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Identification Of Potato Breeding Lines With Tolerance To In Vitro Hydric Stress

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ABSTRACT
Potato breeders from around the world, try to create genotypes with high yield and stress tolerance. In this study it was research tolerance of hydric stress for 27 genotypes by in vitro selection technique. This is based on in vitro tissue culture growth, on medium supplemented with an osmotic agent, allowing selection and regeneration of plants with desired characteristics. To induce in vitro hydric stress was used polyethylene glycol (with 2 concentration 1% and 2%) in nutritive medium and this was compared with basic medium Murashige-Skoog, considered control. Determinations were performed 4 weeks after inoculation of minicuttings belonging to 27 potato breeding lines of plantlets. The parameters analyzed were next: number of leaves, height of plantlets, root length. From obtained results, 1893/5 line can be considered as a genotype capable of extracting the water reserve and finally capable to fight with the effects of drought, so, able to resist in vitro hydric stress conditions. 1893/5 genotype was distinguished by the formation of a high number of leaves/plantlet on the medium with 2% PEG (9.17 leaves). 1893/5 genotype was representative in production of long roots, by using 2% PEG (9.9 cm).
Keywords - Hydric stress, in vitro, nutritive medium, potato breeding line, osmotic agent

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I. INTRODUCTION

Hydric stress is a major abiotic factor that limits plant growth and productivity. Around the world, approximately 40-60% of agricultural and, suffers from drought (Bray 1997 cited by [1]. Hydric stress usually manifested by an osmotic stress, causing morphological, physiological and molecular changes for plants [1].

Drought as phenomenon is difficult to define and plants tolerance to drought even more. On this line, Passioura (1997) said about drought tolerance that "it is like a nebulous which increasing become more as look more carefully", (cited by [2]).

Water deficit associated with temperatures above the thermal threshold, manifested in areas with temperate climates - continental determines several times, to stagnating or even forced maturation [3]. Globally, drought will decrease potato production by 18-32% during 2040-2069 (Hijmans, 2003, cited by [4]). Polyethylene glycol (PEG), it's a polymer, with high molecular weight. It is used to simulate drought for plants, as an agent for lowering the water potential in a way similar to dried soil [5].

In vitro selection is an alternative approach for the development of potato breeding lines with tolerance stress [6]; [7]. Tissue culture is a useful tool in studying mechanisms of stress tolerance under in vitro conditions [8].

In vitro micropropagation is the branch of plant biotechnology that represents the whole of plant propagation methods by using: in vitro cultures of cells, tissues and plant organs [9]. Micropropagation has a particular importance in domain of plant breeding; in vitro cultures are indispensable for branch of plant genetic engineering.

II. MATERIAL AND METHODS

The biological material studied to obtain potato breeding lines with high tolerance to the hydric stress comes from the experimental field of laboratory of genetic breeding and plant selection of NIRDPSB Brasov and was in stage vegetative descendants 1. The potato lines are part of NIRDPSB Brasov breeding program and are obtained by sexually hybridizing, the main method of creating variability. The biological material was selected from the first year on aspects such as adaptability, vigor, aspect, resistance to diseases and pests, etc., both for plants and tubers.

The breeding process besides a careful study of parents, and a well-established procedure,

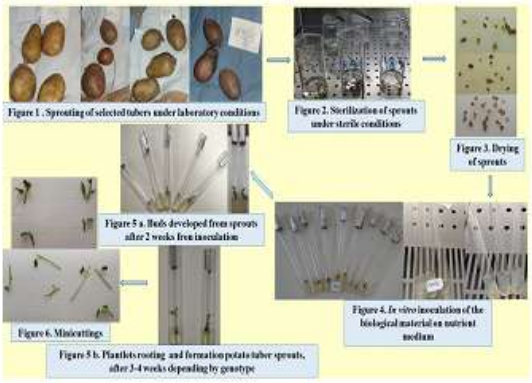
takes 10-12 years, as follows: year I: field of seed; years II-IV: selection field - vegetative hybrid populations (P1-P3); years V-VII: selection field - vegetative descendants (D1 and D2).

