Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 31, issue 2, 2021, pp. 78 - 84

IN VITRO SCREENING OF POTATO VARIETIES FOR DROUGHT STRESS INDUCED WITH DIFFERENT OSMOTIC AGENTS

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Abstract: The objective of this study was to identify potato varieties with tolerance to the drought stress, by introducing in culture medium of different osmotic regulators (mannitol, sorbitol and polyethylene glycol) with various concentrations (0%, 1% and 1.5%). The experiment was performed for three potato Romanian varieties (Marvis, Castrum and Ervant). The determinations were made for different growth and development parameters: plantlets length, number of leaves/plantlets, root length, fresh plantlet and root weight. The nutrient medium with mannitol had as effect reducing of plantlets height (6.93 cm), root length (4.41 cm) and fresh plantlets weight (0.104 g), and PEG (polyethylene glycol) had a negative influence over leaf number (8.33) and weight of the fresh root (0.040 g). The concentration of 1.5% osmotic agent drastically reduced the number of leaves (7.63), the height of plantlets (5.52 cm) and fresh weight of plantlets and root (0.087 g and 0.033 g).

Keywords: potato, *in vitro*, hydric stress, osmotic regulators, drought.

INTRODUCTION

Identification of genotypes adapted to drought conditions is necessary due to the effect of this stress on potato crop production (Taiz and Zeiger, 2006). One of the major challenges for the next decade is attenuation of any effect of climate change on crop, with the main focus on maintaining levels of plant production with low water availability (Obidiegwu et al., 2015). Drought induces a number of morphological, physiological, biochemical and molecular changes (Hossain et al., 2016). During the growing season, plant growth (leaf development and size, plant height, plant branching) decreases with the installation of water stress. In many studies it was tried to find a correlation between root system size, water deficit tolerance and tuber yield (Comas et al., 2013, Iwama et al., 1999, Khan et al., 2016, Lahlou and Ledent, 2005; Villordon et al. 2014, cited by Basu et al., 2016); however, the correlations were not always positive. The culture of plant cells and tissues is a useful tool for studying the mechanisms of stress tolerance *in vitro* conditions. *In vitro* techniques can make it possible to quickly detect a large number of genotypes for stress tolerance (Anithakumari et al., 2011). Identifying a large number of genotypes with drought tolerance in the field is difficult due to the chemical and physical properties of the soil and seasonal fluctuations. *In vitro* identification of potato genotypes with tolerance to water stress is proposed as an alternative approach to expensive, labor-intensive and sometimes problematic field screening (Rahman et al. 2008, cited by Albiski et al., 2012). The effect of water stress or salinity on *in vitro* potato growth has been reported to be similar to that observed in field conditions (Zhang and Donnelly, 1997; Gopal and Iwama, 2007; Aghaei et al., 2008, cited by Albiski et al., 2012). Mannitol is widely used as an osmotic agent for the characterization and selection of genotypes with tolerance to water stress under *in vitro* conditions (Hassanein, 2010). Sorbitol is a sugar alcohol hexahydrate with osmotic effect. Polyethylene glycol (PEG) is widely used *in vitro* to determine water stress. It is a non-penetrating inert osmotic compound (with high molecular weight), which reduces the potential of nutrient solutions, without it being taken up by the plant or presenting phytotoxicity (Hassan et al., 2004). Simulation of osmotic stress *in vitro*, through the use of tissue culture can minimize environmental changes due to multiplication on defined nutrient media, under controlled conditions (Gorji, 2011).

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov.

Induction of osmotic stress for *in vitro* cultures was performed by adding mannitol, sorbitol, PEG reducing the ability of plantlets to absorb water from the nutrient medium. For each osmotic agent, two concentrations of 1 and 1.5% were used, which were compared to control medium (Murashige-Skoog, 1962) with no osmotic agent added.

The three-factor experience (3x3x3), on 3 repetitions had the following factors:

Experimental factor A - osmotic agent (induce water stress), with 3 graduations:

-a_1 - mannitol (considered control);

-a_2 -sorbitol;

-a_3 - polyethylene glycol.

Experimental factor B: concentration (%) of osmotic agents (with 3 graduations):

-b_1 - 0 (as control);

-b_2 - 1;

-b_3 - 1.5.

Experimental factor C: variety, with three graduations:

-c_1 - Marvis;

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-c_2 - Castrum;
-c_3 - Ervant (as control).

