

INFLUENCE OF DIFFERENT *IN VITRO* SIMULATORS FOR HYDRIC STRESS FOR GROWTH AND DEVELOPMENT OF POTATO

Andreea TICAN¹, Mihaela CIOLOCA¹, Nicoleta CHIRU¹, Carmen BĂDĂRĂU^{1,2}

¹National Institute of Research and Development for Potato and Sugar Beet Brasov,
2 Fundaturii Street, Brasov, Romania

²Faculty of Food and Tourism, Transilvania University of Brasov,
148 Castelului Street, Brasov Romania

Corresponding author email: tican_andreea@yahoo.com

Abstract

Necessity of finding genotypes adapted to drought has become urgent due to the effect of this type of stress on potato production. The most important phase, indispensable for improving drought tolerance is to identify genotypes tolerant and sensitive to drought. In this study, to induce in vitro water stress were used polyethylene glycol and sorbitol that was comparable with the basic medium MS, considered control. Determinations were performed 4 weeks after inoculation of mini cuttings belonging to five varieties of plantlets (Ruxandra, Sarmis, Gared, Marvis, Rustic) and the parameters analyzed were next: number of leaves, number of internodes, height of plantlets, root length, weight of fresh plantlet, weight of fresh root. Medium in which was added PEG with different concentrations significantly reduced the average weight of fresh plantlet and root compared with the control medium and medium with sorbitol and significantly reduced the mean number of internodes, the average height of the plantlet, the average root length. This osmotic agent (PEG) can be recommended for in vitro simulation of drought to identify tolerant genotypes to hydric stress.

Key words: hydric stress, in vitro, osmotic agent, potato.

INTRODUCTION

Water deficit, extreme temperatures and low atmospheric humidity lead to drought, which is one of the limiting factors affecting crop quality. The amplitude of the effects of drought on potato production depends on phenological calendar, duration and severity of stress. Two critical periods: sprouting and tuberization affect in final tubers production. Potato crop is often considered sensitive to drought and production during successive episodes of drought may be compromised. Necessity to identify genotypes adapted to drought has become urgent because of the effect this type of stress on the growth of potato and production of this crop. Particularly important for production of potato tubers is the precipitation amount during the growing season and the distribution on the vegetative stages. It is estimated that during the growing season are required 250-400 mm precipitations (Bîlteanu, 2001).

The ability of roots to penetrate the soil depends on the power that can exert roots and may be associated with drought tolerance (Tardieu, 1994). If plants cannot take up water

from the soil needed to compensate for the lost through perspiration, install wilting phenomenon, a consequence of the drought effects (Ianoși, 2002).

Drought reduces the growth of roots, at the time of sprouting. Drought installed after plant sprouting inhibits the stolons development, thereby reducing the number of tubers. These processes are irreversible, even though soil moisture subsequently recovers. Capacity of roots to penetrate into the soil depends on the power that roots can exert and may be associated with drought tolerance.

In vitro tissue culture allowed a deeper understanding of the physiology and biochemistry of plants grown under unfavorable environmental (Benderradji L. and colab., 2012).

The most widely used method for the selection of genotypes tolerant to abiotic stress is the *in vitro* selection pressure technique. This is based on the *in vitro* culture of plant cells, tissues or organs on a medium supplemented with selective agents, allowing selecting and regenerating plants with desirable characteristics (Pérez-Clemente and Gómez-Cadenas, 2012).

Polyethylene glycol (PEG), sucrose, mannitol or sorbitol have been used by several workers as osmotic stress agents for *in vitro* selection.

