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RESEARCHES WITH REFERENCE AT POLYETHYLENE GLYCOL (PEG) OVER "IN VITRO" PLANTS GROWING

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Abstract: The MS (Murashige and Skoog 1962) medium was enriched with vitamins, with 20 gl-1 sucrose supplemented with four levels (0.000, 0.006, 0.012 and 0.018 M) of PEG. The pH of the media was adjusted to 5.6-5.8, and media solidified with 8 g/l agar. The media were poured in test tubes, to generate five treatments, three of which were single layer media, with three levels of PEG, respectively, and three were double-layer media with 0.006, 0.012 of PEG, respectively, in the lower layer medium and 0.000 M PEG in the upper layer medium. The varieties studied were Christian and Roclas.
Key words: PEG, plantlets, leaves, drought stress, nutrition medium

Introduction

Water deficit represents an ordinary stress on potato production, which leads on potato diminution of production and quality. Owing to potato sensibility to drought (Hassapanah and colab., 2008 a, quoted by Sakthivelu and colab., 2008), water is necessary on increasing the quality and production for potato. It is very necessary researching about resistance at drought of different varieties. Owing of temperature changes, water deficit, is a problem in more regions (Sakthivelu and colab., 2008).

Water deficit decreases number of leaves (Frensch, 1997, quoted by Sakthivelu and colab., 2008), leaves area, stem height, tubers number, production, diminishes sugar contain, induces tubers deteriorate and malformation.

Drought is one of the most common environmental stresses affecting plant growth and productivity (Boyer, 1982). Plant cell and tissue culture has been a useful tool to study stress tolerance mechanisms under *in vitro* conditions (Bajji and colab., 2000). Polyethylene glycol (PEG) is used on drought stress simulation for plants. PEG widely used to induce water stress, is a nonionic water polymer, which is not expected to penetrate into plant tissue rapidly (Abd El-Rahman M.F. A-Ansary (2007).

Plant tolerance at drought has a fundamental importance and may present a major subject of research. Potato is very sensitive at drought stress. Drought stress decreases gas changes, inhibits starch synthesis (Geigenberger and colab., 1999, quoted by Pertumbuhan Akar Dan Tajuk and Usman K.J. Suharjo, 2007). It is known that drought stress affects protein synthesis, photosynthesis, breath, inhibit plants development.

PEG presents general formula $HO(CH_2CH_20)_nH$, is known as polyethylene oxide (PEO) or poly oxiethylene .

Molecular formula is: $C_{2n+2}H_{4n+6}O_{n+2}$

In this experiment were studied the variants presented on next table:

Table 1

Studied variables

Var	Variety	Nutrition medium
1	Christian (a1)	b_1
2		b_2
3		b_3
4		b_4
5		b_5
6	Roclas (a2)	b_1
7		b_2
8		b_3
9		b_4
10		b_5
11	Variety average	b_1
12		b_2
13		b_3
14		b_4
15		b_5

From the table number 1, results the fact that the bifactorial experience **axb(3x5) with 15 variants, was realised, using next graduations:**

A factor – variety – with 3 graduations

a_1-Roclas

a_2-Christian

a_3- average of this varieties

The MS (Murashige and Skoog 1962) medium enriched with vitamins with 20 gl^{-1} sucrose supplemented with four levels (0.000, 0.006, 0.012 and 0.018 M) of PEG. The pH of the media was adjusted to 5.6-5.8, and media solidified with 8 g/l agar. The media were poured in test tubes, to generate five treatments, three of which were single layer media (20 ml per test tube), with three levels of PEG, respectively, and three were double-layer media with 0.006, 0.012 of PEG, respectively, in the lower layer medium (10 ml of per test tube) and 0.000 M PEG in the upper layer medium (10 ml per test tube). In test tubes with double layer media, the medium without PEG was poured when the lower layer medium with PEG had solidified. The tubes were closed with tinfoil foil, autoclaved and used for culturing. The test tubes were

taken out of the autoclave after the media had solidified to avoid any mixing of the medium of upper layer with that of the lower layer. Since the medium in the lower layer was heavier than that of the upper layer, there was little mixing of media of two layers.

