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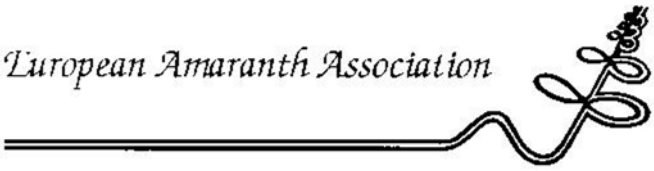

Amaranth - Plant for the Future

Book of Abstracts

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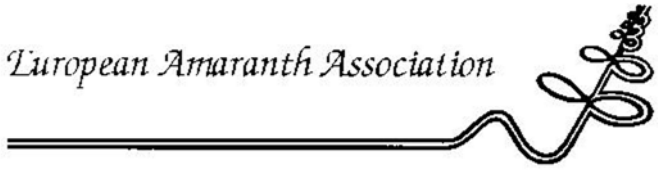

Amaranth - Plant for the Future

organized by

Institute of Plant Genetics and Biotechnology,

Slovak Academy of Sciences, Nitra, Slovak Republic

and

AMR AMARANTH a.s., Blansko, Czech Republic

Plenary session I:
Importance of amaranth in human nutrition - impact on human health, biomedicine and industrial processing

THE AMARANTH: FUTURE-FOOD PROJECT – PRELIMINARY RESULTS ON PHYTOCHEMICALS IN GRAINS OF 12 VARIETIES

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Abstract

The immediate objective of the AMARANTH: FUTURE-FOOD project is to provide the tools for an extensive and sustainable exploitation of amaranth. The project covers research in the following areas: Industrial exploitation of individual amaranth constituents; Evaluation of the health effects on humans and animals of amaranth based food; Identification of genes and gene complexes responsible for resistance to insects, fungi, drought and salinity; Selection of varieties with high competitiveness; Field studies on amaranth cultivation in varying sites; Application of multivariate statistical analysis to the generated data to identify correlated patterns among gene expression; Introduction of amaranth cultivation in Nicaragua to empower Nicaraguan single provider women for obtaining food security. Phytochemicals are chemicals generally of small molecular masses that occur in a high number in plants. They often have health promoting effects, for which reason investigations on these compounds have achieved major interest during the last decade. Thanks to the existence of advanced instrumentation for chemical analysis phytochemical profiles can now be revealed. The present study showed that the concentration levels of phenolic acids and flavonoids varied between varieties and between cultivation sites. The importance of these results will be discussed during the presentation in the meeting.

Key words - secondary metabolites, liquid chromatography, tandem mass spectrometry

Introduction

Health effects of phytochemicals such as flavonoids and phenolic acids have been reported in numerous papers. The occurrence of these compounds in cereals, fruits and vegetables has also been of high interest for decades. Occurrence of these compounds in amaranth seeds or seed flour however until now was only investigated in few samples and reported in scientific literature by Klimczak *et al*., (2002) and Barba de la Rosa *et al*., (2008). The

purpose of this study was to obtain a qualitative and quantitative profile of phytochemicals in seeds of 18 amaranth varieties, grown in Czech Republic, Argentina, Mexico and Spain.

Material and Methods

Seed samples were collected from field trial experiments in Czech Republic (Prague and Olomouc), Argentina, Mexico and Spain. An aliquot of each sample was extracted by accelerated solvent extraction (ASE) and analyzed in Applied Biosystems liquid chromatography tandem mass spectrometry equipment.

Pure compounds of isoquercitrin, nicotiflorin, rutin, caffeic acid, ferulic acid, salicylic acid, syringic acid, sinapic acid, coumaric acid, vanillic acid, 4-hydroxybenzoic acid and protocatechuic acid were used as standards for the quantification. Details on the analytical method can be found in Barba de la Rosa *et al*., (2008).

Results and Discussion

Results from Czech Republic (Prague) and Argentina are shown in Figure 1- 4. Significant differences were seen both between varieties and between cultivation sites. Analysis of the same varieties from Czech Republic (Olomouc), Mexico and Spain are currently taking place in our lab. The results will be presented and discussed in the meeting.

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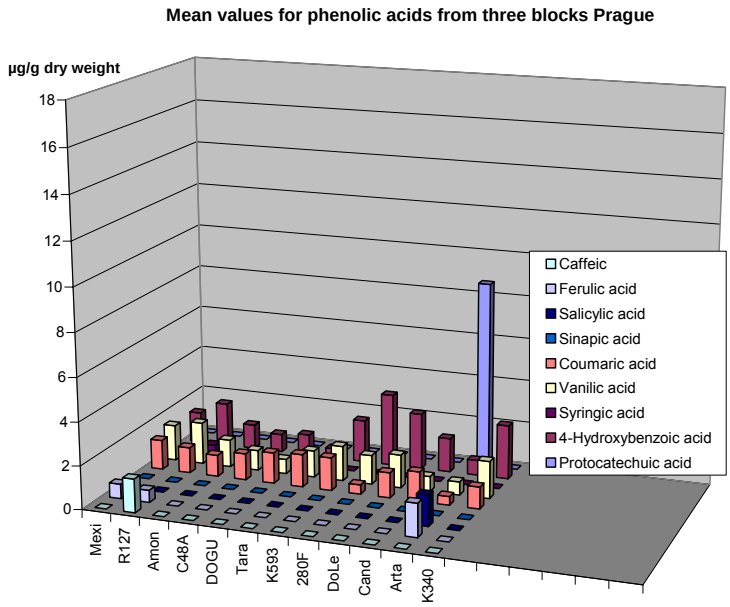

Fig. 1 Results of phenolic acid analysis in amaranth seeds grown in Prague

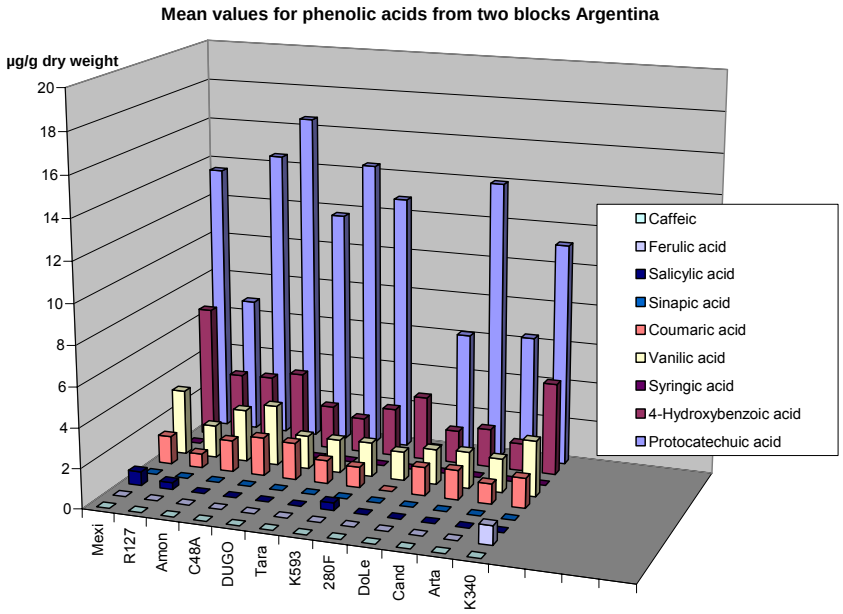

Fig. 2 Results of phenolic acid analysis in amaranth seeds grown in Argentina

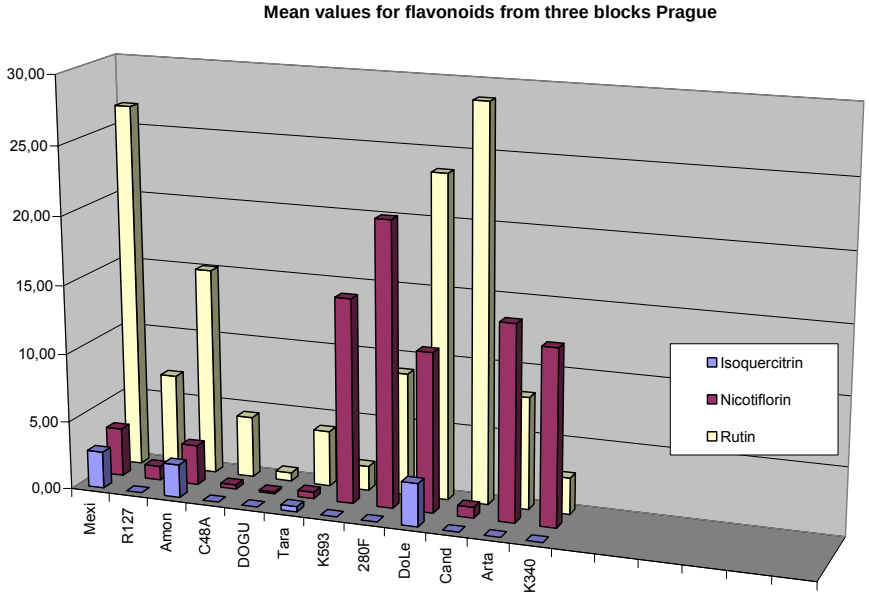

Fig. 3 Results of flavonoid analysis in amaranth seeds grown in Prague

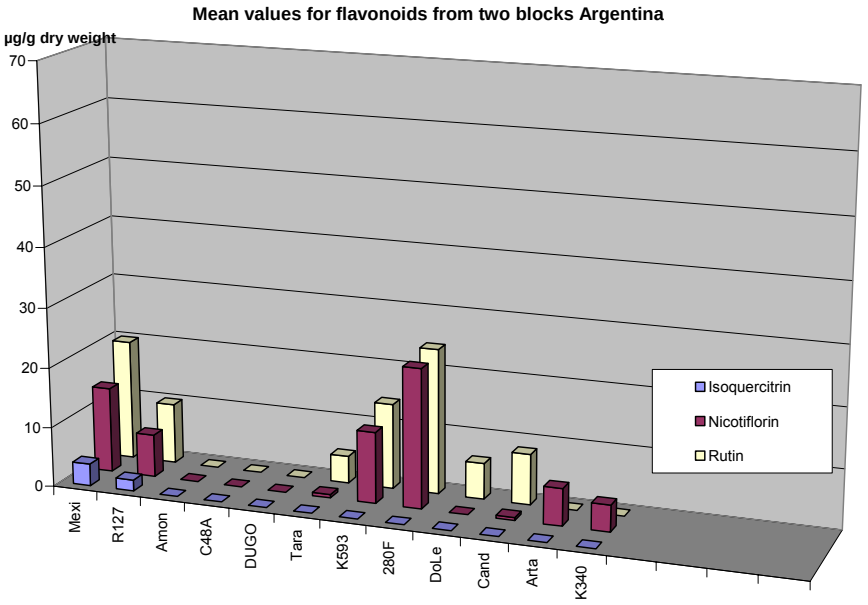

Fig. 4 Results of flavonoid analysis in amaranth seeds grown in Argentina

THE AMINO ACID CONTENT IN AMARANTH SEEDS (*Amaranthus* sp.)

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Abstract
Seeds of amaranth plants contain high portion of amino essential acids - especially LYS (7.59 $g.kg^{-1}$ of dry matter), but also GLX, ASX, LEU, ARG, GLY and SER. *A. hybrid* contains the highest portion of amino essential acids. The differences between genotypes are significant.

Key words - *Amaranthus sp., genotype, variety, amino essential acids*

Introduction

In comparison to cereal crops Amaranth (*Amaranthus sp*.) grain features high nutrition value (Vavreinová, 1997; Jarošová *et al*., 1997). When compared the basic grain composition between particular amaranth varieties grown in the Czech Republic, *A. hypochondriacus* variety and its hybrids tend to generate higher protein content, whereas *A. cruentus* varieties tend to higher fibre material content generation (Jarošová *et al*., 1997). Average protein content in dry matter of grain reach between 17 - 18.5 % (Michalová, 1995). Amaranth protein digestibility reach about 80 % which is very high value when compared to other crops – maize (45 %), wheat (60 %), soya bean (68 %) and products - cow milk (72 %) (Vietmeyer, 1982; Bressani, 1992). According to Michalová (1999) amaranth protein composition is near to the ideal protein recommended by FAO / WHO / UNU (1973). Lysine content in amaranth wholemeal flour is app. trice higher compared to wheat flour (Buchtová *et al*., 1996). The goal of this work is to compare amino-acids content in grown amaranth genotypes.

Material and Methods

Poly-factorial experiments using eight genotypes of tree amaranth species: *Amaranthus hypochondriacus* (No 1008, Koniz), *Amaranthus hybrid* (K 432, K 433, Dakota), *Amaranthus cruentus* (Amar, A 200 D, Olpir) were carried out in years 1997 - 2000 in České Budějovice (389 m. n. m., annual mean temperature 8,2 ˚C, average annual rainfall 570 mm, sandy loam soil) and in Olomouc (220 m. n. m, 8,7 ˚C, 582,8 mm, sandy loam soil). Parcel area - 10 m^2, sowing rate - 2 $kg.ha^{-1}$, row spacing - 25 cm.

Individual amino acids were determined by means of acid hydrolysis and oxidation-acid hydrolysis using Aminoacid analyser T 339 M. Content of amino acids was consequently determined by AAS (Amino Acid Score) calculation (Velíšek, 1999). Determination of amino acid tryptophane was not carried out.

Results and Discussion

MET (32.53%) was found as the limiting amino acid. K 432 genotype showed the highest content of SER (7.33 $g.kg^{-1}$ of dry matter), ARG (7.41 $g.kg^{-1}$ of dry matter), and conversely the lowest content of MET (0.70 $g.kg^{-1}$ of dry matter), K 433 genotype showed highest contents: ASX (11.95 $g.kg^{-1}$ of dry matter), THR (5.78 $g.kg^{-1}$ of dry matter), GLX (23.27 $g.kg^{-1}$ of dry matter), PRO (7.28 $g.kg^{-1}$ of dry matter), GLY (8.34 $g.kg^{-1}$ of dry matter), ALA (6.53 $g.kg^{-1}$ of dry matter), VAL (6.34 $g.kg^{-1}$ of dry matter),

MET (0.88 $g.kg^{-1}$ of dry matter), ILE (5.28 $g.kg^{-1}$ of dry matter), LEU (8.67 $g.kg^{-1}$ of dry matter), TYR (3.27 $g.kg^{-1}$ of dry matter), PHE (6.52 $g.kg^{-1}$ of dry matter), HIS (4.67 $g.kg^{-1}$ of dry matter), LYS (7.98 $g.kg^{-1}$ of dry matter), (Graph 1 and 2). Table 1 presents average contents of amino acids in years 1997 - 2000. Graph 3 shows content in single year. The lowest content variability was found by MET amino acid. Among the monitored species *A. hybrid* showed highest content of: ASX, THR, SER, GLX, PRO, GLY, ALA, LEU, PHE, LYS. VAL, MET, ILE and highest TYR values were found by the species *A. hypochondriacus*. Highest contents of ARG (7.09 $g.kg^{-1}$ of dry matter) was found by variety *A. cruentus* (Graph 4). According to the results there is evident that genotypes No 1008, A 200 D and varieties Olpir (seed size category > 1 mm) proved very steady LYS amino acid content (7.41 – 7.64 $g.kg^{-1}$ of dry matter), in single year. Jamriška (1996) presents practically meaningless differences between chosen genotypes as far as the amino acids content is concerned. However our experiments proved evidential differences between particular genotypes. Average content of LYS amino acid in amaranth grain reached 7.59 $g.kg^{-1}$ of dry matter). Amaranth grain contains high share of essential amino acids, not only LYS (Vietmeyer, 1982; Ostrý, 1998; Michalová, 1999) also GLX, ASX, LEU, ARG, GLY and SER. The highest share of essential amino acids contains *A. hybrid* > *A. hypochondriacus* > *A. cruentus.*

Acknowledgements
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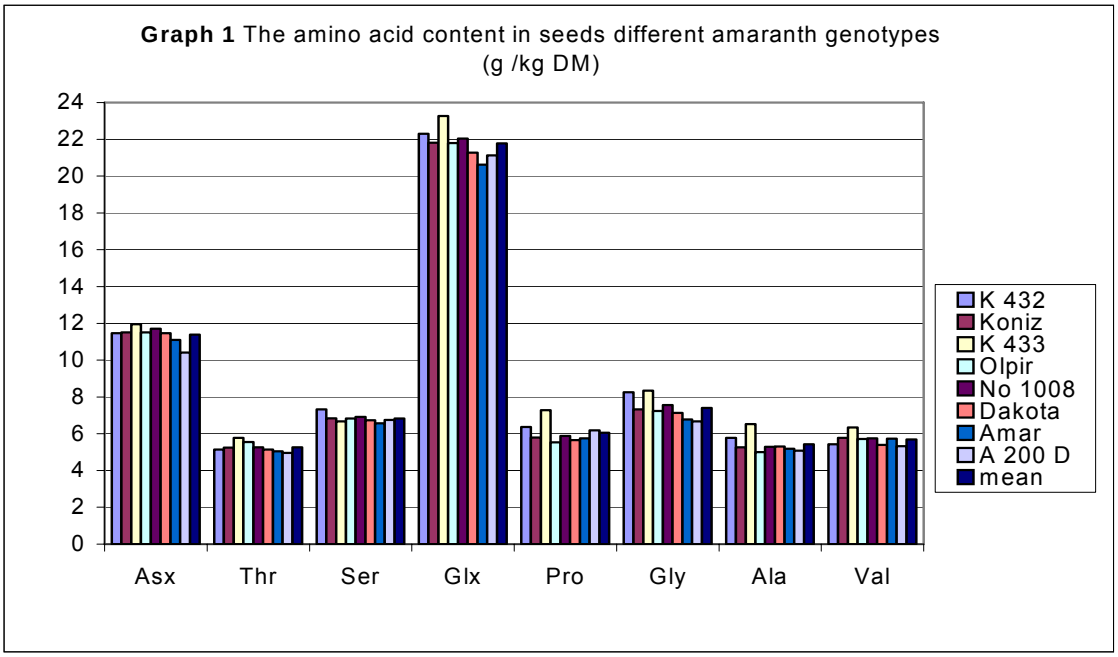
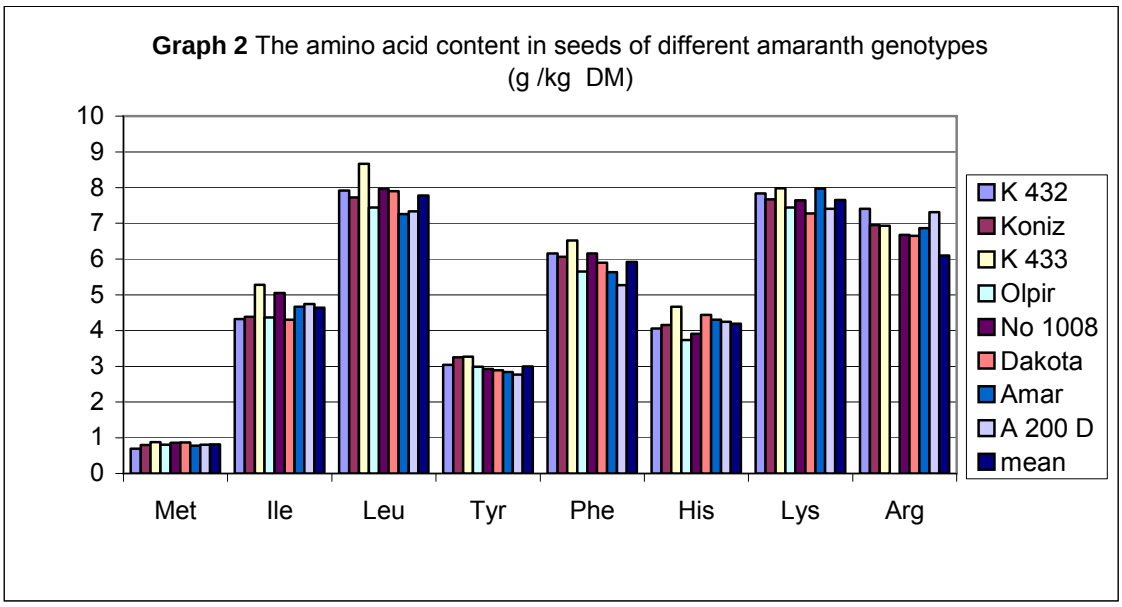
Table 1 The average amino acid content in amaranth seeds (g.kg^{-1} DM)

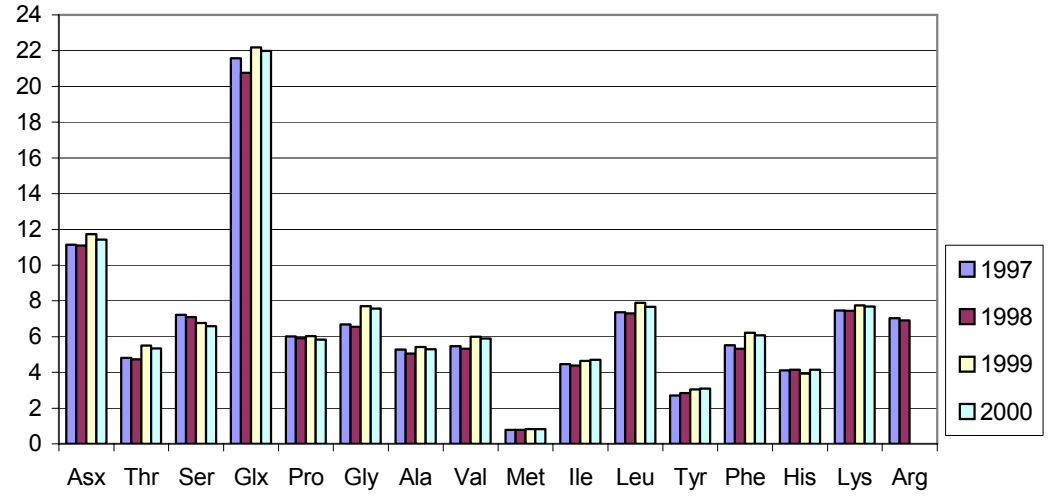
Amino acid	**ASX**	**THR**	**SER**	**GLX**	**PRO**	**GLY**	**ALA**	**VAL**
Amaranth	11,35	5,10	6,92	21,62	5,95	7,13	5,26	5,67
Ideal protein*		0,9 (4,3)						1,3 (5,5)
Amino acid	**MET**	**ILE**	**LEU**	**TYR**	**PHE**	**HIS**	**LYS**	**ARG**
Amaranth	0,80	4,55	7,55	2,92	5,79	4,09	7,59	6,97
Ideal protein*		1,3 (4,6)	1,9 (9,3)	1,9 (7,2)			1,6 (6,6)	

*according to FAO - adults (children)

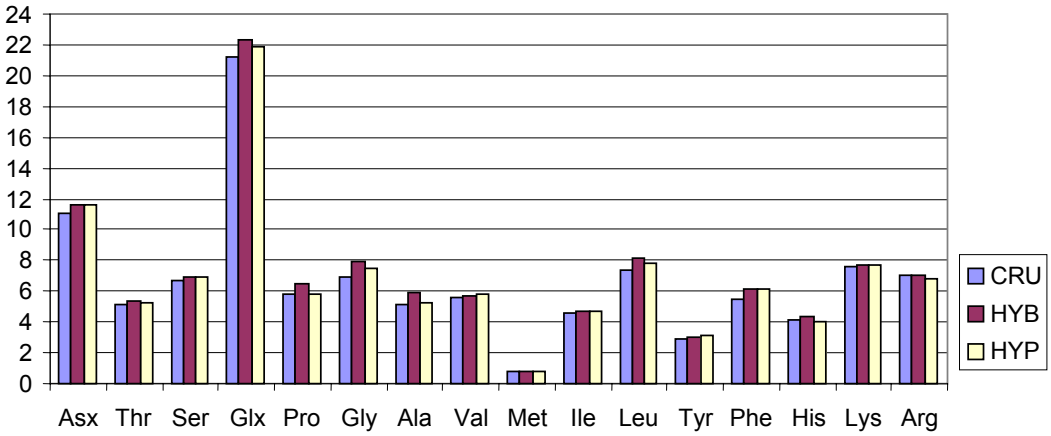




Graph 3 The amino acid content in amaranth seeds in years 1997 - 2000
(g/kg DM)



Graph 4 The amino acid content in seeds of different amaranth species
(g /kg DM)



PROTEIN ISOLATION FROM AMARANTH FLOUR AFTER ENZYMATIC OR MICROBIAL STARCH DEGRADATION

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Abstract

Isolation of proteins from amaranth flour is complicated because of difficulties with separation of the protein and starch fractions. Enzymatic or fermentative starch degradation described in this paper makes possible simple, cheap and ecological method of production of protein concentrates or isolates from amaranth flour applicable on industrial scale. After the starch degradation, protein can be easily separated by isoelectric precipitation.

Complete degradation of amaranth starch to soluble products, dextrins and/or simple sugars, was achieved using industrial amylolytic enzymes BAN 480L α-amylase and AMG 300L amyloglucosidase.

Lactobacillus amylophilus bacterial strain was also used to assimilate amaranth starch. The strain produces only L-form of lactic acid, which is suitable for food purposes, and was also considered as a potential probiotic microorganism. The fermentation can be carried out in non-sterile conditions and without addition of other nutrients.

When used for food purposes, debiterring of the protein products will be necessary.

Key words - amaranth protein isolation, amaranth starch, *Lactobacillus amylophilus*

Introduction

Protein in food is an essential nutrient which comes from animal and plant sources. Most evidence suggests that a shift to largely plant-based protein diets would reduce chronic disease risks among industrialized and rapidly-industrializing populations. High intake of animal protein increases total blood cholesterol, low-density lipoprotein (LDL) cholesterol, obesity, and risks of atherosclerosis and coronary heart disease. On the other hand, many studies report that vegetable protein is associated with low blood cholesterol and the low risk of the diseases aforementioned. The negative association between excessive intake of animal protein and diseases is possibly due to the fact that animal food products are also high in fat content, particularly, saturated fat, and also due to the physical and chemical nature of the animal protein.

Animal protein has a balanced combination of all the essential amino acids, hence it is called complete protein. To the contrary, plant (vegetable) protein is usually incomplete, regarding to the essential amino acids composition. There are several exceptions, such as soya or amaranth proteins, which approximate the recommended optimal essential amino acids profile. However, even soya complete protein is deficient in one of the essential amino acids – methionine. Therefore, with vegetarian diet, to achieve a balanced amino acids intake, a variety of plant protein sources need to be complemented with each other in the diet.

Recently, alarming increase of frequency of intolerant and allergic reactions to the cereal and soya proteins has been observed. Moreover, interest in vegetarianism and plant-based diets in developed countries is on the rise and vegetarians and vegans constitute a significant population group. That is why enlargement of market supply of high-quality food plant-based proteins is very desirable. To accomplish this shift, it will be necessary to overcome market-place barriers and to develop new technologies and policies that will encourage greater consumption of the vegetable proteins. Amaranth is one of the most promising sources of non-allergic and almost complete vegetable proteins.

The crude protein content of grain amaranth ranges from 11 to 17.6 % dry matter (Bressani *et al.*, 1987 a; Imeri *et al*., 1987 b; Bressani *et al.*, 1987 b). This is higher than in most common grains except soybeans. Grain amaranth complete protein contains around 5% lysine and 4% sulphur amino acids, which are the limiting amino acids in other grains. The lysine content is given as the main reason for the high protein quality of amaranth (Saunders *et al*., 1983; Teutonico and Knorr, 1985). Amaranth complete protein also contains significantly more sulphur amino acids than soya complete protein. The amino acid composition of amaranth protein compares well with the FAD/WHO protein standard.

True digestibility of the raw complete amaranth protein is in the range 74 - 80 %. However, the digestibility and the protein efficiency ratio are significantly improved if the grain is heat processed (Garcia *et al*., 1987). At the heat treatment, trypsin inhibitors and other antinutritional substances are denatured (Imeri *et al*., 1987).

The complete amaranth protein contains two fractions of albumins, three globulins (7S, 11S and P), glutelins and minor amounts of prolamins. Albumins and globulins represent about 60% of total nitrogen. Globulin-P is probably a major amaranth protein fraction with peculiar properties (Konishi *et al*., 1985).

Amaranth protein isolates have good functional properties, such as gel and foam forming and whipping characteristics (Marcone and Kakuda, 1999).

Amaranth grain doesn't contain gluten and no allergenicity of amaranth proteins has been recorded until now. Amaranth is unrelated to any other food crops that are commonly consumed, which makes it less likely to cause problems to people who have built up allergies due to repeated consumption of the same foods.

