REASEARCH ON PROTEIN CONTENT OF *Amaranthus cruentus* CALLUS

MAXIMILIAN Carmen, DĂNĂILĂ GUIDEA Silvana Mihaela, RĂDUCAN Cristina, BĂBEANU Narcisa Elena

Abstract. The species *Amaranthus cruentus* belongs to the family *Amaranthaceae* and is considered a pseudocereal. The seeds of *Amaranthus* sp. have a high nutritional value due to the presence of a large amount of important biochemical compounds for human consumption. Most biochemical constituents of proteins, lipids, minerals, vitamins are present in larger amount compared to other cereal species. The aim of our research is to evaluate the protein content of *Amaranthus cruentus* calli. The obtaining of calli fragments of somatic tissues of different origins (hypocotyls, roots) was achieved starting from aseptic germinated seedlings. The suitable culture media for obtaining calli culture were basal medium Murashige-Skoog 1962 supplemented with different plant growth regulators (PGR) α-naphthaleneacetic acid (NAA) and kinetin (kin) (V1) and basal medium Murashige-Skoog 1962 supplemented with NAA, kin, 2,4-dichlorophenoxyacetic acid (2,4 D) and enzymatically hydrolysed casein (V2); the explant source was hypocotyl from cotyledonary node. The protein content using Bradford (1976) method and evaluation of protein electrophoretic spectra was accomplished.

Keywords: amaranth, callus, protein content.

Rezumat. Studii privind determinarea conținutului proteic în calusul de *Amaranthus cruentus*. Specia *Amaranthus cruentus* considerată o pseudocereală aparține familiei *Amaranthaceae.* Semințele de *Amaranthus* sp. au o valoare nutritivă ridicată datorită prezenței într-o cantitate mare, a unor compuși biochimici importanți pentru alimentația umană. Majoritatea componenților biochimici, proteine, lipide, substanțe minerale, vitamine, sunt prezenți în cantitate mai mare, comparativ cu alte specii de cereale. Scopul lucrării a fost determinarea conținutul proteic al calusului de *Amaranthus cruentus.* Obținerea calusului s-a realizat pornind de la fragmente de țesuturi somatice cu diferite origini (hipocotil, rădăcini) provenite de la plante obținute prin germinarea aseptică a semințelor. Mediile de cultură care au asigurat o bună proliferare celulară au fost mediul bazal Murashige-Skoog, 1962 adiționat cu diferiți fitohormoni acjd α naftilacetic (ANA) si kinetină Kin (V1) și mediul bazal Murashige-Skoog, 1962 adiționat cu ANA, kin, acid diclorofenoxiacetic (2,4 D) și hidrolizat de cazeină, sursa de explant fiind hipocotil cu zona de nod cotiledonar. Cele două tipuri de calus au fost evaluate în ceea ce privește conținutul proteic prin dozarea -metoda Bradford (1976) și respectiv evidențierea spectrului electroforetic.

Cuvinte cheie: amarant, calus, conținut proteic.

INTRODUCTION

In the current global agricultural development, in order to protect biodiversity and to practice a more friendly environmental agriculture and due to the changes in diet patterns, the diversification of agricultural production and to the consumers demands for traditional and enhanced nutritional quality products, the attention of the professionals–has moved to other less known and less cultivated crops as pseudo-cereals: Amaranth, quinoa, buckwheat, wild rice, which can become an alternative to the traditional cultivated species. (AKUBUGWO et al., 2008).

Such a culture is the *Amaranthus* spp. from Amaranthaceae family. *Amaranthus* species are known crops, used for a long time, which currently have high potential as a food resource for humans or animals because seeds are rich in protein, with high content of quality starch, and also nitrogen and at the same time, easily digestible vegetative tissues.

Cultures of the species *Amaranthus gangeticus, A. tricolor, A. viridis, A. palmeri, A. blitum*, etc. are currently widespread in South America, North America, Asia and Africa in order to valorize the nutritional and therapeutic potential of their vegetal biomass. *A. cruentus* L. and *A. hypochondriacus* L. are native from Mexico and Guatemala. *A. caudatus* L. is native from the Andean regions of Ecuador, Peru, and Bolivia (SAUER, 1967). In the current publications, there are published the results related to different elements of the culture technology, the obtaining of improved plant varieties, the chemical composition and to the nutritional value of the seed *Amaranthus* spp. varieties, also to the high content in essential amino acids, the oil production and the oil composition (6-7% squalene: antioxidant / anticancer).

Amaranthus species are grown also as ornamental plants, pseudo-cereals and leafy vegetables. According to the results of the researches published in the year 1990 by KAUFFMAN & WEBER, in this scientific area it is recommended a cultivation technology depending on the climatic conditions of the specific area.