In vitro simulation drought was made to identify varieties with optimum tolerance at drought. Observations showed that was obtained a slowing of regeneration cuttings. Sensitivity to drought was not uniform for varieties analyzed. For *in vitro* selection PEG, sucrose, mannitol and sorbitol were analyzed in several research papers, as agents of osmotic stress. Sorbitol is a sugar alcohol hexahydrate with osmotic effect. Widely water stress in vitro simulation is used polyethylene glycol. Culture media which contain PEG, imitate dry soil, rather than the culture media which have low molecular weight compounds. With the increasing amount of sorbitol and PEG, water absorption becomes difficult for plantlets from nutrient medium and thus is simulate the effect of drought. *In vitro* culture technique minimizes external environmental variations due to nutrient medium defined and controlled conditions and homogeneity of stress applied.

MATERIALS AND METHODS

In Laborator of Vegetal Tissue Culture, of NIRDPSB Brasov (2015) was made a study for identify the adequate agente for induce water stress. Microplantlets from the culture collection were multiplied to each internode and cuttings and were inoculated on Murashige-Skoog medium. Plantlets which were developed were multiplied to obtain nodal cuttings. Cuttings of these plantlets were used as explants, for further multiplication *in vitro*. As a basic medium was used Murashige-Skoog medium, naphthylacetic acid, sucrose, agar (this was considered control medium). Both PEG and sorbitol were added in the culture medium in 4 concentrations (0.5%; 1.0%; 1.5%; 2.0%). Test tubes with mini cuttings of varieties proposed for *in vitro* water stress test, were placed in the growth chamber by ensuring light and temperature regime required for growth and development of plantlets. After 4 weeks determinations were made for analysis of the following parameters, for a part of plantlets of these varieties: number of leaves and internodes / plantlet and plantlet height, root length, fresh weight of plantlet and root

(figures 1, 2, 3, 4, 5). The results were processed by analysis of variance and the significance of differences was determined using the method of multiple comparisons, respectively Duncan test. Experimental differences higher than 5% are considered significant (Săulescu and Săulescu, 1967).

For determine the effect of water stress on developing plantlets the study consisted in an bifactorial experience of two factors (5 x 3), 4 repetitions, including the following factors:

Experimental factor A: variety, with 5 graduations:

- a₁- Ruxandra;
- a₂- Sarmis;
- a₃- Gared;
- a₄- Marvis;
- a₅- Rustic.

Experimental factor B – nutrient media used with 3 graduations:

- b₁-control medium MS, to which was made no addition of osmotic agent;
- b₂- MS medium, to which was added PEG;
- b₃- MS medium, to which was added sorbitol.

RESULTS AND DISCUSSIONS

Treatments performed with PEG significantly reduced the average weight of plantlet and of root and compared to sorbitol and nutrient medium, considered control (MS) and significantly reduced the average number of internodes, the height of plantlet, root length. From Table 1 it is noted that PEG and sorbitol have no negative influence on the average number of leaves, but PEG reduces the average number of internodes compared to control medium.

For the first element in the study, Duncan test analysis indicates a proximity of values, with no significant differences between the number of leaves formed on MS medium (8.8 leaves) and on medium that contained sorbitol 1% (8.4 leaves), so compared with PEG, sorbitol has a less stressful effect on plantlets, there is a stronger competition in formation of plant leaves depending on the concentration (Table 2). PEG on maximum concentration of 2% had as result hydric stres for leaves formatic, these are in lowest number (7.40).



Fig. 1. Effects of water stress simulators (PEG and sorbitol) on growth and development of plantlets and roots for Ruxandra variety

