (*Ipomoea batatas* L.). As such, taro flour was used in infant formula and canned baby food. Taro leaves provide plenty of vitamin A, which was necessary for good growth, eye health and disease prevention. They also contained vitamin C [4]. Besides its nutritional value, taro was used as a medicinal plant and provides bioactive compounds used as anticancer drugs [5,6]. Taro was traditionally propagated vegetatively using suckers and bulbs. This method of producing planting material was slow and seasonal, while the shortage of planting material is a big problem to be solved for plantations and for increasing production. This traditional practice common to many tuber plants imposes on farmers a reserve of a not insignificant quantity of edible tuber [4]. Also, this multiplication strategy was a route for the dissemination of microorganisms. Planting material could also transmit pathogens, especially viruses, from one planting season to another, resulting in reduced growth and lower yield potential of next-generation crops [7,8]. Indeed, the sanitary quality of seeds would be affected if a palliative measure did not taken for the production of seeds. Thus, it was essential to foresee the path of plant biotechnology, more precisely in vitro culture through micropropagation, the advantage of which was to ensure the rapid production of planting material free of disease and uniform [9]. Tissue culture had been applied for the propagation of more than 1000 different plant species [4,10,11,]. The success of these techniques

involves certain exogenous factors including an adequate combination of auxin and cytokinin; and endogenous, including the type of implant [12,13]. In Benin, these techniques were already used with success for the micropropagation of elite cultivars of plants with roots and tubers such as Dioscorea spp [12,14]. For this purpose, [15] used MS medium supplemented with benzylamino purine (BAP) and indolebutyric acid (AIB) to establish the protocol for micropropagation of taro from apical buds. Also, [4] they established a protocol for micropropagation of taro from apical buds with MS medium supplemented with benzyladenine (BA) and indole-acetic acid (AIA) as culture media. Neither author established a protocol by combining benzylamino purine (BAP) and naphthalene acetic acid. The present study aims to determine the effect of hormonal combinations of naphthalene acetic acid and benzylamino purine on micropropagation and organogenesis in vitro from apical explants of taro cultivars produced in Benin.

Materials and Methods

Materials

The experiments were carried out in 2018 at the Central Laboratory for Plant Biotechnologies and Improvement of Plants (LCBVAP) of the University of Abomey-Calavi in the Republic of Benin. Two cultivars of taro, one with white flesh collected in the field of a producer in the municipality of Dangbo and the other with pink flesh collected in the field of a producer in the municipality of Zè, both located in the south from Benin.

Methods

Disinfection of explants

The disinfection protocol applied was that of [16]. Indeed, these fragments of taro corm comprising the apical bud were used as explants were rinsed with tap water and were transferred to the culture chamber under a hood. They were then immersed in 70% ethanol for 1 min and then immersed in a 0.1% mercuric chloride solution containing two drops of Tween 80 for 5 min. The explants were rinsed three times with sterile distilled water for five minutes per rinse. The disinfected explants were removed from their necrotic parts by a scalpel.

Culture media

Eight MS media (17) were designed by adding different concentrations of naphthalene acetic acid (NAA) and purine benzylamine (BAP) (Table 1). The pH of the medium is adjusted to 5.7 ± 0.1. The media supplemented with 30 g.L-1 of sucrose and 8 g.L-1of agar were distributed in tubes (25 mm x 150 mm) sterilized at 121 °C for 15 minutes in an autoclave and transferred to the culture chamber. The inoculated media were placed in the culture chamber at 27 ± 1 °C and were subjected to a photoperiod of 16 hours per day under a light intensity of 5000 lux supplied by the Philips TLD18W and Sibalec lamps. Relative humidity is maintained at 80%. The duration of the experiment is twenty-eight (28) days.

