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**Neglected and Under-utilized Species Research in 21<sup>st</sup> Century**

Book of Abstracts

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# Neglected and under-utilized crops for sustainable food production

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Human population is growing at the alarming rate in the developing countries and food availability to newly fed mouth could gradually become a serious problem. The agriculture production is badly affected as a result of environmental pollution, rapid industrialization, water scarcity, soil erosion of top fertile soil, no further scope of expanding arable land, lack of improvement of local plant types, erosion of genetic diversity, and dependence on few crop species for food supply worldwide (Ochatt and Jain, 2007). Only 30 plant species are used to meet 95% of the world's food requirements, which are considered as the 'major crops' These crops are widely and intensively cultivated and selected from a large agro-biodiversity pool, and yet the exploitation of our plant genetic resources is far below that would allow all high potential of exploitation of crop improvement. Several factors such as physical appearance, taste, nutritional properties, cultivation methods, processing qualities, economic gains, and others are responsible for the promotion and acceptance of these crops. The breeding programs of these crops has been very much dependent on the readily availability of genetic variation, either spontaneous or induced ones. The neglected /under-utilized or minor crops are traditionally grown in centres of their origin or centres of diversity by farmers, and have lesser importance in terms of global production and consumption systems. They are locally well adapted to marginal lands and constitute an important part of the local diet providing valuable nutritional elements such as proteins, vitamins, and minerals and species, often lack in staple diet. Their role in traditional medicine is also well known. In addition, these crops are important sources of resistance genes for biotic and abiotic stress breeding that can be utilized also for the genetic improvement of commodity crops. As compared to the major crops, they require relatively low inputs and, therefore, contribute to sustainable agricultural production. The researchers have recognized their great potential towards food security, sustainable agriculture and improving the socio-economic aspect in the poor rural sector. Plant tissue culture has a great potential in plant improvement, provided plants can be readily regenerated in large numbers. It provides the options to reduce costs in generating the useful traits and pre-breeding materials for plant breeders, as well as shortening the screening program, e.g. for salt and drought tolerance. Induced mutations with physical and chemical mutagens have been quite effective for a significant increase in plant production among both seed and vegetative propagated crops. More than 3000 mutant varieties have officially been released in many countries (<http://www-mvd.iaea.org/>). However, there is not enough work done on plant breeding, mutagenesis, biotechnology and molecular biology in neglected and underutilized crops, which is mainly due to shortage of funds. The potential of nuclear applications together with tissue culture and molecular tools in neglected crop improvement will be discussed, and prospects of food security and nutrition. The main crops included are: *Jatropha*, cassava, bambara groundnut, amaranthus, grass pea, quinoa, yam, bitter potato, okra, and taro

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# Enzyme polymorphism analysis in irradiated amaranth (*Amaranthus spp.*) lines

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**Key words:** amaranth, polymorphism, isoenzyme, starch gel electrophoresis

## Introduction

Grain amaranth (*Amaranthus spp.*) is a widely known pseudocereal with interesting nutritional characteristics including proteins, well suited to human nutritional needs. Depending on cultivation conditions and properties of the species, this crop is used as food, fodder, medicinal, and ornamental plant in many countries (Yudina *et al.*, 2005). Crude grain protein content ranges from 12.5 to 22.5 % on a dry matter basis, and is relatively rich in the essential amino acid lysine normally limited in other cereal crops. In addition, amaranth has higher mineral content as compared to cereal grains.

Amaranth belongs to the group of plants, which genetics is poorly studied. Such situation is explained by a complexity of its hybridization, caused by an extremely small size of its reproductive organs and a special constitution of its inflorescences. Application of isozyme analysis, widely used in various plant species will allow bypassing these difficulties and assist in investigation of amaranth evolution, taxonomy and genetics (Yudina, 2010).

## Material and Methods

Two grain amaranth accessions were used for the irradiation treatment - *Amaranthus cruentus* genotype Ficha and product of interspecific hybridization (*A. hypochondriacus* x *A. hybridus*) hybrid K-433. During the years 1998 – 2011, thirteen generations of mutant lines with their untreated counterparts were established. Finally, 4 mutant lines of *A. cruentus* and 3 lines of hybrid K-433 with significantly increased WTS were selected with an obvious tendency to stabilization of this trait when compared to untreated controls and to the samples of the previous generations (Gajdošová *et al.*, 2007).

In our experiment amaranth seeds, undifferentiated seedlings and leaves were analysed for isoenzymes. We used 100 mg of sample and 50 µl of extract solution. For comparing mobility of enzyme activities of amaranth with maize coleoptile section enzyme activities, extract from coleoptile section after five days cultivation of maize single-cross hybrid grain (Sc 3098 x 3150, Sempol Holding Inc., Trnava, Slovak Republic) under the same cultivation condition have been used (Múdry *et al.*, 2011). Extracts were done from maize coleoptile sections, amaranth dry seeds, seedlings and leaves by hand crashing using glass stick in mortar on ice with several grains of pure sand for amaranth seed samples. Extraction solution consisted of 0.84 g of sucrose and 0.42 g of sodium ascorbate dissolved in 5 ml of water.

The standard technique of horizontal starch gel electrophoresis according to Stuber *et al.* (1988) and Múdry *et al.* (2011) was used for analysis of amaranth enzyme polymorphism in acid phosphatase (ACP, E.C.3.1.3.2.), alcohol dehydrogenase (ADH, E.C. 1.1.1.1.), catalase (CAT, E.C. 1.11.1.6.), diaphorase (DIA, E.C. 1.6.99.), β-glucosidase (GLU, E.C.

3.2.1.21), glutamateoxaloacetate transaminase (GOT, E.C. 2.6.1.1.), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42.), malate dehydrogenase (MDH, E.C. 1.1.1.37.), 6-phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44.), phosphoglucoisomerase (PGI, E.C. 5.3.1.9.), and phosphoglucomutase (PGM, E.C. 2.7.5.1).

After electrophoretic separation gels were horizontally cut into thin slices and placed into boxes for chemical staining of enzymatic activity zones. The composition of gel buffer solutions, optimal conditions for gel systems and staining solutions were described before (Múdry *et al.*, 2011).

The amaranth isoenzymes activity zones have been photographed and expressed by factors of relative mobility ( $R_m$ ) and diagram constructions.

## Result and Discussion

Many factors affect isoenzyme analysis and correct interpretation of results, for example extraction, preparing of starch gel, electrophoresis conditions, gel staining etc. For our experiment we chose eleven frequently studied enzymes in plant breeding and seed improvement. We detected no differences between putative mutant lines with their reference sample.

**Acid phosphatase** polymorphism of amaranth was monomorphic in all analysed samples. In isozymogram were detected seven to eight electrophoretic pattern of isozymes. **Alcoholdehydrogenase** is the most studied enzyme in plants, the genetic control and properties of this enzyme are similar in many plant species (Yudina *et al.*, 2010). According to Múdry *et al.* (2011) and Yudina *et al.* (2010), the amaranth ADH spectrum has one activity zone with fast-migrating (*Adh F*) or slow-migrating (*Adh S*) variants of the enzyme. We haven't detected any ADH activity in amaranth leaves. On the other hand, isozyme patterns of ADH in amaranth seeds and seedlings were monomorphic and one-banded.

Isozymograms of amaranth **catalase** were weak, smudged with one elliptical spot. In addition, isozymograms of  **$\beta$ -glucosidase** absented for all analysed samples of amaranth. **Diaphorase** is characteristic with two loci *Dia 1* (three alleles) and *Dia 2* (five alleles). Our analysis showed unclear fingerprints, because of low diaphorase activity and dark background of gel plate. The spots in **glutamateoxaloacetate transaminase** were weak, monomorphic, one of two banded. Polymorphism of **isocitrate dehydrogenase** in all analyzed samples was monomorphic with one of the two-banded phenotypes (Múdry *et al.*, 2011).

**Malate dehydrogenase** belongs to good studied enzyme. In different plant species there is different number of MDH loci, level of polymorphism and interaction between alleles and loci (Goodman *et al.*, 1980; Benito *et al.*, 1983). The isozyme spectrum of MDH has two zones of activity, fast-migrating and slow-migrating (Yudina *et al.*, 2010). Yudina *et al.* (2008) suggested that MDH is controlled by two non-allelic genes monomorphic *Mdh 1* and polymorphic *Mdh 2* that has three alleles. We detected, that all samples were monomorphic with five banded phenotypes. Isozyme patterns of amaranth **6-phosphogluconate dehydrogenase** were one-banded and monomorphic. Amaranth **phosphoglucoisomerase** phenotypes were four-banded and also monomorphic.

As wrote Múdry *et al.* (2011) polymorphism of amaranth **phosphoglucomutase** belongs to poorer studied enzymes. In our amaranth samples we detected four-banded phenotypes with very weak uppermost band.

From the isozymograms of eleven enzymes, we can conclude, that there are no significant differences between irradiated amaranth seeds, seedlings and leaves compared to untreated reference samples.

However, from protein and amino acid analysis results (Hricová *et al.*, 2011) we predict, that this plant material can be considered as valuable matrix useful in further amaranth breeding programme.

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# Progress in sequencing *Amaranthus tricolor* and chloroplast genome phylogenetics

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**Keywords:** *Amaranthus*, barcoding, whole genome sequencing, indigenous leafy vegetable, beta-carotene

## Introduction

*Amaranthus* species (amaranths) are an emerging and highly promising nutritious traditional leafy vegetable food source in Africa, and have high protein content, elevated vitamin A ( $\beta$ -carotene) and C, calcium and phosphorous levels. Amaranth grains are reported to have higher protein, starch and lysine content than more conventional cereal grains such as wheat (*Triticum aestivum* L.) and pearl millet [*Pennisetum glaucum* (L.) R. Br.]. The aims of the current study were to phylogenetically characterize unknown South African Amaranth accessions, using inter and intraspecies informative chloroplast barcoding genes suggested by the Barcoding of Life initiative (*MatK* and *rbcL*). Known international Amaranth accessions were included, and used to identify and classify accessions collected in different regions of South Africa.