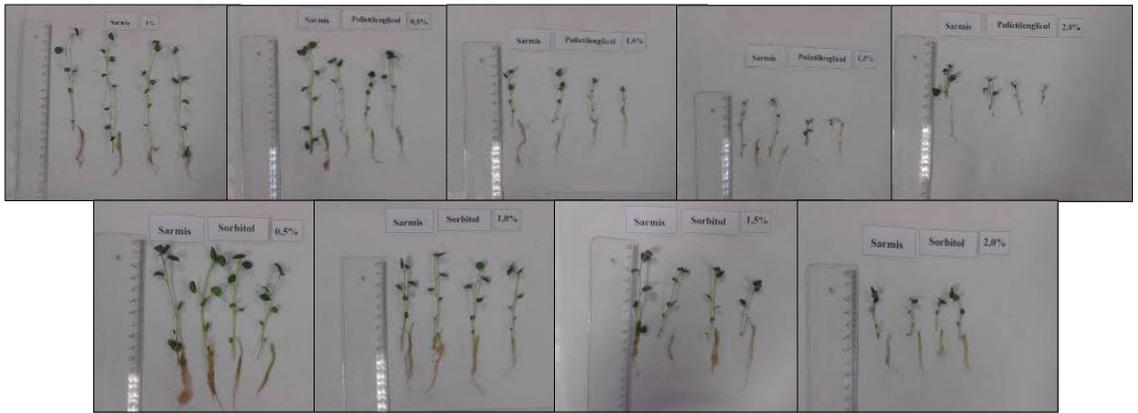


Fig. 2. Effects of water stress simulators (PEG and sorbitol) on growth and development of plantlets and roots for Sarmis variety



Fig. 3. Effects of water stress simulators (PEG and sorbitol) on growth and development of plantlets and roots for Gared variety



Fig. 4. Effects of water stress simulators (PEG and sorbitol) on growth and development of plantlets and roots for Marvis variety

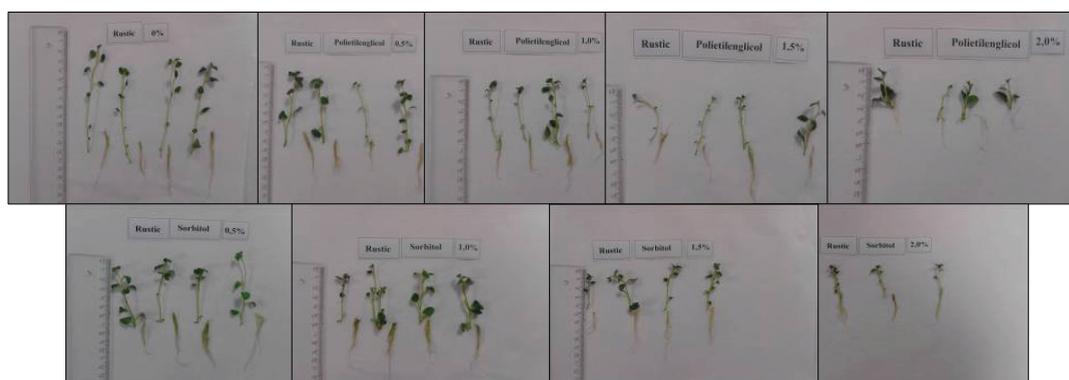


Fig. 5. Effects of water stress simulators (PEG and sorbitol) on growth and development of plantlets and roots for Rustic variety

The average number of internodes / plant indicates the effect of hydric stress attenuation *in vitro* is root length (cm). Concentrations of 1 and 1.5% for sorbitol substance does not describe a relevant effect of water stress (there are no significant differences) with values close to this value for nutrient medium on which sorbitol was used at concentrations 0.5; 1; 1.5% (BC). Sorbitol 2% is so drastic as 1 and 1.5% PEG (Duncan test D and CD) for inducing *in vitro* drought.

The average height of plantlets (cm) is similarly influenced by the first concentration of two chemicals of water stress accelerators (7.725 and 7.685 respectively) (B), while the last two concentrations studied, indicating a similar effect, but severely on growth *in vitro* plantlets (5.515 cm, with decreasing at 3.645 cm for PEG and respectively 5.29 cm, with decreasing to 3.685 cm by application of sorbitol).

A very decisive indicator of drought simulation *in vitro* is root length (cm). Concentrations of 1 and 1.5% for sorbitol substance does not describe a relevant effect of water stress (there are no significant differences) with values close to this value for nutrient medium on which sorbitol was used at concentrations 0.5; 1; 1.5% (BC). Sorbitol 2% is so drastic as 1 and 1.5% PEG (Duncan test D and CD) for inducing *in vitro* drought.

