IN VITRO RESPONSE TO DROUGHT TOLERANCE FOR DIFFERENT POTATO VARIETIES

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Abstract

Current study describes the response of artificial drought stress on six Romanian potato cultivars (Amicii, Astral, Christian, Gared, Kronstad, Nativ) at plantlets stage. Murashige and Skoog basal medium supplemented with different levels (0, 4.8, 9.6% w/v) of polyethylene glycol (PEG-6000) were used for in vitro drought simulation. The starting point consisted of microcuttings belonging to plantlets free of virus. Six weeks after minicuttings inoculation, they formed plantlets and were analyzed following parameters: number of leaves, internodes/plantlets and stem length, root length, fresh and dry weight stem, fresh and dry weight root. Results from experiments showed that plant growth decreased with increasing PEG concentrations. In addition, there were differences between the cultivars in terms of their response to drought. The experimental studies showed that PEG (6000) can be used as water stress agent under in vitro conditions and Kronstad and Christian cultivars present a tolerance to in vitro simulation. Gared cultivar was a sensitive one showing lower values for the parameters analyzed.

Key words: potato plantlets, drought, in vitro conditions polyethylene glycol

INTRODUCTION

Water deficit is a common stress in potato production, which leads to decrease in tuber quality and yield. Because of potato susceptibility to drought (Hassanpanah et al., 2008), preparing sufficient water is very important for increasing potato quality and quantity. By applying conventional and the unconventional techniques, is trying to create genotypes with high productivity and drought tolerance. To improve the yield of potato, from non-conventional techniques, the *in vitro* culture or tissue culture is used on a large scale, in order to produce in a very short time, a large number of healthy plants by micropropagation; thus aims to accelerate the production, because supplying of new varieties on the market and to maintain seed stock material free of disease for future multiplications. *In vitro* selection is an alternative approach for the development of lines with tolerance stress (Jayashankar et al., 2000; Ganesan and Jayabalan, 2006).

Environmental constraints such as drought or low temperatures can have adverse effects on plants and therefore can induce stress reactions at

the molecular and physiological level (Shinozaki, 2000), which often lead to a decreased productivity. Tissue culture is a useful tool in studying mechanisms of stress tolerance under *in vitro* conditions (Bajji et al., 2000).

In vitro culture techniques minimize environmental changes due to nutrient medium, under controlled conditions. 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 (Larher et al., 1993). Production may be adversely affected due to biotic and abiotic stress factors. Damage caused by these stresses is responsible for enormous economic losses worldwide.

Drought is actually a meteorological event which implies the absence of rainfall for a period of time, sufficient to cause a reduction of soil moisture, a water deficit and a reduction in water potential in the tissues of plants (Mitra, 2001). Drought is an environmental stress causing significant agricultural loss, especially in arid and semi-arid areas. Taking into account the importance of water stress on potato tuber development, this study was undertaken to evaluate the effect of polyethylene glycol on six potato cultivars against water stress in *in vitro* conditions.

MATERIAL AND METHOD

Microcuttings, that belonging to plantlets free of virus, were inoculated into test tubes containing Murashige & Skoog (MS) medium enriched with naphthyl acetic acid, 20 g / l sugar and agarized with 9g / l phyto-agar. The medium pH was reduced to 5.6-5.8. For all cultivars, drought was simulated by the addition of polyethylene glycol (molecular weight 6000) at concentrations of 4.8 % and 9.6 % (w/v) to the media; also, it was used a medium that did not contain PEG. The nutrient medium was sterilized by autoclaving for 20 minutes.

Test tubes with microcuttings were placed in the growth chamber, ensuring the light and temperature regime necessary for plantlets growing. After 6 weeks, measurements were made of the following parameters: number of leaves, internodes/plantlets and stem length, root length, fresh and dry weight stem, fresh and dry weight root. We analyzed the regenerative capacity and growth potential of plantlets. The results were studied by analysis of variance and significance of differences was determined by the method of multiple comparisons, respectively Duncan test (significance of differences was determined by the least significant difference (LSD) of testing at a 0.05 probability level).

The variants studied

Experience was made in Laboratory of Vegetal Tissue Culture, NIRDPSB Brasov, in 2014. By simulating water stress, we studied the influence of PEG on plantlets belonging to Romanian cultivars: Amicii, Astral, Christian, Gared, Kronstad, Nativ. Bifactorial experience, such as 6x2, contained the following factors, over 9 repetitions:

Experimental factor A: the cultivar, with **six graduations:**
-a_1- Amicii; -a_2- Astral; -a_3- Christian; -a_4- Gared; -a_5- Kronstad; - a_6- Nativ.

