RESULTS REGARDING NEW ROMANIAN POTATO (*Solanum tuberosum* L.) CULTIVARS REACTION TO *IN VITRO* CULTURE CONDITIONS

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Abstract. The *Solanum* genus presents a great importance for research due to its economical importance being a great aim to different breeding programs. It is the best represented genus from the *Solanaceae* family, part of this family species producing tubers. Working method used in our experimentations was double node fragments culture. Our studies aimed regeneration and multiplication of four potato cultivars (*Desirée, Redsec, Ts. 95-1161-66* and *Ts. 94-1117-98*) in order to improve the multiplication and pathogen free material obtaining protocols. Biometrical determinations performed for explants and new plantlets made possible to conclude as the best *in vitro* response was given by the cultivar *Redsec* both regarding the regeneration, growth and multiplication capacity and in foliar surface evolution, overcoming even the control (*Desiree* variety) and the cultivar *Redsec* can be recommended for other *in vitro* experimentations being a potent cultivar for this type of culture.

Keywords: potato varieties, *in vitro* cultures, regeneration and micropropagation

INTRODUCTION

Potato represents an important culture being situated on the second place after cereals both in Romania and in the world culture, where it is cultured on about 18 millions ha. This is one of the reasons that the potato is considered as a national importance culture, being nominee in the Romanian Government European Integration Program. Potato represents a basic aliment for the third millennium and constitutes the second bred for human's alimentation [10].

Due to the great potato importance in the national economy and in the breeding creations dissemination raises the rapid potato multiplication problem of the Romanian cultivars. *In vitro* cultures are also important in obtaining virus and pathogen free plantlets used in seed material production [2, 4].

Potato conventional breeding progress is quite slow and leaded to the necessity of approaching this plant as a genetic system open to biotechnologies and breeding modern methods at the cellular and molecular level. Thus, due to its *in vitro* regeneration capacity the potato was introduced in the tissue cultures experiments [1].

Potato *in vitro* culture in Romania was practiced in our country in the last years due to its technological fiability either as one certain mean that permits virus affected genotypes regeneration either as a simple technique that rapidly contribute to potato seed material production and/or as alternative to long time potato cultivars conservation [2, 4].

Work method used in experimentation was constituted by the two nodes fragments culture [8]. The experiment aim was establishing the *in vitro* culture response of the four potato cultivars tested (*Desirée, Redsec, Ts. 95-1161-66* and *Ts. 94-1117-98*).

MATERIALS AND METHODS

In vitro culture was initiated starting from shoots germinated *in vitro*.

Choosing this explants type was done due to the reason that the *in vitro* descendants would be genetically identical to the parental material. One of this method advantages is the fact that *in vitro* culture can be done all over the year if the tubers have passed a short vegetative resting period. Many culture media have been tested, but the results obtained on the propagation medium [9] are presented in the results (Table 1).

The biological material used in micropropagation was constituted of the two nodes micro-cuttings proceeded from vitro-shoots of potato (*Solanum tuberosum* L.) obtained on propagation medium [9] from the cultivars: *Desirée, Redsec, Ts. 95-1161-66, Ts. 94-1117-98.*

Two nodes micro-cuttings of about 10-15 mm were generated from shoots regenerated *in vitro*, in aseptic conditions, in the sterile air laminar flow cabinet perimeter, with adequate instrumentation. Cultures incubation was done in growing room with white fluorescent light of 1700 lux and photoperiodism of 16 h light/8 h darkness and 19°C temperature.

Table 1. Potato multiplication culture media constituents [9].

Constituents	PM	Constituents	PM
Macroelements	**mg/l**	**Microelements**	**mg/l**
KNO_3	1.900	$CuSO_4$ x 5 H_2O	0.03
NH_4NO_3	1.650	$CoCl_2$ x 6 H_2O	0.03
$CaCl_2$ x 2 H_2O	440	$FeSO_4$ x 7 H_2O	28
$MgSO_4$ x 7 H_2O	370	Na_2EDTA	37.3
KH_2PO_4	170	**Vitamins**	**mg/l**
$ZnSO_4$ x 7 H_2O	8.6	Myo- inositol	100
$MnSO_4$ x 4 H_2O	22.3	Thiamine HCl	0.4
H_3BO_3	6.2	Pyridoxine HCl	0.5
KI	0.83	Acid nicotinic	0.5
Na_2MoO_4 x 2 H_2O	0.25	Glycine	2
NaH_2PO_4	170	Sucrose	30,000.0
pH: 5.4 – 5.6			

Observation have been made one week after inoculation and continued during 35 days (until plantlets reached the upper part of the vegetation dishes). Notes consisted of date of root formation, date of organogenesis start and every seven days observations and determinations related to plant height (cm), caulia formation (nodes/internodes) and foliar system (cm^2) have been made. Mean leaf diameter was

measured for plantlets grown on vegetation dishes in order to finally compare the development of the foliar system.

