NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 37, 2020
Print ISSN 1222–4227; Online ISSN 2067–5720

IDENTIFICATION OF POTATO BREEDING LINES WITH TOLERANCE TO HYDRIC STRESS UNDER *IN VITRO* CONDITIONS

Maria Ștefan[1], Andreea Tican[1*], Carmen Bădărău[1,2], Delia Paraschiv[1]

[1]National Institute of Research and Development for Potato and Sugar Beet Braşov, 2 Fundăturii str., 500470 Braşov, Braşov County, Romania

[2]Transilvania University, Faculty of Food and Tourism Braşov, 148 Castelului str., 500014 Braşov, Braşov County, Romania

[*]Corresponding author. E-mail: tican_andreea@yahoo.com

ABSTRACT

In this research our aim was to identify genotypes with hydric stress tolerance *in vitro* among 44 potato breeding lines. The study involved the number of leaves / plantlet, plantlet length and root growth of plantlets. Polyethylene glycol (6000) was used for exerting the hydric deficiency stress on the *in vitro* plantlets. Different levels of hydric stress were induced using two concentrations (1% and 2%) of polyethylene glycol (PEG) added in Murashige-Skoog medium. The values obtained for the mentioned traits analyzed on medium with PEG were compared with Murashige-Skoog medium (control). Under osmotic stress, all the measurements for number of leaves / plantlet, plantlet length and root growth of plantlets presented lower values, compared to the control medium (MS), with very significant negative differences. Genotypes 1893/5 and 1901/3 were less affected by the treatment with PEG (with the highest concentration) for all parameters taken in the study. These genotypes, which showed good tolerance to the hydric stress *in vitro* will be tested in next years in the field, to establish how close is the correlation with field performance under drought.

Keywords: potato, breeding line, *in vitro* multiplication, hydric stress, polyethylene glycol.

INTRODUCTION

The current concept of creating new potato varieties is on the way to be transformed, by passing from the tendency to create very intensive varieties, which requires the continuous allocation of resources, to create varieties which efficiently use the resources produced by the biosphere, in order to restore the ecological balance as close to the natural one. Potato is a relatively susceptible crop to drought and water stress tolerance has been a constant in the activity of breeders (Van der Linden et al., 2011; Anithakumari et al., 2012).

The suboptimal growth conditions associated with global warming and climate change have a negative impact on plant growth, survival and crop yield (Lesk et al., 2016). Climate change in Romania, especially in recent years, have subjected potato crops to strong thermo-hydric stresses. The high temperatures and the lack of rainfall affected the potato crops in all areas, except the irrigated ones. Predictions regarding the limitation of water consumption in agriculture require not only essential changes in farm management, but also in the objectives of genetic breeding, whereas genetic resource is regarded as the first pylon in solving multiple aspects. Breeding potato for drought tolerance could reduce water consumption and would increase the area of cultivation in drought prone regions of the world.

Hassanpanah (2010) based on scientific literature established that in the potato crop water deficit decreased: the number of leaves, plant water potentials, leaf area, plant height, ground coverage, tuber number, growth and yield, canopy radiation interception and only to a lesser extent the radiation use efficiency, tuber dry matter concentration, root and leaf weight, root number and root dry weight.

Breeding of new cultivars with excellent root quality that ensures absorption of water from deeper soil layers and under low soil moisture will help in more efficient utilization of water for potato production (Iwama, 2008).

Received 16 December 2019; accepted 8 January 2020. First Online: January, 2020. DII 2067-5720 RAR 2020-30

Biotechnology, like tissue culture technology, offers rapid alternative in crop improvement. In recent years, tissue culture based on *in vitro* selection has emerged as a feasible and cost-effective tool for developing stress tolerant plants. Plants tolerant to abiotic stresses can be selected by applying in the culture medium selecting agents such as PEG for drought tolerance (Errabii et al., 2008). Such assays that can identify genotypes based on osmotic stress tolerance, are less costly, less time consuming than field trials, and easier to multiplicate (Gopal and Iwama, 2007). PEG of high molecular weight has been long used as a non-penetrating non-ionic inert osmotic lowering the water potential of nutrient solutions without being taken up or being phytotoxic (Hassan et al., 2004). The amount of PEG solution to hold water depends on its molecular weight and concentration. PEG is soluble in water, not toxic to plants and is not easily absorbed, making PEG an effective compound to simulate drought conditions (Mullahey et al., 1996).

