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In Vitro Experimental Researches Regarding the Treatment with Phytoregulators of Orach (*Atriplex hortensis* L.)

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Abstract. Orach (*Atriplex hortensis* L.) is a halophyte plant, cultivated ever since the ancient times, used in the food industry, biotherapy, human medicine and apiculture. But, then it has a germinal capacity of about 25% and a duration of two years. These characteristics make the *A. hortensis* L. a plant with economic potential exploitable *via* biotechnological methods. These methods use phytoregulators as biochemical instruments capable of modeling somatic type explants cultivated *in vitro* for the purposes of the induction, generation and materialization of the properties characteristic of stem type cells. These properties are especially relevant by the use of auxinic or cytokininic type phytoregulators.

The experimental researches described in this study has aimed at testing treatments with exogenous auxins or exogenous cytokinins of *A. hortensis* L., on somatic type explants cultivated *in vitro,* for the purposes of the induction, generation and materialization of properties characteristic of stem type cells, transformed in the form of callogenesis, caulogenesis and rhizogenesis.

The vegetal biological material used for the testing of treatments with exogenous auxins (IAA or 2,4,5-T) or exogenous cytokinins (TDZ or 2-iP) has consisted in two varieties of orach (*A. hortensis* L. var. *rubra* and *A. hortensis* L. var. *viridis*) and five types of somatic explants (leaf, cotyledon, epicotyl, hypocotyl and radicle). The research methods have been concordance with the pursued aim, and accord with the plant biotechnologies.

Out of the treatments with exogenous auxins, the best result has been obtained using the 2,4,5-T 0.50mg/l exogenous auxin on *A. hortensis* L. var. *viridis*, on radicle type explants, since it has allowed the induction, generation and materialization of the callogenesis and indirect rhizogenesis processes. At the same time, out of the treatments with exogenous cytokinins, the treatment with TDZ 0.75mg/l exogenous cytokinins has generated the best result on *A. hortensis* L. var. *rubra*, on cotyledon type explants, since it has allowed the induction, generation and materialization of the indirect callogenesis and caulogenesis processes.

The results of the experimental researches described in this study demonstrate that the application of treatments with exogenous auxins or exogenous cytokinins on orach (*A. hortensis* L.), on somatic type explants cultivated *in vitro,* allows the induction, generation and materialization of properties characteristic of stem type cells, transformed in the form of callogenesis, indirect caulogenesis and indirect rhizogenesis.

Keywords: orach, *Atriplex hortensis* L., exogenous auxins, exogenous cytokinins

INTRODUCTION

Orach (*Atriplex hortensis* L.) is a halophyte plant part of the *Amaranthaceae* family, which has the economic potential necessary for the purposes of being exploited *via* callogenesis, as well as *via* caulogenesis, rhizogenesis or embryogenesis. Its exploitation *via* callogenesis could be an alternative to the biosynthesis of substances with tinctorial properties used in various industries (food, pharmaceutical, etc.). While its exploitation *via* caulogenesis, rhizogenesis or embryogenesis may constitute both a way of obtaining a sufficiently high number of vitroplants to overcome the disadvantage of a germinal capacity reduced to about

25% and over only two years (Pârvu, 1997), as well as an alternative to the conventional food sources (Carlsson and Clarke, 1983).

The reviewed specialized literature presents a series of scientific works indicating the use of orach (*Atriplex hortensis* L.) both in genetic engineering experiments aimed at obtaining transgenic maize plants (*Zea mays* L.) with improved performance in respect of the tolerance to the saline stress (Yi-Guo *et al.,* 2002), as well as in experiments aimed at decoding the mechanisms involved in the response to the saline stress (Sai Kachout *et al.,* 2009), and hydric stress (Sai Kachout *et al.,* 2011).

In this context, the experimental research described in this study has aimed at testing treatments with exogenous auxins or exogenous cytokinins of two species of orach (*Atriplex hortensis* L.), leaving from various somatic type explants cultivated *in vitro,* for the purposes of the induction, generation and materialization of properties characteristic for the stem type cells, transformed in the form of callogenesis, caulogenesis and rhizogenesis.

