Scientific Papers. Series A. Agronomy, Vol. LXVI, No. 2, 2023
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE INFLUENCE OF PLANT HORMONAL COMBINATION OVER DIFFERENT PARAMETERS OF GROWING AND DEVELOPMENT FOR POTATO PLANTLETS

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Abstract

The main objective of this experience was monitoring the effect of plant growth regulators and their combinations on potato plantlets development, respectively on the following parameters: plantlets height, leaves number, root length, weight of fresh plantlet and of root. Thus, during in vitro multiplication process, two auxins were tested: naphthalene acetic acid (NAA) and indolyl acetic acid (IAA) (in two concentrations 0.05% and 0.1%, for both auxins), together with a gibberellin: gibberellic acid (GA3) (0.02%) and in vitro behavior was observed for three potato varieties: Marvis, Castrum, Ervant under the influence of these growth hormones. The nutrient medium supplemented with IAA 0.05% determined plantlets obtaining with high height and with the highest value of fresh root weight. The culture medium containing 0.1% NAA was effective in forming the number of leaves, showing a positive influence. NAA 0.1% + 0.02% GA3 and IAA 0.05% + 0.02% GA3 combinations had a beneficial effect on root length and fresh plantlets weight.

Key words*: hormonal combination, in vitro, multiplication, potato, plantlets.*

INTRODUCTION

Micropropagation can be defined as in vitro clonal multiplication through tissue culture and rapid multiplication of plant material in order to obtain a large number of descending plants (Mohapatra, 2017).

Rapid multiplication is an extensive method used to increase the nuclear stock of seed potatoes. This technique is widely used in many countries (Murashige, 1974; Hussey, 1981) is very flexible and offers a high rate of multiplication. Also, by rapid multiplication, it is possible to produce the disease-free seed potato from the disease-carrying seed (Roca et al., 1978; Wang, 1982).

In general, the effectiveness of micropropagation depends on the explants and their source, the treatment of the explants during their preparation for *in vitro* culture, the composition of the culture media, the micropropagation and the performance of the regenerated seedlings. The organ that it will the source of tissue depends on the physiological or ontogenic age of the organ, the season in which the explants are taken, their size and the general quality of the parent plant (Murashige, 1974, cited by Mohapatra & Batra, 2017).

For plantlets production on the nutrient medium, different techniques have been used over the years, the basic methods being similar in most laboratories and is based on the rapid growth of a minicutting on a sterilized liquid or solid culture medium (Ranalli, 1997).

Minicuttings with a single leaf are inoculated on the surface of a solid medium (Espinoza et al., 1984). The axillary/apical buds grow quickly, so in 3-4 weeks a new plantlet regenerates that can be further subculture in a fresh environment. The use of nodal cuttings in vitro is probably the most common method of propagation, applied in the early stages of commercial seed potato production (Pruski, 2007).

Very important factors in micropropagation are: the photoperiod, the temperature in the growth room, the balanced combination of growth hormones. Successful *in vitro* multiplication depends on the presence of an appropriate combination of auxins with gibberellic acid (GA3) in the propagation medium (Kumlay, 2014). Roest and Bokelmann, cited by Kumlay, 2014, suggested that a lower concentration of auxin with GA3 (0.25 mg l^{-1}) had a positive impact on the development of potato plantlets shoots and roots. Zhang et al. (2005) (cited by Kumlay, 2014) suggest that increasing plantlets

length has been promoted among potato explants with increasing concentrations of IAA; however, the stimulatory effect of IAA was enhanced by the addition of GA3.
Auxins are involved in many physiological processes: they interact with other endogenous substances and, of course, with other phytohormones, especially cytokinins, gibberellins and ethylene (Cachiță-Cosma & Sand, 2000). Auxin synthesis is located in the very young leaves, in the active buds. At low concentrations, physiologically stimulating, auxins exert a beneficial effect on growth, while at higher concentrations, they can become toxic (Cachiță-Cosma, 2000). The physiological action of gibberellins, in general, can be summarized as follows: gibberellins elongate the internodes of the stems; plays an important role in regulating the endogenous level of auxin, etc. (Cachiță-Cosma, 2000).