B- factor: nutrition medium, with 5 graduations

b_1 – testifier medium - Murashige - Skoog, enriched with vitamins, 20 g/l sucrose, 5 g/l agar;

b_2- M_2- contains a single layer of PEG 0.006 M: Murashige - Skoog, enriched with vitamins, 20 g/l sucrose, 8 g/l agar, 0,006 M PEG

b_3 – M_3 contains double level: medium of lower layer was Murashige - Skoog, enriched with vitamins, 20 g/l sucrose, 8g/l agar, 0,006 M PEG and upper layer was poured after the lower layer medium and it was without PEG;

b_4- M_4- contains a single layer of PEG 0.012 M: Murashige - Skoog, enriched with vitamins, 20 g/l sucrose, 8 g/l agar,

b_5- contains a single layer of PEG 0.012 M: Murashige - Skoog, enriched with vitamins, 20 g/l sucrose, 8 g/l agar, and upper layer was poured after the lower layer medium with PEG was solidified

OBJECTIVE OF RESEARCH

The goal of this research was to define the possible reactions of plantlets at drought "in vitro", by determining the height of plantlets and number of leaves/plantlets.

Placing of experience

This experience was place on laboratory of NIRDPSB, Brasov. The experimented variants pursue the same stages, excepting the fact that medium Murashige-Skoog with 20g/l sucrose was supplemented with polyethylene glycol with different concentrations: 0M, 0.006 M and 0.012 M (molecular weight 6000). The pH of medium was adjusted at 5.6-5.8 before autoclaving.

MATERIALS AND METHOD

The research was effectuated for Romanian potato varieties **(*Solanum tuberosum* L.)** Christian and Roclas.

Other materials: medium Murashige-Skoog with 20 g/l sucrose, supplemented with polyethylene glycol with different concentration: 0M, 0.006M and 0.012 M, test tubes.

Observations and determinations:the effectuated determinations were refer to plantlets variation height and number of leaves, grown on modified media, comparative with medium MS, unsupplemented with polyethylene glycol.

RESULTS AND DISCUSSIONS

A factor: variety with 2 graduations

Table 2

The influence of variety and nutrition medium over plantlets height

Variant	Variety (a)	Nutrition medium (b)	Plantlet height (cm)	Difference towards the control (average)	Duncan test
V1	Christian	M_1	12.53	1.1	a
		Average **M_1 (Ct)**	**11.43**	-	a
V2		M_2	2.53	-8.9	d
V3		M_3	4.43	-7.0	b
V4		M_4	2.27	-9.16	e
V5		M_5	3.60	-7.83	c
V6	Roclas	M_1	10.33	-1.1	a
V7		M_2	2.27	-9.16	e
V8		M_3	4.27	-7.16	b
V9		M_4	1.53	-9.9	f
V10		M_5	3.43	-8.0	c

Limit differences (bifactorial):

LSD 5 % (a) =0.64 cm

LSD 5 % (b) =0.53 cm

LSD 5 % (axb) =0.87 cm

LSD 5 % (bxa) =0.75 cm

From table 2, we may observe that both varieties behave similar, regarding plantlets height. The greatest values of plantlets height were finding on first Medium, after that this are decreasing on second Medium, increase on thirst Medium, decrease on fourth Medium and increase on fifth Medium. The values registered by Christian variety are bigger than the values of Roclas variety. This are exceeded on two points, at second Medium and thirst Medium (are presented 3 values for each medium).

Table 3

Variety influence over number leaves/plantlets obtained "in vitro"

Variant	Variety	Nutrition medium (b)	Leaves number/plantlet	Difference towards control	Duncan test
1	Christian	M_1	15.27	0.93	a
2	Average (Ct)		**14.34**	**-**	**a**
3	Roclas		13.40	-0.94	a
4	Christian	M_2	3.53	-10.81	d
6	Roclas		3.33	-11.01	d
7	Christian	M_3	8.33	-6.01	a
9	Roclas		6.47	-7.87	b
10	Christian	M_4	2.27	-12.07	e
12	Roclas		2.47	-11.87	e
13	Christian	M_5	5.13	-9.21	c
15	Roclas		4.47	-9.87	c

LSD 5 % (a) =0.77 (leaves number)
LSD 5 % (b) =0.35 (leaves number)
LSD 5 % (axb) =0.84 (leaves number)
LSD 5 % (bxa) =0.50 (leaves number)

From table 3, we may establish that both varieties are behaved similarly, concerning the number of leaves/ plantlet. The biggest values of leaves number/plantlets are regarding on first medium. After that this are decrease on second medium, increase on third medium, decrease on forth medium and increase on fifth medium. The values registered by Christian variety are biggest than Roclas variety. Second medium and forth medium are the most unfavourable for forming and increasing the leaves number/plantlet.