Several methods of isolation and fractionation of amaranth proteins from grain have been described, based on Osborn fractionation and sonication (Paredes-Lopez *et al*., 2002; Búcaro and Bressani, 2002), alkaline extraction - isoelectric precipitation (Martínez and Añón, 1996; Salcedo-Chávez *et al.*, 2002) and micellisation (Cordero-de-los-Santos *et al*., 2005).

The methods described above are not suitable for industrial purposes, mainly because of difficulties with separation of the protein and starch fractions. The aim of this study was to suggest a simple and cheap method of isolation of amaranth complete protein applicable on industrial scale.

Material and Methods

Preparation of amaranth milk by alkali extraction. 1 kg of amaranth flour defatted by supercritical extraction was suspended in 5 litres of distilled water and pH of the suspension was adjusted with KOH to 8.7. The suspension was heated to 50 ºC and stirred at the same temperature 30 min. Fibre sediment was separated by centrifugation at 10 000 x g, 20 min. The amaranth milk obtained as a supernatant was spray-dried.

The dried amaranth milk, containing protein and starch fractions, was used to the following experiments.

Starch degradation in the amaranth milk with amylolytic enzymes. Two suspensions were prepared by addition of 1000 ml of distilled water to 50 g of the dried amaranth milk. Values of pH of the suspensions were adjusted to 5.0 and 6.0 by addition of saturated citric acid solution. The suspensions were sterilised at 121 °C, 20 min. To the first suspension (pH 5.0) 0.2ml AMG 300L (Novozymes A/S, Denmark) amyloglucosidase, to the second one (pH 6.0) 0.02 ml BAN 480L (Novozymes A/S, Denmark) α-amylase were added.

Enzymatic starch degradations proceeded during 72 h at 70°C under slow stirring. After 72 h the suspensions were centrifuged at 10 000 x g, 20 min. Samples of supernatants were collected before and after the enzymatic hydrolysis for GPC analysis. The remaining supernatant was kept in a freezer.

Starch degradation in the amaranth milk by fermentation with Lactobacillus amylophilus. Freeze-dried *Lactobacillus amylophilus* (CCM 7001) was purchased from the Czech Collection of microorganisms and cultivated in Petri dishes using B6 – *Lactobacillus* MRS medium (Difco laboratories, USA) at 37°C, 5 days. Inoculum for the fermentation experiments was routinely prepared by transferring the bacterial colonies to sterile saline solution and homogenisation.

50 g of the dried amaranth milk was suspended in 1000 ml of distilled water in an EM flask and pH of the suspension was adjusted to 6.3 by addition of saturated citric acid solution. The suspension was sterilised at 121 °C, 20 min. The sterilised suspension was cooled, inoculated by the bacterial culture under sterile conditions and incubated under semiaerobic conditions on a shaker (250 rpm) for 2 days at 37 °C.

After 24 and 48 h, pH of the suspension was measured and lactic acid produced during the cultivation was neutralised by addition of a solution containing 12.5g of $CaCO_3$ and 3.13g of $MgCO_3$ in 100ml of distilled water up to pH 6.3. At the same time samples for determination of vital bacterial cell number and GPC analysis were also collected.

After the cultivation a part of the suspension was freeze-dried and vital bacterial cell number was determined in the dry powder. The second part of the suspension was used for protein isolation. The protein concentrates or isolates prepared in this manner can be enriched by Mg and Ca lactates and vital bacterial culture with the potential probiotic properties.

Determination of vital bacterial cell number. Vital bacterial cell numbers were determined by a standard method based on counting of colony forming units (CFU) in test tubes using a special lactobacilli MRS agar M641 (Hi Media Laboratories, India).

Protein isolation from the amaranth milks after starch degradation. Complete protein was isolated from the samples of the amaranth milks after the enzymatic or fermentative starch degradation by isoelectric precipitation. The samples were acidified with citric acid to pH 5.0. The precipitated protein was separated by centrifugation and freeze-dried.

Protein content in the freeze-dried sample was determined by the Kejldahl method using Behrotest InKjel M (Behr, Labor-Technik, Germany) digestion and titration units.

Gel permeation chromatography (GPC). Samples were centrifuged at 15000 x g before the separations. Superdex 75 10/300 GL (300x10 mm) column (Amersham Biosciences AB, Sweden) was used for the separations.

Isocratic elution was performed at 30°C with 0,1M Na_2HPO_4 as eluent at a flow rate of 0.5 $ml.min^{-1}$. Volume of the samples injected to the column was 20 µl.

Results and Discussion

Starch degradation in the amaranth milk with amylolytic enzymes. Almost complete starch degradation (the peak with retention time 12 - 17 min) was achieved using both the industrial amylolytic enzymes used (see the Fig. 1). A small peak of high molecular dextrins (retention time 15 - 20 min) and oligodextrins accumulation (retention time 34 - 40 min) are visible on the chromatogram of the amaranth milk after hydrolysis with BAN 480L α-amylase. Accumulation of low molecular sugars is visible on the chromatogram of the amaranth milk after hydrolysis with AMG 300L amyloglucosidase (especially the peak with retention time about 40 min).

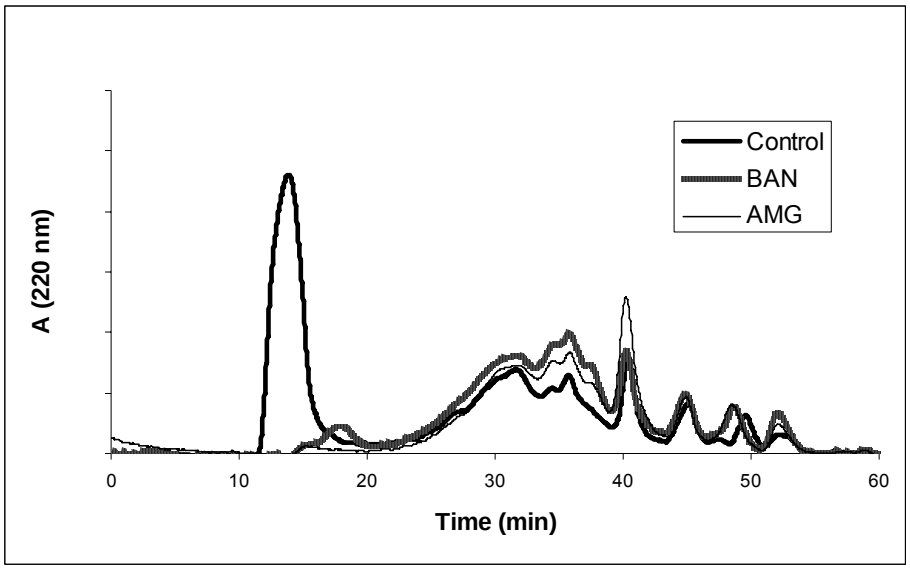

Fig. 1 GPC chromatograms of amaranth milk before (control) and after 72 h enzymatic hydrolysis with BAN 480L α-amylase and AMG 300L amyloglukosidase

Starch degradation in the amaranth milk by fermentation with Lactobacillus amylophilus. A significant decrease of the starch peak (retention time 12-17 min) during the fermentation, about 90% after 48 h, is visible on the chromatograms in Figure 2. An increase of vital bacterial cell number accompanied by a decrease of pH value because of a production of lactic acid during the fermentation was also observed (see Table 1). No significant changes of the chromatographic peaks corresponding to the protein fraction (retention time between 24 and 39 min) were observed. It shows that the protein fraction of the amaranth milk probably stays intact during the fermentation.

A significant vital bacterial cell number, 6.10^9 $CFU.g^{-1}$ were also found in the freeze-dried samples of the fermented amaranth milk.

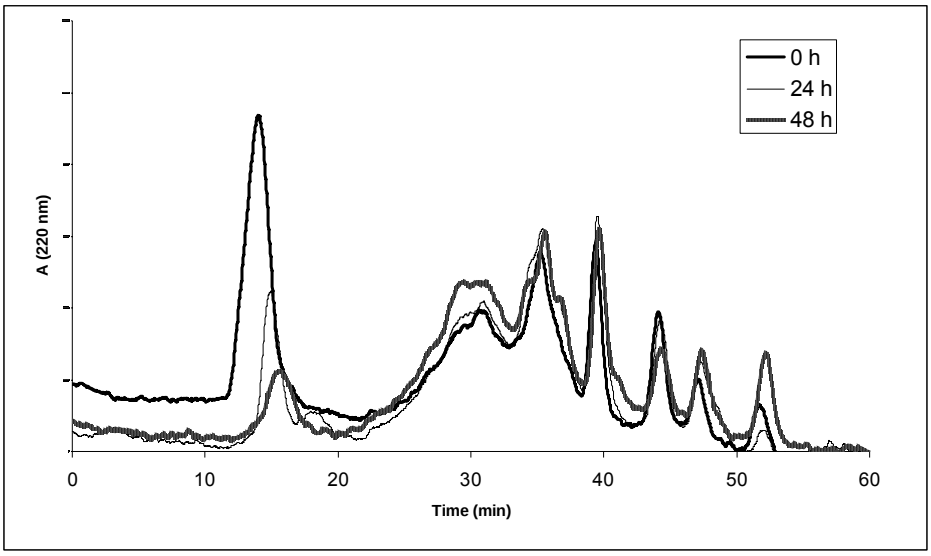

Fig. 2 GPC chromatograms of samples of the amaranth milk collected before and during semiaerobic fermentation with *Lactobacillus amylophilus*

Table 1 Changes of pH and vital bacterial cell number during the fermentation

	pH / CFU.ml^{-1}
0 h	6,3 / 10^5
24 h	5,1 / 10^8 *
48 h	4,7 / 10^9 **

** pH adjusted to 6.3 by addition 5 ml of the solution of $MgCO_3$ and $CaCO_3$*
*** pH adjusted to 6.3 by addition 20 ml of the solution of $MgCO_3$ and $CaCO_3$ (composition described above)*

Protein isolation from the amaranth milks after starch degradation. Protein contents in the freeze-dried protein concentrates prepared by isoelectric precipitation from the amaranth milks after enzymatic starch degradation were 72.4 % (w/w) with BAN α-amylase and 74.4 % (w/w) with AMG amyloglucosidase. The protein concentrate obtained from the fermented amaranth milk contained 69.4 % (w/w) of protein.

The protein concentrates have bitter taste and have to be debittered if used for food purposes.

Acknowledgements
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THE DIFFERENT UTILIZATION OF AMARANTH IN INDUSTRY

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Amaranth as a unique plant with a long and mysterious history is the object for science and also for business. Its characteristics such as absence of gluten and special composition of oil lead this plant to the position of very important plant for the future. The research of amaranth has the results, which bring the expectation of its utilization in different industry. In the Czech Republic, AMR AMARANTH Company is the only one commercial subject, which utilizes amaranth as a raw material for food, cosmetic, feed and special nutrition purposes.

The Food Industry

The very important question for actual days is the food safety On the one hand, there is a fact of accessibility the raw material and on the other side is the nutritional health promoting effect of the food. The European Commission creates the Rapid Alert System for Food and Feed (RASFF) to pay attention about problematic foods. Problem is the innutrition and also obesity, increasing number of people with the food allergy and the effect of increasing number of GMO food. People more require high-quality food. And people starts looking for a new resources, alternative and BIO foods. The utilization of amaranth in food industry is very often topic on the internet. We can find many websites with amaranth recipes. One of the aims of AMARANTH: FUTURE-FOOD, which is the project supported by European Commission, is the collection of amaranth recipes from all around the world in one Amaranth Cook Book, distributed in four languages.

The utilization of amaranth starts with the milling of grain by the specific mill to gain amaranth flour. This is basic raw material for bakery production. Amaranth grain is very often used in the poppy machine, which made a poppy amaranth by the thermal shock. Poppy amaranth is used for the cereal muesli, for the muesli bars and also for bakery products for cover bread and rolls. The most positive property of poppy seeds is the low weight and the water absorption effect. Bread and rolls are longer time soft and supple. The defatted amaranth flour is the most often raw material used in food industry in the Czech Republic in the year 2008. It is the secondary product after amaranth oil extraction with the content max 1% of oil. Defatted flour has big advantage for producers – the price is lower than wholegrain flour and the smell is more gently because of low oil content. The small disadvantage is that the flour is very powdery and dusty for the production.

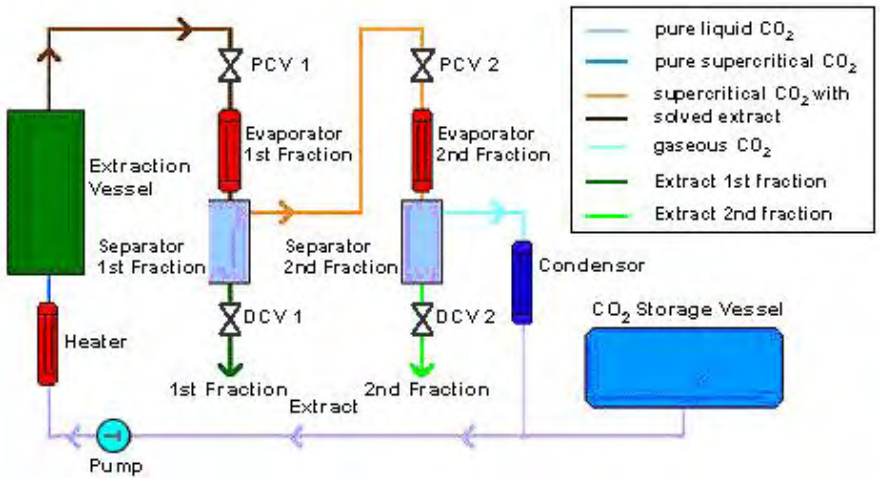
The Advantages of Amaranth for Food Industry:

- Gluten-free
- High-quality protein
- Non Food-allergy
- Thickening property of amaranth starch

The Cosmetic Industry

Amaranth grain oil is very special in the content. There is a unique composition of unsaturated fatty acids and the significant squalene content. There are different methods

for obtaining oil from amaranth grain. We can use pressing the seeds as most simple way without costly equipment. This method is used in Peru. Disadvantage is low recovery factor. There is a method of extraction by organic solvent – the most used is hexane. This is very often way for obtaining oils from grain, but the method is not acceptable for all producers of nature cosmetics, the costs are not low, but you can obtain oil with the 99% success. The last method is supercritical carbon dioxide extraction. This method is expensive, but very clean and acceptable for nature cosmetics. There is an only 1% residue of oil in the flour after extraction.



Source: www.**101herbs.com/co2-extraction.htm**

Fig. 1 The supercritical extraction process

The amaranth oil is used in cosmetics in the pure form or with added tocopherol and it is given under argon for stabilisation. Amaranth oil is added in products for skin and hair (face cream, shampoo, balsams) in actual days. The extract from inflorescence with bioflavonoid content, mainly rutin and amaranth grain after thermal treatment should be also used for cosmetics.

The Advantages of Amaranth oil for Cosmetic Industry:

- Squalene content has a preserve function for skin and hair against sun and radiation
- Available for very sensitive skin
- Non- Allergy amaranth oil on skin

Table 1 Typical fatty acids composition of amaranth, flax and hemp oil

		Amaranth oil	Flax oil	Hemp oil
Fatty Acid		Area%	Area%	Area%
14	Myristic	0,15	0,04	0,03
15		0,06	0,02	0,02
C15:1		0,05	--	--
16	Palmitic	17,01	4,95	5,95
C16:1n7		0,14	0,06	0,12
17		0,12	0,06	0,06
C17:1		0,04	0,03	0,03
18	Stearic	3,29	4,46	2,73
C18:1n9	Oleic	18,34	15,08	10,52
C18:1n7	cis-Vaccenic	0,7	0,55	0,53
C18:2n6	Linolic	45,53	14,65	56,55
	gamma-Linolenic	--	--	3,44
19		0,4	--	--
C18:3n3	alfa-Linolenic	0,93	58,23	16,44
20	Arachidic	0,81	0,14	0,85
C20:1n9		0,25	0,15	0,42
20:2n9		0,02	0,03	0,07
21		0,03	--	--
20:3n3		0,06	0,05	--
22	Behenic	0,32	0,16	0,32
22:1n9	Erucidic	0,04	--	0,02
C22:6n3	Eicosahexaenic	--	--	0,20
23		--	--	0,04
24		0,2	0,10	0,13

Source: Food Research Institute in Prague

The Food Supplement and Functional Food Industry

Amaranth biomass and grain with its specific composition is ideal crop for health promoting products. We can utilize a grain or biomass. Utilization of amaranth biomass starts on the field. It is necessary to harvest the inflorescence, put into pellets and dry. These pellets should be stored until next harvest. Pellets are put into water and the bioflavonoids extract is obtaining by the fractionalisation equipment. The rest – biomass fibre should be dried as a tea and packed or dry in the drying houses and capsuled/tableted as a food supplement. Extract is thicken and stabilised with fructose and the amaranth syrup as a food supplement is ready.

Utilization of grain starts by milling grains into flour and produce a first fraction – amaranth oil. Amaranth oil could be used as a raw material for functional food. Amaranth oil is filled into capsules and used as a food supplement – the content of squalene, which is a precursor of cholesterol helped people with hypertension diseases and support the body against environmental impact. When the amaranth oil is injected to the ham or meat, it could decrease the intussusceptions of cholesterol in the alimentary tract of people.

Second fraction is an amaranth fibre. It is produced by fractionalisation equipment and there are three main kinds of fibre. High-Protein Fibre - with the 20-24% of protein content, Bakery Fibre – with the 55-60% of starch content – utilize mainly for fortification bakery and on milk based products with the aim of special or function foods, and also Pharma Fibre, which is homogenized with additional nutrients and capsuled or tableted for the food supplement production. Amaranth Fibre increases the peristalsis of the intestines and supports the defecation. The rest from production of fibre is mix of protein and starch. This mix could be separated on the separator and transform into powder by spray drying system. Amaranth protein

has the anti-tumoric activity (Fomsgaard, 2008), which could be used in food supplement as a powder or functional food by the adding into meat, milk or bakery products.

Advantages of Amaranth Protein as a Food Supplement:

- Non allergy
- Gluten-free
- High digestibility
- Nutrition composition is recommended for vegetarian
- Lysine content for children nervous system

The Feed Industry

For the feeding animals could be used biomass or the rest of food production. Chicken were fed by amaranth grain and biomass and thermal treated amaranth. The tests showed that chicken could be fed by amaranth and should the meat-bone flour replaced (Matoušová, 2007). There is a problem with the anti-nutrient effect in the rats feeding. The swelled grain should be used as a fodder for young carps. The comparison with the control group and standard fodder were tested (Table 2).

Table 2 The results of comparison I young carps feeding

Feed	Beginning of experiment			Loss		Real live weight gain		Experiment duration
Kind	Number	Total weight (g)	Weight per capita (g)	Number	%	Total weight (g)	Weight per capita (g)	Number of days
Amaranth	200	22630	113,15	10	5	4055	21,34	142
KP-2	200	22905	114,52	12	6	219	1,16	142
Control	200	24040	120,2	145	72,5	0	0	142
Σ	**600**	**69575**	**115,9**	**167**	**27,8**	**4274**	**11,25**	**-**

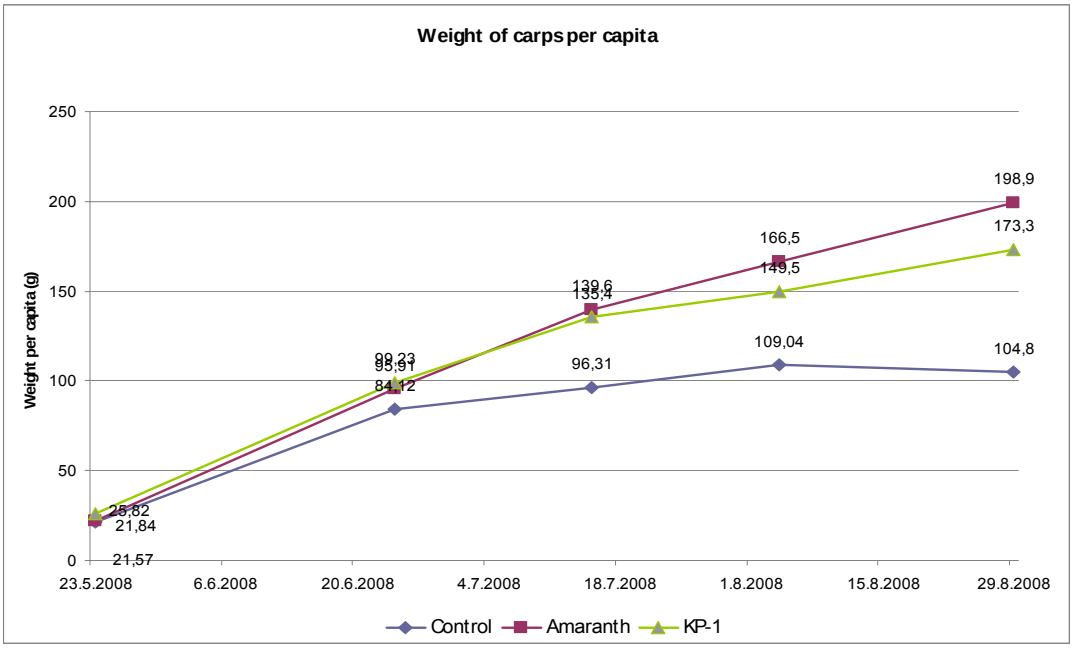


Fig. 2 The graph of weight comparison of young carps

The Energetic Industry

In these days, energetic industry is very often put to context with renewable alternative sources of energy from the biomass. There are a discussion about the coherency between the growing plants for bio fuels and the food security in the underdeveloped countries (Brasil).

Amaranth has a big advantage, because it could be use by two ways - biomass for the bio fuel production and grain for food production. There is a question, which genotype for which country is the best.

In the Czech Republic were tested 18 genotypes in the frame of the Amaranth: Future-Food project. One of tested genotype *Amaranthus hypochondriacus* "Rosita /Rojita", originated in Mexico was chosen as the ideal for the testing in thermal power station for burning process. The reason was the yield of biomass. Disadvantage is impossibility of obtaining seeds from this genotype in the climatic condition of the Czech Republic.

Conclusion

Amaranth is the plant with the sad history, but with the possibility of the very successful future. Its utilization in the food industry is not difficult and it brings a big benefit for the health of people due to unique composition. There are a sufficient number of genotypes for the growing in different climatic conditions. The wide range of utilization of amaranth grain and biomass is the good new for the world, full of food allergies and illness from food, un-stabilized weather climate and increasing number of stock diseases.

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SOME AGROTECHNICAL ASPECTS OF AMARANTH CULTIVATION

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I would like to inform you about the results of the research project 1/6231/99-VEGA "Deepening of theoretical knowledge on the production and utilization of *Amaranthus* in Slovakia".

During project duration polyfactorial field experiments were performed at the experimental fields of Agricultural University in Nitra in the period 1999-2001. The following aspects and parameters were searched:
- testing of different cultivars
- testing of different doses of fertilization
- testing of distance between rows
- testing of influence of different desiccants
- testing of several aspects regarding the harvest and processing of amaranth seed were tested such as different modification of harvest equipment
- testing of storage of amaranth seeds
- testing of use of amaranth for beer production.

The models of technological systems for amaranth cultivation were elaborated for different fields of amaranth application considering the economical and energetical aspects.

Following traits and parameters were studied during amaranth cultivation:

1. Botanical and morphological characterization of different species and hybrids (the high of the plants, size of the leaf area, size and shape of inflorescence).
2. Agrotechnical parameters of cultivation: the optimal distance between rows was 375 mm with the yield obtained 3.95 t/ha. When distance 125 mm was used, the yield was about 30% lower.
3. Testing of influence of desiccants: DAM-390, Roundap.
4. Yield of the seeds at the different variants of the fertilization:
   - without nitrogen (N) – the yield was 3.94 t/ha;
   - with 60 kg nitrogen the yield was 4.4 t/ha;
   - with 120 kg nitrogen 5.16 t/ha;
   - with 120 kg N +120 kg N the yield was 5.7 t/ha.
5. The most suitable seed collection was achieved when the threshing basket was modificated by milling and by use of grooved threshing with the gaps at entrance 12-15 mm and at exit 7-9 mm.
6. Use of amaranth seeds for beer production was quite successful. During processing 20 % of the barley malt was replaced by amaranth. The senzoric properties of the beer were the same as for beer with 100 % barley malt.
7. The best storage for amaranth seeds was proved to be in storage bin.
8. The technological systems of amaranth cultivation were elaborated with regards of seed production, phytomas production and technical purposes.
9. As the most suitable grain amaranths for cultivation in condition of Slovakia are *Amaranthus hypochondriacus* and *Amaranthus cruentus*.

10. For the energetical purposes the cultivation of giant amaranth is recommended (*A.cruentus* genotype from Peru).
11. Collection and maintenance of amaranth genetic resources and their characterization was performed.

Trends

From the results of this research as well as from the knowledge of other authors we can indicate the following future research tasks and trends:

- study of the amaranth reaction on climatic changes
- breeding new cultivars with enhanced productivity (3t/ha) with maintaining or increasing of quality traits
- breeding for the higher quality of food and feed
- breeding for different industrial utilization (in pharmacy, cosmetic industry)
- application in phytoremediation
- as a renewable energy resource
- use of marginal less quality soils with positive environmental and economical aspects
- increasing of social and medicinal aspects (small grower's income, special dietary food)
- development of good processing industry for amaranth seed and biomass.

EFFECT OF *Amaranthus cruentus* SEEDS ON OXIDATIVE STATUS IN PLASMA AND SELECTED TISSUES OF RATS FED WITH HIGH DOSES OF FRUCTOSE

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Abstract

Oxidative stress plays an important role as a mediator of damage produced by fructose. This work was designed to investigate the effect of amaranth seeds on oxidative stress in plasma, hearts, kidneys and pancreas of fructose-administered rats. Fructose administration (310g/kg fodder for 5 weeks) caused oxidative damage that was manifested by the increase in plasma malondialdehyde (MDA) and by the decrease in the enzymatic antioxidant capacity in plasma and selected tissues. Co-administration of amaranth seeds (310 and 155g/kg fodder) restored the activities of some enzymes. It also influenced the oxidative stress as was evidenced by decreasing MDA and increasing FRAP in plasma, and the activities of antioxidant enzymes (erythrocyte superoxide dismutase – eSOD, catalase – CAT, glutathione peroxidase – GPX). The findings demonstrate that amaranth seeds, in dose dependent manner, can act as a moderate protective agent against fructose-induced changes in rats by reducing lipid peroxidation and by enhancing the antioxidant capacity.

Key words - amaranth seeds, malondialdehyde, catalase, glutathione peroxidase, supeoxide oxidase

Pseudocereals such as amaranth seeds (AM) contain remarkable amounts of antioxidant phytochemicals including phenols, flavonoids, anthocyanins, fat-soluble vitamins, fatty acids, squalene and other compounds (non-enzymatic antioxidants) which exert a protective mechanism against the oxidative stress and damage of tissues.

On the other hand, it is well known that the administration of fodders enriched with fructose to rats induces oxidative stress leading to insulin resistance, hypertriglyceridemia, heart disease and obesity (Basciano *et al.,* 2005). Therefore, in our work the experimental model with addition 31% of fructose was applied in aim to induce oxidative stress.

A number of oxygenated compounds are produced during the attack of free radicals against membrane lipoproteins and polyunsaturated fatty acids (PUFA). Malondialdehyde (MDA) is one of the aldehydes produced during this attack from PUFA. MDA may be an indicator of oxidative stress, as its plasma concentration increases in accordance with the rate of free-radical processes. The antioxidative system enables trans-

formation of oxygen reactive forms into inactive and harmless compounds. The antioxidant enzymes produced in the body provide an important defense against free radical. Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) are the most important antioxidant enzymes. SOD can selectively scavenge superoxide radicals by catalyzing its dismutation to hydrogen peroxide and molecular oxygen. Other antioxidative enzymes GPX and CAT serve to decompose hydrogen peroxide to water.

The aim of this study was to assess the influence of fructose addition (31%) and addition of amaranth seeds to fodder on the antioxidant status in selected rats' tissues and plasma.

Material and Methods

Plant material. Amaranth seeds (*Amarantus cruentus*) were cropped in eastern Poland.

