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THE EFFECT OF ANTIVIRAL TREATMENTS FOR *IN VITRO* POTATO CULTURE ON THE GROWTH AND DEVELOPMENT OF PLANTLETS AND ON THE ELIMINATION OF THE *Potato virus* S

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Abstract

During in vitro potato multiplication process, of three Romanian potato varieties (Sarmis, Foresta and Castrum) infected with Potato Virus S (PVS), detected by ELISA test, an experiment was performed with reference of influence of salicylic acid and antiviral ribavirin over two plantlets parameters: height (cm) and leaves number. The trifactorial experience (2 x 3 x 3), on 3 repetitions had the following factors: experimental factor A- the culture medium used before antiviral treatment, with two graduations: a1 - classical medium (as control) Murashige -Skoog (1962); a2 - MS+ salicylic acid (100 mg/l); experimental factor B - the variety, with three graduations: b1 - Sarmis (as control), b2 - Foresta, b3 - Castrum; experimental factor C - ribavirin concentration: c1 - 0 mg/l (as control); c2 - 50 mg/l; c3 - 100 mg/l. The objective of the study is to eradicate PVS. Using of ribavirin drastically decreased the height of the plantlets and the number of leaves, causing very significant negative differences for the two parameters (for both concentrations).

Key words*: potato, in vitro multiplication, plantlets, chemotherapy, virus elimination.*

INTRODUCTION

Vegetative propagation of potato results in transmission of the virus from one generation to the next, with virus titers accumulating as a result of repeated propagation (Thomas-Sharma et al., 2016; Priegnitz et al., 2020). Viral diseases, in addition to inducing increased susceptibility to other pathogens, cause economic losses due to their negative impact on tuber production and quality (Lin et al., 2014; Adolf et al., 2020).

The types and concentrations of antiviral agents used in chemotherapy, the duration of chemotherapy application, as well as the tip sizes of excised shoots, can affect the success of virus eradication (Al Maarri et al., 2012; Kushnarenko et al., 2017; Waswa et al., 2017; Magyar- Tábori et al., 2021, cited by Bettoni et al., 2022). Standardization of virus eradication methodology is therefore important, especially when plants have mixed infections. Several antivirus chemicals were available against plant viruses (Wang et al., 2018). Ribavirin is the most frequently used antimetabolite. hemotherapy is dedicated for elimination of PVM, PVS and PVX. The most difficult virus to remove from infected plants is PVS (Dajmund, 2017).

Salicylic acid participates in the regulation of the plant's response to a series of environmental stresses such as extreme temperatures, salinity, and oxidative condition of potato growth, so it is necessary to determine a safe application dosage for potato in field conditions (Contreras-Liza S., Vargas-Luna, 2022). Salicylic acid (SA) is a molecule related to the stress response in plants (Hayat and Ahmad 2007, quoted by Contreras-Liza and Vargas-Luna, 2022) and is therefore considered a candidate for exogenous applications as an activator of induced systemic resistance. Salicylic acid (SA) is a phenolic derivative, distributed in a wide range of plant species. It is a natural product of phenylpropanoid metabolism. SA has direct involvement in plant growth, thermogenesis, flower induction and uptake of ions (Hayat et al., 2007). Salicylic acid (SA) is an important phytohormone that serves as a critical signal molecule mediating immunity and plant growth (Vlot et al., 2009; Rivas-San Vicente and Plasencia, 2011, quoted by Li et al., 2022). SA plays crucial roles in regulating cell division and cell expansion, the

key processes that determines the final stature of plant (Li et al., 2022). SA is best known as a defence-related hormone. The first observations that SA was involved in plant immunity were reported by Raymond F. White in 1979, who described that the application of aspirin (acetyl-SA) in virus-susceptible tobacco (*Nicotiana tabacum* cv. Xanthi-nc) conferred resistance against tobacco mosaic virus (TMV). This indicated a protective role of SA in plant resistance (Pingtao and Yuli, 2020).
SA is an important mediator of the plant defence response to pathogens (Popova et al., 1997).