Biological material contains in next potato breeding lines 1896/1; 1897/2; 1896/5; 1890/7; 1901/7; 1909/1; 1901/3; 1891/2; 1890/5; 1885/1; 1899/4; 1901/9; 1901/11; 1895/4; 1899/11; 1895/3; 1901/12; 1889/2; 1901/6; 1890/3; 1890/8; 1896/2; 1893/5; 1890/12; 1895/1; 1889/1; 1890/4.

Research regarding identification of potato breeding lines with tolerance to in vitro hydric stress was made in Laboratory of Vegetal Tissue Culture, NIRDPSB Brasov, in 2018. By simulating in vitro hydric stress, we studied the influence of PEG on plantlets belonging to potato breeding lines above. Biological material consisted in sprouts tubers, types of cultures were compound from minicuttings, plantlets.

For in vitro inoculation of biological material, tubers were selected for sprouting under laboratory conditions (Fig. 1). For sprouts sterilizations (Fig. 2) was used sodium hypochlorite (NaOCl) solution, for 10 minutes, followed by washing three times with sterilized distilled water, sterilization with refined alcohol 96^0 for 3 minutes; washing three times with sterilized distilled water; drying them (Fig. 3) on sterilized paper in laminar flow hood. Sprouts were inoculated (Fig. 4) in culture medium, and the test tubes were transferred to the growing room. After 3-4 weeks, plantlets (Fig. 5 a, b) were regenerated, which are multiplied. Sprouts inoculation was made in laminar flow hood in which sterilization with UV lamp and alcohol was previously made. Instruments used for inoculation are sterilized in oven. After the inoculation of sprouts of each genotype, the instruments were flamed, followed by sterilization in the oven at the end of the procedure. After four weeks, plantlets (Fig. 5b) that were developed, were multiplied to obtain nodal cuttings (Fig. 6).

For determination of drought tolerance Murashige-Skoog (1962) [10] medium enriched with vitamins, naphthyl acetic acid (0.5 mg), sucrose, agar (considered as a control medium) was used as basic medium. For in vitro rapid identification of genotypes by hydric stress tolerance, as a hydric stress inducer, PEG 6000 was chosen. There were 3 variants of nutrient medium: control variant (MS medium enriched with vitamins, to which no osmotic agent was added), variants of the nutrient medium in which PEG was added in 2 concentrations (1%; 2%). PEG was added to MS base medium before pH adjustment. Approximately 4 ml of the nutrient medium of each category was put into the culture test tubes. These tubes were obturate with aluminum foil and sterilized in autoclave. Test tubes were placed in the growing room, ensuring light and temperature regime necessary for plant growth and development. After 4 weeks, determinations were made following the parameters: leaves number, plantlets height and roots length.



Variants studied: for determine resistance to in vitro hydric stress, the study consisted of a bifactorial experience (27x3) on 8 repetitions but from which the minimum and maximum variance values were eliminated, remaining 6 repetitions, comprising the following factors: experimental factor A: potato line with 27 graduations; experimental factor B - nutrient medium with three graduations: b1- control medium MS (with no osmotic agent); b_2-MS medium with 1% PEG; b_3-MS medium with 2% PEG.

III. RESULTS AND DISCUSSION

In vitro drought simulation was performed with the aim of identifying genotypes with optimal drought tolerance. During the experiment, a number of morphological aspects were assessed: leaves number, plantlets length, root length. By applying PEG to the nutrient medium, it acts as a simulator of in vitro hydric stress, having as a result reduction of nutrients needed for plant growth and the absorption of water through the roots. Explants depend on carbon assimilation coming from content of growing medium which contain sucrose. Thus, by reducing water absorption, there is a reducing in carbon consumption. Drought tolerant genotypes, capable of absorbing more water, have better growth than those sensitive to drought.

Regarding the first parameter analyzed on the MS medium, leaves number / plant, 1890/5 and 1890/7 genotypes are distinguished, with superior values of 11.33 and 11.17 leaves/ plant, and very significant positive differences, statistically assured of 3.23 and 3.07 cm, compared to mean values

