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EFFECT OF HORMONE BALANCE ON THE *IN VITRO* GROWTH OF POTATO PLANTLETS

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Abstract: The hormone balance played a determining role in the initiation, maintenance of viability of cultures and of the regenerative capacity of potato caulinary apices. The combined action of auxins and cytokinins proved to be more efficient in the regeneration, growth and multiplication of potato plantlets of the varieties Desirée, Ostara and Sante, than phytohormones used individually. Of all tested combinations and variants of phytohormones, the MS medium supplemented with kinetin 1 mg/l, indolylbutyric acid 0.5 mg/l, and glycine 1 mg/l proved to be the most efficient for the *in vitro* regeneration and growth of potato plantlets varieties Desirée, Ostara and Sante.

Introduction

Conventional methods for the conservation of biological plant material in gene banks, either in the form of seeds, pollen or plant multiplication organs, or as clone collections in the field, do not ensure the conservation of the whole range of plant material of theoretic and practical interest [4,8]. On the other hand, plants preserved as collections in the field, at high densities, are at risk to be destroyed apathogenic agents, unfavorable climatic factors, as well as by natural disasters [5].

The impossibility to solve these disadvantages of conventional conservation methods, as well as the necessity to preserve plant resources, have led to the elaboration of unconventional methods for their storage [1,2,3,9,10].

In addition to the major implications in achieving genetic variability, in obtaining pharmacologically active substances, as well as virosis free plants and plants resistant to diseases and pests, tissue cultures also offer the possibility to preserve plants with particular qualities in order to maintain unaltered the genetic characteristic of plants submitted to conservation, and subsequently, the possibility to multiply them by micropropagation [7,11,12].

This paper refers to the induction and multiplication of *in vitro* culture in three potato varieties (*Solanum tuberosum* L, cv. Desirée, Ostara and Sante).

Material and methods

The plant material used in experiments for the initiation of in vitro potato cultures *(Solanum tuberosum* L*)* consisted of shoots grown from the buds existing on potato tubercles. In order for shoots to grow, tubercles were kept in the dark at the temperature of 25°C. When they reached approximately 5-6 cm, shoots were detached from the tubercle in order to initiate the in vitro culture. For this purpose, shoots were

kept under running water for 60 minutes, after which they were desinfected with 5% calcium hypochlorite for 30 minutes and washed several times with sterile water. Subsequently, shoots were shaped into uninodal explants that were inoculated on the MS [6] culture medium supplemented with vitamins (thiamine HCl, pyridoxine HCl and nicotinic acid) 1 mg/l of each, meso-inositol 100 mg/l, saccharose 20 g/l and agar 7 g/l (noted as V_0 = medium free of growth regulators); the pH of the medium was adjusted before autoclaving at 5.6. Medium V_0 was used as control medium compared to the media containing different growth regulators. The conditions of incubation of the cultures for the testing of their regenerative capacity consisted of a photoperiodic regime of 16 light hours/ 24 hours and a light intensity of 39 $\mu E.m^{-2}.s^{-1}$; environmental temperature corresponded to 25±1°C.

In order to enhance regeneration of potato plantlets, the regenerative efficiency of a number of culture media variants with cytokinins was tested, the composition of which is given below: V_0 = MS without growth regulators; V_1 = MS + BA (benzyladenine) 0.1 mg/l; V_2 = MS + BA 0.5 mg/l; V_3 = MS + BA 1 mg/l; V_4 = MS + BA 1.5 mg/l; V_5 = MS + K (kinetin) 0.1 mg/l; V_6 = MS + K 0.5 mg/l; V_7 = MS + K 1 mg/l; V_8 = MS + K 1.5 mg/l; V_9 = MS + Z (zeatin) 0.1 mg/l; V_{10} = MS + Z 0.5 mg/l; V_{11} = MS + Z 1 mg/l; V_{12} = MS + Z 1.5 mg/l.

