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Determination of Optimal Doses of Plant Growth Regulators for *In vitro* Propagation of Four Potato Varieties (*Solanum tuberosum*) in Niger

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Authors' contributions

Author DO supervised all the work, from its conception to the final drafting of this publication; Author SDAR conducted the experiment in the field, collected and processed the data, wrote the first draft of the manuscript; Author LMN directed the experimental device and performed statistical analyses; Author MB and SSI supported the data collection and the first computer processing; Author BM read and approved the final manuscript.

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ABSTRACT

Aims: The main limitation of the production of potatoes (*Solanum tuberosum* L.) in Niger is the external dependence on the supply of quality seeds. In the context of a national potato seed system, one of the critical phases is the rapid *in vitro* multiplication of virus-free good quality potato plantlets. Fifteen hormonal combinations were formulated and tested to determine a suitable hormonal combination and optimum concentrations for the *in vitro* production of a high number of plantlets for four farmer-preferred varieties in Niger (ATLAS, PAMELA, STEMSTER and YONA).
Study Design: 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, at 5% threshold.

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Place and Duration of Study: 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), for 28 days.
Methodology: Uninodal stem explants of *in vitro* plantlets were cultured on full-strength Murashige and Skoog media (MS) supplemented with fourteen different combinations of α-Naphthalene Acetic Acid NAA (0; 0.25; 0.50; 1.0; 2.0 mg/l) and Benzyl Amino Purine BAP (0; 0.25; 0.50 mg/l), for 28 days.
Results: Statistical analysis of the results showed that the variety and hormonal significantly influenced the height of the plant, the number of leaves and 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 the roots and from 1 mg/l there is an increase in roots length. PAMELA and ATLAS cultivars showed better root production.

Keywords: 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, ranging from 300 to 400 million tons, is achieved in countries with large populations, led by China, India, Ukraine, Russia and the United States. 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 increased to 7,623 tons in 2000, then to 97,510 in 2014 and peaked in 2018 at 168,000 tons [2]. Yields still low, vary between 7 and 15 tons per hectare.

In Niger, most potato production takes place in the highlands of north of the country, by small farmers who use traditional means of potato propagation by using all-comers tubers. The increase in production is severely limited by external dependence 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 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 Medium (MS) [3], 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 regulators is known to greatly stimulate micopropagation. However, the result is variable, depending on the variety [4,5,6]. This requires, as a prerequisite, 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 to improve the *in vitro* micopropagation of four desired varieties, as part of a potato seed production scheme in Niger.

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* 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 tube each containing 20 ml of MS medium supplemented with 30 g/l sucrose and 7 g/l agar. The pH of the culture medium was 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 were added to the MS medium, according to Table 1. 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 1mg NAA/ml of solution. Likewise, BAP was dissolved in ethanol (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

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

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 parameters evaluated in the experiment were plant height (cm), number of leaves, root length (cm) and number of roots.

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 statistically different. Results of all parameters were expressed as means from four replications with standard error (± SE).

3. RESULTS AND DISCUSSION

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

3.1 Results

3.1.1 Effect of different combination of NAA and BAP on plantlet height

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-T_{02} (9.1 cm) and the smallest height with Stemster-T_{10} (0.6 cm). Treatments without BAP showed the strongest growth (Fig. 1). The analysis of variance indicates a very significant difference between treatments (*P<.001*), as well as between varieties (*P=.003*). The two varieties Atlas and PAMELA had the fastest growth. T_{02} and T_{03} treatments are the best media for the rapid growth of the vitro-plants stem for the studied varieties.

3.1.2 Effects of different combinations of NAA and BAP combinations on the number of leaves per plantlet

The general average obtained for the number of leaves, independent of variety and treatment, is 10.6 (Table 3). The highest number of leaves was obtained in the variety PAMELA-T_{21} (20.8) and lowest in STEMSTER-T_{11} (3.0). The analysis of variance reveals no significant difference between the four varieties, but shows a very significant difference between the culture media (*P<.001*). T_{21}, T_{22} and T_{23} treatments, which combine 0.5 mg/l BAP with 0.25 to 1 mg/l NAA are the most effective.

