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POTATO MICROTUBERISATION UNDER THE INFLUENCE OF CERTAIN ORGANIC ACIDS

Andreea NISTOR, Nicoleta CHIRU, Mihaela CIOLOCA, Monica POPA
NIRDPSB Brasov

ABSTRACT. The use of in vitro tubers (microtubers) as the final products of potato micropropagation, has several advantages in seed tuber production. In this study we tried to produce in vitro virus free microtubers, using nodal segments. Potato microtubers are usually induced in media containing growth regulators, typically cytokinins and growth retardants. The potential acid-induction of in vitro-mass tuberization was investigated as an alternative. For this purpose: two varieties (Christian and Roclas) and three types of organic acids (iasmonic acid, acetylsalicylic and salicylic acid) were established, in comparison with a classic medium, with a content of growth regulators as kinetin and coumarin. All organic tested under the different experiments caused tuberization. The performance of the microtubers obtained, as compared to those induced under growth regulators treatments, should be investigated.

Keywords: microtubers, coumarin, kinetin, iasmonic acid, acetylsalicylic and salicylic, minicuttings

INTRODUCTION

The motivation of choosing this theme started from the necessity to develop in perspective the production potato seed, having as starting point different techniques in vitro and especially starts from the need to intensify preoccupations in the direction of production in vitro on large scale, of initial material free of disease (microplantlets, microtubers, minitubers).It was taken into account the development of effective methods for "in vitro" regeneration of valuable potato genotypes required by the market.

The aim of this research was to find methods of production, determining influence of genotype and microtuberisation medium on the number of microtubers/plantlet and on average weight of a microtuber.

Microtubers or vitrotubers are small minitubers (3-8 mm), spherical or elongated shape, weighing 0.05 to 0.15 grams (Chiru,1998) and with protein nitrogen content 2.5 times higher than normal minitubers.

First report of microtuberization was made (Baker, 1953), using a medium containing 80 g / l sucrose. Since then, the use of phytohormones was the objective of numerous studies on "in vitro" tuberization.

Microtubers are produced in vitro in a plethora of different growing systems with varying environment, media constituents, and storage intervals. Many of the interactions between growth parameters in vitro and subsequent productivity appear to be genotype-specific. Accordingly, microtubers come in different sizes, have different dormancy requirements, and differ widely in relative growth potential and productivity. Despite these differences, there is evidence for strong analogies in growth responses between fieldgrown tubers and microtubers (Donnellyet al., 2003).

Several factors such as the use of growth regulators in microtu-ber induction and growth media, the mixotropic nature of the in vitro system, and cultivar-specific responses have led to interpretive difficulties (Coleman, 2001).

Recent researches have shown that microtubers and minitubers (nuclear tubers) have production potential and can be successfully integrated in seed potato production program (Khuri, Moorby, 1996; Pruskiet et al., 2003; Struik, Wiersema, 1999).

MATERIALS AND METHODS

The experiment was conducted at the tissue vegetal culture laboratory of NIRDPSB Brasov, Romania, during January 2011. Several experiments were mounted in order to produce microtubers from the varieties Christian and Roclas.

This experience included three repetitions: in each vessel were inoculated 15 minicuttings. To accomplish the experience, two variants were studied in bifactorial experience.

From Table 1, it appears that the bifactorial experience with 2 experimental variants was performed using the following graduations:

- Experimental factor – a – variety with 2 graduations:
- a1-Christian (control variety);
- a2- Roclas.

As control, was fixed variant 1, representative for Romanian varieties.

- Experimental factor – b – tuberization liquid medium with 4 graduations:
- b1 – control tuberization medium (using coumarin and as cytokinin: kinetin);
- b2 – tuberization medium with iasmonic acid;
- b3 – tuberization medium with salicylic acid;
- b4 – tuberization medium with acetylsalycil acid.

The major function of iasmonic acid is to regulate plants to biotic and abiotic stress, and their growth and development. Iasmonic acid is a phenolic phytohormones, responsible for the formation of tubers

**Correspondence:* *NIRDPSB Brasov, Street Fundăturii no 2, City: Brasov, Postal code: 500470, Phone: 0268/476795, Fax: 0268/476608, Romania, tican_andreea@yahoo.com*
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for potatoes and onions. Salicylic acid is found in plants, with role in growth and development.