The concentration of 0.5% sorbitol is less conclusive, because should be expected to induce less attenuating stress for increasing root length of microplant, but this concentration results in the formation of a roots with an average length less (6.15 cm), but with a lower average weight close to that obtained using the control medium.

For concentration of 0.5% sorbitol, it would be expected to result in a less pronounced stress on the plant, on the increase in root length, but in fact lead shortening roots, but with a value of average root weight (79,275mg) (B), close to

the value obtained by using of control medium (89,595mg) (A).

PEG-induced very well drought at concentrations of 1.5 and 2%; produces very little roots with weight of 18.610 mg 23.785 mg (Duncan test G, H).

From Table 3 Marvis variety combat drought effect by forming a large number of leaves (with an average of 9.083 leaves). This is followed by Rustic variety (8.222 leaf / plantlet). The average number of internodes grouped in a closely way Marvis, Ruxandra and Rustic varieties (A), followed by Gared and Sarmis varieties (B). For plant height significantly detaches Ruxandra variety which registered a value of 7.269 cm (A), followed by Sarmis variety, which registered 6.892 cm (B). The root has a higher resistance to drought than the plant. It is noted in this sense Ruxandra and Gared varieties, with high and close values (7.111 cm; 7.006 cm) (A), followed by Marvis variety (5.758 cm). On the opposite side the

Sarmis variety had a tendency of slow root growth (only 4.6222 cm). For fresh plantlet weight (mg) is distinguished Ruxandra variety (126.142 mg), followed by Rustic variety (120.397 mg).

Gared variety, which in terms of microplants size is located on last place (with an average height of 5.506 cm), also recorded an average low weight of microplant 92.019 mg (situated on the last place). Regarding the average weight of fresh root (mg) detaches Ruxandra variety, with a very high value of 83.9 mg (A), followed by Gared (50.489 mg) and Sarmis variety (48,986). Regarding to this variety from previous analyzes, it appears that shows a tolerance to hydric stress, by producing microplants with a high height (6.892 cm) and with a high number of internodes (4.556). Rustic variety take last place, form roots with an average length small (5.244 cm) and a low weight (34.256 mg) indicating an inability of variety to fight with *in vitro* hydric stress.

Table 1 - Simulators osmotic stress influence compared to the control medium for the elements of growth and development for microplants

The treatment made	Average number of leaves	Average number of internodes	The average height of plantlets (cm)	The average length of root (cm)	The average weight of fresh plantlet (mg)	The average root weight (mg)
Control medium (MS)	8.800 A	6.200 A	10.70 A	7.025 A	207.535 A	89.595 A
MS+PEG	7.713 A	4.663 B	5.708 B	5.166 B	86.626 C	35.750 C
MS+sorbitol	8.013 A	4.950 AB	5.779 B	6.711 AB	99.928 B	60.750 B

Media that we are in the same column followed by the same letters are not significant, according to Duncan test

LSD=1.246 LSD=1.416 LSD=1.812 cm LSD=1.252 cm LSD=9.386 mg LSD=4.865 mg

Table 2 - Influence of osmotic stress concentrations simulators on the elements of growth and development of microplants

The treatment made	Hydric stress simulator concentration (%)	Average number of leaves	Average number of internodes	The average height of plantlets (cm)	The average length of root (cm)	The average weight of fresh plantlet (mg)	The average root weight (mg)
Control medium (MS)	-	8.80 A	6.20 A	10.70 A	7.030 A	207.535 A	89.595 A
MS+PEG	0.5	7.95 CD	5.30 B	7.725 B	6.085 C	144.200 C	56.235 C
	1	7.65 DE	4.55 D	5.945 D	5.025 D	83.285 F	44.370 E
	1.5	7.85 CD	4.90 CD	5.515 E	4.825 D	71.245 G	23.785 G
	2	7.40 E	3.90 E	3.645 F	4.730 D	47.775 H	18.610 H
MS+sorbitol	0.5	7.75 CDE	5.15 BC	7.685 B	6.150 C	153.085 B	79.275 B
	1	8.10 BC	5.05 BC	6.455 C	6.835 A	118.695 D	78.275 B
	1.5	8.40 AB	5.05 BC	5.29 E	7.210 A	88.445 E	54.360 D
	2	7.80 CDE	4.55 D	3.685 F	6.650 B	39.485 I	31.090 F