## Methodology

Using the Illumina HiScanSQ high-throughput sequencing platform, a draft whole genome, including complete chloroplast sequence, was generated for *A. tricolor* and assembled using CLC Genomics Workbench analysis software. The complete chloroplast sequence and selected chloroplast genes were used to resolve the phylogeny of several angiosperm species and to further correctly classify South African amaranth collections alongside a representative global amaranth germplasm. The maturase K (*matK*) and the large subunit of RUBISCO (*rbcL*) gene regions were amplified and sequenced for the unknown South African accessions, as well as known international specimens as reference species. Maximum parsimony and neighbour-joining algorithms were used for the identification and classification of unknown *Amaranthus* species by grouping accessions based on their genetic similarity.

## Results

Progress to date on whole genome sequencing includes a draft genome assembly of *A. tricolor* with approximately 78 Gb of sequence data. The assembly incorporated roughly 963 million reads, spanning 365 Mb of genomic region, thus representing approximately 18% of the genome. By using reference mapping algorithms, it was possible to reconstruct 97% of the chloroplast genome by mapping 2.4 million reads to sugarbeet (*Beta vulgaris* L.), used as a reference chloroplast. The estimated size of the chloroplast was found to be approximately 145 249 base pairs (excluding gaps), with a GC content of 37.04% and 115 open reading frames (ORF). Amaranth seems to be closely related to sugarbeet and spinach (*Spinacia oleraceae*), and this grouping is supported by 100% bootstrap values. Further closely related species include *Arabidopsis thaliana*, turnip (*Brassica rapa*), tomato (*Solanum lycopersicum*), grapes (*Vitis vinifera*) and melons (*Cucumis sativus*). The monocot species, sorghum (*Sorghum bicolor*) and rice (*Oryza sativa*), form a completely separate group, supported by 100% bootstrap. Phylogenetic tree construction using barcoding genes reveals species

relationships between *Amaranthus* accessions. Unknown accessions were assigned putative species names, and the relationship between accessions uncovered using chloroplast barcoding analysis.

# Genetic analysis of *Amaranthus hypochondriacus* L. genotypes using Inter-Simple Sequence Repeats

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**Key words:** *Amaranthus hypochondriacus*, genetic analysis, ISSR

## Introduction

The genus *Amaranthus* L. contains approximately 60 species, including cultivated and wild species. Cultivated amaranths are used for food grain, leafy vegetables, forage, ornamental gardening and other potential uses (Brenner *et al.*, 2000). Three species of *Amaranthus* are commonly cultivated for grain production: *A. hypochondriacus* L., *A. cruentus* L. and *A. caudatus* L. (Lanoue *et al.*, 1996). Nowadays, molecular analyses methods provides a base for a wide range of plant biological material assessment. They are involved in different methods from epidemiological changes evaluation (Zeľňáková *et al.*, 2012), continuing in authentication of food (Židek *et al.*, 2012) up to the analyses of biodiversity of wild and cultivated plants (Oslovičová *et al.*, 2010, Vivodík *et al.*, 2011, Ražná, Žiarovská, 2011). Internal transcribed spacer (ITS) of nuclear ribosomal DNA, amplified fragment length polymorphism (AFLP), and double-primer fluorescent inter-simple sequence repeat (ISSR) were employed to re-examine the taxonomic status and phylogenetic relationships of grain amaranths and their wild relatives. Both, AFLP and double-primer fluorescent ISSR have a great potential for generating a large number of informative characters for phylogenetic analysis of closely related species (Xu, Sun, 2001). The aim of presented study was (1) to consider applicable primers for ISSR, (2) to optimize PCR conditions for reproducible results, (3) to assess the use of ISSR markers as a tool for genetic analysis of *Amaranthus hypochondriacus* germplasm collection in dependence on the center of origin.

## Material and Methods

The seeds of 21 *Amaranthus hypochondriacus* L. genotypes (table 1) with different origin were obtained from North Central Regional PI Station (NC 7), Iowa State University, Ames.

The amaranth seedlings were cultivated under *in vitro* conditions on Murashige, Skoog (1962) cultivation medium. DNA from fresh leaves was isolated according to Rogers, Bendich *et al.* (1994) optimized protocol. Each genotype was represented by ten individuals. The amount of DNA in reaction (10-80 ng in 25  $\mu$ l total volume), the primer concentration (0,2-1,5  $\mu$ mol.dm<sup>-3</sup>), MgCl<sub>2</sub> concentration (0,75-1,5 mmol.dm<sup>-3</sup>), deoxyribonucleotides concentration (0,1-0,2 mmol.dm<sup>-3</sup>) and the annealing temperature (45-60 °C) were optimized. All optimization reactions were repeated three times. According to the optimization, PCR conditions were assigned 20 mmol.dm<sup>-3</sup> Tris-HCl, pH 8,0 (Invitrogen™, Life Technologies), 50 mmol.dm<sup>-3</sup> KCl (Invitrogen™, Life Technologies), 1 U Taq polymerase (Invitrogen™, Life Technologies), 3 mmol.dm<sup>-3</sup> MgCl<sub>2</sub> (Invitrogen™, Life Technologies), 0,1 mmol.dm<sup>-3</sup> deoxyribonucleotides (Promega), 0,2  $\mu$ mol.dm<sup>-3</sup> primer (Invitrogen™, Life Technologies, table 2), 20 ng DNA in 25  $\mu$ l total reaction volume. The PCR cycling conditions were as follows: 94 °C for 2 minutes (initial denaturation), then followed by 45 cycles at 94 °C for 1 minute (denaturation), 50 °C for 1 minute (annealing), 72 °C for 2 minutes (polymerisation) with a final 7 min extension at 72 °C and then cool down to 4 °C. The PCR products were separated by electrophoresis on 2 % agarose gel (3:1, Amresco) containing 0,5  $\mu$ g.ml<sup>-1</sup> ethidium bromide in a 1  $\times$  TBE buffer and then photographed under UV light using G-box

(Syngene). The ISSR bands were scored using the binary scoring system that recorded the presence and absence of bands as 1 and 0 using GeneSnap (Syngene) program. Genetic similarity was calculated on the basis of Nei, Li (1979) similarity index. The resulting matrix of genetic similarity was used to construct the dendrogram through UPGMA with statistic program SYNTAX.

## Results and Discussion

Studies on the evolution relationships of the genus *Amaranthus* species cultivated and their wild relatives have been made especially in the last 20 years, by applying various techniques. These studies have allowed the development of some hypotheses about the geographical origin of species and establish phylogenetic links between them (Popa *et al.*, 2010). Most of the current methods to determine genetic diversity are based on polymerase chain reaction (Powell *et al.*, 1995).

Twentyone *Amaranthus hypochondriacus* L. genotypes were analysed using eleven ISSR primers. The primers had 50–72 % GC content. Seven from eleven primers generated consistent profiles. The number of band levels ranged from 10 [primer ISLA-(CT)<sub>8</sub>AC] to 21 [primer ISLA-(GAG)<sub>3</sub>GC]. An average of 6.9 loci per primer was produced, ranging from a minimum of 3.24 loci using ISLA-(CA)<sub>6</sub>AG to a maximum of 11.81 loci using ISLA-(GTG)<sub>3</sub>GC. The average similarity index among all the *A. hypochondriacus* genotypes ranged from 0.58 to 0.84 with a mean of 0.73 indicating variation among amaranth genotypes. *A. hypochondriacus* Ames 21046 Annapurna from India and PI 481464 EC-18626 from Nepal were similarly clustered with the value of Euclidian Distance Averages of Clusters (EDAC) 0,000E+00 using primer ISLA-(CA)<sub>6</sub>AG, ISLA-(CA)<sub>6</sub>GG, ISLA-(GAG)<sub>3</sub>GC and ISLA-(GA)<sub>6</sub>CC. Two genotypes originated from Nepal clustered at EDAC 0,000E+00 using ISLA-(GTG)<sub>3</sub>GC and ISLA-(CT)<sub>8</sub>AC indicating high genetic similarity. Two primers were able to distinguish four genotypes originated from Mexico from other genotypes used in the study. Genotypes PI 477916 RRC 1023 with Ames 5209 RRC 457 and Ames 5132 RRC 363 with Ames 5321 RRC 539 were clustered with the 0,000E+00 EDAC value using ISLA-(GA)<sub>6</sub>CC in PCR. *A. hypochodriacus* Ames 2086 RRC 149 originated from Nepal clustered as a single cluster with differend EDAC values ranging from 0,159E+0,2 to 0,377E+02 with four ISSR primers idicating less genetic similarity in comparison to other genotypes. Primer ISLA-(GTG)<sub>3</sub>GC was able to distinguish 62 % of *A. hypochondriacus* genotypes.

## Conclusions

Markers ISSR have a great potential for generating a large number of informative characters for phylogenetic analysis. Set of two primers distinguished *Amarahnus hypochondriacus* genotypes originated from Mexico. Four primers used in ISSR were able to differentiate *A. hypochondriacus* genotype originated from Nepal.