After 4 weeks from the inoculation of mini-cuttings (coming from the uninodal segmentation of healthy plantlets) in the culture medium with added osmotic agents, some physiological traits were determinates: number of leaves, plantlets height (cm), root length (cm), fresh plantlets and root weight (g). The obtained results were processed by the analysis of variance (according to the methodology set out in the "Field of Experience", Săulescu and Săulescu, 1967).

RESULTS AND DISCUSSION

By using in the culture medium the concentration of 1.5% osmotic agent (Table 1), the number of leaves (-2.93), the height of the plantlets (-3.93 cm), the weight of the fresh plantlets (-0.061) are drastically reduced, with very significant negative differences. This concentration also had a negative effect on the weight of fresh root (0.033 g) with a distinctly significant negative difference (-0.023 g) and root length (4.09 cm), but without significant difference.

Plantlet's height indicates the ability of the variety to continue growing and developing under conditions of water stress. Castrum variety detaches (Table 2), recording a plantlets height of 8.74 cm with a very significant positive difference (2.02 cm), followed by the Marvis variety, which achieves 7.35 cm (but no significant difference from the control variety, Ervant). Regarding the root length, the Castrum and Marvis varieties are remarked, with high and close values (5.56 cm; 5.11 cm) and very significant positive differences (2.89 and 2.44 cm). At the opposite pole, the control variety Ervant shows a tendency to slow down the root growing (2.67 cm). Regarding the weight of the fresh root, the Marvis variety is observed, with a value of 0.052 g, and a significant positive difference (0.0130 g), followed by the Castrum variety (0.043 g), but without a significant difference (0.0041 g) compared to the control variety.

The combined influence of the osmotic agent and the variety on leaves number/ plantlet (Table 3) shows Castrum variety superiority, by using mannitol and PEG in the culture medium (9.33 and 9.22 leaves). The Marvis variety presents a negative, but insignificant differences from the control variety for all osmotic agents used in the culture medium. By comparing PEG and sorbitol, a significant negative difference is observed for the Ervant variety (-1.78 leaves), PEG affecting the leaf formation for this variety.

Table 1.

The influence of the nutrient medium with osmotic agents, on the growth and development element

Osmotic agent	Leaves no.	Diff./ Sign.	Plantlet height (cm)	Diff. (cm)/ Sign.	Root length (cm)	Diff. (cm)/ Sign.	Weight of fresh plantlet (g)	Diff. (g)/ Sign.	Weight of fresh root (g)	Diff. (g)/ Sign.
Mannitol (a_1) (Ct)	8.67	-	6.93	-	4.41	-	0.104	-	0.044	-
Sorbitol (a_2)	9.07	0.41 ns	8.22	1.30 **	4.50	0.09 ns	0.127	0.023 ns	0.052	0.008 ns
PEG (a_3)	8.33	-0.33 ns	7.67	0.74 ns	4.43	0.02 ns	0.121	0.016 ns	0.040	-0.004 ns
LSD 5% = 0.85; LSD 1% = 1.41; LSD 0.1% = 2.64.			LSD 5% = 0.76 cm; LSD 1% = 1.27 cm; LSD 0.1% = 2.37 cm.		LSD 5% = 0.85 cm; LSD 1% = 1.41 cm; LSD 0.1% = 2.64 cm.		LSD 5% = 0.024 g; LSD 1% = 0.039 g; LSD 0.1% = 0.073 g.		LSD 5% = 0.012 g; LSD 1% = 0.021 g; LSD 0.1% = 0.039 g.	

Table 2.

The influence of concentrations of osmotic agents applied in the nutritional medium on the elements of growth and development

Conc. (%)	Leaves no.	Diff./ Sign.	Plantlet height (cm)	Diff. (cm)/ Sign.	Root length (cm)	Diff. (cm)/ Sign.	Weight of fresh plantlet (g)	Diff. (g)/ Sign.	Weight of fresh root (g)	Diff. (g)/ Sign.
0 (b_1) (Ct)	10.56	-	9.44	-	4.28	-	0.149	-	0.057	-
1 (b_2)	7.89	-2.67 ooo	7.85	-1.59 oo	4.96	0.69 *	0.116	-0.032 oo	0.046	-0.011 ns
1.5 (b_3)	7.63	-2.93 ooo	5.52	-3.93 ooo	4.09	-0.19 ns	0.087	-0.061 ooo	0.033	-0.023 oo
LSD 5% = 0.85; LSD 1% = 1.20; LSD 0.1% =1.69.			LSD 5% = 0.83 cm; LSD 1% = 1.16 cm; LSD 0.1% = 1.64 cm.		LSD 5% = 0.61 cm; LSD 1% = 0.86 cm; LSD 0.1% = 1.22 cm.		LSD 5% = 0.016 g; LSD 1% = 0.023 g; LSD 0.1% = 0.033 g.		LSD 5% = 0.014 g; LSD 1% = 0.020 g; LSD 0.1% = 0.028 g.	

