

## RESEARCH ABOUT INFLUENCE OF VARIETY, CULTURE SUBSTRATE AND NUTRIENT SPACE ON POTATO MINITUBERS PRODUCTION

Andreea TICAN<sup>1</sup>, Mihaela CIOLOCA<sup>1</sup>, Carmen Liliana BĂDĂRĂU<sup>1,2</sup>, Monica POPA<sup>1</sup>

<sup>1</sup>National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania

<sup>2</sup>Transilvania University, Faculty of Food and Tourism Brasov, 148 Castelului Street, 500014, Brasov County, Romania

Corresponding author email: tican\_andreea@yahoo.com

### Abstract

*This study was conducted to determine the minituberization capacity of three Romanian potato varieties. The three-factor experiment included the following factors: A - culture substrate with two graduations: a<sub>1</sub> - 80.00% red peat, 9.90% black peat and 10.10% perlite, a<sub>2</sub> - 84.03% red peat, 7.9% black peat and 8.06% perlite; B: nutrition space, with two graduations: b<sub>1</sub> - 1.5 l, b<sub>2</sub> - 2 l; C: variety, with three graduations: c<sub>1</sub> - Marvis; c<sub>2</sub> - Castrum; c<sub>3</sub> - Ervant. Examination of the results on the number and weight of minitubers/plant suggests the high capacity of the Castrum variety for the production of minitubers, when using increased space nutrition and the substrate consisting of a smaller concentration of red peat (80.00%). Regarding the minitubers number, when it was used the substrate with a higher concentration of red peat (84.03%) the Castrum variety was distinguished in a bigger nutrition space (2 l).*

**Key words:** potato minitubers, variety, nutrition space, substrate.

### INTRODUCTION

Plant propagation through the method of tissue culture, a facet of biotechnology, has developed an important industry with considerable potential for the future (Vasil and Vasil, 1986, Yoon Kab Seog, 2000, Zimmerman et al., 1986, quoted by Yoon Kab Seog, 2000).

By the conventional method, the potato is often the target of pathogens, such as fungi, bacteria and viruses, which leads to poor quality and low yield (FAO, 2008).

For seed potatoes production it is essential to use a healthy and high-quality organic material, in order to obtain an increase in potato production at an optimal level (Parrot, 2010). Seed tuber quality is the most important determinant factor for production (Struik and Wiersema, 2012).

Development of a virus eradication technology and establishment of an *in vitro* collection of virus-free germplasm is an important premise for healthy seed potatoes production (Mozafari and Pazhouhandeh, 2000).

Starting from the meristem culture, with or without the application of thermotherapy and/or chemotherapy, healthy plants are obtained in a short period of time, by using micropropagation. The production of potato

seed material is based on the use of pathogen-free microplants and establishment of nuclear stock (germplasm collection) and then minitubers production in protected areas.

Minituber production through planting of micropropagated plantlets in soil can be regarded as a quick effective approach for potato seed tuber production (Ahloowalia, 1994).

Minitubers are principally used for the production of pre-basic or basic seed by direct field planting (Ritter et al., 2001).

### MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania. The technology of minitubers producing is based on rapid multiplication. Rapid multiplication has as its main objective the obtaining of disease-free biological material and starts from the meristem located at the apex of growth. Meristeme culture involves the following steps: selection of biological material estimated to be programmed for *in vitro* multiplication; preparation of biological material and culture medium; their sterilization; meristematic

sampling followed by inoculation; culture maintenance (Figure 1).

To obtain minitubers, the starting point was represented by meristem culture, which was prelevated from studied varieties. After 6-8 month from meristem inoculation plantlets were developed. To ensure the phytosanitary quality, *in vitro* material is tested by the DAS ELISA technique and healthy *in vitro* material was transferred in April-May into “insect-proof” space (Figure 2).

Statistical analysis was performed to determine the influence of genotype, culture substrate and nutrition space in obtaining minitubers number/plant and their weight. The three-factor experiment (2 x 2 x 3), on 6 repetitions,

included the following factors: Experimental factor A - the culture substrate with two graduations: a<sub>1</sub> - 80.00% red peat, 9.90% black peat and 10.10% perlite (considered control); a<sub>2</sub> - 84.03% red peat, 7.9% black peat and 8.06% perlite; Experimental factor B: nutrition space, with two graduations: b<sub>1</sub> - 1.5 l (considered control); b<sub>2</sub> - 2 l; Experimental factor C: variety, with three graduations: c<sub>1</sub> - Marvis; c<sub>2</sub> - Castrum; c<sub>3</sub> - Ervant (as control).