Also, leaves, stems or flowers can be used both as feed and as a source of natural food colours (XU & SUN, 2001; BARBA DE LA ROSA et al., 2009).

Also, in two recent publications in Romania, there have been studied the agro-technical aspects of the production of seeds and leaves of 12 varieties of *Amaranthus* spp. (MARIN et al., 2011; RUSU et al., 2010).

Certain researchers (BENNICI et al., 1992; BENNICI et al., 1997) published some important papers with the results about the induction of the callus development, aspects that were investigated starting from different types of amaranth explants: hypocotyl and epicotyl cultures of four agronomical interesting species of *Amaranthus* (*A. caudatus, A. cruentus, A. hybridus, A. hypochondriacus).* However, callus and production of adventitious shoots from callus or primary explants (hypocotyl) have been previous reported by FLORES et al. (1982) and FLORES & TEUTONICO (1986). Previous studies conducted by FLORES et al. (1982) and BENNICI et al. (1992) on several species and varieties of the genus *Amaranthus* showed the potential of explants with regard to dedifferentiation and morphogenetic *in vitro* processes.

MATERIAL AND METHODS

Plant material

To initiate the "*in vitro*" culture experiments in our research we used the seeds collected from the genotypes *A. cruentus* in 2015 as a source of biological material. The seeds were surface sterilized for 15 minutes with a 10% w / v solution of commercial bleach concentrated sodium hypochlorite (minimum 5%).

They were aseptically germinated on MURASHIGE & SKOOG (1962) medium with GA_3 (gibberellic acid) (0.5 mg/l), for 2 weeks. The experiments for callus obtaining were initiated from seedling explant sources and were carried out using 10 -15 days old plantlets obtained by germinating seeds in aseptic condition (Fig. 1).

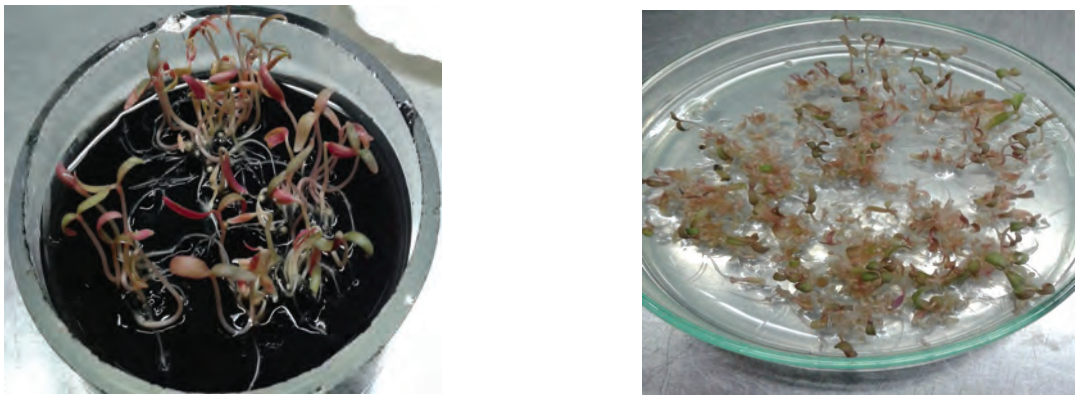

Figure 1. The seeds of *A. cruentus* variety "Amont" germinated under aseptic conditions on MURASHIGE & SKOOG (1962) supplemented with GA_3 (left supplemented with charcoal, right without charcoal) (original photo, Laboratory of Plant biotechnology from the Faculty of Biotechnology U.S.A.M.V. Bucharest).

In order to stimulate "*in vitro*" callus induction and development morphogenetic process from hypocotyl the following PGR variants in 2 different combinations (Table 1) were added: V1, V2 and repeated subculture on fresh media with the same composition at every 3 weeks during 3 months. The callus regenerated from roots was not proliferative. The calli proliferated on V1 and V2 media was used for biochemical analyses: protein electrophoresis and protein assay.

Table 1. Variant of culture medium used for *A. cruentus* "Amont" explants cultured "*in vitro*".

Variant	Composition media	Explant
V1 ("*C2*")	M&S (1962)+20 g/l sucrose+7 g/l agar+2.0 mg/l NAA+1.0 mg/l Kin;	hypocotyl
V2 ("*C3*")	M&S (1962) +30 g/l sucrose +8 g/l agar+1.0 mg/l NAA + 0.5 mg/l 2,4-D+0.5 mg/l Kin + 200 mg/l CH	Hypocotyls, roots

Legend: MS *Abbreviations:* M&S – Murashige &Skoog basal culture medium (1962); NAA – α-naphthaleneacetic acid; 2,4-D – 2,4-dichlorophenoxyacetic acid; Kin– kinetin, CH = enzymatically hydrolysed casein

