BEHAVIOUR OF DIFFERENT POTATO VARIETIES BY SIMULATING IN VITRO OF HYDRIC STRESS CONDITIONS

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Abstract

The water deficit, extreme temperatures and low atmospheric humidity lead to drought, which is one of the limiting factors affecting crop quality and quantity. *In vitro* selection may shorten considerably the time selection of desirable traits and completes selection in the field. *In vitro* tissue culture can be used to determine the drought tolerance of the various varieties assuming that there is a correlation between plant cells *in vitro* and *in vivo*. For determining the resistance to water stress, the research was conducted in the Laboratory of Vegetal Tissue Culture and experience consisted of two factors: variety and nutritive medium with different osmotic agents. With the increasing amount of sorbitol and PEG, water absorption becomes difficult for plantlets from nutrient medium and thus was simulate the effect of drought over microplants. With the increasing amount of sorbitol or PEG into the growth medium, the drought intensity was bigger. Observations were made for different parameters: the average number of leaves; the average number of internodes; the average height of plantlet (cm); the average root length (cm); the average weight of fresh plantlet (mg); the average fresh root weight (mg). This study was performed in order to determine the best osmotic agent put into the *in vitro* medium for simulate the water stress, as well as for for testing the particular genotypes genotypes on different culture media causing selection pressure of water stress.

Key words: water stress, *in vitro*, culture medium, PEG, sorbitol

Drought is one of the most important constraints for ecosystems productivity, in many regions around the world and water availability becomes more scarce for many agricultural areas. Among the factors influencing production can include insufficient rainfall, high levels of salts in the soil. Breeding varieties with drought tolerance is a challenge of great priority in the program of biotechnology. *In vitro* selection may shorten considerably time for selection of desirable traits and completes selection the field.

Drought can be defined in relation with rainfall deficit compared to potential evapotranspiration in a year, a specific season, or in relation with the exigencies of a particular culture at a certain moment. Also, drought may occurs in areas where annual and periodic rainfall are ,,normal" or more or less nearby to the multiannual average, but the water comes from rainfall rare with high intensities (mm / min) and do not permit its accumulation in the soil. This type of drought is more common in southern and eastern Romania with a tendency of extending in central and western country (Petcu, 2008).

Actions for a short period of stress induce reactions in plants of protection, adaptation (response) to respective conditions. In case of long action of stressogenic factors, reversible initial changes may get out of plant genome control and go into irreversible changes that lead to destruction of plant (Popovici A. *et al*, 2013).

Water deficit associated with temperatures over thermal threshold manifested in areas with climate temperate - continental leads several times to stagnating or even forced maturation (Morar, 1999).

There is some evidence that the roots are the primary sensors of water deficit in the soil, causing physiological and biochemical perturbations in stems; growth reduction can generally be interconnected with changes in plant nutrition.

Capacity of roots to pervade the soil depends on the power that roots can exert and may be associated with drought tolerance (Tardieu, 1994).

The effect of water stress on tuber formation depends on the stage of crop development. During stolonization phenophase, water stress is crucial. It reduces the number of tubers and total production,

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due to a decrease in stolons number (Usman, 2012).

Appearence of new approaches involving the use of high efficiency of several technologies (by applying several techniques: genetic, physiological, biochemical, molecular and biotechnology) offers hope for interesting innovations to maintain food security, poverty reduction and mitigating risk agriculture farmers in vulnerable environments (Obidiegwe *et al*, 2015).

In vitro selection may shorten considerably time for selection of desirable traits and selection completes in the field. For *in vitro* selection PEG, sucrose, mannitol and sorbitol were analyzed in several research papers, as agents of osmotic stress.

Taking into account the stress of drought on potato at NIRDPSB Brasov, Laboratory of Vegetal Cultures Tissue was conducted a study to explore tolerance / resistance to drought *in vitro* of five potato varieties.

With increasing amount of sorbitol and PEG, water absorption becomes difficult for plantlets from nutrient medium and thus simulates the effect of drought on microplants. With the higher amount of sorbitol or PEG into the growth medium, the drought intensity will be higher.

In vitro culture techniques minimizes external environmental changes due to nutrient medium defined and controlled conditions applying stress homogeneity.

MATERIAL AND METHOD

Microplants from the culture collection (belonging to five varieties) were multiplied to each internode and minicuttings were inoculated on Murashige-Skoog medium (1962) enriched with vitamins. After four weeks, the plants which were formed were multiplied to obtain nodal cuttings. Cuttings of these plants were used as explants for further multiplication *in vitro*. For rapid identification of genotype with resistance / tolerance to water stress was chosen two simulators of water stress. One of them was polyethylene glycol (PEG 6000) and the second was sorbitol (growth inhibitors). Both of them were separately added in medium using 4 concentrations.