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Table 3.

In vitro behavior of the studied varieties regarding the growth and development elements

Variety	Leaves no.	Diff./ Sign.	Plantlet height (cm)	Diff. (cm)/ Sign.	Root length (cm)	Diff. (cm)/ Sign.	Weight of fresh plantlet (g)	Diff. (g)/ Sign.	Weight of fresh root (g)	Diff. (g)/ Sign.
Marvis (c1)	8.07	-0.81 ns	7.35	0.63 ns	5.11	2.44 ***	0.13	0.02 ns	0.052	0.0130 *
Castrum (c2)	9.11	0.22 ns	8.74	2.02 ***	5.56	2.89 ***	0.11	-0.01 ns	0.043	0.0041 ns
Ervant (c3) (Ct)	8.89	-	6.72	-	2.67	-	0.11	-	0.039	-
LSD 5% = 1.16; LSD 1% = 1.55; LSD 0.1% = 2.05.		LSD 5% = 0.82 cm; LSD 1% = 1.10 cm; LSD 0.1% =1.46 cm.		LSD 5% = 0.55 cm; LSD 1% = 0.73 cm; LSD 0.1% = 0.96 cm.		LSD 5% = 0.03 g; LSD 1% = 0.04 g; LSD 0.1% = 0.05 g.		LSD 5% = 0.0128 g; LSD 1% = 0.0171 g; LSD 0.1% = 0.0226 g.		

Table 4.

The combined influence of the osmotic agent and variety on leaves number/plantlet

Osmotic agent (a)/variety (c)	Mannitol (a_1)		Sorbitol (a_2)		PEG (a_3)		a_2-a_1/ Sign.	a_3-a_1/ Sign.	a_3-a_2/ Sign.
	Leaves no.	Diff. / Sign.	Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.			
Marvis (c_1)	7.78	-1.11 ns	8.67	-1.11 ns	7.78	-0.22 ns	0.89 ns	0.00 ns	-0.89 ns
Castrum (c_2)	9.33	0.44 ns	8.78	-1.00 ns	9.22	1.22 ns	-0.56 ns	-0.11 ns	0.44 ns
Ervant (c_3) (Ct)	8.89	-	9.78	-	8.00	-	0.89 ns	-0.89 ns	-1.78 o
LSD 5% = 2.00; 1% = 2.69; 0.1% = 3.55 leaves.			LSD 5% = 1.77; 1% = 2.47; 0.1% = 3.53 leaves.						

Table 5.

The combined influence of the osmotic agent and the variety on the plantlet's height

Osmotic agent (a)/variety (c)	Mannitol (a_1)		Sorbitol (a_2)		PEG (a_3)		a_2-a_1 (cm) / Sign.	a_3-a_1 (cm) / Sign.	a_3-a_2 (cm) / Sign.
	Plantlet's height (cm)	Diff (cm) / Sign.	Plantlet's height (cm)	Diff (cm) / Sign.	Plantlet's height (cm)	Diff (cm) / Sign.			
Marvis (c_1)	6.83	0.56 ns	7.67	0.00 ns	7.56	1.33 ns	0.83 ns	0.72 ns	-0.11 ns
Castrum (c_2)	7.67	1.39 ns	9.33	1.67 *	9.22	3.00 ***	1.67 *	1.56 *	-0.11 ns
Ervant (c_3) (Ct)	6.28	-	7.67	-	6.22	-	1.39 *	-0.06 ns	-1.44 o
LSD 5% = 1.43 cm; 1% = 1.91 cm; 0.1% = 2.52 cm.			LSD 5% = 1.31 cm; 1% = 1.86 cm; 0.1% = 2.74 cm.						

Table 6.

