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Estimation of Genotype, Explant Size and Microbial Enzymes Influence on Regenerative Capacity of Potatoes.

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ABSTRACT

In this study effects of genotype, explants size and the composition of the culture medium on regeneration capacity of apexes of etiolated potato tubers sprouts were estimated. We observed positive correlation among survival, growth and development of plant tissues *in vitro* and the size of plant explants. When the cultivation medium was supplemented by RNase A (1-10 μg/ml) and ribonucleases from *Bacillus pumilus* (RNAse Bp, 1 μg/ml) 35% increase of the number of the regenerants was observed. Enzymes with ribonuclease activity at low concentrations (1-10 μg/ml) stimulated regenerative and morphogenic processes. In contrast neither plant regeneration dynamic nor plant morphogenesis were changed when cultivation medium was supplemented by other *Bacillus pumilus* proteolytic enzymes (subtilisin-like protease (AprBp), glutamyl endopeptidase (GseBp) and metalloendopeptidase (MprBp) in concentration of 1 μg/ml.

Keywords: potato, plant regeneration, *Bacillus pumilus,* ribonuclease, subtilisin-like protease, metalloendopeptidase, glutamyl endopeptidase.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is world-wide important crop plant. Propagation of potato occurs vegetatively. Most of potatoes diseases infect tubers that result in reduction of the nutrition value and the quality of planting material. There is a seed potato production system including the obtaining of infection-free potato plants, its propagation and planting under infection-free conditions [1]. In this case, *in vitro* technique of plant tissue culture is used [2]. However, since isolated fragments of plant tissues have low regeneration capacity and virus-free plant output, a screening of microexplant culture cultivation conditions is required. In this study the impact of genotype and the size of isolated explants on regeneration capacity of potato was investigated. Application of various biologically active compounds, *i.q.* enzymes, is a novel biotechnological approach to increase the efficiency of plant regeneration and plant morphogenesis [3]. A set of extracellular enzymes of *Bacillus pumilus* such as ribonuclease (RNase Bp) [4], subtilis-like protease (AprBp) [5], glutamyl endopeptidase (GseBp) [6] and metalloendopeptidase (MprBp) [7] were assessed in this study.

MATERIALS AND METHODS

We have used etiolated potato tuber sprouts. The potato tubers were subjected to purification and sterilization by (40%) ethanol solution. Potato sprouts 5 mm in length were used for the explants isolation.

The potato sprouts were sterilized in 0.5% sodium hypochlorite solution for 15 min followed by 10 min incubation in 70% ethanol solution. The sprouts then were washed 3 times in sterile distilled water. The explants with different size were isolated by scalpel under a binocular microscope at 24x magnification. After isolation, all samples were transferred by the needle tip into the nutrient medium MSM1 [3] for regeneration procedure. Isolated explants were incubated at 24-25°C under 2 000 Lux light intensity with 70% humidity during 16 hours. The proteolytic enzymes were added in sterile MSM1 cultivation medium through a bacterial filter.

After regeneration the potato sprouts with one-two leaves were transferred in the MSM2 culture medium [3]. Regenerated plants with 5-6 leaves were micropropagated subjected to and cultivated at the Murashige-Skoog (MS) cultivation medium [8] at room temperature for 16/8 h photoperiod.

The data were calculated using the Statistica 6.0 software. The significance of the data was estimated using Fisher's exact test.

RESULTS AND DISCUSSION

After the transfer of etiolated potato sprout apexes into the MSM1 cultivation medium, regeneration process of plant was observed and plant morphogenesis was occurred in the MSM2 cultivation medium (Figure 1).