The protein extraction was performed by grinding the calli tissue in 50 mM potassium phosphate buffer, pH 7.2 mM EDTA, pH = 6.8, pvp (polyvinyl pyrrolidone) 4%, (in relation to 1 g / 0.5 ml, dry weight / buffer) at 4°C for 24 hours. The extract was centrifuged at 18.000 rpm for 20 min and the supernatant was used for electrophoresis analysis. Electrophoresis was carried out in 10% polyacrylamide gel (running gel) with 0.5% SDS and the running buffer was Tris-Base-glycine, SDS pH 8,3 (LAEMMLI, 1970). Samples were loaded into each well and then electrophoresis was made at 10 mA through the stacking gel for 30 min and 15 mA through the running gel for 2h. The gels were stained in Coomassie Blue and then in destaining solution (45% ethanol, 10% acetic acid).

For detecting the molecular weight of the proteins from protein extract a Gene Tools software was used. The photos were taken from Syngene, a basic gel doc system, capturing digital images. The broad Range Protein Molecular weight Marker delivered from Promega was used. This molecular marker consists of nine proteins with a molecular weight of 225,150,100,75,50,25,35,25,15 and 10 kDa. Each protein is present in concentration of 0.1 mg/ml, except for the 50kDa, which is present at 0.3mg/ml.

The protein concentration was carried out using BRADFORD method (1976) based on binding of protein by Coomassie Blue and measurement of the absorbance of protein-dye complex.

RESULTS AND DISCUSSIONS

Callus induction and growth

The observations related to the effect of PGR on the evolution of *Amaranthus cruentus* "Amont" explants cultured "*in vitro*" after 3 weeks from the inoculation, led to conclusion that the auxins such as α –naphthalene-acetic acid (NAA) 3-indolyl-acetic acid (IAA) and 2,4-D diclorofenoxic acid (2,4-D), stimulated, in varying proportions, the explant hypertrophy and the cytokines type - kinetin (Kin) caused the elongation of up to 5-10 cm of the preformed main apical shoot or axillary buds of explants, with a start of the development of multiple shoots in the inoculated cotyledonary node explants (Fig. 2).

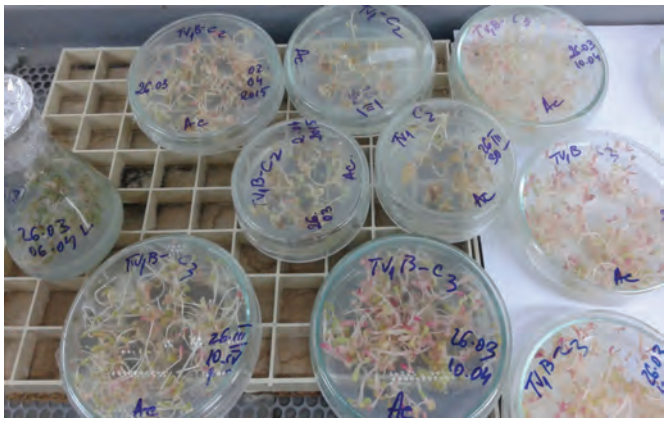

Figure 2. Explant morphogenesis induction on *A. cruentus* under the influence of the combinations of plant hormones used in variants V1 and V2 (original photo).

First developed on the edges of the cut explant, the callus gradually covered, over periodic transfers, the whole mass of the original tissue hypocotyl fragment type and root fragments. After the induction and the establishment of the "*in vitro*" callus cultures on V1 and V2 variants of the cultivation medium, the material was transplanted two times (after the 10^{th} and 20^{th} day) and the results were collected on the 30^{th} day as number of explants with callus and regenerated shoots (or roots).

The results for callus growth capacity were revealed over a period of 1 month. During periodic transfers at intervals of 3 weeks for 3 months on nutritional formulas V1, V2 in the presence of moderate concentrations of auxin and cytokinin, it was obtained a very good multiplication rate of 100% on V2 variant; there was developed callus from fragments of hypocotyl type, as well as multiple shoots from each apex elongated from the initial explant of the cotyledonary node; there were registered weak results on version V1, at which the phytohormonal supplement was represented by kinetin and α naphthyl acetic acid.

The transfer media used for "*in vitro*" *Amaranthus* species culture were represented both by the recipes of phytohormones of the initial variants **V1** (2 mg/l NAA + 1 mg/l Kin) and **V2** (1 mg/l NAA + 0.5 mg/l 2.4-D + 0.5 mg/l Kin) that were supplemented by the addition of 200 mg/l casein hydrolysate in the basal medium Murashige &Skoog (1962). This organic complex ingredient stimulates the caulogenesis process by the contribution of the amino acids, vitamins and growth regulators to the composition of the cultivation medium.

