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EFFECT OF SIMULATED HYDRIC STRESS IN VITRO OVER BEHAVIOR OF DIFFERENT POTATO VARIETIES PLANTLETS

Andreea NISTOR*, Nicoleta CHIRU, Mihaela CIOLOCA, Monica POPA
NIRDPSB Brasov

ABSTRACT: There is a great need for research aimed at understanding drought tolerance and creating breeders of varieties with tolerance / resistance to water stress. The present study had as goal to establish an effective *in vitro* method for determination of hydric stress over potato (*Solanum tuberosum* L.) plantlets, from different varieties. It was used as osmotic stress agent the agar with different levels of concentrations applied in Murashige Skoog medium. Hydric stress in culture affected plantlet growth and the responses varied with genotype. Roclas variety was less affected than Christian and Rustic.

Keywords: *in vitro,* agar, hydric stress, plantlets, *Solanum tuberosum* L.

INTRODUCTION

Abiotic stress is in many parts of the world, one of the most serious threats resulting in increased desertification (Hassanein, 2010). Hydric stress is a major abiotic factor that limits plant growth and productivity. Around the world, approximately 40-60% of agricultural land, suffers from drought (Bray 1997 cited by Hassanein, 2010). It is usually manifested as osmotic stress, causing several morphological, physiological and molecular changes in plants (Hassanein, 2010). Worldwide, there are currently described more than 4.000 species, which can be adapted to different soils and climates, may be associated with genetic broad diversity, of this culture. Drought is a factor that causes severe yield losses in the areas where the water availability to this crop is scarce. Potato breeders around the world are trying to create genotypes with high yield and stress tolerance. In order to achieve these objectives are applied conventional and unconventional methods. From the non-conventional techniques, *in vitro* multiplication method is widely used for the production of a large number of plantlets in a short time, to accelerate the supply of new varieties on market and keeping potato seed stock, the disease free.

In vitro culture techniques minimize environmental changes due to nutrient medium defined, controlled conditions and constant manner of stress simulation.

The objective of research was initiation of an effective and practical *in vitro* method, which can be used in evaluating the tolerance to drought of three potato genotypes. For inducing water stress, different concentrations of agar in Murashige-Skoog (1962) medium it was used.

When it is increases the quantity of agar (in the culture medium), it also increases the bonding force between the water molecules and the agar, which ultimately makes it difficult for the plantlet to draw water from the solution and a state of drought is created for the plantlet *in vitro* (Shafqatullah, 2007). The greater the amount of agar in the medium the higher will be the intensity of drought. To evaluate the effects of agar concentrations on potato plantlets an experiment was made.

MATERIALS AND METHOD

Microcuttings resulting from uninodal segmentation were inoculated into test tubes containing MS medium, supplemented with naphthyl acetic acid, 20 g / l sucrose and different concentrations of agar; tubes were placed in the growing room ensuring light and temperature regime needed for growth and development of plantlets; after 4-5 weeks, were performed measurements for the following parameters: number of leaves and internodes / plantlets and height of the plantlets. The results were processed by variation analysis, and significance of differences was determined by the method of multiple comparisons (Duncan test).

In the Laboratory of Research for Vegetal Tissue Cultures were experimented Christian, Roclas and Rustic varieties (figure 1), included in a bifactorial experience (on the type 2x6), containing the following factors (distribute in 3 repetitions):

- experimental factor A: variety, with 3 graduations:
  - a_1- Christian;
  - a_2- Roclas;
  - a_3-Rustic.
- experimental factor B – agar concentration (%), with 6 graduations:
  - b_1- 0.9;
  - b_2- 1.2;
  - b_3- 1.4;
  - b_4-1.6;
  - b_5- 1.8;
  - b_6-2.0.