Media that we are in the same column followed by the same letters are not significant, according to Duncan test

LSD=0.4218 LSD=0.3740 LSD=0.3778 cm LSD=0.4015 cm LSD=12.277mg LSD=1.783mg

Table 3 - Behaviour the varieties tested under the influence of treatments made to induce water stress

Variety	Average number of leaves	Average number of internodes	The average height of plantlets (cm)	The average length of root (cm)	The average weight of fresh plantlet (mg)	The average root weight (mg)
Ruxandra	7.528 CD	5.194 A	7.269 A	7.111 A	126.142 A	83.9 A
Sarmis	7.222 D	4.556 B	6.892 B	4.6222 D	95.925 C	48.986 C
Gared	7.778 C	4.667 B	5.506 D	7.006 A	92.019 D	50.489 B
Marvis	9.083 A	5.333 A	5.700 D	5.758 B	95.378 C	46.572 D
Rustic	8.222 B	5.056 A	6.103 C	5.244 C	120.397 B	34.256 E

Media that we are in the same column followed by the same letters are not significant, according to Duncan test

LSD=0.3266 LSD=0.3183 LSD=0.3229 cm LSD=0.3638 cm LSD=1.9722 mg LSD=1.458 mg

CONCLUSIONS

Medium with different concentrations in which was additional PEG significantly reduced the weight of fresh plantlets and fresh root as compared with the control medium and sorbitol and also significantly reduced the number of internodes, the height of the plantlet, the root length. The smallest influence of water stress on plantlet height was observed for Ruxandra variety. By using PEG in culture medium, potato plantlets reach 7.83 cm. The roots are the primary sensors of water deficit. Gared and Ruxandra varieties showed a good tolerance to water stress for root length, by applying sorbitol on nutritive medium, plantlet root reaching an average length of 8.13 and 7.22 cm. By addition of PEG, it appears that the same varieties Ruxandra and Gared, shows tolerance to water stress, but PEG being an agent stronger the levels were lower.

REFERENCES

- Benderradji L., Brini F., Kellou K., Ykhelf N., Djekoun A., Masmoudi K., Bouzerour. H., 2012. Callus induction, proliferation, and plantlets regeneration of two bread wheat (*Triticum Aestivum* L.) genotypes under saline and heat stress conditions. ISRN Agronomy, Article ID 367851. Bobr. Plant Cell Tissue and Organ Culture; 102 387–395.
- Bîlteanu Gheorghe, 2001. Fitotehnie, Volumul 2, Editura Ceres, București.
- Ianoși, S. I., 2002. Bazele cultivării cartofului pentru consum, Editura Phoenix, Brașov.
- Pérez-Clemente R. M and Gómez-Cadenas A., 2012. *In vitro* Tissue Culture, a Tool for the Study and Breeding of Plants Subjected to Abiotic Stress Conditions. In: Annarita Leva and Laura M. R. Rinaldi(Eds.) Agricultural and Biological Sciences ."Recent Advances in Plant *in vitro* Culture", Chapter 5, 91-108.
- Săulescu, N.A, Săulescu, N.N., 1967. Cîmpul de experiență, Întreprinderea poligrafică Sibiu.
- Tardieu F., 1994. Growth and functioning of roots and of root systems subject to compaction towards a system with multiple signalling. Soil and Tillage Research 30, 217–43.

FOOD BIOTECHNOLOGY