MATERIALS AND METHODS

This study took place in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania. During in vitro multiplication process, an experiment was performed with reference to the influence of two auxins: naphthalene acetic acid (NAA) and indolyl acetic acid (IAA) (in two concentrations 0.05% and 0.1%, for both auxins), together with a gibberellin: gibberellic acid (GA3) (0.02%).
Thus, the bifactorial experience (3 x 8), on 3 repetitions had the following factors: experimental factor A - variety, with three graduations: a1 - Marvis, a2 - Castrum, a3 - Ervant and experimental factor B - multiplication medium (with 8 graduations): - b1 - naphthalene acetic acid (0.05%); b2 - naphthalene acetic acid (0.1%); b3 - indolyl acetic acid (0.05%); b4 - indolyl acetic acid (0.1%); b5 - naphthalene acetic acid (0.05%) with gibberellic acid (0.02%); b6 - acetic naphthalene acid (0.1%) with gibberellic acid (0.02%); b7 - indolyl acetic acid (0.05%) with gibberellic acid (0.02%); b8 - indolyl acetic acid (0.1%) with gibberellic acid (0.02%).
The experimental variants can be seen in the Figure 1.

Organizing the experience:

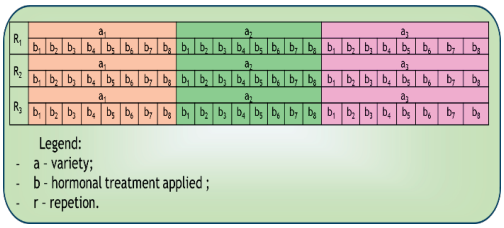

Figure 1. The scheme of experimental variants arranging

Microplants are used in seed potato production system. From healthy developed microplants (Figure 2) from each internode were obtained minicutings (Figure 3).

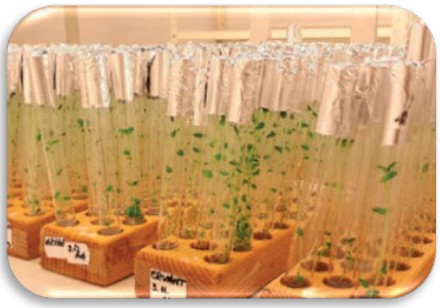

Figure 2. Developed plantlets

Figure 3. Minicuttings

All operations must be performed in the laminar air flow hood (Figure 4). The axillary/ apical buds inoculated (Figure 5) grow rapidly, so that in 3-4 weeks a plantlet regenerates which can be further subcultured on a fresh medium. After 30 days from the minicuttings inoculation on the 8 variants of culture medium, the following determinations were performed: plantlets length (cm), number of leaves/plantlets, root length

(cm), fresh plantlet weight (mg) and weight fresh root (mg).

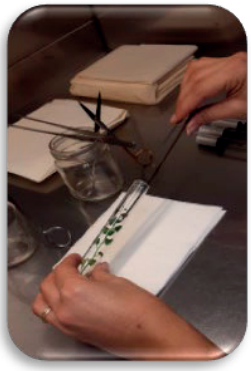

Figure 4. Multiplication in the laminar air flow hood

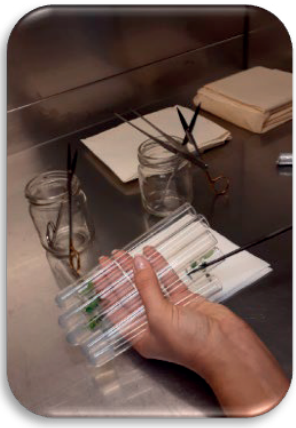

Figure 5. Inoculation of minicuttings

RESULTS AND DISCUSSIONS

Results were analyzed using MSTAT-C statistical package. Differences among the means were compared by the Duncan's Multiple Range Test at 1 level of significant.

Table 1 shows the behavior of the three experimented varieties, analyzed through the prism of the Duncan test. Regarding the height of the plantlets, Castrum variety (10.02 cm) can be distinguished, which differs significantly from the Marvis and Ervant varieties (7.92 and 7.31 cm).

For the second element analyzed, respectively, the number of leaves, Marvis variety (10.54) stands out, a variety that differs significantly from the Castrum variety (8.08 leaves).