Experimental conditions were that from specific growth chamber of plantlets provided in the working protocol; inoculation room of minicuttings is sterilized with laminar flow of sterile air; sterilization of the tubes is carried out in an oven at 180˚C, and the culture medium sterilized must be inserted into test tubes in an autoclave at 120˚C.

The results were examined by analysis of variance. Significance of differences was determined by method of multiple comparisons, respectively Duncan test, stating the significance of any differences obtained by executing all comparisons possible, with the significant difference corresponding remoteness found in classification variants compared. Experimental differences higher than 5% are considered significant (Săulescu, N. A, Săulescu, N. N., 1967).

Experience included the following factors:

Experimental factor A: variety, with 5 graduations**:**

-a_1- Ruxandra;

-a_2- Sarmis;

-a_3- Gared;

-a_4- Marvis;

-a_5- Rustic.

Experimental factor B **–** nutrient media used (with different simulators of water stress) with 3 graduations**:**

-b_1-control medium MS, which contain was no osmotic agent;

-b_2- MS medium, to which was added PEG;

-b_3- MS medium, to which was added sorbitol.

Test tubes with minicuttings varieties proposed for *in vitro* testing of water stress (***figure 1***) were placed in the growth chamber, ensuring light and temperature regime needed growth and development of plantlets; after 4 weeks measurements were made for analysis of the following parameters: number of leaves and internodes/plantlet and plantlets height, root length and root fresh plant weight (mg).

Figure 1 **Fresh inoculatated minicuttings on MS medium to which was added on simulators of water stress**

RESULTS AND DISCUSSIONS

Simulation drought *in vitro* was performed in order to identify the best varieties tolerant to drought. Observations revealed that was obtained a slowdown in regeneration cuttings.

By ample analysis of variety and nutritive medium (MS) with different osmotic agents on four concentrations (*table 1*), Duncan test indicated by multiple analysis, the different behavior for all variants tested.

Regarding the average number of leaves, the highest value was achieved by using control medium for Marvis variety (11 leaves) and by using sorbitol 1.5%, leaves obtained were on a

number of 11.25 (not differ statistically by control medium), but on maximum concentration of PEG 2% leaves were registered on a number of 10.5, which indicated that the variety has a strong tendency to resist on drought, by simulating the formation of a larger number of leaves. At the opposite pole was Sarmis variety, which for both inducers of water stress at concentrations of 2% number of leaves decreased dramatically (6 leaves). Water stress affected the absorption of nutrients from the nutrient medium, reduced photosynthesis process and had as effect decreasing the number of leaves.

The average number of internodes highlighted the Ruxandra variety on control medium, which recorded the highest number of internodes (6.75), but also was observed for Marvis variety tendency, to combat the effect o drought, which by application of sorbitol 1.5% in Murashige-Skoog nutrient medium, produced a greater number of internodes (6.50).

The average height of plantlet (cm) analyzed from the interaction of two factors - variety and nutrient medium with different concentrations of the two simulators of drought highlighted the use of control nutrient medium, with higher value for Sarmis variety of 13.625 cm (A), followed Marvis and Rustic varieties of 10.375 cm and 11.00 cm respectively.

In the case of water stress, plant root growth stops, water absorption can not be performed. Gared variety proved the root elongation, tendency to eliminate the effect of drought, produced by using sorbitol 1.5 and 1% (values of 10.750 cm and respectively 8.375 cm), exceeding even the medium option in which was not apply specific treatment for simulation drought (version control medium: 7.625 cm), indicating the combating drought. The same variety (Gared) by using PEG 1.5% for the induction of water stress, registered a high value for the average root length (7.125 cm), with no significant differences compared to root formed on control medium (7.625 cm). So, this variety resisted very well to water stress, by root elongation „ in search of water. "

The average weight of fresh plant, is contained in the general table of with interactions of variety and medium plus concentrations of drought simulators. The values were between 247.900 mg for Marvis variety (A) and 244.70 mg (A) for Ruxandra variety, both on control medium and 21.60 mg and 23.50 mg (values obtained for the same varieties Marvis and Ruxandra, but were used different osmotic agents, but with the same concentration (2%) PEG and sorbitol, indicating that although Marvis and Ruxandra varieties obtained greater weight on control medium, PEG and sorbitol, at maximum concentrations had inhibitory effect on vigor plantlets and drastically reduced their weight (ex. even 10.4 times for variety Ruxandra).