MATERIAL AND METHODS

The biological material analyzed in order to identify potato breeding lines with high tolerance to the hydric stress is part of NIRDPSB Braşov breeding program. The material was in stage of vegetative descendants 1 and was obtained by sexual hybridizing, the main method of creating variability. The genotypes were selected from the first year based on adaptability, vigor, aspect, resistance to pests and diseases, etc., both for plants and tubers.

The breeding process besides a careful study of parents, and a well-established procedure, takes 10-12 years, as follows: year I: field of seed; years II-IV: selection field - vegetative hybrid populations (P1-P3); years V-VII: selection field - vegetative descendants (D1 and D2); years VIII-IX: comparative orientation trials - years I and II; years X-XII: competition yield trials.

Biological material contained 44 potato breeding lines: 1890/7, 1899/14, 1899/1, 1885/11, 1890/1, 1901/10, 1901/12, 1896/4, 1899/18, 1885/5, 1886/1, 1901/11, 1899/3, 1895/3, 1899/19, 1899/13, 1895/1, 1901/7, 1891/2, 1899/4, 1890/3, 1891/1, 1899/11, 1890/8, 1890/4, 1901/1, 1896/1, 1897/2, 1895/4, 1890/13, 1896/5, 1909/1, 1889/1, 1899/7, 1889/2, 1901/6, 1901/3, 1896/2, 1890/12, 1901/9, 1885/1, 1893/5, 1901/8, 1890/5.

The starting point was represented by the sprouts of selected potato tubers, belonging to the mentioned breeding lines. Potato sprouts were sterilized, following the next steps:

- using sodium hypochlorite solution (NaOCl), for 10-15 minutes (depending on sprouts size);
- washing three times with sterile distilled water;
- passing by solution of ethyl alcohol for three minutes;
- washing again three times with sterile distilled water.

After all steps (selection and sterilization) they were dried on sterilized paper in the laminar flow hood.

Initially, potato sprouts were inoculated into test tubes with classical culture medium (Murashige and Skoog, 1962), to develop plantlets. The test tubes were transferred to the growth chamber, under controlled conditions of temperature and light.

After a period of 4 weeks from potato sprouts inoculated, plantlets developed in test tubes and *in vitro* multiplication procedure was performed.

Over the years, different techniques have been used for obtaining plantlets on the nutritional medium, the basic method being similar in most laboratories. It is based on the rapid growth of mini-cuttings (obtained by multiplying the plantlet at the level of each internode) on a sterilized culture medium.

For rapid identification of genotype(s) with *in vitro* hydric stress tolerance, the osmotic agent PEG 6000 was chosen, which was applied in the Murashige and Skoog (MS) culture medium in two concentrations PEG 1% and PEG 2%, before autoclaving the nutrient medium. So, the mini-cuttings obtained from plantlets developed from

potato sprouts were inoculated on three medium variants.

To determine the tolerance to *in vitro* hydric stress, the study consisted of a bifactorial test (3 x 44) on 6 repetitions, containing the following factors:

The experimental factor A: culture medium with three graduations:

– a_1 - Murashige and Skoog control medium, to which no osmotic agent was added;

– a_2 - Murashige and Skoog medium, which contained PEG 1%;

– a_3 - Murashige and Skoog medium, to which PEG 2% was added.

The experimental factor B - potato breeding lines, with 44 graduations.

Statistical analysis was performed by comparing to the mean of all 44 genotypes on each studied culture medium.

RESULTS AND DISCUSSION

The treatments performed with PEG reduced very significantly the number of leaves, plantlet length, root length, compared to the control medium (Table 1). Adding PEG in culture medium drastically decreased the proliferation of plant tissue, plantlet length and root length. PEG acted as an osmotic agent resulting in reduced root water and nutrients absorption. Explants depend on the assimilation of carbon from sucrose in culture medium. Thus, by reducing water absorption, carbon consumption is reduced.

Table 1. The influence of culture medium to which the osmotic stress inducer was added, compared to the control medium, on the elements of growth and development studied

Culture medium	Number of leaves/pl.	Diff.	Sign.	Length of plantlet (cm)	Diff.	Sign.	Length of root (cm)	Diff.	Sign.
MS	7.56	-	-	7.27	-	-	6.85	-	-
PEG 1%	6.30	-1.25	ooo	5.64	-1.62	ooo	5.94	-0.91	ooo
PEG 2%	4.78	-2.78	ooo	4.01	-3.26	ooo	3.87	-2.98	ooo
LSD 5% = 0.272 leaves				LSD 5% = 0,214 cm			LSD 5% = 0.304 cm		
LSD 1% = 0.386 leaves				LSD 1% = 0,304 cm			LSD 1% = 0.433 cm		
LSD 0,1% = 0.559 leaves				LSD 0.1% = 0,441 cm			LSD 0.1% = 0.626 cm		

Research over the combined influence of genotype and culture medium on leaves number / plantlet shows that by adding PEG 2% in the culture medium, high values of leaf number were obtained for genotypes 1901/8, 1901/3, 1893/5. These presented very significant, positive differences (5.05 leaves; 4.22 leaves; 4.22 leaves) (Table 2).

