Original Research Article

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

Abstract

The main limitation of potato (*Solanum tuberosum* L.) production in Niger is external dependence for the supply of quality seeds. In the context of a national seed potato system, one of the critical phases is the rapid multiplication of virus-free *in-vitro* potato good quality 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 this ~~these~~ work is to determine a suitable hormonal combination and optimum concentrations for production of a high number of In-vitro plantlets for four farmer-preferred varieties ATLAS, PAMELA, STEMSTER and YONA, in Niger. Uninodal stem explant of vitro plantlets were cultured on full-strength Murashige and Skoog media (MS) fortified with fourteen different combinations of α-Naphthalene Acetic Acid NAA (0; 0,25; 0,50; 1,0; 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 showed that the varieties, BAP and NAA combinations and varieties vs combinations were highly significant for plant height, number of leaves and number of roots. Treatments with NAA alone without BAP stimulated rhizogenesis. From 0.25 to 0.5 mg/l there is a proliferation of the roots and from 1 mg/l there is an elongation of the roots length. Cultivars Pamela and Atlas showed better root production.

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

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1. Introduction

The potato plays a key role in the global food system. According to FAO DATA [1], annual production of potato, ranging from 300 to 400 million tons, is achieved in countries with large populations, with 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 to 7,623 tons in 2000, then to 97,510 in 2014 and peaked in 2018 at 168,000 tons [Ref]. Yields still low, vary between 7 and 15 tons per hectare.

In Niger country, most potato production takes place in the highlands in the north of the country (Agadez), by small scale farmers using traditional means to propagate potato through the use of all-comers tubers. The extension of production is strongly limited by external dependence for 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 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 Murachige and Skoog [2] medium, potato plantlet has the ability to grow without exogenous growth hormones. But the use of MS supplemented with various combinations of exogenous plant growth regulator is known to boost highly micopropagation. However, the result is variable, depending on the variety [3, 4, and 5]. This requires, as a prerequisite, to conduct 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

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of *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* 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 tube containing each 20 ml of MS medium, supplemented with 30 g/l of sucrose and 7 g/l of agar. The pH was set 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 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 1mg NAA/ml of solution. Likewise, BAP was dissolved in ethanol (96°). 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/m^2/s). The results of

experiment were recorded as plant eight (cm), leaf number, root length (cm) and root number. The fresh weights of the leaves and roots were measured and the dry weights determined after a passage in the oven for 48 hours at 105°C.

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

Table 1: Different combinations of NAA and BAP added to 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 to the culture media. This result appreciates the additional effects of exogenous hormones added to MS medium.

3.1. Average 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 (Figure 1). The analysis of variance indicates 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 (table 3). The treatments T_{02} and T_{03} are the best media for rapid stem growth of vitro-plants between studied varieties (table 4).

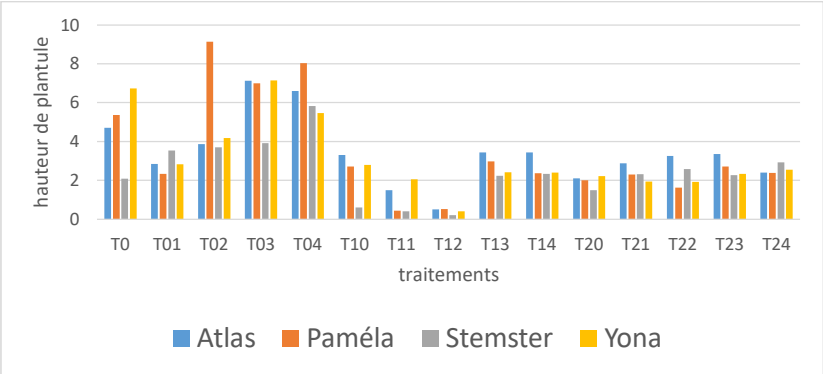

Figure 1: vitro-plantlet height as affected by NAA and BAP combination in MS medium

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

	Treatments																	
Varieties		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	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)

Table 3: Plantlet height by genotype of four 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

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]
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 NAA and BAP combinations on number of plantlets leaves

The general average obtained for the number of leaves, independently of the variety and the treatment, is 10.6 (Table 5). The greatest number of leaves was obtained with the variety Pamela-T_{21} (20.8) and the least with Stemster-T_{11} (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 T_{21}, T_{22} and T_{23}, combining 0.5 mg/l of BAP with 0.25 to 1 mg/l of NAA are the most effective (table 6).