Culture media	Growth regulator compositions
M_0	MS
M_1	MS + 0,5 mgl^{-1} d'ANA
M_2	MS + 1 mgl^{-1} d'ANA
M_3	MS + 2 mgl^{-1} de BAP
M_4	MS + 4 mgl^{-1} de BAP
M_5	MS + 2 mgl^{-1} de BAP + 0,5 mgl^{-1} d'ANA
M_6	MS + 2 mgl^{-1} de BAP + 1 mgl^{-1} d'ANA
M_7	MS + 4 mgl^{-1} de BAP + 0,5 mgl^{-1} d'ANA
M_8	MS + 4 mgl^{-1} de BAP + 1 mgl^{-1} d'ANA

Table 1: Composition of culture media

Parameters evaluated and statistical analysis

A completely random device was adopted for the tests. The binary logistic regression test was used to see if leaf formation of roots, suckers and shoots depended on cultivars and growing media. This test allows us to estimate the strength of the association between the dependent variable and each of the explanatory variables, while taking into account the simultaneous effect of all of the other explanatory variables included in the model. Data on the number of roots, leaves, suckers and shoot height were analyzed for variance to compare these parameters to the 5% threshold. The descriptive statistic was used to obtain the average by environment. Xlstat version 14 software was used to analyze the results.

Result

Effect of benzylamino purine associated with naphthalene acetic acid on the number of leaves formed

Table 2 showed the results of the logistic regression reveals, according to the probabilities associated with the Chi-square tests ($p < 0.0001$) that the cultivar variables, culture media and their interaction had a very highly significant influence on the formation of leaves.

Figure 1 showed the number of leaves formed from the two taro cultivars in the presence of the eight hormonal combinations used. In the pink-fleshed cultivar this number of formed leaves varied from 0 to 4.2 while for the white-fleshed cultivar this number of formed leaves varied from 0 to 7.4.

The pink-fleshed cultivar had the lowest number of leaves (0) on MS, MS + 0.5 mg / L ANA and MS + 1 mg / L ANA media and the highest number of leaves (4 , 2) on MS medium + 2 mgl-1 of BAP + 1 mgl-1 of ANA while the white flesh cultivar shows the lowest number of leaves formed (0) on MS, MS + 0.5 media mg / L of ANA and the large number of leaves (7.4) on the MS medium + 4 mg / L of BAP + 0.5 mg / L of ANA. Thus, for all the hormonal combinations used, the white flesh cultivar had a higher number of formed leaves than pink flesh cultivar. The letters a, b, c and d indicated the existence of a very highly significant difference of the eight hormonal combinations on each of the two cultivars. For the white-fleshed cultivar, the combination MS + 4 mg / L of BAP + 0.5 mg / L of ANA was more suitable for leaf formation while the combination of MS + 2 mgl-1 of BAP + 1 ANA mgl-1 was more suitable for leaf formation in the pink-fleshed cultivar.

Source	DDL	Khi²(Wald)	Pr > Wald	Khi ²(LR)	Pr > LR	R ²adjusté
Cultivars	1	16,04444444	1	18,39490445	< 0,0001	0,82***
Culture media	8	340,8888888	1	48,85350318	< 0,0001	
Morphotype* Culture media	8	20,75555555	1	2,974522292	< 0,0001	

Table 2: Effect of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) and cultivar type on the number of leaves formed: result of the binary logistic model