Table 4

Medium influence over plantlets height "in vitro" obtained

Nutrition medium	Plantlets height (cm)	Relative height (%)	Difference towards the control (M1) (cm)	Significance	Duncan test	Classification variants
M1	11.13	100.0	-	-	a	1
M2	2.4	21.56	-8.73	000	c,d	4
M3	4.35	39.08	-16.74	000	b	2
M4	1.9	17.07	-9.23	000	d	5
M5	3.51	31.53	-7.62	000	b,c	3

LSD 5 % = 1.862
LSD 1% = 2.708
LSD 0.1% = 3.910

In this table is presented the effect of nutrition medium over plantlets height "in vitro" obtained. It was established that the bigger height (cm) was realized using medium M1, containing medium Murashige-Skoog, enriched with vitamins, 20 g/l sucrose, 8g/l agar. It is considered witness.

Adding PEG, in all 4 medium variants, the height of plantlets was reduced.

The smaller height of plantlets was on medium 4, which is composed of one layer of 0.012M PEG, Murashige-Skoog, enriched with vitamins, 20 g/l sucrose, 8 g/l agar, 0.012 M PEG. Small height was also using M2, on single layer 0.006 M PEG: Murashige-Skoog, enriched with vitamins, 20 g/l sucrose, 8 g/l agar, 0.006 M PEG. Results that by decreasing of PEG at 0.006M from 0.012 M, increase plantlets height with 0.5 cm, on medium 5, which consist on double layer, the medium of lower layer was Murashige-Skoog, enriched with vitamins 20g/l, 8g/l sucrose, 0,012 M PEG, and the upper layer was without PEG (this was poured after the first layer was solidified).

It is obviously that decreasing of PEG at 0.006 induces increasing of height, both at lack of double layer, and the presence of this. Using of PEG causes decreasing of plantlets height.

Table 5

The genotype influence over plantlets height "in vitro"obtained

Variety	Plantlets height (cm)	Relative height (%)	Difference (cm)	Significance	Duncan test	Classification variants
Christian	4,95	100,0	-	-	a	1
Roclas	4,36	88	0,59	-	a	2
Average /variety	4,66	94	0,29	-	a	-

LSD 5 % = 7,46 (cm) LSD 1 % = 37,37 (cm)

From this table of analyze variant, we may establish that Christian variety has biggest values of plantlets height, exceeding with 0.59 cm Roclas variety. The differences are not significant for DL5% and 1%. Results reading by Duncan test, show the fact that the differences are not significant for the two varieties (has a letter).

Table 6

Influence of nutrition medium and genotype over plantlets height "in vitro" obtained

Nutrition medium	Variety	Plantlets height (cm)	Relative height (%)	Difference towards the control (M1) (cm)	Significance
M1	Christian	12.53	100.0	-	-
	Roclas	10.33	82.44	-2.2	-
M2	Christian	2.53	100.0	-	-
	Roclas	2.27	89.72	-0.26	-
M3	Christian	4.43	100.0	-	-
	Roclas	4.27	96.4	-0.16	-
M4	Christian	2.27	100.0	-	-
	Roclas	1.53	67.4	-0.74	-
M5	Christian	3.60	100.0	-	-
	Roclas	3.43	95.2	-0.17	-

LSD 5 %=11.032 (cm) LSD 1% = 55.257 (cm)

From data presented on this table, is evident the influence of medium M1 over plantlets height.

Table7

Influence of nutrition medium over plantlets number "in vitro" obtained

Nutrition medium	Number of leaves/plantlets	Relative number (%)	Difference towards the control	Significance	Duncan test	Classification variants
M1	14.33	100.0	-	-	a	1
M2	3.43	23.9	-10.9	000	d	4
M3	7.4	5.16	-6.93	000	b	2
M4	2.37	1.65	-11.96	000	d	5
M5	4.8	3.35	-9.53	000	c	3

LSD 5 % = 3.586 (leaves number)
LSD 1% = 5.215 (leaves number)
LSD 0.1 % = 6. 929 (leaves number)

Discussing about the medium influence over leaves/plantlet growth, is establish that Medium M1 produce the biggest number of leaves/plantlet (14.33), toward the M2, M3, M4, M5 media which produce the smallest number of leaves/plantlet. The differences, statistic analyzed, are negative distinct significant.