Table 5: Number of leaves by plantlet of potato genotypes 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	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 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}
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 NAA and BAP combinations on roots number

The general average, 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. Treatment T_{12} and T_{02}, with high concentration of NAA (0.5 mg/l) showed the maximum number of roots (Table 8 and fig. 2). Atlas and Pamela varieties produce more roots than Yona and Stemster varieties (Table 9 and figure 3). The results show a large difference between the T_{02} (16.6) treatment and the rest of the treatments. The smallest average is obtained by treatment T_{10} (0.05) and the Treatments T_{13}, T_{14}, T_{23} and T_{24} did not produce a root.

<u>Table 7:</u> Number of root by plantlet as affected by genotype and NAA and BAP combination in MS media

	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	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: Roots number by plantlet as affected by NAA and BAP combinations in MS media

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

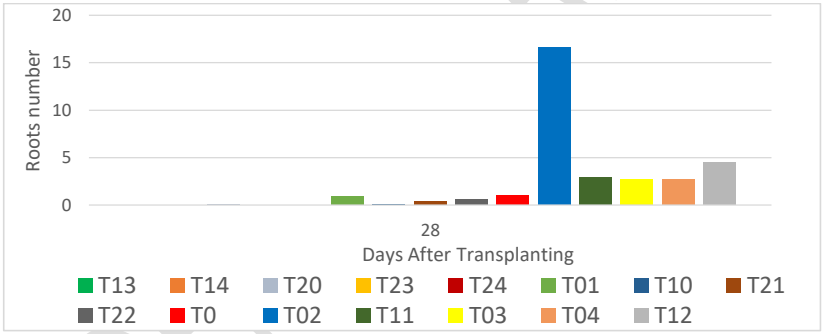

Figure 2: Number of roots as affected by treatments in potato, 28 days after transplanting

Table 9: Root number by plantlet of potato genotypes

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: roots proliferation in potatoes as affected by NAA and BAP in addition to MS media.

Left: Pamela with 0.5 mg/l of NAA. Right: Pamela with 1-2 mg/l de NAA

3.4. Effects of NAA and BAP combinations on roots length

The general average root length, all varieties and all treatments combined, is 1.3 cm (Table 10). 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 the treatments (*P<.001*), between the four varieties (*P<.001*) and for the variety-culture medium interaction (*P=.006*). NAA alone, at highest doses of 1 to 2 mg/L, stimulates root length extension (fig. 4).

The dose of 0.5 mg/l alone increases the number of small roots. Doses below 0.5 mgL-1 were not effective. The association NAA and BAP was shown to be depressive for the growth in root length. The ATLAS and YONA varieties were the best (Tables 11).

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 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

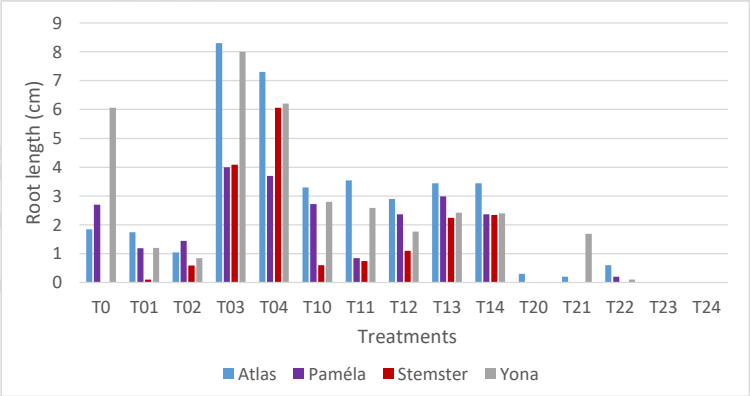

Figure 4: Potato plantlet Roots length as affected by different combinations of NAA and BAP added in MS media