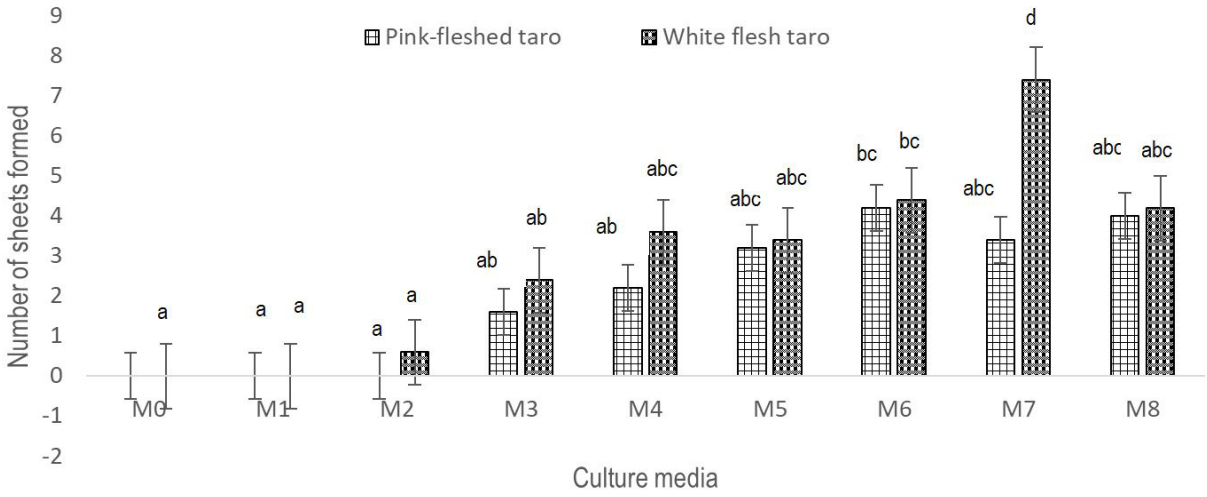

Figure 2: Influence of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) on the number of leaves formed from two cultivars of taro (pink-fleshed taro and white flesh) after 28 days of culture on Murashige and Skoog (MS) medium. The means followed by similar letters do not differ significantly at the 5% level

Effect of benzylamino purine (BAP) in combination with naphthalene acetic acid (ANA) on the number of roots formed

Table 3 showed the results of the logistic regression reveals, according to the probabilities associated with the Chi-square tests ($p < 0.0001$) that the cultivar variables, culture media and their interaction very significantly influence root formation. Figure 2 showed the number of roots formed from the two taro cultivars in the presence of the two hormonal combinations used. In the pink-fleshed cultivar this number of formed leaves varied from 0 to 2.6 while for the white-fleshed cultivar this number of formed leaves varied from 0 to 3.6. The two cultivars had the lowest number of roots formed (0) on MS, MS + 0.5 mg / L ANA and MS + 1 mg / L ANA media and the highest number of roots formed respectively 2 , 6 for the pink-fleshed cultivar and 3.6 for the white-fleshed cultivar on MS medium + 4 mg / L BAP + 0.5 mg / L ANA. The cultivar with white flesh showed for all the hormonal combinations used a number of roots formed higher than that of the cultivar with pink flesh. The letters a, b, c and d indicated the existence of a very highly significant difference of the eight hormonal combinations on each of the two cultivars. For these two cultivars, the combination of MS + 4 mg / L BAP + 0.5 mg / L ANA was more suitable for root formation (Figure 2).

Source	DDL	Khi ²(Wald)	Pr > Wald	Khi ²(LR)	Pr > LR	R ²adjusté
Cultivars	1	4,01111111	1	14,73469387	< 0,0001	0,80***
Culture media	8	95,4	1	43,80612244	< 0,0001	
Morphotype* Culture media	8	3,88888888	1	1,785714285	< 0,0001	

Table 3: Effect of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) and cultivar type on the number of roots formed: result of the binary logistic model

