AcademicPres

Available online: www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2016, 8(4):511-519. DOI: 10.15835/nsb.8.4.9920

Notulae Scientia Biologicae

Original Article

The Growth Response of Several Potato Genotypes (*Solanum tuberosum* L.) to Induced Water Stress Using Sorbitol and Polyethylene Glycol

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Abstract

The current paper aimed to study the *in vitro* response of potato genotypes to water stress induced by adding sorbitol and polyethylene glycol in the culture medium. The biological material analysed in the experiment was represented by a Romanian line 'LP 11-1525/1' and two isogenic lines 'LI 101' and 'LI 102'. For cultures initiation, the line 'LP 11-1525/1' was started from meristems and for the other two genotypes true potato seeds were used. The studied potato genotypes behaved differently depending on the analysed parameters and on the treatment applied for drought tolerance. It was noted that the line 'LP 11-1525/1' achieved good results for most of the growth parameters studied, and also the lines derived from true potato seeds behaved well, in some cases even exceeding the line derived from meristems. Of the lines derived from true potato seeds, the best performance was noted for line 'LI 101-6' in all the analysed parameters, both on sorbitol and PEG medium. In addition, lines 'LI 101-7' and 'LI 102-4' achieved good results on both variants of medium used to mediate water stress. Therefore, establishing drought tolerance individuals within populations derived from true potato seeds using sorbitol and polyethylene glycol might be applied.

Keywords: drought, in vitro, *Solanum tuberosum*, stress, true potato seed

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important vegetables in the world (Albiski *et al.*, 2012). There are currently numerous potato varieties that are adapted to diverse environments with different soil types and climates (Barra *et al.*, 2013). According to the FAO, these characteristics lead the crop to play an important role in world food security (FAO, 2011, cited by Barra, 2013).

The identification of new potato genotypes tolerant to abiotic stress is currently needed since climate change is associated with an increase in temperature of the planet and a decrease in precipitation (Lobell *et al.*, 2011, cited by Barra, 2013).

In the past years Romania was also faced with droughts which affected crop species, including potato, especially when drought was installed in the early stages of the growing season and was extended for a longer time. In this context, Romanian researchers work was oriented to create new potato varieties tolerant to adverse environmental conditions.

High temperatures and lack of rain are abiotic stresses that cause significant decreases in potato production (Arvin and Donnelly, 2008). Abiotic stress refers to the negative impact of environmental factors on plant growth and development. Among these factors it can be mention: extreme temperatures, flooding, high winds, drought, fire, stoniness ground, pollution, soil acidity etc. (Marron *et al.*, 2008). These factors are unavoidable and when they are pulled together cause even greater damages (Mittler, 2006). For example, crop plants are largely dependent on the availability of moisture in the top 10 cm of the soil profile. Drought stress occurs when soil moisture status is low, relative humidity is low and temperature is high. If the drought persists, plants dry up, and productivity of crops gets badly affected (Pareek *et al.*, 2010). Salinity is also considered as a major abiotic stress and significantly affecting crop production all over the world, especially in arid and semiarid regions (Khajeh-Hosseini *et al.*, 2003). Various physiological injuries have been observed under high temperatures, such as scorching of leaves and stems, leaf abscission and senescence, shoot and root growth inhibition or fruit damage (Vollenweider and Günthardt-Goerg, 2005).

In regard of this, the potato production is adjusted towards new regions of the world where high temperatures and limited water resources, or both, are restrictive factors for achieving quality productions, but also a challenging task for researchers is to find new potato genotypes tolerant of those limiting factors mentioned above (Levy and Veilleux, 2007).

Received: 29 Sep 2016. Received in revised form: 21 Nov 2016. Accepted: 01 Dec 2016. Published online: 16 Dec 2016.

The drought is a major environmental factor that determines the plant growth, the yield and the distribution of plants (Rukundo *et al.*, 2012). Currently, the drought represents the most worldwide crop reducing stress in agriculture (Ober, 2008 cited by Rukundo, 2012). The drought affects more than 10% of arable soil (Bray *et al*., 2000; Zidenga, 2006, cited by Rukundo, 2012) and continue to increase, due to the explosive expansion of world population, continuous deterioration of arable land, shortage of fresh water, and the current climate change.