(8.09), considered control, obtained for all 27 potato breeding lines. These two potato breeding lines are followed by 1893/5 and 1885/1 genotypes, which were distinguished by a significant difference of 1.57 leaves compared to control value. On the opposite position are 1901/11, 1895/3 and 1901/12 potato breeding lines, which records a lower leaves number, respectively: 6.83, 6.33, 5.5 with a significant negative difference (-1.27 leaves), distinctly significantly negative (-1.77 leaves) and very significant negative (-2.60 leaves) (Table 1). Treatment with 1% PEG reduced the leaves number compared to the nutrient medium (MS). 1901/9 genotype presented a very significant positive difference of leaves number / plant (3.07), reported to mean of all potato breeding lines studied (6.2593) with value of 9.33, so it can be considered that this is a potato breeding line with in vitro hydric stress tolerance. Also, 1893/5 and 1901/6 potato breeding lines showed tolerance to in vitro simulated hydric stress, by producing a high leaves number: 8.83 and 8.0, with distinct significant positive differences (2.57 and 1.74). The lowest values of the leaves number were found on 1895/3 and 1901/12 potato breeding lines, for 1% PEG osmotic agent, which had an inhibitory role in leaves production under the effect of in vitro hydric stress, respectively 4 and 3.83, with distinct significant negative differences (-2.26 and -2.43 leaves). Statistical analysis made to determine the influence of genotype, on the leaves number / plant developed on medium to which for in vitro drought simulation was added 2% PEG, indicates the superiority of 1893/5 and 1901/3 potato breeding lines, followed by 1885/1 potato line, which obtain very significant, positive differences (4.46 and 4.30 leaves), respectively a significant positive difference (1.96) compared to the control (mean of all 27 genotypes: 4.7037 leaves). Noteworthy, as for this element analyzed, the number of leaves, 1893/5 potato line showed tolerance for 1% PEG, with a distinct significant positive difference (2.57 leaves). The lowest values for the number of leaves, at 2% PEG concentration, was meet to: 1896/1, 1895/3, 1889/2, 1896/2 genotypes (2,6667; 2,6667; 2,6667; 2,3333 leaves) indicating a strong effect of this agent in drought inducing. 1895/3 potato line had a low tolerance to in vitro hydric stress and for medium to which 1% PEG was added, recording a distinct significant negative difference (-2.26 leaves) (Table 1).

Regarding the second parameter analyzed, plantlets length developed on the MS medium was distinguished 1889/1 genotype, with a superior value of 12.50 cm, a distinct significant positive difference, statistically ensured of 3.52 cm, compared to mean values (8.977 cm), considered as a control, obtained at the 27 potato breeding lines. This genotype is followed by the potato breeding lines: 1889/2 and 1901/9, which recorded values of 11.7 and 11.6 cm and significant positive differences of 2.69 and respectively: 2.61 cm. On the MS nutrient medium, the lowest values of the length of plantlets had the following potato breeding lines: 1901/12, 1901/1 with significant negative differences (-2.56, -3.23 cm) and 1890/12 with a distinct significant negative difference (-4.23 cm) (Table 2). The mean height of plantlet (cm), indicates the ability of the genotype to continue growth and development under stressful hydric conditions. 1901/3 and 1901/9 genotypes were significantly detached, recording a plantlets height on the medium to which 1% PEG was added, 11.9 cm and 8.5, with very significant, positive differences by 7.46 and 4.05 cm, reported to all mean values (4.4519). These are followed by 1893/5 genotype, which presented mean height plantlets by 7.3 cm and a significant difference by 2.80 cm (Table 2). It can be noted for 1893/5 and 1901/3 potato breeding lines, a tolerance to drought, because when was applied 2% PEG in the nutrient medium, the height of developed plantlets showed high values of 6.3333 and 5.0833 cm with very significant positive differences of 4.06 and 2.81 cm. These potato breeding lines are followed by 1901/12 line which has an average length of 4 cm, with a significant positive difference of 1.73 cm. 2% PEG produced negative effects on plant height for 1890/12 and 1896/1 potato breeding lines, followed by 1896/2 potato line, showing the lowest values (0.8833, 0.75, 0.4667 cm) (Table 2).

Statistical analysis made for establish genotype influence on mean length of plantlets roots on the MS growing medium, indicates superiority of 1901/3 and 1901/12 potato breeding lines, which obtained very significant, positive differences (4.35 and 3.94 cm) compared to the control (mean of 27 genotypes studied with value: 7.8123 cm) (Table3).