Correspondence: Andreea Nistor, NIRDPSB Brasov, Street Fundăturii no 2, City : Brasov, Postal code: 500470, Phone: 0268/476795, Fax: 0268/476608, Romania, tican_andreea@yahoo.com
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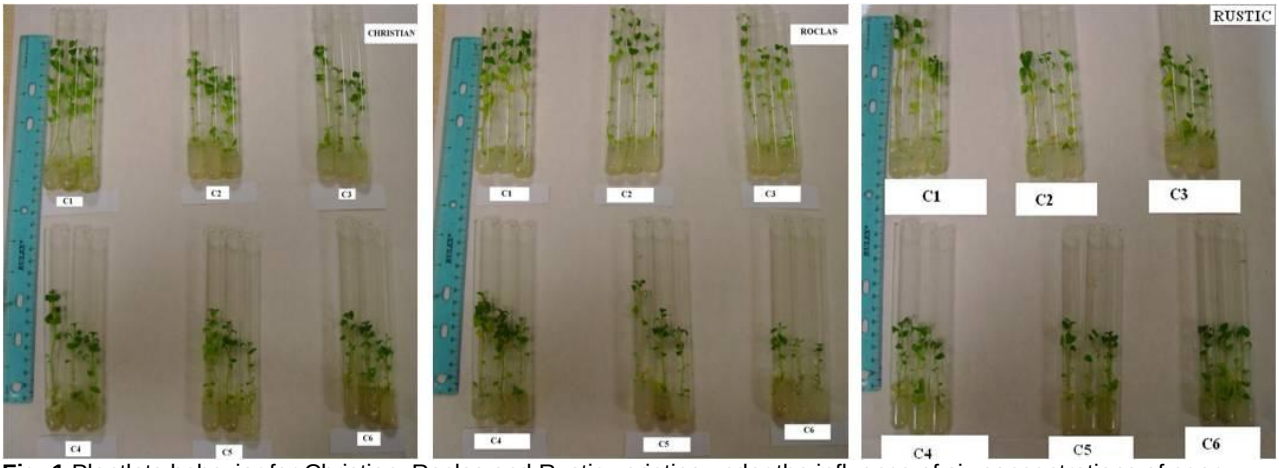

Fig. 1 Plantlets behavior for Christian, Roclas and Rustic varieties under the influence of six concentrations of agar

RESULTS AND DISCUSSION

In vitro simulation of drought was approached as an alternative of expensive determinations, of intensive labor force and sometimes problematic in the field. The lowest concentration of agar (0.9%) is the classical; the other concentrations were used to simulate *in vitro* drought. Statistical interpretation of drought tolerance was performed taking into account two factors, namely the varieties used in the experiment and the different concentrations of agar.

By simulating *in vitro* drought, it can be observed the superiority of Roclas variety (table 1), which differs significantly from the other two varieties, this showing tolerance to water stress, being followed by Christian variety, for all analyzed parameters.

Analyzing the height of plantlets, there is similar behavior of Christian and Rustic varieties, which do not differ significantly.

In general, the registered values of morphological parameters for studied varieties decrease with increasing agar concentration in nutrient medium. The presence of higher concentrations of agar in the multiplication medium unfavored parameters analyzed.

Table 1
Behaviour variety regarding the analyzed elements

Variety	No. of leaves/pl.	Duncan test	No. of internodes /pl.	Duncan test	Height of pl. (cm)
Christian	9.83	B	7.56	B	7.01
Roclas	11.22	A	9	A	8.24
Rustic	8.22	C	6	C	6.69
		LSD = 0.7164	LSD = 0.7658	LSD = 0.7383	

Means within same column followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

The concentration of 9% agar (classical) is commonly used in composition of multiplication medium and in our case can be considered control sample, for which the values of the studied parameters are optimized (table 2).

Although increasing the concentration of agar induces reduction of leaves number, the studied variants reacts significantly different, the concentrations of 2, 3, 4 are more permissive for the inhibitory effect over formation of the leaves.

The formation of the leaf number was specifically inhibited by concentrations of 1.8 and 2.0% agar, leading to a decreased number of leaves, decreasing with the same degree of significance; this warns that increasing concentrations up to this level leads to accentuate water stress and plant suffering without these can still submit drought tolerance. The number of nodes formed on the stem was subjected to the same unfavorable influence of the concentration of agar and hence the simulation of drought effect, plant can tolerate easier levels 2, 3 and 4, the last two of the mentioned, having substantially the same effect in nodes formation. The drastic decrease of nodes number was recorded at concentrations 5 and 6, leading to halving them, compared to control sample.

Regarding the influence of agar concentration and hence the water stress on plant growth (height) is found at concentrations of 1.2 and 1.4% plants do not have an accentuat water-stressed, although these variants are significantly different.

The most drastic water stress manifested by inhibiting plant growth is observed at concentrations of 4, 5, 6 of which the first two mentioned (4 and 5) have the same meaning by using the method of multiply comparisons (Duncan test).

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Table 2

The influence of the agar concentration regarding growth and development elements

Variants	Agar concentration (%)	No. Of leaves /pl.	Duncan test	No. of internodes /pl.	Duncan test	Height of pl. (cm)	Duncan test
c_1	0,9	13.11	A	10.89	A	10.77	A
c_2	1.2	11.22	B	8.78	B	9.02	B
c_3	1.4	10.11	C	7.67	C	7.72	C
c_4	1.6	8.89	D	7	C	6.22	D
c_5	1.8	8.00	E	5.89	D	5.69	D
c_6	2.0	7.22	E	4.89	E	4.47	E

LSD = 0.779 LSD = 0.747 LSD = 0.757

Means within same column followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

Table 3

The influence of agar concentrations and varieties analyzed, regarding the elements for growth and development

