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STUDY REGARDING INFLUENCE OF VARIETY, BIOLOGICAL MATERIAL AND NUTRIENT SPACE ON POTATO MINITUBERS PRODUCTION

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Abstract

The main purpose of this research was to follow the minituberization process for 3 Romanian potato varieties, created at NIRDPSB Brasov. The biological material called "minitubers" is part of the first links of the national system of potato production for seed. The trifactorial experience of this research (3x2x2), on 3 repetitions, included the following factors: experimental factor A: variety, with three gradations: a1 - Marvis; a2 - Castrum; a3 - Ervant (considered control); experimental factor B: biological material used for planting, with two gradations: b1 - plantlets (considered control); b2- microtubers; experimental factor C: the volume of the nutrition space, with two graduations: c1-1.5 l (considered control) and c2- 2 l. The determinations were made for number and weight of minitubers/plant, in function of experimental factors. The average number of minitubers obtained/nutrition space was between 5.75 (Marvis variety) and 10.25 (Ervant variety), and the weight of minitubers ranged from 28.22 g (Ervant variety) to 93.53 (Castrum variety). By increasing nutrition is noted very significant positive differences for both analysed parameters.

Key words*: genotype, microtubers, minitubers, plantlets, potato, rapid multiplication, seed.*

INTRODUCTION

Potato is most important cultivated food crop and is believed to contribute significantly to sustain future global food security (Kumar et al., 2021).

The profitability of agricultural production is largely determined by the quality and yield properties of seeds and planting material. In potato seed production, a promising direction at the current stage of development of the industry is microclonal propagation, the advantage of which is the ability to get healthy planting material in large quantities in a short time (Filippova et al., 2020).

The production of healthy seed is very crucial to sustain the production and productivity of potato (Sadawarti et al., 2020). Nowadays potato can be rapidly multiplied using nodal cuttings produced in vitro and involving following minitubers production (Dimante and Gaile, 2014). Use of tissue culture technique in seed production has resulted in mass production of potato in a very short period of time. The system is characterized by very flexible and rapid multiplication giving a higher planting propagule (Beukema and Van de Zaag, 1990; Pruski, 2001; Muthuraj et al., 2016, quoted by Sadawarti et al., 2020).

Seed potato production involving minituber production systems has found its place all over the world. This system creates a bridge between the in vitro rapid multiplication and the field multiplication of seed tubers and is thus a classical way to multiply or acclimatize in vitro material before its use in the open field (Sharma and Pandey, 2013).

Soil planting of micropropagated plants has been reported to be a rapid and efficient method for producing potato mini-tubers (Ahloowalia, 1994, quoted by Sharma and Pandey, 2013).

High yields of potato (*Solanum tuberosum* L.) in the field can be ensured by use of high-quality seed potato (Calori et al., 2017, quoted by Sadawarti et al., 2020)

The screen house minitubers production is aimed to get a large number of diseases free mini- tubers in the short time and low cost of production to restore the farmer who used his/her own rotated seed potatoes for extended years in open field with stored pathogens that resulted in high yield and quality losses (Tolessa, 2021). Minitubers are usually defined

as the progeny tubers produced on in vitro derived plantlets or microtubers (Rykaczewska, 2016). Minitubers are small seed potato tubers that can be produced year-round in glasshouses on in vitro propagated plantlets planted at high density (Lommen, 1995). They are considered to be the most suitable propagule to reduce the number of field multiplications in a seed programme (Lommen & Struik, 1992, quoted by Lommen, 1995). The production of mini-tubers consists of two phases: the multiplication of plantlets in vitro and the production of minitubers on these plantlets in the glasshouse.