MATERIALS AND METHODS

This study took place in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania. During *in vitro* potato multiplication process, of three potato varieties (Sarmis, Foresta and Castrum) infected with Potato Virus S (PVS), detected by ELISA test, an experiment was performed with reference of influence of salicylic acid and antiviral ribavirin over two plantlets parameters: height (cm) and leaves number. The objective of the study is to eradicate PVS. First the minicuttings of infected plantlets were inoculated on a medium without salicylic acid (SA), as control and on a medium with 100 mg SA. On medium with SA was observed poor root development, thus 100 mg of SA affected the development of plantlets. After 30 days from minicuttings inoculation, developed plantlets were multiplied again, and minicutings were put on medium with different concentration of ribavirin.
Thus, the trifactorial experience (2 x 3 x 3), on 3 repetitions had the following factors: experimental factor A- the culture medium used before antiviral treatment, with two graduations: a1 - classical medium (as control) Murashige -Skoog (1962); a2 - MS+ salicylic acid (100 mg/l); experimental factor B - the variety, with three graduations: b1 - Sarmis (as control), b2 - Foresta, b3 - Castrum; experimental factor C- ribavirin concentration: c1 - 0 mg/l (as control); c2 - 50 mg/l; c3 - 100 mg/l.
The experimental variants can be seen in the Figure 1. Eighteen experimental variants were analysed.

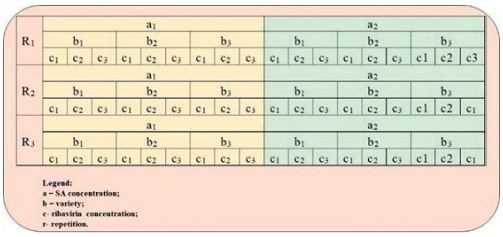

Figure 1. Experimental variants

RESULTS AND DISCUSSIONS

After 40 days from inoculation on medium with antiviral were made determinations on plantlets height (cm) and leaves number. Antiviral treatment inhibited plant growth.
Results were analysed using ANOVA Polifact.

Table 1. The influence of salicylic acid treatment, applied before the antiviral treatment, on plantlets height (cm) and on the number of leaves formed/plantlet

Concentration of salicylic acid (mg/l) (a)	Plantlets height (cm)	Diff. (cm)/ Sign.	Number of leaves	Diff. / Sign.
0 (Ct)	6.35	-	7.67	-
100	6.00	-0.5 ns	7.50	-0,17 ns
	LSD (p 5%) = 1.25 cm; LSD (p 1%) = 2.89 cm; LSD (p 0.1%) = 9.20 cm.		LSD (p 5%) =1.49; LSD (p 1%) = 3.45; LSD (p 0.1%) = 10.96.	

From Table 1, with reference to the influence of salicylic acid applied prior to antiviral treatment, it can be seen that the values obtained for both analysed parameter were close, the differences being insignificant.

Table 2. The influence of the cultivar on plantlets height (cm) and on the number of leaves formed/plantlet

Cultivar (b)	Plantlets height (cm)	Diff. (cm)/ Sign.	Number of leaves	Diff./ Sign.
Sarmis (Ct)	7.24	-	8.39	-
Foresta	6.67	-0.57 ns	8.22	-0.17 ns
Castrum	4.62	-2.62 ooo	6.14	-2.25 ooo
	LSD (p 5%) =1.00 cm; LSD (p 1%) = 1.46 cm; LSD (p 0.1%) = 2.19 cm.		LSD (p 5%) = 0.61; LSD (p 1%) = 0.89; LSD (p 0.1%) = 1.34.	

Regarding the cultivar influence on plantlets development, it can be observed that Sarmis variety (Ct) had obtained superior values for both parameters, at the last place Castrum variety is ranked, which registers very significant negative differences (Table 2).

