

Original Research Article

Determination of ~~the~~ optimal doses of plant growth regulators for *in vitro* propagation of four ~~varieties of~~ potato **varieties (*Solanum tuberosum*) in Niger**

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Abstract

The main limitation of ~~the production of~~ potatoes (*Solanum tuberosum* L.) ~~production~~ in Niger is ~~the~~ external dependence ~~for on~~ the supply of quality seeds. In the context of a national ~~seed~~ potato ~~seed~~ system, one of the critical phases is the rapid *in vitro* multiplication of virus-free, ~~in vitro~~ potato good quality ~~potato~~ plantlets. Fifteen hormonal combinations were formulated and tested using a completely randomized design with two factors and four replicates in the tissue culture laboratory of the Department of Radio-Agronomy, Institute of Radio-Isotopes, University ABDOU MOUMOUNI, Niamey, Niger. The aim of ~~these~~ ~~this~~ work is to determine a suitable hormonal combination and optimum concentrations for ~~the~~ *in vitro* production of a high number of ~~In vitro~~ plantlets for four farmer-preferred varieties ~~in Niger~~ (ATLAS, PAMELA, STEMSTER, and YONA), ~~in Niger~~. Uninodal stem explants of *in vitro* plantlets were cultured on full-strength Murashige and Skoog media (MS) ~~fortified~~ ~~supplemented~~ with ~~fourteen~~ different combinations of α -Naphthalene Acetic Acid NAA (0; 0.25; 0.50; 1; 2 mg/l) and Benzyl Amino Purine BAP (0; 0.25; 0.50 mg/l), ~~in the tissue culture laboratory~~ for 28 days. Statistical analysis ~~of the results~~ showed that the varieties, ~~BAP and NAA~~ and hormonal combinations ~~and varieties vs combinations~~ were highly significantly ~~influenced the~~ ~~for~~ plant height of the plant, ~~the~~ number of leaves, and ~~the~~ number of roots. Treatments with NAA alone, without BAP, stimulated rhizogenesis. From ~~a~~ **NAA concentration of** 0.25 to 0.5 mg/l there is a proliferation of

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the roots and from 1 mg/l there is an ~~elongation~~ **increase in** ~~of the roots~~ length. Cultivars Pamela and Atlas **cultivars** showed better root production.

Key Words: Plant growth regulator; Potato; NAA; BAP

1. Introduction

The potato plays a key role in the global food system. According to FAO DATA [1], annual **potato** production ~~of potato~~, ranging from 300 to 400 million tons, is achieved in countries with large populations, ~~with lead by~~ China, India, Ukraine, Russia, ~~and the United States~~ **in the lead**. Potato production in Niger is still low, but with great potential currently highlighted by increasingly regular cereal deficits, linked to unfavorable climatic conditions. From 1,400 tons in 1985, production ~~rose~~ **increased** to 7,623 tons in 2000, then to 97,510 in 2014, and peaked in 2018 at 168,000 tons. **Yields still low, vary between 7 and 15 tons per hectare.**

In Niger ~~country~~, most potato production takes place in the highlands ~~in of~~ the north of the country (Agadez), by small ~~scale~~ farmers ~~who using~~ traditional means ~~to propagate of~~ potato **propagation** ~~through the use by using of~~ all-comers tubers. The ~~extension increase of in~~ production is ~~strongly~~ **severely** limited by external dependence ~~for on~~ the supply of quality potato seed (high cost, delay in delivery, limited choice of varieties, etc.). The good profitability of potato farms requires the establishment of a local seed production program ~~from in~~ tissue culture laboratories, which is an absolute necessity to ensure a regular supply of high quality and disease-free seed potato tubers. In addition, a tissue culture technique in a seed potato system allows a higher flexibility for scheduling, less testing for health status, and a higher rate of multiplication. *In vitro*, on Murashige and Skoog [2] medium (MS)[2], ~~the~~ potato plantlet has the ability to grow without exogenous growth hormones. But the use of MS **medium** supplemented with various combinations of exogenous plant growth regulator, is known to **boost**

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highly ~~greatly stimulate~~ micropropagation. However, the result is variable, depending on the variety [3, 4, and 5]. This requires, as a prerequisite, ~~to conducting~~ a preliminary study, according to the varieties desired locally. Also, the purpose of this work is to help define the best combinations of NAA and BAP ~~on improvement of~~ to improve the *in vitro* micropropagation of four desired varieties, as part of a potato seed production scheme in Niger.