Experimental factor B – the polyethylene glycol concentration (%), with **three graduations:** -b_1- 0; -b_2- 4,8; -b_3- 9,6.

RESULTS AND DISCUSSIONS

*In vitro s*imulation of drought was performed in order to identify cultivars with high productivity and optimal tolerance to drought. It was studied the behaviour *in vitro* of potato micro cuttings, from Romanian cultivars created in Braşov, TârguSecuiesc, Miercurea Ciuc and Suceava.

In table 1 is presented the statistical analysis of cultivars. Kronstad cultivar is the most tolerant to water stress, showing for the most analyzed parameters, the highest values (leaf number: 9.074, internodes: 6.926, stem length: 11.28 cm, fresh stem weight: 178.80 g and dry stem weight: 47.71 g). Christian cultivar, also presented drought tolerance, recording for fresh root weight and dry root weight, the highest values (4.559 and 2.630 g); concerning the stem fresh weight, no significant difference was observed between Christian and Kronstad. The cultivar which had no tolerance to water stress was Gared (this present for 7 parameters the smallest values). Astral variety behaved like Gared variety regarding fresh stem weight (98.63 g), dry stem weight (14.68 g), dry root weight (1.341 g).

The difference between the mean values of analysis elements, under the influence of PEG (the nutrient media contained PEG with a concentration of 4.8 and 9.6%) and the ones studied in the absence of PEG, showed that simulation of water stress induced a decreasing for values of parameter analyzed. The presence of PEG in the multiplication medium had a negative effect for all elements studied. There was a proportional decreasing values for studied elements with an increasing of PEG concentrations (table 2).

Table 1

Behaviour of experimented varieties concerning growing and development elements under the influence of water stress

Cultivar	Leaves number	Internodes number	Stem length (cm)	Root length (cm)	Stem fresh weight (g)	Root fresh weight (g)	Stem dry weight (g)	Root dry weight (g)
Amicii	6.481 c	5.296 d	6.759 c	5.444 a	64.27 b	3.452 b	21.24 c	2.271 ab
Astral	7.630 b	5.926 c	6.074 d	6.685 b	98.63 b	3.596 b	14.68 d	1.341c
Christian	7.556 b	6.370 b	6.148 d	5.185 bc	110.40 ab	4.559 a	26.16 b	2.630 a
Gared	5.407 d	3.963 e	4.167 e	4.519 d	49.89 b	2.026 c	15.57 d	1.035 c
Kronstad	9.074 a	6.926 a	11.280 a	4.593 d	178.80 a	3.881 b	47.71 a	2.285 ab
Nativ	6.519 c	5.444 d	7.500 b	5.000 c	81.16 b	3.373 b	20.75 c	2.050 b

LSD=0.4408 LSD=0.4246 LSD=0.4577 cm LSD=0.4577 cm LSD=81.29 g LSD=0.516 g LSD=1.180 g LSD=0.4205 g

Table 2

Influence of PEG concentrations used in the nutrient medium on the parameters studied

Variants	PEG concentration (%)	Leaves number	Internodes number	Stem length (cm)	Root length (cm)	Stem fresh weight (g)	Root fresh weight (g)	Stem dry weight (g)	Root dry weight (g)
V_1	**0**	11.56 a	9.704 a	12.37 a	6.583 a	194.20 a	6.918 a	58.42 a	4.01 a
V_2	**4.8**	6.019 b	4.556 b	5.417 b	5.093 b	67.090 b	2.169 b	14.83 b	1.08 b
V_3	**9.6**	3.759 c	2.704 c	3.176 c	4.037 c	34.77 b	1.357 c	5.803 c	0.716 b

LSD=0.4181 LSD=0.3041 LSD=0.4434 LSD=0.3731 cm LSD=78.12 cm LSD=0.3731 g LSD=1.249 g LSD=0.4231 g

From interactions cultivar-PEG concentration can be observed an amplitude of significant differences for most parameters analyzed. When the plantlets had grown on the medium containing no PEG, Christian cultivar showed the best values for 4 parameters (number of leaves: 13.33, number of internodes: 11.67, root fresh weight: 10.280 g and the dry root: 6.244 g) of the 8 analyzed. At the same variety, there are significant differences in the induction of water stress using PEG (4.8% and 9.6%) for the items mentioned above. Also in the absence of PEG in nutrient media, Kronstad cultivar, showed the best values too, for three elements analyzed: stem length (15.89 cm), stem fresh weight (353.7 g), stem dry weight (87.59 g). In case of leaves number and stem fresh weight the values for plantlets (which have grown in a medium that did not contain PEG) belonging to Christian and Kronstad have not significantly differentiated.