Castrum and Marvis varieties (5.13 cm and 4.92 cm) stood out for their root length, differing significantly from the Ervant variety (2 cm).

Examining the results in terms of plantlets weight, we draw attention to the Marvis variety, which had a high capacity in plantlets mass formation (253.81 mg), differing significantly from the varieties Castrum and Ervant (170.96 and 120.68 mg).

From the analysis of the variety's behavior regarding the fresh root weight, Marvis and Castrum varieties can be noticed, which determine obtaining of high values (95.73 mg and 90.42 mg), detaching significantly from the Ervant variety (50.79 mg). Marvis and Castrum varieties have also been identified with high values in terms of root length.

Nutrient variants containing (Table 2) indolyl acetic acid (0.05%) together with gibberellic acid (0.02%), naphthalene acetic acid (0.05%), indolyl acetic acid (0.05%) had a positive influence on plantlets formation, which determined plantlets development with high height (9.83 cm; 9.44 cm; 9.22 cm), favorable in the process of multiplication. Naphthalene acetic acid (0.05%) together with gibberellic acid (0.02%) showed a strong inhibitory effect on plantlet growth (6.78 cm).

The hormonal combinations studied did not have a significant effect on plantlets formation leaves. By using 1% naphthalene acetic acid, plantlet with the highest number of leaves were obtained (10.44), so this hormone had the greatest beneficial influence in leaf formation, although there were no significant differences between nutritive medium variants. The lowest value is observed when applying NAA (0.1%) + GA3 (0.02%) combination in the culture medium, but as it is mentioned above there are no statistical differences between the variants with hormonal treatments.

The hormonal combination of naphthalene acetic acid 0.1% and gibberellic acid (0.02%) induced the formation of a root with the highest value of root length (5.06 cm), without differing from the combinations: naphthalene acetic acid (0.05%) - gibberellic acid (0.02%): 4.94 cm; naphthalene acetic acid (0.1%): 4.78 cm and naphthalene acetic acid (0.05%): 4.56 cm. The lowest value was recorded when applying indolyl acetic acid (0.1%) to the culture medium: 2.61 cm without statistically

differentiating it from the influence of indolyl acetic acid (0.1%) and gibberellic acid (0.02%): 3.11 cm.

The combination of indolyl acetic acid (0.05%) and gibberellic acid (0.02%) hormones had as effect plantlets obtaining with the highest weight (237.22 mg), followed by the combination of indolyl acetic acid (0.1%) and gibberellic acid (0.02%) (214.57). At the opposite pole was using of 0.1% naphthalene acetic acid in the nutrient medium, leading to the lowest value of plantlets weight.

The highest value of root weight was recorded when supplementing the culture medium with indolyl acetic acid (0.05%): 106.68 mg. The minimum value of the root weight was obtained under the influence of 0.05% naphthalene acetic acid: 44.86 mg.

The maximum value of plantlets height was determined for the Castrum variety (13.17 cm), by using in the culture medium the hormonal combination IAA (0.05%) + GA3 (0.02%) (Table 3), without statistically differentiating by value recorded by applying NAA 0.05% (12.67 cm).

NAA (0.05%) + GA3 (0.02%) combination had a negative influence on plantlets formation for the Ervant variety (5.67 cm), strongly affecting their growth.

Regarding leaves number/plantlets, 0.1% naphthalene acetic acid was effective, resulting in the highest value (13) for Marvis variety. For the Ervant variety, a positive influence (11 leaves) showed indolyl acetic acid (0.1%), without significantly differentiating by the effect of naphthalene acetic acid on leaf formation for Marvis variety. IAA 0.05% plant growth regulator and the combination NAA (0.05%) + GA3 (0.02%) had a beneficial effect in leaf formation for Castrum variety, without statistical difference. Instead, using NAA (0.1%) + GA3 and IAA (0.1%) + GA3 combinations had a very pronounced negative effect on leaf formation for Castrum and Ervant varieties (7 leaves).

Regarding the root length, the highest value is observed for Castrum variety (7.5 cm) by applying NAA (0.1%) + GA3 (0.02 %) combination. Also, this hormonal combination, had a positive influence on Marvis variety, without statistically differentiating the recorded values (6.3 cm). For Ervant variety, NAA (0.1%) + GA3 (0.02%) treatment had a negative effect, inhibitory on plantlets root formation (1.3 cm).

