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THE GROWTH AND DEVELOPMENT OF "IN VITRO"' POTATO PLANTLETS BELONGING TO DIFFERENT ROMANIAN VARIETIES UNDER THE INFLUENCE OF WATER STRESS CAUSED BY MANNITOL

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ABSTRACT: This paper had as principal objective identification of potato varieties with tolerance to water stress by using "in vitro" tissue culture and mannitol, in different concentration, as an agent to induce water stress simulation. Mannitol added to the culture nutritive medium had five levels (0.36, 0.73, 1.11, 1.46, 1.92%). The starting point was represented by plantlets culture collection, belonging to Romanian varieties: Rustic, Tampa, Ruxandra, Robusta, Gared. Plantlets were multiplied between two internodes to obtain microcuttings (in sterile condition), which were inoculated on medium established by Murashighe & Skoog (1962), enriched with vitamins to which is added sucrose, agar, growth regulators (for control media). There were other variants of media, to which was added at each mannitol concentrations. After 4 weeks was made the determination of values for different morphological parameters (plantlets height, number of leaves / plantlet and fresh weight of plantlets) - which were statistically analyzed by Duncan test. Water stress affected plantlets length, number of leaves and plantlets fresh weight. The varieties showed different responses. Plantlets height decreased dramatically by exposure to high concentrations of mannitol. This is due to a possible mechanism of water stress intolerance. "In vitro" simulation of water stress for Romanian potato varieties, under the influence of a high osmotic potential, may provide an efficient system of differentiation for varieties tolerant to drought, under field conditions.

Keywords: potato, water stress, mannitol, height of plantlets, weight of fresh plantlets.

INTRODUCTION

Drought involves decreasing soil moisture for a period of time, due to deficiency of water. Resistance to water stress by conventional methods takes time, is costly and labor-intensive. "In vitro" culture makes it possible to save required time to identify genotypes tolerant to water stress. Selected variants "in vitro" should be tested under real field to confirm characteristics of "in vitro" selected genotypes. Development of an "in vitro" selection technology offers the possibility for improving the tolerance to water stress.

Water stress is a major factor, which affecting production significantly. Evaluation of tolerance to water stress is an essential step in amelioration. Plants that grow in natural habitats are exposed in environment to several types of stress, including abiotic and biotic factors. Adapting way and responding to these types of stress are extremely complex, determining changes at the molecular level, cellular and physiological processes. Abiotic stress factors such as heat, drought and salinity have a significant impact on potato (*Solanum tuberosum* L.), affecting production, quality of tubers and market price (Wang Pruski şi Schofield, 2012).

Osmotic stress simulation "in vitro" using tissue culture can minimize environmental changes, as a result of multiplication of the defined nutrient media under controlled conditions (Gorji, 2011).

Simulated stress "in vitro" offer a support for research on drought tolerance and selection of resistant genotypes.

Mannitol is widely used as an osmotic agent for characterization and selection of genotypes with tolerance to "in vitro" water stress (Hassanein, 2010).

Snow (1905) was among the first researchers which observed in his studies, the inhibitory effect of mannitol in plant growth. As an answer to the action of mannitol were made many researches over time with this osmotic agent (Wright şi Jordan, 1970; Erb şi colab., 1988; Karunaratne şi colab., 1991; Hsissou şi Bouharmont, 1994; Hassanein, 2010). Alternative strategies, especially those that are based on micropropagation offer faster ways of identification of varieties tolerant to water stress.

The objective of this study is assessment the tolerance of potato varieties to water deficiency, by "in vitro" simulation, to attempt their cultivation, in regions under the influence thermohidric stress caused by climate change.

MATERIAL AND METHODS

In this paper, Laboratory of research for vegetal tissues culture, NIRDPSB Brasov, Romania, in 2014, carried out an "in vitro" study to determine the tolerance to water stress, under controlled conditions. Was studied behavior of potato varieties plantlets Rustic, Tampa, Ruxandra, Robusta and Gared. The common characteristic is that the first four varieties it is included in semilate varieties and Gared variety is a late variety.