The last analyzed element, fresh root weight (mg), had values between 163.625 mg for Ruxandra variety (A) on control medium and 123.625 mg by application sorbitol 0.5% (the same variety). Forming a vigorous root system, well branched in nutrient medium provides an access to nutrients substances.

Is was observed a drastic decrease in root weight, making them very fragile, using PEG at maximum concentration with values between from 4.1 mg (W) for Sarmis variety, 5.925 mg (W) for Marvis variety and 6,275 mg (W) for Rustic variety. That indicated that Sarmis, Marvis, Rustic varieties perceived on addition of PEG 2% as the most stressful factor for the growth of root for the weight values provided by multiple analysis by Duncan's test from A and B, and reached W and described the best effect of water stress for plantlets.

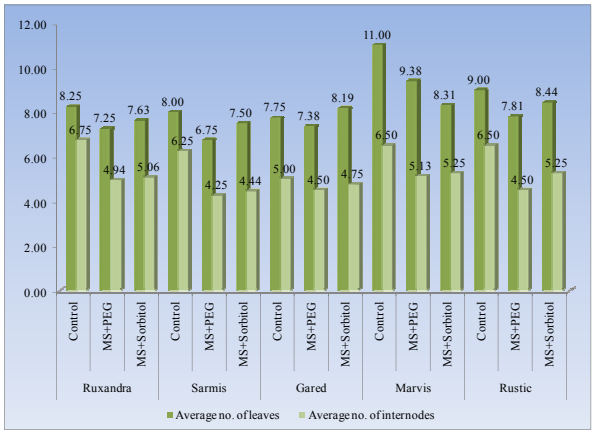

Figure 2 **Effects of water stress stimulators (PEG and sorbitol) on average number of leaves and internodes in comparison with control medium**

The process of forming leaves (*figure 2*) highlighted Marvis variety, both on control medium (with a number of 11 leaves), as well as media that had PEG (it were obtained 9.38 leaves) and sorbitol (8, 31 leaves).

Regarding the average number of internodes Rustic and Marvis varieties had the best resistance/tolerance by using sorbitol (forming 5.25 internodes / plant for both varieties).

Table 1

The combined influence of variety and inducer medium of *in vitro* water stress over the elements of growth and development