Animals and diets. Groups of 6 male Wistar rats (body weight 245.7±2.8 g) were kept for 5 weeks. The groups of animals were combined into pairs: in every pair one group was fed a diet enriched with amaranth seeds, without fructose, while the second group was fed with the fodder enriched with amaranth seeds, in which 31% of starch was replaced with fructose. The fodder of control group contained corn starch instead of fructose (C; CF). Water and food intake and rats' body weight changes were monitored in the course of the experiment. Diets were formulated according to a following scheme: (compounds in constant amounts, $g.kg^{-1}$ fodder): casein 200, rapeseed oil 50, chalk 28, calcium monophosphate 29, lecithin 10, sodium chlorate 3, cellulose 50, mixture of vitamins and microelements 10 (Premix LPM, BASF, Poland); amaranth seeds were added in varied amounts, depending on the type of diet: the first group - 310 $g.kg^{-1}$ fodder (subgroups: AMH, AMHF), the second group – 155 $g.kg^{-1}$ fodder (subgroups: AML, AMLF).

Samples collection. The material used in MDA, FRAP and SOD analysis, were rats' plasma. Blood samples were taken from abdominal aorta under general anaesthetic with tiopenthal via intraperitoneal. The organs were isolated and stored at –20°C. All organs were frozen immediately after the animal was sacrificed. Before the analysis, the samples were defrozen and homogenized in phosphate buffered saline pH=7.4. In all tissues, the activity of GPX and CAT were determined.

Measurement of MDA levels. Determination of MDA levels was based on the coupling of MDA (Zagrodzki *et al., 2007*). Results were expressed as $\mu mol.l^{-1}$ plasma.

Determination of FRAP activity. FRAP (Ferric Reducing Ability of Plasma) assay was conducted at 37°C and pH 3.6. Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction causes formation of intensive blue colored ferrous-tripyridyl-s-triazine complex with absorbance maximum at 593 nm. Absorbance was measured after 60 minutes and was proportional to the combined ferric reducing/antioxidant power of the antioxidants in plasma (Benzie *et al.,* 1996).

Measurement of antioxidant enzymes activity. Catalase (CAT, EC 1.11.1.6) activity was determined using the kinetic method by Aebi. Glutathione peroxidase (GPX, EC 1.11.1.19) activity was determined by the modified method of Paglia and Valentine. Superoxide dismutase (SOD, 1.15.1.1.) was determined in red blood cells (Zagrodzki *et al., 2007*).

Statistical analysis. Kruskal-Wallis test was applied to check for any differences between different groups of animals. Differences with $p<0.05$ were considered to be statistically significant.

Results and Discussion

The studies confirmed the disadvantageous effect of the administered dose of fructose upon the antioxidative system in rats' plasma. In comparison with control group (C), the fructose caused in CF group statistically significant increase in MDA level ($p<0.05$) testifying to intensified lipid peroxidation. That group also experienced a decrease by 5.6% of GPX activity in plasma, 14.5% of SOD activity in red blood cells (SODE) and increase by 7.2% of FRAP activity in plasma (changes not statistically significant) in comparison with the C group. The administration of amaranth in lower dosage did not protect plasma against peroxidation (MDA increased $p<0.05$). We also observed increase of GPX, FRAP in plasma and SODE but these changes were not statistically significant. The administration of amaranth in higher dosage protected plasma against peroxidation (MDA decreased $p<0.05$), and we observed significant decrease in SODE activity and increase in FRAP and GPX activity in plasma (still not significant; Fig. 1). There are no reports on the influence of amaranth seeds upon the antioxidative status in animals. It results from our studies that more affective is the supplementation with amaranth in higher dose. The administration of vitamin E caused increased activity of SOD (Błaszczyk *et al.,*, 2008) but amaranth seeds had a disadvantageous effect on the SODE activity. Perhaps, significant decrease in SODE activity could be associated with high content of methionine in amaranth seeds compared to normal diet, as this effect was observed previously, when administration of amino acid lowered the concentrations of Cu and Zn (Patra and Swarup, 2004). In CF group fructose decreased not significantly the activities of CAT and GPX in kidneys (Tab. 1), and similar effect was previously observed (Wongmekiat *et al.,* 2008). Only in rats with lower dosage of seeds fructose induced significant decrease in activity of GPX ($p<0.03$), which suggests that this dosage was not enough to prevent kidney against stress. The administration of amaranth seeds (AMHL) with fructose increased the CAT activity and decreased GPX activity in kidneys (not significantly). There were also evidences demonstrating nephroprotective effect of polyphenolic in several experimental models associated with oxidative stress. Polyphenolic compounds, which are present in amaranth seeds have been shown to attenuate the renal dysfunction and improve the morphological renal cytoarchitecture (Wongmekiat *et al.,* 2008).

In comparison with C group, fructose in hearts caused only not significant increase in CAT activity and not significant decrease in GPX activity. The administration of amaranth seeds (AMH) with fructose did not influence GPX activity but decreased CAT activity ($p<0.006$) and the levels of these both enzymes were significantly higher in this group than in C or CF. In the second group (AML) fructose did not cause significant changes in the activity of both enzymes. In comparison with C group, fructose in pancreases caused significant decrease in CAT activity ($p<0.04$) and not significant decrease in GPX activity. The administration of amaranth seeds (AMH) with fructose increased significantly GPX activity ($p<0.01$) and CAT activity (not significantly).

In conclusion: The administration of amaranth seeds reduced peroxidation of lipids and changed the activity of antioxidative enzymes in plasma and selected organs in dose dependent manner. Heart tissue had less antioxidant enzyme activity compared to the liver (5), therefore it may be more sensitive to prooxidative damage. Our results suggest that amaranth seeds could be good additives to the common diet and could for some extent protect heart against free radicals.

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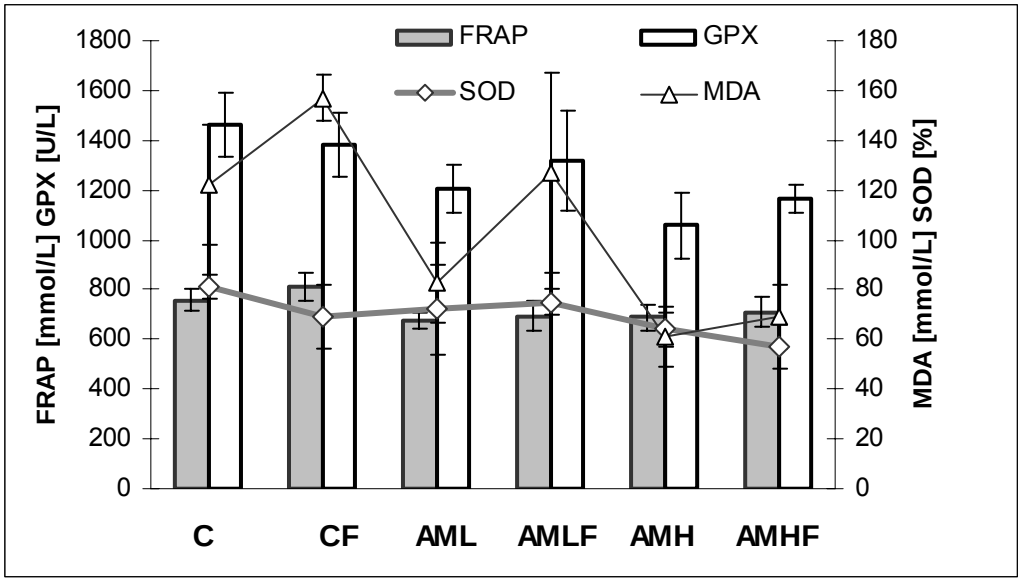

The values are medians±SD for 6 rats per group. C-control group, CF- control group +31% fructose, AML – lower dose of amaranth seeds, AMLF – lower dose of amaranth seeds + 31% fructose, AMH - higher dose of amaranth seeds, AMHF dose of amaranth seeds +31% fructose; MDA in mmole.l^{-1}, FRAP in mmole.l^{-1}, GPX in U/L; SOD in %.

Fig. 1 Antioxidant status of rats evaluated in plasma and erythrocytes

Table 1 Antioxidant enzymes activity in different tissues of rats

GROUPS	KIDNEY		HEART		PANCREAS	
	GPX	CAT	GPX	CAT	GPX	CAT
C	0.32±0.06	19.8±3.4	0.33±0.05	3.6±0.5	0.36±0.06	1.5±1
CF	0.29±0.09	16.6±4.2	0.30±0.04	3.9±0.8	0.34±0.05	0.8±0.3
AML	0.36±0.26	22.5±2.2	0.29±0.08	6.8±0.9	0.21±0.07	0.26±0.25
AMLF	0.26±0.03	21.3±3.4	0.27±0.14	5.0±2.1	0.20±0.02	0.38±0.2
AMH	0.29±0.14	16.4±3.3	0.37±0.05	13.5±2.8	0.20±0.02	0.4±0.4
AMHF	0.33±0.03	20.6±4.5	0.36±0.06	5.2±3.1	0.32±0.07	0.9±0.5

The values are medians±SD of 6 rats per group. GPX - activity of glutathione peroxidase – GPX [U/10 mg protein], CAT- activity of catalase [U/g protein], C - control group, CF- control group +31% fructose, AML – lower dose of amaranth seeds, AMLF – lower dose of amaranth seeds + 31% fructose, AMH - higher dose of amaranth seeds, AMHF – higher dose of amaranth seeds +31% fructose.

ISOLATION AND APPLICATION OF AMARANTH STARCH

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Abstract
Isolation process of fine amaranth starch was developed and tested in pilot scale trials. Starch granules structure was compared to the other starches and possibilities of its applications were searched. The isolated and purified starch was tested for food as well as the non-food applications.

Introduction

The unique aspect of amaranth grain starch is that the size of the starch granules (1 to 3 μm) is much smaller than found in other cereal grains, which influence also its physiochemical characteristics. Amaranth round shape granule contributes to silky texture, uniform cell structure, excellent moisture retention, good clarity, slightly cohesive properties, viscosity stability, forms spherical bodies when co-dried with other natural gums, enhances body and fat-like features in reduced fat goods, and offers non-hazardous (environmentally safe) replacements of coating and solvent agents in tape and paper applications. Amaranth irreplaceable qualitative starch is a natural GRAS ingredient which has good dispersability, exhibits good water binding properties, and supports/enhances dispersions of other coating and spacing ingredients. The amaranth starch is especially suitable for people with indigestion celiac disease patients. Because of its light solubility, smooth surface and size of 1 micron it is ideal for use in dermatology and cosmetics. Amaranth starch posse unique gelatinization and freeze/thaw characteristics which could be of benefit to the food industry. The retrogradation of gelatinized starch is a phenomenon of great importance to the food industry. The amount of retrogradation that occurs in a starch-containing food can influence the texture and acceptability of that food.

A lot of applications of amaranth starch were published during last period, e.g. for custards, pastes, and salad dressing. Small particle and granular starches are desirable as plastic film fillers, biodegradable fillers in LDPE films, starch-filled polypropylene, as a thickener in the printing of textiles, cross-linked starch, sizing agents, and modified amaranth starch are proposed as a fat substitute in foods and extrusion aids. Amaranth starch has good assumption for applying as natural filler in rubber compositions, however it will be need to solve its combinations with the other rubber compounds and also to consider eventual methods of its modification.

Results

The amaranth starch was isolated from amaranth flour (see flowsheet diagram - Fig. 1) and its characterization was performed (determination of pores and specific surface using Hg porosizimeter, type POROZIMETRO 1500 – Carlo Erba (Fig. 2). The study was oriented to the possibility of the specific surface enlargement. As can be seen from the results (Fig. 3) after temeperatutre exposition of amaranth starch (160 oC, 10 min.), the specific surface increased from 5.37 m2.g-1, to 6.44 m2.g-1, i.e. by 20%. However, this enlargement was probably caused by the creation of the starch granules agglomerates.

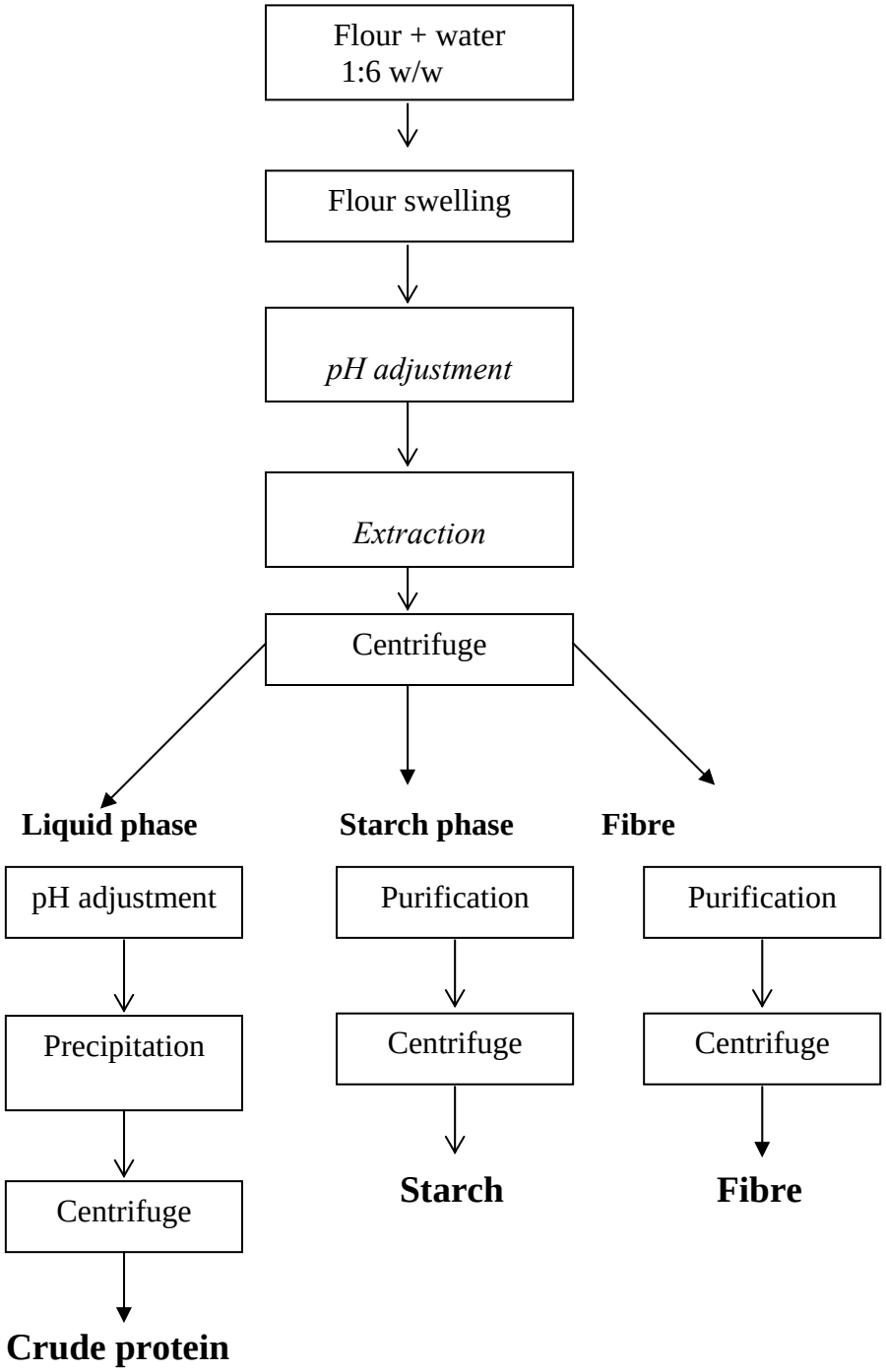
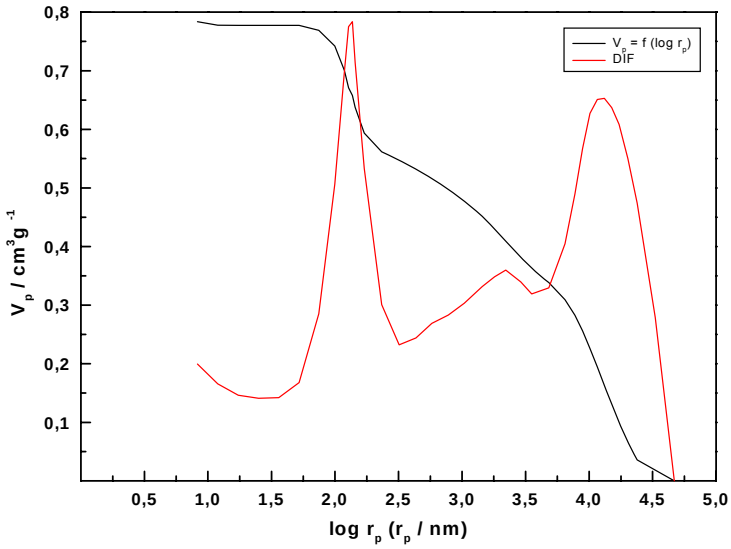


Fig. 1 Flow-sheet diagram of amaranth flour fractionation



V_p – specific pore volume ($cm^3.g^{-1}$)
log r_p – (nm)
V_p = f (log r_p) integral dependence
DIF = differential dependence

Fig. 2 Integral and differential dependence of pore distribution in amaranth starch

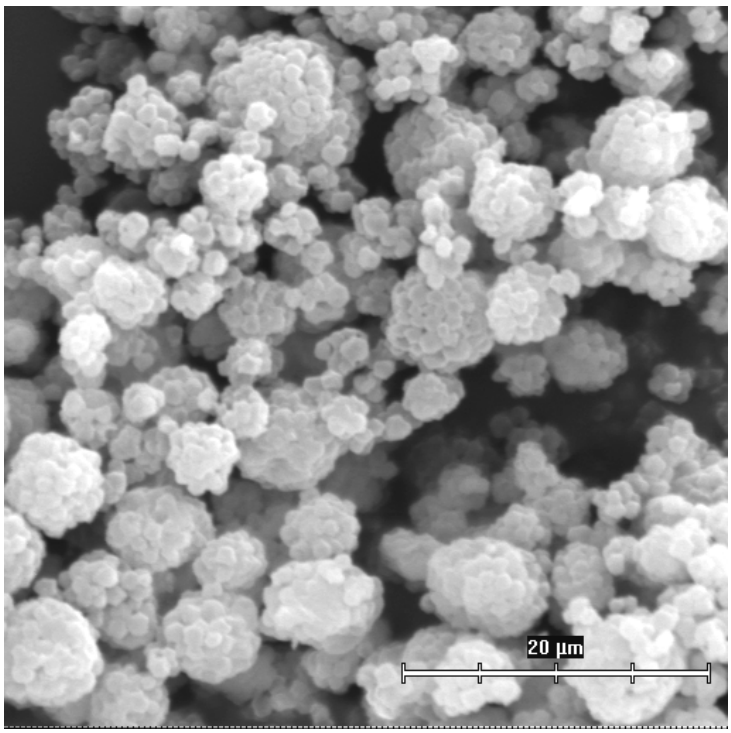


Fig. 3 Electron microphotograph of amaranth starch

Plenary session II:
Agrotechnical aspects of amaranth cultivation

THE CULTIVATION OF AMARANTH IN MÉXICO

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Abstract
Amaranth is a native crop of Mexico, from pre-Columbian times have been planted mainly in the central states. It is a marginal crop and underutilized despite the wealth of protein grain ranging around 14-16% and a good balance of amino acids, from grain is prepared flour, pinole, atole, tamales, granola, cookies and candy known as joy. Various problems that led to the cultivation of amaranth was relegated to marginal areas, where it persists and is grown either under direct seeding and transplanting. On the agricultural system of direct seeding is done in dry regions. Under this agricultural system, the species that are planted are: *Amaranthus cruentus* and *Amaranthus hypochondriacus*, within this species there is great variability genotypic and phenotypic which manifests itself in great variation in growing cycles, colored leaf, panicle and color seed. The planting system of transplant is performed in Tulyehualco, D. F. This system began in the area chinampas that is where establishing the almácigo during April and when the plants reach 20-30 cm transplant in June to areas narrow-minded at the start of the rains. *Amaranthus hypochondriacus* is grown in this region whose growing cycle is six months and plants reach around 180-250 cm height.

Key words - Amaranth, germplasm, floating gardens, agricultural systems

Introduction

Amaranth is a crop originating in Mexico, its domestication is around six thousand years (Sauer, 1994), used their native cultures as much as grain for human consumption as ceremonial. Due to the discovery of the New World brought many crops in Europe traveled to several crops native americas. Amaranth was one of the crops that suffered such a situation that as a grain used in the preparation of idols in the Aztec culture, its cultivation was relegated to the most inhospitable areas of the areas they occupied domains from the Aztec Empire. In pre-Columbian times amaranth had great significance since it was planted thousands of acres and came to constitute the fourth largest crop in the domains of the Aztec Empire, after corn, beans, chile and squash. At present the cultivated areas has been relegated to small areas of temporary state in the centre of the country such as: State of Mexico City. Tlaxcala, Morelos Guerrero, Oaxaca, Puebla, to a lesser surface in Chiapas, Jalisco and foothills of the Sierra Madre Occidental covering the states of Chihuahua, Sonora, Durango and Nayarit. The crop is planted for grain consumption as either as flour, granola, Pinole, atole but the main intended use grain trap is to produce the sweet joy either alone or mixed with walnuts, pine nuts or peanuts Recently in Mexico there has been an interest in the amaranth on their use in new forms of use. Species that are used for grain are: *Amaranthus cruentus* and *Amaranthus hypochondriacus*, each species has its range specified in the Mexican Republic which ranges from 300 meters to 2600 meters above sea level. *Amaranthus cruentus* thrives best in warmer climates from 300 m to 1800 m, however *Amaranthus hypochondriacus* develops better in temperate climates ranging from 1500 to 2600 meters above sea level. In both species there is great variation in terms of length of the

growing cycle, in some regions it is possible to make two growing cycles a year especially among warm areas cropping systems are different depending on. The landraces show great variation in color seed panicles color, color of stem, leaf color and shape.

Material and Methods

For several years were made trips exploration and germplasm collections in most regions where it is planted in the traditional way amaranth, were agro-ecological conditions of the crop, planting systems, as well as collecting germplasm which were collected individual panicles what the once flailed were placed in paper bags which were subsequently dried and then place them in plastic bottles.

Results and Discussion

The cultivation of amaranth in Mexico takes place under direct sowing of the seed when it starts the period of rains that began in May or June depending on the state and low transplant, the system of direct seeding is done in most regions where amaranth is cultivated as are the states of Guerrero, Mexico, Michoacan, Morelos, Oaxaca, Puebla and Tlaxcala. In these states plantings begin in May, starting with preparing the ground, because they are small areas of less than one hectare is used for animal traction, in other cases is planted in home gardens, removes the peasant land using hoes, seeds are placed in the bottom of the groove then lightly covered with a layer of earth, in the case of a water source requires a watering relief, otherwise they are expected to rain. The seed emerge after five days, during the first fifteen days are required for the first weeding amaranth seedlings are free of weeds, carried out this activity weeds invade the drills amaranth and prevent it from her.

THE OCCURRENCE OF SPOTS OF STEMS ON *Amaranthus* spp.

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The research took place in Pawłowice near Wrocław and Łosiów near Brzeg. The observations of healthiness of *Amaranthus cruentus* and *A. retroflexus* were conducted during three years of experiments (2003-2005) from June to November. There was concluded occurring of dark spots on stems. The estimate of damage was conducted in phase of green ripeness of seeds, when the intensity of symptoms of disease which appeared on stems, was the highest. The main species isolated from infected stems was *Phomopsis amaranticola*. Except for this species, there were also isolated *Fusarium spp*. and *Alternaria alternata*. *P. amaranticola* was isolated also from *Amaranthus retroflexus* which was growing in cultivation of cropped *Amaranthus* in Pawłowice as well as Łosiów.

PRODUCTIVITY OF GRAIN AMARANTH *A. cruentus* 'G6' AS AFFECTED BY DROUGHT OCCURING AT DIFFERENT GROWTH STAGES

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Abstract
A greenhouse pot experiment was conducted to study the effect of drought induced at different phenological stages on growth, biomass production and yield performance of grain amaranth *Amaranthus cruentus* 'G6'. After emergence seedlings were exposed to different soil water regimes: constant adequate moisture (W1) and drought (W2) throughout the growing period, drought initiated at crop inflorescence formation (W3), drought condition during pre-inflorescence formation (W4) and treatment W5 where drought condition occurred in the period from the beginning of inflorescence formation to the beginning of flowering. Crop samples were taken at the maturity. The growth and yield performance of amaranth were assessed by measuring root length, stem height and inflorescence length, and by evaluating fresh and dry weight of plant parts, grain yield and harvest index. Drought stress iniciated at different phenological stages affected the evaluated morphological parameters, assimilate allocation and grain yield. Drought throughout the growing period resuted in grain and biomass yield reduction for 51% and 50%, respectively. Water deficit during inflorescence formation appears to be critical growing stage influencing grain yield, while soil drying in the vegetative growth stages improve the assimilate allocation to the above-ground biomass and particularly to the grain.

Keywords - grain amaranth, *Amaranthus cruentus*, drought, biomass allocation, grain yield

Introduction
Soil drought is one of the major limiting factors in crop production and it will become increasingly important due to global climate changes. Instead of nutritional quality (Bavec and Bavec, 2006) grain amaranth (*Amaranthus* spp.), as a plant possessing C_4-photosynthesis pathaway, was also recognised as drought tolerant crop (Kigel, 1994). Johnson and Henderson (2002) reported, for examined grain amaranth cultivars, mean total water use (TWU) of 267 mm and grain water use efficiency (WUE) value of 5.9 kg ha^{-1} mm^{-1}. The values obtained in the study were comparable to other drought adapted crops. The development responsiveness to soil water has only been reported in pot experiments on vegetable amaranth (Liu and Stützel**,** 2002; Liu and Stützel**,** 2004; Ommami and Hammes 2006) and yield formation of grain amaranth under drought conditions is still unclear. Therefore, the objective of the study, which was a part of a broader national research project on grain amaranth, was to quantify the effects of soil water deficit occurred at different phenological stages on biomass allocation and productivity of *Amaranthus cruentus* 'G6'.

Materials and Methods

A pot experiment was conducted in the conditions of plastic greenhouse at the University Agricultural Centre of Faculty of Agriculture, Maribor in 2007. The pipes tubes (75 cm in high, 14 cm in diameter), filed with native top soil were exploited as the experimental pots. After pot's filling grain amaranth *Amaranthus cruentus* 'G6' was over sown and tensiometers tube (30 cm long) were placed randomly into four pots per treatment. Used genotype was proved as perspective and most suitable for NE Slovenian growth condition among the tested germplasm (Grobelnik Mlakar and Bavec, 2000; Bavec and Grobelnik Mlakar, 2002; Grobelnik Mlakar, 2006). According to performance in extremely drought condition of the year 2003 (crop sown in may yielded 2,032 kg ha^{-1} of grain and 20,457 kg ha^{-1} of above-ground biomass), genotype is also presumed to be drought tolerant (Grobelnik Mlakar, unpublished data). Amaranth seedlings were thinned to one plant per pot in the stage of emergence. After sowing pots were subjected to two different soil water tensions: – 0.4 MPa considered as adequate moisture, and – 0.7 MPa considered as drought condition. Soil water regime (SWR) treatments, comprised of a given tensions, initiated at different phenological stages were: constant adequate moisture (W1) and drought (W2) throughout the growing period, drought initiated at crop inflorescence formation (W3), drought condition until the inflorescence formation (W4) and treatment W5 where drought conditions occurred in the period from the beginning of inflorescence formation to the beginning of flowering. The stated phenological stages were considered to begin when inflorescence was visible and when pollen started to shed on 75% of the plants. Soil water tension was monitored every second day during the course of the experiment using the puncture tensimeter instrument (SDEC France, SMS 2500S) and tap water was added according to soil water retention curve defined by six points from 2.5 to 4.2 pF.

Nine plants per treatment were taken at the time of harvest, and root depth, plant height and fresh biomass (separately for root, stem, leaves, inflorescence and grain) were recorded. Root material was obtained by extracting the plant out of tube and washing off the soil. All plant parts were oven-dried at 70 ºC for three days, and dry weights were determined. Harvest indexes, HI_r and HI, as the ratios of grain to biomass yield in dry matter with and without root mass were determined, respectively.

The experiment was arranged in randomised block design with three replications. Obtained data were subjected to analysis of variance (ANOVA) procedures (Statgraphic, 2005) to ascertain significant differences between treatments ($P \leq 0.05$). Differences between means were revealed by Duncan's multiple range test ($\alpha = 0.05$).