The combined influence of the osmotic agent and the variety on the root length

Osmotic agent (a)/variety (c)	Mannitol (a_1)		Sorbitol (a_2)		PEG (a_3)		a_2-a_1 (cm)/ Sign.	a_3-a_1 (cm)/ Sign.	a_3-a_2 (cm) Sign.
	Root length (cm)	Diff (cm)/ Sign.	Root length (cm)	Diff (cm)/ Sign.	Root length (cm)	Diff (cm)/ Sign.			
Marvis (c_1)	5.28	2.17 ***	4.83	2.28 ***	5.22	2.89 ***	-0.44 ns	-0.06 ns	0.39 ns
Castrum (c_2)	4.83	1.72 ***	6.11	3.56 ***	5.72	3.39 ***	1.28 *	0.89 ns	-0.39 ns
Ervant (c_3) (Ct)	3.11	-	2.56	-	2.33	-	-0.56 ns	-0.78 ns	-0.22 ns
LSD 5% = 0.94 cm; 1% = 1.27 cm; 0.1% = 1.67 cm.			LSD 5% = 1.02 cm; 1% = 1.52 cm; 0.1% = 2.44 cm.						

Table 7.

The combined influence of the osmotic agent and the variety on fresh weight of the plantlets

Osmotic agent (a)/variety (c)	Mannitol (a_1)		Sorbitol (a_2)		PEG (a_3)		a_2-a_1 (g)/ Sign.	a_3-a_1 (g)/ Sign.	a_3-a_2 (g)/ Sign.
	Plantlet weight (g)	Diff. (g) /Sign.	Plantlet weight (g)	Diff. (g)/ Sign.	Plantlet weight (g)	Diff. (g)/ Sign.			
Marvis (c_1)	0.114	-0.005 ns	0.133	0.016 ns	0.154	0.052 *	0.019 ns	0.040 ns	0.020 ns
Castrum (c_2)	0.080	-0.040 ns	0.131	0.013 ns	0.106	0.005 ns	0.051 *	0.027 ns	-0.024 ns
Ervant (c_3) (Ct)	0.119	-	0.117	-	0.102	-	-0.002 ns	-0.018 ns	-0.016 ns

LSD 5% = 0.049 g; 1% = 0.066 g; 0.1% = 0.087 g. LSD 5% = 0.044 g; 1% = 0.062 g; 0.1% = 0.090 g.

Table 8.

The combined influence of the osmotic agent and the variety on fresh root weight

Osmotic agent (a)/variety (c)	Mannitol (a_1)		Sorbitol (a_2)		PEG (a_3)		a_2-a_1 (g)/ Sign	a_3-a_1 (g)/ Sign	a_3-a_2 (g)/ Sign
	Root weight (g)	Diff. (g) /Sign.	Root weight (g)	Dif. (g)/ Sign.	Root weight (g)	Diff. (g) /Sign.			
Marvis (c_1)	0.057	0.015 ns	0.046	0.001 ns	0.054	0.023 *	-0.010 ns	-0.003 ns	0.008 ns
Castrum (c_2)	0.032	-0.010 ns	0.063	0.018 ns	0.035	0.004 ns	0.031 **	0.002 ns	-0.028 o
Ervant (c_3) (Ct)	0.042	-	0.045	-	0.031	-	0.003 ns	-0.011 ns	-0.014 ns

LSD 5% = 0.022 g; 1% =0.030 g; 0.1% = 0.039 g. LSD 5% = 0.021 g; 1% = 0.029 g; 0.1% = 0.044 g.

The combined influence of osmotic agent and variety on the height of the plantlets shows the high capacity of the Castrum variety to form plantlets with a bigger height than the other two varieties (Table 5). When applying PEG, the height of Castrum plantlets registers a value of 9.22 cm and a very significant positive difference (3 cm); by using sorbitol the plantlets height of this variety reaches the value of 9.33 cm (with a significant, positive difference 1.67 cm). Also, by using mannitol as osmotic agent for this variety, a high value of plantlet height is obtained (7.67 cm), but without significant difference. Thus, the Castrum variety has tolerance to water stress, registering high values of plantlets height when using the three osmotic agents. By applying osmotic agents in the growth medium, they act as simulators of water stress, resulting in the reduction of nutrients needed for plant growth and water absorption through the roots. Explants depend on the assimilation of carbon from sucrose in the growing medium. Thus, by reducing water absorption, there is also a reduction in carbon consumption. From the examination of the differences between sorbitol and mannitol (regarding plantlet's height), it is found that the Castrum and Ervant varieties register significant positive differences (1.67 cm and 1.39 cm). The difference between PEG and mannitol highlights the Castrum variety, with a significant positive difference (1.56 cm). The Castrum variety under water stress conditions will form plants with high stems, able to continue growing and developing.