The line 1901/10 was noticed, with a distinctly significant positive difference (2.5 leaves), by using PEG 1% compared to control medium MS.

Table 2. The combined influence of genotype and culture medium on the number of leaves / plantlet

Genotype	MS medium (a1)			MS + PEG 1% medium (a2)			MS + PEG 2% medium (a3)			a2-a1	Sign.	a3-a1	Sign.
	No leaves/pl.	Diff.	Sign.	No leaves/pl.	Diff.	Sign.	No leaves/pl.	Diff.	Sign.				
1896/1	7.50	-0.057	ns	4.17	-2.14	oo	2.67	-2.11	oo	-3.333	ooo	-4.833	ooo
1897/2	7.50	-0.057	ns	5.67	-0.64	ns	5.17	0.39	ns	-1.833	o	-2.333	oo
1896/5	7.83	0.277	ns	5.33	-0.97	ns	4.17	-0.61	ns	-2.500	oo	-3.667	ooo
1890/7	11.33	3.777	***	7.17	0.86	ns	5.50	0.72	ns	-4.167	ooo	-5.833	ooo
1901/7	7.00	-0.557	ns	6.50	0.20	ns	4.83	0.05	ns	-0.500	ns	-2.167	oo
1909/1	8.33	0.777	ns	5.83	-0.47	ns	5.50	0.72	ns	-2.500	oo	-2.833	ooo
1901/3	9.00	1.443	ns	7.83	1.53	ns	9.00	4.22	***	-1.167	ns	0.000	ns
1891/2	7.17	-0.390	ns	7.50	1.20	ns	5.00	0.22	ns	0.333	ns	-2.167	oo
1890/5	11.83	4.277	***	5.50	-0.80	ns	4.50	-0.28	ns	-6.333	ooo	-7.333	ooo
1885/1	9.67	2.110	**	7.83	1.53	ns	6.67	1.89	*	-1.833	o	-3.000	ooo
1899/4	7.67	0.110	ns	4.67	-1.64	o	4.00	-0.78	ns	-3.000	ooo	-3.667	ooo
1901/9	8.83	1.277	ns	9.33	3.03	***	5.50	0.72	ns	0.500	ns	-3.333	ooo
1901/11	6.83	-0.723	ns	6.50	0.20	ns	5.17	0.39	ns	-0.333	ns	-1.667	o
1895/4	7.83	0.277	ns	5.67	-0.64	ns	5.17	0.39	ns	-2.167	oo	-2.667	oo
1899/11	7.33	-0.223	ns	5.83	-0.47	ns	5.00	0.22	ns	-1.500	ns	-2.333	oo
1895/3	6.33	-1.223	ns	4.00	-2.30	oo	2.67	-2.11	oo	-2.333	oo	-3.667	ooo
1901/12	5.50	-2.057	o	4.67	-1.64	o	3.83	-0.95	ns	-0.833	ns	-1.667	o
1889/2	8.17	0.610	ns	6.50	0.20	ns	3.17	-1.61	o	-1.667	o	-5.000	ooo
1901/6	8.83	1.277	ns	8.00	1.70	*	4.67	-0.11	ns	-0.833	ns	-4.167	ooo
1890/3	7.67	0.110	ns	6.83	0.53	ns	4.00	-0.78	ns	-0.833	ns	-3.667	ooo
1890/8	7.17	-0.390	ns	4.83	-1.47	ns	3.33	-1.45	ns	-2.333	oo	-3.833	ooo
1896/2	8.67	1.110	ns	6.33	0.03	ns	2.33	-2.45	oo	-2.333	oo	-6.333	ooo
1893/5	9.67	2.110	**	8.83	2.53	**	9.00	4.22	***	-0.833	ns	-0.667	ns
1890/12	8.67	1.110	ns	5.50	-0.80	ns	3.00	-1.78	o	-3.167	ooo	-5.667	ooo
1895/1	7.00	-0.557	ns	6.17	-0.14	ns	3.83	-0.95	ns	-0.833	ns	-3.167	ooo
1889/1	8.50	0.943	ns	6.33	0.03	ns	5.33	0.55	ns	-2.167	oo	-3.167	ooo
1890/4	7.83	0.277	ns	6.50	0.20	ns	4.33	-0.45	ns	-1.333	ns	-3.500	ooo
1899/7	8.17	0.610	ns	8.50	2.20	**	8.33	3.55	***	0.333	ns	0.167	ns
1901/10	6.17	-1.390	ns	8.67	2.36	**	6.17	1.39	ns	2.500	**	0.000	ns
1901/1	7.00	-0.557	ns	6.33	0.03	ns	5.50	0.72	ns	-0.667	ns	-1.500	ns
1901/8	10.00	2.443	**	7.33	1.03	ns	9.83	5.05	***	-2.667	oo	-0.167	ns
1899/3	6.50	-1.057	ns	5.33	-0.97	ns	7.67	2.89	***	-1.167	ns	1.167	ns
1891/1	7.17	-0.390	ns	6.83	0.53	ns	5.17	0.39	ns	-0.333	ns	-2.000	o
1899/19	6.67	-0.890	ns	5.00	-1.30	ns	3.67	-1.11	ns	-1.667	o	-3.000	ooo
1885/5	6.17	0.443	ns	6.17	-0.14	ns	2.67	-2.11	oo	0.000	ns	-3.500	ooo
1886/1	6.50	-1.057	ns	6.00	-0.30	ns	6.33	1.55	ns	-0.500	ns	-0.167	ns
1896/4	6.00	-1.557	ns	7.00	0.70	ns	4.33	-0.45	ns	1.000	ns	-1.667	o
1899/13	6.67	-0.890	ns	4.33	-1.97	o	3.67	-1.11	ns	-2.333	oo	-3.000	ooo
1899/14	5.33	-2.223	oo	4.33	-1.97	o	3.33	-1.45	ns	-1.000	ns	-2.000	o
1890/1	5.00	-2.557	oo	4.83	-1.47	ns	3.17	-1.61	o	-0.167	ns	-1.833	o
1885/11	4.83	-2.723	ooo	6.33	0.03	ns	2.67	-2.11	oo	1.500	ns	-2.167	oo
1899/1	6.83	-0.723	ns	6.83	0.53	ns	3.50	-1.28	ns	0.000	ns	-3.333	ooo
1890/13	7.83	0.277	ns	6.00	-0.30	ns	3.67	-1.11	ns	-1.833	o	-4.167	ooo
1899/18	6.00	-1.557	ns	7.67	1.36	ns	3.33	-1.45	ns	1.667	*	-2.667	oo
Mean	7.56	-		6.30	-		4.78			-1.254		-2.777	