By transferring the "*in vitro*" callus cultures on the V2 transfer recipes variants, the callus biomass increases at every 3 weeks were assessed by the number of explants/culture dish and there were on an average basis similar values for the three used *Amaranth* sp. genotypes (Fig. 3.)

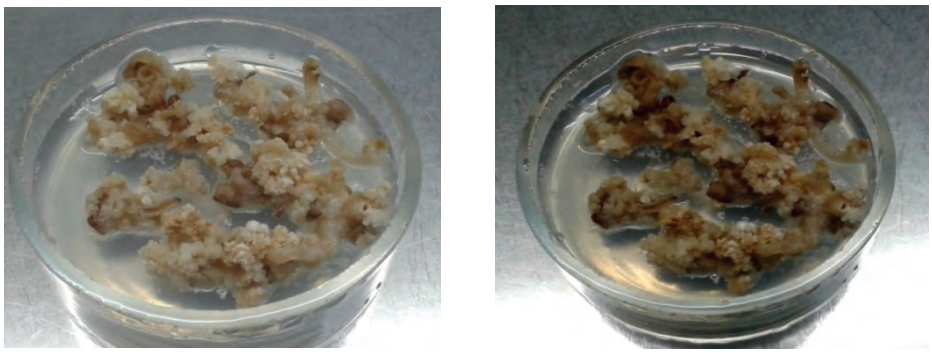

Figure 3. Appearance of callus inoculated explants obtained at: *A. cruentus* "Amont"
(original photo, 2016- Plant Biotechnology Laboratory of the Faculty of Biotechnology U.S.A.M.V. Bucharest).

Biochemical analyses

For the electrophoretic spectrum 3 repetitions (represented by track 2, track 3 and track 4) of calli cultivated on V1 media were used. The electrophoretic spectrum revealed a number of 14 bands (isoforms) for the callus cultivated on V1 medium (Fig. 4).

The first track (track 1) represents the molecular marker. (Fig. 5).

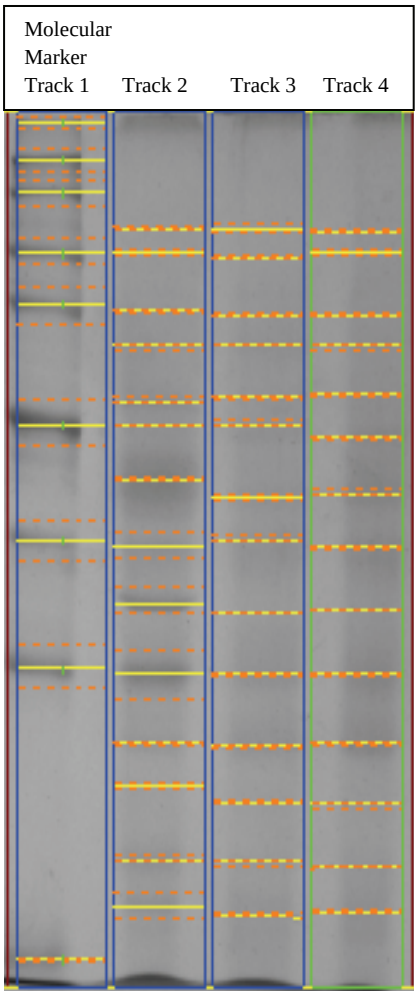

Figure 4. Electrophoretic spectrum of the protein extracted from the callus proliferated on V1 medium (original).

Track 1			
No.	**Molecular Weight (KDa)**	**Height**	**Raw vol.**
1	225.00	9.488	6322.56
2	150.00	30.027	23675.61
3	100.00	23.542	19350.38
4	75.00	19.848	19938.31
5	50.00	19.466	27820.39
6	35.00	38.235	68176.79
7	25.00	16.779	26205.49
8	15.00	21.624	32926.24
9	10.00	7.639	2040.43

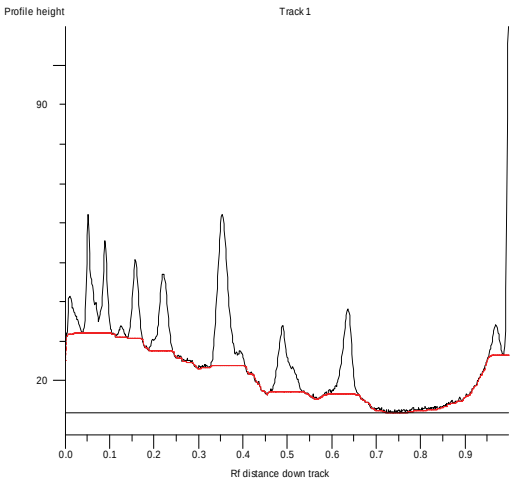

Figure 5. Protein Molecular weight Marker represented by track 1 and profile height of the peak according to the distance proteins migration. (Rf)

The molecular weight of the protein from the electrophoretic bands varied from 84.15 Kda to 10.79 kDa for track 2 (Fig. 6), 83.66 to 10.65 kda for track 3, 83.18 to 10.7 Kda for track 4.