Table 2. *In vitro* plant height as affected by genotype and combination of NAA and BAP in MS medium

	Treatments/Plant height (cm)																	
Varieties	**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}**	**Aver.**	**LSD**	***P***
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^{b}	0.68	0.003
PAMELA	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^{b}		
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^{a}		
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^{b}		
Aver.	4.7^{b}	2.9^{a}	5.2^{bc}	6.3^{c}	6.5^{c}	2.4^{a}	1.9^{a}	2.0^{a}	2.8^{a}	2.6^{a}	2.0^{a}	2.4^{a}	2.3^{a}	2.8^{a}	2.6^{a}	**3.3**		
LSD	1.31																	
P	<.001																	

Student-Newman-Kheul, Least significant differences of means (5% level). The values followed by the same letters (on the same line or on the same column) are not significantly different at the 5% level

Table 3. Number of leaves per plantlet as affected by MS media supplemented by different combinations of NAA and BAP

	Treatments																
Varieties	**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}**	**Aver.**	**LSD**
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^{ab}	8.5^{ab}	9.3^{abc}	10.3^{abcd}	9.8^{abcd}	8.3^{ab}	5.6^{a}	7.0^{ab}	11.1^{abcd}	9.8^{abcd}	12.1^{bcde}	16.2^{e}	14.9^{de}	14.5^{cde}	12.4^{bcde}	10.6	
LSD	3.47																
P	<.001																

Student-Newman-Kheul, Least significant differences of means (5% level). The values followed by the same letters (on the same line or on the same column) are not significantly different at the 5% level

Table 4. Number of roots per plantlet as affected by genotype and NAA and BAP combination in MS media

	Treatments/number of roots																	
Varieties	**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}**	**Aver.**	**LSD**	***P***
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^{b}	1.06	<.001
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^{b}		
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^{a}		
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^{a}		
Average	1.1^{a}	1.0^{a}	16.6^{c}	2.8^{ab}	2.7^{ab}	0.1^{a}	3.0^{ab}	4.5^{b}	0.0^{a}	0.0^{a}	0.1^{a}	0.4^{a}	0.6^{a}	0.0^{a}	0.0^{a}	2.2		
LSD	2.05																	
P	<.001																	

Student-Newman-Kheul, Least significant differences of means (5% level). The values followed by the same letters (on the same line or on the same column) are not significantly different at the 5% level

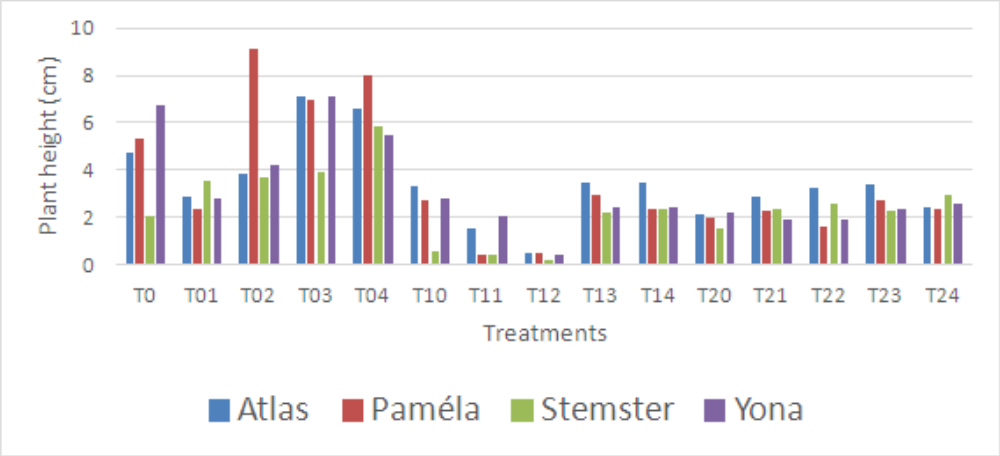

Fig. 1.The height of vitroplant as affected by combination of NAA and BAP in MS medium

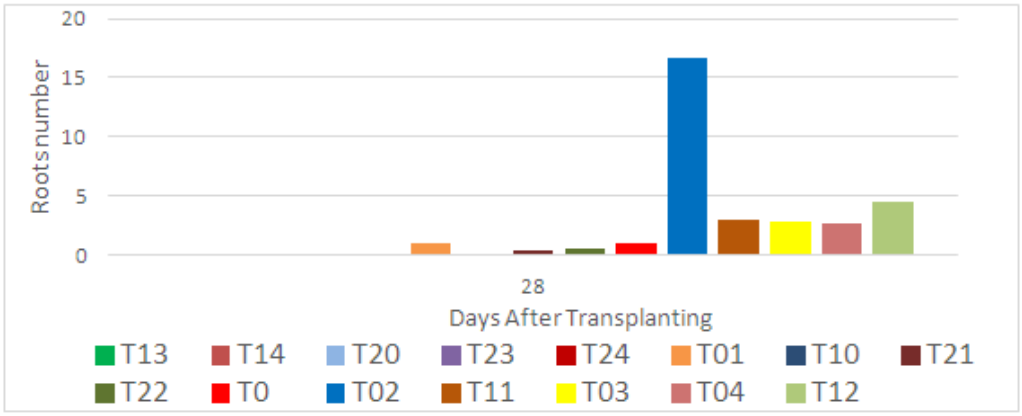

Fig. 2. Number of roots as affected by treatments in potato, 28 days after transplanting