Agar concentration (%)	Variety	No. of leaves /pl.	Duncan test	No. of internodes /pl.	Duncan test	Height of pl. (cm)	Duncan test
0,9	Christian	13	BC	10.667	B	10.73	AB
	Roclas	14.67	A	13	A	11.73	A
	Rustic	11.67	CD	9	C	9.83	B
1,2	Christian	10.67	DE	8.667	CD	7.167	DE
	Roclas	13.67	AB	10.67	B	11.57	A
	Rustic	9.33	EFGH	7	EF	8.33	CD
1,4	Christian	9.67	EFGH	7.33	DE	7	DE
	Roclas	12.33	BC	10	BC	9.67	BC
	Rustic	8.33	GHIJ	5.67	FGH	6.5	EF
1,6	Christian	9	FGHI	6.67	EFG	6.17	EFG
	Roclas	10.33	DEF	9	C	7	DE
	Rustic	7.33	JKL	5.33	GHI	5.5	FG
1,8	Christian	8.67	GHIJ	6.33	EFGH	6.07	EFG
	Roclas	8.67	GHIJ	6.33	EFGH	6	EFG
	Rustic	6.67	KL	5	HI	5	G
2,0	Christian	8	HIJK	5.67	FGH	4.9	GH
	Roclas	7.67	IJK	5	HI	3.5	H
	Rustic	6	L	4	I	5	G

LSD = 1.422 LSD = 1.520 LSD = 1.422

Means within same column followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

From interactions variety and agar concentrations of used through the multiple comparisons, analyzed through multiple comparisons, is found a high amplitude of significances for all parameters analyzed; the significances are very distant for the number of leaves (from A to L), less distant for nodes number (A-I) and closer to the plant height (A-H), indicating that the studied varieties behave differently to water stress simulated; by using the concentrations of the agar, the biggest differences are obtaining in the formation of the leaves (table 3).

If at classical concentration (0.9%), control considered, Roclas variety shows superiority (A -with a number of 14,67 leaves), same variety similar behaves by increasing concentration from 1.2 to 1.4%. Christian variety has similar behavior with Roclas variety only for control sample (0.9%), followed closely by Rustic variety. We may say that 1.4% concentration is tolerated by Roclas variety (BC), variety which tolerates 1.6% concentration in same way with Christian (for 1.2%) and Rustic (for 0.9%) varieties; this means that Roclas is a drought tolerant variety with a resistance level close with that of the Rustic

variety (but for standard concentration) or Christian variety (but at a lower threshold of water stress, this variety forming a number of leaves that can ensure growth and the development of plantlets). Concentrations of 1.8 and 2.0%, remove very much plantlets from optimum of growth and development, that the three varieties lose their ability of forming leaves, reaching only 6 leaves for Rustic.

Concerning the number of nodes far away Roclas variety detaches, as significant at standard sample, with a number of 13nodes, value that is not found for any variety/concentration. Roclas (1.2%) and Christian (0.9%) varieties have similar behavior, and very similar with Roclas (1.4% and 1.6) and equaled by 0.9% Rustic; this confirms the idea that it is a very drought tolerant variety and supporting in development of plant water stress more pronounced, behavior that Rustic variety do not expresses only for control sample.

Increasing of plant height gives great information, depending on the variety and concentration used. If Roclas variety similarly behaves for 0.9 and 1.2% agar, closely followed by Christian variety for 0.9% agar, same variety - Roclas has good behavior for 1.4% concentration (recorded a plantlet height of 9,67 cm), this height is reached by the Rustic variety, only for standard sample. Again is confirmed for Roclas variety, which continued plant growth even in very drought stressful media (1,6%); this value does not differ significantly from that of the Christian variety or 1.2% to 1.4% or Rustic variety for agar concentration 1.2%.

Agar concentrations of 1.8 and 2.0% are very damaging for plantlets, the values recorded for growing plant height, drastically decreasing at 3.5 cm for Roclas, or 5 for Rustic variety. We want to indicate the constancy in behavior of Rustic variety, which similarly tolerate agar concentrations of 1.6, 1.8, 2.0%, but also for Christian variety, which for concentrations 1.4, 1.6, 1.8%, forming a similar number of leaves, providing information distinguished in drought tolerance, even when it is very pronounced.

CONCLUSIONS

From research carried out by simulating *in vitro* drought following conclusions arise:

- Roclas variety was highlighted as the most tolerant at simulation water stress, with the best results for all three parameters analyzed;

- plantlets more easily tolerated agar concentrations of 1.2, 1.4, 1.6%, registering for researched elements, values close to those of the control sample;

- regarding interaction variety - agar concentrations, there is a large amplitude of significances for all parameters analyzed, indicating that the studied varieties behave differently to simulated water stress, using the agar concentrations, biggest differences were obtain in formation of the leaves

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