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2. Materials and methods

The experiment was carried out at the laboratory of Biotechnology and plant improvement of the institute of radio-isotopes in the University Abdou Moumouni of Niamey, in Niger (latitude 13°29' North and longitude 2°10' Est). The explants used in this study were uni-nodal segments, derived from the stems of three weeks old *in vitro* ~~growing vitro~~ plants of the four royalty-free varieties of potatoes: Atlas, Pamela, Stemter, and Yona. All the transplanting operations took place under a laminar flow hood, in totally sterile conditions. All metal instruments were sterilized in an oven at 200°C for 4 hours. The explants were cultured in sterilized test glass tubes ~~each~~ each containing ~~each~~ 20 ml of MS medium, supplemented with 30 g/l ~~of~~ sucrose and 7 g/l ~~of~~ agar. The pH of the culture medium was ~~set~~ adjusted to 5.8 before adding agar and autoclaving. The culture media were sterilized by autoclaving at 121°C (pressure of 1 bar) for 20 min, in aliquots of 1 liter volume. ~~Before autoclaving, the required doses of plant growth regulators (NAA and BAP) for each treatment~~ was were added to the MS medium, according to ~~the~~ Table 1. ~~After autoclaving, the sterilized culture media are distributed in test glass tubes, at a rate of 20 ml per tube, under the laminar flow hood before solidification.~~ NAA was dissolved in NaOH (1N), 16 mg NAA in 1 ml NaOH supplemented with 15 ml of distilled water after dissolution. Final solution is 1 mg NAA/ml of solution. Likewise, BAP was dissolved in ethanol

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(96°). After autoclaving, the sterilized culture media are distributed in test glass tubes, at a rate of 20 ml per tube, under the laminar flow hood before solidification. After cooling and hardening of the medium, each tube receives a single-node segment. The test glass tubes were closed with polycarbonate caps and were placed in a growth chamber set at 25°C and 16 h photoperiod for 4 weeks, under the light of white fluorescent tubes (2,500 lux, 35 µmol/m²/s). ~~The results of The parameters evaluated in the experiment were recorded as plant height (cm), leaf number of leaves, root length (cm), and root number of roots. The fresh weights of the leaves and roots were measured and the dry weights were determined after a passage in the oven for 48 hours at 105°C.~~

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A completely randomized design (CRD) was employed to reveal the performance of four potato varieties, as affected by fourteen combinations of NAA and BAP with four replications. Results of the study were subjected to the analysis of variance, and significant differences among treatments were determined using GENSTAT12.01. Segregation between means was made according to the Student Newman-Keuls test. All the probabilities were assessed at the 5% threshold. Data presented by various letters in the same column are ~~different~~ statistically different. Results ~~on~~ of all parameters were expressed as means from four replications with standard error (± SE).

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Table 1: Different combinations of NAA and BAP added to the MS medium for each treatment

Treatments	NAA (mg/l)	BAP (mg/l)
T01	0.25	0
T02	0.5	0
T03	1	0

T04	2	0
T10	0	0.25
T20	0	0.5
T11	0.25	0.25
T22	0.5	0.5
T12	0.5	0.25
T13	1	0.25
T14	2	0.25
T23	1	0.5
T24	2	0.5
T21	0.25	0.5

3. Results

It is well known that organogenesis is dependent on the hormonal balance between endogenous growth hormones with each other and with ~~added~~ exogenous growth regulators ~~added~~ to the culture media. This ~~result appreciates~~ ~~study evaluate~~ the additional effects of exogenous hormones added to ~~the~~ MS medium.

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3.1. ~~Effects of different combinations of NAA and BAP on~~ Average plantlet height

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After 28 days of growth, the average height of the plants for all treatments and all varieties combined is 3.3 cm (Table 2). The largest height was obtained with Pamela-T₀₂ (9.1 cm) and the smallest height with Stemster-T₁₀ (0.6 cm). Treatments without BAP showed the strongest growth (Figure 1). The analysis of variance indicates ~~a~~ very ~~highly~~ significant difference between ~~the~~ treatments ($P < .001$), as well as between ~~the~~ varieties ($P = .003$). The two varieties Atlas and Pamela had the fastest growth (~~t~~Table 3). ~~The treatments~~ T₀₂ and T₀₃ ~~treatments~~ are the

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best media for the rapid stem growth of the vitro-plants stem between for the studied varieties (Table 4).

