

## RESEARCHES REGARDING THE MINITUBERS PRODUCTION FROM POTATO LINES

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### Abstract

In this study was done researches about minitubers production from 14 potato lines studied: TS 12-1488-1574, TS 12-1489-1574, TS 12-1497-1573, TS 11-1475-1633, TS 12-1502-1675, TS 11-1472-1633, TS 11-1468-1633, TS 09-1441-1525, TS 11-1486-1642, TS 12-1501-1582, TS 11-1480-1633, TS 96 -1207-169, TS 11-1467-1633, TS 09-1442-1525. The experience was monofactorial, in which the analyzed factor was the genotype, consisted of 14 variants, divided into 3 repetitions. As a control, the average of the determined values was determined for each parameter taken in the study. The parameters analyzed were: minitubers number / plant and minitubers weight minitubers / pl. TS 12-1489-1574 potato line was the most representative, being a productive line, which was distinguished by a number of 11.83 minitubers / plant and a significantly positive difference of + 4.21 comparative with control taken in the study. Weight of minitubers / plant was influenced by genotype, proving superiority TS11-1468-1633 potato line which had a good capacity to produce minitubers with high weight (295.17 g) (and a distinct significant difference, positive, to control +114.83). Thus, this potato line has a high potential for producing high yields.

**Key words:** mersitem, micropropagation, plantlets, “insect-proof” space, minitubers

*In vitro* multiplication is a basic method in the production of seed potatoes which has been introduced rapidly in almost all the seed potato producing regions.

*In vitro* culture of plants is regarded as the science and art of growing cells, tissues or plant organs in an artificial medium (Badr, 2011).

In micropropagation program an essential condition is to use as a starting point a free pathogenic material. By conventional method, potato is often pathogens aim such as fungi, bacteria and viruses and all of these have as results poor quality and low yields (FAO, 2008). Micropropagation is a tissue culture method used for rapid and true to type multiplication of plants (Naik and Karihaloo, 2007). Potato can be rapidly multiplied using nodal cuttings produced *in vitro* and involving following minitubers production (Diamante Ilze and Gaile Zinta, 2014). Plantlets are very small plants produced under completely sterile conditions (called *in vitro* conditions) (Struik, Wiersema, 1999). Minitubers can be obtained from *in vitro* plantlets planted under *in vivo* condition after planting them in soil. Minituber is an intermediate step of seed potato production between laboratory micropropagation

and field multiplication (Hagman J., 1990). Minitubers are principally used for the production of pre-basic or basic seed by direct field planting (Ritter A., Riga H. Relluso J. and San M.J., 2001). Minituber production through planting of micropropagated plantlets in soil can be regarded as a quick effective approach for potato seed tuber production (Ahloowalia, 1994).

Using of a high quality planting material is very important. In general, there is a direct link between the quality of tubers used as seed and the productivity of the crop. The quality of seed tubers is the most important factor for production (Struik and Wiersema, 2012).

### MATERIAL AND METHOD

In period 2015-2018, NIRDPSB Brasov as a partner of project ADER 221: Optimizing and modernization the seed potato production system of superior biological categories by eliminating the risk of contamination with damaging quarantine organisms and identifying of new potato varieties / lines resistant to nematodes with cysts of potato *Globodera spp.* (European populations), coordinated by SCDC Targu Secuiesc, obtain a

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biological material, free of viruses, considered Prebase (minitubers).

*In vitro propagation of plantlets*

To obtain minitubers, the starting point was represented by meristem culture, which were inoculate on nutritive medium. The meristematic explants were taken from tubers sprouts of 14 lines which are presented in table 1. The lines are created at SCDC Targu Secuiesc. The meristematic explant is taken with the first pair of foliar primers and introduced into the culture medium which contains as growing agent gibberelic acid. After 2-3 months, depending on the genotype, meristems (figures 1, 2) begin to develop small buds, from which plantlets are formed.



Figure 1. Culture of meristems in the growing room



Figure 2. Inoculated meristem

A variety of factors, such as: explant size, meristem position, and variety affect the success of virus eradication through the meristem culture. The smaller is the size of the meristem (0.2 mm long), the higher is the chances to be free of virus (Wang and Hu, 1980) (quote by Naik and Karihaloo, 2007).