The experience included 12 variants. In the Figure 3 is presented the scheme of experimental variants. The experimental conditions were those specific to the isolated “insect-proof” space.

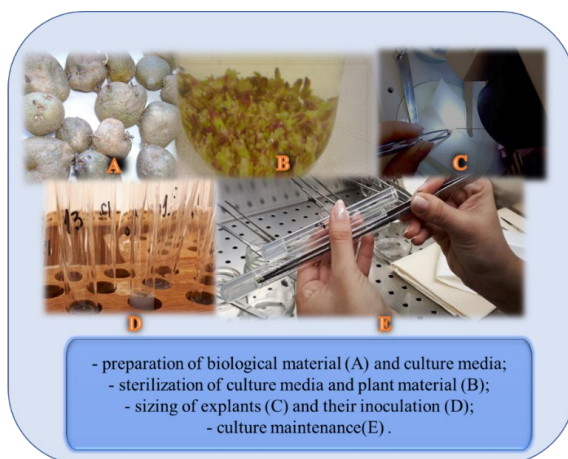


Figure 1. The necessary steps for initiating the meristem culture

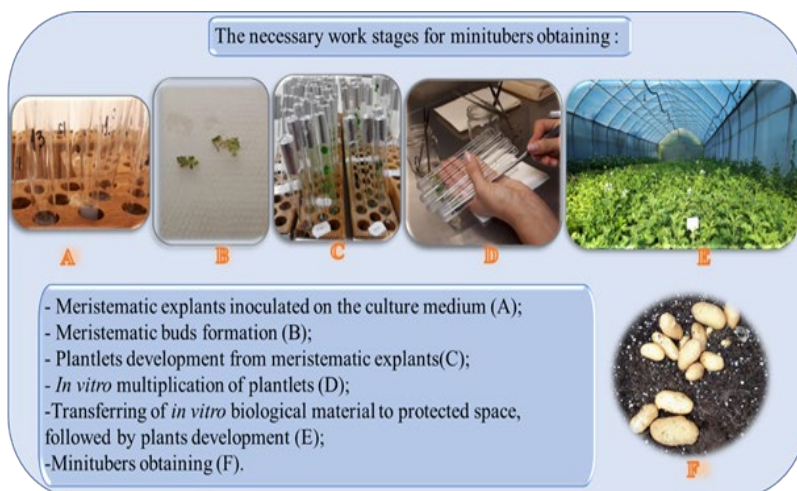


Figure 2. Steps for minitubers obtaining

R <sub>1</sub>	a <sub>1</sub>						a <sub>2</sub>					
	b <sub>1</sub>			b <sub>2</sub>			b <sub>1</sub>			b <sub>2</sub>		
	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>
R <sub>2</sub>	a <sub>1</sub>						a <sub>2</sub>					
	b <sub>1</sub>			b <sub>2</sub>			b <sub>1</sub>			b <sub>2</sub>		
	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>
R <sub>3</sub>	a <sub>1</sub>						a <sub>2</sub>					
	b <sub>1</sub>			b <sub>2</sub>			b <sub>1</sub>			b <sub>2</sub>		
	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>
R <sub>4</sub>	a <sub>1</sub>						a <sub>2</sub>					
	b <sub>1</sub>			b <sub>2</sub>			b <sub>1</sub>			b <sub>2</sub>		
	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>
R <sub>5</sub>	a <sub>1</sub>						a <sub>2</sub>					
	b <sub>1</sub>			b <sub>2</sub>			b <sub>1</sub>			b <sub>2</sub>		
	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>
R <sub>6</sub>	a <sub>1</sub>						a <sub>2</sub>					
	b <sub>1</sub>			b <sub>2</sub>			b <sub>1</sub>			b <sub>2</sub>		
	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>	c <sub>1</sub>	c <sub>2</sub>	c <sub>3</sub>

Figure 3. The scheme of experimental variants

## RESULTS AND DISCUSSIONS

From the analysis of the influence of the culture substrate over minitubers/plant and on weight of them, insignificant differences are observed, the two types of substrates not influencing the parameters studied. On a more detailed analysis of the minituberization process, by using the culture substrate with a higher concentration of red peat, we find a higher value of the number of minitubers (5.89), compared to the control substrate, with a positive difference close to the first threshold of differences limit. Also, the positive effect of the substrate with a higher % of red peat is reflected in obtaining a higher value of the minitubers weight (44.94), compared to the control substrate (Table 1).