Table 3. The influence of ribavirin concentration applied in culture medium on plantlets height (cm) and on the number of leaves formed/ plantlet

Ribavirin concentration (mg/l) (c)	Plantlets height (cm)	Diff. (cm)/ Sign.	Number of leaves	Diff. / Sign.
0 (c1) (Ct)	12.03	-	11.58	-
50 (c2)	4.71	-7.32 ooo	7.47	-4.11 ooo
100 (c3)	1.79	-10.24 ooo	3.69	-7.89 ooo
	LSD (p 5%) = 1.00 cm; LSD (p 1%) = 1.37 cm; LSD (p 0.1%) = 1.83 cm.		LSD (p 5%) =0.74; LSD (p 1%) = 1.01; LSD (p 0.1%) = 1.35.	

Using of ribavirin in the two concentrations drastically decreased the height of the plantlets and the number of leaves, causing very significant negative differences for the two parameters (Table 3).

Table 4. Combined influence of salicylic acid treatment and variety on plantlets height (cm)

Concentration of salicylic acid (mg/l) (a) / cultivar (b)	0 (a_1) (Ct)		100 (a_2)		Diff. (cm)/ Sign. (a_2-a_1)
	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.	
Sarmis (b_1) (Ct)	6.94	-	7.53	-	0.58 ns
Foresta (b_2)	7.27	0.32 ns	6.07	-1.46 o	-1.20 ns
Castrum (b_3)	4.84	-2.11 oo	4.40	-3.13 ooo	-0.44 ns
	LSD (p 5%) = 1.42 cm; LSD (p 1%) = 2.06 cm; LSD (p 0.1%) = 3.09 cm.		LSD (p 5%) = 1.63 cm; LSD (p 1%) = 2.91 cm; LSD (p 0.1%) = 6.80 cm.		

Analysis of the combined influence of treatment with salicylic acid (Table 4) applied *in vitro*, prior to treatment with antiviral, and of cultivar on plantlets height, draws our attention to Castrum variety, which registers a distinctly significant negative difference (-2.11 cm) on control medium (without salicylic acid). Plantlets from medium with 100 mg SA were drastically affected for Castrum and Foresta varieties, with very significant and significant negative differences (-3.13 and -1.46 cm).

Table 5. Combined influence of salicylic acid treatment and ribavirin concentration on plantlets height (cm)

Concentration of salicylic acid (mg/l) (a)/ Concentration of ribavirin (mg/l) (c)	0 (a_1) (Ct)		100 (a_2)		Diff. (cm)/ Sign. (a_2-a_1)
	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.	
0 (c1) (Ct)	12.83	-	11.22	-	-1.61 o
50 (c2)	5.06	-7.78 ooo	4.36	-6.86 ooo	-0.69 ns
100 (c3)	1.16	-11.67 ooo	2.41 ooo	-8.81 ooo	1.25 ns
	LSD (p 5%) = 1.42 cm; LSD (p 1%) = 1.93 cm; LSD (p 0.1%) = 2.59 cm.		LSD (p 5%) = 1.60 cm; LSD (p 1%) = 2.73 cm; LSD (p 0.1%) = 6.10 cm.		

Combined influence of salicylic acid treatment and ribavirin concentration on plantlets height underline the negative effect of medium with SA over plantlets height, with a negative difference (-1.61 cm) compared to control medium. Also, the antiviral treatment, for both concentrations, strongly influenced the growth of plantlets, causing very significant negative differences (Table 5).

Ribavirin (50 and 100 mg) strongly inhibited plantlets height for all studied varieties, with very significant negative differences (Table 6).

The combined analysis of the treatment with salicylic acid applied before the antiviral treatment (Table 7), of the variety and ribavirin shows us a distinctly significant positive difference for the Sarmis variety (4.42 cm), when using 100 mg of ribavirin for culture medium that contained salicylic acid, compared to the medium without salicylic acid. For the same variety the previous treatment with salicylic acid determined a distinctly significant negative difference for control medium (without ribavirin).