MATERIALS AND METHODS

The research took place in the Research Laboratory for Vegetal Tissue Culture of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania, in 2022. The activity of the laboratory has as its main objective: obtaining of a virus-free biological material, starting from meristem culture. After 6-8 months from meristem inoculation, plantlets are developed. The multiplication phase extends over several subcultures, with a duration of 3-4 weeks/subculture until the required number of plantlets is reached. An alternative for in vitro production of healthy material is obtaining microtubers, but in different conditions compared to plantlets. In spring, in period April-May, the biological material obtain *in vitro* (plantlets or microtubers) conditions is transferred in "insect-proof" space. All the stages of minitubers production are shown in Figure 1. The main purpose of this research was to follow the minituberization process for three Romanian potato varieties, created at NIRDPSB Brasov. Marvis, Castrum and Ervant. As biological material it was used plantlets and microtubers. The culture vessels in which they were planted had different volume (1.5 l and 2.5 l).

The trifactorial experience (3 x 2 x 2), on three repetitions, included the following factors:

- experimental factor A - variety, with three gradations: a1 - Marvis; a2 - Castrum; a3 - Ervant (considered control);
- experimental factor B: biological material used for planting, with two gradations: b1 – plantlets (considered control); b2- microtubers;
- experimental factor C: the volume of the nutrition space, with two graduations: c1-1.5 l (considered control) and c2- 2 l.

The results recorded after minitubers harvesting (which represent Prebase material in the production of seed potatoes) were processed by statistical program ANOVA POLIFACT.

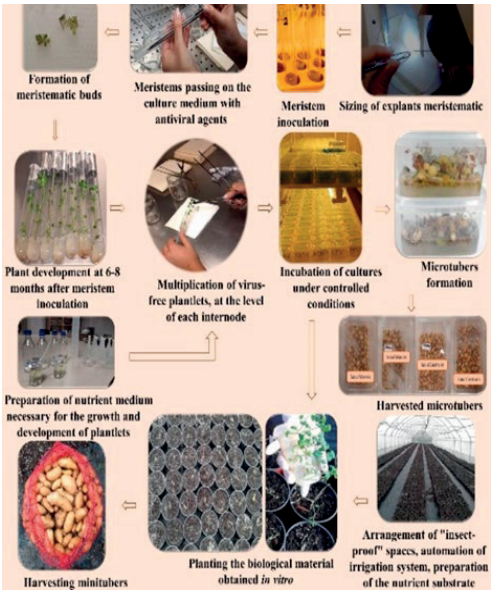

Figure 1. Schematic presentation of the technology for obtaining potato minitubers in "insect-proof" spaces

RESULTS AND DISCUSSIONS

Regarding the influence of variety (Table 1) on the average minitubers number/plant, distinctly significant negative differences are observed for Marvis (-4.50 minitubers) and Castrum varieties (-4.33 minitubers), compared to the control genotype. The Ervant variety stands out with a high value of minitubers number (10.25 g).

When studying the influence of variety on minitubers weight, distinctly significant positive differences are highlighted for Castrum (65.31 g) and Marvis varieties (55.69 g), compared to the control variety, which is at a lower level of the minitubers weight value (28.22 g). Thus, this variety presented a high production capacity of minitubers, but their weight was low.

Table 1. Influence of variety on the average number of minitubers obtained/plant and on the average weight (g) of minitubers/plant

Variety (a)	Minitubers number /pl.	Diff. (g)	Sign.	Minitubers weight/pl. (g)	Diff. (g)	Sign.
Marvis (a_1)	5.75	-4.50	oo	83.91	55.69	**
Castrum (a_2)	5.92	-4.33	oo	93.53	65.31	**
Ervant (a_3) (Ct)	10.25	-		28.22	-	

LSD 5% = 2.48; 1% = 4.10; 0.1% = 7.68. LSD 5% = 24.45g; 1%= 40.45g; 0.1% = 75.71g.

From the analysis of biological material influence on minitubers number/plant and on their weight, insignificant differences are observed, the two types of biological materials not influencing the parameters studied.

On a detailed analysis of the minituberization process, by using microtubers at planting, we find a higher value of minitubers number, compared to the biological material considered as control (plantlets).