The combined influence of variety and treatments with growth regulators performed on plantlets weight highlights the IAA (0.05%) + GA3 (0.02%) treatment, for Marvis variety, leading to the highest value (352.1 mg). The Castrum variety is distinguished by a high plantlets weight (257.3 mg) by applying the same treatment as for Marvis variety, respectively IAA (0.05%) + GA3 (0.02%). For this parameter, 0.1% NAA had a negative influence on the Castrum variety (92.1 mg).

Combined influence examination for variety and hormonal treatments on root weight reveals the beneficial effect of the combination IAA (0.05%) + GA3 (0.02%) for the Marvis variety, resulting in the highest value (140.1 mg). For Castrum and Ervant varieties, IAA growth regulator 0.05% showed a positive influence (128.9 mg and 102.9 mg), without statistical difference. At the opposite pole is the effect of NAA (0.1%) + GA3 combination for Ervant variety which recorded the lowest value of root weight (10.2 mg).

Table 1. The influence of genotype on the elements of growth and development under the influence of *in vitro* treatments with growth regulators, NIRDPSB Brasov (2022)

Variety	Plantlet's height (cm)	Leaves number	Root length (cm)	Weight of fresh plantlet (mg)	Weight of fresh root (mg)
Marvis (a_1)	7.92 B	10.54 A	4.92 A	253.81 A	95.73 A
Castrum (a_2)	10.02 A	8.08 B	5.13 A	170.96 B	90.42 A
Ervant (a_3)	7.31 B	9.46 AB	2.00 B	120.68 B	50.79 B
	LSD 5%=1.29	LSD 5%=1.56	LSD 5%=0.63	LSD 5%=63.87	LSD 5% = 31.33

For each column, the averages followed by the same letter are not significantly different according to the Duncan multiple comparison test ($p < 0.01$).

Table 2. The influence of growth hormones in the culture medium on the elements of growth and development, NIRDPSB Braşov (2022)

Plant regulators growth treatment	Plantlet height (cm)	Leaves number	Root length (cm)	Weight of fresh plantlet (mg)	Weight of fresh root (mg)
NAA (0.05%) (b_1)	9.44 A	9.22 A	4.56 A	179.74 BCD	44.86 D
NAA (0.1%) (b_2)	7.78 CD	10.44 A	4.78 A	141.1 D	66.19 BCD
IAA (0.05%) (b_3)	9.22 A	10.22 A	3.72 B	165.59 CD	106.68 A
IAA (0.1%) (b_4)	8.89 AB	9.33 A	2.61 C	194.29 BC	93.17 ABC
NAA (0.05%) +GA_3 (0.02%) (b_5)	6.78 D	8.89 A	4.94 A	153.77 CD	72.58 ABCD
NAA (0.1%) + GA_3 (0.02%)(b_6)	7.94 BC	8.67 A	5.06 A	168.17 CD	60.94 CD
IAA (0.05%) +GA_3 (0.02%) (b_7)	9.83 A	9.33 A	3.33 B	237.22 A	87.33 ABC
IAA (0.1%) + GA_3 (0.02%) (b_8)	7.44 CD	8.78 A	3.11 C	214.57 AB	100.09 AB
	LSD=1.09	LSD=2.09	LSD=0.81	LSD=41.97	LSD=34.22

For each column, the averages followed by the same letter are not significantly different according to the Duncan multiple comparison test ($p < 0.01$).

Table 3. The combined influence of variety and growth hormones used in the culture medium on the elements of growth and development, NIRDPSB Braşov (2022)