Microtubers are usually induced using microtuberization medium that contains growth regulators. Because these substances can create an imbalance in the physiology of explants and cause adverse effects on subsequent formation of microtubers, was investigated as a potential alternative, induction of acids in microtuberization medium. Research on hormone-free microtuberization received little attention; probably because the few published studies (Garner and Blake, 1989, Hussey and Stacey, 1984) suggests that the process would be slow or inefficient.

However, the availability of a tuberization hormone-free system "in vitro", lead to producing microtubers without further problems related to hormonal balance changed, and would be commercially useful.

It was demonstrated that under certain conditions, salicylic and acetylsalicylic acids (Koda and colab., 1992) can induce "in vitro" tuberization. These compounds exert regulatory functions in hormone levels and interaction with other hormones (Gaspar and colab., 1996).

This paper examines the possibility of establishing a system of microtuberization without hormones, on the basis of acids applications.

For each medium variant, in the first three weeks, the medium used was MS (supplemented with sucrose 20%, 8% agar, NAA 0.5 mg / l), then was added to each pot 70 ml ½ MS liquid medium supplemented with 80g / l sucrose. For b1, control medium (classic medium) was added 2.5 mg / l kinetin and 25 mg / l coumarin. Other medium variants containing acid concentrations are found in Table 2.

For each medium variant, the pH was adjusted to 5.8 and sterilized by autoclaving at 1210C for 20 minutes at pressure of 1.25 atmospheres.

The objective of experience: - elaboration of efficient methods of in vitro regeneration to achieve a biological material free of disease by microtuberization, conducted by devirozation of Romanian valuable genotypes and characterization of these varieties in terms of microtuberization of varieties, using applications with growth regulators and organic acids.

Organization (location) of experience: experience was organized according to the protocol of producing microtubers, performed in laboratory, in special culture vessel, grouped into 3 groups (repetitions) (fig. 1).

Table 1. Experimental variations on different microtuberization medium

Variant	Variety (a)	Microtuberization medium (b)
V_1	Christian (a_1)	Classic medium (b_1)
V_2		Tuberization medium with iasmonic acid (b_2)
V_3		Tuberization medium with salicylic acid (b_3)
V_4		Tuberization medium with acetylsalycil acid (b_4)
V_4	Roclas (a_2)	Classic medium (b_1)
V_5		Tuberization medium with iasmonic acid (b_2)
V_6		Tuberization medium with salicylic acid (b_3)
V_8		Tuberization medium with acetylsalycil acid (b_4)

Table 2. Experimental variants depending on the concentration of acids used

The acids used	Basic medium	Acid concentration (mM)
iasmonic acid	MS/2 + 80g/l sucrose	0,13
salicylic acid		0,18
acetylsalycil acid		0,14

	a1				a2			
r1	b1	b2	b3	b4	b1	b2	b3	b4
r2	b1	b2	b3	b4	b1	b2	b3	b4
r3	b1	b2	b3	b4	b1	b2	b3	b4

Fig.1. Location sketch of experimental variants made for the two varieties and four nutrition media

Legend:
- a – variety
- b – type of medium
- r – repetition

Working mode: The experience was mounted in the laboratory, in conditions required by technology in vitro; the condition of testing were the specific from growing room; for potato tubers shooting, temperature was 180C, on light until the buds reach 2-3 cm long; room for aseptic dissection of meristems is sterilized with niche sterile air laminar flow, sterilization tubes are made in the drying chamber at 1800C, and the culture medium which had to be introduced into test tubes, was sterilized by autoclaving at 1200C; conservation of microtubers was made in refrigerators at 40C.

Growth chamber is equipped with racks, light regime is 4000 lux, the period of 16 hours light and 8 hours dark at a temperature of 200C day and 18 – 200C at night.

The biological material used to achieve experiences: In experience, was working with Romanian varieties, Christian and Roclas described in the Official Catalogue of varieties (hybrids) of crop plants in Romania (2000).

The experience has as starting point plantlets of Roclas and Christian, Romanian varieties, created at NIRDPSB Brasov.

Meristems taken from the potato buds are "in vitro" micropropagated to achieve a healthy pre-basic material. Micropropagation presumes meristem development stage, as a minicutting and then a regeneration of this, in a plantlet, which is then divided into minicuttings. This is a special technique that allows rapid multiplication, protected from contamination of any kind, mostly viral . These minicuttings develop themselves in plantlets (Chiru, 1998).

To obtain microtubers, belonging to these varieties, were used minicuttings (fig. 2) obtained from plantlets, which were then inoculated on the vessels. Each vessel contains 15 minicuttings. In the first three weeks, medium used was MS (supplemented with sucrose 20%, 8% agar, NAA 0.5 mg / l), then added to each vessel 70 ml of liquid medium of tuberization for each medium variant.