Initially, in the first stage, plantlets from the culture collection were multiplicated at each internode; microcuttings were inoculated on a medium for multiplication MS (1962), enriched with vitamins,

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supplemented with agar 9 g/l, sucrose 20 g/l, naftil acetic acid (NAA) 0,05 mg. The pH of the medium was adjusted to 5.6-5.8, and then the medium was autoclaved at 120^0C for 20 minutes. Cultures were maintained at 20^0C in the growth chamber. In the second stage, after 4 weeks, developed plantlets were multiplied and microcuttings were placed on MS medium enriched with vitamins and other components (described above) with different concentrations of mannitol (0,36, 0,73, 1,09, 1,46, 1,92 %), to achieve an exposure to an artificial drought. It was determined for each variety the average values for plantlets length, number of leaves / plantlet and fresh weight of plantlets. These decreased with increasing mannitol concentrations.

Mannitol was used to exert a water deficiency in the nutrient medium necessary for growth and development of the plantlet with the purpose of cause changes of growth, similar to those produced by the drying of the soil.

Bifactorial experience, by type 6*5, with a number of 30 variations, in three repetitions studied, the following degrees presented:

The experimental factor a - mannitol concentration (%), with six graduations:

- a_1: 0.00;
- a_2: 0.36;
- a_3: 0.73;
- a_4: 1.09;
- a_5: 1.46;
- a_6: 1.92.

The experimental factor b – variety, with five graduations:

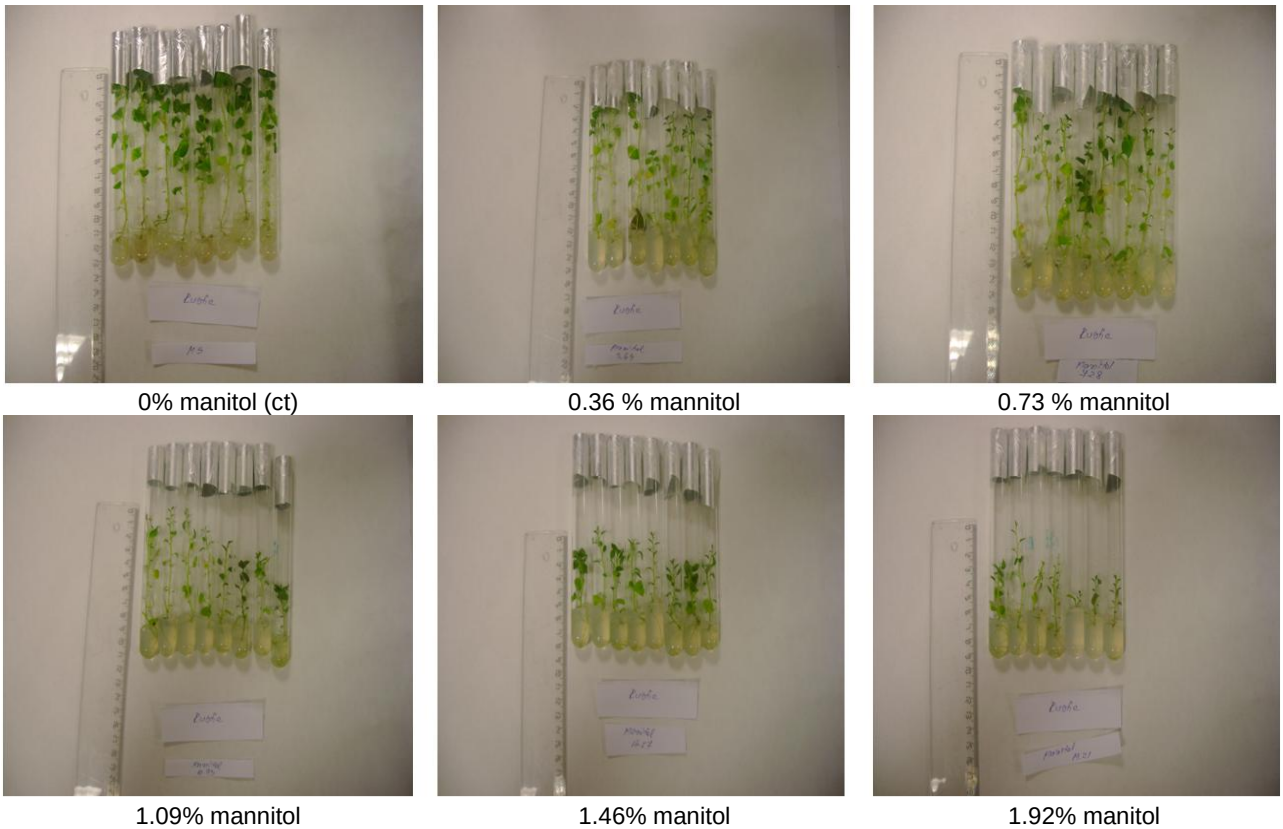
- b_1: Rustic
- b_2: Tâmpa
- b_3: Ruxandra
- b_4: Robusta
- b_5: Gared