Variety	Plant regulators growth treatment	Plantlet's height (cm)		Leaves number		Root lenght (cm)		Weight of fresh plantlet (mg)		Weight of fresh root (mg)	
Marvis	NAA (0.05%)	8.3	DEF	11	ABC	5.7	BC	242.3	BCD	58.9	DEFG
	NAA (0.1%	7.8	DEF	13	A	6.3	AB	190.0	CDEFG	90.7	ABCDE
	IAA (0.05%)	8.7	DE	12	AB	4.0	DEF	233.9	BCDE	88.3	ABCDE
	IAA (0.1%)	8.7	DE	9	BCD	2.7	FGHI	236.1	BCDE	67.7	CDEFG
	NAA (0.05%) + GA_3	7.0	EFG	9	BCD	5.8	BC	219.8	BCDEF	94.7	ABCDE
	NAA (0.1%) + GA_3	7.5	DEFG	9	BCD	6.3	AB	264.2	B	93.9	ABCDE
	IAA (0.05%) + GA_3	8.7	DE	12	AB	4.5	CDE	352.1	A	140.1	A
	IAA (0.1%) + GA_3	6.7	FG	11	ABC	4.0	DEF	292.1	AB	131.5	AB
Castrum	NAA (0.05%)	12.67	A	8	CD	6.0	B	179.5	DEFGH	55.7	DEFG
	NAA (0.1%)	7.67	DEF	8	BCD	5.8	BC	92.1	J	49.9	DEFG
	IAA (0.05%)	12.00	AB	9	BCD	5.2	BCD	160.2	FGHIJ	128.9	AB
	IAA (0.1%)	10.67	BC	8	CD	3.2	EFGH	178.9	DEFGH	128.2	AB
	NAA (0.05%) + GA_3	7.67	DEF	9	BCD	6.3	AB	138.3	GHIJ	73.1	BCDEF
	NAA (0.1%) + GA_3	7.17	EFG	7	D	7.5	A	123.7	GHIJ	78.7	BCDEF
	IAA (0.05%) + GA_3	13.17	A	8	CD	3.7	EF	257.3	BC	82.2	ABCDE
	IAA (0.1%) + GA_3	9.17	CD	8	CD	3.3	EFG	237.6	BCDE	126.5	ABC
Ervant	NAA (0.05%)	7.33	DEFG	9	BCD	2.0	GHI	117.4	GHIJ	19.9	FG
	NAA (0.1%)	7.83	DEF	10	ABCD	2.2	GHI	141.4	GHIJ	57.9	DEFG
	IAA (0.05%)	7.00	EFG	10	ABCD	2.0	GHI	102.7	IJ	102.9	ABCD
	IAA (0.1%)	7.33	DEFG	11	ABC	2.0	GHI	167.9	EFGHI	83.5	ABCDE
	NAA (0.05%) + GA_3	5.67	G	9	ABCD	2.7	FGHI	103.2	IJ	49.9	DEFG
	NAA (0.1%) + GA_3	9.17	CD	10	ABCD	1.3	I	116.6	HIJ	10.2	G
	IAA (0.05%) + GA_3	7.67	DEF	9	BCD	1.8	HI	102.3	IJ	39.7	EFG
	IAA (0.1%) + GA_3	6.50	FG	7	D	2.0	GHI	114.0	HIJ	42.3	EFG
		LSD=1.9		LSD=3.6		LSD=1.4		LSD=72.7		LSD=59.3	

For each column, the averages followed by the same letter are not significantly different according to the Duncan multiple comparison test ($p < 0.01$).

CONCLUSIONS

The effect of hormonal combinations in the multiplication process varied from cultivar to cultivar, according to the parameters studied.

The combination of IAA (0.05%) + GA3 (0.02%) resulted in a pronounced growth of plantlets for the Castrum variety. The NAA 0.1% growth regulator favoured the Marvis variety in leaf formation. Examination of the results regarding the root length suggests the high capacity of Castrum variety to form them, determining the highest value by using NAA 0.1% + GA3 0.02%.

For IAA (0.05%) + GA3 (0.02%) combination there is a positive influence on the weight of plantlets and their root for the Marvis variety.

The nutrient medium supplemented with IAA 0.05% determined plantlets obtaining with high height and with the highest value of fresh root weight. The culture medium containing 0.1% NAA was effective in forming the number of leaves, showing a positive influence.

NAA 0.1% + 0.02% GA3 and IAA 0.05% + 0.02% GA3 combinations had a beneficial effect on root length and fresh plantlets weight.

ACKNOWLEDGEMENTS

This research work was carried out with the support of National Institute of Research and Development for Potato and Sugar Beet Brasov and also was financed from ADER 5.1.2. project Research on the production of minitubers under specific isolation conditions.

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