Number of leaves was greatly affected with *in vitro* treatments of drought simulation, especially for the Amicii cultivar, which obtained a very low number of leaves, 2.67 when PEG concentration increased to 9.6%. This value was not significantly differentiated by the number of leaves

formed Christian varieties (2.89) and Gared (3.22) at the same level of PEG concentration (9.6%).

Table 3

Influence of cultivars studied and PEG concentrations regarding elements of growth and development

Cultivar	PEG conc (%)	Leaves number	Internodes number	Stem length (cm)	Root length (cm)	Stem fresh weight (g)	Root fresh weight (g)	Stem dry weight (g)	Root dry weight (g)
Amicii	**0**	11.78 c	10.22 b	15.060 a	7.333 b	156.0 bcd	8.478 b	49.47 c	6.022 a
	4.8	5.00 hi	3.78 f	3.278 jk	5.444 def	20.00 bcd	1.200 gh	10.90 g	0.49 ghi
	9.6	2.67 l	1.89 h	1.944 m	3.556 j	16.77 cd	0.678 h	3.333 ij	0.30 hi
Astral	**0**	12.33 bc	10.56 b	11.110 cd	8.778 a	170.6 bc	5.633 c	22.24 e	2.011 de
	4.8	7.00 f	4.78 e	4.270 hi	6.000 cd	77.61 bcd	4.033 ef	16.02 f	1.422 ef
	9.6	3.56 jk	2.48 gh	2.833 jkl	5.278 def	47.64 cd	1.122 gh	5.778 hi	0.589 fghi
Christian	**0**	13.44 a	11.67 a	10.61 d	6.444 c	226.5 ab	10.280 a	65.76 b	6.244 a
	4.8	6.33 fg	5.11 e	5.556 fg	4.778 fgh	60.01 cd	2.000 g	10.77 g	1.078 fgh
	9.6	2.89 kl	2.33 gh	2.278 lm	4.333 hi	44.58 cd	1.400 gh	1.967 j	0.567 ghi
Gared	**0**	9.44 d	7.22 d	7.556 e	4.778 fgh	121.1 bcd	4.311 de	40.73 d	2.272 cd
	4.8	3.56 jk	2.78 g	2.611 klm	4.444 ghi	19.67 cd	0.500 h	3.444 ij	2.233 i
	9.6	3.22 kl	1.89 h	2.333 lm	4.333 hi	8.93 d	1.267 gh	2.544 j	0.60 fghi
Kronstad	**0**	12.89 ab	10.44 b	15.89 a	5.667 de	353.7 a	5.189 cd	87.59 a	2.900 c
	4.8	8.44 e	6.44 d	11.89 c	5.111 efg	163.2 bcd	3.367 ef	10.40 d	2.033 de
	9.6	5.89 g	3.89 f	6.056 f	3.000 j	46.52 cd	3.089 f	15.44 f	1.922 de
Nativ	**0**	9.44 d	8.11 c	14.000 b	6.500 c	137.3 bcd	7.691 b	48.74 c	4.611 b
	4.8	5.78 gh	4.44 ef	4.889 gh	4.778 fgh	62.08 cd	1.911 g	7.756 h	1.222 efg
	9.6	4.33 ig	3.78 f	3.611 ij	3.722 ij	44.16 cd	0.589 h	5.744 hi	0.316 hi
		LSD=0.8310	LSD=0.8162	LSD=0.8803	LSD=0.7406	LSD=0.1554	LSD=1.010	LSD=2.479	LSD=0.840

Also, concerning the internodes number, increasing the PEG concentration to 9.6 % had a negative effect for Amicii and Gared cultivars, presenting the lowest values for this parameter (1.89 - had no significant difference). Kronstad cultivar showed a better growing of the plantlets (the length stem: 6.056 cm on a PEG concentration of 9.6%). Astral cultivar presented a less affected root length (5.278 cm) using 9.6% PEG in the

nutrient medium. We may remark that Kronstad and Astral presented tolerance to drought stress.When examining fresh root weight Amicii, Astral, Christian, Gared, Nativ cultivars, it were no significant differences for 9.6% PEG concentration. Plant dry weight was not significantly different for Amicii, Christian and Gared cultivars, being most affected with increasing of PEG concentration (9.6%). The parameter root dry weight did not differ significant for analyzed cultivars (to PEG concentration 9.6%) (table 3).

CONCLUSIONS

In recent years, considerable progress has been made regarding the varieties drought tolerant *in vitro* technique. The addition of PEG to the MS medium decreased the water potential of the media inducing water stress that adversely affected the plantlets growing and development. The potato cv. Kronstad showed better tolerance by PEG-induced water stress compared to Gared.

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