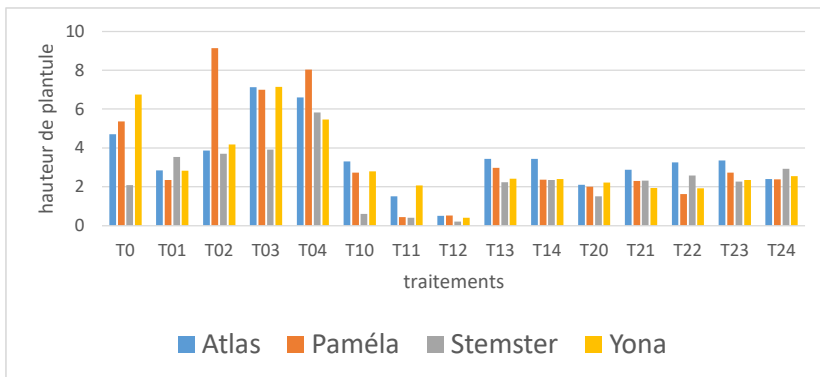


Figure 1: *in vitro* plantlet The height of vitroplantlets, as affected by the combination of NAA and BAP combination in MS medium

Table 2: *In vitro* plant height, as affected by genotype and combinations of NAA and BAP combinations, in MS medium

Varieties	Treatments / Plantlet height (cm)															Aver.	LSD
	T ₀₁	T ₀₂	T ₀₃	T ₀₄	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₂₀	T ₂₁	T ₂₂	T ₂₃	T ₂₄			
Atlas	4.7	2.8	3.9	7.2	6.6	3.3	3.5	2.9	3.4	3.4	2.1	2.9	3.3	3.4	2.4	3.7	0.68
Paméla	5.4	2.3	9.1	7.0	8.0	2.7	0.8	2.4	3.0	2.4	2.0	2.3	1.6	2.7	2.4	3.6	
Stemster	2.1	3.5	3.7	3.9	5.8	0.6	0.7	1.1	2.2	2.3	1.5	2.3	2.6	2.9	2.9	2.6	
Yona	6.7	2.8	4.2	7.1	5.5	2.8	2.6	1.8	2.4	2.4	2.2	1.9	1.9	2.3	2.5	3.3	
Aver.	4.7	2.9	5.2	6.3	6.5	2.4	1.9	2.0	2.8	2.6	2.0	2.4	2.3	2.8	2.6	3.3	
LSD	1.31																

Student-Newman-Kheul, Least significant differences of means (5% level)

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Table 3: Plantlet height by genotype of four potato varieties of potatoes

Varieties	Average plantlet height (cm)
Stemster	2.55 ^a
Yona	3.28 ^b
Paméla	3.61 ^b
Atlas	3.72 ^b
Average	3.30
LSD	0.68
Probability	P=0.003

Comment [A6]: The results in Table 3 are also found in Table 2.

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Table 4: Plantlet height of potatoes as affected by MS media supplemented by different combinations of NAA and BAP

Treatment	Average Plantlet height (cm)
T11	1.92 ^a
T20	1.96 ^a
T12	2.03 ^a
T22	2.34 ^a
T10	2.34 ^a
T21	2.36 ^a
T24	2.56 ^a
T14	2.63 ^a

Comment [A7]: The results in Table 4 are also found in Table 2.

T13	2.77 ^a
T23	2.82 ^a
T01	2.88 ^a
T0	4.72 ^b
T02	5.22 ^{bc}
T03	6.31 ^c
T04	6.48 ^c
Average	3.30
LSD	1.31
Probability	<.001

3.2. Effects of different combinations of NAA and BAP combinations on the number of plantlets leaves per plantlet

The general average obtained for the number of leaves, independently of the variety and the treatment, is 10.6 (Table 5). The greatest highest number of leaves was obtained with in the variety Pamela-T₂₁ (20.8) and the least lowest with in Stemster-T₁₁ (3.0). The analysis of variance reveals no significant difference between the four varieties, but shows a very highly significant difference between the culture media ($P < .001$). Treatments T₂₁, T₂₂, and T₂₃ treatments, combining which combine 0.5 mg/l of BAP with 0.25 to 1 mg/l of NAA are the most effective (Table 6).