The variants of auxin media were the following: V_0 = MS without growth regulators; V_1 = MS + NAA (1-naftaleneacetic acid) 0.1 mg/l; V_2 = MS + NAA 0.5 mg/l; V_3 = MS + NAA 1 mg/l; V_4 = MS + NAA 1.5 mg/l; V_5 = MS + IAA (indolylacetic acid) 0.1 mg/l; V_6 = MS + IAA 0.5 mg/l; V_7 = MS + IAA 1 mg/l; V_8 = MS + IAA 1.5 mg/l; V_9 = MS + IBA (indolylbutyric acid) 0.1 mg/l; V_{10} = MS + IBA 0.5 mg/l; V_{11} = MS + IBA 1 mg/l; V_{12} = MS + IBA 1.5 mg/l.

The observations were made four weeks after inoculation, and the length of the regenerated plantlets, the number and length of roots were evaluated.

Results and discussion

Role of cytokinins in regeneration and organogenesis

In vitro cytokinins promote differentiation and multiplication of cells and tissues, they induce caulogenesis processes, having an antagonic action against auxins.

Regeneration of plantlets from apical explants taken from potato plantlets of different varieties was realised on the MS medium supplemented with cytokinins, described in the chapter Material and methods.

Results regarding the influence of cytokinins on the growth of potato plantlets included in the study can be seen in Fig. 1a, b, c.

In the case of the Desirée variety (Fig. 1a), the best results regarding plantlet lengths of 9.9 cm and 9.2 cm were recorded on media zeatin in the case of the 0.5 mg/l and 1 mg/l concentrations. A similar result was obtained by using the 0.5 mg/l kinetin medium, where the length of the plantlets was 9.8 cm.

In the case of the Ostara variety (Fig. 1b), the best results for plantlet length were obtained on 0.5 mg/l zeatin media (10.4 cm) and 1 mg/l kinetin media (10.25

cm) and. Also, zeatin was found to cause good growth in length of plantlets regardless of the concentration used of 0.1 mg/l, 0.5 mg/l, 1 mg/l or 1.5 mg/l. Of the tested cytokinins, benzyladenine in 1.5 mg/l concentration had the weakest effect on growth in potato plantlets, their length being of 3.4 cm.