Also, the positive effect of microtubers used for planting is reflected in obtaining a higher value of the weight of minitubers (82.23 g), compared to the control biological material (54.88 g), with a positive difference, close to the first threshold of limit differences (Table 2).

Table 2. The influence of biological material on the average number of minitubers obtained/plant and on the average weight of minitubers/plant (g)

Biological material (b)	Minitubers number/pl.	Diff. (g)	Sign.	Minitubers weight/pl. (g)	Diff. (g)	Sign.
Microtubers(b_1)	7.56	0.50	ns	82.23	27.35	ns
Plantlets (b_2) (Ct)	7.06	-		54.88	-	

LSD 5% = 1.52; 1% = 2.30; 0.1% = 3.70. LSD 5% = 29.31g; 1%= 44.38 g; 0.1% = 71.29 g.

By comparing the experimental differences with the limit differences calculated in studying the influence of nutrition space volume on the average number of minitubers/plant, the beneficial effect of the increased nutrition space is noted, leading to a very significant positive difference (3.28 minitubers). Examining the results regarding the minitubers weight /plant highlights the positive influence of the increased nutrition space (Table 3), expressed by a highly significant positive difference (39.73 g).

Table 3. The influence of nutrition space volume on the average number of minitubers obtained/plant and on the weight of minitubers/plant (g)

Nutrition space volume (l) (c)	Minitubers number /pl.	Diff.(g)	Sign.	Minitubers weight/pl. (g)	Diff.(g)	Sign.
1.5 (c_1) (Ct)	5.67	-		48.69	-	
2 (c_2)	8.94	3.28	***	88.42	39.73	***

LSD 5% = 0.80; 1% = 1.13; 0.1% = 1.59. LSD 5% = 11.83 g; 1%= 16.61 g; 0.1% = 23.44 g.

Ervant variety is distinguished by high values of minitubers number for both biological materials used for planting (Table 4). For the Castrum variety, microtubers using showed a positive effect in the formation of minitubers number, with a difference close to the first threshold of the limit differences (2.50 minitubers). When comparing the differences obtained between the Marvis variety and the control, negative differences are found: distinctly significant (-4.83 minitubers) when using microtubers and significant (-4.17 minitubers) when plantlets were used as biological material.

By comparing the differences obtained between the second variety and the control genotype, negative differences are observed: significant for microtubers (-3.00) and distinctly significant (-5.67 minitubers) for control biological material.

Table 4. The combined influence of variety and biological material on the average number of minitubers obtained/plant

Variety/Biological material	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
Microtubers (b_1)	5.33	-0.83 ns	7.17	2.50 ns	10.17	-0.17 ns	-4.83 oo	-3.00 o
Plantlets (b_2) (Ct)	6.17	-	4.67	-	10.33	-	-4.17 o	-5.67 oo

LSD 5% = 2.64; 1%= 3.99; 0.1% = 6.41. LSD 5% = 2.61; 1%= 4.18; 0.1% = 7.41

Ervant and Castrum varieties are distinguished by high values of minitubers number for the increased nutrition space, determining the registration of positive, very significant differences of 8.83 g and 3.832 g. Thus, the nutrition space increasing strongly influenced the mini-

tubers formation for these varieties (Table 5). The increased nutrition space showed a less beneficial effect for Marvis variety, which obtained a lower value of minitubers number (4.33 minitubers) with a highly significant negative difference (-2.83 minitubers).

Table 5. The combined influence of variety and volume of nutrition space on the average number of minitubers obtained/plant

Variety (a)/ Nutrition space volume (l) (c)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/Sign.	a_2-a_3/Sign.
	Minitub. no./pl.	Diff./ Sign.	Minitub. no./pl.	Diff./ Sign.	Minitub. no./pl.	Diff./ Sign.		
1.5 (c_1) (Ct)	7.17	-	4.00	-	5.83	-	1.33 ns	-1.83 ns
2 (c_2)	4.33	-2.83 ooo	7.83	3.832 ***	14.67	8.83 ***	-10.33 ooo	-6.83 ooo

LSD 5% = 1.39; 1%= 1.95; 0.1% = 2.76. LSD 5% = 2.06; 1%= 3.33; 0.1% = 6.00.