The increase in drought stress threatens the global agriculture production and food availability (Rokundo *et al.*, 2012). It has been estimated that two thirds of the yield potential of major crops are routinely lost due to drought stress (Bray *et al.*, 2000; Lafitte *et al.*, 2004; Zidenga, 2006; Magombeyi and Taigbenu, 2008, cited by Rukundo, 2012). Therefore, the sustainability of production will depend on the identification and development of new drought tolerant varieties (Cochard *et al.*, 2008, cited by Rukundo, 2012).

Polyethylene glycol (PEG) is a polymer produced in a range of molecular weights. PEG of higher molecular weight (4,000 to 8,000) was commonly used in physiological experiments to induce controlled drought stress in nutrient solution cultures (Hassanpanah, 2009). Polyethylene glycol (PEG), sucrose, mannitol or sorbitol has been used by several researches as osmotic stress agents for *in vitro* selection (Hassan *et al.*, 2004). However, PEG has been the most extensively used to stimulate water stress in plants. This compound of high molecular weight is a non-penetrating inert osmoticum that reduces water potential of nutrient solutions without being taken up by the plant or being phytotoxic (Hassan *et al.*, 2004).

Sorbitol, a six carbon sugar alcohol, is one of the most frequently found polyols in plants. It is a direct product of photosynthesis in mature leaves, in parallel with sucrose, whereas both serve similar functions, such as translocation of carbon skeletons and energy between sources and sink organs (Jain *et al.*, 2010). Increased transport of polyols, both in the xylem and phloem, occurs frequently as a result of salt or drought stress (Noiraud *et al.*, 2001).

The purpose of the present study was to determine the response to water stress of nine potato genotypes, by measuring the morphological characteristics associated with the vegetative growth of potato plantlets *in vitro* using PEG and sorbitol as a water stress inductors. In order to achieve high productions and biological quality for improved potato crops, it is important to produce local potato varieties tolerant to drought. In the hereby study it was tried the identification of potato genotypes tolerant to water stress, obtained both through vegetative (meristems) and generative (true potato seeds) propagation.

Materials and Methods

Plant material

The study was carried out at the National Institute of Research and Development for Potato and Sugar Beet Brasov (Romania), Research Laboratory for Plant Tissue Culture. The biological material analysed in the current experiment was represented by a Romanian line 'LP 11-1525/1' and two isogenic lines from Dutch company Bejo Zaden: 'LI 101' and 'LI 102'.

The line 'LP 11-1525/1' was started from meristems for cultures initiation, and for the other two genotypes true potato seeds were used. In the case of isogenic lines, were selected for the study those individuals who have a favourable outcome in a medium supplemented with 15 g/L mannitol. The line 'LP 11-1525/1' seedlings were grown on the Murashige-Skoog medium.

All the materials were checked for the PVX (potato virus X), PVS (potato virus S), PVY (potato virus Y) and PVM (potato virus M). After ELISA testing, results showed that the plants were healthy.

Water stress tolerance and growth parameters

To multiply and test water stress tolerance *in vitro*, there were used 1 cm long explants containing an axillary bud with related leaf and half of neighbouring internodes. After inoculation, the cultures were incubated in a growth chamber (temperature 20 ± 2 °C, 16:8 photoperiod) for 6 weeks. During this period a series of observations and notations were made at 2, 4, and 6 weeks on the following parameters: stem length, number of leaves, leaf aspect, rooting, plantlet fresh and dry weight, root length, fresh and dry root weight.

Within the observations made at 2 and 4 weeks of *in vitro* culture for leaf aspect assessment a grading scale with seven levels was used, as follows: 1 – very small; 2 – very small - small; 3 – small; 4 – small - medium; 5 – medium; 6 – medium - large; 7 – large.

Observations concerning the level of rooting was done similarly to the method of leaf aspect assessment and for this purpose a rating scale with values ranging from 1 to 5 was used as follows: 1 – no roots; 2 – very poorly developed roots; 3 – weakly developed roots; 4 – well developed roots.

In order to determine the response of the potato plantlets to artificially induced water stress conditions, three different variants were used: Murashige-Skoog (MS) added with 48 g/L PEG, MS medium added with 40 g/L sorbitol and MS medium containing 20 g/L sucrose, as a control. For solidification of all three variants, 9 g/L agar was used.

The pH of the solutions was adjusted at 5.7 with a pH meter. About 5 ml of nutrient medium was distributed in tubes of 2 cm diameter and 15 cm height, which were covered with aluminium foil caps.