LSD 5% = 1.592 leaves; 1% = 2.103 leaves; 0.1% = 2.688 leaves. LSD 5% = 1.587 leaves; 1% = 2.102 leaves; 0.1% = 2.696 leaves

Regarding the length of the plantlets, the breeding lines 1899/4 and 1896/2 were noted with very significant positive differences (3.689 cm; 3.189 cm), for the culture medium to which PEG 1% was added. Potato breeding lines 1893/5, 1901/3 and 1901/8 were distinguished with very significant positive differences (5.153 cm; 4.987 cm; 4.403 cm) for the culture medium that contained PEG 2% (Table 3).

These breeding lines can produce plants with high stem, capable of continuing growth and development under drought conditions.

Regarding the treatments applied to induce drought *in vitro*, the results suggested

tolerance to osmotic stress for breeding lines 1899/7 and 1901/10, which registered distinctly significant positive differences (2.417 cm; 2.167 cm) for the medium that contained PEG 1%, compared to the control medium (MS). Also, it can be observed that the differences obtained between the medium that contained PEG 1% versus the control medium were significant, for the breeding lines 1885/11 (2.000 cm), 1899/4 (1.667 cm) and 1899/18 (1.667 cm).

Table 3. The combined influence of genotype and culture medium on plantlet length (cm)

Genotype	MS medium (a1)			MS + PEG 1% medium (a2)			MS + PEG 2% medium (a3)			a2-a1	Sign.	a3-a1	Sign.
	Plantlet length	Diff.	Sign.	Plantlet length	Diff.	Sign.	Plantlet length	Diff.	Sign.				
1896/1	7.50	0.231	ns	4.17	-1.478	ns	2.67	-1.347	ns	-3.333	ooo	-4.833	ooo
1897/2	7.50	0.231	ns	5.67	0.022	ns	5.17	1.153	ns	-1.833	o	-2.333	oo
1896/5	7.83	0.565	ns	5.33	-0.311	ns	4.17	0.153	ns	-2.500	oo	-3.667	ooo
1890/7	11.17	3.898	***	7.17	1.522	ns	5.50	1.487	ns	-4.000	ooo	-5.667	ooo
1901/7	7.00	-0.269	ns	6.50	0.855	ns	4.83	0.820	ns	-0.500	ns	-2.167	oo
1909/1	8.33	1.065	ns	5.83	0.189	ns	5.50	1.487	ns	-2.500	oo	-2.833	ooo
1901/3	9.00	1.731	*	7.50	1.855	*	9.00	4.987	***	-1.500	ns	0.000	ns
1891/2	7.17	-0.102	ns	5.50	-0.145	ns	5.00	0.987	ns	-1.667	o	-2.167	oo
1890/5	11.83	4.565	***	7.83	2.189	**	4.50	0.487	ns	-4.000	ooo	-7.333	ooo
1885/1	9.67	2.398	**	4.67	-0.978	ns	6.67	2.653	**	-5.000	ooo	-3.000	ooo
1899/4	7.67	0.398	ns	9.33	3.689	***	4.00	-0.013	ns	1.667	*	-3.667	ooo
1901/9	8.83	1.565	ns	6.50	0.855	ns	5.50	1.487	ns	-2.333	oo	-3.333	ooo