Each meristem receives a number, which at multiplication is called the clone.

After 6-8 months, is made a determination of virus infection for plantlets, by DAS-ELISA test. Only virus indexed plantlets with no virus diseases detected were subjected to further micropropagation. Subculturing of microplants was performed once every 4 weeks. For the development of the axillary bud, the optimum photoperiod used is 16 h light and 8 h dark.

The Murashige-Skoog medium (1962) is the most widely used in micropropagation of potatoes. The agar is applied in the basic medium ( 9 g/l) as a gelling agent.

*Planting of in vitro plantlets and minitubers harvesting*

Vitroplants were planted on an adequate substrate consisting of peat and pearl in "insect-proof" spaces (spaces where aphids cannot penetrate - main vectors of viruses from external sources). Planting (figure 3) was made at 15.05.2018 and harvesting was done in 3.09.2018.



Figure 3. Planting the in vitro material

This research was made to determine the most productive line, experience contained 3 repetitions, was monofactorial, the factor analysed being the potato line.

The minitubers production for potato is the classical intermediate stage to use *in vitro* plant material. Techniques used to produce minitubers (figure 4) are diverse but rely mainly on replicating microplants on a classic substrate.

Variants studied		Table 1
Variants	Line	
V <sub>1</sub>	TS 12-1489-1574	
V <sub>2</sub>	TS 11-1467-1633	
V <sub>3</sub>	TS 96-1207-169	
V <sub>4</sub>	TS 11-1468-1633	
V <sub>5</sub>	TS 11-1475-1633	
V <sub>6</sub>	TS 12-1497-1573	
V <sub>7</sub>	TS 11-1486-1642	
V <sub>8</sub>	TS 12-1501-1582	
V <sub>9</sub>	TS 11-1480-1633	
V <sub>10</sub>	TS 12-1502-1675	
V <sub>11</sub>	TS 11-1472-1633	
V <sub>12</sub>	TS 12-1488-1574	
V <sub>13</sub>	TS 09-1442-1525	
V <sub>14</sub>	TS 09-1441-1525	