Table 8

Variety influence over number leaves/plantlets obtained "in vitro"

Variety	Number of leaves/ plantlets	Relative number (%)	Difference towards the control	Significance	Duncan test	Classification variants
Christian	6.91	106.8	0.44	-	a	1
Average/cultivar	6.47	100.0	-	-	a	2
Roclas	6.03	93.2	0.44	-	a	3

LSD 5 % = 14.368 (leaves number) LSD 1 % = 71.959 (leaves number)

The Christian variety has biggest values of leaves number, exceeding with 0.88 cm Roclas variety and with 0.44 the average of this. The differences are not significant for DL 5% and 1%. The interpretation of results by Duncan Test mark the fact the differences are not significant for values achieved on leaves number of two varieties (with a letter).

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Table 9

The influence of nutrition medium and varieties over leaves number/plantlets "in vitro" obtain

Nutrition medium	Variety	Leaves number/ plantlets	Relative number (%)	Difference towards the control M1	Significance towards Christian variety for each variety	Difference towards Christian variety M1
M1	Christian	15.27	100.0	-	-	
	Roclas	13.40	87.8	-1.87	-	-1.87
M2	Christian	3.53	100.0	-	-	-11.74
	Roclas	3.33	94.3	-0.22	-	-11.94
M3	Christian	8.33	100.0	-	-	-6.94
	Roclas	6.47	77.7	-1.86	-	-8.8
M4	Christian	2.27	100.0	-	-	13.0
	Roclas	2.47	108.8	0.2	-	12.8
M5	Christian	5.13	100.0	-	-	10.14
	Roclas	4.47	87.13	-0.66	-	15.27

LSD 5 %=21.244 (leaves number) LSD 1% = 106.408 (leaves number)

From statistic analyze of medium influence over leaves on plantlets developing is obvious the positive influence of witness M1 medium over both varieties, which produce, on average 15.27 and respective 13.40 leaves/plantlets.

CONCLUSIONS

1. Using of different types of media, containing different concentration of PEG is influenced the increasing of plantlets. Using of different types of medium influences the plantlets height different.
2. Adding on nutrition medium of 0.006M and 0.012M, is feeling over plantlets height the influence of hydric stress.
3. The medium which present elevated concentration of PEG, has a negative influence of plantlets, imprinting of this a hydric stress and inhibition of growing.
4. Utilised media, containing different amounts of PEG, affect the forming of leaves number/ plantlets "in vitro" generated.
5. Adding on nutrition medium of 0.006M and 0.012 M, is feeling over leaves number, the influence of hydric stress.
6. The medium which presents elevated concentration of PEG, has a negative influence over plantlets leaves number, imprinting hydric stress and implicitly, diminution of leaves number /plantlet.
7. Using of 2 layers has a benefit effect towards the using of a single number of layer, and the effect of this is amplified by using at 0.006M.

REFERENCES:

Abd El-Rahman M.F. Al-Ansary, Aida A. Rizkalla and Awatef M. Badr-Elden, 2007. Micropropagation and Biochemical Genetic Markers detection for Drought and Salt Tolerance of Pear Rootstock. Australian Journal of Basic and Applied Sciences, 1(4): 625-636, 2007 ISSN 1991-8178;

Bajji, M., S. Lutts, J. M. Kinet, 2000. Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus culture issued from durum wheat (*Triticum durum*) cultivars differing in drought resistance. J. Plant Physiol., 156, 75–83;

Boyer J.S. 1982. Plant productivity and environment. Science 218: 443-448;

Pertumbuhan Akar Dan Tajuk and Usman K.J. Suharjo, 2007. Use of polyethylene glycol (PEG) 8000 for rapid screening of potato (solanum tuberosum l.) genotypes for water stress tolerance: III. Root and shoot growth. Jurnal Akta Agrosia Edisi Khusus No. 1 hlm 11-18, 2007;

Sakthivelu, G., Akitha, Devi, M.K., Giridhar, P., Rajasekaran, T., Ravishankaran, G.,A., Nedev, T., Kosturkova, G., *2008:* D**rought-induced alterations in growth, osmotic potential and "in vitro" regeneration of soybean cultivar,** *Gen. Appl. Plant physiology, 2008, special issue, 34 (1-2), 103-112.*