1901/11	6.83	-0.435	ns	5.67	0.022	ns	5.17	1.153	ns	-1.167	ns	-1.667	o
1895/4	7.83	0.565	ns	5.83	0.189	ns	5.17	1.153	ns	-2.000	o	-2.667	oo
1899/11	7.33	0.065	ns	4.00	-1.645	o	5.00	0.987	ns	-3.333	ooo	-2.333	oo
1895/3	6.33	-0.935	ns	4.60	-1.045	ns	2.67	-1.347	ns	-1.733	oo	-3.667	ooo
1901/12	5.50	-1.769	o	6.33	0.689	ns	3.83	-0.180	ns	0.833	ns	-1.667	o
1889/2	8.17	0.898	ns	8.00	2.355	**	3.20	-0.813	ns	-0.167	ns	-4.967	ooo
1901/6	8.83	1.565	ns	6.83	1.189	ns	4.67	0.653	ns	-2.000	o	-4.167	ooo
1890/3	7.67	0.398	ns	4.83	-0.811	ns	4.00	-0.013	ns	-2.833	ooo	-3.667	ooo
1890/8	7.17	-0.102	ns	6.33	0.689	ns	3.33	-0.680	ns	-0.833	ns	-3.833	ooo
1896/2	8.67	1.398	ns	8.83	3.189	***	2.33	-1.680	o	0.167	ns	-6.333	ooo
1893/5	9.67	2.398	**	5.50	-0.145	ns	9.17	5.153	***	-4.167	ooo	-0.500	ns
1890/12	8.67	1.398	ns	6.17	0.522	ns	3.00	-1.013	ns	-2.500	oo	-5.667	ooo
1895/1	7.00	-0.269	ns	6.33	0.689	ns	3.83	-0.180	ns	-0.667	ns	-3.167	ooo
1889/1	8.50	1.231	ns	6.50	0.855	ns	5.33	1.320	ns	-2.000	o	-3.167	ooo
1890/4	7.83	0.565	ns	3.17	-2.478	oo	4.33	0.320	ns	-4.667	ooo	-3.500	ooo
1899/7	3.25	-4.019	***	5.67	0.022	ns	1.83	-2.180	**	2.417	**	-1.417	ns
1901/10	4.67	-2.602	oo	6.83	1.189	ns	2.58	-1.430	ns	2.167	**	-2.083	o
1901/1	7.00	-0.269	ns	5.25	-0.395	ns	4.08	0.070	ns	-1.750	o	-2.917	ooo
1901/8	11.67	4.398	***	3.33	-2.311	oo	8.42	4.403	***	-8.333	ooo	-3.250	ooo
1899/3	5.50	-1.769	o	3.67	-1.978	o	2.42	-1.597	ns	-1.833	o	-3.083	ooo
1891/1	11.42	4.148	***	2.75	-2.895	ooo	2.50	-1.513	ns	-8.667	ooo	-8.917	ooo
1899/19	5.17	-2.102	o	2.42	-3.228	ooo	1.07	-2.947	***	-2.750	ooo	-4.100	ooo
1885/5	3.83	5.731	***	3.42	-2.228	oo	0.68	-3.330	***	-0.417	ns	-3.150	ooo
1886/1	3.42	-3.852	ooo	3.42	-2.228	oo	2.67	-1.347	ns	0.000	ns	-0.750	ns
1896/4	4.08	-3.185	ooo	2.95	-2.695	oo	1.50	-2.513	**	-1.133	ns	-2.583	oo
1899/13	4.50	-2.769	ooo	4.40	-1.245	ns	1.65	-2.363	**	-0.100	ns	-2.850	ooo
1899/14	5.40	-1.869	o	4.83	-1.245	ns	3.33	-0.680	ns	-0.567	ns	-2.067	o
1890/1	5.00	-2.269	oo	6.33	0.689	ns	3.17	-0.847	ns	1.333	ns	-1.833	o
1885/11	4.83	-2.435	oo	6.83	1.189	ns	2.58	-1.430	ns	2.000	*	-2.250	oo
1899/1	6.75	-0.519	ns	6.00	0.355	ns	3.50	-0.513	ns	-0.750	ns	-3.250	ooo
1890/13	7.83	0.565	ns	7.67	2.022	*	3.67	-0.347	ns	-0.167	ns	-4.167	ooo
1899/18	6.00	-1.269	ns	7.67	2.022	*	3.40	-0.613	ns	1.667	*	-2.600	oo
Mean	7.27	-		5.64	-		4.01			-1.624		-3.255	