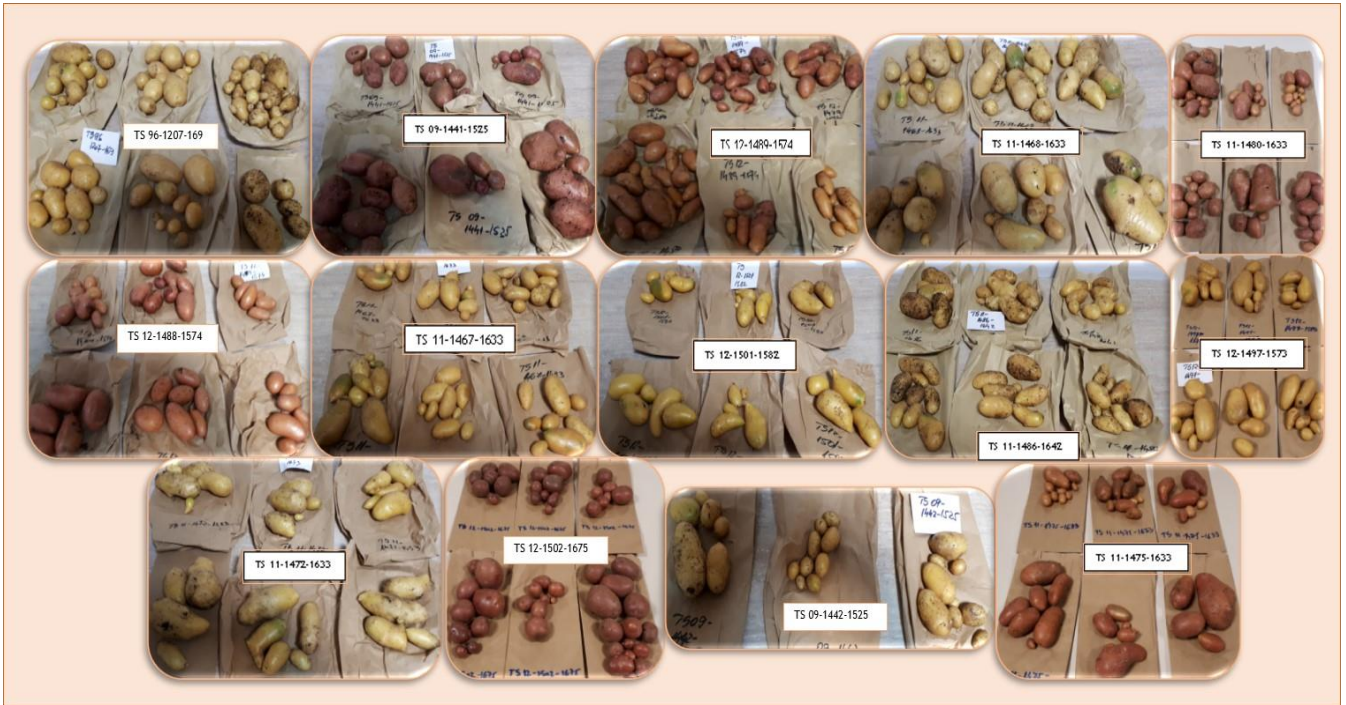


Figure 4. Samples of minitubers harvested from the analysed lines

**RESULTS AND DISCUSSIONS**

**Results on obtaining minitubers from tested potato lines**

*Statistical interpretation of the results obtained*

The obtained results are statistically significantly positive (table 2) for the TS 12-1489-1574 line, which had a number of 11.83 minitubers / plant and a difference of +4.21 compared to control from the study (mean values for all breeding potato lines). The value of TS 12-1501-1582 potato line (4.33 minitubers) indicates a low number of minitubers with an insignificant difference (-3.29 minitubers).

Regarding the minitubers obtaining/ plant, by size assortment (table 3), by comparing genotypes, we can observe that for 15-25 mm caliber, the highest percentage recorded the genotype TS 09-1442-1525 (48.80%), followed by TS 11-1467-1633 (45.44%) and TS 11-1486-1642 (40.69%). For the 25-35 mm size class, the TS 12-1501-1582 potato line obtained the highest percentage (57.74%), followed by TS 12-1497-1573 (51.46%) and TS 96-1207 -169 (40.01%). For the 35-45 mm size assortment, the highest percentage of obtained minitubers was recorded by TS 09-1441-1525 genotype (33.33%) and on the next place was TS 11-1472-1633 potato line (32.12%).

**Average number of harvested minitubers / plant (NIRDPSB Brasov, 2018)**

**Table 2**

Potato line	Number of minitubers / plant		Diff.	Signif.
		(%)		
TS 12-1489-1574	11.83	155.31	4.21	*
TS 96-1207-169	10.83	142.19	3.21	ns
TS 11-1486-1642	9.83	129.06	2.21	ns
TS 11-1480-1633	9.17	120.31	1.55	ns
TS 11-1467-1633	9.17	120.31	1.55	ns
TS 12-1502-1675	9.00	118.13	1.38	ns
<b>Mean (Ct)</b>	<b>7.62</b>	<b>100.00</b>	<b>-</b>	<b>-</b>
TS 11-1468-1633	7.50	98.44	-0.12	ns
TS 11-1475-1633	7.00	91.88	-0.62	ns
TS 09-1442-1525	6.83	89.69	-0.79	ns
TS 09-1441-1525	6.00	78.75	-1.79	ns
TS 12-1497-1573	5.83	76.56	-1.79	ns
TS 12-1488-1577	4.67	61.25	-2.95	ns
TS 11-1472-1633	4.67	61.25	-2.95	ns
TS 12-1501-1582	4.33	56.88	-3.29	ns