Track 2			
No.	**Mol. Weight (KDa)**	**Height**	**Raw vol.**
1	84.15	1.044	167.65
2	75.00	0.359	74.92
3	48.94	1.677	363.78
4	44.29	2.033	662.77
5	37.32	5.491	1665.87
6	34.76	0.415	38.06
7	29.53	14.444	3725.20
8	24.42	6.650	8107.16
9	19.41	18.074	21722.87
10	14.93	21.209	39536.51
11	13.57	4.351	767.13
12	12.74	1.069	225.24
13	11.50	6.321	2583.70
14	10.79	5.807	7312.16

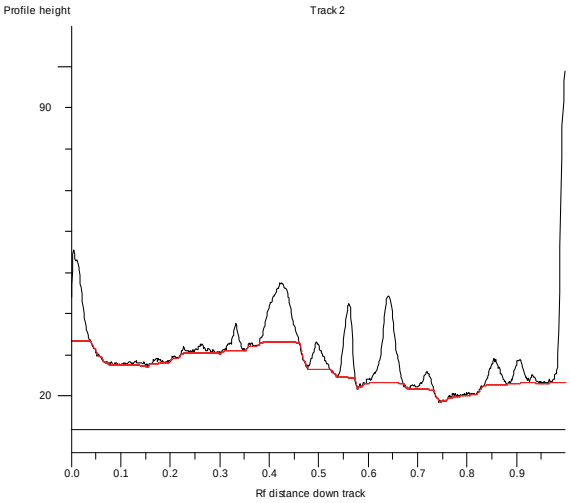

Figure 6. Protein Molecular weight represented by track 2 and profile height of the peak according to the distance proteins migration (Rf).

For the electrophoretic spectrum 3 repetitions (represented by track 6, track 8 and track 10) of the calli cultivated on V2 media were used. The electrophoretic spectrum revealed a number of 20 bands for the callus cultivated on V2 medium (Fig. 7).

The first track (track 1) represents the molecular marker (Fig. 8). The molecular weight of the protein from the electrophoretic bands varied from 61.67 Kda to 10.42 kDa for track 6 (Fig. 9), 62.55 to 13.57 kda for track 8, 64.73 to 10.57 Kda for track 10. The electrophoretic spectrum of the protein revealed a number of 20 bands for each repetition (Track 6, 8, 10).

Track 1			
Number	**Mol. weight**	**Height**	**Raw vol.**
1	225.00	5.219	5299.04
2	150.00	11.895	70184.31
3	100.00	9.429	73699.91
4	75.00	8.329	64820.17
5	50.00	5.741	43205.61
6	35.00	5.506	18085.32
7	25.00	6.939	55423.96
8	15.00	4.331	11398.76
9	10.00	5.799	31542.06

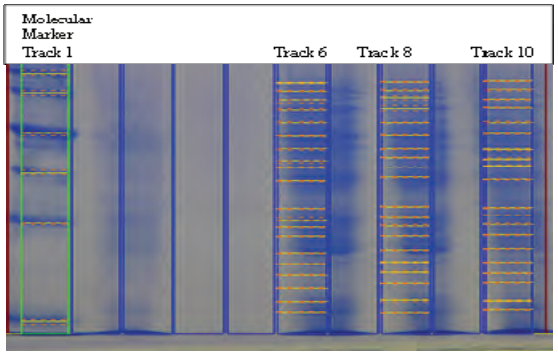

Figure 7. Electrophoretic spectrum of the protein extracted from the callus proliferated on V2 medium.

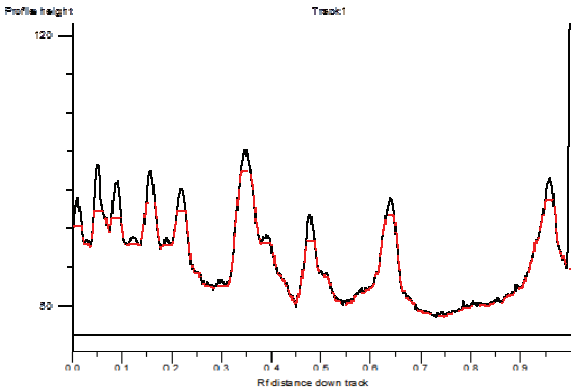

Figure 8. Protein Molecular weight Marker represented by track 1 and profile height of peak according to the distance proteins migration (Rf).