Results and Discussion

Soil water stress affected all studied parameters (Table 1). Plants subjected to soil water stress exhibited slightly increse in rooting depth. In comparison to control (W1), the differences were not significant except for treatment W5, where drought occurred during inflorescence formation and tap root was longer for 34%. Results are in accordance to Johnson and Henderson (2002) who reported grain amaranth apparent ability to respond to water stress also by increasing rooting depth what makes it a potentially useful crop where soil moisture conditions vary considerably among growing seasons. Plant growth decreased according to drought duration. However, in comparison to constant adequate moisture lasted throughout the growing period, the stem height was not affected and inflorescence was even longer when drought occurred in the period of vegetative growth stages (W4). As morphological traits, biomass partitioning and

accumulation, expressed on the fresh weight basis, was the least affected by drought occurred in the period before inflorescence formation.

Dry biomass production and assimilate allocation was altered in response to water supply regimes. Plant total dry mass decreased in case of plants exposed to drought throughout growing cycle, while plants water-stressed only for a certain periods were similar and not differed significantly to the control. Among water-stressed treatments, the reduction in assimilate allocation into the root was the most pronounced in treatment W4 and W2, and similar patterns occurred in respect to stem weight and root to shoot ratio.

In comparison to the control, grain yield of *A. cruentus* 'G6' plants growing under permanent water stress was strongly reduced (for 51% on fresh and dry weight basis). Drough stress during inflorescence formation (W3, W5) had also negative effect on grain yield, although adequate soil moisture status in treatment W5 was restored at the beginning of flowering. Grain yield of plants grown under drough condition until the inflorescence formation (W4) was statistically the same, but in average higher than that of control.

Although, the harvest index (HI) obtained in the pot experiment (Table 1) could not be extrapolated to field conditions, the parameter evaluated for *A. cruentus* 'G6' in field trials is also relatively low and ranged between 0.19 and 0.34, with regard to growing season and date of sowing (Grobelnik Mlakar, 2006). Those low values show the low efficiency of biomass allocation into the seed of species, and improvement of traits increased the harvest index is important breeding goal for this versatile and highly phenotypically plastic crop (Brenner, 2000). However, water regime affected both calculated harvest indexes which values were higher in W4 treatment in comparison to the control (in respect to HI, the difference is pronounced, but not significant). Described increase of grain yield, harvest indexes and decrease in root to shoot ratio in treatment W4 showed higher assimilation efficiency after plant re-watering in the period of generative growth stages. Therefore, soil drying in the growing period from the sowing to the beginning of inflorescence formation seams to provoke and improve the assimilate allocation to the above-ground biomass and particularly to the grain.

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Table 1 Plant growth, biomass production and yield performance of *A. cruentus* 'G6' determined at the time of maturity in response to drought initiated at different growth stages

	ANOVA[1]	Treatments mean (cm, g, cm^2 per plant)[2]				
	SWR	W1	W2	W3	W4	W5
Root length	*	47.4**b**	50.2**b**	50.6**b**	48.4**b**	63.7**a**
Stem height	**	103.8**a**	65.7**c**	82.1**b**	104.8**a**	86.7**b**
Inflorescence length	**	29.4 **b**	15.4**d**	17.6**cd**	33.3**a**	19.9**c**
Root weight (fresh)	**	13.0**a**	5.6**b**	10.2**ab**	8.5**bc**	13.1**a**
Stem weight (fresh)	**	32.7**a**	13.7**c**	23.2**b**	28.1**ab**	33.3**a**
Inflorescence weight (f.)	**	25.3**a**	10.3**b**	15.3**b**	30.4**a**	14.0**b**
Leaves weight (fresh)	**	18.0**ab**	7.5**d**	10.3**cd**	19.5**a**	13.8**bc**
Grain weight (fresh)	**	6.6**b**	3.2**c**	4.6**c**	8.5**a**	3.9**c**
Biomass (fresh)	**	95.6**a**	40.4**c**	63.6**b**	95.1**a**	78.1**ab**
Root weight (dry)	**	5.3**a**	2.3**c**	4.3**ab**	2.9**c**	4.0**ab**
Stem weight (dry)	*	8.8**a**	4.2**b**	6.9**ab**	5.9**ab**	8.0**a**
Inflorescence weight (dry)	**	5.6**a**	2.3**c**	3.4**bc**	4.8**ab**	4.2**ab**
Leaves weight (dry)	*	4.9**ab**	3.6**c**	5.3**a**	4.3**ab**	3.7**c**
Grain weight (dry)	**	3.9**a**	1.9**c**	2.8**b**	4.6**a**	2.5**bc**
Biomass (dry)	**	28.5**a**	14.2**b**	22.8**a**	22.5**a**	22.4**a**
R:S	*	0.23**a**	0.19**ab**	0.23**a**	0.15**b**	0.22**a**
HI	*	0.19**ab**	0.16**b**	0.16**b**	0.24**a**	0.14**b**
HIr	**	0.15**b**	0.14**b**	0.13**b**	0.21**a**	0.12**b**

[1] ***, * - Significant at the 0.01and 0.05 probability level, respectively*
[2] *Means within each row followed by different letters are significantly different (Duncan, α = 0.05)*

ASSESSMENT OF THE ARTHROPODS ASSOCIATED WITH THE AMARANTH CROP IN THE CENTRAL ARGENTINA

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Abstract
The plant of amaranth is characterised by the high nutritional value of their leaves and a contained multifaceted high place in the grain. The aim of this work was to study the arthropods associated with the above mentioned culture. The trial was performed on an amaranth crop sown in a plot of the experimental field in the Faculty of Agronomy of the UNLPam, Santa Rosa (La Pampa), Argentina. The essay was carried out by means of a completely ramdomized blocks design (N = 4) with 18 treatments (varieties) per block. Sampling of the arthropods consisted on inspecting 6 whole plants (stem, petioles and both sides of the leaf) in each weekly treatment during the whole development of the crop. The insects sampled were sorted into ten orders with 21 families and 23 genera. The orders with the largest number of families were Coleoptera (6) and Hemiptera (4).During germination the phytophagous species with highest population was *Acromyrmex striatus* (Roger) (Hymenoptera: Formicidae). During the vegetative stages the most abundant were *Epicauta adspersa* (Kluj) (Coleoptera: Meloidae), *Nezara viridula* (L.) and *Edessa meditabunda* (F.) (Hemiptera: Pentatomidae), whereas in the reproductive stages the population of *Tetranychus urticae* (Koch) (Acari: Tetranychidae) prevailed.

Key words - *Amaranthus*, pest, natural enemies

Introduction

The genus *Amaranthus* is composed by approximately 60 species, nevertheless, only a limited number of them are cultivated, whereas many are considered to be weeds. The germplasm of *Amaranthu*s is available in 11 countries (Sauer, 1967; Toll *et. al*., 1982).

The most common problems of insects and deseases of Amaranth were described in the Amaranth Grain Production Guide (Weber *et al*. 1990), and the Insects and Disease pests of Amaranth (Wilson, 1990). In Montana (USA), extensive damage was observed in the lines of sowing caused by *Epitrix cucumeris* (Coleoptera: Curculionidae) (Stallknecht and Schulz-Schaeffer, 1993). Also problems of virus were detected transmitted by leafhoppers *Circulifer temellus* (Homoptera: Cicadellidae) (Stallknecht *et al*., 1990). Ten species of insects associated with *Amaranthus retroflexus* L. were studied in Turkey as potential candidates for biological control of these weeds (Aslan *et al*., 2002).

In Argentina there are fewer precedents referred to animal plagues in plants of amaranth. Bosq (1943) and Hayward (1944) quoted *Conotrachelus histrio* HBK (Coleoptera: Curculionidae) the presence of stem borers in several species of amaranth. Vasicek *et al*. (1998) evaluated the behaviour of this stem borer in *A. hybridus* var. *quitensis* in soybean crops with the goal of using it as biological control of the above mentioned weeds. In La Pampa province only there is a record of arthropods associated with the culture elaborated for Ves Losada and Covas in 1987, therefore the aim of this work it was to investigate the damage that these organisms produce on the plants, to

evaluate the above mentioned damages and to determine the natural enemies (parasitoides and/or predators) of the species harmful to the crop.

Material and Methods

The trial was carried out in the Faculty of Agronomy of the UNLPam, located in Ruta Nacional 35 km 334, Santa Rosa (La Pampa), Argentina, during summer 2006-2007. The trial was performed on an amaranth crop sown in a plot of the experimental field of the above mentioned Faculty. The soil is an Entic Haplustol with sandy loam texture. The assay was carried out by means of a completely ramdomized blocks design (N = 4) with 18 treatments (varieties) per block. For each variety 5 rows of 12 metres were sown with a spacing of 0.30 m between rows. The density of sowing was 3 $Kg.ha^{-1}$.

Species and varieties sowed:

1. *Amaranthus cruentus* Mexicano (INTA Anguil, L.P.)
2. *Amaranthus cruentus* R 127 (Origen: México) (Don.: USA AMES 2244)
3. *Amaranthus pumilus* RAFIN K 340 (Don.: Rep. Checa)
4. *Amaranthus cruentus* L. var. Amont (Origen: USA) (Don.: USA PI 538255)
5. *Amaranthus caudatus* L. CAC 48A (Origen: Perú) (Don.: Alemania)
6. *Amaranthus cruentus* cv. Don Guiem (Año: 2003) (INTA Anguil, L.P.)
7. *Amaranthus hypochondriacus* var. Revancha Morfotipo Mercado (Origen: México)
8. *Amaranthus hypochondriacus* var. Nutrisol Morfotipo Azteca
9. *Amaranthus cruentus* var. Tarasca Morfotipo Mexicano (Origen: México)
10. *Amaranthus cruentus* var. Morelos (Origen: México)
11. *Amaranthus hybridus* K 593 (USA) PI 538325
12. *Amaranthus hypochondriacus* 280 FK-FH1 (Origen y Don. Hungría)
13. *Amaranthus cruentus* cv. Don León
14. *Amaranthus cruentus* cv. Candil (Origen: Río Cuarto)
15. *Amaranthus hypochondriacus* San Antonio (Origen: México)
16. *Amaranthus hypochondriacus* Rojita/Rosita (Origen: México)
17. *Amaranthus mantegazzianus* cv. Don Juan (Año: 2006) (INTA Anguil, L.P.)
18. *Amaranthus hypochondriacus* cv. Artasa 9122 (Año: 2006) (INTA Anguil, L.P.)

Detection of organisms on the plant: sampling of the arthropods consisted of inspecting 6 whole plants (stem, petioles and both sides of the leaf) in each treatment weekly during the whole development of the crop. We registered: arthropod species, stage of the insect, number of individuals, place in the plant, the growth stage. The gathered material was taken to the laboratory for identification. The parts of damaged plants were botanized and the adult insects gathered were collected in entomological boxes.

The identification was carried out by the use of specific bibliography with keys (Borror *et al*., 1989; De Santis, 1969; Gauld, 1980; Lanteri, 1994; Quintanilla, 1976; Stehr, 1987; Stehr, 1991; Wharton *et al*., 1997) and consulting with specialists.

Results and Discussion

The insects sampled were sorted into ten orders with 21 families and 23 genera. The orders with the largest number of families were Coleoptera (6) and Hemiptera (4).

Within the Order Coleoptera the most represented families were Curculionidae and Coccinelidae; while in Hemiptera the family Pentatomidae predominated. During

germination the phytophagous species with highest population was *Acromyrmex striatus* (Roger) (Hymenoptera: Formicidae).

During the vegetative stages the most abundant were *Epicauta adspersa* (Kluj) (Coleoptera: Meloidae), *Nezara viridula* (L.) and *Edessa meditabunda* (F.) (Hemiptera: Pentatomidae), whereas in the reproductive stages the population of *Tetranychus urticae* (Koch) (Acari: Tetranychidae) prevailed.

Behaviour of the genotypes of *Amaranthus*

Differences between genotypes regarding their degree of infection with plagues were observed. The species with least number of insects was the genotype *Amaranthus hypochondriacus* 280 FK-FH1 (genotype 12). This low amount of insects can partly be due to its short growth stage (60 days). This genotype was not affected by insects such as *Tretanychus urticae* (Koch), thrips, *Spodoptera frugiperda* (Smith) and *Rachiplusia nu* (Guenée) since these species appeared when the genotype *Amaranthus hypochondriacus* 280 FK-FH1 had already been harvested.

The varieties that had the highest amount of insects were the genotypes *Amaranthus cruentus* var. Monster morfotipo Mexican (genotype 9) and *Amaranthus hypochondriacus* San Antonio (genotype 15) with a rank between 41 and 36 insects in two blocks. Other genotypes presented a similar behaviour with an average of 23, 93 insects.

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Table 1 Species of insects associated with *Amaranthus*

Orden	**Family**	**Species**	**Food habit**
Coleoptera	Chrysomelidae	*Diabrotica speciosa* (Germar)	Phytophagous
	Coccinelidae	*Hippodamia convergens* (Guerin-Meneville)	Predator
		Eriopis conexa (Germar)	Predator
		Harmonia axiridis (Pallas)	Predator
	Curculionidae	*Pantomorus auripes (*Hustache)	Phytophagous
		Conotrachelus spp.	Phytophagous
	Elateridae	*Monocrepidius spp.*	Phytophagous
	Melyridae	*Astylus atromaculatus* Blanch	Phytophagous
	Meloidae	*Epicauta adspersa* Klug	Phytophagous
Lepidoptera	Noctuidae	not identified species	
		Helicoverpa zea (Boddie)	Phytophagous
		Pseudaletia unipuncta (Haworth)	Phytophagous
		Rachiplusia nu (Guenée)	Phytophagous
		*Spodoptera frugiperda (*Smith)	Phytophagous
Diptera		not identified species	
Hymenoptera	Pompilidae	*Anoplius* sp.	Predator
	Mutilidae	not identified species	
	Formicidae	*Acromyrmex lobicornis*(Emery)	Phytophagous
		Acromyrmex striatus (Roger)	Phytophagous
	Braconidae	*Bracon sp*.	Parasitoid
		Alabargus sp.	Parasitoid
	Encyrtidae	*Copidosoma floridanum (*Ashmead)	Parasitoid
Thysanoptera			
Neuroptera		*Chrysopa lannata* (Banks)	Predator
Homoptera	Cicadelidae	not identified species	Phytophagous
	Aphididae	not identified species	Phytophagous
Hemiptera	Largidae	*Largus fasciatus* (Berg)	Phytophagous
	Rhopallidae	not identified species	Phytophagous
	Pentatomidae	*Nezara viridula* (L.)	Phytophagous
		Edessa meditabunda (F.)	Phytophagous
	Coreidae	not identified species	Phytophagous
Orthoptera	Acrididae	*Trimerotropis pallidipennis* (Burmeister)	Phytophagous
Acarina	Tetranichidae	*Tetranychus telarius* (Koch)	Phytophagous

AGRONOMIC CHARACTERISTICS RELATIONSHIP WITH YIELD SEED OF SIXTEEN GENOTYPES OF *Amaranthus* GROWN IN ARGENTINA

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Abstract
In this work was compared the agronomic characteristics associated with the production of grain of sixteen *Amaranthus* genotypes grown in the Faculty of Agronomy UNLPam., Argentina. Plant height and long inflorescence and yield grain of the harvest was measured. The harvest index (HI) was calculated thus: HI (%) = (economic yield /biological yield) x 100. The weight of 1000 seeds and density was determined. *A. hypochondriacus* Artaza had shorter height plant than the rest and good yield seed. *A. cruentus*, R 127, *A. caudatus* CAC 48 A, *A. cruentus* Don Juan, *A. pumilus* Rafin K 340, *A. hypochondriacus* 280 FK-FH1 and *A. cruentus* var. Amont had HI around 20 but only the last two had good grain yield. The rest HI was between 15.2 and 18.5. *A. hypochondriacus* 280 FK-FH1 is the only genotype that is the length of inflorescence significantly higher than the rest. The weight of 1000 seeds varied between 0.67 and 0.90 g and the density between 61.15 and 84.20 kg hL^{-1}

Key words - amaranth, grain, harvest index, weight seed

Introduction
The genus *Amaranthus*, family *Amaranthaceae*, has 65 member species, some 50 of which are native to the Americas. Some species are cultivated for their grain, other as vegetables or forage and still others for their pigments. Some species are weeds (Granjero Colin *et al*., 1994; Kigel, 1994). Their genetic variability has afforded them exceptional adaptability to a wide range of environmental conditions, although being C_4 plants they do require high temperatures (35 ºC) (Kulakow and Hauptli, 1994) and strong light (Putman, 1990).

Amaranth grain has received special attention in North America because of its high protein and lysine contents. Its starch and lipids have also been studied for potential use in the food and cosmetics industries (Henderson *et al.*, 2000). In Argentina, amaranth cultivation could be an alternative for some 5 million ha north of Patagonia in the semi-arid region of the country (Covas, 1994). However, this crop is not traditionally grown in the region (Fresentese, 1987) and its cultivation needs to undergo extensive experimentation. The objective of this work was compared agronomic characteristics associated with the production of grain of sixteen *Amaranthus* genotypes grown in the Faculty of Agronomy UNLPam., Argentina.

Material and Methods
Sowed genotypes are on the table 1. Sowing of genotype was in to the Experimental Field of the Facultad of Agronomy UNLPam. (S: 36º 32,726' W: 64º 18,721', 220 m height on the level of the sea), located to 5 km of Santa Rosa, capital city of the County of The Pampas, Argentina.

Sowing was performed by horticultural machine of density approximately 3 $kg.ha^{-1}$. The maximum sowing depth was 1.5 cm. The used plots were 12 m long and 1.5 m

wide with six rows. The two extern rows are parcel border and they were discarded. We selected ten plants on the second row to measure plant height and inflorescence long at harvest. Yield seed was measured in the third, fourth and fifth row, discarding the two extremes of 0.5 m. The harvest index (HI) was calculated thus: HI (%) = (economic yield /biological yield) x 100. It was determined the weight of 1000 seeds and density. The plots were maintained free of weeds by a mixture of mechanical (between plots) and manual (between rows) methods during the juvenile stage of plant growth (until 25 days after emergence).

The experiment had a randomized block design with four replicates. ANOVA and Tukey test was used for detected significance and compared the mean.

Sowed genotypes:

Amaranthus cruentus Mexicano (INTA Anguil, L.P.)
Amaranthus cruentus R 127 (Origen: México) (Don.: USA AMES 2244)
Amaranthus pumilus RAFIN K 340 (Don.: Rep. Checa)
Amaranthus cruentus L. var. Amont (Origen: USA) (Don.: USA PI 538255)
Amaranthus caudatus L. CAC 48A (Origen: Perú) (Don.: Alemania)
Amaranthus cruentus cv. Don Guiem (Año: 2003) (INTA Anguil, L.P.)
Amaranthus hypochondriacus var. Nutrisol Morfotipo Azteca
Amaranthus hypochondriacus var. Tarasca Morfotipo Mexicano (Origen: México)
Amaranthus cruentus var. Morelos (Origen: México)
Amaranthus hybridus K 593 (USA PI 538325)
Amaranthus hypochondriacus 280 FK-FH1 (Origen y Don.: Hungría)
Amaranthus cruentus cv. Don León (INTA Anguil, L.P.)
Amaranthus cruentus cv. Candil (Origen: Río Cuarto)
Amaranthus hypochondriacus Rojita/Rosita (Origen: México)
Amaranthus mantegazzianus cv. Don Juan (Año: 2006) (INTA Anguil, L.P.)
Amaranthus hypochondriacus cv. Artasa 9122 (Año: 2006) (INTA Anguil, L.P.)

Results and Discussion

The average performance of the variables measures are in Table 1 and Figure 1. In the ANOVAs of all variables were found highly significant differences ($P < 0.01$) between genotypes, we did not find significant differences between blocks. *A. hypochondriacus* Artaza had shorter height plant (1.286 m) than the rest similar to report by Troiani *et al.* (2004) for the same species. This is desired condition for mechanical harvesting. It had good yield seed (4147 $kg.ha^{-1}$). *A. mantegazzianus* had the biggest plant height (2.381 m). *A. hypochondriacus* 280 FK-FH1 is the only genotype that is the length of inflorescence significantly higher than the rest. Its yield grain was lower caused by its lax and branched inflorescence.

A. cruentus, R 127, *A. caudatus* CAC 48 A, *A. cruentus* Don Juan, *A. pumilus* Rafin K 340, *A. hypochondriacus* 280 FK-FH1 and *A. cruentus* var. Amont had HI around 20 but only the last two had good grain yield. The rest HI varied between 12.3 and 18.6.

The weight of 1000 seeds is a partial indicator of its size. The small size of amaranth seed hinders the same management and the establishment of the crop because of the need for intimate contact with moisture of the soil.

The weight of 1000 seeds varied between 0.37 and 1.21 g of Amaranth (Espitia-Rangel, 2000). Big seeds have greater force, better handling and pop, by increasing the endosperm (starch portion) to the detriment of the cotyledons, which would decrease

proportionally the protein content (Brenner *et al*., 2000). In this work the weight of 1000 seeds varied between 0.67 and 0.90 g.
The seed density (kg hL^{-1}) can be influenced by the size, shape, roughness and moisture grain. Amaranth has no density standards as the rest of cereals, 78 kg hL^{-1} has been established as a basis for *Triticum durum* by the European Economic Community.

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Table 1 Mean of the plant height, long inflorescence, sees size and seed density of the genotype sowed in Argentina

Genotype	Plant height (mm)	Long inflorescence (mm)	Seed Size (g)	Seed Dens. (kg hL^{-1})
A. cruentus Mexicano	2255 d e	358 a	0,90 f	62.47 a b
A. cruentus R 127	2176 c d e	342 a	0,79 d e	64.95 a b c d
A. pumilus RAFIN K 340	1544 a b	404 a	0,80 d e	78.80 d e f
A. cruentus var. Amont	1859 b c	392 a	0,73 b c d	75.85b c d e f
A. caudatus L. CAC 48A	1966 c d	324 a	0,77 d e	66.57 a b c d e
A. cruentus Don Guien	2135 c d e	414 a	0,82 e	62.52 a b
A. hypochond. var. Nutrisol Morfotipo Azteca	2094 c d e	460 a	0,65 a b	69.70 a b c d e
A. hypochond. var. Tarasca	1977 c d	359 a	0,79 d e	77.37 c d e f
A. cruentus var.Morelos	2051 c d e	397 a	0,78 de	61.15 a
A. hybridus K 593	1496 a	311 a	0,63 a	73.00a b c d ef
*A. hypoch*ond. 280 FK-FH1	1546 a b	665 b	0,75 c d e	84.20
A. cruentus cv. Don León	2125 c d e	402 a	0,77 d e	63.70 a b c
A. cruentus cv. Candil	2075 c d e	354 a	0,75 c d e	67.62 a b c d e
A. hypochond. Rojita/Rosita	2211 d e	423 a	0,80 d e	66.57 a b c d e
A. mantegaz. cv. Don Juan	2381 e	427 a	0,76 c d e	63.52 a b c
A. hypoch. cv. Artasa 9122	1286 a	330 a	0,69 a b c	80.72 f

Number followed by the same letter are not significantly different (P <0.05)

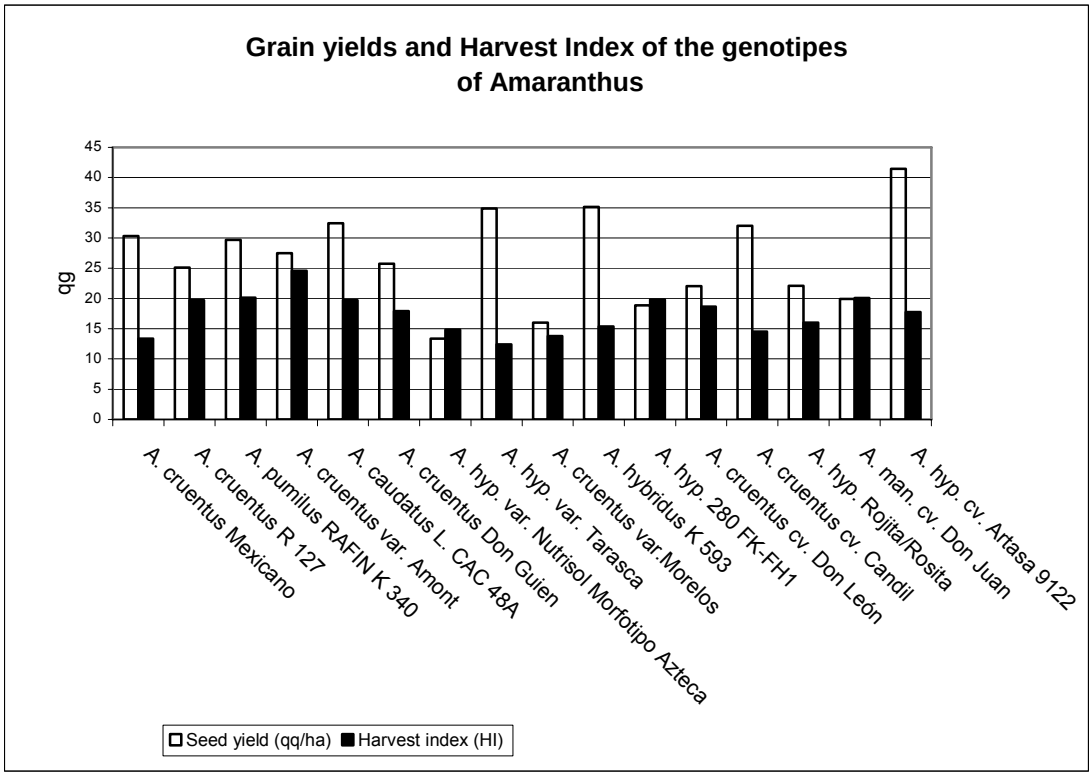

Figure 1 Mean of grain yields and harvest index of the genotype of amaranth sowed in Argentina

Plenary session III:
Amaranth genetic resources – environmental, nutritional and molecular evaluation

GRAIN AMARANTH GENOTYPES (*Amaranthus cruentus, Amaranthus hypochondriacus*) ADAPTED TO EASTERN AUSTRIA

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Abstract

Amaranth genotypes adapted to semiarid conditions in Eastern Austria were examined. Hand-harvested grain yields of the tested amaranth lines ranged between 200 and 300 g. m^{-2}. The best yielding genotypes "Neuer Typ" and "Amar" reached 240 to 300 $g.m^{-2}$ depending on environment. Thousand seed weight of the tested genotypes ranged between 0.59 and 0.96 g. While the yield of "Neuer Typ" is due to heavy seeds "Amar" is characterised by a large number of seeds per plant. Mechanical harvest carried out by a plot combine resulted in yields between 160 and 260 $g.m^{-2}$. Grain moisture at harvest of the earliest type "Neuer Typ" ranged between 21 and 24%, that of the latest type "Amar" between 30 and 38%.

Key words - grain amaranth, genotypes, yield, harvest index, seed weight

Introduction

Selection and breeding of grain amaranth in the past give rise to the assumption that relevant agronomic characters can be further improved. In Austria, selection and breeding activities were started in 1988 by Dr. Georg Dobos (Dobos, 1997). Important breeding objectives are high grain yield, early plant dry down and large seeds. The aim of the following study was to characterise genotypes adapted to semiarid conditions with respect to potential and harvestable grain yield, grain maturity and seed weight.

Material and Methods

Field experiments were carried out under semiarid conditions on a czernosem at the Experimental Farm Groß-Enzersdorf in Eastern Austria between 2002 and 2005. Seeds of genotypes adapted to the Pannonian climate were provided by the breeder Dr. Georg Dobos (ZENO PROJEKTE, Vienna). The genotypes belong to different species and differ with respect to plant morphology and time to maturity (Figure 1, Table 1). "Neuer Typ" is a strongly branching, early-maturing, semi-dwarf type. "Anderer Typ" is an early-maturing, rather short type with a compact inflorescence. "Mittlerer Typ" is medium in maturity and tall with little tendency to branch. "Amar" is a tall, sparsely branching, late-maturing type with compact inflorescences. The experiment was arranged in a split-plot design in four replications with three genotypes on main plots and five crop densities on subplots. Table 2 shows the dates of sowing and harvesting. No fertiliser was added due to high mineral nitrogen in the soil. Plots were hand-harvested (1 m^2) and combined by a plot combine.