Cultures were then incubated in the dark at a temperature of 18-20 ° C for 12-14 weeks. After the tuberization period of (12-14 weeks), potato plantlets were removed from culture test tubes and microtubers (figures 3,4,5) were harvested, washed to remove traces of medium and to avoid further infections that may occur during their storage. Then microtubers (figures 6, 7) were dry, calibrated, numbered and put in refrigerator for storage at temperatures of 40C in the dark.

Fig. 2. Minicuttings

Fig.3 Microtuberization

Fig.4 Microtuberization: Christian variety

Fig.5 Microtuberization: Roclas variety

Fig.6 Microtubers: Christian variety

Fig.7 Microtubers: Roclas variety

Legend:

1 – control medium of tuberization (using phytochemical coumarin and cytokin: kinetin);
2 – medium of tuberization with iasmonic acid;
3 – medium of tuberization with salicylic acid;
4 – medium of tuberization with acetylsalicilic acid.

Observations and determinations: determining the number and average weight of microtubers by weighing individual, depending by variety and acid used in medium of microtuberization.

RESULTS AND DICUSSIONS

From observations it was found that plantlets obtained in vitro from Romanian varieties, Christian and Roclas, had a different behavior in microtuberization. There were differences between the two varieties in terms of number and weight of microtubers obtained. All tested acids induced tuberization.

Number of microtubers obtained/plantlet

As a result of research in obtaining microtubers using different media, these had influenced the number of microtubers different. From data analysis it is observed that the best results were obtained when was used jasmonic acid and salicylic acid for Christian and Roclas varieties, who had obtained a total of 1.38 microtubers / plantlet for jasmonic acid (for Christian variety) and 1.31 microtubers / plantlet for salicylic acid (for Roclas variety) (fig. 8).

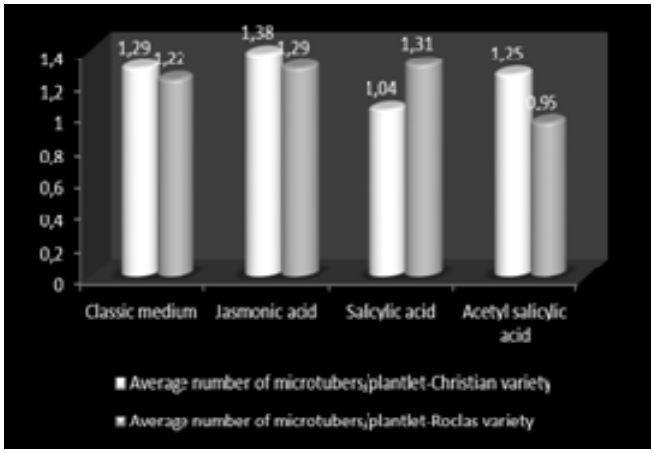

Fig. 8. Number of microtubers plantlet, average values per variety

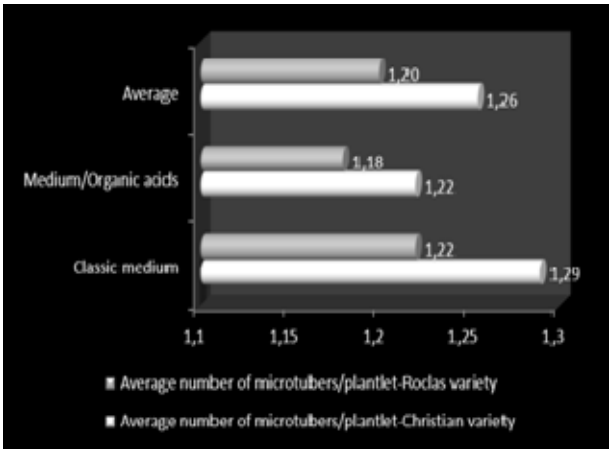

Fig. 9. Number of microtubers/ and medium of tuberization

A few number of microtubers was obtained for Christian variety, when was used salicylic acid (1.04 microtubers / plantlets) and acetylsalicylic acid for Roclas variety (0.95 microtubers / plantlet) (fig. 8).

In case of the influence of variety, the best results were obtained for Christian variety (1.26 microtubers / plantlet) (fig. 9).