The sterilization was carried out in the autoclave at 121 °C for 20 minutes. After sterilization, the medium was removed from the autoclave and allowed to cool and solidify until the next day when inoculation of stem explants has been started.

Statistical analyses

The study was design as a randomized complete block with three replicates; factors included 3 potato genotypes ('LI 101', 'LI 102' and 'LP 11-1525/1'), 3 variants of medium (MS supplement with 40 g/L sorbitol, MS supplement with 48 g/L PEG and MS as control) and three measurement times (2, 4 and 6 weeks).

The data were statistically analysed using SPSS program. The response of each potato genotype to PEG and sorbitol induced water stress was measured for each morphological parameter and its value under stress conditions was related to its respective control, in three different periods of time. The statistical analysis was performed by comparing the means, specifying standard deviation. For comparing the obtained means, ANOVA procedure and Duncan's multiple range test

was applied. Correlation between all the characters has been made. Correlation of measured parameters was performed using Pearson correlation.

Results and Discussion

Regarding the stem length of the studied potato genotypes, Duncan test analysis indicated close values, thus no significant differences in the medium with sorbitol or PEG (Table 1). Throughout observations, water stress caused a decrease in stem length of all lines. However, the lines 'LI 101-6' and 'LI 101-7' have achieved good and consistent results for all the time periods, both on sorbitol and PEG medium (Table 1).

Table 2 presents the behaviour of potato genotypes regarding the number of leaves. Several lines results on medium added with sorbitol and PEG were higher or similar to those obtained on control. Lines 'LI 101-6', 'LI 101-7' and 'LI 102-4' have achieved good and consistent results both on sorbitol and PEG medium (Table 2).

Table 3 shows the response of potato genotypes regarding the leaf aspect. Throughout observations, leaf aspect decreased due to drought in all lines. However, the lines LI '101-6' and 'LI 101-7' have achieved good and consistent results both on sorbitol and PEG medium (Table 3).

Table 1. Stem length of the studied potato genotypes under induced water stress

Line (cod)	Stem length (cm)						Relative response compared to the control (%)	
	Control		Sorbitol 4%		PEG 4.8%		Sorbitol 4%	PEG 4.8%
2 weeks								
'LI 101-3'	3.62	bcd	0.52	f	0.80	f	14.4	22.1
'LI 101-6'	3.46	bcd	0.60	f	0.78	f	17.3	22.5
'LI 101-7'	2.80	cde	0.44	f	0.72	f	15.7	25.7
'LI 102-2'	2.24	def	0.54	f	0.88	f	24.1	39.3
'LI 102-3'	4.90	ab	0.80	f	0.30	f	16.3	6.1
'LI 102-4'	4.12	abc	0.78	f	0.46	f	18.9	11.2
'LP 11-1525/1-1'	5.20	ab	0.44	f	0.68	f	8.5	13.1
'LP 11-1525/1-2'	5.88	a	0.66	f	1.12	ef	11.2	19.1
'LP 11-1525/1-3'	5.68	a	1.08	ef	0.62	f	19.0	10.9
4 weeks								
'LI 101-3'	9.00	ab	1.04	c	1.34	c	11.6	14.9
'LI 101-6'	7.44	b	1.54	c	1.40	c	20.7	18.8
'LI 101-7'	7.52	b	1.54	c	1.62	c	20.5	21.5
'LI 102-2'	8.20	ab	0.60	c	2.76	c	7.3	33.7
'LI 102-3'	10.80	a	2.62	c	0.56	c	24.3	5.2
'LI 102-4'	8.48	ab	1.06	c	0.92	c	12.5	10.9
'LP 11-1525/1-1'	7.50	b	1.78	c	2.30	c	23.7	30.7
'LP 11-1525/1-2'	8.02	ab	1.88	c	3.02	c	23.4	37.7
'LP 11-1525/1-3'	9.38	ab	2.10	c	2.20	c	22.4	23.5
6 weeks								
'LI 101-3'	13.94	ab	1.72	d	1.38	d	12.3	9.9
'LI 101-6'	9.12	c	2.62	d	2.22	d	28.7	24.3
'LI 101-7'	12.62	ab	2.54	d	1.72	d	20.1	13.6
'LI 102-2'	13.58	ab	0.76	d	3.80	d	5.6	28.0
'LI 102-3'	15.66	a	4.46	d	1.08	d	28.5	6.9
'LI 102-4'	11.56	bc	1.54	d	1.18	d	13.3	10.2
'LP 11-1525/1-1'	8.38	c	2.12	d	3.30	d	25.3	39.4
'LP 11-1525/1-2'	8.98	c	2.20	d	3.50	d	24.5	39.0
'LP 11-1525/1-3'	10.58	bc	2.80	d	3.12	d	26.5	29.5
Mean								
2 weeks	4.21	a	0.65	b	0.70	b	16.2±4.6	18.9±10.0
4 weeks	8.48	a	1.57	b	1.79	b	18.5±6.3	21.9±10.8
6 weeks	11.60	a	2.31	b	2.37	b	20.5±8.3	22.3±12.6