MATERIALS AND METHODS

The vegetal biological material used for the testing of treatments with exogenous auxins or exogenous cytokinins of orach (*Atriplex hortensis* L.), was procured from a commercial source, and consisted in two species recognized as cultivated vegetable plants. The selection criterion used as distinctive element between these two species was the color of the plant, being red in the case of the *A. hortensis* L. var. *rubra* and green in the case of the *A. hortensis* L. var. *viridis*. The primary source of somatic explants (leaf, cotyledon, epicotyl, hypocotyl and radiclet) consisted in plantules obtained from the germination of seeds in *in vitro* conditions.

The method of surface aseptization of the vegetal biological material used for the testing of treatments with exogenous auxins or exogenous cytokinins of orach (*Atriplex hortensis* L.), consisted in the pre-aseptization of the seeds by maintaining them under continuous tap water jet for 1 h, and the actual aseptization of the seeds by their successive treatment with ethyl alcohol ($C_6H_{12}O_6$) 80% for 30 sec. and with sodium hypochlorite (NaClO) 1.5% for 30 min. (Moghaddam *et al.,* 2000). The removal of the toxic residues of the used chemical agents was achieved by three successive washings with aseptized distilled water, for 10'/each washing (Badea and Săndulescu, 2001; Cachiță-Cosma *et al.,* 2004), while stirring continuously.

Culture conditions used for the testing of treatments with exogenous auxins or exogenous cytokinins of orach (*Atriplex hortensis* L.). The inoculated seeds and the explants were incubated at $23^oC \pm 2^oC$ temperature/light period and $20^oC \pm 2^oC$ temperature/dark period, with a photoperiod of 16 h of light, and a light intensity of 1700 luxes. Subcultivations were regularly achieved at a time span of 2 to 4 weeks (Takaiama, 1990).

The artificial nutritive mediums used for the testing of treatments with exogenous auxins or exogenous cytokinins of orach (*Atriplex hortensis* L.) were achieved on the basis of the nutritive formula of Murashige and Skoog medium (Murashige and Skoog, 1962), supplemented by agar 8 g/l and sucrose 30 g/l. Several variants of artificial nutritive mediums were achieved leaving from this base medium, having a various composition depending on the content of phytoregulators (*Tab. 1*), respectively exogenous auxins (IAA or 2,4,5-T) or exogenous cytokinins (TDZ or 2-iP). The pH value was adjusted to 5.3 in respect of each artificial nutritive medium variant before the autoclaving.

Each experimental variant consisted in three repetitions with 20 explants/ repetition. Measurements were performed in respect of each individual inoculum. The average value and standard error were determined in respect of each parameter analyzed

via the Duncan's multiple range test ($p<0.05$). The experimental results are shown in the form of average value ± standard error.

Tab. 1

Variants of artificial nutritive mediums used for the testing of treatments with exogenous auxins or exogenous cytokinins of orach (*Atriplex hortensis* L.) on somatic type explants cultivated *in vitro*

Variants of artificial nutritive mediums	Exogenous auxins (mg/l)		Exogenous cytokinins (mg/l)	
	IAA	2,4,5-T	TDZ	2-iP
MS 0	0.00 mg/l	0.00 mg/l	0.00 mg/l	0.00 mg/l
MS 1	0.25 mg/l	0.00 mg/l	0.00 mg/l	0.00 mg/l
MS 2	0.50 mg/l	0.00 mg/l	0.00 mg/l	0.00 mg/l
MS 3	0.75 mg/l	0.00 mg/l	0.00 mg/l	0.00 mg/l
MS 4	1.00 mg/l	0.00 mg/l	0.00 mg/l	0.00 mg/l
MS 5	0.00 mg/l	0.25 mg/l	0.00 mg/l	0.00 mg/l
MS 6	0.00 mg/l	0.50 mg/l	0.00 mg/l	0.00 mg/l
MS 7	0.00 mg/l	0.75 mg/l	0.00 mg/l	0.00 mg/l
MS 8	0.00 mg/l	1.00 mg/l	0.00 mg/l	0.00 mg/l
MS 9	0.00 mg/l	0.00 mg/l	0.25 mg/l	0.00 mg/l
MS 10	0.00 mg/l	0.00 mg/l	0.50 mg/l	0.00 mg/l
MS 11	0.00 mg/l	0.00 mg/l	0.75 mg/l	0.00 mg/l
MS 12	0.00 mg/l	0.00 mg/l	1.00 mg/l	0.00 mg/l
MS 13	0.00 mg/l	0.00 mg/l	0.00 mg/l	0.25 mg/l
MS 14	0.00 mg/l	0.00 mg/l	0.00 mg/l	0.50 mg/l
MS 15	0.00 mg/l	0.00 mg/l	0.00 mg/l	0.75 mg/l
MS 16	**0.00 mg/l**	**0.00 mg/l**	**0.00 mg/l**	**1.00 mg/l**

Abbreviations: MS=Murashige and Skoog (1962); IAA=indole-3-acetic acid; 2,4,5-T=2,4,5-Trichlorophenoxyacetic acid; TDZ=1-phenyl-3-(1,2,3 thiadiazol-5-yl)urea; 2-iP=N^6-(2-isopentenyl)adenine.