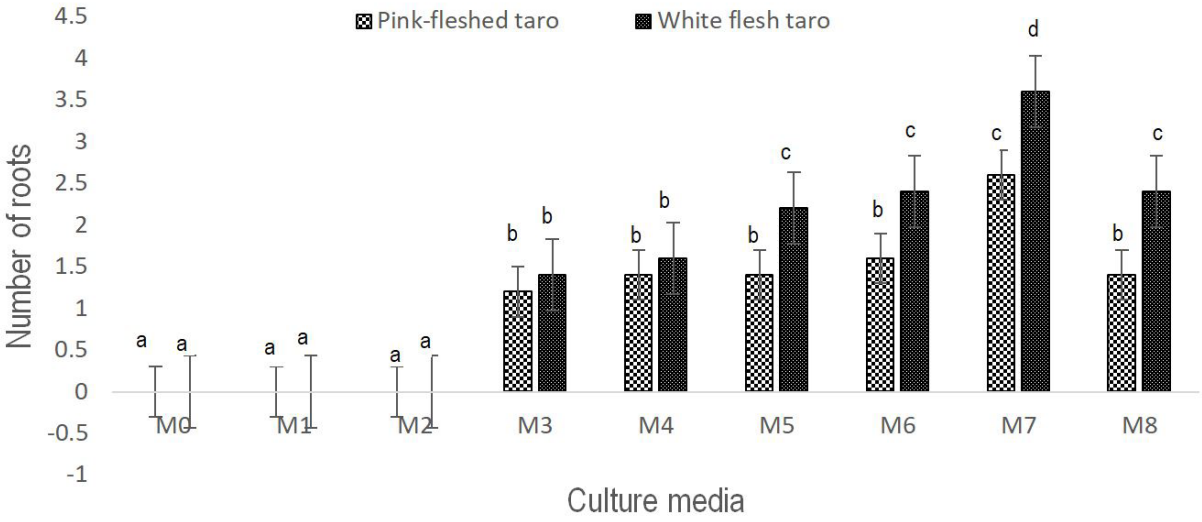

Figure 2: Influence of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) on the number of roots formed from two cultivars of taro (pink-fleshed taro and white flesh) after 28 days of culture on Murashige and Skoog (MS) medium. The means followed by similar letters do not differ significantly at the 5% level

Effect of benzylamino purine associated with naphthalene acetic acid on seedling height

Table 4 showed the results of the logistic regression reveals, according to the probabilities associated with the chi-square tests ($p < 0.0001$) that the cultivar variables, culture media and their interaction very significantly influenced the height of the seedlings.

Source	DDL	Khi ²(Wald)	Pr > Wald	Khi ²(LR)	Pr > LR	R ²adjusté
Cultivars	1	12,39511111	1	42,98054324	< 0,0001	0,84***
Culture media	8	127,5522222	1	55,28655365	< 0,0001	
Morphotype* Culture media	8	6,266888888	1	2,716335966	< 0,0001	

Table 4: Effect of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) and cultivar type on seedling height: result of the binary logistic model

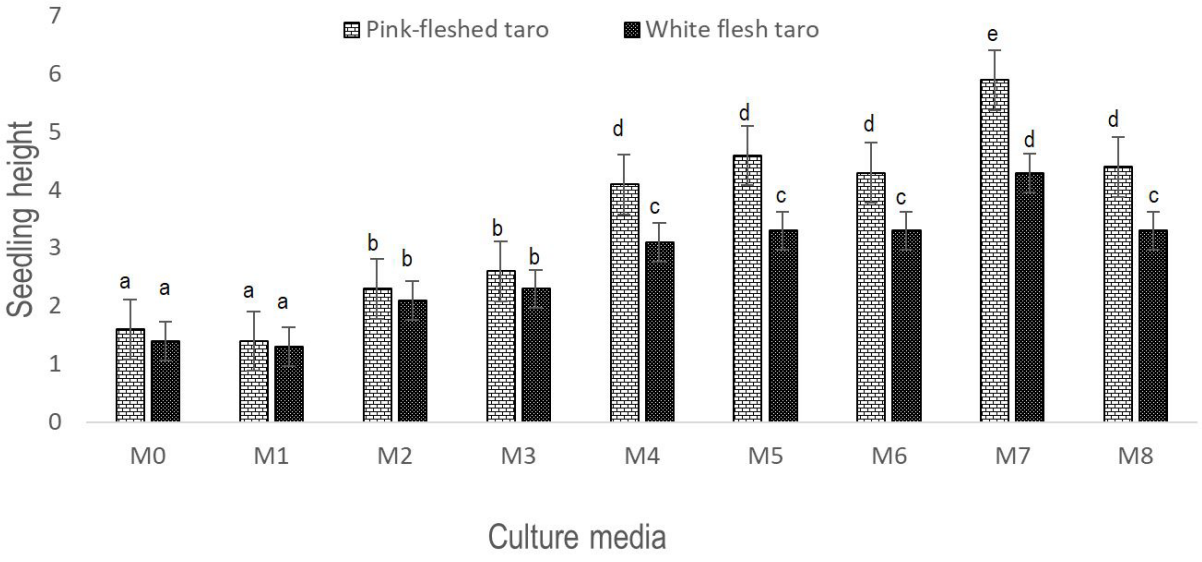

Figure 3: Influence of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) on the height of seedlings of two taro cultivars (pink-fleshed taro and fleshed taro white) after 28 days of culture on Murashige and Skoog (MS) medium. The means followed by similar letters do not differ significantly at the 5% level