The statistical analysis regarding the influence of the salicylic acid concentration and the variety shows that from the point of view of the analysed variety, Castrum variety determines obtaining of a low number of leaves/plantlets (with a significant negative difference for the medium without salicylic acid (-1.00) and a very significant negative difference for medium with 100 mg of salicylic acid (-3.50). Between the medium with salicylic acid (100 mg) and the one without salicylic acid, there were no significant differences for the three varieties (Table 8).

The combined influence of salicylic acid treatment and ribavirin concentration on the number of leaves shows very significant negative differences for the antiviral treatment added to the culture medium (50 and 100 mg) compared to control (0 mg), both for plantlets that came from a culture medium without salicylic acid and for those with salicylic acid. Regarding the number of leaves/plants, there were no significant differences between plantlets derived from with and without salicylic acid in culture medium (Table 9).

Table 6. Combined influence of ribavirin concentration and cultivar on plantlets height (cm)

Cultivar (b) / Concentration of ribavirin (mg/l) (c)	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		Diff. (cm)/ Sign. (b_2-b_1)	Diff. (cm)/ Sign. (b_3-b_1)
	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.		
0 (c_1) (Ct)	11.58	-	14.08	-	10.42	-	2.50 **	-1.16 ns
50 (c_2)	5.92	-5.67 ooo	5.29	-8.79 ooo	2.92	-7.50 ooo	-0.63 ns	-3.00 oo
100 (c_3)	4.21	-7.38 ooo	0.63	-13.46 ooo	0.53	-9.89 ooo	-3.58 ooo	-3.68 ooo

LSD (p 5%) = 1.74 cm; LSD (p 1%) = 2.36 cm; LSD (p 0.1%) = 3.17 cm.

LSD (p 5%) =1.74 cm; LSD (p 1%) = 2.41 cm; LSD (p 0.1%) = 3.35 cm.

Table 7. Combined influence of salicylic acid treatment, of variety and of ribavirin concentration on plantlets height (cm)

Conc. of SA (mg/l) (a) / Variety (b) / Conc. of ribavirin (mg/l) (c)	0 (a_1) (Ct)						Diff. (cm)/ Sign.		100 (a_2)						Dif (cm)/Semn.		Diff. (cm)/ Sign. ($a_2b_1c_1$ - $a_1b_1c_1$)	Diff. (cm)/ Sign. ($a_2b_2c_1$ - $a_1b_2c_1$)	Diff. (cm)/ Sign. ($a_2b_3c_1$ - $a_1b_3c_1$)
	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)				Sarmis (Ct) (b_1)		Foresta (b_2)		Castrum (b_3)		b_2-b_1	b_3-b_1			
	Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.	b_2-b_1	b_3-b_1	Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.					
0 (c_1) (Ct)	13.58	-	15.00	-	9.92	-	1.42 ns	-3.67 oo	9.58	-	13.17	-	10.92	-	3.58 ***	1.33 ns	-4.00 oo	-1.83 ns	1.00 ns
50 (c_2)	5.25	-8.33 ooo	6.00	-9.00 ooo	3.92	-6.00 ooo	0.75 ns	-1.33 ns	6.58	-3.00 o	4.58	-8.58 ooo	1.92	-9.00 ooo	-2.00 ns	-4.67 oo	1.33 ns	-1.42 ns	-2.00 ns
100 mg (c_3)	2.00	-11.58 ooo	0.80	-14.20 ooo	0.68	-9.23 ooo	-1.20 ns	-1.32 ns	6.42	-3.17 o	0.45	-12.72 ooo	0.37	-10.55 ooo	-5.97 ooo	-6.05 ooo	4.42 **	-0.35 ns	-0.32 ns

LSD (p 5%) = 2.46 cm; LSD (p 1%) = 3.34 cm; LSD (p 0.1%) = 4.48 cm.

LSD (p 5%) = 2.46 cm; LSD (p 1%) = 3.41 cm; LSD (p 0.1%) = 4.74 cm

SD (p 5%) = 2.56 cm; SD (p 1%) = 3.83 cm; SD (p 0.1%) = 6.62 cm.