Track 6			
No.	**Molecular Weight (kDa)**	**Height**	**Raw vol.**
1	61.67	0.501	355.17
2	51.95	1.512	1486.71
3	46.98	3.495	12723.67
4	43.05	4.262	14637.58
5	38.07	0.896	1220.59
6	34.02	1.211	1702.51
7	30.74	1.293	3043.72
8	27.84	1.329	2193.27
9	26.24	0.641	1084.61
10	23.11	0.830	914.25
11	17.51	0.911	1007.82
12	15.41	1.954	6047.99
13	14.77	0.903	986.99
14	14.23	0.296	210.31
15	13.30	0.242	177.45
16	12.71	1.415	2641.54
17	12.111	0.810	696.16
18	11.48	0.697	976.12
19	10.88	0.680	634.05
20	10.42	2.295	4286.15

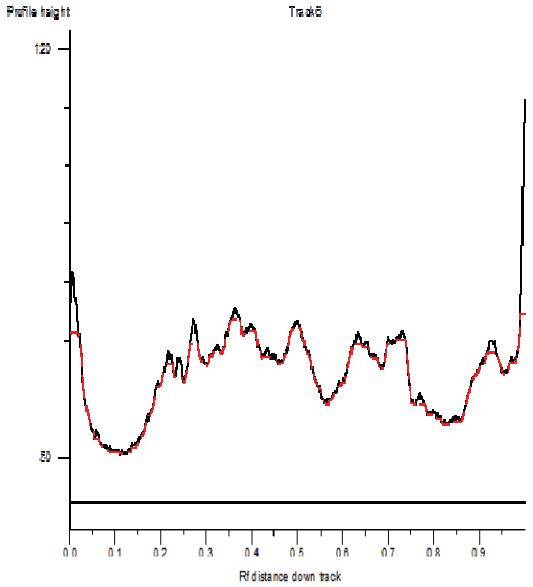

Figure 9. Protein Molecular weight represented by track 6 and profile height of peak according to the distance proteins migration (Rf).

For the protein assay 3 repetitions of calli cultivated on V2 media were used. Our result emphasized a concentration of the protein between 307.25 and 361.11 µg/ml /g fresh weight callus.

Our result emphasized a concentration of the protein from the callus proliferated on V1 medium between 628.9 and 495.66 µg/ml /g fresh weight callus and 361.11 and 307.25 µg/ml /g fresh weight callus for V2 medium emphasized using Bradford method (1976) (Fig. 10).

The content of the protein in the callus cultivated on V1 medium was higher comparative with the callus proliferated on V2 medium (Figure 10). The number of electrophoretic bands of the callus cultivated on V1 medium (14) was lower than the callus cultivated on V2 medium (20). It is possible that PGR existing in V1 medium to amplify the protein biosynthesis and increase the concentration and callus biomass, but inhibit certain types of proteins with specific molecular weight.

These results can offer more information regarding the effect of PGR in culture media used in our experiments.

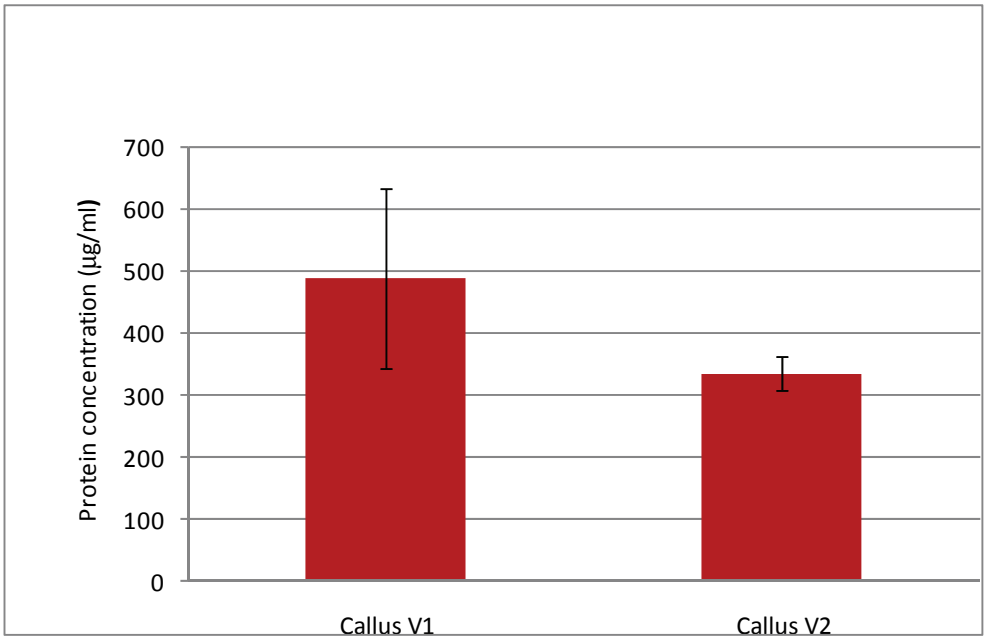

Figure 10. Variation of protein concentration of *A. cruentus* calli cultivated on V1 medium and V2 medium.