Figure 3 showed the height of the seedlings of the two taro cultivars in the presence of the two hormonal combinations used. In the pink-fleshed cultivar, the height of the seedlings varied from 1.4 cm to 5.9 cm while for the white-fleshed cultivar, this height varied from 1.3 cm to 4.3 cm. The two cultivars had the smallest height respectively 1.4 cm for the pink-fleshed cultivar and 1.3 cm for the white-fleshed cultivar on MS medium + 1 mg / L ANA and the greatest height respectively 5.9 cm for the pink-fleshed cultivar and 4.3 cm for the white-fleshed cultivar on MS medium + 4 mg / L BAP + 0.5 mg / L ANA. For all hormonal combinations used, the height of seedlings of the pink-fleshed cultivar was greater than that of the white-fleshed seedlings. The letters a, b, c, d, e and f indicated the existence of a very highly significant difference of the eight hormonal combinations on each of the two cultivars. For these two cultivars, the combination of MS + 4 mg / L BAP + 0.5 mg / L ANA was more suitable for seedling growth.

Effect of benzylamino purine associated with naphthalene acetic acid on the number of suckers formed

Table 5 showed the results of the logistic regression reveals, according to the probabilities associated with the Chi2 tests ($p < 0.0001$) that the cultivar variables, culture media and their interaction very significantly influenced the number of suckers formed.

Source	DDL	Khi ²(Wald)	Pr > Wald	Khi ²(LR)	Pr > LR	R ²adjusté
Morphotype	1	8,09999999	1	31,02127659	< 0,0001	0,92***
Culture media	8	256,422222	1	122,7553191	< 0,0001	
Morphotype* Culture media	8	9,79999999	1	4,691489361	< 0,0001	

Table 5: Effect of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) and cultivar type on seedling height: result of the binary logistic model

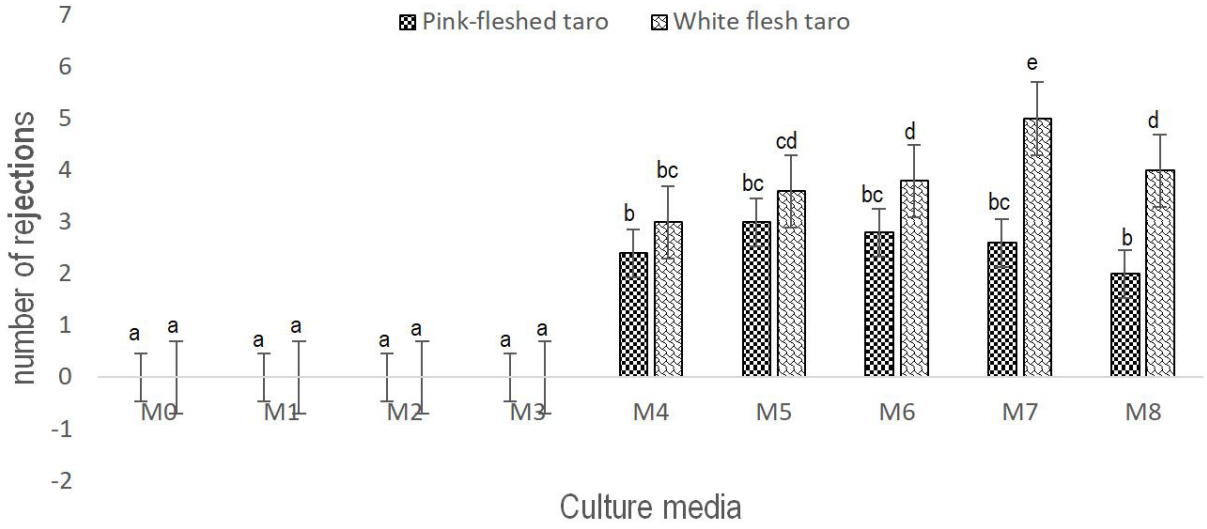

Figure 4: Influence of BAP (2mg / L and 4mg / L) associated with ANA (0.5mg / L and 1mg / L) on the number of suckers formed from two cultivars of taro (pink-fleshed taro and white flesh) after 28 days of culture on Murashige and Skoog (MS) medium. The means followed by similar letters do not differ significantly at the 5% level