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Table 5: Number of leaves by per plantlet of potato genotypes as affected by MS media

supplemented by with different combinations of NAA and BAP

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Varieties	Treatments / Number of leaves per plantlet															Aver.	LSD
	T ₀	T ₀₁	T ₀₂	T ₀₃	T ₀₄	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₂₀	T ₂₁	T ₂₂	T ₂₃	T ₂₄		
Atlas	8,0	6.6	6.6	8.6	7.6	8.8	5.6	8.2	10.4	9.8	10.0	13.4	14.0	11.8	7.8	9.1	1.79
Pamela	7.6	6.2	11.2	9.2	9.4	11.2	4.8	10.8	11.0	11.4	14.8	20.8	13.6	15.6	12.6	11.3	
Stemster	8.6	11.8	10.6	14.0	13.6	4.2	3.0	5.0	10.4	9.2	11.2	16.8	18.6	16.2	12.8	11.1	
Yona	10.6	9.4	9.0	9.6	8.6	9.2	4.2	4.2	12.8	9.0	12.4	13.8	13.4	14.4	16.6	10.8	
Average	8.7	8.5	9.3	10.3	9.8	8.3	5.6	7.0	11.1	9.8	12.1	16.2	14.9	14.5	12.4	10.6	
LSD	3.47																

Student-Newman-Kheul, Least significant differences of means (5% level)

Table 6: Number of leaves by per plantlet as affected by potato genotypes

#Treatments	Number of leaves
T11	5.65 ^a
T12	7.05 ^{ab}
T10	8.35 ^{ab}
T01	8.50 ^{ab}
T0	8.70 ^{ab}
T02	9.35 ^{abc}
T04	9.80 ^{abcd}

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T14	9.85 ^{abcd}
T03	10.35 ^{abcd}
T13	11.15 ^{abcd}
T20	12.10 ^{bcde}
T24	12.45 ^{bcde}
T23	14.50 ^{cde}
T22	14.90 ^{de}
T21	16.20 ^e
Average	10.60
lsd	3.47
Probability	<.001

3.3. Effects of different combinations of NAA and BAP combinations on the number of roots number

The general average, for all treatments and all varieties combined, is 2.2 roots after 28 days of in vitro growth (Table 7). The analysis of variance reveals highly significant difference between the culture media ($P<.001$), and also between the four varieties tested ($P<.001$). Similarly, a highly significant positive interaction between culture media and varieties ($P<.001$) is highlighted.

Treatments T₁₂ and T₀₂, with a high concentration of NAA (0.5 mg/l) showed the maximum number of roots (Table 8 and fig. Figure 2). Atlas and Pamela varieties produced d more roots than Yona and Stemster varieties (Table 9 and Figure 3). The results show a large difference between the T₀₂ treatment (16.6 roots/plantlet) treatment and the rest of the treatments. The smallest

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lowest average is obtained by the treatment T₁₀ (0.05 roots/plantlet) and the treatments T₁₃, T₁₄, T₂₃, and T₂₄ treatments did not produce a roots.

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Table 7: Number of roots by per plantlet as affected by genotype and NAA and BAP combination in MS media

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Varieties	Treatments / Number of roots															Aver.	LSD
	T ₀	T ₀₁	T ₀₂	T ₀₃	T ₀₄	T ₁₀	T ₁₁	T ₁₂	T ₁₃	T ₁₄	T ₂₀	T ₂₁	T ₂₂	T ₂₃	T ₂₄		
Atlas	1.7	2.0	21.6	3.4	3.0	0.0	4.6	8.0	0.0	0.0	0.4	0.6	0.8	0.0	0.0	3.1	1.06
Paméla	0.8	1.4	32.0	1.8	1.6	0.0	1.8	5.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	3.0	
Stemster	0.0	0.2	7.0	2.6	3.0	0.0	2.4	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	
Yona	1.8	0.2	5.8	3.2	3.2	0.2	3.0	4.0	0.0	0.0	0.0	0.8	1.0	0.0	0.0	1.5	
Average	1.1	1.0	16.6	2.8	2.7	0.1	3.0	4.5	0.0	0.0	0.1	0.4	0.6	0.0	0.0	2.2	
LSD	2.05																