Different letters indicate statistically significant differences between different treatments. (Duncan's multiple range test; $P < 0.05$)

Table 2. The number of leaves of the studied potato genotypes under induced water stress

Line (cod)	Number of leaves						Relative response compared to the control (%)	
	Control		Sorbitol 4%		PEG 4.8%		Sorbitol 4%	PEG 4.8%
2 weeks								
'LI 101-3'	6.00	a	2.60	def	3.80	abcde	43.3	63.3
'LI 101-6'	4.20	abcde	3.00	cdef	4.20	abcde	71.4	100.0
'LI 101-7'	5.80	ab	4.20	abcde	3.80	abcde	72.4	65.5
'LI 102-2'	4.00	abcde	1.20	f	3.80	abcde	30.0	95.0
'LI 102-3'	5.40	abc	3.40	bcdef	2.40	ef	63.0	44.4
'LI 102-4'	4.40	abcde	4.40	abcde	3.20	cdef	100.0	72.7
'LP 11-1525/1-1'	5.00	abcd	3.00	cdef	3.40	bcdef	60.0	68.0
'LP 11-1525/1-2'	5.80	ab	3.60	abcdef	4.00	abcde	62.1	69.0
'LP 11-1525/1-3'	5.80	ab	4.40	abcde	3.40	bcdef	75.9	58.6
4 weeks								
'LI 101-3'	9.20	ab	4.60	efgh	5.20	cdefgh	50.0	56.5
'LI 101-6'	6.40	abcdefg	4.20	fgh	6.00	bcdefg	65.6	93.8
'LI 101-7'	9.80	a	6.80	abcdefg	5.00	defgh	69.4	51.0
'LI 102-2'	8.00	abcde	2.40	h	5.80	bcdefg	30.0	72.5
'LI 102-3'	8.00	abcde	6.20	bcdefg	3.80	gh	77.5	47.5
'LI 102-4'	6.80	abcdefg	5.20	cdefgh	4.60	efgh	76.5	67.7
'LP 11-1525/1-1'	8.20	abcd	7.80	abcde	6.00	bcdefg	95.1	73.2
'LP 11-1525/1-2'	9.00	ab	7.00	abcdefg	7.40	abcdef	77.9	83.2
'LP 11-1525/1-3'	8.60	abc	7.20	abcdefg	5.40	cdefgh	83.7	62.8
6 weeks								
'LI 101-3'	9.60	abc	6.00	cdef	8.20	abcde	62.5	85.4
'LI 101-6'	6.00	cdef	5.60	def	7.40	abcdef	93.3	123.3
'LI 101-7'	10.20	a	8.60	abcde	6.20	bcdef	84.3	60.8
'LI 102-2'	9.80	abc	3.60	f	7.80	abcde	36.7	79.6
'LI 102-3'	9.80	abc	8.00	abcde	5.40	ef	81.6	55.1
'LI 102-4'	9.20	abcde	7.20	abcdef	7.20	abcdef	78.3	78.3
'LP 11-1525/1-1'	10.00	ab	8.20	abcde	7.40	abcdef	82.0	74.0
'LP 11-1525/1-2'	10.40	a	9.20	abcde	8.20	abcde	88.5	78.9
'LP 11-1525/1-3'	10.20	a	9.40	abcd	7.40	abcdef	92.2	72.5
Mean								
2 weeks	5.16	a	3.31	b	3.56	b	64.2±19.9	70.7±17.3
4 weeks	8.22	a	5.71	b	5.47	b	69.5±19.4	67.5±14.9
6 weeks	9.47	a	7.31	b	7.24	b	77.7±17.9	78.7±19.3