By comparing the experimental differences with the calculated limit differences in studying over influence of nutrition space on minitubers number/plant, the beneficial effect of increased nutrition space is noted, leading to a statistically positive significant difference (1.83 minitubers). Examination the results over the weight of minitubers/plant highlights the positive influence of the increased nutrition space expressed by a very significant positive difference (23.71 g) (Table 2).

The study of variety influence on minitubers weight/plant draws our attention to Castrum and Marvis varieties which had a very high capacity in minituberization, determining the obtaining of statistically assured results, with a

very significant positive difference for Castrum variety (36.95 g/plant) and a distinctly significant positive difference for the Marvis variety (25.89 g/culture vessel). The number of minitubers/plant ranged from 4.33 (Marvis variety) to 6.21 (Castrum variety), whose differences are statistically insignificant (Table 3).

The combined influence of the culture substrate and the nutrition space minitubers number/plant highlights the substrate consisting of a higher percentage of red peat (84.03%), in an increased nutrition space (2 l), leading to a significant positive difference (2.22) (Table 4).

Castrum variety is distinguished with high values of minitubers number for both culture substrates, followed by the Ervant variety which on both experimented substrates had produced a larger number of minitubers, compared to the Marvis variety (Table 5).

Increasing the nutrient space strongly influences the minitubers formation for Castrum and Marvis varieties, whose distinctly significant (2.75) and significant (2.17) positive differences it detaches from the low nutrition space (Table 6).

Examination of the combined influence of the culture medium and the nutrient space on minitubers weight reveals the beneficial effect of the increased nutrient space on a substrate with a higher % of red peat, resulting in a very significant positive difference (33.37 g) (Table 7). Statistical interpretation of obtained results regarding minitubers weight shows that from combined interaction of the culture substrate and the variety resulted in significant (27.42) and very significant differences for Marvis and Castrum varieties, when it was used the 80.00% red peat, 9.90% black peat and 10.10% perlite substrate and significant and distinctly significant differences for the same varieties by applying the substrate 84.03% red peat, 7.9% black peat and 8.06% perlite (Table 8).

From the analysis of varieties behavior on minitubers weight obtained on nutrition spaces the Castrum variety can be noticed, which determines obtaining of a distinctly significant positive difference for the reduced nutrition space (Table 9).

Table 1. Influence of the culture substrate on minitubers number and on minitubers weight/plant (g)

Culture substrate (a)	Minitubers number/plant	Diff./Sign.	Minitubers weight/pl. (g)	Diff. (g)/Sign.
80.00% red peat, 9.90% black peat and 10.10% perlite (a <sub>1</sub> ) (Ct)	4.72	-	38.58	-
84.03% red peat, 7.9% black peat and 8.06% perlite (a <sub>2</sub> )	5.89	1.17 ns	44.94	6.37 ns

LSD 5% = 1.56; 1% = 2.45; 0.1% = 5.24. LSD 5% = 14.66 g; 1% = 22.99 g; 0.1% = 49.12 g.

Table 2. Influence of culture space on minitubers number/plant and on minitubers weight/plant (g)

Nutrition space volume (l) (b)	Minitubers number/plant	Diff./Sign.	Minitubers weight/plant (g)	Diff. (g)/Sign.
1.5 (b <sub>1</sub> ) (Ct)	4.39	-	29.90	-
2 (b <sub>2</sub> )	6.22	1.83 *	53.61	23.71 ***

LSD 5% = 1.40; 1% = 2.00; 0.1% = 2.89. LSD 5% = 10.27 g; 1% = 14.60 g; 0.1% = 21.14 g.