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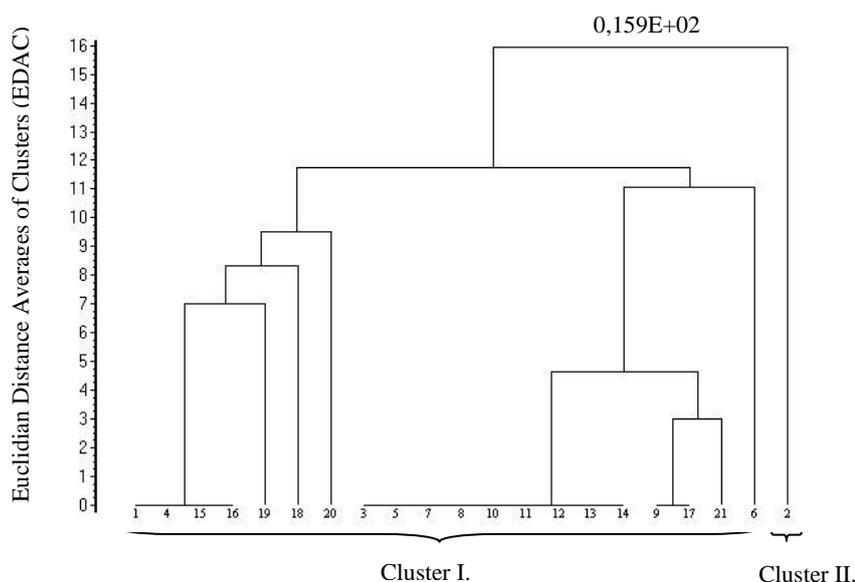
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**Table 1** *Amaranthus hypochondriacus* L. genotypes used in the study

| Code  | Genotypes            | Country of origin       | Note                |
|-------|----------------------|-------------------------|---------------------|
| AH-01 | Ames 2064 RRC 126    | Nepal                   | Breeding material   |
| AH-02 | Ames 2086 RRC 149    | Nepal                   | Unknown origin      |
| AH-03 | Ames 2061 RRC 124    | Nepal                   | Unknown origin      |
| AH-04 | Ames 21046 Annapurna | India                   | Cultivar            |
| AH-05 | PI 481464 EC-18626   | Nepal                   | Unknown origin      |
| AH-06 | PI 538794 AJCO74     | Russia                  | Cultivated material |
| AH-07 | PI 542595            | China                   | Cultivated material |
| AH-08 | PI 568130 DB 926     | USA, Iowa               | Breeding material   |
| AH-09 | PI 511731 HH 104     | Mexico                  | Cultivated material |
| AH-10 | Ames 12744 I 91      | Nepal                   | Unknown origin      |
| AH-11 | Ames 1972 RRC 18 A   | Nigeria                 | Breeding material   |
| AH-12 | PI 274279 RRC 171    | India, Himachal Pradesh | Unknown origin      |
| AH-13 | PI 337611 P 373      | Uganda                  | Landrace            |
| AH-14 | PI 477915 RRC 1008   | India                   | Breeding material   |
| AH-15 | PI 477916 RRC 1023   | Mexico                  | Cultivar            |
| AH-16 | PI 477917 RRC 1024   | Mexico                  | Cultivar            |
| AH-17 | Ames 2178 RRC 266    | Nepal                   | Breeding material   |
| AH-18 | Ames 5132 RRC 363    | Mexico, Chihuahua       | Landrace            |
| AH-19 | Ames 5209 RRC 457    | Mexico, Mexico          | Landrace            |
| AH-20 | Ames 5321 RRC 539    | Mexico, Chihuahua       | Landrace            |
| AH-21 | Ames 5467 RRC 720    | Mexico, Oaxaca          | Unknown origin      |

**Table 2** The list of primers used in the study

| Primer                    | Sequence (5' → 3')         | Primer                     | Sequence (5' → 3')         |
|---------------------------|----------------------------|----------------------------|----------------------------|
| ISLA-(AGC) <sub>4</sub> G | AGC AGC AGC AGC G          | ISLA-(CT) <sub>8</sub> TG  | CT CT CT CT CT CT CT CT TG |
| ISLA-(CA) <sub>6</sub> AG | CA CA CA CA CA CA AG       | ISLA-(GA) <sub>6</sub> CC  | GA GA GA GA GA GA CC       |
| ISLA-(CA) <sub>6</sub> GG | CA CA CA CA CA CA GG       | ISLA-(GAG) <sub>3</sub> GC | GAG GAG GAG GC             |
| ISLA-(CA) <sub>6</sub> GT | CA CA CA CA CA CA GT       | ISLA-(GTG) <sub>3</sub> GC | GTG GTG GTG GC             |
| ISLA-(CT) <sub>8</sub>    | CT CT CT CT CT CT CT CT    | ISLA-(GT) <sub>6</sub> CC  | GT GT GT GT GT GT CC       |
| ISLA-(CT) <sub>8</sub> AC | CT CT CT CT CT CT CT CT AC |                            |                            |



**Picture 1** Dendrogram of *Amaranthus hypochondriacus* L. genotypes constructed with UPGMA method on the basis of ISSR analysis with ISLA-(CT)<sub>8</sub>AC primer.

Annotation: 1 – AH-01, 2 – AH-02, 3 – AH-03, 4 – AH-04, 5 – AH-05, 6 – AH-06, 7 – AH-07, 8 – AH-08, 9 – AH-09, 10 – AH-10, 11 – AH-11, 12 – AH-12, 13 – AH-13, 14 – AH-14, 15 – AH-15, 16 – AH-16, 17 – AH-17, 18 – AH-18, 19 – AH-19, 20 – AH-20, 21 – AH-21.

## Adding value to amaranth through the use of radiation mutagenesis

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**Key words:** *amaranth, mutagenesis, WTS, nutrition*

The genus *Amaranthus* contains about 60 species including weeds, garden flowers and crops. They exhibit a high degree of variability, are resistant to diseases, insects and weeds, also known for their tolerance to drought, salinity and high temperature what makes them a good alternative for conditions of the global climate warming. Amaranths, as C4 plants, can contribute to mitigate CO<sub>2</sub> concentration, the major factor provoking greenhouse effects. Because of high biomass production and ability to accumulate heavy metals they can be used as a renewable energy source and for phytoremediation. Amaranth has a very promising nutritional potential when compared to other grains, whether cereals or food legumes with high impact on human health. Grain amaranth is a widely know pseudocereal with interesting nutritional characteristics including proteins, well suited to human nutritional needs. Its highly nutritious gluten-free grain make a good alternative to cereals or legumes and can be used in gluten-free diet.

We focus our research on the enhancement of the quality and quantity of amaranth grain by use of radiation mutagenesis and biotechnology approaches. Two grain amaranth assessions were used for the irradiation treatment - *Amaranthus cruentus* genotype Ficha and hybrid K-433 (*A. hypochondriacus* x *A. hybridus*). During the years 1998 – 2011, thirteen generations of putative mutants with their untreated counterparts were established. Finally, 4 putative mutant lines of *A. cruentus* and 3 lines of hybrid K-433 with a long-term significantly increased weight of thousand seeds were selected, with obvious tendency to stabilization of this trait compared to non-treated forms and to the samples of the previous generations (1).

Detailed analyses of biochemical traits such as soluble oxalate level, protein and amino acid content, lipid profile or squalene content in the grains of putative mutant lines showed improved nutritional quality over the control varieties (2, 3).

We can conclude, that our mutagenesis-generated lines are considered as valuable matrix for amaranth breeding programme. We have chosen putative mutant line with biggest impact of radiation on monitored traits to register as a new variety.

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# Effect of selenium supplementation on the biological activity of amaranth sprouts

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**Key words:** amaranth sprouts, selenium, antioxidant activity, NFκB, TNFα

## Introduction

In the last decade, the use of amaranth seeds has broadened not only in the common diet, but also in diet of people with celiac disease or allergies to typical cereals (Berti *et al.*, 2005). Amaranth seeds have high nutritional and functional values which are associated with the quality and quantity of their proteins, fats and also antioxidant potential (Gorinstein *et al.*, 2007; Paśko *et al.*, 2009).

The new trend in nutrition, in recent years, is the consumption of various sprouts – the atypical vegetables, which have received attention as functional foods, because of their nutritive value, including amino acids, fibre, trace elements and vitamins as well as flavonoids, and phenolic acids (Paśko *et al.*, 2008). Consumption of seeds and sprouts has become increasingly popular among people interested in improving and maintaining their health status by changing dietary habits. The sprouts are excellent examples of ‘functional food’ defined as a type of food lowering the risk of various diseases and/or exerting health promoting effects in addition to its nutritive value.

On the other hand, it is well known that the content of trace element selenium in daily diets depends on the selenium concentration in the soil and water. The large differences of soil selenium concentrations among different countries cause significant differences in the daily intake of this element. Almost all European countries belong to low-selenium regions. As a consequence, field treatment with selenium or selenium supplementation of food are the examples of actions to improve selenium nutritional status of people (Lintschinger *et al.*, 2000). Evaluation and searching for new plants which could accumulate selenium is highly required.

Most of the recently published papers are focused mainly on the studies of typical sprouts such as broccoli, mung bean, and soybean, which are already easily available on the market. The sprouts of amaranth could be both – “new” vegetables, which can be used in the nutrition of vegans and vegetarians, and a common diet being also the valuable source of selenium. Our goal of present investigation was to describe the best procedure of amaranth harvesting and show which doses of selenium are adequate to improve growing of sprouts. The second aim of our study was to show the nutritional value of amaranth sprouts as a good source of antioxidants and selenium. The antioxidant potential of seeds and sprouts of different edible amaranths was evaluated by the FRAP method. We investigated also total polyphenols (TP) content in obtained material. The last part of our work was associated with determining the influence of obtained amaranth sprouts extracts on the inhibition of inflammation processes in *in vitro* model.

## Material and Methods

Different edible amaranth seeds (*Amaranthus cruentus*, *Amaranthus caudatus*, *Amaranthus paniculatus*, *Amaranthus tricolor*) were used in sprouts harvesting. Amaranth seeds were immersed in water or water with different selenium concentration (10 mg.l<sup>-1</sup> and 15 mg.l<sup>-1</sup>; selenium as a sodium selenite) for 3 h and then put into a clay vessels. Sprouts were grown for 6 days after seeding at fixed temperature of 24±2°C. They were watered every day. All of the culture was stored in natural conditions of the light.