Variety	Inducer medium of *in vitro* water stress	The concentration of water stress simulator (%)	The average number of leaves	The average number of internodes	The average height of plantlet (cm)	The average length of root (cm)	The average weight of fresh plantlet (mg)	The average weight of fresh root (mg)
Ruxandra	Control medium (MS)	-	8,25 CDEFG	6,75 A	11,375 B	7,875 BCDE	244,700 A	163,625 A
	MS medium+PEG	0.5	7,50 FGHIJ	5,25 CDEFG	9,075 CD	7,875 BCDE	165,675 F	76,350 EF
		1	7,00 HIJKL	5,00 DEFGH	8,625 CDE	7,125 CDEFGH	153,200 G	83,300 D
		1.5	7,75 EFGHI	5,75 ABCDE	8,25 CDEF	4,250 PQRST	100,125 KL	41,850 NO
		2	6,75 IJKL	3,75 IJ	5,375 KLMNO	8,000 BCD	65,225 OP	52,650 HIJ
	MS medium +sorbitol	0.5	8,00 DEFGH	5,50 BCDEF	7,925 EFG	6,000 HLM	187,775 D	123,625 B
		1	8,75 CDE	5,50 BCDEF	6,800 HIJ	8,125 BC	113,95 I	100,300 C
		1.5	7,50 FGHIJ	5,0 DEFGH	5,000 KLM	8,000 BCD	81,100 M	79,600 DE
		2	6,25 KL	4,25 GHIJ	3,000 TU	6,750 EFGHIJ	23,525 W	33,800 PQ
Sarmis	Control medium (MS)	-	8,00 DEFGH	6,25 ABC	13,625 A	4,625 NOPQRS	218,650 B	72,525 F
	MS medium +PEG	0.5	7,25 GHIJK	5,00 DEFGH	9,125 C	4,500 OPQRST	111,775 I	53,575 HI
		1	7,25 GHIJK	4,50 FGHI	4,850 MNOPQR	5,000 MNOPQR	42,100 ST	47,225 KLM
		1.5	6,50 JKL	4,25 GHIJ	4,625 NOPQR	4,000 S	35,825 UV	12,275 V
		2	6,00 L	3,25 J	2,750 U	2,875 U	39,250 TU	4,100 W
	MS medium +sorbitol	0.5	8,00 DEFGH	4,75 EFGHI	8,750 CDE	5,625 JKLMNO	171,700 E	96,975 C
		1	8,25 CDEFGH	5,25 CDEFG	8,000 DEFG	5,675 JKLMNO	105,500 J	65,400 G
		1.5	7,75 EFGHI	4,50 FGHI	7,000 GHI	6,175 GHIJKLM	105,650 J	65,700 G
		2	6,00 L	3,25 J	3,300 STU	3,125 T	32,875 V	23,100 ST
Gared	Control medium (MS)	-	7,75 EFGHI	5,00 DEFGH	7,125 GHI	7,625 BCDE	153,075 G	71,450 F
	MS medium +PEG	0.5	7,50 FGHIJ	4,75 EFGHI	5,875 JKLM	5,300 KLMNOPQ	106,075 J	41,725 NO
		1	7,00 HIJKL	4,25 GHIJ	4,925 MNOPQR	4,500 OPQRS	50,025 R	37,525 OP
		1.5	7,50 FGHIJ	5,00 DEFGH	4,875 MNOPQR	7,125 CDEFGH	115,95 I	33,000 PQ
		2	7,50 FGHIJ	4,00 HIJ	4,375 OPQRS	6,000 HIJKLM	48,000 R	24,100 S
	MS medium +sorbitol	0.5	7,75 EFGHI	5,00 DEFGH	7,375 FGH	6,000 HIJKLM	124,850 H	50,850 IJK
		1	7,00 HIJKL	4,00 HIJ	5,625 KLMN	8,375 B	73,850 N	81,625 D
		1.5	7,75 EFGHI	4,25 GHIJ	5,375 KLMNO	10,750 A	95,250 L	66,350 G
		2	10,25 AB	5,75 ABCDE	4,000 KLMNOPQRST	7,375 BCDEF	61,100 PQ	47,770 IJK
Marvis	Control medium (MS)	-	11,00 A	6,50 AB	10,375 B	7,250 BCDEFG	247,900 A	84,125 D
	MS+PEG	0.5	9,00 CD	6,00 ABCD	7,375 FGH	7,625 BCDEF	153,875 G	64,100 G
		1	8,75 CDE	4,50 FGHI	4,950 KLM	4,000 RSTU	67,325 O	24,175 S
		1.5	9,25 BC	4,75 EFGHI	4,875 MNOPQR	4,625 NOPQRS	46,775 RS	13,125 V
		2	10,50 A	5,25 CDEFG	2,975 TU	3,700 STU	21,600 W	5,925 W
	MS+sorbitol	0.5	6,50 KL	5,25 CDEFG	7,250 FGH	6,250 FGHIJKL	124,950 H	82,375 D
		1	7,75 EFGHI	4,75 EFGHI	5,725 JKLMN	6,375 FGHIJK	104,350 JK	95,425 C
		1.5	11,25 A	6,50 AB	3,825 RSTU	5,750 JKLMN	57,600 Q	32,950 PQ
		2	7,75 EFGHI	4,50 FGHI	3,950 KLMNOPQRST	6,250 FGHIJKL	34,025 V	16,950 UV
Rustic	Control medium (MS)	-	9,00 CD	6,50 AB	11,000 B	7,750 BCDE	173,350 E	56,250 H
	MS medium +PEG	0.5	8,50 CDEF	5,50 BCDEF	7,175 FGH	5,125 LMNOPQR	183,600 D	45,425 LMN
		1	8,25 CDEFG	4,50 FGHI	6,375 HIJK	4,500 OPQRS	103,775 JK	29,625 QR
		1.5	8,25 CDEFG	4,75 EFGHI	4,950 MNOPQ	4,125 QRST	57,550 K	18,675 TU
		2	6,25 KL	3,25 J	2,750 U	3,075 T	64,800 OP	6,275 W
	MS medium +sorbitol	0.5	8,50 CDEF	5,25 CDEFG	7,125 GHI	6,875 DEFGHI	156,150 G	42,550 MN
		1	8,75 CDE	5,75 ABCDE	6,125 IJKL	5,625 JKLM	195,825 C	48,475 JKL
		1.5	7,75 EFGHI	5,00 DEFGH	5,250 LMNOP	5,375 KLMNOP	102,625 JK	27,200 RS
		2	8,75 CDE	5,00 DEFGH	4,175 PQRS	4,750 NOPQRS	45,900 RS	33,825 PQ
			LSD=1,246	LSD=1,105	LSD=1,116 cm	LSD=1,1186 cm	LSD=5,047 mg	LSD=4,994 mg

Means that are in the same column followed by the same letters are not significant, according to Duncan test ($p \leq 0.05$).

