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RESEARCH AT NIRDPSB BRASOV ABOUT *IN VITRO* BEHAVIOR OF POTATO PLANTLETS BELONGING TO NEW AMELIORATED LINES AT SRDP TARGU SECUIESC

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Abstract

Through in vitro propagation accomplish a rapid multiplication of biological material free of viruses and so it can be reduce the number of generations in the field. This research is directed on behavior of in vitro plantlets belonging to 14 lines ameliorated at Station of Research and Development for Potatoes Targu Secuiesc and studing of different parametes of these: average length of a plant, average number of leaves/plant, average length of a root, average weight of a plant. Another part includes microtuber production from these lines (and studding two parameters: number of microtubers obtained / plant and wight of microtubers) and highlighting the best lines.

Key words*: potato, in vitro material, plantlets, microtubers, nutrive medium.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a vegetative propagated crop, is inclined to accumulation of infections by bacteria, fungi, viruses. Worldwide, viral diseases are considered as a limiting factor in seed potato production (Meybodi et al., 2011). Micropropagation allows the rapid multiplication of disease-free potato clones in a short time in a controlled environment. Using of micromultiplication techniques has opened new perspectives in for seed potato production and multiplication. These techniques allow a better control and maintenance of growth vigor and more efficient control of the phytosanitary state (Molet, Lavieville, 1990; Fourage, 1991; Senac, 1991, 1992, quoted by Morar, 1999). A huge amount of disease-free potato plantlets can be produced by the micropropagation method (Khurana et al., 2003). Microtubers are small *in vitro* tubers, which can be produced all over year on the complete plantlets or on plants organs (Ranalli, 1997). Over all each plantlet or explant can produce one microtuber with a 3-10 mm diameter and weight of 0.2-0.7 g (Struik, Lommen, 1990).

The aim of this work was to multiply potato plantles *in vitro*, especially to determine different parameters of plantlets and to produce microtubers. Microtubers production is one of the strategies under this perspective because of their small size and weight, which gives them tremendous advantages in terms of disease free, storage, transportation and mechanization Kanwal et al. (2006), in addition to the possibility of being stored for extended periods of time (McCrown, Joyce,1991).

MATERIALS AND METHODS

For research on the *in vitro* behavior of potato plants belonging to the lines studied

In vitro multiplication is an extensive method used to increase seed potato nuclear stock. This technique is widely used in many countries, is very flexible and offers a high rate of multiplication.

The use of tissue culture technology in the rapid multiplication of disease-free planting material has facilitated the production of seed potatoes, being used as a standard methodology in the production of free potato viruses from a valuable stock but with infections. Virus eradication technology and the establishment of an *in vitro* collection of virus-free germplasm is an important prerequisite for the production of seed potatoes, having as its starting point the

culture of meristems. Plantlets free of virus, tested by the DAS ELISA technique, were used to analyze 4 parameters: average length of a plant; average number of leaves/plant; average length of a root; average weight of a plant.
The data were recorded 29 days after inoculation; the monofactorial experience, in which the analyzed factor was the genotype, comprised 14 variants, divided into 3 repetitions.
The minicuttings, containing a single node, were inoculated into culture vessels (test tubes) containing Murashige-Skoog (MS) medium. The axillary/apical bud develops rapidly, so a plant has regenerated in 4 weeks.
As a control, was established for each analyzed parameter the average values.