### Determination of total phenols

Total phenols were determined colorimetrically using the Folin–Ciocalteu reagent, as described previously (Paško *et al.*, 2009). Total phenols assay was conducted by mixing 2.7 ml of de-ionized water, 0.3 ml of extracts, 0.3 ml 7 g/100 g Na<sub>2</sub>CO<sub>3</sub> and 0.15 ml Folin–Ciocalteu reagent. Absorbance of mixture was measured at 725 nm and 760 nm using the spectrophotometer Jasco UV-530. A standard curve was prepared with gallic acid. Final results were given as gallic acid equivalents (GAE).

### FRAP

FRAP assay was carried out according to Benzie and Strain (1996), and modified to 48-well plates and automatic reader (Synergy-2, BioTek/USA) with syringe rapid dispensers. Briefly, the oxidant in the FRAP assay (reagent mixture) consisted of ferric chloride solution (20 mmol.l<sup>-1</sup>), 2,4,6-tripyridyl-striazine (TPTZ) solution (10 mmol.l<sup>-1</sup> TPTZ in 40 mmol.l<sup>-1</sup> HCl) and acetate buffer (pH = 3.6) in a proportion of 5:5:10, respectively, and was freshly prepared. To each plate, 0.4 ml of acetate buffer (pH 3.6) was dispensed, followed by 50 µl of sample (methanol extracts), standard or blank. The plate was conditioned at the temperature of 37°C for 2 min, and then 0.2 ml of reagent mixture was added and shaken for 30 s; afterwards, absorbance at 593 nm was measured with kinetic mode for 15 min. The final results were expressed as mmol Fe<sup>2+</sup>/kg of dry weight.

### Selenium concentration

The 12-positional microwave system MARS X (CEM, Matthews, USA), equipped with temperature (RTP-300) and pressure (ESP-1500 Plus) sensors, was used for the sample preparation. The system allowed programmable control of the pressure and temperature up to 5520 kPa (800 psi) and 220°C, respectively. The Mini-vap device (Sigma Aldrich, Germany) was used for removal of gaseous products with nitrogen.

Analysis: A double-channel atomic fluorescence spectrometer AFS-230 (Beijing Haiguang Instrument Co., China) with flow hydride-generation system was used. The light sources used were cathode lamp (Se-HCL) designed for AFS measurements. The operating current (pulsed value) of these lamps was 100 mA. The argon-shielded gas flow and the carrier gas flow were 800 and 500 ml.min<sup>-1</sup>, respectively. The atomization process occurred in the Ar-H<sub>2</sub> flame at 200°C. The signals were measured for determination of selenium in plants and extracts and processed in the peak area mode with the use of an IBM 586 computer (Beijing Haiguang Instrument Co., China).

Analytical Procedure: A portion of 0.3 ml or 0.2 mg of sample with 7 ml of concentrated HNO<sub>3</sub> was digested at a maximum temperature of 200°C. The digestion condition for the microwave system was applied as 16 min for 960 W, and 8 min for 1080 W. After digestion, the sample solution was cooled in the air to 25°C and then blown under nitrogen flow for 10 min. At the end, a sample was transferred into a 25-ml volumetric flask, dosed by 12.5 ml of 6 mol.l<sup>-1</sup> HCl, and diluted to the mark with water.

### Evaluation of influence on amaranth sprouts on inflammation process

Influence of sprouts extracts on the inhibition of inflammatory status was evaluated in *in vitro* model using mouse leukaemic monocyte macrophage cell line (RAW 264.7). This effect was induced by TNFα addition in the concentration 20 ng.ml<sup>-1</sup> for 24 h. Transport of NFκB to the cell nucleus was estimated.

## Results and Discussion

The highest antioxidant activity by FRAP method and TP were obtained in case of *Amaranthus cruentus* supplemented with 10 mg Se.l<sup>-1</sup>. They were equal 105.3 mmol Fe<sup>2+</sup>.kg<sup>-1</sup> DW, and 12 mcg.g<sup>-1</sup>, respectively. Judging from another results of antioxidant activity, selenium addition to the water did not influence significantly FRAP values of *A. caudatus* and also *A. tricolor*. Evaluation of selenium content in amaranth sprouts apparently indicated that addition of this trace element to the water increased selenium concentration significantly. *Amaranthus* sprouts rinsed with standard water had Se in the range 0.33 – 1.36 mcg.g<sup>-1</sup>, while selenium concentration in amaranth sprouts rinsed with water containing 10 mg Se.l<sup>-1</sup> or 15 mg Se.l<sup>-1</sup> were in the range 35-67 mcg.g<sup>-1</sup>, and 45-82 mcg.g<sup>-1</sup>, respectively.

## Conclusions

Presented results have proved that amaranth sprouts (especially *A. cruentus*) show relatively high antioxidant activity. The results of our investigation have also shown that sprouts have a significantly higher antioxidant activity than seeds, which may be implicated by the difference in the content of polyphenols and other compounds such as selenium compounds. The information about possible anti-inflammatory activity seem to be useful for nutritional recommendation of amaranth sprouts.

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## ***In vitro* cultivated explant type influence on the expression of totipotency in different varieties of *Amaranthus* sp.**

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**Key words:** *pseudo-grain, Amaranth, biomass, explant totipotency*

### **Introduction**

In the current global agricultural development, in order to protect biodiversity and to practice a more friendly environmental agriculture and due to the changes in diet patterns, the diversification of agricultural production and the consumers demands to traditional and enhanced nutritional quality products, the attention of the professionals are moving to other less known and less cultivated crops as pseudo-cereals: Amaranth, quinoa, buckwheat, wild rice, which can could become an alternative to the traditional cultivated species. [1]

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

Also, leaves, stems or flowers can be used both as feed and as a source of natural food colors [2; 7]. *Amaranthus* species are grown as ornamental plants, as pseudo-cereals and leafy vegetables.

In the current publications there are published results related to different elements of the culture technology, the obtaining of improved plant varieties, the chemical composition and to the nutritional value of the seed *Amaranthus* spp varieties, the high content in essential amino acids, the oil production and the oil composition (6-7% squalene : antioxidant / anticancer).

According to the results of the researches presented published in the year 1990 by Kauffman, C.S., and L.E. Weber [3], in this scientific area it is recommended a cultivation technology depending on the climatic conditions of the specific area.

Cultures of species □ *Amaranthus gangeticus*, *Amaranthus tricolor*, *Amaranthus viridis*, *Amaranthus palmeri*, *Amaranthus blitum* etc. are currently widespread in South America, North America, Asia and Africa in order to valorize the nutritional and therapeutic potential of their vegetal biomass. *A. cruentus* L. and *A. hypochondriacus* L. are native from Mexico and Guatemala. *A. caudatus* L. is native from the Andean regions of Ecuador, Peru, and Bolivia [6].

Our research aimed to test the proliferative capacity induced by "*in vitro*" techniques to different somatic explants taken from three genotypes of *Amaranthus* sp., which are subject to various testing and acclimatization studies of to INCD CPT-Fundulea and to the Didactic Farm of Moara Domneasca-Ilfov, Romania,[4] (*Amaranthus cruentus* "Amont" -V3, *Amaranthus hypochondriacus* "Intense Purple" -V7 și *Amaranthus ssp.* "Plenitude" -V10) and also to develop an efficient method for obtaining callus cell biomass from them.

Our experimental data show in the tested variants, the potential of the versions of *Amaranthus* somatic callus cultures to develop further "*in vitro*", the long term, under the effect of optimal phytohormone levels.

Highest values of average callus biomass growth on culture vessel was registered to variant sample "E7", in which basal culture Murashige & Skoog (1962) [5] medium was supplemented with auxine 3-indolil-acetic acid (IAA), in combination with citokinine-

kinetine (Kin) that exhibits a pronounced stimulating effect of callus development and for other species. In this variant there were developed chlorophyll calluses without rhizogenesis capacity, but some times adventitious buds developed.

## Material and Methods

The biological material used in the „*in vitro*“ culture experiments was represented by seeds of the three species of *Amaranthus* (*Amaranthus cruentus* “Amont”, *Amaranthus hypochondriacus* “Intense Purple” and *Amaranthus ssp.* “Plenitude”) germinated in controlled conditions, on a Murashige & Skoog (1962) [5] culture medium, with a composition of macro and micronutrients reduced by half, solid, pH 5.8, 3% sucrose and 0.8% Agar Noble, without the addition of phytohormones.

The culture medium prepared on said basal recipe base, was sterilized by autoclaving and after that it was distributed in aseptic conditions in a laminar flow hood (about 5 ml of culture medium/culture dish) in glass Petri dishes of 7 cm diameter, sterilized in an oven for 1 hour at 180 °C. After a gentle surface sterilization of seeds with a 10% w / v solution from a commercial bleach concentrated sodium hypochlorite (-min 5%) were made three rinses with sterile distilled water.

The *Amaranthus* sterilized seeds were placed on the surface of the solid culture medium, and incubation was carried out in crop growth chamber at  $25 \pm 2$  °C, with a photoperiod of 16/8 h and a light intensity of 3000 lux. After initiation of the cultures there were selectect only healthy seedlings, level at which there were taken these different types of explants □ hipocotil fragments, cotyledons, the entire cotyledonar node and fragments of roots that were inoculated on 3 different initial Murashige & Skoog [5] =M&S (1962) basal culture medium, supplemented with phytohormones to stimulate morphogenetic processes "*in vitro*" as follows:

- V1 = M&S + 20 g.l<sup>-1</sup> sucrose + 7 g.l<sup>-1</sup> agar + 2.0 mg.l<sup>-1</sup> NAA + 1.0 mg.l<sup>-1</sup> Kin;
- V2 = M&S +30 g.l<sup>-1</sup> sucrose + 8 g.l<sup>-1</sup> agar + 1.0 mg.l<sup>-1</sup> NAA + 0.5 mg.l<sup>-1</sup> 2,4-D + 0.5 mg.l<sup>-1</sup> Kin;
- E7 = M&S +30 g.l<sup>-1</sup> sucrose + 1.8 mg.l<sup>-1</sup> IAA + 0.022 mg.l<sup>-1</sup> Kin.