DL 5% = 3.29 minitub. DL 1% = 4.44 minitub. DL 0.1% = 5.93 minitub

Table 3

## Number of minitubers harvested on size assortments / plant (NIRDPSB Brasov, 2018)

Assortment of size for harvested material (mm)/potato line		<15	15-25	25-35	35-45	>45	TOTAL
		Number	%	Number	%	Number	%
TS 12-1489-1574	Number	1.83	4.83	4.00	1.17	0.00	11.83
	%	15.50	40.86	33.81	9.86	0.00	100.00
TS 96-1207-169	Number	2.00	3.50	4.33	0.83	0.17	10.83
	%	18.47	32.32	40.01	7.69	1.54	100.00
TS 11-1486-1642	Number	1.83	4.00	2.83	1.17	0.00	9.83
	%	18.65	40.69	28.82	11.87	0.00	100.00
TS 11-1480-1633	Number	1.50	2.83	3.33	1.00	0.50	9.17
	%	16.36	30.90	36.35	10.91	5.45	100.00
TS 11-1467-1633	Number	0.83	4.17	3.50	0.67	0.00	9.17
	%	9.09	45.44	38.17	7.27	0.00	100.00
TS 12-1502-1675	Number	0.50	3.33	3.17	2.00	0.00	9.00
	%	5.56	37.04	35.19	22.22	0.00	100.00
TS 11-1468-1633	Number	1.00	2.00	2.83	1.17	0.50	7.50
	%	13.33	26.67	37.78	15.56	6.67	100.00
TS 11-1475-1633	Number	1.67	2.00	2.50	0.67	0.17	7.00
	%	23.81	28.57	35.71	9.52	2.38	100.00
TS 09-1442-1525	Number	0.67	3.33	2.33	0.50	0.00	6.83
	%	9.76	48.80	34.16	7.32	0.00	100.00
TS 09-1441-1525	Number	0.67	1.17	1.83	2.00	0.33	6.00
	%	11.11	19.44	30.56	33.33	5.56	100.00
TS 12-1497-1573	Number	0.50	1.67	3.00	0.67	0.00	5.83
	%	8.58	28.59	51.46	11.44	0.00	100.00
TS 12-1488-1577	Number	0.67	1.33	1.83	0.50	0.33	4.67
	%	14.28	28.55	39.26	10.71	7.14	100.00
TS 11-1472-1633	Number	0.50	1.00	1.33	1.50	0.33	4.67
	%	10.71	21.41	28.55	32.12	7.14	100.00
TS 12-1501-1582	Number	0.50	0.67	2.50	0.67	0.00	4.33
	%	11.55	15.40	57.74	15.40	0.00	100.00

### Results on the weight of harvested minitubers / plant

The genotypes capacity to produce high weight minitubers is found at TS 11-1468-1633

potato line, represented by distinctly significant positive difference (114,83 g), this proving a high potential for minituberization (table 4).

Table 4

## Results on average weight of harvested minitubers / plant (NIRDPSB Brasov, 2018)

Line	The average weight of minitubers / plants		Diff.	Signif.
	g	(%)		
TS 11-1468-1633	295.17	163.67	114.83	**
TS 09-1441-1525	245.25	135.99	64.91	ns
TS 12-1489-1574	221.55	122.85	41.21	ns
TS 96-1207-169	195.88	108.62	15.54	ns
TS 11-1472-1633	192.82	106.92	12.48	ns
TS 11-1480-1633	188.66	104.61	8.32	ns
TS 11-1486-1642	187.37	103.90	7.03	ns
Mean (Ct)	180.34	100.00	-	-
TS 12-1502-1675	172.95	95.90	-7.39	ns
TS 11-1467-1633	164.55	91.24	-15.79	ns
TS 12-1497-1573	159.96	88.70	-20.38	ns
TS 11-1475-1633	134.58	74.63	-45.76	ns
TS 12-1488-1577	130.20	72.20	-50.14	ns
TS 12-1501-1582	129.77	71.96	-50.57	ns
TS 09-1442-1525	106.07	58.81	-74.27	ns

DL 5% = 77.61 g DL 1% = 104.73 g DL 0.1% = 139.77 g

### Results on the weight of minitubers harvested on size assortments

It can be seen from the table 5 that the best results for the average weight of the minitubers / plant are obtained at 35-45 mm. In this size class,

potato lines in the top positions are: TS 09-1441-1525 with an average weight of 163.97 followed by TS 11-1472-1633 with 51.79 g and TS 12-1502-1675 potato lines with 50.82. For the 25-35 mm size fraction, superior results obtained TS 12-1501-1582 genotypes (84.67 g), TS 12-1497-1573 (58.76 g). We can say that for distribution of the

harvest obtained on size assortments we have an interpretable distribution on certain sizes of minitubers formed and on a certain genotype, suggesting the superiority of the fraction of 35 - 45 mm.