For research on potato microtubers production belonging to the lines studied

In the Vegetable Tissue Culture Laboratory of NIRDPSB Brasov in period September-October 2017. Inoculation of minicuttings was made to study two parameters: number of microtubers obtained/plantlet and average weight of a microtuber.
Potato uninodal segments (microcuttings) are inoculated into a solid base medium Murashige-Skoog (1962), specific to the growth and rooting phase. The microcuttings cultures are kept in the growth room under controlled conditions of light and temperature for 30 days. The temperature is 22 ± 1°C / day and 18 ± 1°C / night, with a photoperiod of 16 hours light and 8 hours dark.
The pH of the nutrient medium was adjusted to 5.7 with 0.1N NaOH or HCl before autoclaving at 121°C for 15 minutes, pressure 1.1 kg/cm^2.
After microcuttings formed plantlets well-developed, 80 ml liquid tuberization medium was introduced/recipient.
The liquid microtuberization medium contains the same substances from Murashige-Skoog medium but in a reduced amount by half. Instead have an increased energy source: sucrose is higher (80 g/l) (20 g/l was in phase of formation and rooting of plantlets) and also in this phase is added coumarin and kinetin.
Cultures of plantlets were incubated in the climatic chamber in the dark at a temperature of 20°C for about 12 weeks. After microtuberization period (Figure 1), in January 2018 the data were recorded.
Potato plantlets were extracted from the culture recipents and the harvested microtubers were washed to remove all traces of the medium to avoid further infections that might occur during their storage. Microtubers obtained were harvested (Figure 2), washed, treated with fungicide, counted and stored for refrigeration at temperatures of 4-5^{o}C in the dark.
The experience was monofactorial. The studied factor being the genotype, which comprised 14 variants, divided into 3 repetitions. As a control, the average of the determined values was determined for each analyzed parameter.
The aim of the researches was *in vitro* identification of genotypes with valuable characteristics.

Figure 1. Microtubers obtained in culture recipients

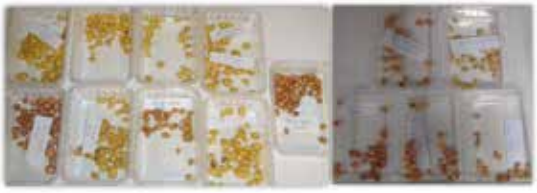

Figure 2. Harvested microtubers

RESULTS AND DISCUSSIONS

For *in vitro* behavior of potato plants belonging to the lines studied

By comparing variants studied differences to control variant with DL of 5, 1 and 0.1%, it appears that a single line, TS 12-1489-1574, presented superiority in the growth of the plantlets'lenght, with a significant difference (statistically ensured) by 1.44 cm; TS 11-1475-1633 line has registered a slowdown in plant growth, deviating by -1.43 cm (a significant negative difference) from mean (control).

It is found that the other lines are quite constant in plant growth, indicating stability in their behavior towards environmental factors (*in vitro* specific growth climate).

TS 12-1489-1574 line will provide in the future a possible biologically advantageous material in sense that it will develop a plant with high height (Table 1).

Table 1. Influence of genotype on the mean length of a potato plantlets/line

Line	Length of the plantlets		Diff. (cm)	Sign.
	(cm)	(%)		
TS 12-1489-1574	10.27	116.27	1.44	*
TS 11-1480-1633	9.83	111.36	1.00	ns
TS 11-1468-1633	9.33	105.70	0.50	ns
TS 11-1467-1633	9.27	104.95	0.44	ns
TS 11-1486-1642	9.17	103.81	0.34	ns
TS 12-1502-1675	9.07	102.68	0.24	ns
TS 12-1497-1573	9.00	101.93	0.17	ns
Mean (Ct)	8.83	100.00	-	-
TS 11-1472-1633	8.77	99.28	-0.06	ns
TS 96-1207-169	8.50	96.26	-0.27	ns
TS 12-1488-1574	8.50	96.26	-0.33	ns
TS 09-1442-1525	8.50	96.26	-0.33	ns
TS 09-1441-1525	8.17	92.49	-0.66	ns
TS 12-1501-1582	7.83	88.71	-1.00	ns
TS 11-1475-1633	7.40	83.81	-1.43	o