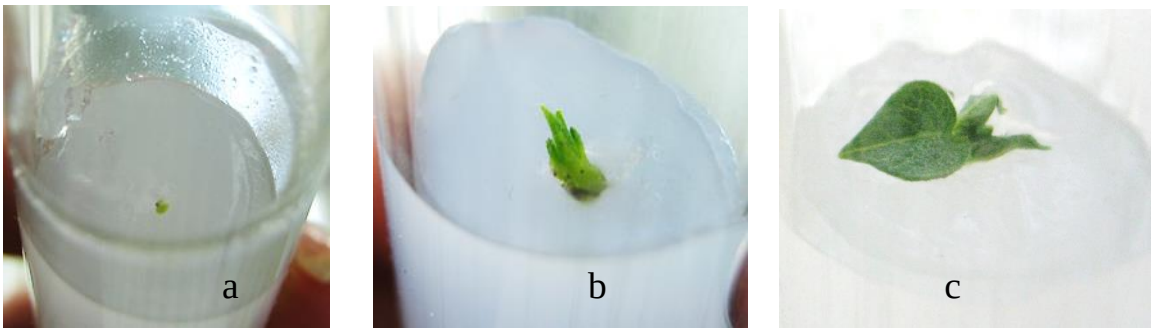

Figure 1: Development stages of potato tuber etiolated sprout apexes of breeding line 3-23-2: (a) explant after the transfer to the culture medium MSM1; (b) development of adventitious buds on a culture medium MSM1; (c) formation of adventitious shoots on the medium MSM2.

The regeneration of plant explants was studied on a potato cultivar Arosa and breeding lines 3-23-2, 3-43-6, 8-28-38, 8-22-9, 8-12-1, 8-32-4, 8-8-6, 8-5-3 and 8-20-3. The experimental results are shown in Table 1.

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In total, 551 plant explants were isolated from the 187 potato tubers. Among them, 167 plant explants have survived. The survival rate of the plant explants was 30%.

Table 1: The results of the regeneration investigated samples of potato

№	Sample	Number of tubers, pcs.	Number of isolated explants, pcs.	Explant size, mm	Number of survived explants	
					pcs.	%
1	8-28-38	9	21	0,5-1	0	0
2	8-32-4	10	31	0,5-1	0	0
3	8-22-9	10	33	0,5-1	2	6
4	8-12-1	4	12	0,5-1	1	8
5	8-20-3	7	7	0,5-1	0	0
6	8-8-6	10	48	0,5-1	0	0
7	8-5-3	10	34	0,5-1	1	3
8	Arosa	64	203	2-4	72	35
9	3-23-2	34	100	2-4	47	47
10	3-43-6	29	62	2-4	44	71

