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PRELIMINARY STUDIES ON *IN VITRO* BEHAVIOR OF VARIOUS SOMATIC EXPLANTS FROM SOME CULTIVATED *AMARANTHUS* GENOTYPES

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Abstract

The recent renewal of interest in underutilized crops of nutritional and economic potential for the agriculture of the future stimulated the research in Amaranthus sp. cultivation and breeding. In vitro systems have important practical applications not only for rapid breeding of this rediscovered crop but also for producing cell biomass to be used as source of phytochemicals of practical interest. The response of explants from hypocotyl, root and cotyledon node of three varieties of Amaranthus species (Amaranthus cruentus"Amont", Amaranthus hypochondriacus"Intense Purple"and Amaranthus ssp."Plenitude") were recorded, upon their cultivation "in vitro" on media supplemented with different combinations of auxins and cytokinins.Our experimental results pointed out that the explant type, the auxin supplement and the genotype were the most important factors in callus initiation. Calluses were induced most frequently on Murashige&Skoog-MS (1962) basal medium with 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D),whereas the root development occurred in the presence of NAA 1-2 mg/l(α-naphthaleneacetic acid). Proliferative callus derived from cotyledons and hypocotyls of the studied Amaranthus species were transferred to MS media enriched with hydolyzated casein, NAA and kinetin in order to compare their morphogenetic capacity for plant regeneration.

Key words**:** *Amaranthus species, callus initiation -tissue culture*

INTRODUCTION

The nowadays renewal of interest in underexploited crops resulted in intensive efforts made for identification / cultivation and quality evaluation of many plant species that for a long time enjoyed only a local importance. A suitable plant alternative crop must have reduced requirements for inputs (especially water and energy-intensive fertilizers), must withstand adverse environmental conditions and have a high growth rate, a higher energy efficiency and produce substances that can be used in energy, food and industry [5].

In this context, the attention focused mainly on three ancient *Amaranthus* species: *Amaranthus caudatus* (L), *Amaranthus hypochondriacus* (L) and *Amaranthus cruentus* (L) which are at present cultivated worldwide because of their exceptional nutritional value of both seeds and leaves. Additionally, the species are widespread ornamentals and also have a potential as forage crops and as sources of red food colorants, of antioxidant compounds and of other valuable phytochemicals such as α-amylase trypsin inhibitors and other active compounds with important uses in medicine. Besides, this re-discovered crop has some agricultural advantages and noted ability to grow successfully in adverse environmental conditions, such as high irradiance, temperature and drought. [2, 3, 10].

"Grain amaranth" is a name commonly used for certain lines of at least three species of the family Amaranthaceae, viz. *A. hypochondriacus* A., *A. caudatus* L. and *A. cruentus* L. Though centuries ago grain amaranth was a staple food in Aztec and other Mexican Indian diets, only in the 1970s some research reports revealed the nutritional value of these tiny grains.

The seed contain about 17-19% (of seed dry eight) high quality protein (5% lysine and 4% sulfur-containing amino acids) and 63% easily digestible carbohydrates, as compared to more traditional crops that have an average of approx.10% proteins. In *Amaranthus* 50% of the total seed proteins at maturity are globulin and albumin [6,9].

Some varieties of *Amaranthus* species (AMA 5, V2, AMA 18, VOP, etc..), were recorded as containing different concentrations of vitamin C, nitrogen and minerals [7]. Extensive scientific research was lately conducted on the biology, ecology, biomass accumulation, and harvest quality Amaranthus species (*Amaranthus cruentus* L., *A. hypochondriacus* L., *A. Caudatus* L.). The relatively high content of essential aminoacids in Amaranthus recommended it as a possible substitute for meat. Thus, the use of Amaranthus grain may have implications both in food intended for human consumption and in the diet for certain special categories of consumers. Starting with the 8th decade of the twentieth century, Amaranthus species began to be exploited commercially, but its market is still limited to people allergic to gluten products, local traditional medicine and regional restaurants. In the relevant scientific literature there were published results on the cultivation technologies, elements of culture, acclimatization methods, the chemical composition, oil production and its composition [6].

The underexploited crops, including amaranth, offer a special challenge for the use of *in vitro* approaches, because extensive efforts are required from the plant breeders to select and improve this plant material.