The statistical calculations were used for analysis of the individual variance, the values with central tendency and their deviation ($\bar{x} \pm s_{\bar{x}}$) as well as the differences and their deviation ($\bar{d} \pm s_{\bar{d}}$) being assessed.

For multiple comparisons we have used specific methods for single factor analysis.

RESULTS

Ten days after inoculation organogenesis started to be obvious, but no differences were observed between the experimental variants (Table 2).

Explants growth in high was registered 7, 14, 21, 28 and 35 days after inoculation. Plants reached more than 11 cm high after 35 days on culture and no more measurements were possible.

Table 2. The height growth of *in vitro* cultivated potato plantlets, *Desirée, Redsec, Ts. 95-1161-66, Ts. 94-1117-98* varieties, on propagation medium.

Statistics evaluation	Days of observation				
	7 days	14 days	21 days	28 days	35 days
C – *Desiree*					
$\bar{x} \pm s_{\bar{x}}$	1.52 ± 0.10	3.03 ± 0.33	4.34 ± 0.32	5.67 ± 0.45	6.01 ± 0.55
$\bar{d} \pm s_{\bar{d}}$	-	1.51 ± 0.35	1.31 ± 0.46	1.33 ± 0.55	0.34 ± 0.71
V_1 – ***Redsec***					
$\bar{x} \pm s_{\bar{x}}$	1.55 ± 0.07	3.84 ± 0.31	5.25 ± 0.47	6.18 ± 0.96	6.46 ± 0.37
$\bar{d} \pm s_{\bar{d}}$	-	2.29 ± 0.32	1.41 ± 0.57	0.29 ± 1.07	0.62 ± 1.02
$\bar{d}$ **V_2-C**	+ 0.30 ± 0.12 **(ns)**	+ 0.81 ± 0.45 **(ns)**	+ 0.91 ± 0.57 **(ns)**	+ 0.51 ± 1.05 **(ns)**	+ 0.45 ± 0.67 **(ns)**
V_2 – ***Ts. 95-1161-66***					
$\bar{x} \pm s_{\bar{x}}$	1.42 ± 0.06	3.52 ± 0.33	4.78 ± 0.56	5.29 ± 0.58	6.13 ± 0.10
$\bar{d} \pm s_{\bar{d}}$	-	2.1 ± 0.34	1.26 ± 0.65	0.51 ± 0.81	2.04 ± 0.59
$\bar{d}$ **V_3-C**	- 0.10 ± 0.12 **(ns)**	+ 0.49 ± 0.47 **(ns)**	+ 0.43 ± 0.64 **(ns)**	- 0.41 ± 0.74 **(ns)**	+ 0.12 ± 0.56 **(ns)**
V_3 – ***Ts. 94-1117-98***					
$\bar{x} \pm s_{\bar{x}}$	1.47 ± 0.21	2.53 ± 0.28	4.42 ± 0.45	5.30 ± 0.16	5.73 ± 0.19
$\bar{d} \pm s_{\bar{d}}$	-	1.06 ± 0.35	1.89 ± 0.53	1.52 ± 0.48	0.79 ± 0.25
$\bar{d}$ **V_4-C**	- 0.05 ± 0.24 **(ns)**	- 0.51 ± 0.44 **(ns)**	+ 0.08 ± 0.55 **(ns)**	- 0.37 ± 0.48 **(ns)**	- 0.28 ± 0.59 **(ns)**

Note: $\bar{X} \pm S_{\bar{X}}$ (average ± standard deviation of the average), $\bar{d} \pm s_{\bar{d}}$ (difference ± standard deviation of difference), $\bar{d}$ $V_{1 \ldots 3}$ -C (difference between variant and control), ns – nonsignificant.

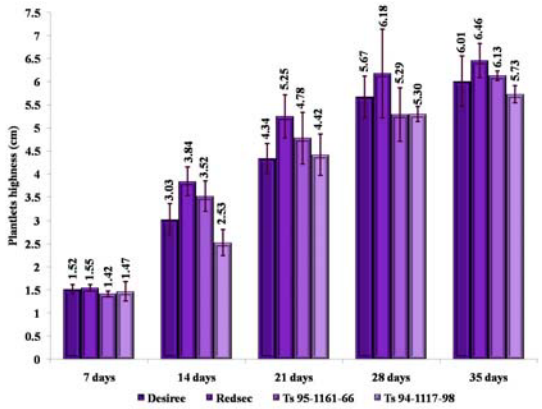

Figure 1. Growth rate of *in vitro* cultivated potato plantlets, *Desirée, Redsec, Ts. 95-1161-66, Ts. 94-1117-98* varieties on propagation medium.