RESULTS AND DISCUSSIONS

According to the specialized literature the current vegetal biotechnologies use the phytoregulators (exogenous auxins, exogenous cytokinins, etc.), as biochemical instruments aimed at modeling the vegetal biological material *via* various methods. These methods generally metamorphose various explants with a view to obtaining vegetal biological structures with the highest complexity possible, that is: vegetal biomass *via* callogenesis, unipolar vegetal structures via caulogenesis or rhizogenesis, or bipolar vegetal structures *via* embryogenesis (Badea and Săndulescu, 2001; Cachiță-Cosma *et al.*, 2004).

This scientific study presents the experimental results recorded pursuant to the experimental research achieved with a view to testing treatments with exogenous auxins or exogenous cytokinins of orach (*Atriplex hortensis* L.), on somatic type explants cultivated *in vitro*, for the purposes of the induction, generation and materialization of the properties characteristic of stem type cells, transformed in the form of callogenesis, caulogenesis and rhizogenesis.

The testing of treatments with exogenous auxins of orach (*Atriplex hortensis* L.) allowed the conversion of the differentiated somatic cells of four types of explants (hypocotyl, cotyledon, epicotyl and radicle) into stem cells capable of inducing, generating and proliferating the vegetal callus, only in the case of the red orach species (*A. hortensis* L. *rubra*), and only under the influence of IAA exogenous auxin having a concentration of 1.00 mg/l (*Fig. 1*). At the same time, only three types of explants (hypocotyl, cotyledon and

radicle) resulted in similar processes of obtaining vegetal callus, under the influence of 2,4,5-T exogenous auxin. However, the callogenesis obtained under the influence of 2,4,5-T exogenous auxin was achieved in respect of both orach species (*A. hortensis* L. *rubra* and *A. hortensis* L. *viridis*). Furthermore, 2,4,5-T exogenous auxin allowed, as compared to IAA exogenous auxin, the obtaining of the callogenesis as well at concentrations smaller than 1.00 mg/l (*Fig. 2*).