Table 8. The combined influence of salicylic acid treatment and variety on the number of leaves/plantlets

Concentration of salicylic acid (mg/l) (a)/ Variety (b)	0 (a_1) (Ct)		100 (a_2)		Diff./Sign.
	Leaves number	Diff. / Sign.	Leaves number	Dif. / Semnif.	a_2-a_1
Sarmis (b_1) (Ct)	7.78	-	9.00	-	1.22 ns
Foresta (b_2)	8.44	0.67 ns	8.00	-1.00 o	-0.44 ns
Castrum (b_3)	6.78	-1.00 o	5.50	-3.50 ooo	-1.28 ns

LSD (p 5%) = 0.87 leaves; LSD (p 5%) = 1.59 leaves;
LSD (p 1%) = 1.26 leaves; LSD (p 1%) = 3.26 leaves;
LSD (p 0.1%) = 1.89 leaves. LSD (p 0.1%) = 9.24 leaves.

Table 9. The combined influence of salicylic acid treatment and ribavirin concentration on the number of leaves

Concentration of salicylic acid (mg/l) (a)/ Concentration of ribavirin (mg/l) (c)	0 (a_1) (Ct)		100 AS (a_2)		Diff./ Sign.
	Leaves number	Diff. / Sign.	Leaves number	Diff. / Sign.	a_2-a_1
0 (c_1) (Ct)	11.72	-	11.44	-	-0.28 ns
50 (c_2)	8.11	-3.61 ooo	6.83	-4.61 ooo	-1.28 ns
100 (c_3)	3.17	-8.56 ooo	4.22	-7.22 ooo	1.06 ns

LSD (p 5%) = 1.05 leaves; LSD (p 5%) = 1.61 leaves;
LSD (p 1%) = 1.43 leaves; LSD (p 1%) = 3.10 leaves;
LSD (p 0.1%) = 1.9 leaves. LSD (p 0.1%) = 8.21 leaves.

For all cultivars (Table 10), chemotherapy (50 and 100 mg ribavirin) negatively influenced the leaves number/plant with very significant differences, negative. Foresta cultivar showed a high capacity to form leaves both on the control medium (without antiviral) and on the medium with 50 mg/l ribavirin, compared to the control cultivar (Sarmis). Instead for this variety, 100 mg/l ribavirin strongly inhibited leaf formation, compared to Sarmis cultivar (with a very significant negative difference -3,50 leaves). Castrum variety compared to the control variety showed negative differences, both for medium without antiviral (-1.92 leaves, a distinctly significant difference) and for 50 (-1.42 leaves, a significant negative difference) and 100 mg ribavirin (-3.42, a very significant negative difference).

For the plantlets of all varieties taken into study, which came from the culture medium without salicylic acid, chemotherapy strongly influenced, in a negative sense, the formation of leaves (Table 11). Only Castrum variety registered a significant difference when using 50 mg/l ribavirin (-2.17 leaves), compared to the medium with 0 mg/l ribavirin. The others cultivar presents with very significant negative differences (for both 50 and 100 mg/l), compared to culture medium without ribavirin, even Castrum variety for 100 mg/l ribavirin. Foresta variety had a superior behavior in leaves formation compared to the control variety when 50 mg/l ribavirin was applied (with a significant positive difference 2.33 leaves). For plantlets that came from the culture medium with 100 mg/l salicylic acid, the chemotherapy negatively influenced the formation of the number of leaves: thus, distinctly significant differences were obtained for the Sarmis variety (using 100 mg/l ribavirin), compared to from culture medium without ribavirin and very significant differences for the Sarmis variety (using 50 mg/l ribavirin), as well as for the other varieties treated with ribavirin (50 and 100 mg). When comparing the leaves number/plantlets from the nutrient medium with salicylic acid, compared to those without salicylic acid, a positive influence is observed for Sarmis variety for the medium with 100 mg/l ribavirin (4.33 leaves, with a distinctly significant positive difference).