Neuer Typ Anderer Typ Mittlerer Typ Amar

Fig. 1 Amaranth genotypes

Table 1 Genetic background and observed characteristics of the tested genotypes at the experimental site

Genotype	Genetic background	Plant height (cm)	Inflorescence	Temp. sum sowing to harvest (°C d)
Neuer Typ (*A. hypochondriacus*)	Selection of a crossbred	70–100	Highly branching	1950–2200
Anderer Typ (*A. hypochondriacus*)	Selection of a crossbred	80–140	Rather compact spike	2100–2300
Mittlerer Typ (*A. hypochondriacus*)	Selection of a crossbred (by B. Baji, Inst. for Agrobot, Tapioszele, Hungary)	120–170	Loose spike, red-coloured	2350–2550
Amar (*A. cruentus*, Mexican type)	Selection of RRC 1041 (Rodale Research Center)	120–170	Compact spike	2400–2700

Table 2 Dates of sowing and harvesting

Growing season	Sowing	Harvesting			
		Neuer Typ	Anderer Typ	Mittlerer Typ	Amar
2002	April 29	Aug. 19	-	Sept. 5	Sept. 18
2004	May 27	Sept. 9	Sept. 13	-	Oct. 5
2005	May 11	Sept. 5	Sept. 7	-	Oct. 3

Results and Discussion

The tested genotypes do not only differ with respect to plant morphology but also in grain yield and grain yield components. Under climatic conditions of Eastern Austria, the tested amaranth genotypes yielded between 200 and 300 $g.m^{-2}$ when harvested manually (Table 3). It is similar to yields obtained in South Western Germany (Kübler, 2002) and Slovakia (Jamriška, 1998). Crop density hardly affected grain yield

Table 3 Potential grain yield, harvest index and grain yield components

Year	Genotype	Grain yield * (hand-harvested) ($g.m^{-2}$)	Harvest index	Observed density range (plants m^{-2})	Number of seeds per plant	Thousand seed weight * (g)
2002	Neuer Typ	239 a	0.39 a	8–135	16000 a	0.96 a
	Mittlerer Typ	219 a	0.26 b	7–86	21900 a	0.63 b
	Amar	216 a	0.25 b	6–119	20000 a	0.66 b
2004	Neuer Typ	295 a	0.38 a	10–80	16500 b	0.86 a
	Anderer Typ	204 b	0.26 b	9–82	11700 c	0.83 a
	Amar	290 a	0.27 b	8–92	22000 a	0.60 b
2005	Neuer Typ	241 a	0.37 a	7–54	16200 b	0.91 a
	Anderer Typ	201 b	0.31 b	8–63	12100 b	0.89 a
	Amar	235 a	0.25 c	8–52	24300 a	0.59 b
*	*dry weight*					
	SNK, P=0.05					

(data not shown), but crop stands at low densities proved to show a slight yield advantage (Gimplinger et al., 2008). The semi-dwarf “Neuer Typ” and the tall “Amar” were the best yielding cultivars. In general, the harvest index of amaranth is low compared to common cereals. While the genotypes “Anderer Typ”, “Mittlerer Typ” and “Amar” were characterised by low harvest indices between 0.25 and 0.27, the semi-dwarf “Neuer Typ” reached noticeable harvest indices between 0.37 and 0.39. Grain yield per area is the product of plants per area, seeds per plant and seed weight. The lines “Neuer Typ” and “Anderer Typ” produce large, heavy seeds while “Amar” and “Mittlerer Typ” form substantially smaller seeds. Yield components of the best yielding lines differed: The grain yield of “Neuer Typ” was based on large, heavy seeds but a lower number of seeds per plant, while the grain yield of the small-seeded “Amar” was due to a higher number of seeds per plant.

Combine harvest resulted in average yields between 170–260 g m^{-2} (Table 4). Seed losses during combining, calculated as the difference between hand-harvested and combine-harvested grain yield, proved to be highly variable and amounted up to 40%.

Table 4 Harvestable grain yield, seed losses and grain moisture at harvest

		Grain yield * (combined) ($g.m^{-2}$)	Seed losses (%)	Grain moisture at harvest (%)
2002	Neuer Typ	181 b	32	24 c
	Mittlerer Typ	156 c	40	27 b
	Amar	238 a	-9	32 a
2004	Neuer Typ	252 a	17	21 b
	Anderer Typ	209 b	-2	22 b
	Amar	264 a	10	30 a
2005	Neuer Typ	222 a	9	23 b
	Anderer Typ	207 ab	-3	24 b
	Amar	173 b	36	38 a
*	*dry weight*			
	SNK, P=0.05			

High seed losses of “Neuer Typ” in 2002 and “Amar” in 2005 were probably due to moist weather conditions at harvest. Crop density also affects seed losses (data not shown): Dense crop stands of more than 50 plants m^{-2} could be combined more easily and resulted in smaller seed losses (Gimplinger et al., 2008).

Time to maturity and grain moisture at harvest of the tested genotypes showed clear differences. “Neuer Typ” could be harvested four weeks, “Anderer Typ” three weeks earlier than the late-maturing “Amar”.

In conclusion, the semi-dwarf “Neuer Typ” showed a number of agronomic advantages: It was characterised by high grain yield, large seeds and early grain maturation.

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GENE BANK OF SLOVAKIA

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Gene Bank of RIPP Piešťany, that was established in year 1996 provides principal task at conservation of seed species of the plants. The Gene Bank is specific technical institution for medium-term and long-term storage of the seeds in vital state with storing capacity of 50 000 samples. Inseparable part of the Gene Bank activity is monitoring on viability and supply of the seeds, distribution of the samples to the users, information system management, identification and verification of PGR. The Gene Bank is the only institution of this kind in Slovakia.

The task solution of the National Programme can resume onto following items: a) gathering and evaluation of biodiversity of original domestic gene pool of Slovakia; b) conservation of individual collections of genotypes *in vitro, ex situ* and *in situ* in the gene bank or in field collections – repositories, respectively; c) acquisition of rare foreign genotypes into the collection of NP; d) creation of passport and descriptive databases; e) participation in international cooperation; f) education and edification; g) offering biological material for breeding, research, study purposes and for exchange to other gene banks.

Key words - Gene Bank, conservation, evaluation

MANAGEMENT OF GENE BANK IN THE CZECH REPUBLIC WITH RESPECT TO MINOR CROPS

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Introduction

Plant genetic resources for food and agriculture (PGRFA) are the biological basis of world food security and, directly or indirectly, support the livelihoods of every person on Earth. Plant biodiversity represents the primary source for food, feed, shelter, medicines and many other products and means that make life on Earth possible and enjoyable (FAO, 1996; WCMC, 1992; UNEP 1995). Plant genetic resources are maintained particularly *ex situ* in gene banks.

In the Czech Republic the gene bank coordinates National Programme on Plant Genetic Resources Conservation and Utilization launched by the Ministry of Agriculture in 1993. Since then twelve research institutes and universities joined the programme. In its activities the gene bank follows international agreements and recommendations. Convention on Biodiversity (CBD) was acceded by the Czech Republic in 1994 and adopted into the Czech legal system as the Act No. 134/1999. To reflect principles of CBD and to strengthen the care on agro-biodiversity, the Act No. 148/2003 on "Conservation and Utilization of Genetic Resources of Plants and Micro-organisms Important for Food and Agriculture" passed in 2003, followed by the Regulation 458/2003, which carries out this Act. Then the Czech Republic joined International Treaty on Plant Genetic Resources for Food and Agriculture (IT PGRFA).

The Ministry of Agriculture of the Czech Republic updated in 2007 the "National Programme on Plant Genetic Resources and Agro-biodiversity Conservation and Utilization" issued in January 2004. Among others, access to plant genetic resources and sharing of benefits (ABS) are important issues of the Programme. To fulfil this purpose, the national Material Transfer Agreement (MTA) was worked out in 2005 and since that time it is used by the gene bank and all participants of the National programme are prompted to use it as well.

Minor Crops in the Czech Gene Bank

The number of plant species used by humans around the world is only one third of the number of species, which generations of diverse cultures around the world have drawn upon to develop crops that would meet specific needs (Zeven & de Wet, 1982). Under the term "Minor crops" can be included underutilized, neglected, new or alternative crops. Motivations for utilization of minor crops originate from a variety of concerns and/or expectations ranging from ethical, social to economic:

- Contribute to agricultural diversification
- Contribute to a greater use of marginal lands and changing environments
- Contribute to greater food security
- Contribute to a more balanced diet
- Contribute to a better safeguard of whole the agrobiodiversity heritage
- Contribute towards a better preservation of cultural identities and traditions

- Contribute to enhance self-reliance of agricultural systems, particularly in disadvantaged areas
- Provide additional and /or diversified sources of income to farmers
- Provide opportunities of employment in agriculture and related sectors (Padulousi, 1998)

Czech institutions, which now take part in the National Programme hold in their collections over 49,000 accessions (Figure 1), among them 18%, belong to vegetatively propagated species. Among minor crops there are included 812 accessions (Tab. 1). The gene bank has in addition its main activities in the framework of the National programme also responsibility for certain collections of PGRFA. Among them minor crops play important role. Some of the minor crops have been collected for a long time as parts of major crop e.g. hulled species of wheat. Other collections have been established relatively recently (*Amaranthus*, quinoa).

Table 1 List of Minor crops in collections of Gene Bank in the Czech Republic

Species	English name	Number of accessions
Triticum monococcum	Einkorn	54
Triticum dicoccon	Emmer	115
Triticum spelta	Spelt wheat	77
Sorghum bicolor	Sorghum	40
Panicum miliaceum	Common millet	179
Setaria italica	Foxtail millet	41
Echinochloa frumentacea	Barnyard millet	1
Digitaria sanguinalis	Crabgrass	3
Fagopyrum esculentum	Common buckwheat	126
Fagopyrum tataricum	Tartary buckwheat	49
Amaranthus sp.	Amaranths	124
Chenopodium quinoa	Quinoa	3

Evaluation and characterisation of minor crops

Evaluation of PGR in GB keeps to the common methodology

1) small seed samples – preliminary evaluation
2) second phase – experimental plots (3 years)
3) third phase – cooperation of breeding company or manufacturer

The first year of evaluation is mainly for multiplication of imported or exchanged seed samples and for their preliminary evaluation. Small amounts of seed is sown in a few rows and after that unsuitable samples (for example quarantine disease, formation of no flowers caused by long-day light etc) are excluded from collection. Suitable samples receive a national accession number (ECN).

Experiments on plots planted in the second phase are used as a source of evaluation data. During the vegetation period, plant morphology, growth and development data are registered according to international descriptors. This procedure is repeated also the following year. After third year of evaluation, seed samples of self-pollinated plants are ready to store them in gene bank. At first, the seeds must be clean to achieve the maximum purity of sample (e.g. without any admixtures, rests of inflorescences or flowers, etc.). The next step is evaluation of germination ability on Petri dish. If the samples fulfil all steps of procedure in the best quality, they can be stored in gene bank.

In case of cross-pollinated plants, it is necessary to obtain “clean”, not cross-pollinated seed samples. For this reason, the fourth year they are multiplicated in the isolation cages. Next steps are the same as in case of self-pollinated plants – cleaning, evaluation for germination ability etc.

Management of minor crop accessions in the Czech Gene Bank

Minor crops belong to the group of seed propagated crops which are stored in the gene bank, under two different temperature regimes -18°C for base collection and -5°C for active collection or -18°C for sensitive accessions respectively. The active collection is kept as a medium term and it is used mainly for seed distribution to users. Seed samples and relevant information are distributed according to requests for purposes of research, breeding and education under conditions listed in MTA. All accessions are stored in the active collection. The base collection is kept as a long-term reserve of the most valuable accessions for the future. In the base collection are also stored original indigenous or rare accessions. The amount of seeds depends on way of pollination; for self-pollinated species, there are stored 4,000 and for cross-pollinated 12,000 viable seeds; the minimum amount is 1,000 seeds. For storing are suitable only accessions with 98% of purity without admixture, fully mature and visibly healthy, without any treatment. The initial germination ability of seeds should be 95% and more. The moisture content depends on species, in case of minor crops the moisture content range 6-9%. Before storing, sample is documented, labelled and put into glass containers with silica gel and located in the storage. After 10 years the regular viability test is performed.

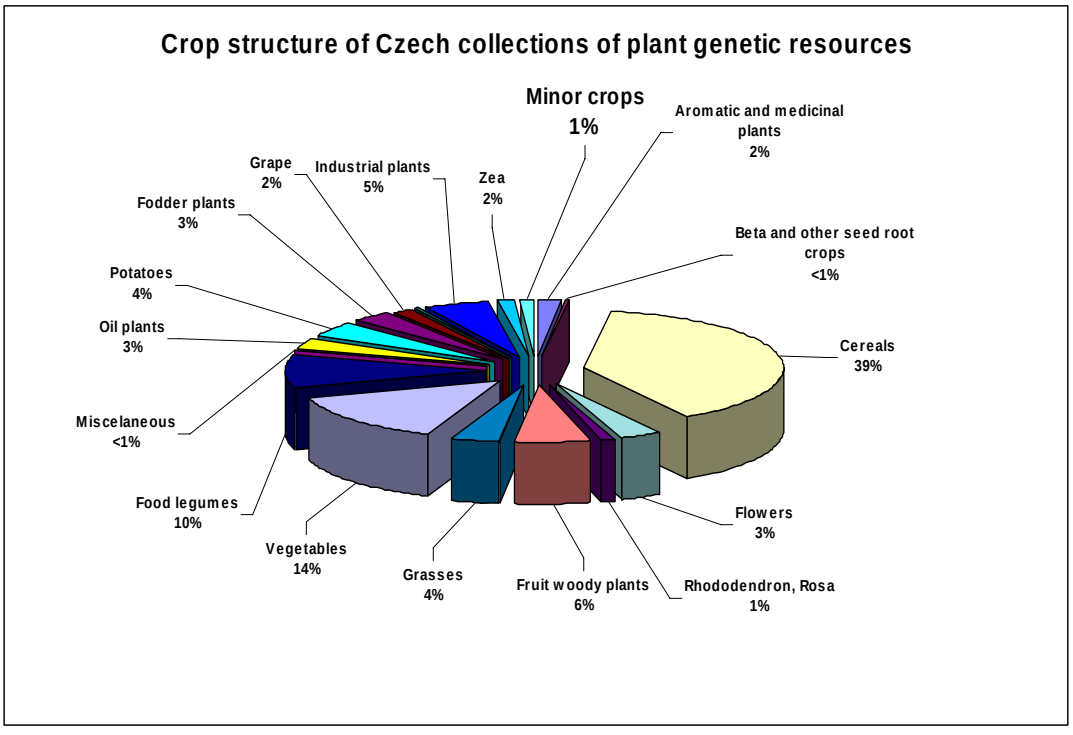

Fig. 1 Crop structure of Czech collection of plant genetic resources

Acknowledgement

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FARINOGRAPH PROPERTIES AND BREAD QUALITY OF AMARANTH-WHEAT AND AMARANTH-SPELT COMPOSITE FLOURS

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Abstract
The study was conducted to research the quality of bread made from organic wheat and spelt based composite flours with different rates of wholemeal grain amaranth flour replacement. Amaranth flour replaced basic flours in ratios of 10, 20 and 30% (w/w). The composite flours were analysed on their farinograph properties, and resulted composite breads on weight, volume and specific volume of the loaf. The breads were sensory evaluated by a non-trained panellists according to 10-point hedonic evaluation procedure. Sole wheat and spelt flours (both type 550) were used for comparison. Increasing levels of amaranth flour in the blends increased water absorption of composite flours and farinograph quality number mainly by increased development time, stability of dough and decreased degree of softening in blends with amaranth levels above 10%. Composite breads made of 10% amaranth flour had higher loaf volume and specific volume than breads made of sole basic flours, but further increasing of amaranth rate in blends resulted in decreasing of values. Sensory results revealed that the incorporation of amaranth into the dough had a significantly positive effect on the loaf colour (wheat-composite bread) and overall acceptability (spelt-composite bread).

Keywords - grain amaranth, composite flour, wheat, spelt, farinograph, bread quality

Introduction
Due to the unique seed composition, the grain amaranth (*Amaranthus* spp.) represents also an interesting alternative material in cereal-based composite flours for breadmaking purposes (Schnetzler and Breene, 1994; Berghofer and Schoenlechner, 2002; Bavec and Bavec, 2006). Among studies on amaranth containing composite breads there are only few reports on their farinographic and dought properties, baking performance and bread sensory characteristics (Lorenz, 1981; Breene, 1991; Burisova *et al.*, 2001; Ayo, 2001; Tosi *et al.*, 2002; Silva-Sanches *et al.*, 2004; Sindhuja *et al.*, 2005). However, studies carried out so far have concentrated only on wheat and virtually no research has been found on composite spelt-amaranth flour. Therefore, the aim of the present study, which was a part of a broader national research project on grain amaranth (project L4-6349-0482-06), was to investigate the farinograph properties of wheat and spelt based amaranth-composite flours, loaf apperence and bread quality.

Materials and Methods
White wheat, white spelt flour (*Triticum spelta* L.) and wholemeal amaranth flour were produced and processed in accordance with the EU Council Regulation (2092/91) for Organic farming. Composite flours were prepared by mixing wholemeal amaranth flour

to wheat and spelt white flour (both type 550) at rates of 0, 10, 20 and 30% (w/w). Rheological properties of dough made from each cereal flour and different blends were determined by Brabander farinograph using standard procedures ICC No. 115/1 (1998) for wheat. The dough was prepared only with salt, yeast and water according to farinograph water absorption values. Farinograph and baking tests were done in three replications for each treatment. Quality analyses of cooled bread samples (6 hours after baking) were carried out by measuring weight, loaf volume (determined by displacement of millet seeds in a constructed loaf volume meter) and calculating specific volume as a ratio of loaf volume to loaf weight. Hedonic sensory evaluation (scored from 1-unacceptable to 10-excellent) by non-trained panellists were conducted the following day after baking. Uniform sized bread slices of pre-coded samples were presented, and the following sensory atributes were evaluated: bread colour, flavour, aroma, texture, and overall acceptability. Product is considered acceptable when its mean score for overall acceptability was above 5.

Statistical analyses were performed using the Statgraphics Centurion XV (2005) statistical program, with the significance level set at $P < 0.05$. Duncan test was used to determine significance of differences among means.

Results and Discussion

Farinograph results (Table 1) show that water absorption of composite flours was affected and increased with amaranth substitution in case of both tested basic flours. Parameter increase from 53.0 to 55.9% and from 60.0 to 62.5% as amaranth flour substituted wheat and spelt flour, respectively. Dough development time and mixing stability were higher when amaranth flour was presented in the composite flours. Results on water absorption are in accordance to Lorenz (1981), but not on development time and stability. However, higher dough development time and stability was reported by Silva-Sanches *et al.* (2004) in treatments where wheat flour was supplemented with 1% amaranth albumin isolate. In the research of Tosi *et al.* (2002) where hyperproteic whole amaranth flour and hyperproteic defatted amaranth flour were added to wheat flour in rations of 4, 8 and 12%, the increased ratio of amaranth flour in the blend increased the water absorption, dough development time and decreased farinographic stability. Increasing levels of amaranth flour in the blends increased farinograph quality number mainly by increased development time, stability of dough and decreased degree of softening with amaranth levels above 10%. Development time and stability values are indicators of flour strength, thus higher values suggesting performance of stronger dough when amaranth flour is added.

Loaf weight, loaf volume and specific volume of bread are presented in Table 2. Loaf weigh recorded after baking was not influenced by amaranth addition to both cereal flours. Breads containing 10% of amaranth flour had the highest loaf volume and thus the highest specific volume, but further increasing of amaranth rate in composite flour resulted in decreasing of specific volume of the loaf. Similarly the baking potential of *Amaranthus hypohondriacus* was evaluated by Lorenz (1981). The results of his study showed that at the substitution with amaranth flour at levels of 10 and 15% did not significantly change baking properties, although the bread specific volume is lower for 7 and 10%, respectively. In the research where hyperproteic whole amaranth flour and hyperproteic defatted amaranth flour were added to wheat flour in rations of 4, 8 and 12%, the increased ratio of amaranth flour in the mixture increase the concentration of protein and lysine whereas volume and specific volume decreased (Tosi *et al.*, 2002)

Ayo (2001) reported the significant decreasing of loaf specific volume only when amaranth supplements the wheat flour above the rate of 20%.

The effects of grain amaranth flour addition on the bread sensory characteristics are shown in Table 3. When amaranth subsitited wheat flour, the significant differences were observed only between the colour of the control loaf and those of amaranth flour supplemented breads. In terms of loaf colour, the breads from wheat-composite flours were evaluated as more acceptable (values from 7.5 to 7.7) than the control (evaluated with 6.7). In case of spelt-composite breads, the amaranth substitution influenced overal acceptability and only bread containing 30% of amaranth flour was evaluated significantlly lower. All other evaluated sensory attributes of breads were not significantly influenced by an amaranth flour addition. The results are in accordance with both Lorenz (1981) and Saunders and Becker (1984) who described wheat-amaranth breads as being pleasant, nutty-tasting, and preferred by a taste panel over the flavour of white bread. The bread was more open, the texture not as silky, and the crumb colour slightly darker. Ayo (2001) reported decrease of flavour mean score at above 15%, and texture mean score at above 10% supplementation of wheat in composite flour. The crumb colour was not significantly influenced by amaranth flour replacement to 50%. As reported Burisova *et al.* (2001), the sensory quality of bread slowly decreases with increasing rate of amaranth, significantlly with substitution higher than 20%. Authors also reported the improved bread porosity for products where amaranth substituted wheat flour by 10 and 15%.

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Table 1 Farinogram values of tested composite and control flours[1]

Composite flour (cereal:amaranth in %)	Water absorption (%)	Development (min)	Stability (min)	Degree of softening 10'	Quality number
White wheat flour	**	**	**	**	**
100:0	53.0**c**	1.3**c**	1.40**c**	93.3**a**	23**c**
90:10	55.2**b**	1.9**c**	3.90**b**	64.3**b**	57**b**
80:20	55.5**b**	4.4**b**	5.70**a**	59.7**b**	75**a**
70:30	55.9**a**	5.3**a**	3.46**b**	69.7**b**	74**a**
White spelt flour	**	**	**	*P*=(.057)*	**
100:0	60.0**d**	2.4**c**	1.93**c**	100.0**a**	36**d**
90:10	61.2**c**	3.7**b**	3.03**b**	86.0**b**	54**c**
80:20	61.8**b**	4.0**b**	3.40**a**	85.0**b**	62**b**
70:30	62.5**a**	4.8**a**	2.97**b**	83.7**b**	67**a**

[1] Means within a column and among individual cereal followed by different letter are significantly different (Duncan, $\alpha = 0.05$)
**, * - Significant at the 0.01and 0.05 probability level, respectively

Table 2 Weight, volume and specific volume of loafs made from sole cereals (wheat and spelt)[1] and from composite flours

Composite flour (cereal:amaranth in %)	Loaf weight (g)	Loaf volume (cm^3)	Specific volume (cm^3/g)
White wheat flour	NS	**	**
100:0	724	1844**b**	2.55**b**
90:10	712	1937**a**	2.48**a**
80:20	716	1772**c**	2.72**c**
70:30	715	1633**d**	2.28**d**
White spelt flour	NS	**	**
100:0	713	2046**b**	2.87**b**
90:10	710	2188**a**	3.08**a**
80:20	736	2030**c**	2.76**c**
70:30	732	1946**c**	2.66**d**

[1] Means within a column and among individual cereal followed by different letter are significantly different (Duncan, $\alpha = 0.05$)
**, *, NS - Significant at the 0.01, 0.05 probability level and not significant, respectively

Table 3 Scores of sensory attributes of loaves made from sole cereals[1] and from different amaranth-cereal composite flours

Composite flour (cereal:amaranth in %)	Loaf colour (crumb and crust)	Bread flavour	Bread aroma	Crumb texture and appearance	Overall acceptability
White wheat flour	*	NS	NS	NS	NS
100:0	6.7**b**	7.2	7.4	8.0	7.2
90:10	7.6**a**	7.1	7.5	7.7	7.3
80:20	7.5**a**	6.8	7.5	7.5	7.2
70:30	7.7**a**	6.6	7.0	7.5	6.8
White spelt flour	NS	NS	NS	NS	*
100:0	7.9	8.1	8.1	8.1	7.8**a**
90:10	8.4	7.0	8.2	8.8	8.2**a**
80:20	8.8	7.9	7.8	8.2	7.9**a**
70:30	8.3	7.2	7.5	7.8	6.9**b**

[1] Means within a column and among individual cereal followed by different letter are significantly different (Duncan, $\alpha = 0.05$)
**, *, NS - Significant at the 0.01, 0.05 probability level and not significant, respectively

CHARACTERISATION OF DROUGHT TOLERANT *Amaranthus tricolor* MUTANT PLANTS

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Abstract

Amaranthus tricolor is a nutritious vegetable crop that is used as a subsistence or cash crop in the rural areas in Africa. Its yield and production is severely limited by abiotic stresses such as drought. Mutation technology was previously employed as a tool to create genetic variation in order to select for lines with improved drought tolerance. *A. tricolor* seeds were subjected to different doses of gamma radiation and 160 Gy was selected in order to ensure subsequent seed germination. The resulting mutant lines were screened over several generations under field and greenhouse conditions and seven promising drought tolerant lines were selected. Here we report on further physiological and morphological studies on two of these mutant *Amaranthus* lines. Compared with the wild type these mutant lines showed increased recovery after withdrawal of water for two weeks. Both mutant lines also showed increased protein content per gram dry weight.

Key words - *Amaranthus tricolor*, gamma irradiation, drought tolerance

Introduction

Amaranthus is a promising vegetable species often adapted to limiting growing conditions such as low nutrient soils. *Amaranthus* is well-adapted to both arid and humid environments (Liu *et al*., 2004). It has many species which are used as leafy vegetables for example: *Amaranthus tricolor, Amaranthus tristis* and *Amaranthus viridis*, and bears edible protein rich seeds. The flour ground from these seeds mixes well with cereal flours and increases the protein content (van Wyk *et al.*, 2000). Leaves of *A. tricolor* are rich in vitamins, protein and minerals hence it serves as a nutritious leafy vegetable that can be further improved to enhance the quality of life of people dependent on it for subsistence (Vietmeyer, 1984). Improvement of the seeds and development of new varieties of *A. tricolor* plants were previously reported at the ARC Vegetable and Ornamental Plant Institute, South Africa where seeds were subjected to different doses of gamma radiation in order to induce increased drought tolerance. Radiation is known to cause changes to the plant genome, but plant performance between the wild type and mutant *Amaranthus* plants may however, be similar (Jie *et al.*, 1993). Hence, this study seeks to compare the phenotypic performance of irradiated and wild type *Amaranthus* lines during drought conditions. Two mutant lines were compared to a wild type and the parameters investigated include plant height, relative water content, protein content of the leaves and finding differences between DNA genomes of the two mutants and the wild type using RAPD analysis and to determine if

they are associated with the increased drought tolerance traits observed in the mutant lines.

Materials and Methods

The *Amaranthus tricolor* lines used during this study were the two mutant lines, mutant #2 and mutant #5, as well as a wild type *Amaranthus* line. The plants were grown in a seed tray containing a germination mix with fertilizer for 3 weeks. After 3 weeks, water was withheld from half of the plants for 19 days; plants were rehydrated after the 19 days of drought treatment.

The plant height of wild type, mutant #2 and mutant #5 *Amaranthus* plants were measured every three days under well watered and drought stress conditions using a measuring tape. Measurements were made from the bottom of the stem (where it emerged from the soil) to the tip of the stem's growth point.

Protein content of the leaves of the stressed and non-stressed plants was determined using Bradford's reagent (Biorad Laboratories, 2000 Alfred Novel Drive, Hercules, California 94547). A standard curve was prepared using bovine serum albumin (BSA), distilled water and Biorad protein reagent (colouring). Protein measurements were performed every three days for both the control and drought stress plants by cutting a leaf from each plant in half and determining its fresh weight (FW). Care was taken to ensure that the size of the leaves were similar for each time point. After weighing the leaves, it was oven-dried overnight at 70ºC. Thereafter the dried leaves were weighed again in order to obtain the dry weight (DW).

During the protein assay the leaves from mutant #5, mutant #2 and wild type were homogenized separately in 0.5 ml of 50mM Tris-HCl, pH 8.0 using a pestle and mortar. Ten µl of this sample was added to 790 µl dH_2O and 200 µl Biorad protein assay. The samples were mixed well and left at room temperature for 5-10 minutes. The absorbance of the solutions was measured at 595 nm in 1.5 ml cuvettes using a spectrophotometer. These absorbance values were used to determine the total protein content using the BSA standard curve.

Relative water content was determined by sampling three pots per line every third morning. Immediately after harvesting, leaf disks were cut using a cork borer and weighed to obtain the fresh weight (FW). The samples were rehydrated with 3 ml of distilled water for 4 hours. Thereafter the leaf disks were dried on paper towel and weighed again to obtain the turgid weight (TW). The samples were oven dried overnight at 70ºC, cooled during the next day at room temperature and weighed again to obtain the dry weight (DW). Relative water content (RWC) was calculated as follows: RWC = [(FW-DW) / (TW- DW)] * 100.