The results were processed by analysis of variance and the significance of differences was determined by the method of multiple comparisons, Duncan test respectively.

RESULTS AND DISCUSSION

The data were statistically analyzed, using the MSTAT-C program and to determine significant values mean values were compared with LSD. The length is significantly lower with each increase in the concentration of mannitol, in medium of drought simulation.

In general, the recorded values of the investigated parameters decrease with increasing concentration of mannitol in the nutrient medium. By using of high concentrations of mannitol this leads to a low parameter values in the study. It can be seen in figures 1-5 decreases in plantlets height and number of leaves, with increasing concentrations of mannitol.



0% manitol (ct) | 0.36 % mannitol | 0.73 % mannitol

1.09% mannitol | 1.46% manitol | 1.92% manitol

Fig.1. Plantlets developed under the influence of mannitol concentrations for Rustic variety

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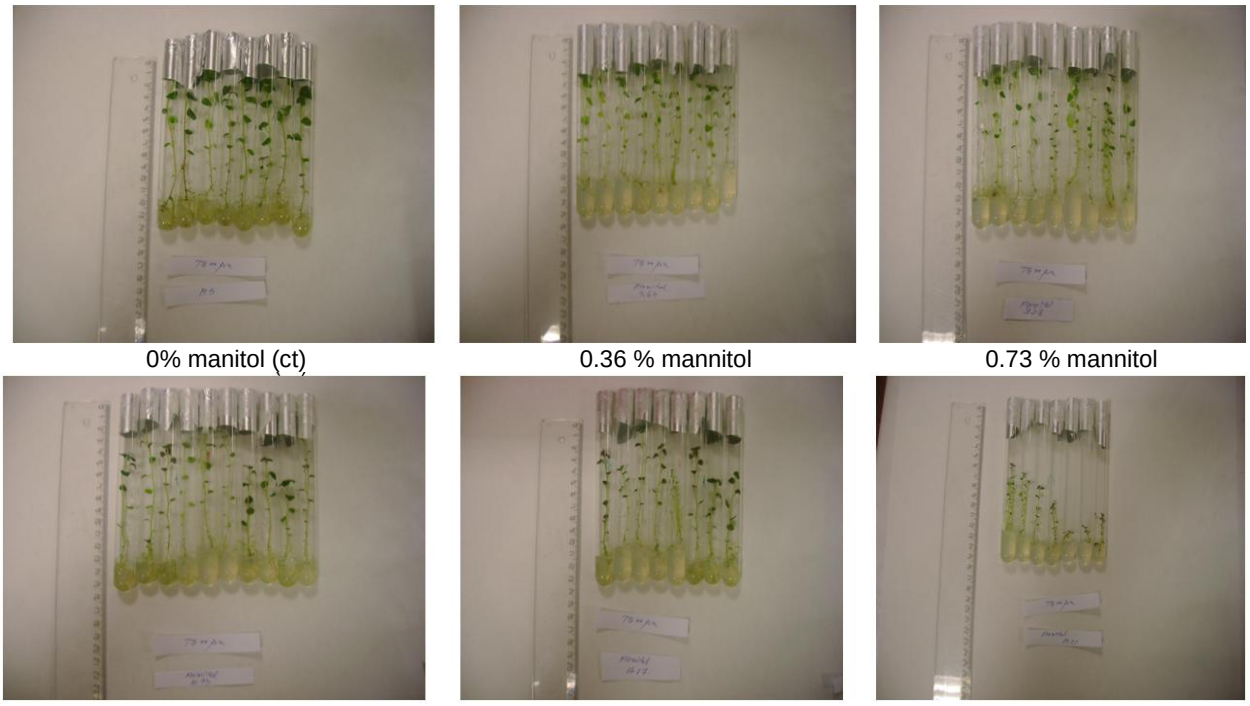
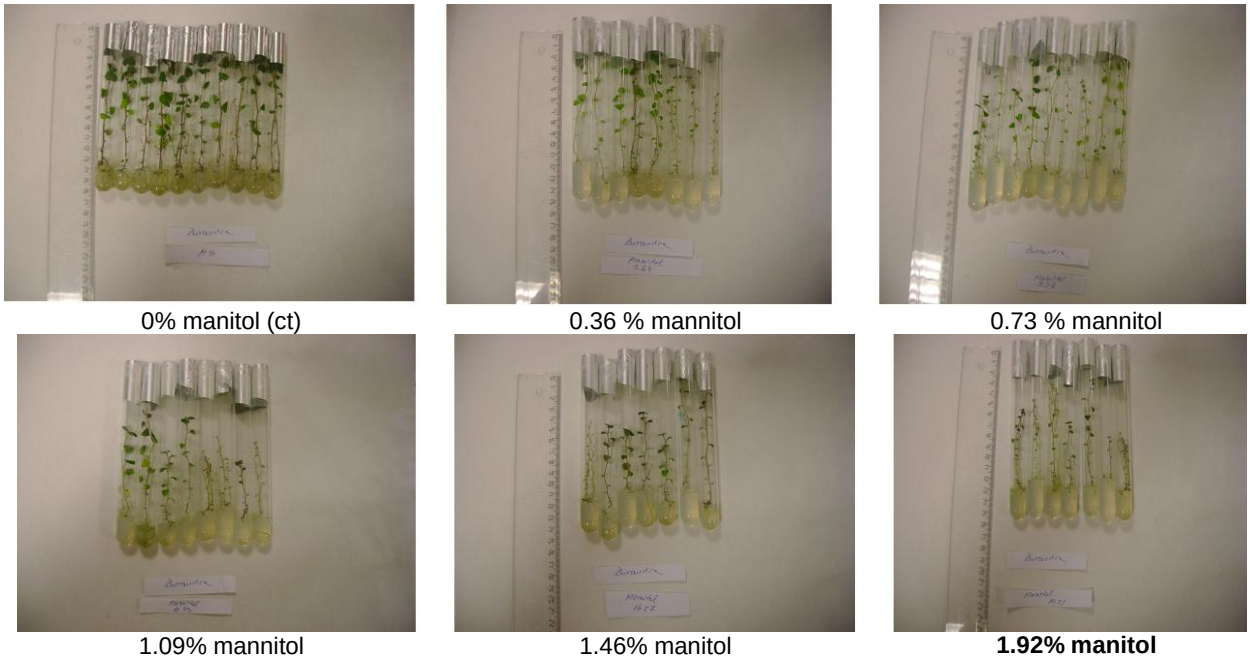


0% manitol (ct) 0.36 % mannitol 0.73 % mannitol

1.09% mannitol 1.46% manitol 1.92% manitol

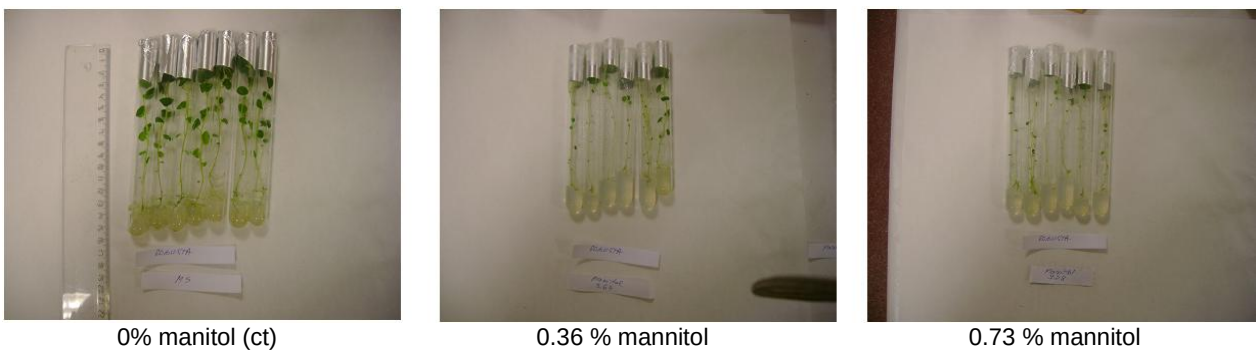
Fig.2. Plantlets developed under the influence of mannitol concentrations for Tampa variety