Fig. 3. Potato roots proliferation as affected by the combination of NAA and BAP. *Left: Pamela with 0.5 mg/l of NAA. Right: Pamela with 1-2 mg/l de NAA*

3.1.3 Effects of different combinations of NAA and BAP on the number of roots

The general average, for all treatments and all varieties combined, is 2.2 roots after 28 days of *in vitro* growth (Table 4). 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. Treatment T_{12} and T_{02}, with a high concentration of NAA (0.5 mg/l) showed the maximum number of roots (Figure 2). Atlas and Pamela varieties produced more roots than YONA and STEMSTER varieties (Figure 3). The results show a large difference between the T_{02} treatment (16.6 roots/plantlet) and the rest of the treatments. The lowest average is obtained by the treatment T_{10} (0.05 roots/plantlet) and the T_{13}, T_{14}, T_{23} and T_{24} treatments did not produce roots.

3.1.4 Effects of different combination of NAA and BAP on roots length

The general average root length, for all varieties and all treatments combined, is 1.3 cm (Table 5). Root lengths vary between 0 cm (T_{13}, T_{14} and T_{24}) and 8.3 cm for ATLAS and YONA (T_{03} and T_{04}). The analysis of variance reveals highly significant differences between 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 (Figure 4). The dose of 0.5 mg/l NAA alone, increases the number of small roots. Doses below 0.5 mg/l were not effective. The combination of NAA and BAP has been shown to have inhibitory effect on root growth. The ATLAS and YONA varieties were the best (Tables 6).

3.2 Discussions

3.2.1 Plantlet height

Stem elongation is important, increasing the rate of multiplication over time and shortening the time required for subculturing regenerated plantlet *in vitro.* The combinations of NAA and BAP affected the stem length with highly significant difference among them and among varieties. The sizes of the vitroplants are larger from T0 to T04. In these four treatments, the hormonal balance is in favor of NAA. All other treatments with BAP, even at a concentration of 0.25 mg/l,

Table 5. Root length of potato plantlet as affected by the combination of NAA and BAP

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 6. Root length as affected by potato genotype

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

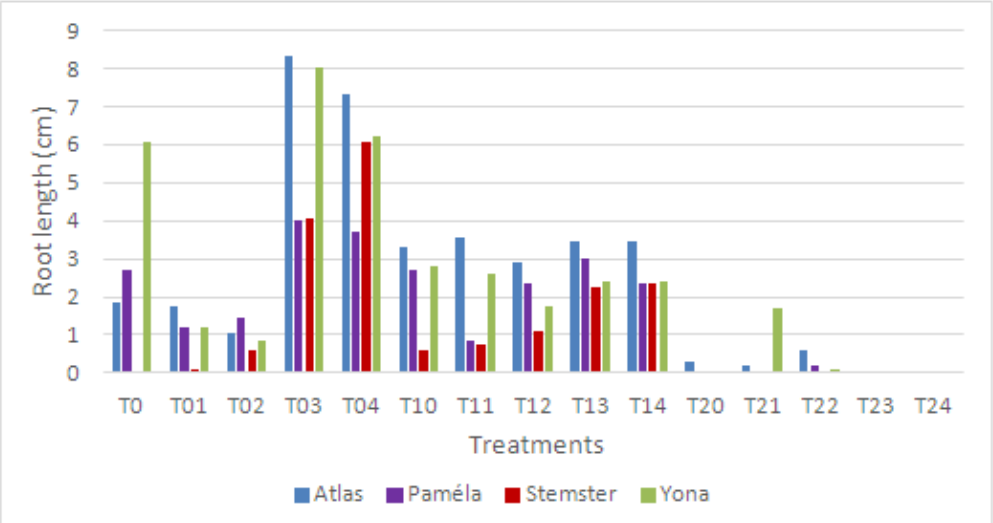

Fig. 4. Potato plantlet roots length as affected by different combinations of NAA and BAP