The regular transfers on fresh culture media were performed every 3 weeks, with respect to all aseptic measures to prevent accidental contamination with microorganisms.

The observations related to the effect of phytohormones on the evolution of *Amaranthus* sp explants cultured "*in vitro*" after 3 weeks from the inoculation, led to conclude that the experimented types of phytohormones, from the auxines category,  $\alpha$  -naphthalene-acetic acid (NAA) and 3-indolil-acetic acid (IAA), stimulated in varying proportions, the explants hipertrophy and the cytokines of benzil 6-amino purine (BAP) and kinetine (Kin) type caused the elongation of up to 5-10 cm of preformed main apical shoot or axillary buds of explants, with a start of development of multiple shoots in the inoculated cotyledonar node explants.

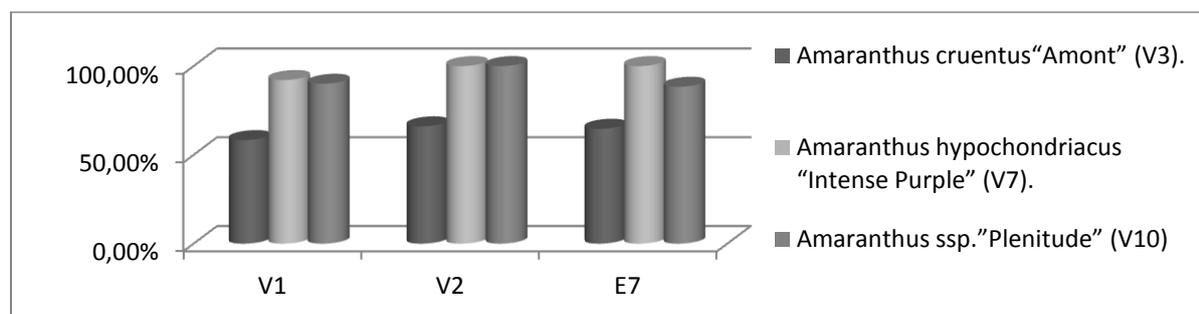
First developed on the edges cut the explant, callus covered gradually over periodic transfers, the whole mass of the original tissue hipocotil fragment type and root fragments. The results of average increases for callus biomass / Petri dish and also the evaluation of the morphogenetic processes (hypertrophy, appearance of adventitious roots) were analysed at the time of performance of the periodic transfer at every 3 weeks, depending on the inoculated explant type and also on the genotype of the explant used for the inoculation from the three *Amaranthus* sp.selected genotypes.

## Results and Discussion

- By the present research initiated for the three varieties. (*Amaranthus cruentus* “Amont”, *Amaranthus hypochondriacus* “Intense Purple” and *Amaranthus ssp.* “Plenitude”) we monitorise the comparative effect of experimental variants of used recipes of phytohormones

in connection with the analysed genotype of *Amaranthus* sp. and the results are presented in **Figure 1**.

- During periodic transfers at intervals of 3 weeks for 3 months on nutritional formulas V1, V2 and E7, in the presence of moderate concentrations of auxine and cytokinine, it was obtained a very good multiplication rate of 100% in V2 and E7 variants (**Figure 1**), there were developed even multiple shoots from each apex, and a weak version **V1**= M&S (1962)+20 g/l sucrose+7 g/l agar+2.0 mg/l NAA+1.0 mg/l Kin, at which the phytohormonal supplement constitutes in kinetin and naphthyl acetic acid, respectively.



**Figure 1** Morphogenetic response of explants of *Amaranthus* sp. due to the phytohormones recipes (V1, V2 and E7) used to initiate the experiments.

- The used transfer media were represented both by the variants of initiation (V1, V2 and E7) and also by the V3 variants (2 mg /l NAA + 1 mg /l Kin) and **V4** (1 mg/ l NAA + -0,5 mg/ l 2,4 D + 0,5 mg/ L Kin)), each supplemented by the addition of 200 mg/l casein hydrolyzate in basal medium Murashige & Skoog (1962) [5]. This ingredient is stimulating organic complex for caulogenesis through the contribution of amino acids, vitamins and growth regulators to the composition of the cultivation medium.

- By transferring the "*in vitro*" callus cultures on the V3 and V4 transfer variants, the callus biomass increases at every 3 weeks were assessed by the number of explants / culture dish and there were on average similar values for the three used *Amaranth* sp. genotypes. (**Figure 2**.)

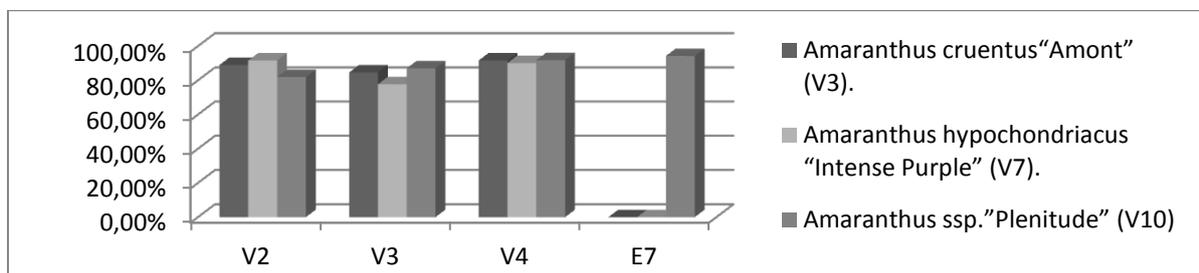
- Based on these results, we conclude that after the induction and the establishment of the "*in vitro*" callus cultures on V1 and V2 variants of the cultivation medium, the E7 variant, with a phytohormonal content consisting in the addition of 1.8 mg/l IAA + 0.022 mg/l Kin stimulated explants callusogenesis at a 94.28% rate, based on the inoculated explants, so it was demonstrated to be most effective in order to achieve a significant increase of the callus biomass / Petri dish, in a relatively short period of time (3 months). (**Figure 3**.)

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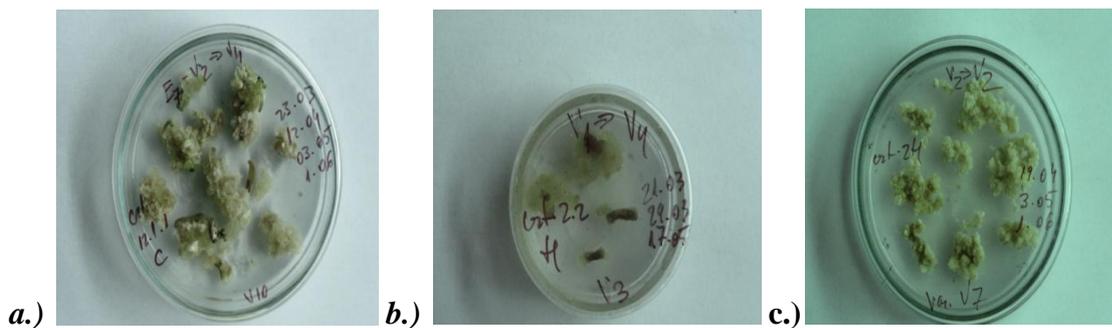
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**Figure 2** Morphogenetic response of the former plant of *Amaranthus* sp., due to the recipes of phytohormones (V2, V3, V4 and E7) used to transfer cultures



**Figure 3.** Influence of phytohormones recipes used to the transfer of the crops (V2, V3, V4 and E7) in relation to the analysed genotype of *Amaranthus* sp.: **a.)** *Amaranthus cruentus* "Amont" - V3, **b.)** *Amaranthus hypochondriacus* "Intense Purple"-V7 and **c.)** *Amaranthus* ssp. "Plenitude"- V10)



# Field evaluation of genetic resources of minor crops in the Czech gene bank

Dagmar JANOVSKÁ & Anna PROHASKOVÁ

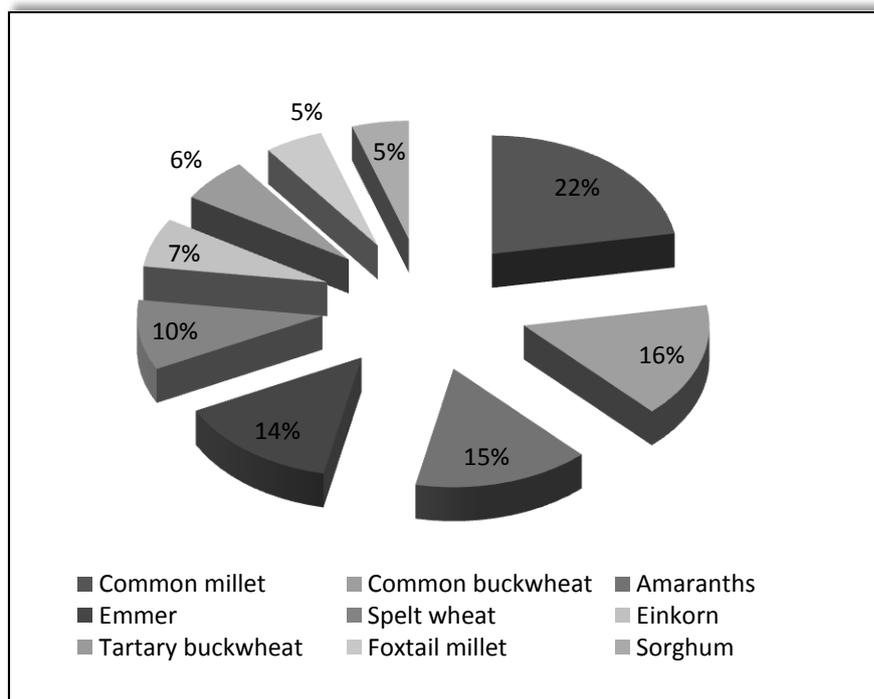
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**Key words:** minor crops, genetic resources, evaluation, gene bank

## Introduction

Genetic diversity of plant genetic resources (PGR) for food and agriculture is the unique and irreplaceable source for further genetic improvement of crops and for increasing the diversity of crops and cultivars in agriculture (Dotlačil *et al.*, 2003). According to FAO (1993) PGR are a reservoir of genetic adaptability, which acts as a buffer against potentially harmful environmental and economic changes. Their conservation and utilization work as a safeguard against an unpredictable future. The main technique of PGR conservation is their storing in the gene bank. There are store about 90% of the total accessions held *ex situ* (FAO, 1997). Characterization and evaluation of PGR are provided mainly through the international descriptors. Essential information such as morphological and basic agronomic traits are obtained (Perry & Ayad, 1995).