Assortment of size for harvested material (mm)/potato line		<15	15-25	25-35	35-45	>45	TOTAL
TS 11-1468-1633	g	1.08	45.30	49.32	118.40	154.93	369.03
	%	0.29	12.28	13.36	32.08	41.98	100.00
TS 12-1489-1574	g	1.80	51.75	116.08	132.87	45.47	347.97
	%	0.52	14.87	33.36	38.18	13.07	100.00
TS 12-1502-1675	g	1.43	23.40	53.37	112.85	76.92	267.97
	%	0.53	8.73	19.92	42.11	28.70	100.00
TS 11-1475-1633	g	5.87	19.18	77.43	55.77	73.20	231.45
	%	2.53	8.29	33.46	24.09	31.63	100.00
TS 96-1207-169	g	4.55	23.63	70.32	90.13	34.83	223.47
	%	2.04	10.58	31.47	40.33	15.59	100.00
TS 11-1480-1633	g	0.32	18.93	73.68	84.35	29.85	207.13
	%	0.15	9.14	35.57	40.72	14.41	100.00
TS 12-1497-1573	g	1.63	18.43	56.52	70.78	50.75	198.12
	%	0.82	9.30	28.53	35.73	25.62	100.00
TS 12-1488-1577	g	1.05	10.15	40.20	96.77	47.85	196.02
	%	0.54	5.18	20.51	49.37	24.41	100.00
TS 09-1442-1525	g	0.00	11.98	47.35	85.27	30.77	175.37
	%	0.00	6.83	27.00	48.62	17.54	100.00
TS 11-1486-1642	g	1.42	35.85	77.80	46.70	0.00	161.77
	%	0.88	22.16	48.09	28.87	0.00	100.00
TS 11-1467-1633	g	3.42	25.72	74.63	53.57	0.00	157.33
	%	2.17	16.35	47.44	34.05	0.00	100.00
TS 11-1472-1633	g	0.87	19.83	45.42	36.07	32.75	134.93
	%	0.64	14.70	33.66	26.73	24.27	100.00
TS 09-1141-1525	g	0.68	11.20	21.95	44.57	49.52	127.92
	%	0.53	8.76	17.16	34.84	38.71	100.00
TS 12-1501-1582	g	0.95	23.18	43.85	7.57	17.08	92.63
	%	1.03	25.03	47.34	8.17	18.44	100.00

## CONCLUSIONS

TS 12-1489-1574 potato line was the most representative, being a productive line, which was distinguished by a number of 11.83 minitubers / plant and a significantly positive difference of +4.21 comparative with control taken in the study.

For the 25-35 mm size class, minitubers number obtained / plant are found on the TS 12-1501-1582 and TS 12-1497-1573 lines obtaining the highest weight (57.74 and 51.46%).

Weight of minitubers / plant was influenced by genotype, proving superiority TS11-1468-1633 potato line which had a good capacity to produce minitubers with high weight (295.17 g) (and a distinct significant difference, positive, to control +114,83). Thus, this potato line has a high potential for producing high yields.

In the future, we recommend to be used TS 12-1489-1574 potato line and to obtain patent as a

variety because this potato line has been identified in previous studies such as: *in vitro* by producing a high number of meristems, plantlets height was superior to the other potato lines, number of microtubers produced for this potato line showed a significant positive difference (+0.41 g). For minitubers production, in "insect-proof" space, in both years of study this line was representative. During the research of the ADER 221Project, TS 11-1468-1633 potato line was also highlighted by: obtaining *in vitro* a high number of clones, obtaining high-weight microtubers, and for two years of minituberization, this potato line has obtained minitubers, with remarkable minitubers weight values (369.03 g / pl for 2017 and 295.17 g / pl for 2018).

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