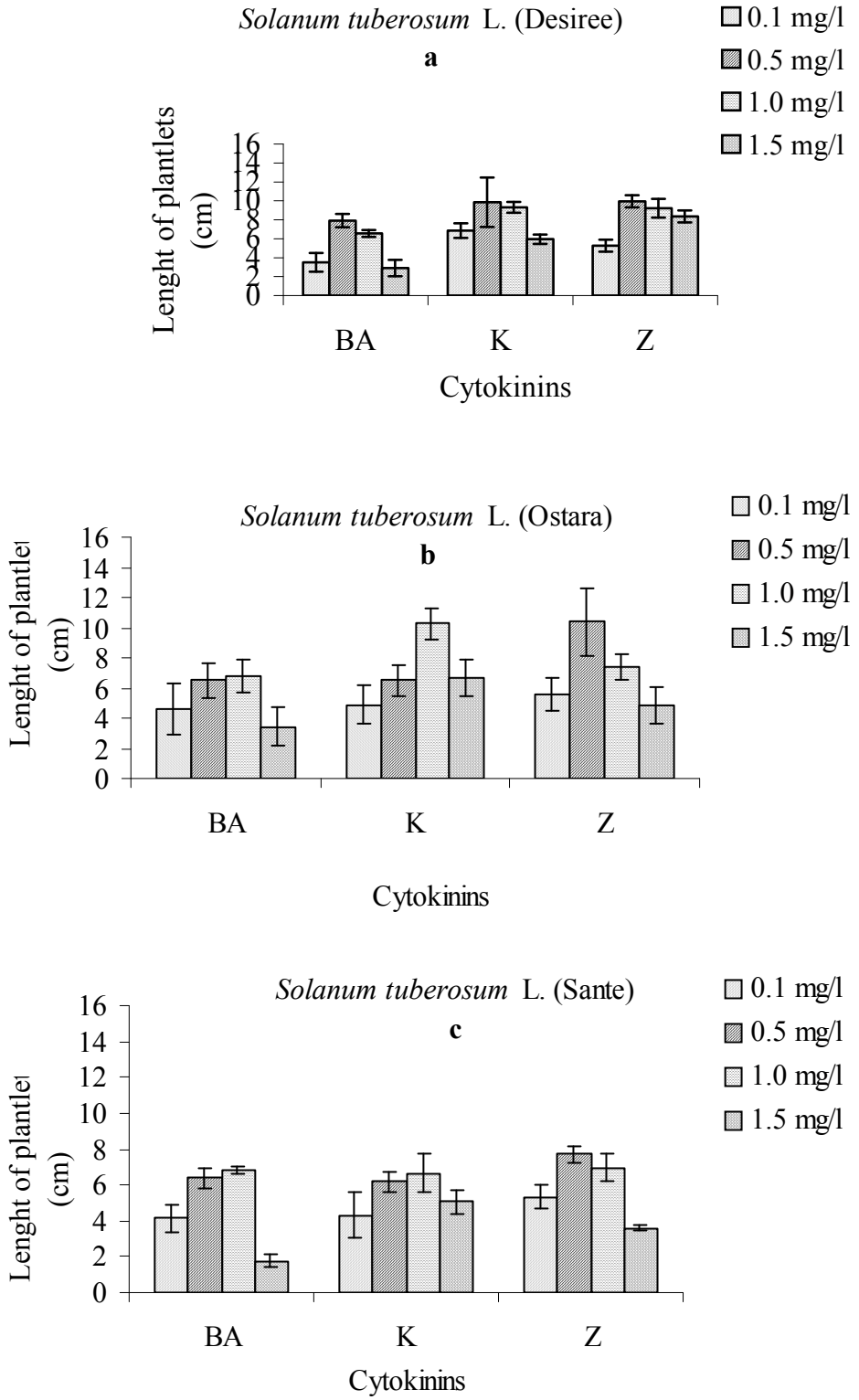

Fig. 1a, b, c.: Influence of cytokinins on the in vitro regeneration and growht of potato plantlets (Solanum tuberosum L.) cv. Desirée (a), Ostara (b) and Sante (c).

The plantlets of the Sante variety (Fig. 1c) showed a weaker growth in length compared to the two other varieties tested, regardless of the cytokinin type, benzyladenine, kinetin or zeatin. Results concerning the length of plantlets were relatively similar. Thus, values ranged between 6.2 cm and 7.7 cm in all cytokinins tested at concentrations of 0.5 mg/l and 1 mg/l.

It is known that for plantlet regeneration and growth *in vitro*, in addition to cytokinins, which stimulate growth through cell divisions, auxins are equally necessary, as they stimulate both growth, by cell elongation, and rhizogenesis.

Role of auxins in regeneration and organogenesis

In the case of *in vitro* cultures, auxins favor rhizogenesis and callus formation.

The *in vitro* growth of potato plantlets (varieties Desirée, Ostara and Sante) on media containing the mentioned auxins (Material and methods) is presented in Fig. 2a, b, c.

Regarding the Desirée variety (Fig. 2a) auxins NAA and IBA exerted a similar action on the growth in length of plantlets regardless of the tested concentration, values ranging between 3.3 cm (NAA 1 mg/l) and 4.4 cm (IBA 0.5 mg/l). The best result was obtained on 0.5 mg/l IAA media, 5.6 cm.

In the Ostara variety (Fig. 2b), an aspect that should be mentioned is the fact that in the case of the three auxins used (NAA, IAA and IBA), the concentrations of 1 mg/l and especially 1.5 mg/l had a lower effect on the growth in length of plantlets.