DL 5% = 1.41 cm DL 1% = 1.90 cm DL 0.1%= 2.54 cm

Another element studied was the average leaf number on the plantlet (Table 2), to which it was again chosen as control mean values of number of leaves/plantlets, situated at 8.38 leaves. This average is found to be 5th place, following a number of 10 lines, with a smaller number of leaves of which 9 lines have insignificant differences and only one line (TS 12-1488-1574) a significant negative difference of -1.38 leaves. Positive results get the lines: TS 11-1472-1633, TS 12-1502-1675 , which provides valuable genetic material, with a large number of leaves of 11.00 and 10.67, respectively, contributing with statistically positive differences by 2.62 considered very significant and 2.29, respectively, distinctly significant; these two lines give us valuable information on the creation of superior genetic material which will form a well-developed plant, with a large number of leaves and thus with a large assimilation surface. A number of 11 lines obtain results closely to mean variants, with insignificant differences indicating and by this time the stability of genetic material created at Station of Research and Development for Potatoes Targu Secuiesc.

Mean length analysis (Table 3) of the roots formed by plantlets belonging of the 14 genotypes (lines) studied, compared to their mean positioned control in the middle of these variants, values of limit differences compared to DL of 5, 1 and 0.1 %, indicating the starting lines TS 09-1442-1525 and final TS 12-1489-1574 with a diametrically opposed behavior, which get differences from significantly higher (3.55 cm) to significantly lower (-3.18 cm). Among these, 12 lines obtain insignificant results of differences in root length. TS 09-1442-1525 line which obtained the longest root length, but also the 6 lines that follow to the

level of the control, can be considered as valuable genotypes, viewed from the perspective of the formation of an elongated root, capable of extracting water from the soil and finally to fight against drought, therefore, to resist in and water stress conditions. Mean weight analysis (Table 4) of potato plantlets freshly taken gives us indications about ability of these genotypes to form well-developed plantlets, capable of growing and for later to develop vigorous and, of course, productive plants. It is distinguished TS 11-1480-1633 line which records the greatest weight of the plantlet (245.37 mg) with a significant positive difference by 70.29 mg to control (mean of values). Although the differences recorded by the other lines compared to control are insignificant they give us some indications over *in vitro* plantlets regeneration capacity, these values of weights being between 218.10 mg (TS 09-1442-1525) and 121.93 mg (TS 11-1486-1642).

Table 2. Results on average number of leaves/plant

Line	Average number of leaves		Diff.	Sign.
		(%)		
TS 11-1472-1633	11.00	131.26	2.62	***
TS 12-1502-1675	10.67	127.29	2.29	**
TS 11-1480-1633	8.67	103.42	0.29	ns
TS 09-1442-1525	8.67	103.42	0.29	ns
Mean (Ct)	8.38	100.00	-	-
TS 11-1467-1633	8.33	99.44	-0.05	ns
TS 11-1475-1633	8.33	99.44	-0.05	ns
TS 12-1489-1574	8.00	95.47	-0.38	ns
TS 96-1207-169	8.00	95.47	-0.38	ns
TS 11-1468-1633	8.00	95.47	-0.38	ns
TS 09-1441-1525	8.00	95.47	-0.38	ns
TS 11-1486-1642	7.67	91.49	-0.71	ns
TS 12-1501-1582	7.67	91.49	-0.71	ns
TS 12-1497-1573	7.33	87.51	-1.05	ns
TS 12-1488-1574	7.00	83.53	-1.38	o

DL 5% = 1.35 DL 1% = 1.82 DL 0.1% = 2.43

Table 3. Results on the average length of root/line

Line	Length of the plant		Diff. (cm)	Sign.
	(cm)	(%)		
TS 09-1442-1525	11.50	144.65	3.55	*
TS 11-1475-1633	10.00	125.79	2.05	ns
TS 11-1472-1633	9.17	115.30	1.22	ns
TS 12-1488-1574	9.00	113.21	1.05	ns
TS 12-1502-1675	8.67	109.01	0.72	ns
TS 12-1497-1573	8.33	104.82	0.38	ns
TS 11-1486-1642	8.17	102.73	0.22	ns
Mean (Ct)	7.95	100.00	-	-
TS 11-1468-1633	7.67	96.44	-0.28	ns
TS 11-1480-1633	7.33	92.24	-0.62	ns
TS 12-1501-1582	7.17	90.15	-0.78	ns
TS 11-1467-1633	7.00	88.05	-0.95	ns
TS 96-1207-169	6.33	79.66	-1.62	ns
TS 09-1441-1525	6.17	77.57	-1.78	ns
TS 12-1489-1574	4.77	59.96	-3.18	o