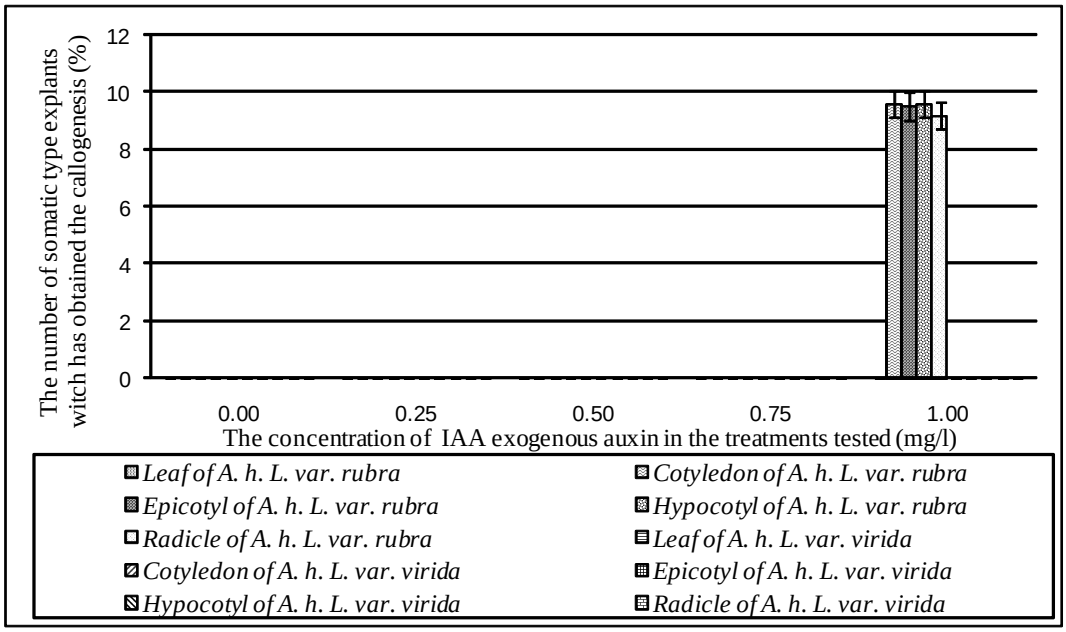

Fig. 1. Results of the testing of treatments with IAA exogenous auxin of orach (*Atriplex hortensis* L.) on somatic type explants cultivated *in vitro* for the obtaining of the callogenesis

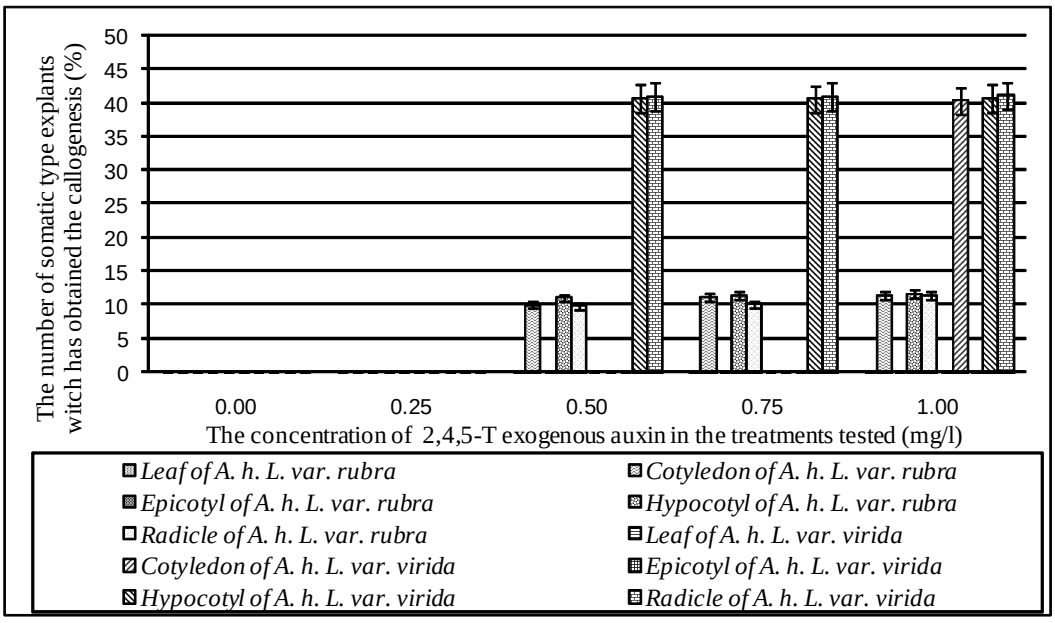

Fig. 2. Results of the testing of treatments with 2,4,5-T exogenous auxin of orach (*Atriplex hortensis* L.) on somatic type explants cultivated *in vitro* for the obtaining of the callogenesis

Out of the treatments with exogenous auxins of orach (*A. hortensis* L.), only the treatment with 2,4,5-T 0.50 mg/l exogenous auxin allowed the induction, generation and materialization of roots from the callus obtained from the radicle type explants, in the case of the white orach species (*A. hortensis* L. *viridis*). The explants, which achieved the indirect rhizogenesis process, represented a percentage of 9.8±0.7 from the number of radicle type explants used as initial inoculum for this species.

Thus, the experimental results recorded pursuant to the testing of treatments with exogenous auxins of orach (*A. hortensis* L.) consisted in the induction, generation and materialization both of the process of callogenesis under the influence of each of the exogenous auxins IAA and 2,4,5–T in respect of both species of orach (*A. hortensis* L.), and of the process of indirect rhizogenesis under the influence of 2,4,5–T exogenous auxin in respect of the white orach species (*A. hortensis* L. *viridis*).