Different letters indicate statistically significant differences between different treatments. (Duncan's multiple range test; $P < 0.05$)

The response of potato genotypes regarding the level of rooting at 2 and 4 weeks, respectively roots length at 6 weeks was illustrated in Table 4. After 2 and 4 weeks drought caused a decrease in level of rooting of all lines. Regarding the roots length, after 6 weeks some lines have achieved better results on the sorbitol medium than control. Thus, in line 'LI 101-3', 'LP 11-1525/1-1', 'LP 11-1525/1-2' and 'LP 11-1525/1-3' the sorbitol treatment resulted in roots length increases (Table 4). Line 'LI 101-6' achieved good results for all time periods both on sorbitol and PEG medium (Table 4).

After 6 weeks of *in vitro* cultivation of potato plantlets in addition to parameters measured at 2 and 4 weeks, other determinations were carried out. Table 5 shows the behaviour of potato genotypes regarding the total plant fresh weight. Induced water stress caused a decrease in fresh weight of all lines (Table 5). However, line 'LI 101-6' has achieved good results both under sorbitol and PEG treatments (Table 5).

Except line 'LI 102-3' which obtained a higher value under sorbitol treatment (0.017 g) and the line 'LI 102-2' which obtained the same amount both under PEG treatment as the control (0.015 g), induced water stress factors determined a lower amount regarding the total dry weight compared to the control (Table 6). Lines 'LI 102-4' and 'LI 101-6' have achieved good results both under sorbitol and PEG treatments (Table 6).

Table 3. Leaf aspect of the studied potato genotypes under induced water stress

Line (cod)	Leaf aspect (scale)						Relative response compared to the control (%)	
	Control		Sorbitol 4%		PEG 4.8%		Sorbitol 4%	PEG 4.8%
2 weeks								
'LI 101-3'	5.0	a	1.0	ef	1.4	ef	20.0	28.0
'LI 101-6'	3.0	abcdef	2.0	def	1.8	ef	66.7	60.0
'LI 101-7'	5.0	a	2.4	bcdef	2.2	cdef	48.0	44.0
'LI 102-2'	2.4	bcdef	0.6	f	1.2	ef	25.0	50.0
'LI 102-3'	3.4	abcde	1.4	ef	1.0	ef	41.2	29.4
'LI 102-4'	3.2	abcde	2.2	cdef	1.0	ef	68.8	31.3
'LP 11-1525/1-1'	4.4	abcd	2.0	def	2.0	def	45.5	45.5
'LP 11-1525/1-2'	4.6	abc	2.0	def	3.0	abcdef	43.5	65.2
'LP 11-1525/1-3'	4.8	ab	3.0	abcdef	2.0	def	62.5	41.7
4 weeks								
'LI 101-3'	5.0	abc	2.2	efgh	1.4	fgh	44.0	28.0
'LI 101-6'	3.0	cdefgh	2.2	efgh	1.6	fgh	73.3	53.3
'LI 101-7'	5.0	abc	3.0	cdefg	3.4	bcdef	60.0	68.0
'LI 102-2'	4.2	abcde	0.6	h	1.2	gh	14.3	28.6
'LI 102-3'	4.2	abcde	2.6	defgh	1.2	gh	61.9	28.6
'LI 102-4'	4.6	abcd	3.0	cdefg	1.4	fgh	65.2	30.4
'LP 11-1525/1-1'	5.0	abc	4.4	abcd	3.0	cdefg	88.0	60.0
'LP 11-1525/1-2'	5.4	ab	4.2	abcde	3.4	bcdef	77.8	63.0
'LP 11-1525/1-3'	6.0	a	5.0	abc	3.2	cdefg	83.3	53.3
6 weeks								
'LI 101-3'	5.8	abcd	1.8	ij	1.8	ij	31.0	31.0
'LI 101-6'	3.0	fghi	2.8	ghij	1.8	ij	93.3	60.0
'LI 101-7'	6.4	ab	4.4	cdefg	3.2	efghi	68.8	50.0
'LI 102-2'	5.0	abcde	1.0	j	2.6	ghij	20.0	52.0
'LI 102-3'	4.8	bcdef	4.2	defg	1.4	ij	87.5	29.2
'LI 102-4'	6.8	a	3.0	fghi	2.2	hij	44.2	32.4
'LP 11-1525/1-1'	6.2	abc	4.0	defgh	2.6	ghij	64.5	41.9
'LP 11-1525/1-2'	5.8	abcd	4.0	defgh	3.0	fghi	69.0	51.7
'LP 11-1525/1-3'	5.8	abcd	4.0	defgh	2.6	ghij	69.0	44.8
Mean								
2 weeks (scale)*	3.98	a	1.84	b	1.73	b	46.8±17.2	43.9±13.1
4 weeks (scale)*	4.71	a	3.02	b	2.20	c	63.1±22.7	45.9±16.8
6 weeks (scale)*	5.51	a	3.24	b	2.36	c	60.8±24.5	43.7±10.9