Studying of combined influence of biological material used at planting and nutrition space volume on the average number of minitubers obtained/plant reveals the beneficial effect of increased nutrition space, both for microtubers and plantlets, with positive differences: very significant (4.89 minitubers) and distinctly significant (1.67 minitubers). By planting microtubers in the increased nutrition space, a distinctly significant positive difference (2.11) is obtained, compared to the use of plantlets (Table 6).

Table 6. The combined influence of biological material used at planting and nutrition space volume on the average number of minitubers obtained/plant

Biological material (b)/ Nutrition space volume (l) (c)	Microtubers(b_1)		Plantlets (b_2)		b_1-b_2/ Sign.
	Minitub. no./pl.	Diff./ Sign.	Minitub. no./pl.	Diff./ Sign.	
1.5 (c_1) (Ct)	5.11	-	6.22	-	-1.11 ns
2 (c_2)	10.00	4.89 ***	7.89	1.67 **	2.11 **

LSD 5% = 1,14; 1% = 1,59; 0,1% = 2,25. LSD 5% = 1.36; 1% = 2.03; 0.1% =3.17.

When examining the combined influence of variety and the biological material used for planting on the weight of minitubers (g) obtained/plant, distinctly significant positive differences are observed for the varieties Marvis (79.23 g) and Castrum (85.63 g), compared to the control genotype, by using microtubers. Also, a significant positive difference is noted for the Castrum variety (44.99 g), compared to the control genotype when using plantlets (Table 7).

Table 7. The combined influence of cultivar and biological material on minitubers weight (g)/plant

Variety (a)/ Biological material (b)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/Sign.	a_2-a_3/Sign.
	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/ pl.	Diff./ Sign.	Minitubers weight (g)/ pl.	Diff./ Sign.		
Microtubers (b_1)	106.50	45.18 ns	112.91	38.75 ns	27.28	-1.89 ns	79.23 **	85.63 **
Plantlets (b_2) (Ct)	61.32	-	74.16	-	29.17	-	32.15 ns	44.99 *

LSD 5% = 50.76 g; 1%= 76.85 g; 0.1% = 123.48 g. LSD 5% =40.38 g; 1%= 62.80 g; 0.1% = 106.02 g.

Examining the combined influence of biological material used for planting and nutrition space volume on minitubers weight (Table 8) reveals the beneficial effect of the increased nutrition space for both biological materials, determining the registration of positive differences: very significant (52,80 g) and distinctly significant (26.65 g).

Table 8. The combined influence of the biological material used at planting and the volume of the nutrition space on the weight of minitubers obtained (g)/plant

Biological material (b)/ Nutrition space volume (l) (c)	Microtubers (b_1)		Plantlets (b_2)		b_1-b_2/ Sign.
	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/pl.	Diff./ Sign.	
1,5 (c_1) (Ct)	55.83	-	41.56	-	14.27 ns
2 (c_2)	108.63	52.80 ***	68.21	26.65 **	40.42 **

LSD 5% = 16.73 g; 1%= 23.48 g; 0.1% = 33.15 g. LSD 5% = 24.16 g; 1%= 36.17 g; 0.1% = 57.08 g

From the analysis of varieties behavior regarding the weight of the minitubers obtained on the two nutrition spaces, Castrum (98.12 g) and Ervant (33.81 g) varieties stand out, which determine the achievement of very significant positive differences for the increased nutrition space.

The variety/nutrition space interaction highlights: very significant positive difference (78.97 g) and positive significant difference (33.16 g), by comparing Marvis and Castrum varieties, with the control variety, by using reduced nutrition space and significant differences (32.41 g) and very significant (97.47 g) positive by comparing the previously mentioned varieties with the control variety, but when increased nutrition space was used (Table 9).