Table 8: Number of roots number by per plantlet as affected by NAA and BAP combinations in MS media

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Treatments	Number of roots
T13	0.00 ^a
T14	0.00 ^a
T23	0.00 ^a
T24	0.00 ^a
T10	0.05 ^a

T20	0.10 ^a
T21	0.35 ^a
T22	0.55 ^a
T01	0.95 ^a
T0	1.07 ^a
T04	2.70 ^{ab}
T03	2.75 ^{ab}
T11	2.95 ^{ab}
T12	4.50 ^b
T02	16.60 ^c
Average	2.20
lsd	2.05
probability	<0.001

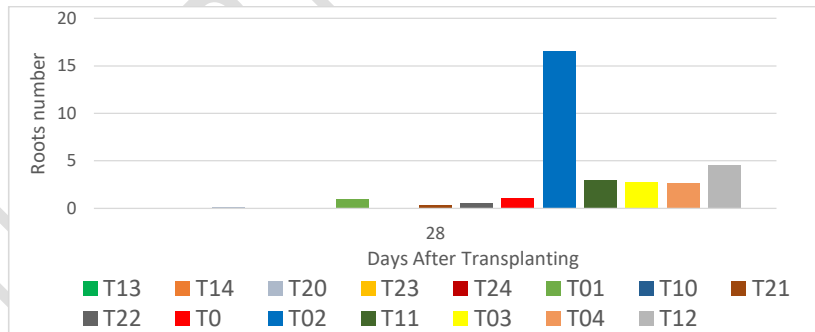


Figure 2: Number of roots as affected by treatments in potato, 28 days after transplanting

Table 9: Root number by plantlet of potato genotypes

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Comment [A11]: The results in Table 9 are also found in Table 7.

variety	Root Number
Stemster	1.08 ^a
Yona	1.55 ^a
Paméla	2.99 ^b
Atlas	3.07 ^b
Average	2.20
lsd	1.06
Probability	<.001



Figure 3: Potato roots proliferation in potatoes as affected by the combination of NAA and BAP in addition to MS media. Left: Pamela with 0.5 mg/l of NAA. Right: Pamela with 1-2 mg/l of NAA

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3.4. Effects of different combinations of NAA and BAP combinations on roots length

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The general average root length, for all varieties and all treatments combined, is 1.3 cm (Table 10). Root lengths vary between 0 cm (T₁₃, T₁₄, and T₂₄) and 8.3 cm for ATLAS and YONA (T₀₃ and T₀₄). The analysis of variance reveals highly significant differences between ~~the~~ treatments ($P < .001$), between the four varieties ($P < .001$) and for the variety-culture medium interaction ($P = .006$). NAA alone, at the highest doses of 1 to 2 mg/L, stimulates root growth length extension (fig. Figure 4). The dose of 0.5 mg/l NAA? alone increases the number of small roots. Doses below lower than 0.5 mg/L + mg/l were not effective. The ~~association~~ combination of NAA and BAP was has been shown to be depressive for have inhibitory effect on the root growth in root length. The ATLAS and YONA varieties were the best (Tables 11).

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Table 10: Root length of potato plantlet as affected by the combination of NAA and BAP added in MS media

Treatment	Root length (cm)
T10	0.00 ^a
T13	0.00 ^a
T14	0.00 ^a
T23	0.00 ^a
T24	0.05 ^a
T20	0.07 ^a
T22	0.22 ^a
T12	0.41 ^a
T21	0.47 ^a

T02	0.98 ^a
T01	1.06 ^a
T11	1.10 ^a
T0	2.65 ^b
T04	5.82 ^c
T03	6.10 ^c
Average	1.30
lsd	1.02
Probability	<.001

Table 11: Root length as affected by the potato genotype of potato

Variety	Root length (cm)
Stemster	0.76 ^a
Pamela	0.95 ^a
Atlas	1.56 ^b
Yona	1.77 ^b
Average	1.30
lsd	0.53
Probability	<.001