Table 1. Average number of leaves/plants

Breeding material inoculated on MS medium				Breeding material inoculated on MS + 1% PEG medium				Breeding material inoculated on MS + 2% PEG medium			
Breeding line	Average leaf number	Dif.	Sign.	Breeding line	Average leaf number	Diff.	Sign.	Breeding line	Mean number of leaves	Diff.	Sign.
1901/12.	6.6	-2.60	ooo	1901/12.	3.3333	-2.43	oo	1896/2.	2.3333	-2.37	o
1895/3.	6.33	-1.77	oo	1895/3.	4	-2.26	oo	1889/2.	2.6667	-2.04	o
1901/11.	6.83	-1.27	o	1896/1.	4.6667	-1.59	ns	1895/3.	2.6667	-2.04	o
1895/1.	7	-1.10	ns	1899/4.	4.6667	-1.59	ns	1896/1.	2.6667	-2.04	o
1899/11.	7	-1.10	ns	1890/8.	4.3333	-1.43	ns	1890/12.	3	-1.70	ns
1901/7.	7	-1.10	ns	1896/5.	5.3333	-0.93	ns	1890/8.	3.3333	-1.37	ns
1890/8.	7.17	-0.93	ns	1897/2.	5.3333	-0.93	ns	1895/1.	3.8333	-0.87	ns
1981/2.	7.17	-0.93	ns	1890/12.	5.8	-0.76	ns	1901/12.	3.8333	-0.87	ns
1896/1.	7.5	-0.60	ns	1890/5.	5.8	-0.76	ns	1890/3.	4	-0.70	ns
1897/2.	7.5	-0.60	ns	1895/4.	5.6667	-0.59	ns	1899/4.	4	-0.70	ns
1890/3.	7.67	-0.43	ns	1899/11.	5.5333	-0.43	ns	1896/5.	4.1667	-0.54	ns
1899/4.	7.67	-0.43	ns	1901/1.	5.5333	-0.43	ns	1890/4.	4.3333	-0.37	ns
1890/4.	7.83	-0.27	ns	1895/1.	6.1667	-0.09	ns	1890/5.	4.5	-0.20	ns
1895/4.	7.83	-0.27	ns	MEAN	6.2593	0.00	-	1901/6.	4.6667	-0.04	ns
1896/5.	7.83	-0.27	ns	1889/1.	6.3333	0.07	ns	MEAN	4.7037	0.00	-
MEAN	8.0988	0.00	-	1889/2.	6.3333	0.07	ns	1901/7.	4.8333	0.13	ns
1889/2.	8.17	0.07	ns	1896/2.	6.3333	0.07	ns	1899/11.	5	0.30	ns
1901/1.	8.33	0.23	ns	1890/4.	6.5	0.24	ns	1981/2.	5	0.30	ns
1889/1.	8.5	0.40	ns	1901/11.	6.5	0.24	ns	1895/4.	5.1667	0.46	ns
1890/12.	8.67	0.57	ns	1901/7.	6.5	0.24	ns	1897/2.	5.1667	0.46	ns
1896/2.	8.67	0.57	ns	1890/3.	6.8333	0.57	ns	1901/11.	5.1667	0.46	ns
1901/6.	8.83	0.73	ns	1899/7.	7.1667	0.91	ns	1889/1.	5.3333	0.63	ns
1901/9.	8.83	0.73	ns	1981/2.	7.5	1.24	ns	1890/7.	5.5	0.80	ns
1901/3.	9	0.90	ns	1885/1.	7.8333	1.57	ns	1901/1.	5.5	0.80	ns
1885/1.	9.67	1.57	*	1901/3.	7.8333	1.57	ns	1901/9.	5.5	0.80	ns
1893/5.	9.67	1.57	*	1901/6.	8	1.74	**	1885/1.	6.6667	1.96	*
1890/7.	11.17	3.07	***	1893/5.	8.8333	2.57	**	1901/3.	9	4.30	***
1890/5.	11.33	3.23	***	1901/9.	9.3333	3.07	***	1893/5.	9.1667	4.46	***
DL 5% = 1,25 DL 1% = 1,66 DL 0.1% = 2,13				DL 5% = 1,65 DL 1% = 2,19 DL 0.1% = 2,81				DL 5% = 1,35 DL 1% = 2,45 DL 0.1% = 3,15			