In the Sante variety (Fig. 2c), we mention as a general aspect a more reduced effect of the three auxin types tested. In the case of IAA and IBA, length values ranged between 1.25 cm (IAA 0.1 mg/l) and 3.6 cm (IBA 0.5 mg/l). The highest value of plantlet length, 4.2 cm, was found in NAA media in 1 mg/l concentration.

Given these results, the next experiments used media with a mixture of cytokinins and auxins, testing various hormone balances.

Combined action of cytokinins, auxins and gibberellin

Based on the results obtained regarding the role of cytokinins and auxins tested individually in the regeneration and growth of potato plantlets from the varieties Desirée, Ostara and Sante, the combined action of these phytohormones was investigated, as well as in combination with gibberellic acid (GA_3).

For the stimulation of regeneration and organogenesis processes, gibberellic acid was used, as in general gibberellins are known to have a synergic action with that of auxins and to suppress the effects of some plant growth inhibitors. Regeneration and growth of potato plantlets (Desirée variety) on media containing auxins, cytokinins and gibberellic acid is shown in Table 1.

The analysis of the data presented in Table 1 shows that the mean number of plantlets/vial is one. Another parameter monitored in addition to the number of plantlets, was the length of plantlets, which showed extremely different evolutions depending on the composition of the culture medium.