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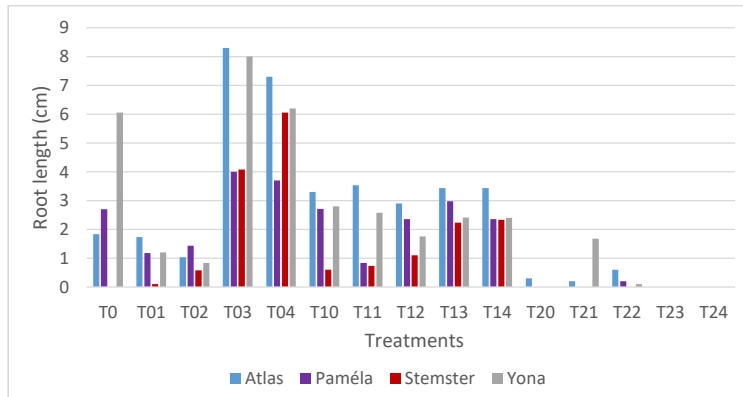


Figure 4: Potato plantlet roots length as affected by different combinations of NAA and BAP added in MS media

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4. Discussion

4.1. Plantlet height

Stem elongation is important, increasing the rate of multiplication over time and shortening the time required for subculturing the tissue culture regenerated plantlets in vitro to be transferred.

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The combinations of NAA and BAP affected the stem length with highly significant difference

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among them and among varieties. The sizes of the vitro-plants are larger from T0 to T04. In

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these four treatments, the hormonal balance is in favor of NAA. All the other treatments with

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BAP, even at a concentration of 0.25 mg/l, significantly inhibit the stem growth. This result is in

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agreement consistent with the data reported by Sota *et al.* [6] who find showed that BAP

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concentration higher concentrations of BAP of than 1 mg/l caused the decrease in biometric

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parameters except for the number of leaves number. Statistical analyzes show that the T02, T03

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and T04 treatments constitute the best hormonal balance for the optimal growth of the vitro-

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plants of the potato varieties studied. Mehmood *et al.* [7] and Xhulaj [8] reported better plantlet

development in terms of shoot height (8.7 cm) with low concentrations of NAA (0.02 mg/L),

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but in the presence of gibberellic Acid (0.2 mg/L). In fact, Gibberellic Acid (GA3), as like others gibberellins, is a plant hormone that regulates various developmental processes, including stem elongation. However, Kumlay *et al.* [5] obtained a reduction in stem growth with the same low concentrations of both hormones NAA (0.1 mg/L) and GA3 (0.1 mg/L). They reported that potato plantlets of potato grown in a culture medium supplemented with Jasmonic Acid (JA) were taller when compared to the other plant growth regulators treatments. The results also showed that the control treatments, without growth hormone, of the different varieties, gave interesting appropriate heights. These results are similar to those of Hamadou [9] and Salifou [10] who obtained the longest highest plantlet height with in the control variant, without growth hormone.

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4.2. Number of Leaves per Plantlet

The number of leaves per plantlet did not differ significantly across between genotypes ($P=0.072$).

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Nevertheless, the PAMELA variety had the highest number of leaves (11.3) and ATLAS the

lowest (9.1). However, there were significantly differences among between hormonal

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combinations ($P<0.001$). Treatments T₂₁, T₂₂, and T₂₃ treatments with the highest dose of BAP

(0.5 mg/L) and NAA between 0.25 to 2 mg/L were the best most efficient. These results

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showed that the doses of BAP under lower than 0.5 mg/L were not efficient but NAA is

necessary. This result is in agreement with those of Mohapatra *et al.* [11] who obtained the best

clumps with BAP in the presence of small amounts of Indole 3-Acetic Acid, just like Hajare *et*

al. [12] who obtained the same result with BAP but in the presence of a large quantity of NAA (3

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mg/L). Kumlay *et al.* [5] found that a single application of NAA and BAP, even in the presence

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of gibberellic acid (GA₃) did not significantly improved the number of leaves per explant. In

their experience, Jasmonic Acid was necessary to boost leaves stimulate leaf proliferation. The highest number of leaves was obtained with the variety PAMELA cultivar (20.8), treatment T₂₁ treatment (NAA: 0.25 mg/L and BAP: 0.5 mg/L). The lowest number of leaves was obtained with the STEMSTER cultivar (3.0 leaves), with treatment T₁₁ (NAA: 0.25 mg/L and BAP: 0.25 mg/L). The results of this research clearly indicated that high doses of BAP (> 0.5 mg/L) in the presence of NAA were required, in presence of NAA, for leaves leaf production for the tested varieties tested.