In the Czech Gene Bank are stored 39 921 accessions of cereals, forage crops, vegetables, medicinal and spice crops etc. The structure of minor crops is shown in Fig.1. This paper shows results of PGR evaluation of amaranths, common and tartary buckwheat and proso millet.



**Figure 1 Structure of minor crops in the Czech Gene Bank**

## Material and Methods

All samples were sown in experimental fields of CRI. The seeds were sown by hand at the beginning of May in double-line 1.5 m long with 0.25 m distance between rows; the row distance was chosen according to the descriptors of each species. Ten reference plants were selected for evaluation. The evaluated traits were specific for each species and were chosen to be informative on the possible utilisation of genetic resources.

## Results

Quantitative data from evaluation of main traits of each species are shown in tables 1, 2 and 3.

**Table 1 Quantitative data of Amaranth evaluations**

|                                      |         | Heading (days) | Flowering (days) | Maturity (days) | HTS (g) | Inflorescence length (cm) | Plant height (cm) | Crude proteins (% d.m.) |
|--------------------------------------|---------|----------------|------------------|-----------------|---------|---------------------------|-------------------|-------------------------|
| <i>Amaranthus</i> (hybrid sp.)       | Min     | 35             | 62               | 97              | 0.66    | 36                        | 96                | 13.75                   |
|                                      | Max     | 48             | 67               | 123             | 0.77    | 49                        | 131               | 16.62                   |
|                                      | Average | 43.80          | 64.50            | 116.00          | 0.73    | 43.33                     | 114.20            | 15.68                   |
|                                      | SD      | 4.58           | 1.80             | 9.59            | 0.04    | 5.44                      | 11.86             | 0.95                    |
| <i>Amaranthus caudatus</i> L.        | Min     | 45             | 63               | 113             | 0.59    | 34                        | 77                | 16.69                   |
|                                      | Max     | 49             | 67               | 118             | 0.89    | 51                        | 159               | 17.19                   |
|                                      | Average | 46.20          | 65.75            | 116.25          | 0.73    | 40.00                     | 112.20            | 16.94                   |
|                                      | SD      | 1.47           | 1.64             | 2.05            | 0.11    | 6.75                      | 28.02             | 0.25                    |
| <i>Amaranthus cruentus</i> L.        | Min     | 43             | 56               | 95              | 0.64    | 34                        | 83                | 16.50                   |
|                                      | Max     | 60             | 74               | 116             | 0.94    | 60                        | 151               | 17.61                   |
|                                      | Average | 48.25          | 64.50            | 104.38          | 0.81    | 41.38                     | 118.00            | 17.06                   |
|                                      | SD      | 4.79           | 5.04             | 7.85            | 0.08    | 6.92                      | 15.74             | 0.52                    |
| <i>Amaranthus hypochondriacus</i> L. | Min     | 35             | 54               | 99              | 0.71    | 29                        | 92                | 16.48                   |
|                                      | Max     | 57             | 83               | 128             | 0.84    | 54                        | 159               | 17.81                   |
|                                      | Average | 47.11          | 70.00            | 112.36          | 0.75    | 40.70                     | 126.31            | 16.97                   |
|                                      | SD      | 7.40           | 8.70             | 8.77            | 0.05    | 8.68                      | 16.18             | 0.50                    |
| <i>Amaranthus</i> L. sp.             | Min     | 28             | 40               | 101             | 0.59    | 30                        | 89                | 15.30                   |
|                                      | Max     | 55             | 69               | 130             | 0.80    | 57                        | 154               | 17.31                   |
|                                      | Average | 44.13          | 58.67            | 113.57          | 0.68    | 43.19                     | 116.59            | 16.23                   |
|                                      | SD      | 6.64           | 8.15             | 9.05            | 0.07    | 7.07                      | 17.02             | 0.57                    |

**Table 2 Quantitative data of buckwheat evaluations**

|                                    |         | Flowering (days) | Maturity (days) | WTA (g) | Plant height (cm) | Crude protein (%) | Rutin content (% d.m.) |         |
|------------------------------------|---------|------------------|-----------------|---------|-------------------|-------------------|------------------------|---------|
|                                    |         |                  |                 |         |                   | Aerial part       |                        | Achenes |
| <i>Fagopyrum esculentum</i> Moench | Min     | 23               | 95              | 17.8    | 68                | 12.84             | 2.15                   | 0.018   |
|                                    | Max     | 41               | 137             | 36.3    | 112               | 14.54             | 3.35                   | 0.026   |
|                                    | Average | 29.90            | 122.60          | 26.00   | 90.80             | 13.64             | 2.80                   | 0.021   |
|                                    | SD      | 3.20             | 6.50            | 3.60    | 9.50              | 0.47              | 0.29                   | 0.002   |
| <i>Fagopyrum tataricum</i> Gaertn. | Min     | 42               | 102             | 8.1     | 67                | 9.55              | 2.85                   | 0.293   |
|                                    | Max     | 54               | 112             | 17.6    | 111               | 11.10             | 4.17                   | 0.413   |
|                                    | Average | 47.19            | 106.63          | 13.19   | 89.13             | 10.30             | 3.44                   | 0.361   |
|                                    | SD      | 4.20             | 2.89            | 2.51    | 11.06             | 0.404             | 0.53                   | 0.044   |

**Table 3 Quantitative data of proso millet evaluations**

|                             |         | Heading (days) | Maturity (days) | WTS (g) | Panicle length (cm) | Plant height (cm) | Crude proteins (% d.m.) |
|-----------------------------|---------|----------------|-----------------|---------|---------------------|-------------------|-------------------------|
| <i>Panicum miliaceum</i> L. | Min     | 33             | 71              | 4.07    | 15                  | 58                | 11.64                   |
|                             | Max     | 79             | 123             | 7.15    | 38                  | 131               | 16.14                   |
|                             | Average | 50.86          | 94.15           | 5.56    | 27.13               | 98.26             | 13.38                   |
|                             | SD      | 8.23           | 11.05           | 0.66    | 4.46                | 14.63             | 1.367                   |

#### *Qualitative data of Amaranths*

Seedlings colour, inflorescence colour and seed colour were treated as qualitative traits.

The colour structure was different according to species. *Amaranthus* (hybrid) had 57% of dark green seedlings, 50% dark amaranthine inflorescence and 100% creamy seeds. *Amaranthus caudatus* had 30% light green seedlings, 30% light amaranthine and 30% dark amaranthine inflorescences and 40% of pink seeds. *Amaranthus cruentus* showed 32% purple green seedlings, 30% dark amaranthine inflorescences and 47% creamy seeds. *Amaranthus hypochondriacus* showed 36% dark green seedlings, 48% of light green inflorescences and 61% creamy seeds. In case of amaranths, which are still undetermined into species, there were 36% of light green seedlings, 38% dark amaranthine and 45% of creamy seeds.

#### *Qualitative data of common buckwheat*

No conclusions could be drawn about tartary buckwheat accessions from colour, although most of genotypes were uniform in colour across accessions.

In case of common buckwheat, stem colour, leaf colour, flower colour and seed colour and shape were evaluated. From the evaluations were obtained following results: 80% of accessions had green red stem colour, 99% green leaf colour, 87% white flower colour and 44% brown seed colour. The shape was predominately (57%) ovate.

#### *Qualitative data of proso millets*

Stem colour, leaf colour, seed colour and seed shape were evaluated in case of proso millet. Predominant colours were following: 97% green stem colour, 85% grey green leaf colour, 39% light brown seed colour. The predominant seed shape was globular (49%).

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## **The evaluation of morphological traits of genetic resources of amaranth (*Amaranthus* L.) and quinoa (*Chenopodium quinoa* Willd.)**

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The aim of this work was to evaluate and compare of phenological, morphological and agronomic characteristics selected varieties of amaranth (*Amaranthus* L.) and quinoa (*Chenopodium quinoa* Willd.) by the international descriptors list. Another important objective this study was to monitor the incidence of diseases and pests on quinoa and the cultural amaranth species. The field experiments with varieties of amaranth and quinoa were established in the year 2007 - 2009 in the Research Centre of Plant Production in Piešťany. Based on the results of the varieties evaluation in the experimental years are suitable for planting in the seed production and use a special plant production variety of amaranth 1008, Burgundy, Golden Giant, Olpir, Koniz, varieties and genotypes of quinoa are Baer, Faro and NSL 106401, NSL 106400. In terms of food plant species are both useful mainly for the production of biscuits. High types are suitable for the production of biomass and biogas amaranth Annapurna and quinoa genotype NSL 106400 and Baer. From the monitoring of biotic factors was not detected significant pest in condition of corn production area. The result of this study was based on all the ratings to choose appropriate varieties of amaranth and quinoa for special use in crop production.