Different letters indicate statistically significant differences between different treatments. (Duncan's multiple range test; $P < 0.05$).
*Leaf aspect: scale from 1 (very small leaves) to 7 (large leaves)

Regarding the treatment for inducing drought in laboratory conditions, the average values of the characteristics indicated that PEG had a stronger effect than sorbitol, except for stem length. The obtained results showed that some lines had higher values on sorbitol medium than PEG one, while others had higher values on PEG medium than sorbitol ones. Thus, for all time periods and for all measured parameters the line 'LI 102-2' obtained better results on PEG medium than sorbitol one and the line 'LI 102-3' perform better on sorbitol than PEG one. Also, lines 'LI 101-3' and 'LI 102-4' obtained generally better results on sorbitol medium than PEG one.

Regarding the correlations between morphological characteristics under induced water stress, the values were significant for all the parameters (Table 7).

Discussion

A good approach to evaluate water stress tolerance is with field trials, but environmental variables are difficult to control (Barra *et al.*, 2013). An alternative to minimize this effect is to develop *in vitro* assays (Gopal *et al.*, 2008; Rahman *et al.*, 2008). Various *in vitro* methods to induce water stress in plants have been described, which use

Table 4. Level of rooting and roots length of the studied potato genotypes under induced water stress

Line (cod)	Level of rooting (scale)						Relative response compared to the control (%)	
	Control		Sorbitol 4%		PEG 4.8%		Sorbitol 4%	PEG 4.8%
2 weeks								
'LI 101-3'	4.4	a	2.8	cdefgh	1.7	ghi	63.6	38.6
'LI 101-6'	3.4	abcde	2.6	defghi	2.0	efghi	76.5	58.8
'LI 101-7'	4.2	abc	2.0	efghi	1.6	ghi	47.6	38.1
'LI 102-2'	3.4	abcde	1.2	i	1.8	fghi	35.3	52.9
'LI 102-3'	3.5	abcd	2.0	efghi	1.4	hi	57.1	40.0
'LI 102-4'	3.2	abcdef	1.8	fghi	1.4	hi	56.3	43.8
'LP 11-1525/1-1'	4.4	a	2.9	bcdefg	1.9	fghi	65.9	43.2
'LP 11-1525/1-2'	4.3	ab	2.7	defgh	2.3	defghi	62.8	53.5
'LP 11-1525/1-3'	4.6	a	3.7	abcd	2.3	defghi	80.4	50.0
4 weeks								
'LI 101-3'	5.0	abc	3.5	bcdefg	2.2	ghij	70.0	44.0
'LI 101-6'	3.0	cdefgh	2.6	efghij	2.5	efghij	68.4	65.8
'LI 101-7'	5.0	abc	2.5	efghij	2.7	efghij	50.0	54.0
'LI 102-2'	4.2	abcde	1.4	j	2.0	hij	35.9	51.3
'LI 102-3'	4.2	abcde	2.8	defghij	1.5	ij	65.1	34.9
'LI 102-4'	4.6	abcd	2.2	ghij	2.2	ghij	53.7	53.7
'LP 11-1525/1-1'	5.0	abc	4.2	abcd	2.4	fghij	89.4	51.1
'LP 11-1525/1-2'	5.4	ab	3.9	abcde	3.1	cdefgh	84.8	67.4
'LP 11-1525/1-3'	6.0	a	4.2	abcd	2.9	cdefghi	87.5	60.4
Root length (cm)								
6 weeks								
'LI 101-3'	10.76	ab	11.04	ab	3.82	defg	102.6	35.5
'LI 101-6'	6.60	abcdefg	5.76	bcdefg	4.08	cdefg	87.3	61.8
'LI 101-7'	11.66	a	6.20	bcdefg	4.06	cdefg	53.2	34.8
'LI 102-2'	9.42	ab	3.24	efg	3.28	efg	34.4	34.8
'LI 102-3'	8.20	abcde	7.12	abcdef	1.48	g	86.8	18.1
'LI 102-4'	9.64	ab	3.38	efg	2.60	fg	35.1	27.0
'LP 11-1525/1-1'	9.26	ab	9.60	ab	2.98	efg	103.7	32.2
'LP 11-1525/1-2'	8.90	abcd	9.96	ab	7.46	abcdef	111.9	83.8
'LP 11-1525/1-3'	9.10	abc	9.72	ab	5.84	bcdefg	106.8	64.2
Mean								
2 weeks (scale)*	3.93	a	2.41	b	1.82	c	60.6±13.8	46.6±7.5
4 weeks (scale)*	4.47	a	3.03	b	2.39	c	67.2±18.3	53.6±10.2
6 weeks (cm)	9.28	a	7.34	b	3.96	c	80.2±31.1	43.6±21.4