LSD 5% = 1.615 cm; 1% = 2.134 cm; 0.1% = 2.727 cm. LSD 5% = 1.605 cm; 1% = 2.124 cm; 0.1% = 2.720 cm.

As representative for the tendency of breeding lines, potential new potato varieties, in tolerance to unfavorable environmental conditions, especially the lack of ground water, is the average root length.

Breeding lines 1893/5, 1901/12, 1901/3 and 1901/9 under the influence of PEG 2% combat the effect of *in vitro* hydric stress by forming plantlets with a medium root length and very significant differences (6.049 cm; 5.049 cm; 4.883 cm; 4.466 cm), proving tolerance to hydric stress, by elongating the root (Table 4).

Table 4. The combined influence of genotype and culture medium on root length (cm)

Genotype	MS medium (a1)			MS + PEG 1% medium (a2)			MS + PEG 2% medium (a3)			a2-a1	Sign.	a3-a1	Sign.
	Root length	Diff.	Sign.	Root length	Diff.	Sign.	Root length	Diff.	Sign.				
1896/1	8.42	1.562	ns	7.08	1.142	ns	3.17	-0.701	ns	-1.333	ns	-5.250	ooo
1897/2	9.83	2.978	**	4.58	-1.358	ns	3.75	-0.118	ns	-5.250	ooo	-6.083	ooo
1896/5	8.83	1.978	ns	6.83	0.892	ns	6.92	3.049	**	-2.000	ns	-1.917	ns
1890/7	7.33	0.478	ns	4.67	-1.275	ns	3.58	-0.284	ns	-2.667	o	-3.750	oo
1901/7	7.33	0.478	ns	7.75	1.809	ns	2.90	-0.968	ns	0.417	ns	-4.433	ooo
1909/1	10.58	3.728	**	6.17	0.225	ns	5.25	1.383	ns	-4.417	ooo	-5.333	ooo
1901/3	12.17	5.312	***	10.25	4.309	ooo	8.75	4.883	***	-1.917	ns	-3.417	oo
1891/2	7.83	0.978	ns	6.08	0.142	ns	5.08	1.216	ns	-1.750	ns	-2.750	o
1890/5	7.92	1.062	ns	7.83	1.892	ns	5.83	1.966	ns	-0.083	ns	-2.083	ns
1885/1	4.75	-2.105	ns	3.00	-2.941	o	2.82	-1.048	ns	-1.750	ns	-1.930	ns
1899/4	8.75	1.895	ns	8.83	2.892	o	5.00	1.133	ns	0.083	ns	-3.750	oo
1901/9	9.25	2.395	*	9.17	3.225	**	8.33	4.466	***	-0.083	ns	-0.917	ns
1901/11	8.92	2.062	ns	6.67	0.725	ns	6.33	2.466	*	-2.250	o	-2.583	o
1895/4	7.33	0.478	ns	6.25	0.309	ns	6.17	2.299	*	-1.083	ns	-1.167	ns
1899/11	9.25	2.395	*	7.67	1.725	ns	5.25	1.383	ns	-1.583	ns	-4.000	ooo
1895/3	10.13	3.278	**	12.50	6.559	***	7.13	3.266	**	2.367	*	-3.000	oo
1901/12	11.75	4.895	***	10.60	4.659	***	8.92	5.049	***	-1.150	ns	-2.833	o