Currently, there are not much published reports on the tissue culture of *Amaranthus* sp.,but among the practical applications of tissue culture in amaranth we shell mention micropropagation of selected genotypes and their subsequent exploitation, rescue of the genetic variation or inducing new variation, phytoremediation studies and using the cell biomass to obtain phytochemicals of practical interest.

Previous studies conducted by H. Flores et al. (1982) and A. Bennici et al. (1992) [1, 4] on several species and varieties of the genus *Amaranthus* showed its potential with regard to dedifferentiation and morphogenetic processes *in vitro,* with emphasis on age dependant competence of explanted tissues and the cytokinin / auxin ratio in the culture medium

With this background, the aim of the present study was to evaluate the growth and morphogenetic responses of various types of explants from three varieties of Amaranthus species cultivated *"in vitro": Amaranthus cruentus* "Amont", *Amaranthus hypochondriacus* "Intense Purple" and *Amaranthus* ssp."Plenitude".

MATERIAL AND METHOD

The seeds belonging to three varieties of *Amaranthus* species (*Amaranthus cruentus* "Amont", *Amaranthus hypochondriacus* "Intense Purple" and *Amaranthus* ssp. "Plenitude" were germinated "*in vitro*", in aseptic conditions, on the basal medium of Murashige-Skoog (1962), having half strength as regards the concentration of macro and microelements, 3% sucrose , 0.8% Agar Noble, pH 5,8, without the addition of hormones (Fig. 1).

For successful aseptic cultivation of explants in "*in vitro*" conditions, an important role is played by the chemical composition of the culture media. Preferential depletion of some elements leads to symptoms of deficiency or toxicity, sometimes with necrosis of the inoculum.

The basal medium Murashige - Skoog (MS), rich in nitrogen meets the nutritional requirements of explants cultured *in vitro* for most species [8]. This was also the optimal formulation for the initiation and for the transfer of *"in vitro"* cultures in *Amaranth*, following the supplementation of the culture medium with several types and combinations of phytohormones (Table 1).

a)

b)

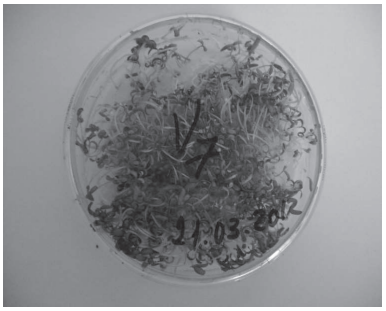

Fig. 1. *Amaranthus* seeds germinated in controlled laboratory conditions:
a.) in the Erlenmeyer flask;
b.) in Petri dishes (photo date: 3/29/2012)

Plant hormones were added before sterilization of the media by autoclaving, which was performed for 20 minutes at 121 °C, and pH correction was made to values of 5.8 to 6.

Table 1. Experimental versions of the culture medium used for in vitro culture initiation and establishment from *Amaranth* tissues

	Growth regulators				Nutritive Supliment
Variants	Auxins– mg/l			Citokinins – mg/l	CH mg/l
	NAA	2,4-D	IAA	Kin	
V1	2	-	-	1	-
V2	1	0,5	-	0.5	-
V3	2	-	-	1	200
V4	1	0,5	-	0.5	200
E7	-	-	1.8	0.022	-

Legend: NAA = acid α –naphthalene-acetic; 2,4-D = acid 2,4-dichlorophenoxyacetic ; IAA acid indolil acetic; Kin = chinetin; CH = casein hydrolyzate of.

Three types of explants (hypocotyl, cotyledon node and roots) were placed on the surface of the culture media (Variants 1 and 2) distributed in 5 cm in diameter Petri plates (containing 5 ml of sterile autoclaved culture medium variants solidified with 8 g/l agar) and the incubation was performed in the growth chamber, at 25± 2 °C, under a 16/8 h photoperiod, with a light intensity of 3000 lux. The periodical transfers on fresh culture media were performed at 3 week intervals.

The mean increasing of callus biomass / petri dish and the other evaluated morphogenetic processes (hypertrophy, appearance of adventitious roots) were recorded at intervals of 3 weeks for 3 months, depending on the type of explant and on the *Amaranthus* genotype from which explants originated.

Three different combinations of citokinins and auxins were used in the first phase of the initiation and establishment of callus cultures (V1, V2 and E7). Callus development was evident at 2 weeks after inoculation on the inductive culture media. First developed on the cut edges, callus covered gradually the whole explant over periodic transfers. The proliferative capacity of the callus cultures increased following the periodic transfers on fresh culture media, every 3 weeks for 3 months (Table 2).