Different letters indicate statistically significant differences between different treatments. (Duncan's multiple range test; $P < 0.05$).
*Level of rooting: scale from 1 (no roots) to 5 (well developed roots)

chemical agents that reduce water potential in the culture medium (Barra *et al.*, 2013). Among these, sorbitol, NaCl, mannitol, agar, polyethylene glycol (PEG) are used. PEG is the most recommended inductor because it does not penetrate plant cells and also reduces the water potential of the medium in which plants develop (Manoj *et al.*, 2011). Data about effects of sorbitol on *in vitro* potato growth are more limited compared with other agents that induce water stress. Some researchers reported that addition of sorbitol on Murashige-Skoog medium decreased water potential, inducing drought stress affecting shoot and root growth (Gopal and Iwama, 2007).

The choice of the inducing water stress agents concentration was based on the results obtained by other researchers. Thus, differences in morphological parameters occurred only at 4% sorbitol, while at 2% sorbitol plant responses were generally similar to the control level. At 6, 8 and 10% sorbitol, plants did not produce stems and leaves (Albiski

Table 5. Effect of sorbitol and PEG on fresh weight of the studied potato genotypes under induced water stress after six weeks of *in vitro* cultivation

Line (cod)	Fresh weight (g)						Relative response compared to the control (%)	
	Control		Sorbitol 4%		PEG 4.8%		Sorbitol 4%	PEG 4.8%
'LI 101-3'	0.368	ab	0.048	ij	0.042	ij	13.0	11.4
'LI 101-6'	0.204	cdefg	0.058	hij	0.050	ij	28.4	24.5
'LI 101-7'	0.292	bcd	0.058	hij	0.050	ij	19.9	17.1
'LI 102-2'	0.188	defgh	0.016	j	0.032	ij	8.5	17.0
'LI 102-3'	0.224	cdef	0.084	ghij	0.018	j	37.5	8.0
'LI 102-4'	0.442	a	0.070	hij	0.098	fghij	15.8	22.2
'LP 11-1525/1-1'	0.260	bcde	0.154	efghi	0.118	fghij	59.2	45.4
'LP 11-1525/1-2'	0.262	bcde	0.110	fghij	0.136	efghij	42.0	51.9
'LP 11-1525/1-3'	0.324	bc	0.156	efghi	0.080	ghij	48.2	24.7
Mean	**0.284**	**a**	**0.083**	**b**	**0.069**	**b**	**30.3±17.5**	**24.7±14.8**

Different letters indicate statistically significant differences between different treatments (Duncan's multiple range test; P < 0.05)

Table 6. Effect of sorbitol and PEG on dry weight of the studied potato genotypes under induced water stress after six weeks of *in vitro* cultivation

Line (cod)	Dry weight (g)						Relative response compared to the control (%)	
	Control		Sorbitol 4%		PEG 4.8%		Sorbitol 4%	PEG 4.8%
'LI 101-3'	0.030	ab	0.009	defg	0.004	fg	30.0	13.3
'LI 101-6'	0.023	abcde	0.009	efg	0.005	fg	39.1	21.7
'LI 101-7'	0.024	abcde	0.006	fg	0.006	fg	25.0	25.0
'LI 102-2'	0.015	bcdefg	0.002	g	0.015	bcdefg	13.3	100.0
'LI 102-3'	0.014	bcdefg	0.017	bcdefg	0.002	g	121.4	14.3
'LI 102-4'	0.029	ab	0.014	bcdefg	0.011	cdefg	48.3	37.9
'LP 11-1525/1-1'	0.026	abcd	0.024	abcde	0.021	abcdef	92.3	80.8
'LP 11-1525/1-2'	0.026	abcd	0.021	abcdef	0.020	abcdef	80.8	76.9
'LP 11-1525/1-3'	0.036	a	0.027	abc	0.019	bcdefg	75.0	52.8
Mean	**0.025**	**a**	**0.014**	**b**	**0.011**	**b**	**58.4±35.9**	**47.0±32.2**