Table 2. Mean values of plantlets length

Breeding material inoculated on MS medium				Breeding material inoculated on MS + 1% PEG medium				Breeding material inoculated on MS + 2% PEG medium			
Breeding line	Length (cm)	Diff.	Sign.	Breeding line	Length (cm)	Diff.	Sign.	Breeding line	Length (cm)	Diff.	Sign.
1890/12.	4.8	-4.23	oo	1890/5.	1.9	-2.54	o	1896/2.	0.4667	-1.81	oo
1901/1.	5.8	-3.23	o	1895/3.	2.1	-2.37	o	1896/1.	0.75	-1.52	o
1901/12.	6.4	-2.56	o	1896/1.	2.6	-1.87	ns	1890/12.	0.8833	-1.39	o
1885/1.	6.8	-2.14	ns	1890/7.	2.7	-1.79	ns	1890/8.	1.1333	-1.14	ns
1981/2.	7.0	-1.98	ns	1890/12.	2.9	-1.54	ns	1890/5.	1.3833	-0.89	ns
1890/7.	7.9	-1.06	ns	1901/1.	3.0	-1.45	ns	1889/2.	1.5	-0.77	ns
1895/4.	8.0	-1.01	ns	1890/8.	3.2	-1.29	ns	1895/3.	1.55	-0.72	ns
1901/6.	8.1	-0.89	ns	1889/2.	3.3	-1.12	ns	1899/4.	1.5833	-0.69	ns
1893/5.	8.3	-0.73	ns	1890/3.	3.8	-0.70	ns	1981/2.	1.6717	-0.60	ns
1896/1.	8.3	-0.73	ns	1901/6.	3.8	-0.62	ns	1901/6.	1.7	-0.57	ns
1895/3.	8.4	-0.56	ns	1890/4.	3.9	-0.54	ns	1890/7.	1.75	-0.52	ns
MEAN	8.977	0.00	-	1895/4.	4.0	-0.50	ns	1895/1.	1.8333	-0.44	ns
1890/3.	9.0	0.02	ns	1896/5.	4.0	-0.45	ns	1896/5.	1.8333	-0.44	ns
1899/11.	9.0	0.02	ns	1897/2.	4.0	-0.45	ns	1901/7.	1.9167	-0.36	ns
1890/4.	9.2	0.24	ns	1981/2.	4.1	-0.37	ns	1890/4.	2.0833	-0.19	ns
1897/2.	9.3	0.36	ns	1896/2.	4.2	-0.29	ns	1899/11.	2.1	-0.17	ns
1895/1.	9.4	0.41	ns	1899/4.	4.2	-0.29	ns	MEAN	2.2743	0.00	-
1896/5.	9.6	0.61	ns	1901/12.	4.2	-0.29	ns	1895/4.	2.2833	0.01	ns
1901/11.	9.7	0.74	ns	1885/1.	4.3	-0.12	ns	1901/11.	2.4833	0.21	ns
1901/3.	9.8	0.86	ns	MEAN	4.4519	0.00	-	1890/3.	2.5167	0.24	ns
1901/7.	9.8	0.86	ns	1899/11.	4.9	0.46	ns	1889/1.	2.5833	0.31	ns
1890/8.	9.9	0.94	ns	1901/11.	5.2	0.71	ns	1897/2.	2.5833	0.31	ns
1896/2.	10.4	1.44	ns	1889/1.	5.3	0.80	ns	1901/1.	2.6667	0.39	ns
1899/4.	10.8	1.86	ns	1895/1.	5.3	0.80	ns	1901/9.	3.2333	0.96	ns
1890/5.	10.9	1.94	ns	1901/7.	5.9	1.46	ns	1885/1.	3.5	1.23	ns
1901/9.	11.6	2.61	*	1893/5.	7.3	2.80	*	1901/12.	4	1.73	*
1889/2.	11.7	2.69	*	1901/9.	8.5	4.05	***	1901/3.	5.0833	2.81	***
1889/1.	12.5	3.52	**	1901/3.	11.9	7.46	***	1893/5.	6.3333	4.06	***
DL 5% = 2,49 cm DL 1% = 3,30 cm DL 0.1% = 4,24 cm				DL 5% = 2,34 cm DL 1% = 3,10 cm DL 0.1% = 3,99 cm				DL 5% = 1,33 cm DL 1% = 1,76 cm DL 0.1% = 2,27 cm			