1889/2	4.58	-2.272	o	4.17	-1.775	ns	1.82	-2.051	ns	-0.417	ns	-2.767	o
1901/6	8.75	1.895	ns	4.25	-1.691	ns	5.25	1.383	ns	-4.500	ooo	-3.500	oo
1890/3	6.00	-0.855	ns	8.08	2.142	ns	5.52	1.649	ns	2.083	ns	-0.483	ns
1890/8	7.17	0.312	ns	5.57	-0.375	ns	0.18	-3.684	oo	-1.600	ns	-6.983	ooo
1896/2	6.08	-0.772	ns	7.33	1.392	ns	1.12	-2.751	o	1.250	ns	-4.967	ooo
1893/5	7.17	0.312	ns	10.33	4.392	***	9.92	6.049	***	3.167	**	2.750	*
1890/12	4.92	-1.938	ns	4.42	-1.525	ns	0.80	-3.068	oo	-0.500	ns	-4.117	ooo
1895/1	4.50	-2.355	o	5.33	-0.608	ns	5.08	1.216	ns	0.833	ns	0.583	ns
1889/1	4.67	-2.188	ns	3.92	-2.025	ns	3.75	-0.118	ns	-0.750	ns	-0.917	ns
1890/4	6.50	-0.355	ns	4.42	-1.525	ns	3.08	-0.784	ns	-2.083	ns	-3.417	oo
1899/7	2.92	-3.938	ooo	4.20	-1.741	ns	1.65	-2.218	ns	1.283	ns	-1.267	ns
1901/10	7.25	0.395	ns	5.08	-0.858	ns	5.75	1.883	ns	-2.167	ns	-1.500	ns
1901/1	7.08	0.228	ns	6.50	0.559	ns	4.83	0.966	ns	-0.583	ns	-2.250	o
1901/8	7.58	0.728	ns	6.67	0.725	ns	4.33	0.466	ns	-0.917	ns	-3.250	oo
1899/3	9.75	2.895	*	5.00	-0.941	ns	5.25	1.383	ns	-4.750	ooo	-4.500	ooo
1891/1	7.50	0.645	ns	4.83	-1.108	ns	2.20	-1.668	ns	-2.667	o	-5.300	ooo
1899/19	4.42	-2.438	o	2.43	-3.508	oo	2.72	-1.151	ns	-1.983	ns	-1.700	ns
1885/5	6.92	1.145	ns	6.50	0.559	ns	0.50	-3.368	oo	-0.417	ns	-6.417	ooo
1886/1	4.50	-2.355	o	5.17	-0.775	ns	3.25	-0.618	ns	0.667	ns	-1.250	ns
1896/4	4.75	-2.105	ns	3.17	-2.775	o	0.80	-3.068	oo	-1.583	ns	-3.950	ooo
1899/13	5.25	-1.605	ns	5.67	-0.275	ns	1.97	-1.901	ns	0.417	ns	-3.283	oo
1899/14	2.75	-4.105	ooo	1.52	-0.275	ns	2.10	-1.768	ns	-1.233	ns	-0.650	ns
1890/1	3.43	-3.422	oo	3.25	-2.691	o	0.10	-3.768	oo	-0.183	ns	-3.333	oo
1885/11	4.83	-2.022	ns	6.83	0.892	ns	0.97	-2.901	o	2.000	ns	-3.867	ooo
1899/1	3.62	-3.238	oo	4.17	-1.775	ns	0.10	-3.768	oo	0.550	ns	-3.517	oo
1890/13	5.50	-1.355	ns	4.50	-1.441	ns	1.30	-2.568	o	-1.000	ns	-4.200	ooo
1899/18	2.85	-4.005	ooo	2.18	-3.758	oo	0.65	-3.218	oo	-0.667	ns	-2.200	ns
Mean	6.85	-		5.94	-		3.87			-0.914		-2.987	

LSD 5% = 2.251 cm; 1% = 2.974 cm; 0.1% = 3.801 cm. LSD 5% = 2.238 cm; 1% = 2.960 cm; 0.1% = 3.792 cm.