Table 2. Comparative effect of the hormonal supplement of the media variants used to initiate and establish "in vitro" cultures from explants from the three genotypes of *Amaranthus* sp..

Recipe	*Amaranthus* sp. genotypes		
	Amaranthus cruentus **"**Amont*"*	***Amaranthus hypochondriacus*** **"**Intense Purple"	***Amaranthus ssp."***Plenitude"
V1	58.33%	92.15%	90.07%
V2	**66.07%**	**100%**	**100%**
E7	64.7%	**100%**	88.36

Legend: V1 (MS-1962 medium,supplemented with: 20 g / l sucrose, 7 g / l agar, 2.0 mg / l NAA and 1.0 mg / l Kin); V2 (MS-1962 medium,supplemented with: 30 g / l sucrose, 8 g / l agar, 1.0 mg / l NAA + 0.5 mg / l and 2,4-D 0.5 mg / l Kin); E7= 1,8 mg/ L IAA+0.022 mg/ l Kin

Calli developed on these variants were yellow-green, partly loose, with morphogenesis expressed by developing adventitious roots in small numbers (to *Amaranthus cruentus* **"**Amont" genotype) in average number (to *Amaranthus* ssp "Plenitude") and in large numbers (to *Amaranthus hypochondriacus* "Intense Purple").

After 3 months of ”in vitro” culture, the recorded results according to the genotype and to the type of the explant were as follows:

I. For the genotype *Amaranthus cruentus* “Amont”:

- from the root explants were obtained: 64% hypertrophied explants, 60% thereof with callus and 46% with adventitious roots;
- from the explants of hypocotyl were obtained: 100% explants were hypertrophied: 62.5% thereof with callus and 34.37% with adventitious roots;
- from the cotyledon node explants were obtained: 88.33% hypertrophied explants, of which 50.0% with callus and 46.91% with adventitious roots.

II. For the genotype *Amaranthus hypochondriacus* “Intense Purple” :

- from the root explants were obtained 100% the hypertrophied explants: 53% thereof with callus and 61.53% with adventives roots;
- from the hypocotyl explants were obtained 100% hypertrophied explants: 100% thereof with morphogenetic callus and without adventitious roots;
- and from the cotyledonary node explants were obtained 100% hypertrophied explants, 100% thereof with callus and 13.6% with adventitious roots.

III. For the genotype *Amaranthus* ssp "Plenitude":

- from the root explants resulted: 100% hypertrophied explants of which: 50.79% with callus and 59.52% with adventitious roots;
- from of hypocotyl explants resulted 96% hypertrophied explants of which: 71% with callus and 26% with adventitious roots;
- and from the cotyledon node inoculated explants resulted 54.48% hypertrophied explants of which: 84.56% with callus and 19.51% with adventitious roots.

Calluses developed from inoculated hypocotyl fragments on hormone supplements medium variants V1 and V2, were yellow-green, friable and expressed morphogenesis by developing adventitious roots, though in a fewer number than in the roots explants (Fig.2).

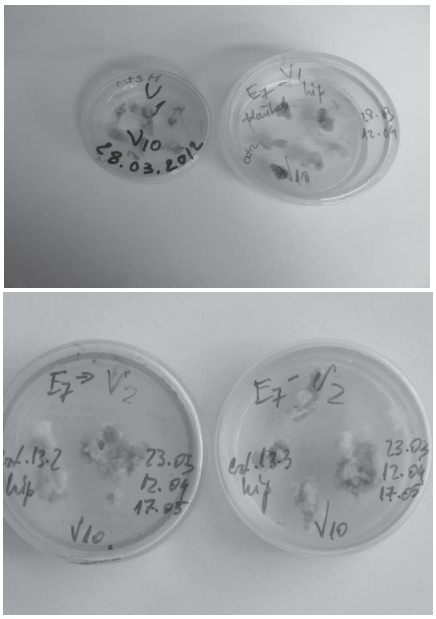

Fig. 2. The influence of explant type on morphogenesis induction in inoculated *Amaranthus* ssp "Plenitude" hypocotyl type of explants: a.) V1 (MS medium,supplemented with: 20 g / l sucrose, 7 g / l agar, 2.0 mg / l NAA and 1.0 mg / l Kin) b.) V2 (MS medium,supplemented with: 30 g / l sucrose, 8 g / l agar, 1.0 mg / l NAA + 0.5 mg / l and 2,4-D 0.5 mg / l Kin).