On the opposite side were 1885/1, 1889/1, 1889/2 and 1895/1 potato breeding lines which had a low capacity to form roots (with very significant negative differences: -3.01; -3.15; -3.23; -3.31) (Table 3). Roots are primary sensors of water deficit. by applying 1% PEG osmotic agent in nutritive medium 1895/3 potato line, obtained highest value of this parameter analyzed - root length, 12.5 cm, with a very significant positive difference (5.78 cm) (Table 3). 1893/5 and 1901/12 potato breeding lines combat effect of in vitro hydric stress by forming microplants with a medium root length (10.3 and 10.2 cm) and distinct significant differences of 3.62 and 3.45 cm, respectively. The smallest values of the root length are recorded at 1889/1 and 1885/1 potato breeding lines, indicating that these genotypes had a poor rooting capacity under in vitro hydric stress conditions with values of 3.9 and 3.0 cm Increasing in length of the root, under the influence of medium with 2% PEG was strongly influenced by it, highlighted by low values, recorded by the analyzed potato breeding lines. In hydric stress case, plant root growth is stopped, water absorption cannot be done anymore. But 1893/5 potato breeding line proved by root elongation, tendency to remove drought effect, with the value of 9.9 cm and very significant positive difference of 5.05 cm (Table 3). 1901/12 and 1901/3 potato breeding lines recorded high values for mean length of root, by 9.1 and 8.8 cm with distinctly significant positive differences by 4.22 and 3.89 cm. Also, 1901/9 potato line resisted to in vitro hydric stress, by root elongation "in water searching", having the value of root length (8.3 cm) over all mean values (4.8648 cm), with a significant positive difference (3.47 cm). For 1890/12 and 1890/8 potato breeding lines a drastic decreasing in root length it was observed, with 0.8 and 0.2 cm values and distinctly significant negative differences, by -4.06 and -4.68 cm.

Table 3. Mean values of plantlets root

Breeding material inoculated on MS medium				Breeding material inoculated on MS + 1% PEG medium				Breeding material inoculated on MS + 2% PEG medium			
Breeding line	Root length (cm)	Diff.	Sign.	Breeding line	Root length (cm)	Diff.	Sign.	Breeding line	Root length (cm)	Diff.	Sign.
1895/1.	4.5	-3.31	000	1885/1.	3.0	-3.72	00	1890/8.	0.2	-4.68	00
1889/2.	4.6	-3.23	000	1889/1.	3.9	-2.80	0	1890/12.	0.8	-4.06	00
1889/1.	4.7	-3.15	000	1889/2.	4.2	-2.55	ns	1896/2.	1.1	-3.75	0
1885/1.	4.8	-3.01	000	1901/6.	4.3	-2.47	ns	1889/2.	1.8	-3.10	0
1890/12.	5.1	-2.73	00	1890/12.	4.4	-2.30	ns	1885/1.	2.4	-2.51	ns
1890/3.	6.0	-1.81	0	1890/4.	4.4	-2.30	ns	1901/7.	2.9	-1.96	ns
1896/2.	6.1	-1.73	0	1897/2.	4.6	-2.13	ns	1890/4.	3.1	-1.78	ns
1890/4.	6.5	-1.31	ns	1890/7.	4.7	-2.05	ns	1896/1.	3.2	-1.70	ns
1890/8.	7.2	-0.66	ns	1895/1.	5.3	-1.38	ns	1890/7.	3.6	-1.28	ns
1893/5.	7.2	-0.65	ns	1890/8.	5.6	-1.15	ns	1889/1.	3.8	-1.11	ns
1890/7.	7.3	-0.48	ns	1981/2.	6.1	-0.63	ns	1897/2.	3.8	-1.11	ns
1895/4.	7.3	-0.48	ns	1901/1.	6.2	-0.55	ns	MEAN	4.8648	0.00	-
1901/7.	7.3	-0.48	ns	1895/4.	6.3	-0.47	ns	1899/4.	5.0	0.14	ns
MEAN	7.8123	0.00	-	1901/11.	6.7	-0.05	ns	1895/1.	5.1	0.22	ns
1981/2.	7.8	0.02	ns	MEAN	6.7164	0.00	-	1981/2.	5.1	0.22	ns
1890/5.	7.9	0.10	ns	1896/5.	6.8	0.12	ns	1899/11.	5.3	0.39	ns
1896/1.	8.4	0.60	ns	1896/1.	7.1	0.37	ns	1901/1.	5.3	0.39	ns
1899/4.	8.8	0.94	ns	1896/2.	7.3	0.62	ns	1901/6.	5.3	0.39	ns
1901/6.	8.8	0.94	ns	1899/11.	7.7	0.95	ns	1890/3.	5.5	0.65	ns
1896/5.	8.8	1.02	ns	1901/7.	7.8	1.03	ns	1890/5.	5.8	0.97	ns
1901/11.	8.9	1.10	ns	1890/5.	7.8	1.12	ns	1895/4.	6.2	1.30	ns
1899/11.	9.3	1.44	ns	1890/3.	8.1	1.37	ns	1901/11.	6.3	1.47	ns
1901/9.	9.3	1.44	ns	1901/3.	8.6	1.87	ns	1896/5.	6.9	2.05	ns
1897/2.	9.8	2.02	*	1899/4.	8.8	2.12	ns	1895/3.	7.1	2.27	ns
1895/3.	10.1	2.32	**	1901/9.	8.8	2.12	ns	1901/9.	8.3	3.47	*
1901/1.	10.6	2.77	**	1901/12.	10.2	3.45	**	1901/3.	8.8	3.89	**
1901/12.	11.8	3.94	***	1893/5.	10.3	3.62	**	1901/12.	9.1	4.22	**
1901/3.	12.2	4.35	***	1895/3.	12.5	5.78	***	1893/5.	9.9	5.05	***
DL 5% = 1,68 cm DL 1% = 2,22cm DL 0.1% = 2,86 cm				DL 5% = 2,76 cm DL 1% = 3,65 cm DL 0.1% = 4,69 cm				DL 5% = 2,94 cm DL 1% = 3,89 cm DL 0.1% = 5,00 cm			