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

Variety (a)/ Nutrition space volume (l) (c)	Marvis (a_1)		Castrum (a_2)		Ervant (a_3)		a_1-a_3/Sign.	a_2-a_3/Sign.
	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/pl.	Diff./ Sign.	Minitubers weight (g)/pl.	Diff./ Sign.		
1.5 (c_1) (Ct)	90.28	-	44.48	-	11.32	-	78.97 ***	33.16 *
2 (c_2)	77.53	-12.75 ns	142.59	98.12 ***	45.13	33.81 ***	32.41 *	97.47 ***

LSD 5% = 20.49 g; 1%= 28.76 g; 0.1% = 40.61 g. LSD 5% = 23.32 g; 1%= 36.77 g; 0.1% = 64.17g.

When comparing the differences between biological materials used, a distinctly significant positive difference is found by increasing the nutrition space (40.42 g). There was an extremely significant positive correlation between number and variety and volume of space nutrition. The weight was positively correlated with volume of space nutrition (Table 10).

Table 10. Correlation Matrix of Pearson Correlation Coefficients

Pearson Correlation	Number	Weight	Variety	Biological material	Volume of nutrition space
Number	1	.093	.457**	-.062	.408**
Weight	.093	1	-.441**	-.265	.385*
Variety	.457**	-.441**	1	.000	.000
Biological material	-.062	-.265	.000	1	.000
Volume of nutrition space	.408**	.385*	.000	.000	1

**. Correlation is significant at the 0.01 level (1-tailed).
*. Correlation is significant at the 0.05 level (1-tailed

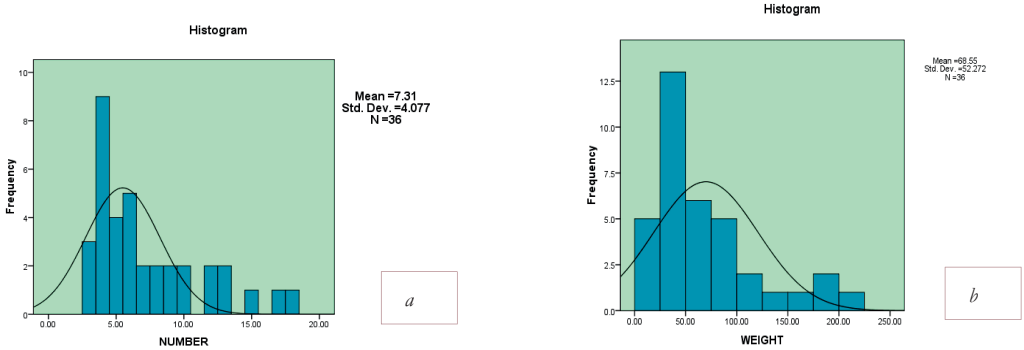

Figure 2. Number distribution (a) and weight (b) for studied genotype

CONCLUSIONS

The average number of minitubers obtained/nutrition space was between 5.75 (Marvis variety) and 10.25 (Ervant variety), and the weight of minitubers ranged from 28.22 g (Ervant variety) to 93.53 (Castrum variety). For the Ervant variety, it can be observed that although it obtained a high number of minitubers/nutrition space (10.25), their weight is low.

The microtubers using as planting material showed a beneficial effect on the average number of minitubers and on the weight, thus

obtaining higher values (7.56 minitubers and 82.23 g), compared to the biological material consisting of plantlets, for which -they recorded 7.06 minitubers and a weight of minitubers/plant of 54.88 g.

Planting the biological material in the increased culture space favored the minituberization process for the two studied parameters (8.94 minitubers and 88.42 g).

The analysis of varieties behaviour in minituberization draws our attention to Ervant (14.67 minitubers) and Castrum varieties (7.83 minitubers), by using the increased nutrition space.

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