As a representative of varieties tolerance to unfavorable environmental conditions, especially the lack of water in the soil, is the length of the root (Table 6). Castrum and Marvis varieties obtain high values of root length for all three types of osmotic agents, with very significant positive differences: when applying mannitol, the values are 4.83 and 5.28 cm, when using sorbitol: 6.11 and 4.83 cm, and for PEG: 5.72 and 5.22 cm. By the comparison of sorbitol with the medium that contained the control osmotic agent (mannitol) it is observed that a significant positive difference is obtained for the Castrum variety (1.28 cm).

For the weight of the fresh plantlets, the Marvis variety (0.154 g) is distinguished by a significant positive difference (0.052 g) (Table 7) for medium with PEG. The differences between the osmotic agents for the three varieties analyzed are small. Only Castrum variety showed a significant positive difference (0.051 g) by using sorbitol in the culture medium, comparing to mannitol (the other differences are insignificant).

When applying PEG in the culture medium, for the weight of the fresh root (Table 8), the same variety is

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observed (as for the fresh weight of plantlet), respectively the Marvis variety (0.054 g) with a significant positive difference (0.023 g). When comparing osmotic agents: sorbitol and mannitol, a distinctly significant positive difference (0.031 g) is observed for the Castrum variety. By comparing the experimental differences with the limit differences obtained regarding the influence of the osmotic agent on the weight of the fresh root, it appears that PEG affected the fresh weight of the root, with a significant negative difference (-0.028 g) for the Castrum variety.

Table 9.

The combined influence of osmotic agent concentration and the variety on leaves number

Concentration of osmotic agent (a)/variety (c)	0 % (b_1)		1 % (b_2)		1.5 % (b_3)		a_2-a_1 (cm)/ Sign.	a_3-a_1 (cm)/ Sign.	a_3-a_2 (cm)/ Sign.
	Leaves no.	Diff./ Sign.	Leaves no.	Diff./ Sign.	Leaves no.	Diff./ Sign.			
Marvis (c_1)	7.33	-4.67 ooo	7.67	-0.33 ns	9.22	2.56 *	0.33 ns	1.89 *	1.56 ns
Castrum (c_2)	12.33	0.33 ns	8.00	0.00 ns	7.00	0.33 ns	-4.33 ooo	-5.33 ooo	-1.00 ns
Ervant (c_3) (Ct)	12.00	-	8.00	-	6.67	-	-4.00 ooo	-5.33 ooo	-1.33 ns

LSD 5% = 2.00; 1% = 2.69; 0.1% = 3.55 leaves. LSD 5% = 1.76; 1% = 2.37; 0.1% = 3.18 leaves.

Table 10.

The combined influence of osmotic agent concentration and the variety on plantlets height

Concentration of osmotic agent (a)/variety (c)	0 % (b_1)		1 % (b_2)		1.5 % (b_3)		a_2-a_1 (cm) /Sign	a_3-a_1 (cm) /Sign	a_3-a_2 (cm)/ Sign
	Plantlet height (cm)	Diff. (cm)/ Sign.	Plantlet height (cm)	Diff. (cm)/ Sign.	Plantlet height (cm)	Diff. (cm)/ Sign			
Marvis (c_1)	8.17	-0.33 ns	7.83	0,78 ns	6,06	1,44 *	-0,33 ns	-2,11 oo	-1,78 o
Castrum (c_2)	11.67	3.17 ***	8.67	1,61 *	5,89	1,28 ns	-3,00 ooo	-5,78 ooo	-2,78 ooo
Ervant (c_3) (Ct)	8.50	-	7.06	-	4,61	-	-1,44 o	-3,89 ooo	-2,44 ooo

LSD 5% = 1.43 cm; 1% = 1.91 cm; 0,1% = 2.52 cm. LSD 5% = 1.31 cm; 1% = 1.79 cm; 0.1% = 2.41 cm.

Table 11.