When examining the culture media, the potato breeding line 1893/5 is highlighted, which obtained a significant positive difference (2.750 cm) in case of using culture medium containing PEG 2% compared to the MS medium. This breeding line had the capacity to tolerate water stress, by forming a well-developed root system. By comparing the PEG 1% medium with the control medium, the same line (1893/5) registered a distinctly significant positive difference (3.167 cm). Regarding root length, the breeding line 1895/3 is also distinguished, with a significant positive difference (2.367 cm) between PEG 1% and control medium.

CONCLUSIONS

Development of modern biotechnology techniques allows obtaining scientific and practical results, materialized in the rapid identification of potato breeding genotypes, with tolerance to *in vitro* hydric stress.

The lack of water, due to the osmotic agent, represents one of the abiotic stresses, which has the effect of inhibiting the growth and development of plantlets.

The potato breeding lines studied had different behavior regarding the tolerance to *in vitro* hydric stress, for the analyzed parameters. Drought-tolerant genotypes capable of absorbing more water had better growth than drought-sensitive ones.

The evaluation of genotypes with tolerance to hydric stress showed that the increased level of osmotic stress, due to the treatment with PEG in the growth medium, led to the reduction of leaves formation, plantlets growth and root length.

Genotypes 1893/5 and 1901/3 were identified with high values and very significant differences in case of medium with PEG in 2% concentration, for all parameters analyzed. At the same time, breeding line 1901/8 presented high values for two of the analyzed parameters: number of leaves / plantlet and plantlet length, when applying PEG 2% in the nutritional medium.

Noteworthy was also the breeding line 1901/10 which registered a distinctly significant positive difference regarding the number of leaves/ plantlets, when using in the culture medium PEG 1%, compared to the control medium. The same breeding line was noted with a distinctly significant positive difference in plantlet length, for PEG 1%, compared to the control medium.

The efficacy of *in vitro* simulation will have to be further tested in field conditions under the influence of water stress, by testing the promising potato genotypes for yield under stress.

We recommend further research on breeding lines 1893/5 and 1901/3, which can provide a valuable genetic material. We estimate that these genotypes will form plants with a large number of leaves and therefore with a large assimilation surface, with vigorous and deep root systems, adapted to water stress.

REFERENCES

Anithakumari, A.M., Karaba, N., Nataraja, Richard, G.F., Visser, van der Linden, C., Gerard, 2012. *Genetic dissection of drought tolerance and recovery potential by quantitative trait locus mapping of a diploid potato population*. Mol. Breed. 30(3): 1413-1429.

Errabii, T., Gandonou, C.B., Essalmani, H., Abrini, J., Idaomar, M., Senhaji, N.S., 2008. *Growth, proline and ion accumulation in sugarcane callus cultures under drought-induced osmotic stress and its subsequent relief*. Afri J. Biotechnol., 5: 1488-1493.

Gopal, J., and Iwama, K., 2007. *In vitro screening of potato against water stress mediated through sorbitol and polyethylene glycol*. Plant Cell Rep., 2007, 26: 693-700.

Hassan, N.S., Shaaban, L.D., El-Sayed, A.H., Seleem, E.E., 2004. *In Vitro Selection for Water Stress Tolerant Callus Line of Helianthus annuus L. Cv Myak*. Int. J. Agric. Biol., 5(3): 125-130.

Hassanpanah, D., 2010. *Evaluation of potato advanced cultivars against water deficit stress under in vitro and in vivo condition*. Biotechnology, 9(2): 164-169, ISSN 1682-296X.

Iwama, K., 2008. *Physiology of the potato: new insights into root system and repercussions for crop management*. Potato Res., 51: 333-353.

Lesk, C., Rowhani, P., Ramankutty, N., 2016. *Influence of extreme weather disasters on global crop production*. Nature, 529: 84-87. 10.1038/nature16467.

Mullahey, J.J., West, S.H., Cornell, J.A., 1996. *Effects of simulated drought by polyethylene glycol on Bahiagrass germination*. Seed Science and Technology, 24: 219-224.

Murashige, T., Skoog, F., 1962. *A revised medium for rapid growth and bioassays with tobacco tissue cultures*. Physiologia Plantarum, 15: 473-497.

Van der Linden, C., Gerard, Anithakumari, A.M., van Culemborg, M., Richard, G.F., Visser, 2011. *Dissecting the genetics of abiotic stress tolerance in potato*. Plant & Animal Genomes XIX Conference, San Diego, CA, January 15-19.