The testing of treatments with exogenous cytokinins of orach (*Atriplex hortensis* L.) facilitated the conversion of the differentiated somatic cells of four types of explants (hypocotyl, cotyledon, epicotyl and radicle) into stem cells capable of inducing, generating and proliferating the vegetal callus, in the case of both orach species (*A. hortensis* L. *rubra* and *A. hortensis* L. *viridis*) under the influence of TDZ exogenous cytokinin at variable concentrations thereof (*Fig. 3*). At the same time, in respect of the same types of explants (hypocotyl, cotyledon, epicotyl and radicle) the callogenesis was materialized in the case of the red orach species (*A. hortensis* L. *rubra*), and under the influence of the treatments based on 2-iP exogenous cytokinin. However, the callogenesis materialized in respect of only three types of explants (hypocotyl, cotyledon and radicle) in the case of the white orach species (*A. hortensis* L. *viridis*) under the influence of the same treatments based on 2-iP exogenous cytokinin (*Fig. 4*).

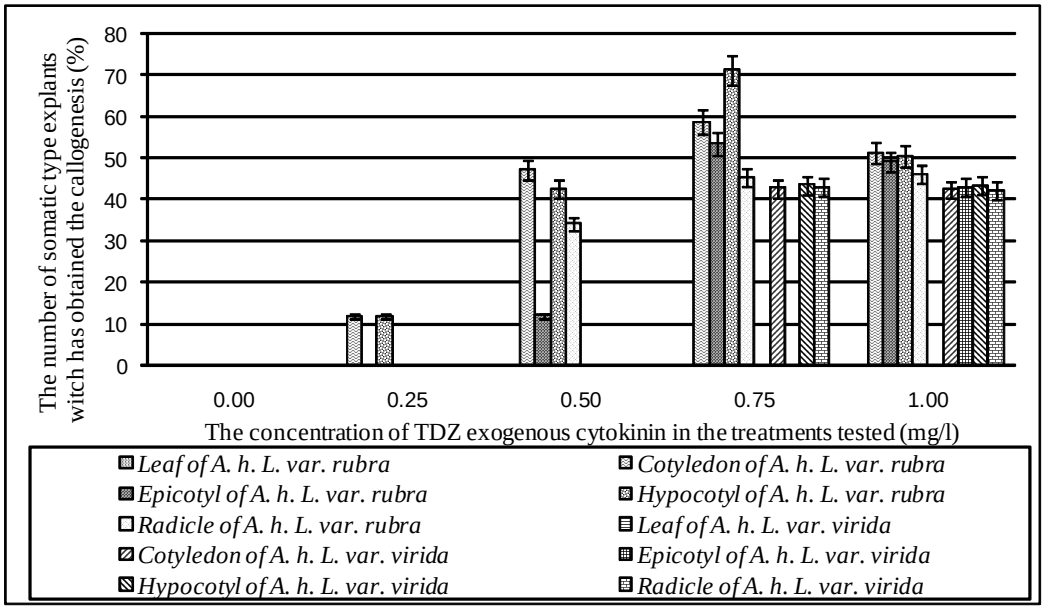

Fig. 3. Results of the testing of treatments with TDZ exogenous cytokinin of orach (*Atriplex hortensis* L.) on somatic type explants cultivated *in vitro* for the obtaining of the callogenesis

Out of the treatments with exogenous cytokinins of orach (*A. hortensis* L.), only the treatment with TDZ 0.75 mg/l exogenous cytokinin allowed the induction, generation and materialization of sprouts from the level of the callus obtained from cotyledon type explants in the case of the red orach species (*A. hortensis* L. *rubra*). The explants, which achieved the

indirect caulogenesis process, represented a percentage of 40.2±6.7a from the number of the cotyledon type explants used as initial inoculum for this species.