Statistical interpretation of results

It was analyzed bifactorial analyses of the number of microtubers obtained using four types of tuberization medium for the two varieties. The first factor considered was the variety, the second factor being tuberization medium.

From point of view of the influence of the variety (Table 3), by comparing the control variety, Christian obtained a greater number of microtubers/ plantlets (1.29), compared with Roclas variety, which leads to a significant, negative difference of -0.09 microtubers/ plantlet.

Statistical analysis for the combined influence of the variety used and microtuberization medium (table 4) shows that the results obtained by in vitro tuberization medium were presented as follows: for Roclas variety, by comparison with Christian variety, the statistical assurance was from distinct significant, in the negative way, at tuberization medium with acetylsalicylic acid (-0.3 microtubers / pl) to significant distinct, in the positive way, at tuberization medium with salicilic acid (0.27 microtubers / pl); between acids used in tuberization medium, limit differences obtained are very significant positive for Christian variety, on acetylsalicylic acid using, by comparing with salicylic acid (0.21 g / minitub. / plant).

Table 3. Influence of variety on the number of obtained microtuberculi / plantlets

Variety	Number of microtubers obtained/plantlet		Differences	Significance
	No.	%		
Christian (Ct)	1,29	100,00	-	
Roclas	1,19	92.69	-0,09	o

LSD 5 % = 0,04 microtub./pl. LSD 1 % =0,10 microtub./pl. LSD 0,1 % = 0,33 microtub./pl

Table 4. Influence of variety and tuberization medium over microtubers number obtained/plantlets

Variety/ Tuberization medium	classic medium		iasmonic acid		salicilic acid		acetylsalicylic acid		Differences					
	No.	Dif. Semnif.	No.	Dif. Semnif.	No.	Dif. Semnif.	No.	Dif. Semnif.	a2-a1	a3-a1	a3-a2	a4-a1	a4-a2	a4-a3
Christian	1,29	-	1,38	-	1,04	-	1,25	-	0,09 *	-0,25 ooo	-0,34 ooo	0,04 ns	-0,13 oo	0,21 ***
Roclas	1,22	-0,07 ns	1,29	-0,09 ns	1,31	0,27 **	0,95	-0,30 oo	0,07 *	0,07 *	-0,02 ns	-0,27 ooo	-0,34 ooo	-0,36 ooo

LSD a 5 % =0,104 microtub./pl. LSD b 5% =0,067 microtub./pl.
LSD a 1 % = 0,220 microtub./pl. LSD b 1% =0,094 microtub./pl.
LSD a 0,1 % = 0,624 microtub./pl. LSD b 0,1% =0,133 microtub./pl.

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Table 5. Influence of in vitro medium of tuberization over microtubers number obtained / plantlet

Medium	Number of microtubers obtained/plantlet		Differences	Significance
	No.	%		
classic	1,26	100,00	-	-
jasmonic acid	1,33	106,10	0,08	***
salicylic acid	1,18	88,13	-0,08	ooo
acetylsalicylic acid	1,10	93,62	-0,16	ooo

LSD b 5 % = 0,004 microtub./pl. LSD b 1 % = 0,006 microtub./pl. LSD b 0,1 % =0,012 microtub./pl.

In case of tuberization medium influence (Table 5), the obtained results indicate a positive very significant difference using jasmonic acid (0.08 microtub. / plantlet) and very significant, negative for acetylsalicylic acid and salicylic acid compared with classic medium, consisting of coumarin and kinetin (-0.08 microtub. / plantlet and -0.16 microtub. / plantlet).

Microtubers weight obtained on plantlets

Analysis the average weight of a microtuber, shows that the highest value for average weight of a microtubers of 0.61 g was obtained in medium containing salicylic acid in medium of microtuberization and the lowest average weight for microtuberization medium containing acetylsalicylic acid (0.24 g) both values was for Christian variety (Figure 10).

Microtuberization medium containing jasmonic acid, because it had product a high number of microtubers, it has a low value of the average weight of a microtuber for both varieties used in experience (0.40 g of a microtub. for Christian variety, and 0.34 g of a microtub. for the variety Roclas).