DNA was isolated from the leaves of *Amaranthus tricolor* using a mini protocol for purification of total DNA from plant tissue from Qiagen DNeasy Plant Handbook (2006) was used for DNA isolation. The concentration of the DNA samples was measured using the ND-1000 Nanodrop®spectrophotometer (Nanodrop technologies) according to the manufacture's instruction. This was followed by amplification of *Amaranthus* plants using a MyCycler™ thermal cycler (Biorad) and random amplified polymorphic DNA (RAPDs) was used to amplify the DNA. Polymerase Chain Reaction (PCR) was performed in 0.2 ml Eppendorf tubes which contained 3.5 mM $MgCl_2$, 0.04 mM dNTP, 0.24 µM each primer(OPA01-OPA17 & OPB01 and OPB02), 0.75 U TaKaRa Taq and 30 ng genomic DNA in a final volume of 16 µl. DNA was amplified using the following parameters: denaturing step of 1 min 30 s at 94.5 ºC followed by 35

cycles consisting of 15 s at 94.5 ºC, 20 s at 37 ºC, 30 sec at 72ºC and final extension of 2 min at 72 ºC, 2 min 10 ºC and stored at 4 ºC. The integrity of the DNA was observed on a 1.2 % agarose gel. A molecular weight marker, control and the samples were loaded on the gel. The gel was run for 1h30min at 80 V and stained in 250 ml of deionised water and 10µl of ethidium bromide for 10-15 min. The result was viewed under UV light and an image was photographed.

Results and Discussion

During this study, several differences were observed between the mutant *Amaranthus* lines, as well as between the mutant and wild type lines in reaction to drought stress. Mutant #5 appeared to grow faster than mutant #2 and the wild type under both drought-stress and non-stress conditions (Figure 1).

Relative water content is the method used to determine the water status of leaves in plants during water deficit periods. The mutant plants retained more water under drought conditions compared to the wild type (results not shown). After rehydration, both mutants recovered better than the wild type from drought stress.

Under drought conditions both mutants and the wild type had smaller, denser leaves, but a significant difference was observed in protein concentration. Protein concentration of the mutant plants under drought conditions was higher than the protein concentration of well-watered mutant plants (Figure 2).

From 19 arbitrary primers used, only two primer sets (OPA07 and OPA16) showed polymorphisms between the *Amaranthus* wild type and the two mutant lines (Figure 3a and b). After DNA amplification of the above mentioned lines using primer OPA07, 5 bands were observed for the wild type and mutant #2 while only 4 bands were detected for mutant #5. The absence of the band could be due to a mutation on the binding site of the primer or loss of an enzyme recognition site. Using primer OPA16, 2 bands were obtained for the wild type and mutant #5, and an additional band was obtained for mutant #2. The differences observed during the RAPD analyses of the two mutants as compared to the wild type, could be indicative of specific genomic areas possibly involved in drought tolerance.

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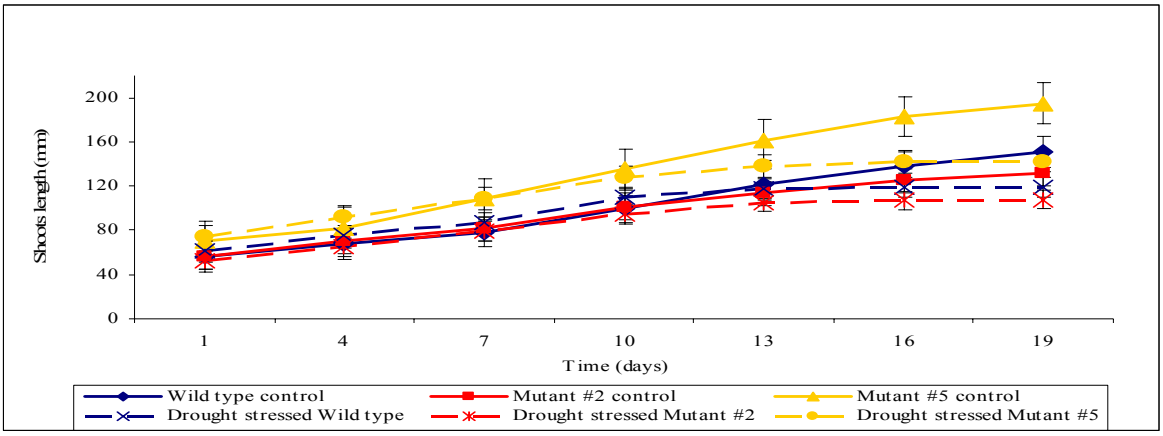

Fig. 1 Shoot growth of the *Amaranthus* wild type, mutant #2 and mutant #5 plants under normal and drought stress conditions in the greenhouse at 25°C

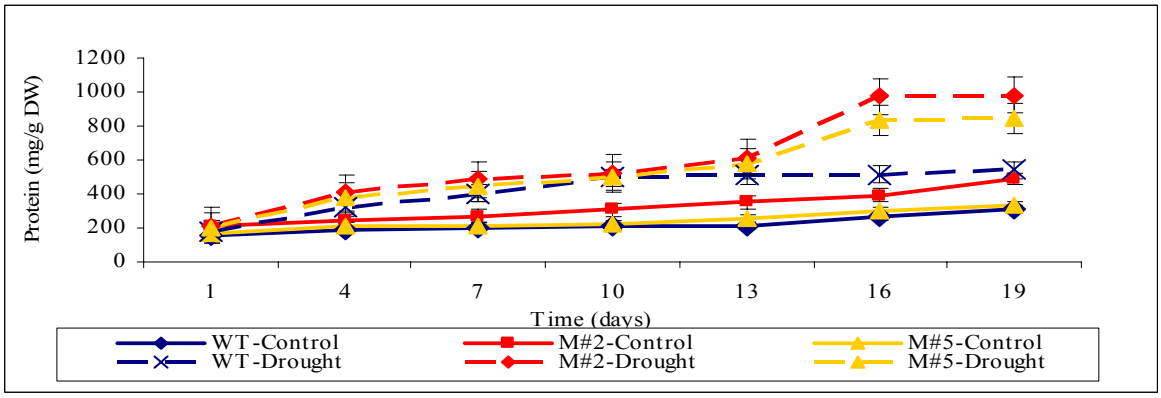

Fig. 2 Protein content from control and drought stressed *Amaranthus* wild type, mutant #2 and mutant #5 plants in the greenhouse at 25°C

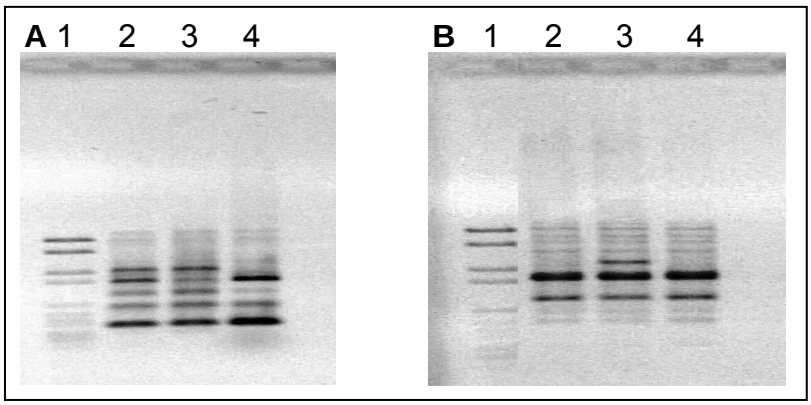

Fig. 3 RAPD polymorphisms detected in *Amaranthus* plants using the random pri- mers OPA07 (A), OPA16 (B). For each figure, lane 1 represents DNA Molecular Weight Marker IV; lane 2, Wild type; lane 3, Mutant #2; lane 4, Mutant #5

FIELD EVALUATION OF AMARANTH IN THE CZECH REPUBLIC

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Abstract
In 2007, the first year of field trials was started. Eighteen amaranth genotypes were evaluated in four countries –Argentina, Mexico, Spain and the Czech Republic. In the Czech Republic, it was performed in two localities – Prague and Olomouc. The seeds were obtained from participants of international project AMARANTH: FUTURE - FOOD. From results follows that growing period in Prague was 159 days and in Olomouc 149 days. The genotypes numbered 7, 8, 10, 15, and 16 originated in Mexico and 17 from Argentina were excluded from evaluation due to their long vegetative period and early frost in the October 2007. The biggest WTS possessed genotypes 1 and 6 from Argentina 0.86 g, 0.83 g respectively. The highest yield had *A. cruentus* Don Guiem 3.641 $t.ha^{-1}$.

Key words – *Amaranthus* sp., field evaluation

Introduction

In recent years, pseudocereals and alternative cereals have been attracting increased interest, both from the agricultural point of view and from aspects regarding processing (Berghofer & Schoenlechner, 2002). One of pseudocereals with the worldwide major importance is amaranth (*Amaranthus* sp.). The genus *Amaranthus* L. consists of about 70 species (Costea *et al.*, 2001) some of them are the world´s most stubborn weeds (Horak&Loughin, 2000); others are cultivated as cereals, vegetables or ornamentals (Brenner *et al*., 2000). Amaranth grain provides an ideal amino acid composition for human nutrition. In particular, the content of lysine is high. Remarkable is also the high content of arginine and histidine, which makes amaranth interesting for child nutrition. The fat of the grain is characterised by a high content of unsaturated fatty acids. Moreover, concentration of minerals is higher than in other cereals. In particular, amaranth grain is rich in calcium, magnesium, iron, potassium and zinc (Berghofer & Schoenlechner, 2002).

The international project of Sixth Framework Programme of European Commision AMARANTH: FUTURE- FOOD contains nine workpackages. One of them is focused on field trials. In the Czech Republic, field trials were performed in two different localities – Prague and Olomouc. This paper is about first year of field trials under conditions in the Czech Republic.

Material and Methods

The field trial was designed to include three blocks in which each genotype was represented at least once, six rows 15 m long and 0.25 m between rows. All varieties were sown randomly. The second row was used to mark the ten reference plants for follow-up evaluation. The Crop Research Institute (Czech Republic), the National University of La Pampa (Argentina) and assorted growers and different Mexican agricultural research institutes (Mexico) provided the seeds (Table 1). The sowing rate

was 3 kg per hectare. Seeds were sown by drill. Field trials were sown at two different times and in two different localities. In Prague was sown in May 11 and in Olomouc was sown in June 6. The differencies in time were caused by different crop rotation system in both places. During trials, the genotypes were evaluated and information was gathered about several morphological traits and phenological phases. Fourteen traits were evaluated in different phenological stages. All evaluations and observations were made in time according to phenological stages of individual genotypes. All weeding was done by hand in both sites, only in time before leaves cover rows; then it was not necessary.

Analysis of variance (ANOVA) and the Tukey HSD test were used for statistical evaluation.

Table 1 Selected genotypes

Genotype Number	Genotype Name
1	*A. cruentus* Mexicano (INTA Anguil, L.P.)
2	*A. cruentus* R 127 (CRI)
3	*Amaranthus* sp. K 340 (CRI)
4	*A. cruentus* Amont (CRI)
5	*A. caudatus* CAC 48A (CRI)
6	*A. cruentus* Don Guiem (INTA Anguil, L.P.)
7	*A. hypochondriacus* Revancha tipo Mercado
8	*A. hypochondriacus*. Nutrisol Morfotipo Azteca (Origin: Mexico)
9	*A. hypochondriacus* Tarasca (Origin: Mexico)
10	*A. cruentus* Morelos (Origin: Mexico)
11	*A. hybridus* K 593 (CRI)
12	*A. hypochondriacus* 280 FK-FH1 (CRI)
13	*A. cruentus* Don Leon (INTA Anguil, L.P.)
14	*A. cruentus* Candil (Origin: Rio Cuarto)
15	*A. hypochondriacus* San Antonio (Origin: Mexico)
16	*A. hypochondriacus*Rosita (Origin: Mexico)
17	*A. mantegazzianus* Don Juan (INTA Anguil, L.P.)
18	*A. hypochondriacus* Artasa 9122 (INTA Anguil)

Results

Table 2 and Table 3 show the results of selected traits from field trials. From results follows that growing period in Prague was 159 days and in Olomouc 149 days. The earliest ripening was the genotype 12. In Prague, it was harvested in 96 days and in Olomouc in 84 days after sowing. The genotypes numbered 7, 8, 10, 15, 16 and 17 were excluded from evaluation due to their long vegetative period and early frost in the October 2007. Higher plants in the time of harvest were in Prague due to higher rainfalls in the time of growing (Figure 1). The bigger yield was recorded in Prague location. The higher yield of seeds from hectar possessed the genotypes number 5, 6 and 9. In Olomouc, the highest yield was in genotypes 4 and 6. While exist the differences between yield in Olomouc and Prague locality, the differences between the localities in case of WTS was very low.

Acknowledgement
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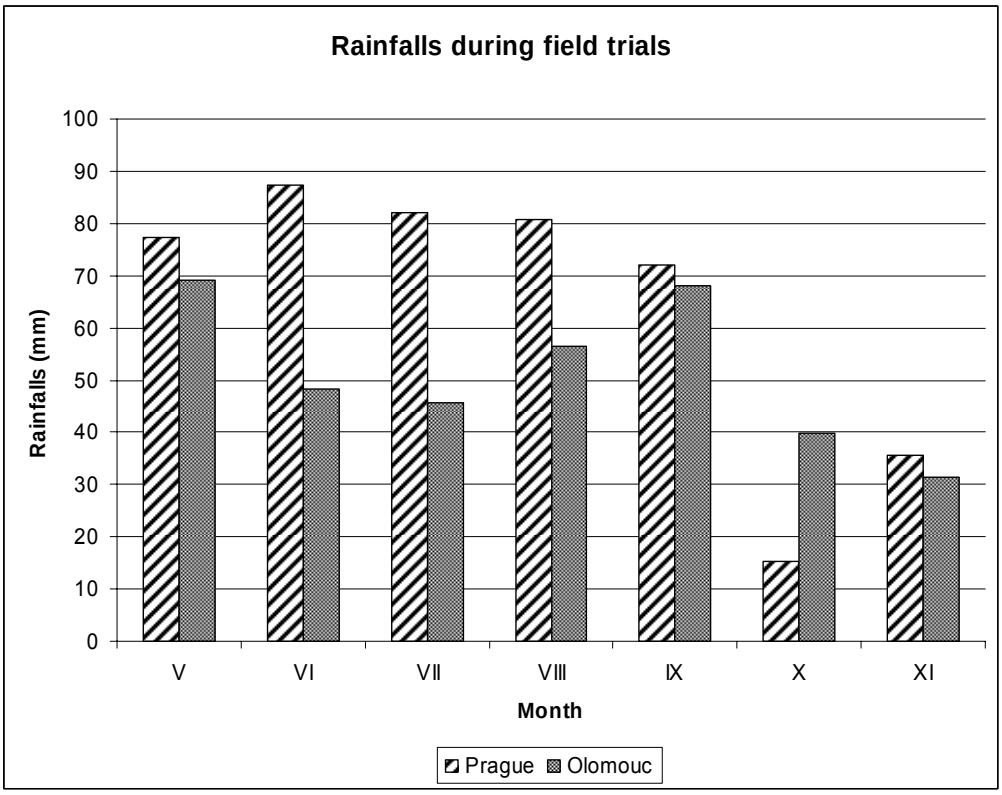

Fig. 1 Precipitation during field trials

Table 2

		Emergency (days)	Days to leaves cover rows (days)	Anthesis (days)	Harverst (days)	Seed Density ($g.l^{-1}$)	WTS (g)	HI	Grain Yield ($kg.ha^{-1}$)
1	*A. cruentus* Mexicano	12.33±5.85ab	26.17±0.41abc	78.00±0.00cd	141.00±7.67ab	859.30±26.72bc	0.86±0.04a	0.26±0.17a	1801.58±835.97a
2	*A. cruentus* R 127	11.17±4.79ab	27.17±3.13a	72.50±9.31abc	139.50±6.02ab	824.17±6.96a	0.76±0.03abc	0.31±0.18a	1654.49±594.67a
3	*Amaranthus* sp. K 340	10.17±3.49ab	26.00±2.19abc	69.00±5.48ab	134.00±2.19a	836.29±10.64ab	0.72±0.04ab	0.26±0.13a	2559.89±2086.83a
4	*A. cruentus* Amont	12.17±5.67ab	29.67±0.82d	72.50.±3.83abc	116.00±9.86c	840.02±9.10abc	0.78±0.07abc	0.28±0.19a	2841.00±1146.96a
5	*Amaranthus* sp. CAC48A	11.17±4.07ab	26.67±1.51ab	75.00±6.57bc	146.00±1.10ab	831.97±10.51ab	0.77±0.04abc	0.32±0.20a	3261.90±1881.27a
6	*A. cruentus* Don Guiem	12.83±5.31b	27.00±1.10ab	72.50±3.83abc	133.50±1.64a	840.88±16.64abc	0.83±0.06cd	0.31±0.21a	3241.31±1814.86a
9	*A. hypochondriacus* Tarasca	11.50±5.21ab	25.00±1.10bc	83.00±15.34d	152.00±7.67b	848.17±14.25abc	0.82±0.01cd	0.28±0.16a	2950.13±1830.12a
11	*A. hybridus* K 593	10.00±3.29a	24.33±1.86c	69.00±5.48ab	140.50±4.93ab	826.14±32.36a	0.71±0.05ab	0.28±0.14a	2688.61±1607.87a
12	*A. hypochondriacus* 280 FK-FH1	10.00±3.29a	25.67±2.58abc	66.00±2.19a	90.00±6.57d	869.61±11.31c	0.77±0.02abc	0.23±0.08a	1896.07±645.80a
13	*A. cruentus* Don Leon	10.00±3.29a	27.50±2.74a	70.00±6.57ab	120.00±14.24c	841.40±15.21abc	0.79±0.06bcd	0.21±0.09a	2301.86±909.08a
14	*A. cruentus* Candil	11.17±5.00ab	26.50±0.55ab	72.50±9.31abc	134.00±2.19a	832.64±12.75ab	0.73±0.04ab	0.31±0.16a	2283.95±1552.07a
18	*A. hypochondriacus* Artasa 9122	10.83±4.58ab	26.50±1.76ab	70.00±6.57ab	151.50±8.22b	826.05±5.41a	0.70±0.04a	0.26±0.20a	1750.28±753.15a
Prague		15.08±2.27b	25.11±1.75a	78.17±6.78b	135.83±15.81b	840.02±19.14a	0.75±0.07a	0.15±0.05b	3314.85±1497.14a
Olomouc		7.14±0.35a	27.92±1.46b	66.83±4.14a	130.50±19.86a	839.42±21.00a	0.79±0.06b	0.40±0.11a	1556.99±427.77b

Table 3

		Height of plants in harvest time (mm)	Lenght of inflorescence in harvest time (mm)
1	*A. cruentus* Mexicano	1333.21±328.70cd	253.81±106.61ad
2	*A. cruentus* R 127	1296.43±371.19acd	326.67±138.19abc
3	*Amaranthus* sp. K 340	1240.00±348.28abcd	341.19±124.51bc
4	*A. cruentus* Amont	1061.67±334.55b	298.33±96.24abc
5	*Amaranthus* sp. CAC48A	1109.52±399.83ab	217.62±136.00d
6	*A. cruentus* Don Guiem	1435.71±345.30d	367.62±126.06c
9	*A. hypochondriacus* Tarasca	1289.52±313.15acd	300.48±102.12abc
11	*A. hybridus* K 593	1082.86±281.24b	299.52±107.11abc
12	*A. hypochondriacus* 280 FK-FH1	1290.95±226.12acd	466.19±129.58e
13	*A. cruentus* Don Leon	1216.43±332.40abc	292.14±93.27abcd
14	*A. cruentus* Candil	1156.67±348.84abc	265.00±102.65abd
18	*A. hypochondriacus* Artasa 9122	1129.05±404.09ab	285.95±122.50abd
Prague		1414.48±326.01b	328.02±137.47b
Olomouc		1025.85±260.19a	291.07±119.65a

GENETIC RESOURCES OF QUINOA (*Chenopodium quinoa* WILL.) IN SLOVAKIA

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Quinoa is an adaptive plant which can be grown in altitude of 0 - 4000m above sea level. In comparison with amaranth (*Amaranthu*s L.), which is suitable for growing in to warmer regions. For European conditions there are suitable genotypes with short vegetation period, unbranched habit, long compact inflorescence, big white - yellow seeds with low saponin content and vegetation period longer than 150 days can consider hazardous for condition of Europe. The aims of the study is to gather, evaluate and select appropriate quinoa genotypes (*Chenopodium quinoa* Willd.) for need of plant production and food industry, to extend diversity of grown crops and recommend their suitable use. Complete research of the quinoa (*Chenopodium quinoa* Willd.) from growing to food production including identification of economically important diseases and pests for this crop in condition of Slovakia will be realized. Detecting nutritional value seeds of quinoa, verify application amaranth and quinoa flour mixtures with wheat flour at production cereal health foods (loaf of bread). For Slovakia are perspective variety Baer, Faro, NSL 106393.

Key words - quinoa, genotype, evaluation

MORPHOLOGICAL EVALUATION OF AMARANTH

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Amaranth (*Amaranthus* L.) is an old crop of American continent. Grain types, which were domesticated in America provide high grain yield and they are suitable for human nutrition. Vegetal types provide high biomass yield and some material for alternative use, for example for fodder plants or energy purposes. According to the Descriptor for amaranth genetic resources, there were studied also genetic resources in Piešťany. Several production traits of amaranth were evaluated: plant height (mm), length of inflorescence (mm), stem pigmentation and pubescence, leaf number and pigmentation, inflorescence colour, maturity, seed colour. We have evaluated species of amaranth and found out its large variability in plant height (700 – 2180mm), leaf width (50-180mm), inflorescence length (300-780mm), and flowering time (30-72 days). Studied task is aimed at Andean pseudo-cereals and possibility of their utilization in innovation of the plant production, in food industry and also in energetic. Amaranth species is known to our farmers and some pharmaceutical firms have manifested their interest in it this field, as well as food factories. The objective of the work is to gather, evaluate and select appropriate amaranth genotypes (*Amaranthus* L.) for need of plant production and food industry, to extend diversity of grown crops and recommend their suitable use.

Key words - amaranth, morphological traits, evaluation

Plenary session IV:
Impact of amaranth cultivation on sustainable agriculture, phytoremediation, forage and biomass production

THE POTENTIAL OF AMARANTH AS AN ENERGY CROP AND ITS ENVIRONMENTAL IMPACT

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Abstract

Perspective of amaranth utilisation in agriculture, food production and in industry fetches simultaneously a demand on a task solution of utilisation of its unprocessed and unexploited mass, and especially in coherence with its growing on land unsuitable for food production. As the most suitable alternative appears exploitation of that sort of amaranth biomass in an energy sector. This solution will reduce as volume as mass portion of waste and hereby will be exploited organic carbon for heat production as well as further noble energies. However at these thermooxidizing processes besides energy, also rise by-products as solid waste and emission, which with their composition are dependent not only on level of treatment technique in technology but as well as on chemical composition of biological material. The contribution deals with qualitative characteristics arising from solid waste during heat generation from this biological - vegetable mass by its thermooxidizing treatment. The main part deals with polluting matter, which are generated by burning of unprocessed amaranth phytomass. Ash analysis showed that the residue ash contains: 1mg.kg^{-1} of cadmium (SK limit is 0.2 mg.kg^{-1}); 5.05 of cobalt (SK limit is 2 mg.kg^{-1}); 11.28 mg.kg^{-1} of copper (SK limit is 5 mg.kg^{-1}); 4.9 mg.kg^{-1} of nickel (SK limit is 2 mg.kg^{-1}); 34.3 mg.kg^{-1} of zinc (SK limit is 5 mg.kg^{-1}). There are also mentioned further analyses besides the above-mentioned elements in the contribution, but these did not overstep the limit of concentrations (SK limit of element concentrations) of topical elements. Results of our analyses, which were aimed at determination of concentration of chosen heavy metals in solid residue after thermal oxidation of amaranth biomass-phytomass, have proved that this plant has a tendency to absorb some heavy metals from the surroundings - the soil in which it was growing and no doubt that they are dangerous by their physiological activity for living organisms.

Key words - *Amaranthus*, energy crop, heating value, ash content, polluting elements

1 Introduction

Utilisation of amaranth biomass as renewable energy source for some technology indicates, however, that this biomass could be a fuel and moreover also a perspective plant having ability to absorb some heavy metals from the surroundings - the soil in which it was growing and therefore it could be considered as a multipurpose crop with great potential in the 21st century (Húska, 1992).

It has become clear, that nowadays knowledge of biomass physical properties especially by new energy crops is not enough for any further optimisation of industrial energy plants or domestic units. For this reason it is necessary to explore biomass especially new energy plant species as fed to the energy plant. Without this, there can

be no guarantee that the fuel will perform satisfactorily under actual operating conditions.

Presented research results of the analyses of an amaranth as a new crop have been obtained in the framework of the project "The Adaptability of Amaranth Growing and Utilisation in Slovakia". Some species of amaranth would be suitable for development as biomass energy crops. The actual and potential yield of amaranth is examined for Slovakia. Chemical analyses and bomb calorimetry of amaranth have been carried out on crops grown by the Slovak Agriculture University in Nitra.

It is clear that our research on amaranth as an energy crop has not included a definitive taxonomic study but it could be a key link in environmental protection.

2 Identification and Relevance of Problematic

The soil horizon as the basic means of non-renewable source is permanently subjected to all negative effects of civilisation and its protection is criterion of society.

According to available information about subdued agriculture in Slovakia (economically and particularly ecological reasons) food products should be limited as an area of 400 000 ha of land, which should be e.g. afforested or grassed.

Experiences from abroad offer possibilities of utilisation of these areas for growing and producing technical biomass, which could be partly used as raw material in industry. Its unworkable and unusable part could be used for energetic purposes. As an introduction phenomenon recultivation, or eventual decontamination of damaged contaminated soil horizont.

It was calculated (Petriková, Roth, 1992), that replacement of traditional fuels (coal) for biomass, produced on an area of five hundred thousand ha land, reduces the load of sulphur by 4 900 tonnes. At present, costs saved by liquidation of results brought about by emissions are about 170 mil. Euro.

2.1 Priority area problems

Under Slovak conditions technological decontamination of soil was not widespread and original work in this field is represented mostly by a team of workers from the Slovak Agricultural University (SPU) in Nitra.

The outcome of their cultivating research show that for these purposes - ecologization of chosen areas by producing different species of amaranth, which under our conditions so far has not its appropriate position in spite of the fact, that it has extraordinary tolerance on its environmental conditions - resistance to stress, dryness and saline soil, particular ability re-detoxification of polluted soil, by radionuclids, heavy metals etc. These qualities lead to wider world interest in its production.

1. The suggestion to use contaminated soil for production of technical biomass of amaranth, which could be partly used for industrial raw material - seeds (for production of high quality starch, essentially fat acid directly used in food industry) and for energetic utilisation of unprocessed biomass, thoughts about biologization within the frame work of technology of soil decontamination are being looked into. Gained raw material from energetically utilisation of technical biomass has suitable and safe usefulness at the building of communications, according to practical experiences abroad - e.g. Denmark.
2. This means of soil recultivation procedure - lowers the content of contamination to an acceptable level, which is not only effective, but also economically attractive, inspite of being timed exacting.
3. About soil recultivation with amaranth the papers (Kona, 1995; Verešová, Hoffmanová, 1995; Víglaský, Blaho, 1994) which was solved by members of the co-

ordination workplace SPU in Nitra, on the task „The Adaptability of Amaranth Growing and Utilisation in the Slovak Republic“.

2.2 Characteristic of process of thermooxidation of biomass treatment

Thermooxidation treatment of biological material from plant sources is at present realised particularly for these reasons:

a) with an aim to use this raw material to produce heat,
b) treatment of waste,
c) simultaneously treatment of waste and to produce heat.

Apart from heat, during burning, unwanted production occurs, which we categorise as emission and waste. The qualitative and quantitative character of the emission is conditioned by chemical components of burnt material, as well as by technical standard of used techniques of technology.