4. Discussion

4.1. Plantlet height

Stem elongation is important, increasing the rate of multiplication over time and shortening the time required for the tissue culture plant to be transferred. Combinations of NAA and BAP affect stem length with highly significant difference among them and among varieties. The sizes of the vitro-plants are larger from T0 to T04. In these four treatments, the hormonal balance is in favor of NAA. All the other treatments with BAP, even at a concentration of 0.25 mg/l, significantly inhibit the stem growth. This result is in agreement with Sota *et al.* [6] who find higher concentrations of BAP of 1 mg/l caused decrease in biometric parameters except leaves number. Statistical analyzes show that the T02, T03 and T04 treatments constitute the best hormonal balance for optimal growth of vitro-plants of the potato varieties studied. Mehmood *et al.* [7] and Xhulaj [8] reported better plantlet development in 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), as others gibberellins, is a plant hormone regulating various developmental processes, including stem elongation. However, Kumlay *et al.* 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 plantlets of potato grown in a medium supplemented with Josmonic 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 heights. These results are similar to those of Hamadou [9] and Salifou [10] who obtained the longest height with the control, without growth hormone.

4.2. Number of Leaves per Plantlet

The number of leaves per plantlet did not differ significantly across genotypes (*P=.072*). Nevertheless, the PAMELA variety had the highest number of leaves (11.3) and ATLAS the lowest (9.1). However, there were significantly different among hormonal combinations (*P<0.001*). Treatments T_{21}, T_{22} and T_{23} with the highest dose of BAP (0.5 mg/L) and NAA between 0.25 to 2 mg/L were the best efficient. These result showed that the doses of BAP under 0.5 mg/L were not efficient but NAA is necessary. This result is in agreement with those of Mohapatra *et al.* [11] who obtain the best clumps with BAP in the presence of small amounts of Indole 3-Acetic Acid, just like Hajare *et al.* [12] who obtain the same result with BAP but in the presence of a large quantity of NAA (3 mg/L). Kumlay *et al.* [5] found that a single application NAA and BAP, even in the presence of gibberellic acid (GA_3) did not significantly improved number of leaves per explant. In their experience, Jasmonic Acid was necessary to boost leaves proliferation. The highest number of leaves was obtained with the variety PAMELA cultivar (20.8), treatment T_{21} (NAA: 0.25 mg/L and BAP: 0.5 mg/L). The lowest number was obtained with STEMSTER cultivar (3.0 leaves), with treatment T_{11} (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) were required, in presence of NAA, for leaves production for the varieties tested.

4.3. Rhizogenesis

Statistical analysis shows a highly significant difference (*P<.001*) between treatments at JAR 28. The MS medium (T_{02}), supplemented with NAA alone at rate of 0.5 mg/L, had 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 with Mohapatra *et al.* [11] who obtained roots proliferation with only Indole 3-Butyric Acid and

Hajare *et al.* [12] which had 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 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. The results also reveal 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 media with high concentration of NAA resulted in less rooting (T_{21}, T_{24}, T_{23}, T14), with 1-2 mg/L de NAA.

Finally, with regard to the length of the roots, the statistical analysis showed a highly significant difference (*P<.001*) in the treatments and the MS medium enriched with only 1mg/l of NAA (T_{03}); followed by the medium MS+2mg/l of NAA (T_{04}) were the most favorable for the length of the roots with respectively 6.1 cm and 5.8 cm. The smallest length is obtained by the T_{20} treatment (0.07cm). This is similar to the results of khadiga *et al.*[15] who obtained the longest roots (13.7cm) using an MS medium supplemented with only IBA at 0.5 mg/l. With the YONA variety, the control gave an interesting result for the length (6.06cm), so even a medium without exogenous growth hormone is favorable for the rooting of 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 MS medium.

5. Conclusion

The response of different combinations of NAA and BAP on *in vitro* micropropagation of the four varieties of potato (PAMELA, ATLAS, YONA and STEMPTER) was evaluated in 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 leaf, root number and length). From the above discussion, it is revealed that a genotype is dependent on *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 that the treatments T_{02} and T_{03} are the best treatments for the four varieties tested. These treatments have shown 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 produce more roots than YONA and STEMSTER varieties. Treatment T_{12} and T_{02}, with high concentration of NAA (0.5 mg/l) showed the maximum number of roots. ATLAS and PAMELA varieties produce more roots than YONA and STEMSTER varieties. NAA alone, at highest doses of 1 to 2 mg/L, stimulates root length extension. The dose of 0.5 mg/l alone increases the number of small roots. Doses below 0.5 mgL-1 were not effective.

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