Figure 5: Effect of growth regulators on taro. A: leaf formation in the pink-fleshed cultivar on MS medium + 2 mgl-1 of BAP + 1 mgl-1 of ANA B: root formation in the white-fleshed cultivar on MS medium + 4 mg / L of BAP + 0.5 mg / L of ANA C: formation of suckers in the white-fleshed cultivar on MS medium + 4 mg / L of BAP + 0.5 mg / L of ANA

Discussion

The response of taro cultivars varied according to the combinations of growth regulators and the different parameters studied in the process of micropropagation and organogenesis. Indeed, there was a very highly significant variation ($p < 0.0001$) between the influence of growth regulators and the number of leaves formed. The MS medium + 4 mg / L of BAP + 0.5 mg / L of ANA induced the highest number of leaves (7.4) for the white-fleshed cultivar while the combination of MS + 2 mg / L of BAP + 1 mgl-1 of ANA gave a large number of leaves (4.2) in the pink-fleshed cultivar. These results showed that the combination of a cytokine (BAP) and an auxin (ANA) promoted leaf formation in taro [*Colocasia esculenta* (L.) Schott]. Our results were consistent with those of (18) who showed that the combination of a cytokinin (BAP or kinetin) with ANA allows better regeneration respectively of different genotypes of Dioscorea spp. and mainioc (Manihot esculenta). However, only hormonal combinations with a BAP / ANA ratio of 2 favored the formation of a large number of leaves. (19) working on Artemisia showed that the percentage of regeneration of leafy shoots was higher with a hormonal combination with a BAP / NAA ratio equal to 1. The effect of BAP and ANA on leaf formation varied according to their dose and species. In addition, the cultivar had a very highly significant ($p < 0.0001$) influence on leaf formation. The number of leaves formed in the cultivar with white flesh was greater than that in the cultivar with pink flesh and this for all hormonal combinations. Thus, the ability of taro buds to regenerate leaves was influenced by the genotype. Also we noted a very highly significant difference ($p < 0.0001$) between the combinations of growth regulators with more leaves formed on the combination MS + 4 mg / L of BAP + 0.5 mg / L of ANA with the white flesh cultivar on the one hand and more leaves formed with the pink flesh cultivar on the combination MS + 2 mgl-1 of BAP + 1 mgl-1 of ANA on the other hand. This showed that there is an interaction between growth regulators and cultivars of taro in cultivation. On the other hand, (18) did they notice an interaction between the genotypes of cassava (Manihot esculenta) and growth regulators using ANA (0.5 mg / l) + BAP (0.5 mg / L) of a part and ANA (0.5 mg / L) + KIN (0.5 mg / L). Relative to leaf formation, BAP (4 mg / L) in combination with ANA (1 mg / L) exerted a negative effect on the number of leaves formed in both cultivars. (4) noticed this negative action on the formation of leaves of certain varieties of taro cultivated in the Philippines with 1 mg / L of BAP associated with 1 mg / L of AIA. The absence of leaves on the MS, MS + 0.5 mgl-1 ANA and MS + 1 mgl-1 ANA media was explained by the inhibition of the lateral buds caused by the auxins produced at the apical meristem at the profit from underground organs. These facts were in accordance with the already known rules of the physiological transport of auxins polarized to the base [20].