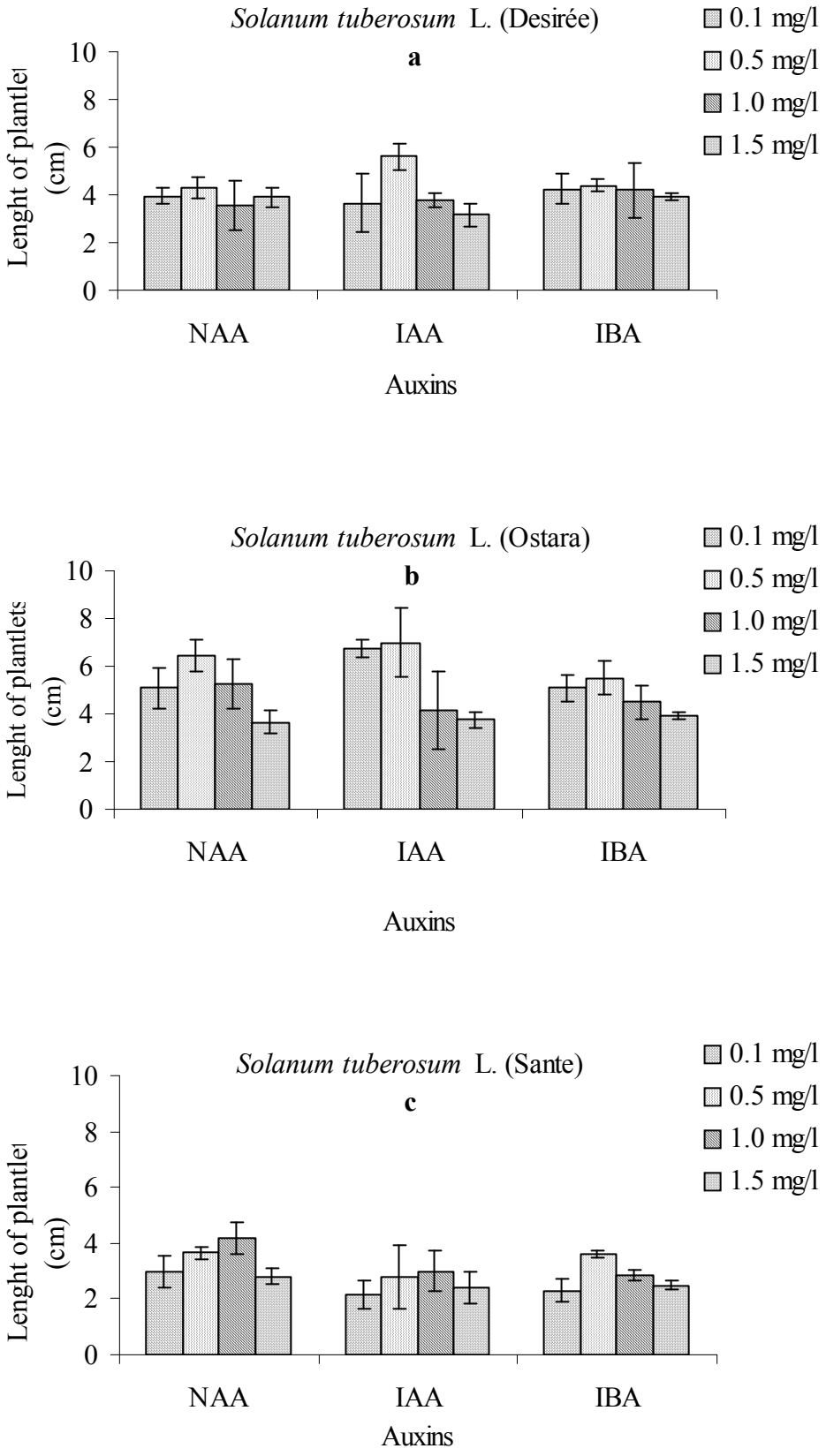

Fig. 2a, b, c: Influence of auxins on the in vitro regeneration and growht of potato plantlets (Solanum tuberosum L.) cv. Desirée (a), Ostara (b) and Sante (c).

The greatest length of plantlets, 9.1 cm, was found on medium V_6 (MS + K 1 mg/l + IBA 0.5 mg/l + glycine 1 mg/l) and the shortest length, 2.9 cm, on medium V_8 (MS + Z 1 mg/l + IAA 0.5 mg/l + glycine 1 mg/l). Regarding the number of roots, the highest value, 7.85 roots/ vial, was obtained on the same medium V_6, on which the maximum length of plantlets was also obtained. In the case of the Desirée variety, roots were absent in plantlets on media V_7 (MS + Z 1 mg/l + NAA 0.5 mg/ + glycine 1 mg/l), V_{10} (MS + GA_3 1 mg/l + NAA 0.5 mg/l + Z 0.5 mg/l + glycine 1 mg/l) and V_{11} (MS + GA_3 1 mg/l + IAA 0.5 mg/l + Z 0.5 mg/l + glycine 1 mg/l). The highest value of root length, 6.55 cm, was found onmedium V_6, and the lowest value, 0.25 cm, on medium V_{12} (MS + GA_3 1 mg/l + IBA 0.5 mg/l + glycine 1 mg/l).

Table 1: Influence of hormonal balance on the maintenance of regenerative and organogenesis capacity in the case of potato plantlets, Desirée variety.