0% manitol (ct) 0.36 % mannitol 0.73 % mannitol

1.09% mannitol 1.46% manitol **1.92% manitol**

Fig. 3. Plantlets developed under the influence of mannitol concentrations for Ruxandra variety



0% manitol (ct) 0.36 % mannitol 0.73 % mannitol

1.09% mannitol 1.46% manitol 1.92% manitol

Fig. 4. Plantlets developed under the influence of mannitol concentrations for Robusta variety

0% manitol (ct) 0.36 % mannitol 0.73 % mannitol

1.09% mannitol 1.46% manitol 1.92% manitol

Fig. 5. Plantlets developed under the influence of mannitol concentrations for Gared variety

At concentrations of 1.46, 1.92% plantlets height was affected alarmingly, mannitol inhibiting the growth of plantlets. Analyzing by variety, Tampa presented the highest tolerance to water stress, recording the highest value/plantlets height (10.38 cm), followed by Robusta variety (9.56 cm). On last place is graded Gared variety (7.65 cm).

The number of leaves is significantly low for plantlets developed on the media containing mannitol in 1.09 to 1.92%. Rustic variety produced the highest number of leaves (14.11 leaves) differing significantly from Tampa, Ruxandra, Robusta varieties (13.17; 13.17; 12.72 leaves).

Regarding the last element analyzed, fresh weight of plantlet decreased dramatically with increasing the mannitol concentration.There are observed significant differences between values of plantlets weight developed on MS medium (259.5 mg) (without mannitol) and those that are formed on a medium for inducing water stress, with mannitol concentrations 1.46, 1.92% (61.33 and 44.37 mg).

Table 1.

Mean values of analyzed elements under the influence of different concentrations of mannitol

Mannitol concentrations (%)	The average height of plantlets (cm)	Duncan test	The average number of leaves / plantlet	Duncan test	The average weight of fresh plantlets (mg)	Duncan test
0	13.60	A	14.00	A	259.50	A
0.36	12.47	B	14.53	A	147.20	AB
0.73	10.45	C	14.60	A	149.20	AB
1.09	7.91	D	13.00	B	137.90	AB
1.46	5.67	E	11.40	C	61.33	B
1.92	3.86	F	10.20	D	44.37	B

Means with the same letter in the same column in each part are not significantly different according to Duncan test ($p \leq 0.05$)

LSD = 0.6736 cm LSD = 0.8066 LSD = 165.3 mg

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Comparing the mean values for the first analyzed parameter, plantlets height, we can notice that Tampa variety presents the highest value (10,38), by Duncan test applying, being a variety with tolerance to water stress and the more sensitive the Gared variety. For the second parameter analyzed, number of leaves/plantlets, superior value registered Rustic variety (14.11), differing significantly by Tâmpa (13.17), Ruxandra (13.17), Robusta (12.72) varieties and these significantly differentiates by Gared variety (11.61).

Table 2.

Mean values of analyzed elements depending the varieties considered for the study

Variety	The average height of plantlets (cm)	Duncan Test	The average number of leaves / plantlet	Duncan test	The average weight of fresh plantlets (mg)	Duncan test
Rustic	8.76	C	14.11	A	163.7	A
Tâmpa	10.38	A	13.17	B	163.9	A
Ruxandra	8.53	C	13.17	B	139.3	A
Robusta	9.56	B	12.72	B	97.86	A
Gared	7.65	D	11.61	C	10.15	A

Means with the same letter in the same column in each part are not significantly different according to Duncan test ($p \leq 0.05$)

LSD = 0.6642 cm LSD = 0.8066 LSD = 144.6 mg

CONCLUSIONS

From research conducted by simulating "in vitro" drought we are reporting the following:

- Tampa and Rustic varieties were the most tolerant to water stress simulation, with the best results for the analyzed parameters;

- plantlets tolerated more easily levels of 0.36 and 0.73% recording for the elements researched values around those of the control sample.

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