4.3. Rhizogenesis

Statistical analysis of our results showed a highly significant difference ($P < .001$) between treatments at JAR 28. The MS medium (T₀₂), supplemented with NAA alone at a rate of 0.5 mg/L (T₀₂), had resulted in the highest number of roots (16.6). It is the best medium for root proliferation. From this dose, increasing the dose of NAA or introducing BAP, considerably reduces rhizogenesis. This result is in according consistent with data reported by Mohapatra *et al.* [11] who which obtained roots proliferation only with only Indole 3-Butyric Acid and Hajare *et al.* [12] which had obtained the best rhizogenesis with a combination of IBA and IAA alone. Note that some authors [13] have shown in the past that supplementation of the media with only one type of auxin was less effective for root induction in inducing roots and obtaining a large number of good quality shoots than the use of a combination of two auxins simultaneously, i.e., NAA together with IBA. All media containing only NAA produced roots except the variety STEMSTER variety. The results also revealed a reduction in the number of roots with the addition of BAP. This is consistent with the results of Motallebi *et al.* [14] who showed that the addition of BAP to a medium containing auxin decreases the number of roots. The culture media

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with a high concentration of NAA (1-2 mg/l) resulted in less a lower rooting (T₂₁, T₂₄, T₂₃, T₁₄); with 1-2 mg/L de NAA.

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Finally, with regard to the length of the roots, the statistical analysis of the results showed a highly significant difference ($P < .001$) in between the treatments; and the MS medium enriched with only 1 mg/l of NAA (T₀₃); followed by the medium MS medium supplemented with +2 mg/l of NAA (T₀₄) were the most favorable for the root length of the roots with respectively the values obtained being 6.1 cm and 5.8 cm respectively. The smallest shortest length is was obtained by the T₂₀ treatment (0.07 cm). This is similar to the results of Khadiga *et al.* [15] who obtained the longest roots (13.7 cm) using an MS medium supplemented only with only IBA at 0.5 mg/l. With In the YONA variety, the control variant gave an interesting satisfactory result for the root length (6.06 cm), so even a medium without exogenous growth hormone is favorable for the rooting of plantlets in this variety. This is in agreement with the results of Belguendouz [16] who showed that root length is not only influenced by the presence of growth regulators in the MS medium.

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5. Conclusion

The response of different combinations of NAA and BAP on the *in vitro* micropropagation of the four potato varieties of potato (PAMELA, ATLAS, YONA, and STEMPER) was evaluated in the present study. Results have shown that there were highly significant differences among treatments and cultivars for most of the growth parameters ($P < .001$) under study (plantlet height,

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number of ~~leaf~~leaves, root number and length ~~of roots~~). From the above discussion, it ~~is revealed~~ appears that a genotype is dependent on ~~the~~ *in vitro* protocol for its micro-propagation. The two varieties ATLAS and PAMELA had the fastest growth. On the basis of results obtained from these experiments, it ~~is recommended~~ can be said that ~~the treatments~~ T₀₂ and T₀₃ treatments are the best treatments for the four varieties tested. These treatments have shown ~~the~~ best performance for most ~~of the~~ growth parameters, particularly for rapid stem growth and roots proliferation. The behavior of the four varieties turns out to be very different for all the parameters studied. Atlas and Pamela varieties produced more roots than YONA and STEMSTER varieties. Treatments T₁₂ and T₀₂, with high concentration of NAA (0.5 mg/l) showed produced the maximum number of roots. ATLAS and PAMELA varieties produced more roots than YONA and STEMSTER varieties. NAA alone, at highest doses of 1 to 2 mg/L, stimulates ~~and the increase in~~ root length extension. The dose of 0.5 mg/l NAA alone increases ~~and~~ the number of small roots. Doses ~~below~~ lower than 0.5 mg/L 1 mg/l were not effective.

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