Table 3. Influence of variety on minitubers number/plant and on minitubers weight/plant (g)

Variety (c)	Minitubers number/plant	Diff./Sign.	Minitubers weight/plant (g)	Diff. (g)/Sign.
Marvis (c <sub>1</sub> )	4.33	-1.04 ns	46.70	25.89 **
Castrum (c <sub>2</sub> )	6.21	0.83 ns	57.76	36.95 ***
Ervant (c <sub>3</sub> ) (Ct)	5.38	-	20.81	-

LSD 5% = 1.48; 1% = 1.97; 0.1% = 2.59. LSD 5% = 16.08 g; 1% = 21.49 g; 0.1% = 28.26 g.

Table 4. The combined influence of culture substrate and nutrition space on minitubers number/plant

Culture substrate (a)/ Nutrition space volume (l) (b)	80.00% red peat, 9.90% black peat and 10.10% perlite (a <sub>1</sub> )		84.03% red peat, 7.9% black peat and 8.06% perlite (a <sub>2</sub> )		a <sub>2</sub> -a <sub>1</sub> / Semn.
	Minitubers number/plant	Diff./ Semn.	Minitubers number/plant	Diff./ Semn.	
1.5 (b <sub>1</sub> ) (Ct)	4.00	-	4.78	-	0.78 ns
2 (b <sub>2</sub> )	5.44	1.44 ns	7.00	2.22 *	1.56 ns

LSD 5% = 1.99; 1% = 2.82; 0.1% = 4.09. LSD 5% = 2.10; 1% = 3.14; 0.1% = 5.72.

Table 5. The combined influence of the culture substrate and variety on minitubers number/plant

Culture substrate (a)/ Variety (c)	80.00% red peat, 9.90% black peat and 10.10% perlite (a <sub>1</sub> )		84.03% red peat, 7.9% black peat and 8.06% perlite (a <sub>2</sub> )		a <sub>2</sub> -a <sub>1</sub> / Semn.
	Minitubers number/plant	Diff./ Semn.	Minitubers number/plant	Diff./ Semn.	
Marvis (c <sub>1</sub> )	3.33	-1.33 ns	5.33	-0.75 ns	2.00 ns
Castrum (c <sub>2</sub> )	6.17	1.50 ns	6.25	0.17 ns	0.08 ns
Ervant (c <sub>3</sub> ) (Ct)	4.67	-	6.08	-	1.42 ns

LSD 5% = 2.09; 1% = 2.79; 0.1% = 3.67. LSD 5% = 2.30; 1% = 3.28; 0.1% = 5.49.

Table 6. The combined influence of space nutrition and variety on minitubers number obtained / plant

Nutrition space volume (l) (b)/ Variety (c)	1.5 (b <sub>1</sub> )		2 (b <sub>2</sub> )		b <sub>2</sub> -b <sub>1</sub> / Semn.
	Minitubers number/plant	Diff./ Sign.	Minitubers number/plant	Diff./ Sign.	
Marvis (c <sub>1</sub> )	3.25	-1.83 ns	5.42	-0.25 ns	2.17 *
Castrum (c <sub>2</sub> )	4.83	-0.25 ns	7.58	1.92 ns	2.75 **
Ervant (c <sub>3</sub> ) (Ct)	5.08	-	5.67	-	0.58 ns

LSD 5% = 2.09; 1% = 2.79; 0.1% = 3.67. LSD 5% = 2.00; 1% = 2.74; 0.1% = 3.74.

Table 7. The combined influence of the culture medium and nutrient space on the average weight of the mini-tubers / plant (g)

Culture substrate (a)/ Nutrition space volume (l) (b)	80.00% red peat, 9.90% black peat and 10.10% perlite (a <sub>1</sub> )		84.03% red peat, 7.9% black peat and 8.06% perlite (a <sub>2</sub> )		a <sub>2</sub> -a <sub>1</sub> (g) / Semn.
	Minitubers weight/pl. (g)	Diff. (g)/ Sign.	Minitubers weight/pl. (g)	Diff. (g)/ Sign.	
1.5 (b <sub>1</sub> ) (Ct)	31.55	-	28.26	-	-3.29 ns
2 (b <sub>2</sub> )	45.60	14.05 ns	61.63	33.37 ***	16.03 ns

LSD% = 14.52 g; 1% = 20.64 g; 0.1% = 29.89 g. LSD 5% = 17.86 g; 1% = 27.06 g; 0.1% = 51.60 g.