Different letters indicate statistically significant differences between different treatments (Duncan's multiple range test; P < 0.05)

Table 7. Pearson correlation of the morphological parameters measured for all time periods

	2 weeks				**4 weeks**				**6 weeks**				
	SL2	NL2	LA2	LR2	SL4	NL4	LA4	LR4	SL6	NL6	LA6	RL6	FW6
NL2	0.71**												
LA2	0.72**	0.78**											
LR2	0.76**	0.67**	0.83**										
SL4	0.89**	0.72**	0.73**	0.76**									
NL4	0.57**	0.83**	0.67**	0.65**	0.71**								
LA4	0.65**	0.73**	0.83**	0.79**	0.71**	0.76**							
LR4	0.67**	0.69**	0.78**	0.90**	0.76**	0.77**	0.87**						
SL6	0.80**	0.69**	0.66**	0.71**	0.97**	0.71**	0.66**	0.73**					
NL6	0.50**	0.78**	0.59**	0.56**	0.61**	0.87**	0.70**	0.66**	0.62**				
LA6	0.66**	0.69**	0.75**	0.73**	0.75**	0.76**	0.84**	0.80**	0.74**	0.72**			
RL6	0.45**	0.54**	0.56**	0.69**	0.56**	0.65**	0.70**	0.79**	0.56**	0.62**	0.69**		
FW6	0.79**	0.66**	0.76**	0.78**	0.84**	0.65**	0.76**	0.80**	0.80**	0.58**	0.79**	0.62**	
DW6	0.56**	0.61**	0.72**	0.71**	0.63**	0.65**	0.74**	0.75**	0.56**	0.59**	0.69**	0.58**	0.78**

** - correlation was significant at P = 0.01 level; N = 135

SL - stem length (cm); NL - number of leaves; LA – leaf aspect; LR - level of rooting; RL – root length (cm); FW – fresh weight (g); DW – dry weight (g)

et al., 2012). Relating to PEG, a concentration of 4.8% allowed better discrimination among genotypes than 9.6% (Barra *et al.*, 2013; Gopal and Iwama, 2007). In several studies (Kosturkova *et al.*, 2008) drought stress was evaluated by using PEG in the following concentrations: 2, 4, 6, 8, 10 and 15% (w/v). Other researchers have used different amounts (128, 188 and 235 g/L^{-1}) of PEG6000 to obtain various drought levels (Bahrami *et al.*, 2012).

Drought leads to decreased tissue water content resulting in inhibited cell elongation (Taiz and Zeiger, 2006). Compared to other species, potato is sensitive to drought because of its shallow root system (Iwama and Yamaguchi, 2006). Drought

slows growth, induces stomatal closure and therefore reduces photosynthesis (Nemeth *et al.*, 2002).

Potato cultivars or clones which are able to maintain relatively high yields at high temperatures have been identified in field trials (Levy, 1984; Malik *et al.*, 1992, cited by Arvin, 2008). In the case of potato, water shortage during the tuberization period reduces yield more than in other development stages (Anithakumari *et al.*, 2011). The major effects of water stress on potato plant are decreases in leaf area and number of leaves, plant height, number of tubers, tuber growth, quality and yield, number of roots and biomass (Tourneux *et al.*, 2003; Schittenhelma *et al.*, 2006; Arvin and Donnelly, 2008; Hassanpanah, 2009).

The present findings suggested that line 'LP 11-1525/1' might be used as potential potato cultivar resistant at drought stress, but further studies and field trials are needed.

Conclusions

From all the studied potato genotypes, the line 'LP 11-1525/1' achieved good results for most of the growth parameters under study. Lines derived from true potato seeds behaved well, in some cases even exceeding the line derived from meristems. In addition, selection of drought tolerance individuals within populations derived from true potato seeds might be applied for further analyses.

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