**Key word:** *amaranth, quinoa, evaluation, morphological traits, rheological traits, diseases, pest*

## Biogas production from amaranth biomass

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The aim of this work was to verify the possibility of large-scale cultivation of energetic varieties of amaranth (*Amaranthus spp.*) and its use as a renewable energy source for biogas plants. The possibility of biogas production using anaerobic co-fermentation of manure and amaranth silage was verified in the experimental horizontal fermentor of 5m<sup>3</sup> volume, working at mesophilic conditions of 38 - 40° C. The goal of the work was also to identify optimum conditions for growth, harvesting and preservation of amaranth biomass, to optimize biogas production process and to examine the waste created in biogas plant as fertilizer. In 2011, the average yield of green amaranth biomass was 51.66 t.ha<sup>-1</sup> with dry matter content of 37%. Based on the reached results it can be concluded that amaranth silage solely or together with another organic materials of agricultural origin is suitable raw material for biogas production.

# **Amaranthus species - a valuable pseudocereal for organic agriculture in Romania**

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**Keywords:** *pseudocereal, Amaranthus sp., new crop, organic agriculture, productivity and yield quality.*

## **Introduction**

In the present world agriculture and feeding framework, specialists focus on the other less popular and less used crops such pseudocereals, respectively *Amaranthus* sp. which may become an alternative to traditional cultivated crops. Importance of these species results from grains chemical composition and nutrition value - high contents in proteins and essential amino acids, mineral elements, lipids - and from the fact they do not have special claims concerning growing conditions, presenting tolerance to diseases and pests, and being able to grow in some harsher climates.

On the other hand, the present situation of Romanian economy and agriculture is very favorable for the extension of the Organic Agriculture sector. The Romanian agriculturists are interested to produce such kind of marketable agriculture products and food for domestic and external market.

In this sense, our research focused to study biological and morphological characteristics of different *Amaranthus* sp. species (*A. cruentus*, *A. hypochondriacus*, *A. caudatus*), a new crop for Romanian agriculture, in order to evaluate the adaptability of these species in the organic agriculture conditions from South Romanian Plain.

## **Material and Methods**

In the Moara Domneasca Experimental Field, near Bucharest, on reddish preluvosoil, there were organized experiments for three species of *Amaranthus* genus, i.e.: *A. cruentus*, *A. hypochondricus*, *A. caudatus*, with seeds coming from the world collection.

According to the organic principles, the previous plant was a legume – the peas. Sowing was done on the 17<sup>th</sup> of April, at a depth of 1-2 cm, with a crop density of 100.000 plant/ha and a distance between rows of 50 cm.

During vegetation period it was a program of biometric measurements regarding: morphology and biology of plants; productivity elements (grains yield per plants, TGW) and grains chemical composition (moisture and dry matter, starch, proteins, lipids, fibres and ash contents). The chemical analyses were performed in the Yield Quality Laboratory of the Field Crops Department, Faculty of Agriculture, University of Agronomic Sciences and Veterinary Medicine Bucharest, with modern equipment, respectively Infrared spectrophotometer NIR Instalab 600.

## **Results and Discussion**

**Phenological observations and biometric measurements.** Related to the phenological aspects, in organic agriculture conditions, the duration of the sowing/emergence period was of 13 days at *A. caudatus* and 20 days at *A. hypochondricus*, much prolonged due to small seeds and shallow sowing.

Referred to the vegetation cycle, *A. hypochondriacus* species has reached the maturity stage on September 18, two weeks earlier than the other two species that have reached maturity on October 2. Thus, the vegetation cycle was of 135 for *A. hypochondriacus* and of 152-154 days for *A. caudatus* and for *A. cruentus*.

**Table 1** Phenological date of *Amaranthus* species in organic agriculture conditions from South Romania

| Genotypes                 | Sowing date               | Emergence date            | Sowing-emergence (days) | Harvesting date               | Vegetative cycle duration (days) |
|---------------------------|---------------------------|---------------------------|-------------------------|-------------------------------|----------------------------------|
| <i>A. cruentus</i>        | 17 <sup>th</sup> of April | 30 <sup>th</sup> of April | 13                      | 2 <sup>nd</sup> of October    | 154                              |
| <i>A. hypochondriacus</i> | 17 <sup>th</sup> of April | 7 <sup>th</sup> of May    | 19                      | 18 <sup>th</sup> of September | 135                              |
| <i>A. caudatus</i>        | 17 <sup>th</sup> of April | 3 <sup>th</sup> of May    | 15                      | 2 <sup>nd</sup> of October    | 152                              |

The highest plant belonged to *A. caudatus* with 108 cm, who had an inflorescence length of 39.6 cm and 21 leaves on main stem (table 2).

Having in view that these plants are almost unknown in Romania, an important objective in our research were some morphological determinations, respectively number, colour and dimensions of leaves, flowers and seeds.

**Table 2** Morphological date for *Amaranthus* species in organic agriculture conditions from South Romania

| Morphologic characteristics      | <i>A. cruentus</i>                            | <i>A. hypochondriacus</i>  | <i>A. caudatus</i>                |
|----------------------------------|---|--|-----------------------------------|
| Plants length (cm)               | 85.5  | 88.7   | 108                               |
| Stem color                       | Light-green                                   | Bright-green   | Red-purple                        |
| Number of leaves/main stem       | 26  | 42   | 21                                |
| Leaves color                     | Yellowish green                               | Slightly reddish green shades  | Reddish with green veins          |
| Inflorescence length (cm)        | 27.4  | 37.7   | 39.6                              |
| Color and shape of inflorescence | Golden, shining; inflorescence erect, compact | Red-purple with light green hues, or yellow, inflorescence erect, gathered | Dark red, inflorescence lax, bent |
| Seeds color                      | White-yellowish                               | White-yellowish  | Shining black                     |
| Vegetative cycle duration        | 154   | 135  | 152                               |

**Grains yields.** Concerning grains yields, table 3 shows that it ranged between 18.5 and 23.4 q.ha<sup>-1</sup>. In examining the data, it can be seen that the highest value was obtained from *A. hypochondriacus* species, 23.4 q grains/ha, provided statistical difference with the average (very significant). In comparison with average value, *A. cruentus* achieved a production increase of only 0.14 q.ha<sup>-1</sup> and the *A. caudatus* species showed the lowest production of 18.50 q.ha<sup>-1</sup>.

These data reflect an important adaptation capacity of *Amaranthus* species to the cropping condition in the area and resistance to drought and high temperatures.

**Table 3** Productivity elements of *Amaranthus* species in organic agriculture conditions from South Romania

| Genotypes                 | Grains weight per plant (g) | Difference from average | TGW (g) | Difference from average |
|---------------------------|-----------------------------|-------------------------|---------|-------------------------|
| <i>A.cruentus</i>         | 21.4                        | 0.1***                  | 1.32    | 0.1***                  |
| <i>A. hypochondriacus</i> | 23.8                        | 2.5***                  | 1.12    | -0.2 <sup>oo</sup>      |
| <i>A. caudatus</i>        | 18.7                        | -2.6 <sup>ooo</sup>     | 1.49    | 0.2***                  |
| Average                   | 21.3                        | -                       | 1.31    | -                       |
| DL 5%                     |                             | 0.09                    |         | 0.01                    |
| DL 1%                     |                             | 0.13                    |         | 0.02                    |
| DL 0.5%                   |                             | 0.19                    |         | 0.03                    |

**Table 4** Grains yields of *Amaranthus* species in organic agriculture conditions from South Romania

| Genotypes                 | Grains yields (q.ha <sup>-1</sup> ) | Relativ production (%) | Difference from average (q.ha <sup>-1</sup> ) | Significant |
|---------------------------|-------------------------------------|------------------------|---|-------------|
| <i>A.cruentus</i>         | 21.00                               | 100.14                 | 0.14  | **          |
| <i>A. hypochondriacus</i> | 23.40                               | 111.58                 | 11.50   | ***         |
| <i>A. caudatus</i>        | 18.50                               | 88.22                  | -11.78  | ooo         |
| Average                   | 20.97                               | 100                    | Control                                       | -           |
| DL 5%                     |                                     |                        | 0.07  |             |
| DL 1%                     |                                     |                        | 0.10  |             |
| DL 0.5%                   |                                     |                        | 0.19  |             |

**Chemical compositions and yield quality.** In the climatic conditions recorded in the South Romanian Plain, *A. hypochondriacus* grains accumulated the highest amount of proteins (17.83%). *A. cruentus* was close to average values for proteins (16.23%), and lower values for lipids (5.81%), fibres (2.21%) and ash (2.56%). The lowest values were for *A. caudatus* which accumulated: 14.43% proteins, 60.34% starch, 6.44% lipids, 5.65% fibres and 4.73% ash.

**Table 4** Chemical composition of *Amaranthus* species grains in organic agriculture conditions from South Romania (%)

| Genotypes                 | Proteins | Starch | Lipids | Fibres | Ash  |
|---------------------------|----------|--------|--------|--------|------|
| <i>A.cruentus</i>         | 16.23    | 60.24  | 5.81   | 2.21   | 2.56 |
| <i>A. hypochondriacus</i> | 17.83    | 62.55  | 6.49   | 4.85   | 3.93 |
| <i>A. caudatus</i>        | 14.43    | 58.24  | 6.44   | 5.65   | 4.73 |
| Average                   | 16.16    | 60.34  | 6.25   | 4.24   | 3.74 |

## Conclusions

As conclusions of our research performed during 2008-2011, in Organic Agriculture conditions of South Romanian Plain, may be emphasised as important:

1. *A. cruentus* had a growing season of 154 days, in comparison with the earliest species *A. hypochondriacus*, with 135 days of vegetation.
2. *A.hypochondriacus* species was characterized by the following morphological characteristics: 88.7 cm stems height; 37.7 cm length of inflorescence; red-purple with light green hues, or yellow and erect inflorescence. Weight of grains per plant was 23.8 g, 1.12 g TGW and grain yields of 23.4 q.ha<sup>-1</sup>.