The combined influence of osmotic agent concentration and the variety on root length

Concentration of osmotic agent (a)/variety (c)	0 % (b_1)		1 % (b_2)		1.5 % (b_3)		a_2-a_1 (cm)/ Sign.	a_3-a_1 (cm)/ Sign.	a_3-a_2 (cm) Sign.
	Root length (cm)	Diff. (cm)/Sign.	Root length (cm)	Diff. (cm)/ Sign.	Root length (cm)	Diff. (cm)/ Sign.			
Marvis (c_1)	4.50	2.00 ***	6.22	3.00 ***	4.61	2.33 ***	1.72 ***	0.11 ns	-1.61 oo
Castrum (c_2)	5.83	5.83 ***	5.44	2.22	5.39	2.28 ***	-0.39 ns	-0.44 ns	-0.06 ns
Ervant (c_3) (Ct)	2.50	-	3.22	-	2.28	-	0.72 ns	-0.22 ns	-0.94 o

LSD 5% = 0.94 cm; 1% = 1.27 cm; 0.1% = 1.67 cm. LSD 5% = 0.89 cm; 1% = 1.22 cm; 0.1% = 1.65 cm.

Table 12.

The combined influence of osmotic agent concentration and the variety on plantlet fresh weight

Concentration of osmotic agent (a)/variety (c)	0 % (b_1)		1 % (b_2)		1.5 % (b_3)		a_2-a_1 (g)/ Sign.	a_3-a_1 (g)/ Sign.	a_3-a_2 (g)/ Sign.
	Plantlet weight (g)	Diff. (g)/ Sign.	Plantlet weight (g)	Diff. (g)/ Sign.	Plantlet weight (g)	Diff. (g) /Sign.			

Marvis (c_1)	0.120	-0.064 o	0.165	0.078 **	0.115	0.05 *	0.045 *	-0.005 ns	-0.050 o
Castrum (c_2)	0.141	-0.043 ns	0.097	0.009 ns	0.079	0.01 ns	-0.044 o	-0.062 oo	-0.018 ns
Ervant (c_3) (Ct)	0.184	-	0.087	-	0.067	-	-0.097 ooo	-0.118 ooo	-0.021 ns

LSD 5% = 0.049 g; 1% = 0.066 g; 0.1% = 0.087 g. LSD 5% = 0.042 g; 1% = 0.057 g; 0.1% = 0.075 g.

Table 13.

The combined influence of osmotic agent concentration and the variety on root fresh weight

Concentration of osmotic agent (a)/variety (c)	0 % (b_1)		1 % (b_2)		1.5 % (b_3)		a_2-a_1 (g)/ Sign.	a_3-a_1 (g)/ Sign.	a_3-a_2 (g)/ Sign.
	Root weight (g)	Diff. (g)/ Sign.	Root weight (g)	Diff. (g)/ Sign.	Root weight (g)	Diff. (g)/ Sign.			
Marvis (c_1)	0.054	-0.013 ns	0.061	0.029 *	0.041	0.02 ns	0.007 ns	-0.013 ns	-0.020 ns
Castrum (c_2)	0.048	-0.020 ns	0.043	0.011 ns	0.040	0.02 ns	-0.005 ns	-0.008 ns	-0.003 ns
Ervant (c_3) (Ct)	0.068	-	0.032	-	0.018	-	-0.035 oo	-0.049 ooo	-0.014 ns

LSD 5% = 0.022 g; 1% = 0.030 g; 0.1% = 0.039 g. LSD 5% = 0.021 g; 1% = 0.028 g; 0.1% = 0.038 g.

The study of the combined influence of the osmotic agent concentration and the variety on number of leaves / plantlet (Table 9) shows that the difference obtained at the highest concentration of 1.5%, for the Marvis variety is significantly positive (2.56), this variety having a good capacity to form leaves under the influence of water stress. Marvis variety shows the lowest number of leaves (7.33, with a very significant negative difference -4.67 leaves), for control concentration 0%. Under water stress conditions, on the highest concentration of osmotic agent (1.5%), this variety has the capacity to form a high number of leaves 9.22 (with a significant positive difference: 2.56 leaves). Regarding the influence of the osmotic agent concentration over leaf formation, the experience with 1.5%, compared to the control determined obtaining a large number of leaves and a significant positive difference (1.89 leaves) for Marvis variety. The Castrum and Ervant varieties for concentrations of 1% (-4.33 and -4.00 leaves) and 1.5% (-5.33 leaves), compared to the control concentration, register very significant negative differences for leaves number, with a drastically decreasing.