Recent studies achieved by ACOSTA et al., 2016 showed that amaranth seed shows protein fractions of globulins and albumins as storage protein. Globulins (7S and 11S), albumins (2S), and glutelins are the major protein fractions in amaranth seeds. He demonstrated using SDS-PAGE electrophoresis that the predominant *Amaranth* storage proteins are globulins 7S and 11S. 7S presents a molecular weight of 41-45 kDa. Recently, globulin 11S from amaranth has been named amaranthine (ROMERO-ZEPEDA & PAREDES-LOPEZ, 1996). This protein has two subunits consisting of an acid polypeptide (AS) (33-36 kDa) and a basic polypeptide (AB) (16-19 kDa). The 2S albumins have been described like a band of low molecular weight near 6-10 kDa.

The protein electrophoretic bands could be a type of globulins: band no. 4 with a molecular weight of 44.29 KDa in the callus proliferated on V1 medium and band no. 4 with a molecular weight of 43.05 kDa in the callus proliferated on V2 medium.

Other studies used the electrophoresis profiles of proteins on SDS-PAGE for the evaluation of amaranth diversity. The first characterization of the protein fraction spectra of amaranth species was performed by GORINSTEIN et al. (1991) and DRZEWIECKI et al. (2003). In 2011, DZUNKOVA et al., set up the methodology for the identification of the amaranth species using glutelin protein fraction. JANOVSKA et al., 2012 compared spectra of storage proteins and their fractions of wild weedy and cultivated species of amaranths and verified the suitability of this method for species identification in the collection of the Czech Gene Bank.

In this respect, we used the SDS PAGE for analysing not only glutelin protein fraction but all proteins from the extract for showing the high number of proteins and nutritional profile of the species. The studies were completed by analyses of protein concentration.

CONCLUSIONS

In the tested variants, our experimental data demonstrated the potential of the versions of *Amaranthus* somatic callus cultures to further develop, under "in vitro" conditions, on long term, under the effect of optimal phytohormonal levels.

The transfer of the callus from V1 medium on V2 medium determined inhibitions of the protein biosynthesis but the number of electrophoretic bands increased.

For obtaining high protein content is suitable to cultivated amaranth callus on V1 medium containing NAA, Kin, 2,4D and CH.

REFERENCES

ACOSTA C., CARPIO C., VILCACUNDO R., CARRILLO W. 2016. Identification of proteins isolate from Amaranth (*Amaranthus caudatus*) by sodium dodecyl sulphate-polyacrylamide gel electrophoresis with water and Nacl 0.1 M solvents, *Asian Journal of Pharmaceutical and Clinical Research.* Innovare Academic Sciences Pvt Ltd. Madhya Pradesh. **9**: 331-333.

AKUBUGWO I. E, OBASI N. A, CHINYERE G. C, UGBOGU A. E. 2008. Mineral and phytochemical contents in leaves of *Amaranthus hybridus* L. and *Solanum nigrum* L. subjected to different processing methods, *African Journal of Biochemistry Research.* Academic Journals, Ebene, Lagos. Nairobi. **2**: 40-44.

BARBA DE LA ROSA, A.P., INGE FOMSGAARD S., LAURSEN B., MORTENSEN A. G., OLVERA-MARTINEZ L., SILVA-SANCHEZ C., MENDOZA-HERRERA A., GONZALEZ-CASTANEDA J., DE LEON-RODRIGUEZ. A. 2009. Amaranth (*Amaranthus hypochondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flavonoids with potential impact on its nutraceutical quality. *Journal of Cereal Science*. Edit. Elsevier. Amsterdam. **49**: 117–121.

BENNICI A., SCHIFF S., BOVELLI R. 1992. *In vitro* culture of species and varietes of four *Amaranthus* L. species. Euphytica. Edit. Springer. Berlin. **62**: 181-186.

BENNICI A., GRIFONI T., SCHIFF S. & BOVELLI R. 1997. Studies on callus growth and morphogenesis in several species and lines of *Amaranthus. Plant Cell, Tissue and Organ Culture*. Edit. Springer-Verlag. Berlin. **49**: 29-33.

BRADFORD MARION. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry.* Edit. Elsevier. Amsterdam. **72**: 248-254.

DRZEWIECKI J., DELGADO-LICON E., HARUENKIT R., PAWELZIK E., MARTIN-BELLOSO O., PARK Y. S., JUNG S. T., TRAKHTENBERG S., GORINSTEIN S. 2003. Identification and Differences of Total Proteins and Their Soluble Fractions in Some Pseudocereals Based on Electrophoretic Patterns. *Journal of Agricultural and Food Chemistry.* ACS Publication, Washington. **51**(26): 7798-7804.