Table 8. The combined influence of the culture medium and the variety on minitubers weight/plant (g)

Culture substrate (a)/Variety (c)	80.00% red peat, 9.90% black peat and 10.10% perlite (a <sub>1</sub> )		84.03% red peat, 7.9% black peat and 8.06% perlite (a <sub>2</sub> )		a <sub>2</sub> -a <sub>1</sub> (g) / Sign.
	Minitubers weight/pl. (g)	Diff. (g)/ Sign.	Minitubers weight/pl. (g)	Diff. (g)/ Sign.	
Marvis (c <sub>1</sub> )	43.45	27.42 *	49.96	24.36 *	6.51 ns
Castrum (c <sub>2</sub> )	56.25	40.22 ***	59.27	33.67 **	3.02 ns
Ervant (c <sub>3</sub> ) (Ct)	16.03	-	25.60	-	9.57 ns

LSD 5%=22.74 g; 1%=30.39g; 0.1%=39.96 g. LSD 5%=23.51 g; 1%=33.21 g; 0.1%=53.62 g

Table 9. The combined influence of nutrition space and variety on minitubers weight / plant (g)

Nutrition space volume (l) (b) / Variety (c)	1.5 (b <sub>1</sub> )		2 (b <sub>2</sub> )		b <sub>2</sub> -b <sub>1</sub> (g) / Sign.
	Minitubers weight/pl. (g)	Diff. (g)/ Sign.	Minitubers weight/pl. (g)	Diff. (g)/Sign.	
Marvis (c <sub>1</sub> )	30.57	17.67 ns	62.84	34.09 **	32.28 **
Castrum (c <sub>2</sub> )	46.27	33.39 **	69.25	40.50 ***	22.98 *
Ervant (c <sub>3</sub> ) (Ct)	12.88	-	28.75	-	15.88 ns

LSD 5%=22.74 g; 1%= 30.39 g; 0.1% = 39.96g. LSD 5%= 20.11 g; 1%= 27.25 g; 0.1% = 36.65 g.

Increasing nutrition space favors Castrum and Marvis varieties, an increase reflected by the achievement of very significant and distinctly significant positive differences. The variety/nutrition space interaction highlights the distinctly significant and significant differences for the Marvis and Castrum varieties, obtained by comparing the two types of culture spaces (Table 9).

Examination of the results on the number and weight of minitubers/plant suggests the high capacity of Castrum variety for the production of minitubers, grown in increased nutrient space and on the substrate consisting of 80.00% red peat, 9.90% black peat and 10.10% perlite. Regarding the number of minitubers, the experimentation on the second type of culture substrate highlights the Castrum variety by using the increased culture space. For this substrate, the Marvis variety records the highest value of the weight of the mini-tubers (74.75 g), followed by the Castrum variety (71.85 g), in an increased nutrition space (Figure 4).

## CONCLUSIONS

Increased nutrition space had a positive influence in minituberization, both for minitubers number, but also, in the total weight of the obtained minitubers/pl.

Regarding the influence of the culture substrate in minituberization, it is found that by using as a substrate the red peat in a higher percentage, high values are obtained for the minitubers number/pl. as well as for their weight/pl.

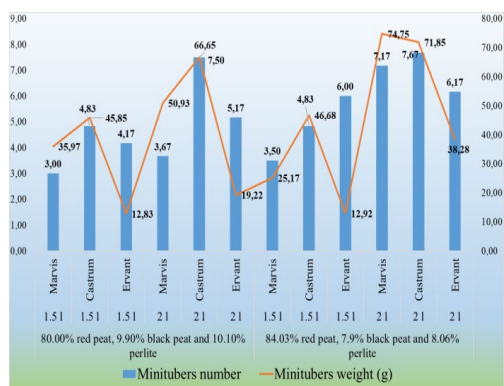


Figure 4. The number and minitubers weight

The Castrum variety stands out for both analyzed parameters (6.21 minitubers/plant and 57.76 g/plant).

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