The medium that had in composition PEG which is a more appropriate simulator of water stress *in vitro*) determined for varieties studied lower values compared with sorbitol.

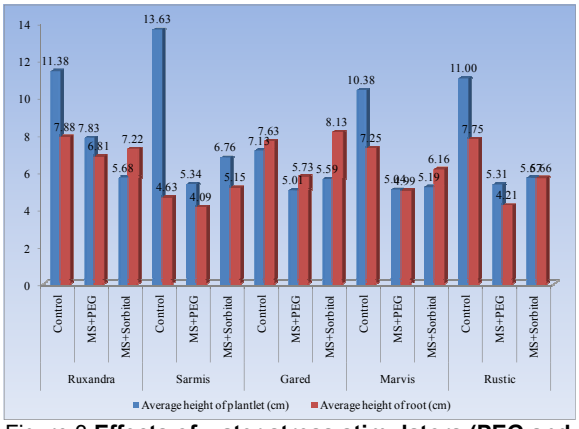

Figure 3 **Effects of water stress stimulators (PEG and sorbitol) on average height of plantlet and root length**

The smallest influence of water stress on plantlets height (*figure 3*) was observed for Ruxandra variety. By using PEG in culture medium, plantlets reached 7.83 cm. For Sarmis variety was observed a very high ratio between height plantlets developed on control medium and medium with drought simulators (MS medium: MS+PEG medium the ratio is 2.55 and MS medium: MS+sorbitol medium ratio is 2.02), so we can conclude low tolerance drought.

A good tolerance to water stress for root length, presented Gared and Ruxandra varieties when was applied sorbitol on growth medium. The root plantlets reached an average length of 8.13 and 7.22 cm. On addition of PEG, it appears that the same varieties Ruxandra and Gared, shows tolerance to water stress, but being a stronger agent, the levels were lower.

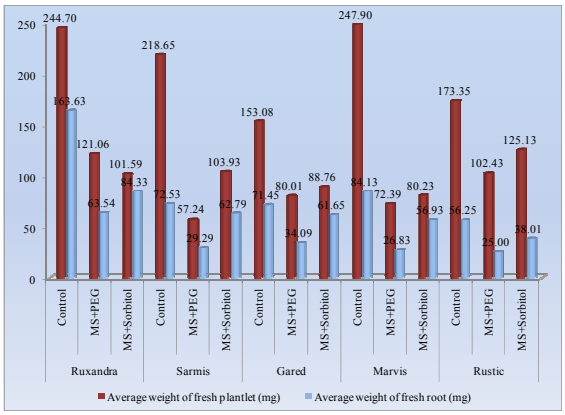

Figure 4 **Effects of water stress stimulators (PEG and sorbitol) on average weight of fresh plantlet and root**

Regarding the average weight of plantlet (*figure 4*) the highest values were observed for Ruxandra variety (121.06 cm) by applying PEG on growth medium and Rustic variety (125.13 cm), by using sorbitol. This varieties presented tolerance to drought.

CONCLUSIONS

Experimental results showed that the sensitivity to drought was not uniform for the analyzed varieties.

Medium in which was added PEG in different concentrations significantly reduced the average weight of fresh plantlet and fresh root compared with the control medium and sorbitol and significantly reduced the mean number of internodes, the average height of the plantlet, the average root length. This osmotic agent (PEG) is recommended for simulation *in vitro* of drought and to identify genotypes tolerant to drought.

In the process of leaves formation Marvis variety is highlighted, showing both for using of control medium, the greater average number of leaves (11) and on nutrient media which is applied to PEG (9.38 leaves) and sorbitol (8.31).

Regarding the average number of internodes, this decreases with the addition of PEG and sorbitol in culture medium.

The influence of variety and concentrations of osmotic agents marks Marvis variety, who under the influence of concentration of 1.5% of sorbitol has the highest value of the average number of leaves (11.25).

The smallest influence of water stress on plantlets height was observed for variety Ruxandra.

Gared and Ruxandra varieties showed good tolerance to water stress on root length, by applying sorbitol in nutritive medium, the root of plantlets reaching an average length of 8.13 and 7.22 cm.

By adding PEG (1.5%) Gared variety is characterized by achieving high values of the average weight of fresh plantlet (115.950 mg).

For average root weight is distinguished Gared variety that by using sorbitol 1% records a value of 81.625 mg (higher than obtained for control medium: 71.45 mg).

For the parameters analyzed, Marvis Gared, Ruxandra varieties, showed the best *in vitro* tolerance to water stress.

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