DZUNKOVA M., JANOVSKA D., HLASNA-CEPKOVA PETRA, PROHASKOVA A., KOLAR M. 2011. Glutelin protein fraction as a tool for clear identification of Amaranth accessions. *Journal of Cereal Science.* Edit. Elsevier, Amsterdam. **53**: 198-205.

FLORES H. E. & TEUTONICO R. A. 1986. Amaranths (Amaranthus spp.): Potential grain and vegetable crops, In: Y.P.S. Bajaj (Ed). *Biotechnology in Agriculture and Forestry*. Crops I. Edit. Springer-Verlag, Berlin, Heidelberg. **2**: 568-577.

FLORES H. E., THEIR A., GALSTON A. W. 1982. In vitro culture of grain and vegetable Amaranths (*Amaranthus* spp.). *American Journal of Botany*. Botanical Society of America. St. Louis. **69**: 1049-1054.

GORINSTEIN S., MOSHE R., GREENE L. J. 1991. Evaluation of four *Amaranthus* species through protein electrophoretic patterns and their amino acid composition. *Journal of Agricultural and Food Chemistry*. ACS Publication. Washington. **39**(5): 851-854.

KAUFFMAN C. S. & WEBER L. E. 1990. Grain amaranth. In: *Advances in new crops.* Eds. J. Janick and J. E. Simon. Edit. Timber Press. Portland. OR: 127-139.

JANOVSKÁ DAGMAR, HLÁSNÁ ČEPKOVÁ PETRA, DŽUNKOVÁ MÁRIA 2012. Characterisation of the Amaranth Genetic Resources in the Czech Gene Bank. *Genetic Diversity in Plants*. Prof. Mahmut Caliskan (Ed.), ISBN: 978-953-51-0185-7, In: *Tech*. Available from: http://www.intechopen.com/books/genetic-diversity-in-plants/characterisation-of-the-amaranth-genetic-resources-in-the-czech-gene-bank (Accesed February 12, 2016).

LAEMMLI U. K. 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature*. Nature Publishing Group, London. **227**(5259): 608-685.

MARIN D. I., BOLOHAN C. M., MIHALACHE RUSU T. 2011. Research on *Amaranthus cruentus* L. and *Amararanthus hypochondriacus* L. species grown in south-eastern Romania (Moara Domnească-Ilfov), *Scientific Papers.* U.A.S.V.M. Bucharest. Series A. **54**: 297-303.

MURASHIGE T. & SKOOG F. 1962. *A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum*. Wiley-Blackwell**,** Hoboken. New Jersey. **15**(3): 473-497.

ROMERO-ZEPEDA H. & PAREDES-LOPEZ O. 1996. Isolation and characterizaton of amaranthine. The 11S amaranth seed globulin. *Journal Food Chemistry*. Edit. Elsevier. Amsterdam. **19**: 329-39.

RUSU T., MARIN D. I., MORARU P. I., BOGDAN I., SOPTEREAN M. L. 2010. Agro-technique studies on foliage and seed production of some *Amaranthu*s cultivars from the Somesan Plateau conditions. *Analele Universităţii din Craiova, seria Agricultură-Montanologie-Cadastru*. **40**. /1.

SAUER J. D. 1967. The grain amaranths and their relatives: a revised taxonomic and geographic survey. *Annals of Missouri Boanical Garden*. Missouri Botanical Garden Press. St Loius. **54**: 103-137.

XU F. & SUN M. 2001. Comparative analysis of phylogenetic relationships of grain amaranths and their wild relatives (*Amaranthus;* Amaranthaceae) using internal transcribed spacer, amplified fragment length polymorphism, and double-primer fluorescent intersimple sequence repeat markers. *Molecular Phylogenetic Evolution.* Edit. Elsevier. Amsterdam. **21**(3): 372-387.

Maximilian Carmen
Institute of Biology, the Romanian Academy,
269 Splaiul Independenței, Bucharest. Romania.
E-mail: carmen.voichita@ibiol.ro

Dănăilă Guidea Silvana Mihaela
University of Agronomic Sciences and Veterinary Medicine Bucharest,
Faculty of Biotechnology, Bucharest, Romania.
E-mail: silvana.danaila@yahoo.com

Răducan Cristina
University of Agronomic Sciences and Veterinary Medicine Bucharest,
Faculty of Biotechnology, Bucharest, Romania.

Babeanu Narcisa Elena
University of Agronomic Sciences and Veterinary Medicine Bucharest,
Faculty of Biotechnology, Bucharest, Romania.

Received: March 31, 2016
Accepted: June 25, 2016