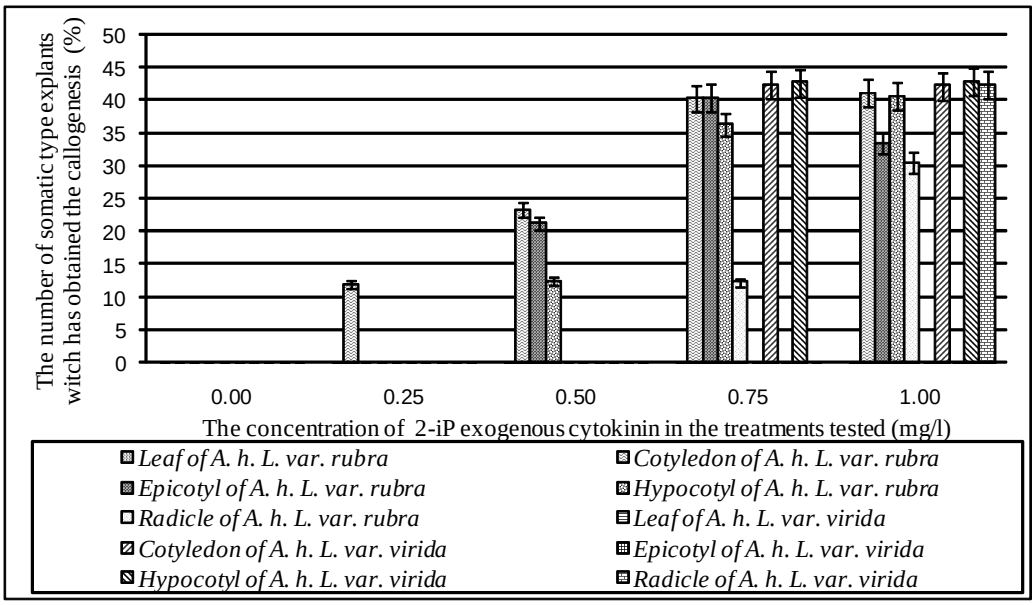

Fig. 4. Results of the testing of treatments with 2-iP exogenous cytokinin of orach (*Atriplex hortensis* L.) on somatic type explants cultivated *in vitro* for the obtaining of the callogenesis

Therefore, the experimental results recorded pursuant to the testing of treatments with exogenous cytokinins of orach (*A. hortensis* L.) consisted in the induction, generation and materialization both of the process of callogenesis under the influence of each of the two exogenous cytokinins used (TDZ or 2-iP) in respect of both species of orach (*A. hortensis* L.), and of the process of indirect caulogenesis under the influence of TDZ exogenous cytokinin in respect of the red orach species (*A. hortensis* L. *rubra*).

An analytical analysis of the experimental results compared with the results obtained by other authors in the specialized literature indicates that these experimental results are in many respects similar to those reported worldwide with reference to the influence of the phytoregulators in respect of the induction, generation and materialization of properties characteristic for stem type vegetal cells, metamorphosed in the form of callogenesis, caulogenesis and rhizogenesis.

Nevertheless, the obtained experimental results complete with new elements the existing information under the specialized literature in respect of the treatments with IAA or 2,4,5–T exogenous auxins and with TDZ or 2-iP exogenous cytokinins of plants, by studying the *in vitro* reactivity of the two species of orach (*A. hortensis* L.), not analyzed before in relation to the modeling of somatic type explants cultivated *in vitro*, with a view to achieving the processes of callogenesis, indirect caulogenesis and indirect rhizogenesis.

CONCLUSION

The experimental results recorded pursuant to the *in vitro* experimental research regarding the testing of treatments with phytoregulators of orach (*Atriplex hortensis* L.) illustrate the influence of the treatments with exogenous auxins (IAA sau 2,4,5–T) or those with exogenous cytokinins (TDZ on 2-iP) on the somatic type explants (leaf, cotyledon, epicotyl, hypocotyl and radicle) with a view to inducing, generating and materializing

properties characteristic for the stem type cells transformed in the form of callogenesis, indirect caulogenesis and indirect rhizogenesis.

An analysis of the experimental results indicates the following:

- ✓ out of the treatments with exogenous auxins the treatment using 2,4,5-T 0.50mg/l exogenous auxin generated the best result in respect of the white orach species (*A. hortensis* L. *viridis*) on radicle type explants, as it allowed the induction, generation and materialization of the callogenesis and indirect caulogenesis processes;
- ✓ whilst out of the treatments based on exogenous cytokinins the treatment using TDZ 0.75mg/l exogenous cytokinin generated the best result in the case of the red orach species (*A. hortensis* L. *rubra*) on cotyledon type explants, since it allowed the induction, generation and materialization of the callogenesis and indirect caulogenesis processes;
- ✓ the induction, generation and materialization of the callogenesis, indirect rhizogenesis and indirect caulogenesis in respect of orach (*A. hortensis* L.) can be modeled by identifying the optimal ratio between the vegetal biological material (species and explant type) and the phytoregulator (type and concentration).

The experimental results performed contribute with new results, which can be useful for the setting in place of a working protocol for the purposes of the *in vitro* propagation in respect of orach (*Atriplex hortensis* L.), and demonstrate that the application of treatments with phytoregulators on somatic type explants cultivated *in vitro* allows the induction, generations and materialization of the callogenesis process in respect of the both studied species.

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