The qualitative and quantitative character of waste depends on the chemical components of burnt material, as well as the content and qualitativeness of component minerals. Organic components as a rule turn completely into gas, which escapes from the furnace into the atmosphere. Their chemical components depend as elementary components of organic parts of burnt material. Plant material is represented to its maximum in organic shares of carbon, which forms up to 50 % of organic mass, further, oxygen and hydrogen. Albumen and organically bases in chlorophyll, nucleosides and others, especially heterocyklite porphyrited derivatives contain nitrogen (Blaho, Víglaský, 1994). Some free amino acids or bound to albumen contain sulphuric acid. The mentioned elements (apart from hydrogen, which produces water) during thermooxidation reaction bring about oxidation of these elements, of different degrees of oxidation. Together with surplice air escape into the free atmosphere of the technical equipment for burning. For higher temperature, which are actual also for the mentioned thermooxidation processes, which are reactive components in the air and a small amount of nitrogen too. This is alongside organic bounded nitrogen source of nitrogen oxide emissions. The process of burning natural biological material takes place mostly under conditions from which arise greater contents of reduced form nitrogen-oxides (N_2O) and carbon (CO).

3. Experimental part

Ash content and its chemical component are one of the most important dates for thorough analyses and classification of potential biomass as a fuel.

3.1 Ash content

This parameter connected with amaranth is not common in literature and published values are considerably different (from 5 - 22 %). This difference is expressive and if it is true it is necessary to explore it by making experiments and give a more direct explanation.

Experimental findings of ash content in amaranth phytomass:

The fixed state of Amaranth ash was carried out as five weighed air dried samples, with material weighing from 1 - 5 grams, annealing at 520 °C, for 48 hours. The solid matter remaining after annealing - ash were determined by gravimetrical method, gained value was 19.09 % (S_x = 0.18) (Viglasky, Blaho, 1994).

Further evaluation of this parameter, gained experimental experiences in 1994, come from Zd. Strasil - Research Institute for Crop Production, CZ - Prague:

A. cruentus - phytomass:

a) 11.36 % - above soil part of plant,

b) 4.84 % - seeds.

3.2 Chemical composition of Ash

Ash from the above experiment was used for fixing state of individual elements, while particular attention was paid to ascertaining the presence of heavy metals, and that was aimed at the following groups of elements:

1. mercury, thallium, cadmium,
2. arsenic, nickel, chromium, cobalt,
3. lead, copper, manganese.

These data are important in view of the proposal, respectively recommended working parameters of the technological equipment, which should use biomass amaranth as a fuel.

Thermooxidal reaction from the given samples of biomass amaranth we carried out in laboratory conditions at controlled burning temperature by means of air oxygen. The surplus, which remained after oxidation samples were gravimetrical, fixed and adapted for analytic fixing of chosen elements.

Adaptation on analytic consisted in preparation of water lye with demonising water and the following process of elution with solid matter in 3 % solution of nitric acid, purified for spectral analysis.

Eluates were analytically prepared and individual elements were fixed by AAS, AAS - ICP methods. Mercury was fixed by mercury analyzator. Results of the analyses were numbered on account of their original solid surplus matter, which remained after thermooxidation reaction and is listed in the Table 1.

Table 1 Representation of chosen elements in water lye and lye of 3 % nitric acid from solid matter after sample annealing at temperatures 800 - 850 °C calculation for 1 kg of solid residue - ash [$mg.kg^{-1}$], (Blaho, Viglasky, 1997)

No.	Element	Element concentration in solid surplus		Sum [$mg.kg^{-1}$]	Emission limit $mg.m^{-3}$
		from water lye	from lye with 3 % HNO_3		
1.	F	< 0.0500	< 0.050	< 0.0500	10
2.	Pb	< 0.1000	1.250	1.2500	5
3.	Cd	< 0.2000	1.000	1.0000	0.2
4.	Cr	< 0.0200	2.500	2.5000	5 (no Cr^{VI})
5.	Co	0.5000	5.050	5.0500	2
6.	Cu	< 0.3000	11.280	11.2800	5
7.	Ni	< 0.1000	4.900	4.9000	2
8.	Zn	< 0.1000	34.300	34.3000	5
9.	As	< 3.0000	< 3.000	< 3.0000	2
10.	Sb	< 0.5000	< 0.500	< 0.5000	5
11.	Sn	< 1.0000	< 1.000	< 1.0000	5
12.	Se	< 5.0000	< 5.000	< 5.0000	2
13.	Hg	0.0134	0.005	0.0184	0.2
14.	B	1.0000	8.250	9.2500	-
15.	Conductivity	5.2100 $\mu S.cm^{-1}$	-	-	-
16.	pH	11.1300	-	-	-

3.3 Calorific value

Bomb calorimetry of amaranth biomass has been carried out on crop samples grown by the Slovak Agriculture University in Nitra.

Calorific value of amaranth dry matter in excess of 16.5 $MJ.kg^{-1}$, heating value is about 14 $MJ.kg^{-1}$ at 10 % moisture content (Viglasky, Blaho, 1994). Different crops are likely to have similar calorific values per unit weight of DM (Grimm, Strehler, 1987).

4. Discussion

The analyses of samples of amaranth were directed on products, which arise during annealing and remain in the experimental system as solid material. Our experiment was placed under laboratory standards and should be the basis for realisation of larger experiments in the 4^{th} prevailing criteria. At this planned experiment, fully fledged analyses should take place, which would be directed also on the study of gas production, which occurs during process of thermal oxidation.

Analyses of solid matter after thermooxidation were made for the reason that, in case of utilisation of parts of Amaranth for technical purposes on production of heat energy is supposed occurrence of solid parts. These from the point of view of evaluation of technological processes in the aspect of environmental protection are part of solid emissions and also solid waste, which belongs from the point of view of waste management to the category of dangerous waste (Anonymous, 1996a, 1996b, 1991).

The realised measurements and analyses showed that, in solid matter after thermooxidation compound elements are found, which have from the health point of view for human organisms unfavourable physiological effects. From elements with cancerous characteristics, cadmium (Cd) was found in solid matter (in ash) in concentration 1 $mg.kg^{-1}$, further chromium (Cr^{VI}), cobalt (Co) and nickel (Ni) which from view of laws about atmosphere [8] and its exacting in G SR No. 92/96 (Anonymous, 1996a, 1996b) belongs to the Group 1 - polluted materials with cancerous activity and their content is with cadmium limited by burning at 0.2 $mg.m^{-3}$ the same goes for chromium, cobalt and nickel at 2 $mg.m^{-3}$.

Another noticeable and dangerous element, was ascertainment of mercury (Hg) whose concentration was found to be at the value of 13.4 $\mu g.kg^{-1}$ which is especially unfavourable, and that Cobalt and Mercury are found in parts and in water soluble form.

Further observed elements, laws protected in the atmosphere and waste materials are copper, lead, zinc and chromium (Cr^{III}). These elements like emissions belong to the Group 3, under the Group 2 polluted materials and their emission limit is fixed at 5 $mg.m^{-3}$ in relief gas. The common concentration reaches up to 50 $mg.m^{-3}$ in ash in the given amaranth samples, which were cultivated in soil with higher contamination content.

The result of our analyses, aimed at fixing concentration of chosen heavy metals in solid matter after thermooxidation of amaranth phytomass, proved that this plant has a tendency to absorb from its surroundings in which it grows, some heavy metals. These with their physiological activity are dangerous for the living organism.

Our results were confirmed by independent measuring carried out at the Department of Gardening AF - Slovak Agriculture University in Nitra. Kona (1995) observed the content of heavy metals under two pedological conditions: on garden earth and on contaminated soil from the region of Rudnan. Cultivation was compared also with green lettuce for eating. From these experiments and results follow, that amaranth can be listed among plants, which have the ability to a higher degree cumulate up to 103 times more Pb, 240 times more Cd and 5.9 times more Hg. The presence of contamination in

seeds was low and at all tests within the norm. This is important especially from the point of view of possibility to use the amaranth seeds in the food industry. In other available home and foreign literature the ability of amaranth to cumulate heavy metal is not evaluated.

Equal success can be evaluated of the growing of amaranth also in regions with burnt fuel containing oxides of sulphur. Verešová and Hoffmanová (1995) carried out tests with amaranth on land near a chemical factory, where they produce ammonium sulphate. Their results were not negative on the growth of amaranth.

5 Conclusions

In western countries today this conception in agriculture is universally supported and has excellent results. Growing technical biomass is most often used for burning and gaining energy, for example heat as well as electric energy or gas as a fuel. By these thermooxidation processes however apart from energy arise accompanying products - solid waste and emissions, which with their compound are often put among dangerous waste, as was proved in the above mentioned experimental results, and therefore it is necessary to handle them in the sense of competent regulations ordered by the Ministry of Environment SR (Anonymous, 1991, 1996a, 1996b).

In certain regions in Slovakia, with higher content of elements observed by legislature as harmful, or polluted materials, it would be very desirable to utilise some areas for the production of technical and energetically products, because they do not enter the food chain and do not endanger the health of people, but on the contrary help detoxicate, respectively recultivate even strongly polluted soil and at the same time can be utilised for energetically purposes if there is suitable technology and techniques available.

Furthermore it is necessary to pay greater attention to the development of effective technology for harvesting, processing and utilisation of technical biomass as well as processing of waste.

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MULTIFUNCTIONAL USE OF AMARANTH PHYTOMASS FOR INDUSTRY AND ENERGY

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World-wide, there is an increasing demand for phytomass for production of renewable CO_2-neutral fuel and as an inexpensive environmentally friendly raw material source for food and industry production. Amaranth species hold much promise in fulfilling these demands. Perspective of amaranth species utilisation in agriculture and subsequently in food production as well as in industry brings about simultaneously a demand on a task solution of utilisation of its unprocessed and unexploited mass, and especially in coherence with its growing on waste land unsuitable for food production. After seed processing, one of the most suitable alternative ways of utilisation of residual amaranth biomass-phytomass would be for energy production. This solution will reduce volume as well as mass portion of waste and therefore organic carbon and hydrogen will be exploited for heat production. However at these thermooxidizing processes, apart from energy, also occur by-products such as solid waste and emissions. Submitted results of amaranth-phytomass ash analyses remarking on significant absorption properties of this plant for some heavy metals. These by-products with their composition are dependent not only on treatment technique level in technology, but as well as on chemical composition of biological material and applied method of processing.

Key words - amaranth phytomass; heating value; ash content; polluting elements; amino acids; starch

1. INTRODUCTION

Amaranth is a small-seeded grain crop with a dramatic history. It was playing a very important role in religious rituals of the ancient Aztecs in Mexico. Once a staple in the diet of the Aztec Indians, today it is grown throughout the world. Amaranth spread from the kingdom of Aztecs and Incas to India, Pakistan, Nepal and China, where people became more interested in the utilisation of the plant than in its native country. In spite of the group of main cereals (wheat, rice, corn) also so-called pseudo-cereals with a favourable nutritive value (some species of amaranth) are becoming more popular than others. The high quality of amaranth is combined with its agronomic productivity; it compares favourably with the more commonly utilised crops and could possibly be used as a forage alternative. The amaranth grains have high content of amino acids (up to 20 %) with very positive amino acids composition and a high content of lysine. In comparison with other cereals, an amaranth seed has a relatively high fat content (5 - 8 %), especially unsaturated fatty acids. Among the mineral substances content, there are in a higher content K, Ca, P and Mg (3 - 4 %). There is a high content of proteins (50 %) in leaves of amaranth, also mineral substances, mainly Fe, Ca and vitamins, especially ascorbic acids. Currently several research projects are being carried out in many countries as well as in the Slovak Republic. The actual and potential yield of

amaranth is investigated for Slovak climate and soil conditions. Yields of amaranth phytomass are in the range of 50-250 tonnes of green matter (or 10-50 tonnes of DM) per hectare and growing season, depending on chosen plant species and location of cultivation inclusive soil condition.

2. AMARANTH MASS UTILISATION

2.1 Energy applications.

Energy generated from phytomass is reducing greenhouse gas (CO_2) emissions and decreasing dependence on drying up supplies of fossil fuels. Amaranth is being evaluated for production of heat by thermal conversion.

2.1-1 Thermal Conversion

Thermal conversion quality is determined by many factors, including water content at harvest, and ash, alkali, Cl and N content. Limited data available shows that as a low input C_4 plant with a low water requirement, amaranth has high ash and alkali content in comparison with a typical wood crop and a typical C_3 crop like grass species.

2.1-2 Using Material and Methods

Crop material. The crop samples of amaranth for chemical analyses and bomb calorimetry were obtained from experimental sites - plots of the Slovak Agricultural University in Nitra.

Methods used for the characterisation of amaranth phytomass. Thermooxidation reactions from the given samples of amaranth phytomass were carried out under lab conditions at controlled burning temperature by means of air oxygen. The surplus that remained after oxidation samples were gravimetricaly fixed and adapted for analytic fixing of chosen elements.

The analysis consisted in preparation of water lye with de-ionised water and the following process of elution with solid matter in 3 % solution of nitric acid, purified for spectral analysis. Eluates were analytically prepared and individual elements were fixed by AAS, AAS - ICP methods. Mercury was fixed by a mercury analyser.

2.2 Non-energy uses

Amaranth utilisation is extensive as well as the grain and the leaves. At the present in the USA approximately 30 products of food fortified with amaranth are on the market. Exceptionally nutritive value of proteins can be found in an amaranth grain which is similar to milk casein.

The leaves are used to prepare a salad, or can be cooked like spinach or fried in butter. Some nations, living in tribes separate certain extracts of colorants from this plant. Amaranth is also used in pharmaceutical industry. Amaranth leaves improve the appetite and have diuretic and antipyretic effects. It is known from literature that *Amaranthus spinosus* and *Amaranthus viridis* are used as dressing on wounds after snakebite.

The grain has abundant use as well. A part from its use in bakery, pasta and biscuit production pancakes or pureé is also prepared. Whole grain can be cooked and used as a vegetable too. In some countries the grain is cooked with milk and sugar. In the State of Bihar the grains are cooked with rice and addition of mustard leaves. In the state of Kashmir the grains are fried, milled and then eaten with milk or water on fast - days.

Industrially amaranth grains are treaded with cooking in water (atmospheric or decreased pressure), by flocculation on a roll, by extrusion, by puffing, by dry milling or pre-germination. Puffed amaranth grain is used as an ingredient for snacks, biscuits, bread and other products, which gives a characteristic taste and smell to these products.

Whole grain mixtures, whole meals and "GRANOLA" cake are further amaranth products. The amaranth grain products are a suitable diet for colitis suffering patients.

Oil with relative high content of unsaturated fatty acids was isolated from an amaranth grain.

Mentioned review deals with the most frequent ways of amaranth utilisation. Amaranth leaves and grains have their use in many other large industries too. Amaranth grain is a perspective raw material in the starch industry, because special properties of amaranth starch is distinct from starch of others cereals. From some species of amaranth we can isolate so-called amylopectin starch. Besides these properties of amaranth leaves and grains it is important to turn attention to antinutritive and native toxic substances which occur in these parts of the amaranth plant.

Amaranth crop (seeds, flower-matter as well as phytomass) could be sold in bulk or processed as raw material to produce biscuits, starch of top quality, natural food dye-stuff as well as pharmaceutical products and forage or fodder, etc - competitive products at current market and price levels.

2.2-1 Amino acids content in an Amaranth grain

The analysed seeds were produced in edaphic-climatic conditions of the Research Experimental Base in Nitra. Experimental plots are located on glued brown soil with medium to good P and K supply (45 - 69 mg $P.kg^{-1}$ and 195 - 260 mg $K.kg^{-1}$), the humus content 2.15 - 2.64 % and pH 5.2 - 6.5. Annual precipitation - 532.5 mm and a year average temperature 9.8 °C.

We have analysed the following *Amaranthus* species: *A. caudatus* L., *A. cruentus* L., *A. hypochondriacus* L., *A. paniculatus* L., and one species of wheat - *Triticum aestivum* L. subs. aestivum. Samples of biological material have represented an average yield sample from 1995, 1996. The biological matter was grinded on the laboratory mill into flour. The content of amino acids in amaranth and wheat flour was determinated on the automatic amino acid analyser AAA T 339 M with fibertec system 1010 heat Extractor-Tecator flushing out of oils by a method prescribed by producer.

The content of amino acids of a whole grain amaranth and wheat flour together with the total sum of amino acids ($g.kg^{-1}$) and the comparison of total amino acid content in percentage is presented in Table 1.

Results were evaluated by the comparative analysis - compare to the daily consumption of amino acids according to FAO recommendations.

The flour from amaranth contains all-important amino acids average values, some of which were slightly or even much higher in comparison with those of whole grain wheat flour. In the comparison to single amaranth species according to the 15 amino acids content, the highest average values were determinated in the case of whole grain flour produced from grains of *Amaranthus hypochondriacus* L.

Cultural species of amaranth can be perspectively used at treatment of some civilisation diseases. Amaranth seed preparations having high biological value are useful for people suffering from low-level resumption of nutrient substances (from people allergic to gluten). From the view of treatment of these allergies amaranth cultures can be evaluated as no entirely new herbs in crop production.

Table 1 Content of amino acids in whole grain amaranth and wheat flour ($g.kg^{-1}$)

Amino-acid	Amaranth species		Average	Wheat	DCAR (by FAO)
	A.HY	A.CA			
Lys	8.92	9.27	917.00	3.04	2.31
Leu	10.61	10.39	10.46	5.30	2.94
Ile	6.79	6.59	6.59	2.74	1.68
Phe	7.79	7.37	7.41	6.10	2.52
Thr	7.65	7.50	7.52	1.30	1.68
Val	8.01	7.93	7.94	4.08	2.10
Ala	6.32	6.70	6.51	2.89	
Arg	18.85	19.22	18.88	3.06	
Gly	14.90	13.63	13.92	3.17	
His	5.30	5.25	5.36	3.24	
Asp	15.80	15.64	15.08	5.12	
Glu	29.23	29.95	29.46	30.82	
Pro	8.13	9.16	8.56	8.89	
Ser	12.98	10.62	11.31	3.80	
Tyr	5.53	7.26	6.28	3.35	
Total ($g.kg^{-1}$)	166.8	166.5	164.5	87.10	
%	100.0	99.8	98.6	52.20	

Legend:
A.HY - *Amaranthus hypochondriacus* L.
A.CA - *Amaranthus caudatus* L.,
We used for average calculation two more amaranth species - *Amaranthus paniculatus* and *Amaranthus cruentus*.
DCAR by FAO - Daily Consumption Amount Recommended by FAO.

2.2-2 *Amaranthus cruentus* starch

Starch from species of amaranth, because of its uniform small granule size, is useful in many areas. Like the common cereal and pseudo-cereal grains, amaranth seeds contain starch as a major constituent. The content of starch in different kinds of amaranth, according to literature varies from 48 to 73 % (Halasová *et al*., 1999).

Chemical composition of starch

To estimation of moisture, ash, protein (Nx5.70), starch and fat was performed as per standard AOAC procedures. The chemical composition of the starch is presented in Table 2. The purity of isolated starch was high - 95.56 %. There was no amylase in this starch. *Amaranthus cruentus* starch is a waxy type of starch.

Table 2 The chemical composition of *Amaranthus cruentus* starch

Components	(% on dry basis)
Moisture	10.47
Lipids	0.98
Proteins	0.85
Ash	0.02
Starch	95.56
Yield of starch	60.00

Water absorption, swelling and solubility of starch

We have studied the physico-chemical properties of the *Amaranthus cruentus* starch. Our results were similar to results of other researchers. We compared our findings with properties of corn and wheat starch. *Amaranthus cruentus* starch has greater solubility, greater water binding capacity and better swelling in comparison to corn and wheat starch.

The gelatinisation temperature range of starch

The gelatinisation temperature range reflects the breakdown in the order and crystallinity of the molecules within the starch granules. The gelatinisation temperature range was followed using a microscope equipped with a hot stage and also from Brabenderov viscograph. Table 2 shows this temperature range for various starches.

Table 3 Gelatinisation temperature range from various starches

Starch source	**Gelatinisation tem. range (°C)**
Corn	62 - 72
Wheat	52 - 63
Amaranth Cruentus	57 - 70

The gelatinisation temperature for *Amaranthus cruentus* starch is lower than that of cornstarch and higher than that of wheat starch.

Scanning electro microscopy examination of starch granules

The starch granules were examined in a JEOL JSM - 840A scanning electron microscope at an accelerating voltage 25 kV. The granules are angular and polygonal in shape, very small in size, having a diameter of about 1 micrometer.

Viscographs of starch

Pasting properties were determined with a Brabender Visco-Amylograph. 6% suspension of Amaranth starch was heated from 25 °C to 95 °C, kept at this temperature for 30 min, then cooled to 50 °C, held at this temperature for 30 min, then cooled to 25 °C and held at this temperature for 30 min. The reference viscidity points are presented in Table 4.

Table 4 References viscidity points of amaranth starch

Viscosity	**(Brabender Units)**
At a peak	935
At 92.5 °C	875
After 30 min. at 92.5 °C	820
On cooling to 50 °C	900
After 30 min. at 50 °C	940
After cooling to 25 °C	960
After 30 min. at 25 °C	980

The drop in viscosity from the peak value to that obtained after holding for 30 min at 92.5 °C indicates the stability of pasta to breakdown. The extent of change in viscosity on cooling with continual mechanical agitation reflects the retrogradation tendency of starch, which is influenced by amylase content. Since *Amaranthus cruentus* starch contained no amylase, it exhibited no increase in viscosity on cooling to 25 °C, indicating weak intermolecular forces within granules. In comparison to the cornstarch,

amaranth starch has weaker intermolecular forces within the granules than that of cornstarch.

2.3 Multifunctional uses

In the Slovak Republic (and elsewhere too) it may be difficult to economically produce biomass crops for energy use in the short term, since the energy price will not compensate for the costs made. One of the possible solutions is to find combinations with other uses.

This combined use is possible on different levels:

a. Fractionation of the crop into a high value component (e.g. protein, natural food dyestuff, starch, oil or pharmaceutical products) and biofuel component.

b. Cultivation of the crop in combination with other functions in the landscape.

Examples of function combinations for amaranth species would be erosion control, vegetation in recreational areas, in buffer zones around environmentally sensitive areas, around water pumping stations, on contaminated soils, etc.

3. RESULTS

Calorific value of amaranth phytomass

The lower and higher heating values were determined according to DIN 51900. Data on proximate and ultimate compositions and heating values are given on dry basis.

HH value of A-th DM varies from 15.5 to 17.0 $MJ.kg^{-1}$, LH value is range 13-14 $MJ.kg^{-1}$ at 10 % MC. The analysis results confirm A-th phytomass to be a low- grade fuel in comparison with coal. A summary of the results obtained in laboratory analyses of A-th phytomass as a fuel is presented in Table 5.

Table 5 Results of lab-analyses of *Amaranthus cruentus* "Giganteus" phytomass

Fuel			**A-th matter, S1**			**A-th matter, S2**
Type			**Inflorescence & leaves**	**Stems**	**Average**	**Stems**
Measured value		**Unit**				
MC as received	Wt-r	%	11.80-19.70	12.80-20.10		14.70-22.40
Ave.	Wt-r	%	15.75	16.45	16.10	18.60
MC in analyt.sample	W-a	%	6.94	6.18	6.56	6.05
Ash content,	A-d	%	15.40	10.17	12.79	13.03
Sulphur content,	St-d	%	0.57	0.11	0.34	0.18
Carbon content,	C-d	%	41.00	43.50	42.25	40.00
Hydrogen content,	H-d	%	5.70	6.20	5.95	5.10
Nitrogen content,	N-d	%	2.50	0.80	1.65	1.20
HHV						
Analytical sample,	Qs-d	MJ/kg	15.72	16.61	16.17	15.48
LHV						
DM	Qi-d	MJ/kg	14.48	15.26	14.87	14.35
"as received", Ave. MC	Qi-r	MJ/kg	11.81	12.34	12.08	11.23

Thermal conversion quality is determined by many factors, including water content at harvest, and ash, alkali, Cl and N content. Limited data available shows that as a low input C_4 plant with a low water requirement, amaranth has high ash and alkali content in comparison with a typical wood crop and a typical C_3 crop like grass species.

Chemical composition of amaranth ash

Ash analysis showed that the residue ash contains 1mg.kg^{-1} of cadmium; 5.05 of cobalt; 11.28 mg.kg^{-1} of copper; 4.9 mg.kg^{-1} of nickel; 34.3 mg.kg^{-1} of zinc. There are also mentioned further analyses besides the above-mentioned elements in the contribution, but these did not overstep the Slovak limit of concentrations of topical elements.

Results of the analyses, which were aimed at determination of concentration of chosen heavy metals in solid residue after thermal oxidation of amaranth phytomass, have proved that this plant has a tendency to absorb some heavy metals from the soil. Which without doubt would be dangerous in their physiological activity for living organisms.

From the environmental point of view it should be mentioned that amaranth is the plant species, which is also suitable for marginal lands and contaminated soils. It is known for its dry or saline land resistance as well as contamination by radioactive dust or other harmful material.

3.1 Amaranth as a crop for sustainable development of agriculture

The search for new crops, which produce both food and energy, together with the development of appropriate technology, is becoming a matter of paramount importance.

Amaranth could be characterised as a high-energy multipurpose C_4 plant, fits the bill as a true „4F crop“ (Food, Feed, Fuel and Fibre) as well as being a short cycle, drought and salinity tolerant crop. The amaranth agro-environmental system is a key link in the sustainable production of agriculture. It will play an important role in „healthy“ food as well as environmental protection in the next century.

Amaranth is one of few plants, which become a model plant and of great interest for many researchers around the world. Crop husbandry methods for amaranth have been researched in many countries of Europe (Germany, Poland, Slovakia - Slovak Agriculture University in Nitra, Russia, Ukraine, etc.) and especially overseas in Central America - countries of Amaranth origin, where its several species have been cultivated by Indians for many centuries.

4. CONCLUSIONS

- Amaranth should be a very attractive biomass-phytomass source because of a high yield under marginal conditions.
- Many successful applications such as healthy food production, in industrial as well as energy sector of amaranth show promise, though research remains to be done.
- The amaranth agro-environmental system is a key link in the sustainable production of agriculture. It will play an important role as raw material source for industrial biofuel production as well as environmental protection in this century.
- Energy generated from amaranth based biofuels has a potential to reduce greenhouse gas (CO_2) emissions and decreasing dependence on drying up supplies of fossil fuels.
- Thermal conversion quality is determined by many factors, including water content at harvest, and ash, alkali, Cl and N content.
- HH value of A-th DM varies from 15.5 to 17.0 MJ.kg^{-1}, LH value is range 13-14 MJ.kg^{-1} at 10 % MC.

These are the main reasons for increasing interest to explore phytomass quality of different A-th species especially giant amaranth and to receive their definitive taxonomic studies. Giant amaranth species e.g. *Amaranthus australis L.* or *Amaranthus cruentus L.* is one of the main crops being considered as source of raw material for solid biomass based production processes to receive one of main products - high quality biofuel.

Presently the economic viability is still uncertain, as is the case for all biomass crops. It will be necessary to exploit the multifunctional uses of amaranth crop species to increase the value per area of land and/or per tonne of biomass-phytomass.

These are the main reasons for increasing interest in investigation of cultivation conditions of amaranth plant species as a sustainable crop of the twenty-first century.

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Plenary session V:
Breeding and biotechnology approaches for amaranth improvement

MUTATION BREEDING IN SELECTED *Amaranthus* spp.

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Amaranths have drawn a great interest in the last years as an agronomic crop, alternative energy source and ornamental plants around the world. Several of them are cultivated as the leaf vegetables, cereals, or as the colorful, quick growing garden plants. Breeding work on grain amaranth is just beginning and shows the necessity of further research (Williams and Brenner, 1995; Brenner *et al*., 2000). Among the problems to be solved in amaranth breeding are the high degree of heterozygosity, low heritability of some traits and susceptibility to some diseases. Goals in improving cultivars of amaranth are determinated by their use: improvement of the grain and biomass yield, grain quality, elimination of antinutritional factors, increasing tolerance to biotic and abiotic stresses, early/late flowering, inflorescence architecture and colour, seed retention, improving harvestibility, etc. (Brenner *et al.*, 2000).

Conventional plant breeding is based on the use of genetic variation and selection of the desired genotypes what requires the screening of relatively large populations. The availability of simple and efficient techniques for inducing genetic variation, such as use of radiation for induction of mutations and selection for desired traits is an essential component of any plant breeding programme. The goal of the research was by use of radiation mutagenesis to enhance quality and quantity of amaranth grain and to evaluate the performance of selected mutants and parent lines.

For this purpose the seeds of two genotypes of grain amaranth *Amaranthus cruentus* genotype 'Ficha' and hybrid K-433 a plant breeding material, product of interspecific hybridization (*A. hypochondriacus x A. hybridus)* were irradiated by γ radiation dose 175 Gy (Gajdošová *et al*., 2007) and positive selection was performed during 8 mutant generations (1998-2008).