DL 5% = 3.16 cm DL 1% = 4.26 cm DL 0.1% = 5.69 cm

Table 4. Results on the average weight of a potato plant on the line

Line	Weight of the plant		Diff. (mg)	Sign.
	(mg)	(%)		
TS 11-1480-1633	245.37	140.15	70.29	*
TS 09-1442-1525	218.10	124.57	43.02	ns
TS 11-1475-1633	214.07	122.27	38.99	ns
TS 09-1441-1525	203.80	116.40	28.72	ns
TS 96-1207-169	201.53	115.11	26.45	ns
TS 12-1489-1574	176.83	101.00	1.75	ns
Mean (Ct)	175.08	100.00	-	-
TS 11-1468-1633	169.1	96.58	-5.98	ns
TS 12-1502-1675	159.37	91.03	-15.71	ns
TS 12-1497-1573	153.8	87.85	-21.28	ns
TS 12-1488-1574	153.23	87.52	-21.85	ns
TS 11-1467-1633	149.57	85.43	-25.51	ns
TS 11-1472-1633	143.93	82.21	-31.15	ns
TS 12-1501-1582	140.47	80.23	-34.61	ns
TS 11-1486-1642	121.93	69.64	-53.15	ns

DL 5% = 59.81 mg DL 1% = 80.71 mg DL 0.1% = 107.72 mg

For behavior of potato lines experimented in microtuberization

The response of varieties analyzed in terms of the number of microtubers (Table 5) shows the superiority of TS 11-1486-1642 genotype compared to the control mean (1.16 microtubers). Which shows an increase in the number of microtubers/plant (1.73). Which is very significant (+ 0.58 microtubers/pl), followed by the genotype TS 12-1489-1574 which recorded 1.57 microtubers/pl. and a distinctly significant difference (+0.41 microtubers/pl.). By comparing the experimental differences with limit differences obtained regarding the influence of the genotype (Table 6) on the average weight of a microtuber, it appears that line TS 11-1468-1633 (0.901 g) was superior to the mean of all values (considered control 0.643 g), showing a significantly positive difference in producing microtubers (+0.258 g), whereas TS 12-1502-1675 and TS 12-1488-1574 presented significant negative differences (-0.218 g and -0.224 g). By comparing the number of microtubers / plant and the average weight of a microtuber, it is noted that the TS 11-1486-1642 line produced the highest number of microtubers/pl. (1.73) with an average weight of 0.646 g. The TS 11-1468-1633 line is distinguished with a high average weight of microtuber (0.901 g), but with a number of them/pl. low (1.03).

Table 5. Influence of the genotype on the number of microtubers obtained/plant

Genotype	Number (microtub.)	%	Diff. (microtub.)	Sign.
TS 11-1486-1642	1.73	149.79	0.58	***
TS 12-1489-1574	1.57	124.80	0.41	**
TS 12-1501-1582	1.20	103.70	0.04	ns
TS 09-1442-1525	1.20	103.70	0.04	ns
Mean (Ct)	1.16	100.00	0.00	-
TS 11-1467-1633	1.13	97.94	-0.02	ns
TS 09-1441-1525	1.10	95.06	-0.06	ns
TS 11-1472-1633	1.07	92.18	-0.09	ns
TS 12-1488-1574	1.07	92.18	-0.09	ns
TS 11-1468-1633	1.03	89.30	-0.12	ns
TS 11-1475-1633	1.03	89.30	-0.12	ns
TS 96-1207-169	1.03	89.30	-0.12	ns
TS 11-1480-1633	1.03	89.30	-0.12	ns
TS 12-1497-1573	1.03	89.30	-0.12	ns
TS 12-1502-1675	0.97	83.54	-0.19	ns

DL 5% = 0.23 microtub. DL 1% = 0.31 microtub. DL 0.1%= 0.42 microtub.