Best response on *in vitro* culture conditions was given by the genotype *Redsec* that was nominee as the cultivar with the highest *in vitro* regenerative and multiplication capacity (Fig. 1).

Comparing the results obtained regarding the four cultivars micropropagation it was observed that differences between the Romanian cultivars (*Redsec, Ts. 95-1161-66, Ts. 94-1117-98*) and the control cultivar *Desirée* are not significant.

The highest positive differences were registered for the cultivar *Redsec* at 14 and 21 days of experimentation ($\bar{d} \pm s_{\bar{d}}$ =+ 0.81 ± 0.45 respectively $\bar{d} \pm s_{\bar{d}}$ = + 0.91 ± 0.57) and the highest negative differences were registered for the cultivar Ts. 94-1117-98 ($\bar{d} \pm s_{\bar{d}}$ =- 0.51 ± 0.44) (Table 2).

These results reflect each genotype influence both on *in vitro* "culturability" [3, 11, 12] and on multiplication in artificial conditions capacity [13].

Comparing the results obtained regarding the foliar surface index (cm^2) for the forth potato cultivars studied was observed that the differences between the Romanian cultivars (*Redsec, Ts. 95-1161-66, Ts. 94-1117-98*) and the control cultivar *Desirée* are not significant in the first seven days of culture, but become significant in different grades during the culture (Table 3 and Fig. 2).

Thus positive differences toward the control were registered for the cultivar *Redsec* (between $\bar{d} \pm s_{\bar{d}}$=+0.03 ± 0.13 cm^2 at 14 days and $\bar{d} \pm s_{\bar{d}}$= + 0.24 ± 0.71 cm^2 at 28 days). The cultivars *Ts. 95-1161-66*

Table 3. Evolution of foliar surface index of new *Solanum tuberosum* L. plantlets, *Desirée, Redsec, Ts. 95-1161-66, Ts. 94-1117-98* varieties, *in vitro* cultivated on propagation medium.

Statistics evaluation	Days of observation				
	7 days	14 days	21 days	28 days	35 days
C – *Desiree*					
$\bar{x} \pm s_{\bar{x}}$	0.11 ± 0.03	0.30 ± 0.07	0.99 ± 0.70	1.21 ± 0.56	1.86 ± 0.40
$\bar{d} \pm s_{\bar{d}}$	-	0.19 ± 0.08	0.69 ± 0.71	0.22 ± 0.90	0.65 ± 0.69
V_1 – ***Redsec***					
$\bar{x} \pm s_{\bar{x}}$	0.10 ± 0.02	0.33 ± 0.11	1.04 ± 0.46	1.44 ± 0.44	2.04 ± 0.41
$\bar{d} \pm s_{\bar{d}}$	-	0.23 ± 0.11	0.71 ± 0.48	0.40 ± 0.64	0.60 ± 0.60
$\bar{d}$ **V_2-C**	-0.01 ± 0.04 **(ns)**	+ 0.03 ± 0.13 **(ns)**	+0.05 ± 0.84 **(ns)**	+0.24 ± 0.71 **(ns)**	+0.18 ± 0.57 **(ns)**
V_2 – ***Ts. 95-1161-66***					
$\bar{x} \pm s_{\bar{x}}$	0.11 ± 0.02	0.17 ± 0.03	0.23 ± 0.05	0.40 ± 0.06	0.63 ± 0.14
$\bar{d} \pm s_{\bar{d}}$	-	0.06 ± 0.04	0.05 ± 0.06	0.05 ± 0.09	0.23 ± 0.15
$\bar{d}$ **V_3-C**	0.00 ± 0.04 **(ns)**	-0.13 ± 0.08 **(ns)**	-0.76 ± 0.71 **(ns)**	-0.81 ± 0.57 **(ns)**	-1.22 ± 0.42 **(o)**
V_3 – ***Ts. 94-1117-98***					
$\bar{x} \pm s_{\bar{x}}$	0.11 ± 0.05	0.20 ± 0.05	0.35 ± 0.06	0.48 ± 0.05	0.66 ± 0.05
$\bar{d} \pm s_{\bar{d}}$	-	0.09 ± 0.07	0.15 ± 0.08	0.31 ± 0.05	0.18 ± 0.05
$\bar{d}$ **V_4-C**	0.00 ± 0.06 **(ns)**	-0.10 ± 0.09 **(ns)**	-0.64 ± 0.71 **(ns)**	-0.73 ± 0.56 **(ns)**	-1.19 ± 0.40 **(o)**

Note: $\bar{x} \pm S_{\bar{x}}$ (average ± standard deviation of the average), $\bar{d} \pm s_{\bar{d}}$ (difference ± standard deviation of difference), $\bar{d}\, V_{1\ldots3}$ -C (difference between variant and control), ns – nonsignificant.