As we can see from Figure 2, Castrum and Foresta varieties suffered the most for both analyzed parameters.

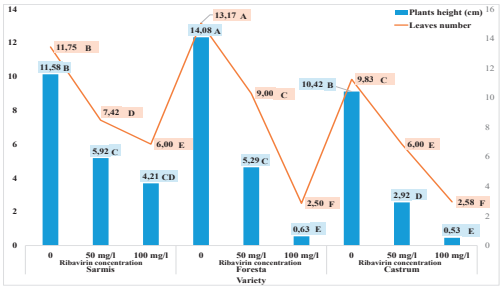

Figure 2. Influence of variety and ribavirin concentration over plants height (cm) and leaves number

Plantlets for Castrum and Foresta cultivars, from growth medium with salicylic acid and treated with ribavirin 100 mg/l, had the strongest growth inhibition obtaining the lowest values (0.37 and 0.45 cm), compared to those that came from nutrition medium without salicylic acid (both varieties: 0.68 and 0.80 cm). Regarding the leaves number, the lowest values were obtained for Foresta variety (2.17) by using 100 mg ribavirin, for the plants that came from the medium with salicylic acid (Figure 3).

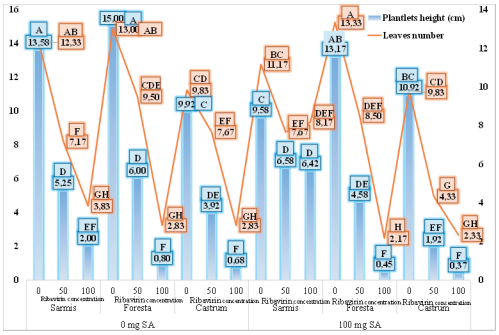

Figure 3. Influence of variety, medium with salycilic acid and ribavirin concentration over plants height (cm) and leaves number

Table 10. The combined influence of cultivar and ribavirin concentrations on leaves number

Cultivar (b) / Concentration of ribavirin (mg/l) (c)	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		Diff. / Sign. (b_2-b_1)	Diff. / Sign. (b_3-b_1)
	Leaves number	Diff./ Sign.	Leaves number	Diff./ Sign.	Leaves number	Diff./ Sign.		
0 (c_1) (Ct)	11.75	-	13.17	-	9.83	-	1.42 *	-1.92 oo
50 (c_2)	7.42	-4.33 ooo	9.00	-4.17 ooo	6.00	-3.83 ooo	1.58 *	-1.42 o
100 (c_3)	6.00	-5.75 ooo	2.50	-10.67 ooo	2.58	-7.25 ooo	-3.50 ooo	-3.42 ooo

LSD (p 5%) =1.29 leaves; LSD (p 5%) =1.22 leaves;
LSD (p 1%) = 1.75 leaves; LSD (p 1%) = 1.68 leaves;
LSD (p 0.1%) = 2.35 leaves. LSD (p 0.1%) = 2.31 leaves.

Table 11. Combined influence of salicylic acid treatment, of variety and of ribavirin concentration on leaves number/plantlet

Conc. of SA (mg/l) (a) / Variety (b) / Conc. of ribavirin (mg/l) (c)	0 (a_1) (Ct)						Diff. / Sign.		100 (a_2)						Diff. / Sign.		Diff. / Sign. ($a_2b_1c_1$-$a_1b_1c_1$)	Diff. / Sign. ($a_2b_2c_1$-$a_1b_2c_1$)	Diff. / Sign. ($a_2b_3c_1$-$a_1b_3c_1$)
	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		b_2 - b_1	b_3 - b_1	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		b_2-b_1	b_3-b_1			
	Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.			Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.					
0 (c_1) (Ct)	12.33	-	13.00	-	9.83	-	0.67 ns	-2.50 oo	11.17	-	13.33	-	9.83	-	2.17 *	-1.33 ns	-1.17 ns	0.33 ns	0.00 ns
50 (c_2)	7.17	-5.17 ooo	9.50	-3.50 ooo	7.67	-2.17 o	2.33 *	0.50 ns	7.67	-3.50 ooo	8.50	-4.83 ooo	4.33	-5.50 ooo	0.83 ns	-3.33 ooo	0.50 ns	-1.00 ns	-3.33 o
100 mg (c_3)	3.83	-8.50 ooo	2.83	-10.17 ooo	2.83	-7.00 ooo	-1.00 ns	-1.00 ns	8.17	-3.00 oo	2.17	-11.17 ooo	2.33	-7.50 ooo	-6.00 ooo	-5.83 ooo	4.33 **	-0.67 ns	-0.50 ns