Nutritive medium (mg/l)	Plantlet formation		Root formation	
	Number of plantlets/vial	Length of plantlets (cm)	Number of roots/vial	Length of roots (cm)
V_0 (MS)	0.9 ± 0.14*	7.8 ± 0.56	2.9 ± 0.14	4.55 ± 0.49
V_1 (BA 1 + NAA 0.5 + glycine 1)	1.15 ± 0.21	5.75 ± 0.21	3.95 ± 0.35	2.25 ± 0.35
V_2 (BA 1 + IAA 0.5 + glycine 1)	1.1 ± 0.14	5 ± 0.28	3.2 ± 0.28	3.6 ± 0.14
V_3 (BA 1 + IBA 0.5 + glycine 1)	1.15 ± 0.21	7.85 ± 0.77	2.85 ± 0.21	2.65 ± 0.35
V_4 (K 1 + NAA 0.5 + glycine 1)	1 ± 0	5.8 ± 1.55	6.05 ± 0.35	5 ± 0.28
V_5 (K 1 + IAA 0.5 + glycine 1)	1.15 ± 0.21	8.9 ± 0.84	3.35 ± 0.49	6.2 ± 0.56
V_6 (K 1 + IBA 0.5 + glycine 1)	1.1 ± 0.14	9.1 ± 1.27	7.85 ± 1.2	6.55 ± 0.63
V_7 (Z 1 + NAA 0.5 + glycine 1)	1.15 ± 0.2	5.05 ± 1.2	0	0
V_8 (+ Z 1 + IAA 0.5 + glycine 1)	1.2 ± 0.28	2.9 ± 0.14	0.6 ± 0.28	0.7 ± 0.14
V_9 (Z 1 + IBA 0.5 + glycine 1)	1.15 ± 0.21	3.35 ± 0.49	0.75 ± 0.35	0.65 ± 0.49
V_{10}(GA_3 1 + NAA 0.5+Z 0.5+glycine 1)	1.15 ± 0.07	4.15 ± 0.91	0	0
V_{11}(GA_3 1 + IAA 0.5+Z 0.5+glycine 1)	1.2 ± 0.14	3.45 ± 0.35	0	0
V_{12}(GA_3 1+ IBA 0.5+Z 0.5+glycine 1)	1.3 ± 0.14	5.85 ± 0.21	1.65 ± 0.49	0.25 ± 0.07

Note: * standard deviation

The phytohormone combination formed by kinetin 1 mg/l, indolylbutyric acid 0.5 mg/l and glycine 1 mg/l (V_6) proved to be the most efficient in ensuring the growth in length of plantlets, as well as in terms of the number and length of roots.

Regeneration and growth of potato plantlets, Ostara variety, on complex media containing auxins, cytokinins as well as gibberellic acid is shown in Tab. 2. The best result in terms of efficiency of the growth in length of plantlets was found in medium V_6 (MS + K 1 mg/l + IBA 0.5 mg/l + glycine 1 mg/l) where the mean plantlet length was 17.89 cm, as well as in medium V_3 (BA 1 mg/l + IBA 0.5 mg/l + glycine 1 mg/l) with a length of plantlets of 13.09 cm.

In the case of the root system, i.e. regarding the number and length of roots, the results obtained were very different according to the phytohormone combination

tested. Thus, the greatest number of roots, 15.84/ vial, was obtained in plantlets from medium V_6 (MS + K 1 mg/l + IBA 0.5 mg/l + glycine 1 mg/l), and the smallest number of roots was obtained in plantlets from media V_8 (MS + Z 1 mg/l + IAA 0.5 mg/l + glycine 1 mg/l), V_9 (MS + Z 1 mg/l + IBA 0.5 mg/l + glycine 1 mg/l) and V_{10} (MS + GA_3 1 mg/l + NAA 0.5 mg/l + Z 0.5 mg/l + glycine 1 mg/l).

Plantlets from variants V_7 (MS + Z 1 mg/l + NAA 0.5 mg/l + glycine 1 mg/l) and V_{11} (MS + GA_3 1 mg/l + IAA 0.5 mg/l + Z 0.5 mg/l + glycine 1 mg/l) are characterized by the absence of the root system. Also, it should be mentioned that on variant V_6, where plantlets with the highest number of roots were obtained, the greatest plantlet length was also found, 8.93 cm. On the other variants of the tested media, lengths of roots ranged between 0.88 cm on V_8 (MS + Z 1 mg/l + IAA 0.5 mg/l + glycine 1 mg/l) and 7.25 cm on V_5 (MS + K 1 mg/l + IAA 0.5 mg/l + glycine 1 mg/l).

Table 2: Influence of hormonal balance on the maintenance of regenerative and organogenesis capacity in the case of potato plantlets, Ostara variety.