IV. CONCLUSIONS

The mean number of leaves / plant oscillated medium with 1% PEG between: 9.33 (1901/9 line) and 3.83 (1901/12 line), and for the medium to which 2% PEG was added the values were between 9.17 (1893/5 line) and 2.33 (line 1896/2). Potato breeding lines 1901/9 and 1893/5 provided a valuable genetic material, which will form a well-developed stem, with a large number of leaves, and thus with a large assimilation surface, with the in vitro hydric stress tolerance. In vitro results on plant height indicated that 1901/3 genotype achieved a preeminent value of 11.9 cm (for 1% PEG). 1901/3 potato breeding line will provide in the future a possible biologically advantageous material, in the sense that it will develop high. A good tolerance to in vitro hydric stress presented when it was used 2% PEG in the nutrient medium: 1893/5 line, which recorded for this concentration the highest value of plantlets length (6.3 cm). For 1% PEG, line 1890/5 had the lowest plantlet length (1.9 cm), proving a low tolerance for in vitro hydric stress. Also, when it was used 2% PEG, for 1896/2 line a low hydric stress tolerance was observed. The statistical analysis performed to determine the influence of genotype on the mean length of roots, indicates the superiority of 1895/3 line, which obtained a very significant positive difference (5.78 cm) on the 1% PEG medium. This is followed by 1893/5 line (for the same concentration of 1% osmotic agent), which recorded a distinctly significant positive difference of 3.62 cm. For 2% PEG, 1893/5 line, obtained the highest value 9.9 cm, with a very significant positive difference (5.05). This line can be considered as a genotype capable of extracting the water reserve and finally capable to fight the effects of drought, so, able to resist in vitro hydric stress conditions. 1890/8 line showed the lowest root length value when 2% PEG (0.2 cm) was used. 1893/5 genotype was distinguished by the formation of a high number of leaves/plantlet on the medium with 2% PEG (9.17 leaves), followed by 1901/3 line (9.00 leaves). 1893/5 was remarked for 2% PEG medium by length of plantlets, superior to the other potato breeding lines analyzed (6.3 cm), followed by the 1901/3 line (5.1 cm).

1893/5 genotype was representative in production of long roots, by using 2% PEG (9.9 cm), followed by 1901/12 line (9.1 cm). In the next years, the selected potato genotypes: 1893/5, 1901/3, 1901/12, which had shown tolerance to hydric stress, will continue in the breeding program of NIRDPSB Brasov in order to obtain new varieties with patent.

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