When evaluating the behavior of the three varieties studied regarding plantlet height (Table 10), it is observed that when it was used the highest concentration (1.5%), Marvis variety registers a significant positive difference (1.44 cm), compared to the control variety. By increasing the osmotic agent concentration to 1.5%, compared to the control, the plantlets height is affected for all three varieties, obtaining distinctly significant negative differences (-2.11 cm) for the Marvis variety and very significant negative (-5.78 cm and -3.89 cm), for Castrum and Ervant varieties.

Research on the combined influence of the variety and concentration of osmotic agent on the root length (Table 11) shows that for the highest concentration, Castrum and Marvis varieties (5.39 and 4.61 cm) produce plantlets that have the value of the root length superior to the control variety (2.28 cm), with very significant positive differences. The differences between the osmotic agents' concentrations used in the culture medium detach the concentration of 1% from the control concentration, for the Marvis variety, obtaining a very significant positive difference (1.72 cm). The same variety has a positive, but insignificant, difference (0.11 cm), by comparing the 1.5% concentration with the control one.

Regarding the fresh plantlet weight, from the analysis of the behavior of the varieties at different concentrations of osmotic agent (Table 12), the Marvis variety can be remarked at the concentration of 1% water stress inducing substance, with a plantlet weight value of 0.165 g (with distinctly significant positive difference: 0.078 g), higher than that recorded for the control concentration (0.120 g), where the variety showed a significant negative difference (0.064 g), compared to the control variety. The same variety is distinguished at 1.5% concentration with a high value of fresh plantlet (0.115 g) and a significant positive difference (0.05 g), compared to the control variety. By analyzing the concentrations used, the superiority of the Marvis variety is found, for the concentration of 1%, compared to the control one. At the opposite pole is the

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Ervant variety, which obtained very significant negative differences for concentrations of 1 and 1.5%, compared to the control concentration (-0.097 g and -0.118 g).

From the comparison of the results obtained between the varieties, for the concentration of 1%, the Marvis variety is highlighted (Table 13), with a significant positive difference (0.029 g) for the weight of the fresh root. The concentrations of 1% and 1.5% osmotic agent, compared to the control concentration, influenced differently the weight of the fresh root for the Ervant variety with distinctly significant and very significant, negative differences (-0.035 g and -0.049).

CONCLUSIONS

The medium in which mannitol was applied reduced plantlets height (6.93 cm), root length (4.41 cm) and fresh plantlets weight (0.104 g), and PEG had a negative influence on leaf number (8.33) and weight of fresh root (0.040 g). These osmotic agents are recommended in *in vitro* simulation drought to identify drought tolerant genotypes. Sorbitol did not affect cell division, under its influence, the highest values were obtained.

The statistical analysis made to establish the influence of the osmotic agent concentration on the studied elements shows the drastic reduction over: number of leaves (7.63), plantlet height (5.52 cm), plantlet and root weight (0.087 g and 0.033 g), for the concentration of 1.5%.

The reaction of the varieties analyzed in terms of plantlet height and root length, shows the superiority of the Castrum variety, which presented the highest values for the mentioned elements (8.74 cm and 5.56 cm), with very significant positive differences, followed by the Marvis variety with a root length of 5.11 cm and a very significant positive difference. These varieties have a good rooting capacity under conditions of water stress.

Marvis and Castrum varieties are distinguished by obtaining plantlet with bigger values for root length at 1.5% and 1% concentrations, compared to the control concentration, showing a good tolerance of water stress *in vitro* induced.

Regarding the weight of the fresh plantlet, the Marvis variety is remarked, compared to the other varieties, by the formation of vigorous plantlet for concentrations of 1 and 1.5%.

Regarding the weight of root, there is the Marvis variety, which at 1% concentration forms a good root system, with a weight value of 0.061 g, higher than value recorded for the control concentration (0.054 g) and a significant difference of 0.029 compared to the control variety.

AUTHORS CONTRIBUTIONS

Conceptualization, A. Tican and M. Cioloca; methodology, M. Cioloca and C.L. Bădărău.; data collection A. Tican and M. Cioloca.; data validation, A. Tican; data processing A. Tican; bibliographic study and translation, N. Bărăscu and M. Hermeziu.

FUNDING

This research work was carried out with the support of National Institute of Research and Development for Potato and Sugar Beet Brasov and also was financed from ADER 5.1.2. project *Research on the production of minitubers under specific isolation conditions*.

CONFLICT OF INTEREST

The authors declare no conflict of interest and no financial interest.

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