As for rhizogenesis, there was a very highly significant influence ($p < 0.0001$) of the media used on the formation of the roots with the greatest number of roots for the two cultivars on the MS + 4 mg / L medium of BAP + 0.5 mg / L ANA, with 2.6 and 3.6 on average roots formed for the pink-fleshed cultivar and the white-fleshed cultivar, respectively. The MS, MS + 0.5 mg / L ANA and MS + 1 mg / L ANA media did not promote root formation for either cultivar. On the other hand, their combination with BAP (2 mg / L and 4 mg / L) favored the formation of roots in both cultivars. These results showed that ANA alone does not allow good rhizogenesis. [21] observed improved root formation in potatoes when the cytokinin / auxin ratio varied from 0.1 to 5. The cytokinin therefore influenced the transport of auxin from the cell to the cell by modifying the expression of several components. of auxin transport and thus modulates the distribution of auxin important for the regulation of root meristem activity and size [22]. In addition, the number of roots formed in the white-fleshed cultivar was greater than that of the pink-fleshed cultivar for all hormonal combinations. This showed that the number of roots formed was influenced by the genotype. The differential response of the two cultivars studied could be related to a difference in the endogenous level of growth substances and their interactions with exogenous inputs of auxin [23]. Both cultivars showed a reduction in the number of roots formed when BAP (4 mg / L) was combined with ANA (1 mg / L). At this concentration, ANA may inhibit the role of cytokinins in cellular auxin transport or may had anti-cytokinin activity [24].

As for the average height of the shoots formed, there was also a very highly significant influence ($p < 0.0001$) of the media used on the height of the shoots formed with the highest average height observed on the MS medium + 4 mg / L of BAP + 0.5 mg / L of ANA for the two cultivars with 5.9 and 4.3 on average respectively for the cultivar with pink flesh and cultivar with white flesh. BAP and ANA favored better growth of the shoots formed. However, MS medium + 4 mg / L BAP + 1 mg / L ANA promoted reduced shoot growth. This could result from using a higher level of auxin (ANA) above the optimum level.

Finally, as for the number of rejects formed, there was also a very highly significant influence ($p < 0.0001$) of the media used on the number of rejects formed. MS media, MS + 0.5 mgl-1 ANA, MS + 1 mgl-1 ANA. ANA alone does not allow the formation of suckers. The MS medium + 2 mgl-1 of BAP and the MS medium + 4 mgl-1 of BAP favored the formation of suckers in the two cultivars, respectively 3 on average per explant for the pink flesh cultivar and 3.6 on average per explant for the white-fleshed cultivar. BAP alone favored the formation of rejects. This could be due to the effect of cytokinin (BAP) in releasing shoots from lateral buds by breaking apical dormancy by inhibiting the effect of a high level of endogenous auxins [25]. On the other hand [26] when working on taro obtained an average of 5.9 rejections per explant using 8 mg / l of BAP. Also, [25] using 10 mg / l BAP recorded an average of 6.44 rejections per taro explant. These differences could be due to the genotypic variation of the plant. For the combinations tested, MS medium + 4 mg / L BAP + 0.5 mgl-1 ANA induced a large number of rejections (5 on average per explant) in the white-fleshed cultivar. So this medium showed a relatively higher response in terms of the number of rejections compared to the other combinations. A comparatively lower response in number of rejections was observed with the combination MS + 4 mg / L of BAP + 1 mg / l of ANA (4 rejections on average per explant). This could be related to the interaction effect of the two growth regulators at this level of concentration. Also in the pink-fleshed cultivar, all combinations resulted in a reduction in the number of suckers. This could be due to the genotypic variation of the different cultivars.

Conclusion

The present research work, which was part of an improvement in seed production of taro cultivars by the micropropagation technique, leads to the conclusion that the BAP associated with the ANA (MS + 4 mg / L of BAP + 0.5 mg / L ANA) promoted the harmonious development of the above-ground and underground organs of the two cultivars. As for the production of suckers, the MS medium + 2 mgl-1 of BAP + 0.5 mgl-1 of ANA is more favorable for the pink-fleshed cultivar and the MS medium + 4 mg / L of BAP + 0, 5 mg / L ANA for the white flesh cultivar. This study offered the opportunity to produce healthy seeds in order to limit the risk of contamination and increase taro production in Benin.

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