Table 6. The influence of genotype on the weight of the microtubers obtained

Genotype	Weight (g)	%	Diff. (g)	Sign.
TS 11-1468-1633	0.901	140.11	0.258	*
TS 12-1501-1582	0.854	132.71	0.210	ns
TS 11-1480-1633	0.784	121.87	0.141	ns
TS 11-1472-1633	0.768	119.35	0.124	ns
TS 11-1467-1633	0.732	113.84	0.089	ns
TS 12-1489-1574	0.682	105.95	0.038	ns
TS 11-1486-1642	0.646	100.45	0.003	ns
Mean (Ct)	0.643	100.00	0.000	-
TS 09-1441-1525	0.624	97.02	-0.019	ns
TS 96-1207-169	0.599	93.16	-0.044	ns
TS 11-1475-1633	0.580	90.12	-0.064	ns
TS 12-1497-1573	0.524	81.51	-0.119	ns
TS 09-1442-1525	0.468	72.70	-0.176	ns
TS 12-1502-1675	0.425	66.05	-0.218	o
TS 12-1488-1574	0.419	65.17	-0.224	o

DL 5% = 0.215 g DL 1% = 0.291 g DL 0.1%= 0.388 g

CONCLUSIONS

The development of modern biotechnology techniques, has allowed to obtained scientific and practical results, concretized in efficient methods of rapid multiplication of new breeding creations and the production of seed material from the first biological links, of higher phytosanitary quality with profitable productions and competitive productions at national and international level.

In vitro results on plant height indicate that genotype TS 12-1489-1574 has achieved a preeminent value of 10.27 cm, with a significant positive difference, statistically assured +1.44 cm, compared to the mean of values (8.83 cm) obtained at the 14 lines. TS 12-1489-1574 will deliver in the future, a possible biologically advantageous material.

The mean number of leaf/plant oscillated between 11.00 (TS 11-1472-1633 line) and 7.00 (TS 12-1488-1574 line), being visible superior characteristics of TS 11-1472-1633 and TS 12-1502-1675 lines, which obtain statistically positive differences by 2.62 considered very significant and +2.29, respectively distinctly significant. They offer a valuable genetic material that will form a well-grown plants with a large number of leaves and so with a large assimilation surface. Statistical analysis made to determine the influence of genotype on mean length of roots, indicates the superiority of TS 09-1442-1525 line, which achieves a significant, positive difference (+3.55 cm). This line can be considered as a genotype capable of extracting soil water reserve and finally fight with effects of drought.

The mean weight of a freshly taken plantlet was inclined in favor of TS 11-1480-1633, which records the greatest weight of the plantlet (245.37 mg) with a significant positive difference by 70.29 mg from the mean (control).

TS 11-1486-1633 line was the most representative, being a productive line, which was distinguished by a number of 1.73 microtubers/plant and a difference very significant positive (+0.58 microtub.) compared to the control. This is followed by TS 12-1489-1574 which recorded a difference distinctly significant (0.41 microtub.), compared to the value of control.

Regarding the weight of microtuber line TS 11-1468-1633, produce big microtubers, with a significant positive difference (+0.258 g).

In the future we can focus on TS 11-1486-1633 and TS 12-1489-1574 lines if our purpose is obtaining a big number of potato tubers, with a weight (0.646 g and 0.682 g) which is over all mean values (control). If we want to obtain potato tuber with high caliber, we recommend TS 11-1468-1633 line.

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