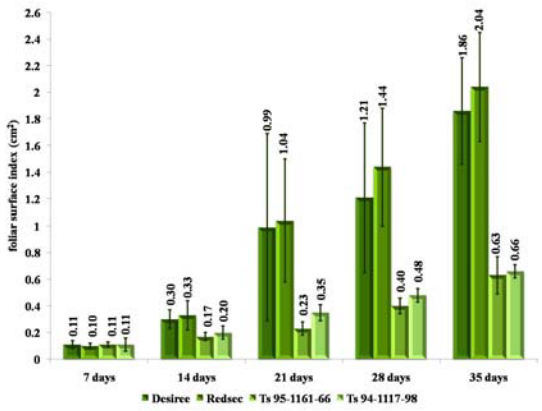

Figure 2. Evolution of foliar surface index of new *Solanum tuberosum* L. plantlets, *Desirée, Redsec, Ts. 95-1161-66, Ts. 94-1117-98* varieties, *in vitro* cultivated on propagation medium.

and *Ts. 94-1117-98* registered lower results comparing with the control regarding the foliar surface index, differences that increased during the *in vitro* culture period. Significantly statistical differences, for these two cultivars were registered in the 35th day of observation and were between $\bar{d} \pm s_{\bar{d}}$=- 1.22 ± 0.42 cm^2 for the cultivar Ts. 95-1161-66 and $\bar{d} \pm s_{\bar{d}}$=- 1.19 ± 0.40 cm^2 for the cultivar Ts. 94-1117-98 respectively.

The results obtained lead us to the conclusion that *Redsec* is the cultivar with the best *in vitro* "culturability" giving best results both in growth rate and in foliar surface evolution during the 35 days of experimentation, overcoming even the control.

DISCUSSIONS

Micropopagation is a rapid multiplication technique used to obtain high quality potato seed tubers, one of its main objectives being to optimize in vitro shoot growth. Considerable research has been done on the nutritional [17, 6] and hormonal [14] aspects of culture media preparation: however, the physical states of the culture media and its effects on the mechanisms that regulate explants growth have received less attention [16].

A number of different approaches have been successfully used in the regeneration of potato plants from *in vitro* cultures. Roest and Bokelman [15] obtained plantlet regeneration from potato stem segments when explants were cultured on a MS medium supplemented with 10 mg/l GA3, 1.0 mg/l BA, and 1.0 mg/l IAA. Espinoza and collaborators [7] have reported on the micropropagation of potato by either nodal section or shake cultures. They found that when nodal sections were inoculated into a MS culture medium supplemented with 0.25 mg/l GA3 and 2.0 mg/l calcium pantothenic acid, the number of nodes increased six fold within 3-4 weeks. When nodal sections were cultured on a liquid MS medium supplemented with 0.4 mg/l GA3, 0.5 mg/l BA, 0.01 mg/l NAA, 2.0 mg/l calcium pantothenic acid, and 2% sucrose, there was a 10- to 20-fold increase in the number of shoos in 2-3 weeks.

Ranalli [14] reported a typical method for potato micropropagation namely single node propagation or micro grafting. The micrografts are constituted of single node cutting 5-10 mm long that inoculated on solid culture medium generate shoots and finally one plant.

Several Romanian potato cultivars were studied for regenerative capacity and recalcitrance for *in vitro* culture was found for some of them [4, 5].

The aim of these studies was to establish the protocols for micropropagation of four important Romanian potato cultivars, *Redsec*, *Ts 95-1161-66* and *Ts 96-1117-98* created at Potato Research – Develop-

ment Station from Targu Secuiesc Romania as against *Desiree* variety as well as control.

Biometrical determinations performed for explants and new plantlets made possible to conclude the followings:

- Organogenesis constituted of roots forming and first leaf appearance took place 10 days after inoculation for all the cultivars taken into experimentation.
- PM medium proved to be the best basic medium for potato micropropagation due to the fact that contains phosphorus and glycine supplementary, necessary for potato plantlets regeneration and growth.
- The best *in vitro* response was given by the cultivar *Redsec* both regarding the regeneration, growth and multiplication capacity and in foliar surface evolution, overcoming even the control.
- The cultivar *Redsec* can be recommended for other *in vitro* experimentations being a potent cultivar for this type of culture.

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