The phenological observations were performed during all vegetation periods and selection on desired traits was done starting in M_2 generation. The negative plants (plants with weak seedling growth, undeterminated plant growth, non-uniform flowering and seed maturation, with abundant leafiness in the inflorescence area, low size of seeds) were removed from the field and only plants with positive traits were collected. The weight of 1000 seeds (WTS) was determined using seed counter Contador (2 x 500 seeds), recorded and statistically evaluated.

Finally, several putative mutant lines of *A. cruentus* and hybrid K-433 were selected characterized by highly significantly increased WTS (in comparison with control) with an obvious tendency to stabilization of this trait when comparing these samples with the samples of the previous generations. At those selected plants genetically fixed WTS can be expected.

Therefore, there exists real assumption that this plant material can be considered as valuable mutation lines useful in further amaranth breeding programme.

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Agrobacterium tumefaciens -MEDIATED TRANSFORMATION OF AMARANTH

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Research in amaranth cultivation and breeding has been reinitiated as part of the recent renewed interest in nutritional and economic potential of underutilized crops. The goal of the research was to improve *in vitro* regeneration system of specific amaranth genotypes in order to benefit the conventional and /or molecular plant breeding work. The genotypes selected for the work were *Amaranthus cruentus* genotype 'Ficha' and hybrid K-433. To improve the *in vitro* regeneration and multiplication system different explants and plant hormone combinations were tested. Application of 3 mg l^{-1} TDZ together with 0.01 mg l^{-1} NAA seems to be the most appropriate for the genotype 'Ficha'. The hybrid K-433 have shown very poor or no regeneration response apart of callus induction.

In attempt to develop a transformation procedure for hypocotyl and leaf segments, the sensitivity of non-transformed tissues to antibiotics was studied. Two groups of antibiotics were tested: antibiotic commonly used to eliminate *Agrobacterium* growth from tissue culture (cefotaxime) and antibiotics for the selection of transformed tissue (kanamycin, G-418). Leaf/hypocotyl explants were placed on regeneration media (MS + 1 mg l^{-1} TDZ) containing 500 mg l^{-1} of cefotaxime combined either with 50, 100, 150, 200, 250, 300 mg l^{-1} of kanamycin or 10, 15, 20, 25, 30 mg l^{-1} of G418.

Our results confirmed that the use of cefotaxime proved to be highly effective in the elimination of *Agrobacterium* (Teixeira da Silva and Fukai, 2001; QingYao *et al*., 2004). Considering the rate of browning and phytotoxicity, the explants showed severe susceptibility to cefotaxime/G418 combination, even at lowest concentration 10 mg l^{-1} of G418. The higher tolerance of tested explants to cefotaxime/kanamycin combination with optimum selection pressure 500/150 mg l^{-1} indicated that of the antibiotics tested kanamycin may be more useful for selection of *nptII*-transformed hypocotyl/leaf explants of amaranth.

The previous experiments indicated that some of the genotypes are susceptible to *Agrobacterium*-mediated transformation (Jofre-Garfias *et al*., 1997), but shoot induction from hypocotyl and leaf segments were unsuccessful. In order to develop reliable transformation strategy for these explants we transformed them by *A. tumefaciens* strain AGL0 harbouring plasmid pTS2 containing *GUS*-intron reporter gene under the control of double dCaMV 35S promoter and *NPT II* selection gene driven by the nos promoter. Hypocotyl and leaf explants were incubated for 2 days in the diluted *Agrobacterium* suspension in presence of acetosyringone (100 mM) in the darkness at 23±2 ^{o}C. Explants were blotted dry and placed on MS medium supplemented with 1 mg l^{-1} TDZ and 500 mg l^{-1} cefotaxime. After 1 week explants were transferred to the same medium but containing also 150-200 mg l^{-1} kanamycin for selection and incubated under conditions described above. Transient GUS activity of transformed cells was determined using histochemical assay and was assayed 5-14 days after transformation (Jefferson *et al*., 1987).

Our preliminary results have shown integration of T-DNA into the plant cells demonstrated by blue spots/patches on the cut ends of hypocotyls as well as on induced callus. Hence, by choosing suitable parameters we are able to transform hypocotyl

explants of amaranth with potential genes yielding certain characteristic traits that are of commercial value.

Acknowledgements
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GRAIN VARIETIES OF AMARANTH DEVELOPED BY SELECTION AT KHARKIV NATIONAL AGRARIAN UNIVERSITY AND THE PERSPECTIVES OF THEIR USE

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Abstract
Characteristics of the most prevailing grain amaranth varieties developed by selection at Kharkov National agrarian university named after V.V. Dokuchajev and verification of the results of their farm value and use perspective are given. It was proved that the grain amaranth varieties at by KNAU selection have high nourishing properties, contain up to 16-18% protein, from 5 to 8 % oil with high pharmacologic properties in their seeds and can be used in food, pharmacology and fodder industries.

Key words - Amaranth, use, variety, grain, oil

Introduction

Creation of varieties with respective farm traits, adapted to certain soil-climatic conditions of domestication has an important significance in solving the problem of introduction of grain amaranth to the cultivation.

Since 1985, in the Kharkiv National Agrarian University named after V.V. Dokuchaev (KhNAU) the work in creation of grain amaranth varieties has being conducted. During this period 4 varieties of grain amaranth such as Ultra, Sem, Lera have been created with applying of different methods selection (selection, induced mutagenesis, remote hybridization). Since 2008, the work has been begun on the study of genetic diversity of amaranth varieties of the Ukrainian selection using the isoenzyme systems.

Variety ULTRA. It is created by processing seeds of variety Belosemyanniy in concentration of 0.012 %. It was included in the Register of Ukraine's plants varieties in 1998. Species – *Amaranthus hybridus*. Plant height is up to 105 centimeters. Leaves – green, absence of downiness. Inflorescence is half-compressed compact panicle, of light-green colour, yellow when mature. Seeds are white. The variety is steady against drowning and falling of grain. It is early-ripening grade – 90-95 days. Seeds productivity is 14 c/h. The content of oil in seeds is up to 5 %. Its oil contains 11.25 % of squalen and 0.28 % of tocopherols.

Variety SEM. This variety is created by individual selection from sample A. hypochondriacus (Panishmen). It was included in the Register of Ukraine's plants varieties in 2002. Species – A*maranthus hypochondriacus*. Plant height – up to 128 centimeters, the stalk and leaves are red. Panicle is red and branchy; its length is 47 centimeters. Its resistance to drought is estimated by 7 points, falling of grain and to drowning – 9 points. It is a mid-ripening grade – 110 days. Seeds contain 19.5 % of a protein and 6.7% of oil. Seeds productivity is up to 25 c/h. Anti-inflammatory action and protective effect of oil concerning development of experimental resistency of insulin has been established.

Variety LERA. It is created by individual selection from sample *A. hypochondriacus* (K-14). It was included in the Register of Ukraine's plants varieties in 2002. Species – *A. hypochondriacus*. The height of the plant is up to 165 centimeters. The stalk is green, leaves are green with red veins. Panicle length is 54 centimeters, it is red and compact. Seeds are white; the mass of 1000 seeds is 0.7 grams. Its resistance to drowning is estimated by 9 points; falling of grain is 8 points. It is a mid-ripening grade – 105 days. Seeds contain 20.6 % of protein and 7.0% of oil. Seeds productivity is up to 22 c/h.

Variety Kharkovskiy 1. Variety developed by individual selection from the population *A. hypochondriacus* (K-7). It was included in the Register of Ukraine's plants varieties in 2001 as medicinal. Species – *A. hypochondriacus*. Plant height is up to 160 centimeters. The stalk and leaves are green, the panicle is white and compact, and its length is 60 centimeters. Seeds are white; the mass of 1000 seeds is 0.65 grams. Seeds contain up to 8% of oil. By the results of medicinal check properties in the Institute of endocrinical pathologies problems named after V.Ya. Danilevsky seeds oil of Kharkovskiy 1 varieties is recommended for the further studying at medical institutions with the purpose of possible clinical application for the prevention and treatment of the second type diabetes and stomach ulcer.

In spite of the amaranth varieties selection for grain use, which is conducted in Ukraine, the questions connected with use of amaranth corn have not been studied sufficiently yet. Therefore the task of our research was the study of forages, food and pharmacological properties of amaranth corn by the example of the KhNAU varieties selection and determination of basic directions to use them.

Materials and Methods

The research was conducted on the experimental field of KhNAU and the farm Ukrainka of the Stock-raising Institute of UAAN in 2000-2007. The content of protein and oil in the corn of amaranth was determined in the laboratory of mass analyses of the KhNAU. Chemical composition of forages with addition of amaranth corn and their nourishing properties were determined in the quality research laboratory of forages and stock-raising products of IS UAAN. The test bakings of bread with addition of flour from the amaranth corn were conducted in the technological laboratory of the plant-growing Institute of UAAN. Antiulcer activity of amaranth oil was determined in the Kharkiv pharmacological therapy institute of endocrine diseases. The field research was conducted on the experimental field of KhNAU, its area-50 m^{-2}, repetition-4-multiple.

Results and Discussion

Comparative study of amaranth varieties Ultra, Kharkovskiy 1, Sem, Lera, from which Ultra variety belongs to the type of *A. hybridus*, and Kharkovskiy 1, Sem and Lera varieties to the type of *A. hypochondriacus*, showed that among the studied varieties the most productive were the varieties Ultra and Kharkovskiy 1. Their productivity on the average for 3 years of study was 22.5 c/h and 24.3 c/h accordingly. Sem and Lera varieties were less productive their productivity was at the level of 21.8 c/h and 21.1 c/h. Variety Ultra was the most precocious, and ripened in the middle of August in the conditions of Left-bank Forest-steppe of Ukraine. It was remarkable for its ripening friendliness and aptitude for mechanized harvest. The most oil content in a corn was in the Sem variety – 8.5%, the least – in Ultra variety - 5%. However, the oil from the corn of Ultra variety had better pharmacological characteristics in comparison with other varieties. In

addition, there was more protein in the corn of Ultra variety - to 18%. While in the corn of other varieties its content was 15-16%.

Research of bakery properties of the corn in different amaranth varieties, conducted by foreign and Russian scientists, testifies about expediency of amaranth flour application for its use in diet therapy (Gilbert and Kaufman, 1981; Goptsiy, 1999). We carried out a tasting of the bread, made of the wheat flour together with adding of the flour of Ultra and Kharkovskiy 1 amaranth varieties in amount from 10 to 20%. It showed that the volume output of bread diminishes with the increase of amaranth flour amount, its original appearance gets worse. At the same time the bread received after addition of amaranth flour, in taste qualities exceeds the wheat one. In addition, it contains more protein and less carbohydrates in comparison with wheat bread.

In the Voronezh state university (Russia) the technology of simultaneous receipt of oil and flour was developed from the corn of Ultra and Kharkovskiy 1 varieties. The technology foresees receiving of embryonic flakes and flour. The embryonic flakes after extraction of oil are a good source of protein, its content in them is 30-32% and it exceeds the protein of cereals and broad bean plants in amino acid balance. The protein of amaranth flakes differs with high content of water and salt soluble fractions and is well assimilated by a human organism. In addition, flakes are rich in mineral substances, such as: potassium, calcium, phosphorus, copper and zinc. The amaranth flakes and flour can be used for production of bakery, pastry, dairy-vegetable products, and also in alcohol industry.

Besides food value, amaranth corn is unique for pharmacological properties and interests pharmacologists as a source of valuable oil (Goptsiy, 1999).

As a result of biochemical analysis of the oil, received from the corn of Ultra variety conducted in the Kharkov national university named after V.N. Karazin (Ukraine) the high content of squalene and tocopherols was determined in it, accordingly – 11.25% and 0.28%, that can testify perspective of its use in pharmaceutical industry.

In the Institute of pharmacological therapy of endocrine diseases we conducted researches of antiulcer and antidiabetic properties of oil from the corn of Kharkovskiy 1, Ultra, Sem amaranth varieties. In this research the rats of Vistar line were used. The study of medical and prophylactic action of amaranth oil was conducted on the model of sharp ethanol ulcer. High antiulcer activity of Ultra variety oil was achieved.

The use of amaranth corn deserves attention for preparation of the mixed fodders to fattening different types of cattle and birds. (Acar *et al*., 1988; Goptsiy, 1999; Laovoravit *et al.*, 1986; Tillman and Waldroup, 1988; Waldroup *et al*., 1985).

In the Institute of stock-raising of UAAN we conducted determination of biological value of the corn of Kharkovskiy 1 amaranth varieties comparatively with other fodder grain crops. As a result of the conducted analysis it was established, that on the protein value of the amaranth corn equals the corn one and can be a good component in mixed fodders production.

The of use efficiency study of amaranth seed extrudate as a part of the mixed fodder in the rations of repair pigs showed that the mixed fodder feeding, in which extrudated amaranth seed amounted from 5 to 15%, helped increase the content of raw and sodden protein lysine and methionine in the ration of 4-6 monthly age pigs, that in turn helped increase average daily mass growth of animals in experimental groups, reduce forage expenses on unit of increase, accordingly by 0.31 and 0.58 forages units, or by 5.7 and 10.7%.

The amaranth corn varieties of KhNAU selection have high nourishing characteristics, contain up to 16-18% of protein, from 5 to 8% oil with high pharmacological properties in their corn and can be used in food, pharmacological industries and forage production.

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Table 1 Corn Productivity of grain amaranth varieties by KhNAU selection, c/h

Varieties	**Year**			**Average**
	2005	**2006**	**2007**	
Ultra	30.3	21.8	24.4	25.5
Sem	25.3	16.1	23.9	21.8
Kharkovskiy 1	29.9	19.1	23.8	24.3
Lera	23.8	17.4	22.2	21.1
LSD_{05}	2.5	1.9	2.0	

Meeting of European Amaranth Association

FROM THE HISTORY OF EUROPEAN AMARANTH ASSOCIATION

Jozef HÚSKA

Dear colleagues,

its my pleasure to welcome you on the meeting of European Amaranth Association. I would like to give you some brief information on the European Amaranth Association.
Our association was grounded in 1992 in the middle of Europe, in the former Czechoslovakia. The first president of association was prof. Magomedov (physiologist) from Russia, followed by Ass.prof. J. Húska (agronomist) from Slovakia (1997-1999). At the present this function is performed by prof. Zdenek Zadák, an expert in the field of medicine, from the Czech Republic (1999- till now).

The EAA from its establishment in 1992 has own history. According to the association rules the EAA should organize symposia every second year. The last one was in Nitra (Slovakia) in 1999. The activities of the association till 1999 were presented on conference in Nitra in 1999. During this conference the actual research results on amaranth were presented and published in 41 research papers. The participants from 6 countries took part on the conference with majority of the participants from Slovakia and Czech Republic. The 5th meeting of association was planned to be held in Hradec Kralove (Czech Republic) where is the workplace of the president Zdenek Zadák. Finally, after disscusion we agreed to organize this 5th conference now in Nitra.

Our interest for amaranth research brought us (together with former rector of Agricultural University in Nitra assoc. Prof. J. Zima, PhD.) at the conference held on the University in Amies, Iowa in 1997. In August 2001 we took part together with prof. Zadak on the conference in Missouri, city Columbia which was organised by Thomas Jefferson Agricultural Institute.

What about the "amaranth situation" in Europe?
There was an intensive research work within several research project in many universities: in Stuttgart (Germany), Vienna (Austria), Warsaw (Poland), Nitra (Slovakia), České Budějovice (Czech Republic) and many other countries (Greece, Italy, Hungary, Yugoslavia, Sweden) during the last years. The important activities can be observed in former Soviet Union countries, especially in Russia, Ukraine, and Kazahstan. There are also organised regular biennial conferences on non-traditional crops, where amaranth takes a key role.

From year to year there are more and more products made from amaranth on the European market. The leading role in processing of the amaranth grain for food industry is played by the Czech Republic followed by Germany, Austria and Poland. Except the processing of the own amaranth seed production, there is also increasing tendency to import the amaranth grain from South Africa.

What about the results of amaranth research in Europe?
During the last years there was paid a special attention to this crop on many European universities and research institutions. In Slovakia the research was focused on solution of research project "Adaptability of cultivation and utilization amaranth in Slovakia"

were about 50 different scientists were involved. From 1999 till 2001 the research continued with new project "Deepening of theoretical knowledge on the production and utilization of amaranth in Slovakia".

During 1999-2001 original results were acquired mainly on the production technology (testing of varieties, fertilization, crop rotation, desiccation, harvest, storage and the *Amaranthus* beer production) as well as the utilization of *Amaranthus* as renewable energy source.

As the conclusions of the research the proposal on the cropping system was elaborated for utilization of amaranth in several directions:

1. Amaranth is a high adaptable crop having great perspectives in production conditions of Europe.
2. The biological yield potential of *Amaranthus* in the conditions of Slovakia is on the level of more than 8 tons per hectare.
3. The most suitable species for grain production in Slovak conditions are *Amaranthus hypochondriacus* and *Amaranthus cruentus*. Several hybrids acquit themselves also very well in the production practice.
4. The production technology contains two very important key periods:
   - sowing has to be done during the most suitable term, i.e. in the end of April, into non-weedy soil with hard seedbed and soft shallow cover (hard bed and soft quilt).
   - harvesting in the middle of biological ripeness, i.e. after 120 days by powerful combine-harvester and an immediate drying and cleaning.
5. The research has verified the technologies for the production of starch, protein, fibre and organic dye-stuff from flowers.
6. We have produced the first amaranth beer in Europe, there were evaluated properties of this beer and in the train of that the beer was recommended as a excellent beverage for special occasion.
7. High performance of the soil moisture were confirmed and the ability to bind heavy metals from the soil.
8. As the most important knowledge can be considered the possibility to use the unique phenomena called "gigantism". Seeds originated from South America (Bolivia, Peru) sowed in our conditions brought extremely high yield of biomass. However, the plants haven't continue into the generative growing phase, they were flowering, but any seeds were created. The yield of biomass was 5 times higher than in the case of corn biomass. This knowledge is the challenge to focus our research effort on the use of biotechnology approaches to solve this problem to be able to produce the giant amaranth as a renewable energy source.
9. Researchers from several European countries (Germany, Slovakia, Czech Republic, Austria, Greece, Italy and Poland) have been in contact during last years and they have elaborated a proposal for research project within the 5FP - EU called "Amaranth (*Amaranthus* L.) Network in Europe: Adaptation, Productivity and Sustainability" in order to study this crop more comprehensive in 2002-2004. Unfortunately, this project finally wasn't funded.

Therefore, the present project, which was introduced by Dr. Inge Fomsgaard we consider as very promising for the intensification of amaranth production and its use for human nutrition not only in Europe but mainly in low-income food deficit countries.

CHARTER

of European Amaranth Association

Chapter 1

Main Principles

p.1

The Title and Location

1. The title of organization: European Amaranth Association (further Association) EaA.
2. The location of Association: Olomouc 772 00

   Address: Volkerova str.31

   Tel: 5413562

   Fax: 5413119

p.2

The Relations Governed by Law

1. The persons were consolidated into the Association on a voluntary basis in object to collect and spread the information about the plants attributed to the Amaranth genus. The main goal of Association is to coordinate the activities of European enterprises as well as some individuals those are engaged in the field of grain production, cultivation and conversion of Amaranth.

p.3

The Association acts in Europe and on the territories of independent states those have sprung up after the disintegration of former Soviet Union. Moreover, the association coordinates its activities on an international scale.

p.4

The Object of Activity

1. The Association coordinates the research and experimental activities within the framework of Amaranth genus.
2. The association established the contacts with the organizations as well as juridical and physical persons those are engaged in the same problems under investigating the Amaranth and similar cultures in the place of location abroad.
3. The association will guarantee a financial supply to create and support the function of European Research Amaranth Center, the project to form this Center being prepared by the association.

4. The association carries out the teaching and consulting. The Educational center will be formed at the European Research Amaranth Center.
5. The association will create and organize the enterprises to ensure the economical independency of association.
6. The international conferences and symposia are carried out and organized by the association. Moreover, some measures are undertaken to popularize the amaranth.
7. The Association will be engaged in the editorial and informational activities to publish the bulletins and collections presenting the association.
8. The association is going to organize the European Amaranth Gene Bank.

Chapter 2
The Membership of the Association and the Duties of Association's Members

p.5

The Origin and Terms of Membership

1. Every juridical and physical person is able to receive the title of the member of Association. The agreement with the goals and aims of Association and its Charter as well as the active realization of Association's goals are the conditions to receive the membership.
2. Anyone who wishes to join the Association, ought to submit the application on the registration form, that should be authenticated by the signature of applicant being the institutional or juridical representative.
3. The receiving of application and passing of solution are carried out in written form.

p.6

The Rights of Association Members

1. Every member of association has the right to take part in the general Meeting.
2. Every member has the right to vote (one vote per each member)
3. Every member has the right to be elected to the Association's agencies, to estimate the activity of association and its Presidium, to make the suggestions, to put the question to the vote.

4. Physical person has one vote during the votation, this person being able to transfer his right to vote for the other member of association.
5. The official spokesman or person being given by a written powers stands proxy for the juridical person.
6. Every member of Association has the rights to participate in any activity of Association including the trade one on condition that this kind of activity would be reasonable to carry out.
7. Every member has to right to be informed about all Association's activities, to participate in them, to take all preferences following from the membership of Association.

p.7

The Duties of Association's

Members

1. Every member is obliged to keep the Charter of the Association, to carry out the resolutions of Presidium and General Meeting.
2. Every member is ought to participate actively in the carrying out of the goals of Association and achieving of its objects.
3. The established dues to be paid out.
4. Every member must protect actively the interests of association and try to attain Association's objects. No steps being in the contradiction with the Association's interests can be undertaken by the members of Association.

p.8

The Cessation of Association's Membership

1. The membership of Association is ceased:
   a. when coming out from Association;
   b. when expelling from the Association;
   c. when liquidating of juridical person
   d. by death of physical person.
2. The withdrawal from the Association.

   The member has the right to withdraw from the Association in one year after the day of admission to the Association. The application is in the force from 31.12. of

corresponding year. The application should be submitted in the written form till 30.09. of corresponding year at the latest. Only written applications can be accepted.

3. The expulsion from the Association:

   a. for the flagrant violation of Charter and Association's agencies resolutions;

   b. if the terms of membership are disappeared (p.5 of Charter);

   c. if the activity of Member undermines the interests or the gravity of the Association.

   The person being expelled from the Association is immediately informed by the Presidium about the resolution to expel him. The member has the right to send in an appeal for 30 days from the day when he was given by the resolution to expel.

p.9

The Conditions to Case the Membership

The Association has to settle up with the person who withdrew or was expelled from the Association for 2 months from the day when the membership was stopped.

p.10

The Defence

If the member of the Association consider any agency resolution to be illegal or to contradict the Charter, he will be able to demand to restore the justice during the trial for 30 days from the day when he was informed about the resolution and for 6 months at the latest.

Chapter 3

The Organizing Structure of Association

1. Following agencies are the Association's ones:

   a. General Meeting;

   b. Presidium.

2. The general Meeting elects the Presidium, that reports back to the General Meeting for its activities.
3. The resolutions of the Association's agencies are obligatory for the all members and the agencies elected by them.
4. The agencies have the right to take the resolutions on condition that more than a half of the members are present.

5. If the resolution is carried by more than a half of the members being represent, the resolution will come into force. The vote of Association's President is decisive while coinciding the votes for the proposal and against one.
6. The resolution is carried out by open voting.
7. The Presidium and the President are elected as a rule for two years. The third part of Presidium may be co-opted during this period.

p.11

General Meeting

1. The General Meeting is the supreme organ of the Association. The members realize their rights and check the Association's activity at the general Meeting.
2. While carring out the congress or symposium the General Meeting convenes a Presidium as a rule once for 2 years.

   3. General Meeting consisting of all Association's members has the right to pass the resolutions in conformity wit p.10 (5^{th} item).
4. Clase of problems those are solved by General Meeting:
   a. to pass the programme and goals of Association's activity;
   b. to estimate the activities of Presidium, President and Secretary for the last period;
   c. to elect the members for the agencies, to release them or to recall them for their function;
   d. to pass the changes and additions in 's Charter;
   e. to pass the financial plan of the Association' and result of its activity;
   f. to confirm the rate of dues;
   g. to carry the resolution to liquidate the Association or to include its into other organization.
5. The programme of session of General Meeting is prepared by Presidium and sent to all registered Association's members no sooner than before 30 days till the beginning of General Meeting session.
6. The President of Association conducts a session of General Meeting, the Presidium authorizing somebody to conduct the session in the President's absence.
7. The representatives check the results of vote.
8. The election of Association's agencies:

a. the vote is carried out as the proposals are made. The mode of votation as well as the coming of resolution into force are established by p.10 (items 3-6). The President and secretary of Association are elected separately;

b. the counting of the votes and report about the results of votation are carried out separately for each part of election;

c. when being given by the report the chosen person about the acceptance of function, one can consider the vote results to come into force.

9. The results to General Meeting session are minuted, the document being signed by the President and other two members of Association.

p.12

The Presidium

1. The Presidium is the authority of the Association.
2. The representatives are sent as a delegate by the delegations of some countries to take part in Association's activity. This representative realize the objects and goals of Associationin participating countries.
3. The Presidium consist of President, Secretary and other members is determined by the number of status being the member of Association.
4. The Presidium and President are elected as a rule for 2 years in conformity with p.10 (item 7).
5. The President and Secretary are directly elected by General Meeting (p.10, item 8a).
6. The class of problems to be solved by Presidium:

   a. to ensure and check the execution of resolutions of General Meeting;

   b. to inform regularly the members about Association's activity;.

   c. to solve all present-day problems those are outside the general meeting's competence.

   d. to call and prepare the general meeting and symposia.

7. The correspondence and telephony have the equal rights as the modes of leadership.

   If the Presidium cannot be convened owing to the lack of time or other reason, the President will be able to make reach to a decision on the strength or written answers of all Presidium's members.

8. The President of Association supervises the scientific studies, represents the Association during the negotiations, has the right to sign documents, prepares the

scientific and technical symposia, draws specialists into work of the Association and research and Teaching Amaranth Centers, finds the means to carry out the activity of these centers and Association. The President conducts the activities of Presidium and secretary.

p.13

Executive Agency of Association

1. All present-day problems are solved by the executive agency of Association, this organ consisting of professional workers – the management of Association.

2. The secretary of Association is in the executive agency of Association, the Secretary being elected and recalled by the general meeting. Other workers are likely to be in the management. The secretary conducts the activities of other workers.

3. The executive Agency of Association:

a. conducts, organizes and carries out the solutions of all present-day problems;

b. carries out the central registration of Association's members and issues a Association's membership cards;

c. fulfils the connections with external subjects and prepares the documents for the negotiations and sessions;

d. creates and ensures the functioning of Association's information net.

e. carries out the managerial activity in conformity with established financial plan of Association;

f. manages the Association's property;

g. executes other activities.

p.14

Working Groups and Committees

The Presidium is able to organize the working groups, committees and sections to solve the special questions.

Chapter 4

The Managerial Activity of Association

p.15

The Property of Association

1. The property of Association (material and financial means) is made due to the dues and gift or contributions of other subjects.
2. Not only the material means but also the results of spiritual activity are the property of Association.
3. No commercial activities are carried out by the Association.

p.16

The Funds of Association

1. The Association creates and uses the Funds, those are necessary for its activity. The Funds are created on the principles of law or due the resolutions of General Meeting.
2. The creating and using funds on the principle of General Meeting's resolution will be guided by original rules, those are prepared by Presidium and confirmed by General Meeting.

p.17

To Sum Up the Economic Activity

1. All economic activity is carried out in conformity with active juridical standards.
2. The President presents the report about economic activity undertaken for the period under review to General Meeting.
3. Owing to the resolution of General Meeting, possible loss can be transferred for the next period.

Chapter 5

Final Principles

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Proxies

On behalf Association, the President, the Secretary and other representative members carried out the negotiations.

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The Liquidation of Association

1. If the resolution to liquidate the Association is passed by two-thirds of all participants of General Meeting, the Association will end its existence

2. As the existence of Association is sopped, the liquidation of Association’s property will be undertaken by commission confirmed by General Meeting. The commission acts in conformity with the legislation and methods passed by general Meeting.

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The Charter of Association

1. This Charter was passed on the constituent assembly of Association.
2. Present Charter can be changed or supplement only in condition that the two-thirds of General Meeting’s participants are agreed.

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