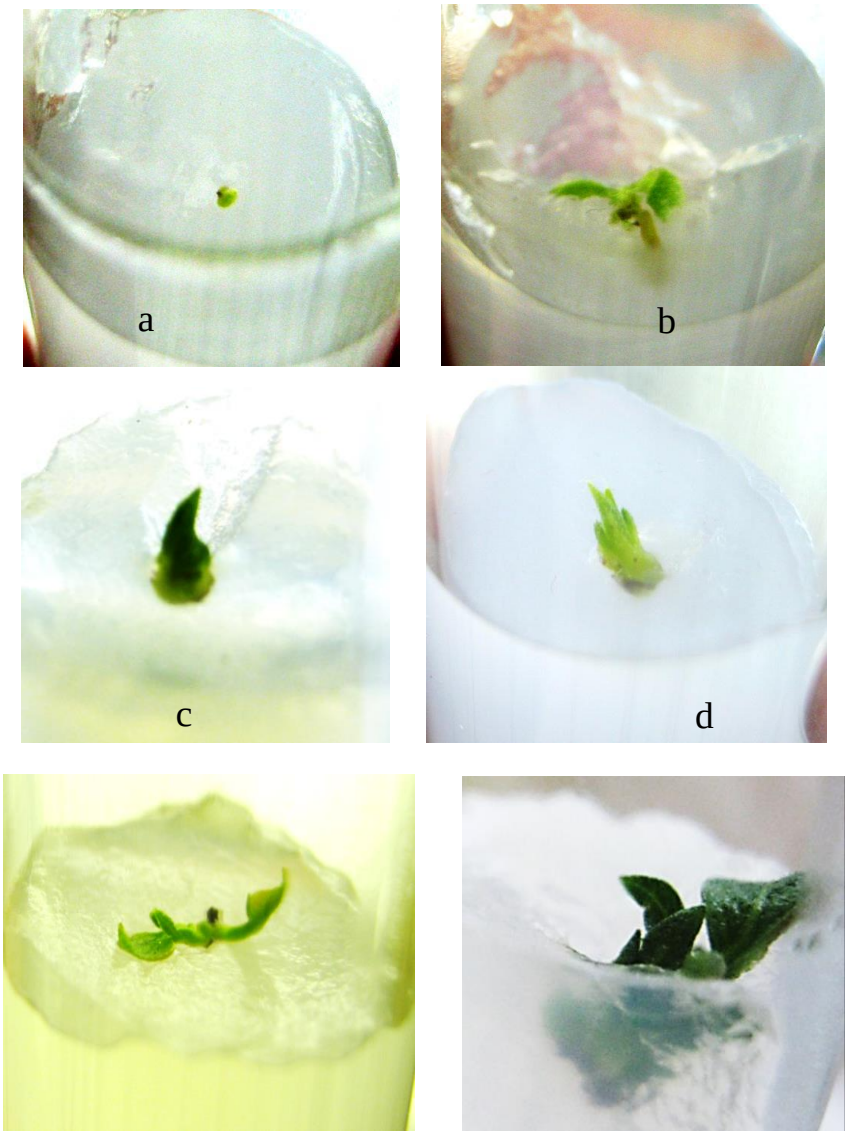

Figure 2: Development of explants (a) breeding line 8-5-3 and (b) breeding line 8-22-9 on culture medium MSM1 at 20-th week of cultivation. Development of explants (c) breeding line 3-43-6 and (d) breeding line 3-23-2 on culture medium MSM1 at 5-th week of cultivation. Development of explants breeding line 3-23-2 on culture medium MSM1 with addition (e) RNase Bp 1 µg/ml and (f) RNase A 1 µg/ml at 3-d week of cultivation.

The survival rate was the explant-size dependent. When the size of plant explants were 0.5-1 mm (for potato breeding lines 8-28-38, 8-22-9, 8-12-1, 8-32-4, 8-8-6, 8-5-3, 8-20-3) the survival rate was ranged from 0 to 8,3% (Table 1). For a variety Arosa potato and breeding lines 3-23-2 and 3-43-6 the size explants was 2-4 mm and the survival rate was 35, 47 and 71% respectively. Thus, size of plant explants is one of the most important factors for the survival. Moreover, it was shown that genotype has influence on the survival of plant explants. This fact should be taking into account during the planning of biotechnology experiments as well as in the biotechnology industry.

The size of plant explants have affected on the speed of plant regeneration. For potato breeding lines 8-28-38, 8-22-9, 8-12-1, 8-32-4, 8-8-6, 8-5-3, 8-20-3, where the size of the plant explant was 0.5-1 mm, the regeneration of plants has been slow (Figure 2a, 2b). For the breeding lines 3-43-6, 3-23-2 and a cultivar Arosa the size of plan explants was 2-4 mm and the plant development was faster (Figure 2c, 2d). These samples were developed normal tissue differentiation and active growth at 5th weeks of the growth.

While the low yield of regenerated plants and the delay in their growth were observed, we further investigated action and biological activity of *B. pumilus* enzymes. Sprouts from potatoes breeding line 3-23-2 were used to assess the impact of the enzymes activity (Table 2). When the cultivation medium was supplemented by RNase Bp (1 µg/ml) the increased amount of plant regenerates were found. An increasing of ribonuclease concentration resulted in a lower number of regenerated plants. At the same time, the calculation of Fisher's exact test showed that identified differences are not statistically significant from control ($p>0.05$).

Table 2: The effect of enzymes on the regeneration efficiency of potato plants

Variant	Enzyme concentration, µg/ml	Number of explants, pcs.	Number of regenerants	
			pcs.	%
Control	0	100	47	47
RNase A	1	17	14	82**
	10	15	12	80*
	50	13	9	69
	100	11	8	73
	500	5	4	80
RNase Bp	1	18	15	83**
	10	17	12	71
	50	9	7	78
	100	4	2	50
	500	5	3	60
AprBp	1	20	11	55
GseBp	1	25	14	56
MprBp	1	19	10	53

* – $p<0.05$; ** – $p<0.01$.

RNase Bp impact was comparable with that of the pancreatic ribonuclease A (RNase A). Stimulation effect on the plant regeneration was previously shown for RNase A [3]. It should be noted that RNase A had a profound impact on both quantitative yield of regenerated plants and speed of plant morphogenesis.

New plant tissues (1-1.5 cm) and formation of leaves were detected in almost all selected samples at 3rd growing week (Figure 2e, 2f). Statistically significant effect of RNase A was observed under its concentration range of 1-10 mg/ml.

When the cultivation medium was supplemented by *B. pumilus* proteolytic enzymes (AprBp, GseBp MprBp) in concentration of 1 µg/ml there was no impact on both the plant regeneration process and the plant morphogenesis.

CONCLUSION

The survival of etiolated potato tuber sprout apexes, its growth and development in the cultivation medium *in vitro* increased in explants-size depended manner (positive correlation). The ribonuclease treatment in low concentrations stimulated potato plant growth and the plant regenerative processes.

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