Nutritive medium (mg/l)	Plantlet formation		Root formation	
	Number of plantlets/vial	Length of plantlets (cm)	Number of roots/vial	Length of roots (cm)
V_0 (MS)	1.5 ± 0.70*	10.72 ± 0.45	4.07 ± 0.19	6.78 ±0.29
V_1 (BA 1 + NAA 0.5 + glycine 1)	1.20 ± 0.13	8.73 ± 0.26	5.81 ± 0.36	1.68 ± 0.27
V_2 (BA 1 + IAA 0.5 + glycine 1)	1.44 ± 0.10	7.91 ± 0.21	4.77 ± 0.19	4.87 ± 0.38
V_3 (BA 1 + IBA 0.5 + glycine 1)	1.93 ± 0.89	13.09 ± 0.16	5.06 ± 0.33	4.99 ± 0.12
V_4 (K 1 + NAA 0.5 + glycine 1)	1.2 ± 0.28	10.97 ± 1.05	7.95 ± 0.50	6.42 ± 1.02
V_5 (K 1 + IAA 0.5 + glycine 1)	1.83 ± 0.79	13.96 ± 1.18	5.92 ± 0.59	7.25 ± 0.27
V_6 (K 1 + IBA 0.5 + glycine 1)	1.22 ± 0.11	17.89 ± 0.7	15.84± 0.89	8.93 ± 0.25
V_7 (Z 1 + NAA 0.5 + glycine 1)	2.27 ± 1.23	7.9 ± 0.46	0	0
V_8 (+ Z 1 + IAA 0.5 + glycine 1)	1.7 ± 0.41	5.82 ± 0.55	0.81 ± 0.56	0.88 ± 0.16
V_9 (Z 1 + IBA 0.5 + glycine 1)	1.2 ± 0.28	5.61 ± 0.57	1.0± 0.21	2.55 ± 1.2
V_{10}(GA_3 1 + NAA 0.5+Z 0.5+glycine 1)	1.2 ± 0.28	6.91 ± 1.37	0.98 ± 0.30	2.35 ± 1.28
V_{11}(GA_3 1 + IAA 0.5+Z 0.5+glycine 1)	1.67 ± 0.76	5.82 ± 0.59	0	0
V_{12}(GA_3 1+ IBA 0.5+Z 0.5+glycine 1)	2.55 ± 0.63	8 ± 0.43	2.87 ± 0.67	1.71 ± 0.5

Note: * standard deviation

Results concerning the influence of hormone balance on the maintenance of regenerative and organogenesis capacity in the case of potato plantlets, Sante variety, are shown in Table 3.

In the Sante variety, a weaker development of plantlets was seen in all medium variants, in the case of all phytohormone combinations tested. The greatest length of plantlets, 8.05 cm, was found on variant V_6 (MS + K 1 mg/l IBA 0.5 mg/l + glycine 1 mg/l) as well as in the case of Desirée and Ostara varieties. The same medium V_6 proved to be the most efficient regarding both the number of roots (6.75) and their length (5.15 cm). Like in the case of the Desirée variety, in

the case of Sante variety the absence of the root system was found in plantlets from media V_7 (MS + Z 1 mg/l + NAA 0.5 mg/l + glycine 1 mg/l), V_{10} (MS + GA_3 1 mg/l + NAA 0.5 mg/l + Z 0.5 mg/l + glycine 1 mg/l) and V_{11} (MS + GA_3 1 mg/l + IAA 0.5 mg/l + Z 0.5 mg/l + glycine 1 mg/l).

Globally, plantlets belonging to the Sante variety presented a lower regenerative and organogenesis capacity compared to that recorded for Desirée and Ostara varieties.

Table 3: Influence of hormone balance on the maintenance of regenerative and organogenesis capacity in the case of potato plantlets, Sante variety.