significantly inhibit stem growth. This result is consistent with the data reported by Sota et al. [7] who showed that BAP concentrations higher than 1 mg/l caused the decrease in biometric parameters except the number of leaves. Statistical analyzes show that the T02, T03 and T04 treatments constitute the best hormonal balance for the optimal growth of the vitroplants of the potato varieties studied. Mehmood et al. [8] and Xhulaj [9] reported better plantlet development in terms of shoot height (8.7 cm) with low concentrations of NAA (0.02 mg/l), but in the presence of gibberellic acid (0.2 mg/l). In fact, gibberellic acid (GA3), like others gibberellins, is a plant hormone that regulates various developmental processes, including stem elongation. However, authors in [6] 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 grown in a culture medium supplemented with jasmonic acid (JA) were taller compared to other plant growth regulators treatments. The results also showed that the control treatments, without growth hormone, of the different varieties, gave appropriate heights. These results are similar to those of Hamadou [10] and Salifou [11] who obtained the highest height in the control variant, without growth hormone.

3.2.2 Number of leaves per plantlet

The number of leaves per plantlet did not differ significantly between genotypes (*P=.072*). Nevertheless, the PAMELA variety had the highest number of leaves (11.3) and ATLAS the lowest (9.1). However, there were significant differences between hormonal combinations (*P<0.001*). T_{21}, T_{22} and T_{23} treatments with the highest dose of BAP (0.5 mg/l) and NAA between 0.25 to 2 mg/l were the most efficient. These results showed that the doses of BAP lower than 0.5 mg/l were not efficient but NAA is necessary. This result is in agreement with those of Mohapatra et al. [12] who obtained the best clumps with BAP in the presence of small amounts of indole 3-acetic acid, just like authors in [13] who obtained the same result with BAP but in the presence of a large quantity of NAA (3 mg/l). Kumlay et al. [6] found that a single application of NAA and BAP, even in the presence of gibberellic acid (GA_3) did not significantly improve the number of leaves per explant. In their experience, jasmonic acid was necessary to stimulate leaf proliferation. The highest number of leaves was obtained with PAMELA cultivar (20.8), T_{21} 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 T_{11} treatment (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 for leaf production for the tested varieties.

3.2.3 Rhizogenesis

Statistical analysis of our results showed a highly significant difference (*P<.001*) between treatments at JAR 28. The MS medium,

supplemented with NAA alone at a rate of 0.5 mg/l (T_{02}), result in the highest number of roots (16.6). It is the best medium for root proliferation. Increasing the dose of NAA or introducing BAP, considerably reduces rhizogenesis. This result is consistent with data reported by Mohapatra et a*l.* [12] which obtained roots proliferation with only indole 3-butyric acid and Hajare et al*.* [13] which obtained the best rhizogenesis with a combination of IBA and IAA alone. Some authors [14] have shown that supplementation of media with only one type of auxin was less effective 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 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. [15] who showed that the addition of BAP to a medium containing auxin decreases the number of roots. The culture media with a high concentration of NAA (1-2 mg/l) resulted in lower rooting (T_{21}, T_{24}, T_{23}, T_{14}).

Finally, with regard to the length of the roots, the statistical analysis of the result showed a highly significant difference (*P<.001*) between the treatments; MS medium enriched with only 1 mg/l NAA (T_{03}) followed by MS+ supplemented with 2 mg/l NAA (T_{04}) were most favorable for root length, the values obtained being 6.1 cm and 5.8 cm respectively. The shortest length was obtained by the T_{20} treatment (0.07cm). This is similar to the results of Khadiga et al*.* [16] who obtained the longest roots (13.7cm) using an MS medium supplemented only with IBA at 0.5 mg/l. In the YONA variety, the control variant gave satisfactory result for root length (6.06cm), so even a medium without exogenous growth hormone is favorable for the rooting of plantlet in this variety. This is in agreement with the results of Belguendouz [17] who showed that root length is not only influenced by the presence of growth regulators in the MS medium.

4. CONCLUSION

The response of different combinations of NAA and BAP on the *in vitro* micropropagation of the four potato varieties (PAMELA, ATLAS, YONA and STEMPTER) 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, number of leaves, number and length of roots). From the above discussion, it 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 can be said that T_{02} and T_{03} treatments are the best treatments for the four varieties tested. These treatments have shown the best performance for most 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 T_{12} and T_{02}, with high concentration of NAA (0.5 mg/l) 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, stimulated the increase in root length. The dose of 0.5 mg/l NAA alone increased the number of small roots. Doses lower than 0.5 mg/l were not effective.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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