Calluses developed from the cotyledon node inoculated explants on variants V1, V2 and E7 hormone supplements medium, were yellow-green, loose, had a low rate of the morphogenetic development and formed adventitious roots in a smaller number of inoculated explants (Fig.3).

During the regular transfers performed every 3 weeks for 3 months on media variants V1, V2 and E7, in the presence of moderate concentrations of auxins and citokinins, a 100% multiplication rate was recorded on the V2 and E7 variants (Table 2). Development of multiple shoots from each apex was lower on V1, which consisted in a combined hormonal supplement of naphthyl acetic acid and kinetin.

By transferring the callus culture developed "in vitro" from hypocotyl and cotyledon node explants on the variants V2,V3, V4 and E7 (Table 3), the callus biomass increased every 3 weeks averaging close values, measured by the number of viable proliferative explants / culture dish.

a)

b)

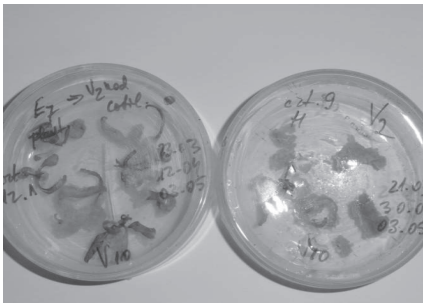

Fig. 3. The influence of explant type (cotyledon node) on morphogenesis induction in *Amaranthus* ssp "Plenitude"; a.) V1 (MS medium supplemented with: 20 g / l sucrose, 7 g / l agar, 2.0 mg / l NAA and 1.0 mg / l Kin) b.) V2 (MS medium supplemented with: 30 g / l sucrose, 8 g / l agar, 1.0 mg / l NAA + 0.5 mg / l and 2,4-D 0.5 mg / l Kin) and E7 (MS medium supplemented with: 30 g / l sucrose, 8 g / l agar, 1.8 mg / l IAA and 0.022 mg / l Kin)

Table 3. Compared effect of the phytohormone combinations (V2, V3, V4 and E7) on callus development after 60 days since the initiation of the experiment

Recipe	*Amaranthus* sp. genotypes		
	Amaranthus cruentus "Amont"	*Amaranthus hypochondriacus* "Intense Purple"	*Amaranthus ssp."*Plenitude"
V2	89.04%	91.66%	81.91%
V3	84.61%	77.7%	87.06%
V4	91.66%	90.12%	91.83%
E7	_	_	94.28%

Legend: V2= (MS medium,supplemented with: 30 g / l sucrose, 8 g / l agar, 1.0 mg / l NAA + 0.5 mg / l and 2,4-D 0.5 mg / l Kin); V3= NAA 2 mg / L + 1 mg / L Kin + 200 mg / L hidrolizat; V4= NAA 1 mg/ L+2,4 D-0,5 mg/ L +Kin- 0,5 mg/ L +200 mg/ L casein hydrolyzate: E7= 1.8 mg/ L IAA+0.022 mg/ l Kin.

Superior results were recorded on the variant V4, with values of calluses /culture vessel ranging from 90.12% to 91.83% for all the tested genotypes, and on variant E7 (94.28%), but only for root explants from *Amaranthus ssp."*Plenitude" genotype.

CONCLUSIONS

The underexploited crops, including amaranth, offer a special challenge for the use of *in vitro* approaches for micropropagation of selected genotypes and their subsequent exploitation, rescue of the genetic variation or inducing new variation, phytoremediation studies and using the cell biomass to obtain phytochemicals of practical interest.

Our observations on the effect of phytohormones on the evolution of *Amaranthus* sp. explants in *"in vitro"* culture conditions after 3 weeks since inoculation led to conclude that the phytohormones such as the auxins NAA, 2,4-D and IAA had a stimulating effect on callus development and morphogenesis expressed by the development of adventitious roots.

Thus, callus induction, callus growth and organogenetic processes expressed by root development were achieved under the effect of moderate concentrations of these auxins, alone or in combination with low concentrations of citokinins (kinetin).

Therefore, our experimental data reveal the potential of somatic explants of *Amaranthus sp.* to develop *in vitro* long-term, continuous callus cultures, under the effect of optimal hormone concentrations, which alongside the genotype and the type of explants represent important factors that influence the obtaining of cell biomass in reliable quantities, to be used for practical purposes.

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