Nutritive medium (mg/l)	Plantlet formation		Root formation	
	Number of plantlets/vial	Length of plantlets (cm)	Number of roots/vial	Length of roots (cm)
V_0 (MS)	0.65 ± 0.21*	6.05 ± 0.21	2.2 ± 0.28	4 ± 0.14
V_1 (BA 1 + NAA 0.5 + glycine 1)	0.75 ± 0.35	5.25 ± 0.49	3.05 ± 0.49	3 ± 0.42
V_2 (BA 1 + IAA 0.5 + glycine 1)	0.8 ± 0.70	6.15 ± 0.35	3.95 ± 0.07	3.9 ± 0.14
V_3 (BA 1 + IBA 0.5 + glycine 1)	1.15 ± 0.21	7 ± 0.42	2.85 ± 1.2	2.9 ± 0.56
V_4 (K 1 + NAA 0.5 + glycine 1)	1.25 ± 0.07	6.8 ± 1.27	6.3 ± 0.42	5 ± 0.42
V_5 (K 1 + IAA 0.5 + glycine 1)	1 ± 0	6.1 ± 0.56	3.55 ± 0.49	4.95 ± 0.35
V_6 (K 1 + IBA 0.5 + glycine 1)	0.4 ± 0.14	8.05 ± 0.91	6.75 ± 0.35	5.15 ± 0.49
V_7 (Z 1 + NAA 0.5 + glycine 1)	1.4 ± 0.14	5.65 ± 0.91	0	0
V_8 (+ Z 1 + IAA 0.5 + glycine 1)	0.9 ± 0.56	3.9 ± 0.98	0.75 ± 0.35	0.6 ± 0.14
V_9 (Z 1 + IBA 0.5 + glycine 1)	0.8 ± 0.56	3.3 ± 0.42	0.4 ± 0.14	0.85 ± 0.63
V_{10}(GA_3 1 + NAA 0.5+Z 0.5+glycine 1)	0.55 ± 0.35	5.5 ± 0.42	0	0
V_{11}(GA_3 1 + IAA 0.5+Z 0.5+glycine 1)	1.4 ± 0.56	4.95 ± 0.35	0	0
V_{12}(GA_3 1+ IBA 0.5+Z 0.5+glycine 1)	1.6 ± 0.14	5 ± 0.42	0.95 ± 0.49	0.75 ± 0.35

Note: * standard deviation

Conclusions

The results obtained following the studies on the role of hormone balance in the initiation, maintenance of culture viability and of the regenerative capacity of potato caulinary apices demonstrated the fact that the combined action of auxins and cytokinins was more efficient in the regeneration, growth and multiplication of potato plantlets belonging to the Desirée, Ostara and Sante varieties, than phytohormones used individually.

Of all tested combinations and variants of phytohormones, the MS medium supplemented with kinetin 1 mg/l, indolylbutyric acid 0.5 mg/l and glycine 1 mg/l proved to be the most efficient for the *in vitro* regeneration and growth of potato plantlets (Desirée, Ostara and Sante varieties).

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EFECTUL BALANȚEI HORMONALE ASUPRA CREŞTERII PLANTULELOR DE CARTOF IN VITRO

(Rezumat)

Prezenta lucrare se referă la inducerea culturii *in vitro* la trei soiuri de cartof (*Solanum tuberosum* L., cv. Desirée, Ostara şi Sante). Balanța hormonală a avut un rol determinant în inițierea, menținerea viabilității culturilor şi a capacității regenerative a apexurilor caulinare de cartof. S-a demonstrat că acțiunea combinată a auxinelor şi citochininelor a fost mai eficientă în atingerea parametrilor urmăriți, respectiv regenerarea, creşterea şi multiplicarea plantulelor de cartof aparținând soiurilor Desirée, Ostara şi Sante, decât fitohormonii utilizați individual. Dintre toate combinațiile şi variantele de fitohormoni testate, cea mai eficientă, în ce priveşte regenerarea şi creşterea *in vitro* a plantulelor de cartof soiurile Desirée, Ostara şi Sante s-a dovedit a fi mediul MS suplimentat cu chinetină 1 mg/l, acid indolilbutiric 0,5 mg/l şi glicină 1 mg/l.