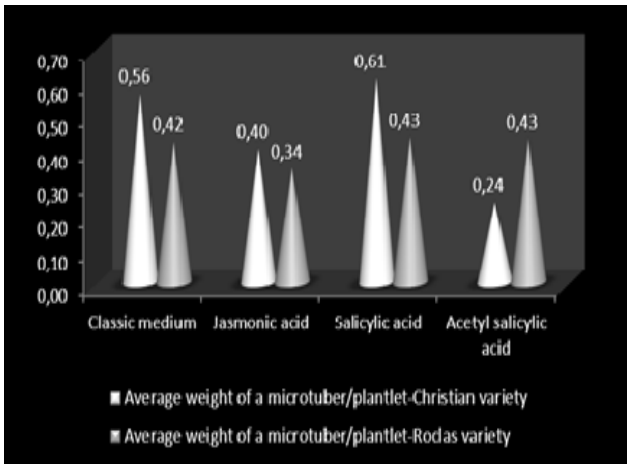

Fig. 10. Microtubers weight (g) obtained / plantlet, average values per variety and medium used

The average weight of a microtuber (Table 6) was significantly influenced for the Roclas variety, with a distinct negative difference of – 0.05 g, statistically assured. Christian variety showed a higher value of average weight by 0.45 g for a microtuber compared to the Roclas variety (0.40 g).

Combined influence of variety and acids used in culture medium for in vitro tuberization shows that the medium has a greater influence on the average weight of a microtuber. When it was used Roclas variety, by comparison with the Christian variety, the results for tuberization medium containing acetylsalicylic acid are distinct significant positive (0.19 g) and for medium with salicylic acid, the differences are distinct significant negative (-0.18 g) statistically assured. Analyzing tuberization media between them, it can be seen that on Christian variety using, tuberization medium containing salicylic acid have positive significant differences (0.21 g) by comparing with medium containing jasmonic acid; for Roclas variety using, media that contain salicylic acid and acetylsalicylic acid in their composition present significantly distinctly positive differences to iasmonic acid (0.09 g).

The results indicate a very significant negative difference using iasmonic acid (-0.12 g) and acetyl salicylic acid (-0.16 g) and significant in medium of microtuberization (0.03 g) using of salicylic acid compared with the control, classic medium (Table 8).

Table 6. The influence of a variety over average weight of a microtuber obtained

Variety	Average weight of a microtuber obtained		Differences (g)	Significance
	g	%		
Christian (Ct)	0,45	100	-	-
Roclas	0,40	89,34	-0,05	oo

LSD 5 % = 0,007 (g) LSD 1 % = 0,017 (g) LSD 0,1 % = 0,053 (g)

Table 7. Influence of variety and medium over the average weight of a microtuber obtained

Variety/ Medium	classic medium		iasmonic acid		salicylic acid		acetylsalicylic acid		Dif.					
	Weight (g)	Dif. Semnif	Weight (g)	Dif. Semnif	Weight (g)	Dif. Semnif	Weight (g)	Dif. Semnif	a2-a1	a3-a1	a3-a2	a4-a1	a4-a2	a4-a3
Christian (Ct)	0,56	-	0,40	-	0,61	-	0,24	-	-0,16 ooo	0,05 *	0,21 ***	-0,32 ooo	-0,16 ooo	-0,37 ooo
Roclas	0,42	-0,14 o	0,34	-0,06 ns	0,43	-0,18 oo	0,43	0,19 **	-0,08 oo	0,01 ns	0,09 **	0,01 ns	0,09 **	-0,01 ns

LSD a 5 % =0,0711 (g) LSD b 5% =0,0480 (g)
LSD a 1 % = 0,1498 (g) LSD b 1% =0,0673 (g)
LSD a 0,1 % = 0,4231 (g) LSD b 0,1% =0,0950 (g)
(Legend ns=not significant)

Table 8. Medium influence of microtuberization over the average weight of a microtuber obtained

Medium	Average weight of a microtuber obtained		Differences (g)	Significance
	Weight (g)	%		
classic medium (Ct)	0,49	100,00	-	-
jasmonic acid	0,37	75,25	-0,12	ooo
salicylic acid	0,52	140,99	0,03	*
acetylsalicylic acid	0,33	63,90	-0,16	ooo

LSD 5 % = 0,022 (g) LSD 1 % = 0,036 (g) LSD 0,1 % = 0,067 (g)

CONCLUSIONS

In experience, in which were used different media for microtuberization, Christian and Roclas varieties behaved differently depending by medium of tuberization, both in the number of microtubers obtained per plantlets and their weight.

When it was used jasmonic acid, in medium of tuberization can get a very significant positive difference, of 0.08 microtubers / plantlet, with assurance statistics, compared to the control, classic medium, containing coumarin and kinetin.

When it was used traditional medium, the highest average weight of a microtuber was recorded at Christian variety of 0.45 g.

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