LSD (p 5%) = 1.82 leaves; LSD (p 5%) = 1.72 leaves; LSD (p 5%) =2.11 leaves;
LSD (p 1%) = 2.48 leaves; LSD (p 1%) = 2.37 leaves; LSD (p 1%) = 3.46 leaves;
LSD (p 0.1%) = 3.32 leaves. LSD (p 0.1%) = 3.27 leaves. LSD (p 0.1%) = 7.27 leaves.

For the Sarmis variety, the application of salicylic acid in the culture medium was effective in eliminating the PVS virus in the variant in which ribavirin was not added to the nutrient medium (Table 12).

Table 12. The influence of the applied treatment on the elimination of PVS virus after viral testing

Variety	Concentration of salycilic acid (mg/l)	Concentration of ribavirin (mg/l)	Virus eradication
Sarmis	0	0	PVS
		50	Free of virus
		100	Free of virus
Foresta		0	PVS
		50	Free of virus
		100	Free of virus
Castrum		0	PVS
		50	PVS
		100	Free of virus
Sarmis	100	0	Free of virus
		50	Free of virus
		100	Free of virus
Foresta		0	PVS
		50	Free of virus
		100	Free of virus
Castrum		0	PVS
		50	PVS
		100	Free of virus

For this variety, both ribavirin (50 and 100 mg/l) and salicylic acid treatment (100 mg/l) in combination with ribavirin (50 and 100 mg/l) resulted in elimination of PVS virus.

By adding salicylic acid prior to ribavirin treatment (50 and 100 mg/l), virus-free plantlets were obtained for Foresta variety. And treatments with ribavirin (50 and 100 mg/l), but without salicylic acid, determined the elimination of this virus.

For the Castrum variety, virus-free plantlets were obtained only in the variants with ribavirin (100 mg/l) both for plantlets that were not treated with salicylic acid and those treated with salicylic acid.

CONCLUSIONS

The addition of salicylic acid to the culture medium led to a slight decrease in plant height and the number of leaves, without significant differences between the two types of medium (with 0 and 100 mg/l salicylic acid).

Using of ribavirin (50 and 100 mg/l) drastically decreased the height of the plantlets and the number of leaves, causing very significant negative differences for the two parameters, compared to the control medium (0 mg/l ribavirin).

Plantlets from medium with 100 mg SA were drastically affected, reducing the height of the plantlets for Castrum and Foresta varieties, with very significant and significant negative differences (-3.13 and -1.46 cm).

Ribavirin (both concentrations) strongly inhibited plantlets height for all studied varieties.

For Castrum variety 100 mg of salicylic acid determines obtaining of a low number of leaves/plantlets.

All cultivars not previously treated with salicylic acid were found to be virus-free plantlets for the ribavirin 100 mg/l medium variant, and for the medium variant with ribavirin 50 mg/l, the virus-free plantlets were for the Sarmis and Foresta varieties.

By treating the plantlets with salicylic acid, the PVS virus was no longer identified at the Sarmis variety (for culture media with 0, 50 and 100 mg/l ribavirin).

The application of 100 mg/l ribavirin in the culture medium led to obtaining virus-free material for the Foresta and Castrum varieties (plantlets previous treated with salicylic acid).

At Foresta variety, the elimination of the PVS virus was also achieved by treating with 50 mg/l ribavirin, for plantlets previously developed on medium with salicylic acid.

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