3. By comparison, *A. caudatus* species was characterized by plants with height over 106 cm, 39.6 cm inflorescence length and inflorescence color was dark red, lax, bent; grains weight/plant was 18.7 g, 1.49 g TGW, and grains yields was of 18.50 q/ha.
4. *A. cruentus* was characterized by the following: plant height of 85.5 cm; length of inflorescence 27.4 cm; golden color, shining; inflorescence erect, compact; grains production per plant was 21.4 g, TWG 1.32 g, and the grains yields production was estimated to 21.4 q.ha<sup>-1</sup>.
5. On chemical compositions, *A. hypochondriacus* and *A. cruentus* grains accumulated the highest amount of proteins (16.23-17.83%) and *A. caudatus* fibres (5.65%) and ash (4.73%).

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## **The amaranth innovation cluster of north Hungary**

**Bálint NAGY**

*Amaranth Innovation Cluster – Honor Consulting Kft., Hungary*

Amaranth Innovation Cluster was founded in 2010 with the aim of bringing together those players of the region who are able to contribute to the utilisation of amaranth. Cluster management organisation is Agrofer Kft. with main office in Hidasnémeti, Hungary and branch office in Miskolc, Hungary. Latter office is the place of business for the cluster management.

There are 23 founding members who are part of the cluster operation from the very beginning. In the past two years the number of members increased dynamically as the cluster management put lot of emphasis on networking and searching for those businesses and other stakeholders who show interest in participating in the utilisation of amaranth.

At the moment there are more than 30 members of the cluster including agricultural firms, breeders, machine manufacturers, dealers, research institutions, consultants.

29 SMEs (13 agricultural), 1 large company and 2 non-profit organisations are full members of the company. The cluster has cooperation agreement with the University of Miskolc, College of Nyíregyháza and Plant Production Research Center Piešťany (Slovakia), Hungarian Academy of Sciences (Martonvásár), Károly Róbert College (Gyöngyös).

The establishment of the cluster is basically a bottom-up initiative supported with a two-year long public funding through national program New Széchenyi Plan. Bottom-up approach is one of the key factors to success. Under success of the past 2 years we mean:

- Better access to information,
- Knowledge transfer between actors,
- Use of synergetic effects
- Stimulation of innovations,
- Creation of new business chances.

Specific objectives of the cluster for the future are:

- stimulate international cooperation (especially cross-border cooperation with Slovakian partners) and support internationalisation (opening of export markets) of cluster member companies
- foster innovation and competitiveness through promoting R&D and cooperation projects

## Mutation breeding of amaranth (*Amaranthus cruentus* L.) - experiment results from locality Prešov

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### Abstract

Purple Amaranth (*Amaranthus cruentus* L.) belongs among the important alternative crops – pseudo-cereals suitable for global warming conditions. The cultural forms of Amaranth are characteristic by high nutritional value of the grain and green mass suitable for human nutrition and feeding. The aim of the research was induction of mutations by gamma radiation. The seeds of *Amaranthus cruentus* L. genotype ‘Ficha’ were treated with 175 Gy. After evaluation of twelve mutant generations (1998 – 2010) two putative mutation lines C26 and C82 were selected for increased weight of thousand seeds. Two selected putative mutant lines C26 and C82, control non - treated *Amaranthus cruentus* ‘Ficha’ and the variety ‘Aztek’ were tested on the experimental field of Prešov university in Prešov. Morphological characteristics of tested variants were evaluated according to test guide UPOV 247/1 on the plots 2.5 m<sup>2</sup> (one experimental variant) in four repetitions. The aim of this evaluation was to estimate differences between breeding material (putative mutant lines), the control variety ‘Aztek’ and control non-treated *Amaranthus cruentus* ‘Ficha’ and to reach homogenization of putative mutant lines in order to achieve their uniformity and trait stability. Differences were observed between putative mutant lines and variety ‘Aztek’ in 7 characteristics: leaf - central blotch, color on the lower side, blade color, inflorescences – color, density of glomerules, length of bract relative to utricle and weight of thousand seeds (WTS). Compared to the starting material ‘Ficha’ the statistically significant differences were found in WTS. The weight of thousand seeds is the most important attribute for grain yield. This attribute was influenced by irradiation and genetically fixed by selection. The WTS for putative mutation lines C82 and C26 was 0.9967 g and 0.94165 g, respectively, for control non - treated variety ‘Ficha’ 0.8821 g and for variety ‘Aztek’ 0.7647 g. Among the all experiment variants statistically significant differences on WTS (method 95 % LSD) were achieved.

**Keywords:** *Amaranthus cruentus* L., morphological characteristics, mutation breeding, weight of thousand seeds (WTS).

**Acknowledgement:** The work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, the project: 00162-0001 (MS SR-3634/2010-11) “Isolation of Natural Plant Substances by Lyophilisation and Change of their Qualitative-Quantitative Properties”, by CRP IAEA Vienna, Austria and by VEGA project no. 2/0109/09.

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# Characterization of chemical composition, protein composition, rheological and technological properties of sweet sorghum flour

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**Key words:** *sorghum, protein, kafirin, rheology, composition*

## Introduction

As a key trend of the most recent years, heat and electric energy is increasingly produced from renewable resources and the proportion of so-called bio-fuels in the use of fuel for transport is constantly growing. The use of renewable base materials in the chemical industry is less significant but rapidly increasing. One of the possible sources of biofuel base materials is sweet sorghum. This high sugar containing plant grows extremely fast and so produces high amount of biomass. The fundamental objective of our project is to develop a model system for the production, processing and utilization of sweet sorghum which can provide optimum income for agricultural and industrial entrepreneurs and, at the same time, ensure the sustainability of production. A part of this project was the investigation of the possible food industrial utilization of sweet sorghum seeds.

Sorghum is a pseudo cereal that is possibly applicable in the bakery industry as functional food base material. As it does not contain gluten, it can also be built in the diet of consumers suffering from coeliac disease. Furthermore, the main storage protein of sorghum (kafirin) is likely to be suitable for preparing biofilms and packaging material. In this respect several studies have been done with different agricultural products. Zein, the main storage protein of maize seemed to be applicable for this purpose because of its high hydrophobicity. As kafirin is more hydrophobic than zein and triggers no allergenic reactions, sorghum could be a better base material in biofilm and packaging material production than maize.

## Material and Methods

In the present study compositional, rheological and end-product examinations were done on different Hungarian sorghum varieties for reveal the potential of sorghum seed flour as non-allergenic, functional food base material. In compositional aspect crude protein, crude fibre, ash and crude fat content were examined. Farinograph-type z-arm mixer and Rapid Visco Analyzer were used for examining rheological properties of sorghum flour. As end-product examinations sorghum containing bread and pasta products were prepared and analyzed. For rheological and end-product tests different wheat-sorghum blends were prepared and tested as well. Protein isolation was prepared using sodium hydroxide, sodium metabisulfite and ethanol. Based on the literature two different extraction methods were tested. Protein characterization was carried out with Lab-on-a-chip, SDS-PAGE and RP-HPLC methods.

## Results and Discussion

Considerable differences were revealed among the sorghum cultivars in terms of major chemical composition. Protein content was found between 7.71 % and 11.62 %; crude fat content between 2.36 % – 3.13 %; crude fiber content between 4.15 % – 4.69 %.

It was revealed that sorghum flour does not show any resistance against the mixing action of z-arm mixer. This occurs probably due to the high hydrophobicity of the kafirin proteins, which causes weak hydration and hinders dough-forming. Experiments with wheat-sorghum flour blends revealed the exact effect of sorghum addition on the dough-forming behavior of the flour. Longer dough development time, increased breakdown were observed at the investigated sorghum flours. Considerable varietal differences were observed at pasting properties and non-linear behaviour was observed at increasing sorghum flour addition. At Rapid Visco Analyzer significantly lower pasting time, peak viscosity and breakdown, considerably higher final viscosity and setback were observed compared to wheat flour.

It was shown that addition of sorghum flour affects significantly the end-product quality of wheat flour-based products. Sorghum flour addition resulted in decreased loaf volume at bread and weaker consistency at noodles.

Optimization of protein isolation procedure was accomplished. All of the applied methods were applicable for characterization of kafirin proteins. Using RP-HPLC soluble and insoluble protein fraction were examined as well (Figure 1). Based on literary sources different kafirin subunits were identified using the SDS-PAGE and LOC results.  $\alpha$ - and  $\gamma$ -kafirin subunits were found at 26000-30000 Da,  $\beta$ - kafirins at 18000-21000 Da (Figure 2). Trimers were identified at 62000-66000 Da. In some cases dimers were also found in low concentration. Apart of these protein subunits, others were found at lower molecular weight interval (14500 - 18000 Da). In terms of protein profile only quantitative differences were revealed among the investigated varieties (Figure 2).

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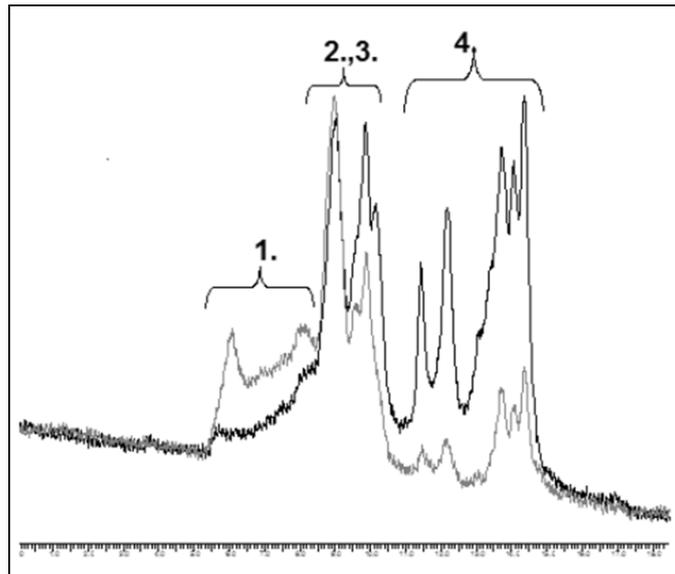


Figure 1. RP-HPLC chromatogram of soluble (black) and insoluble (grey) protein fraction of sorghum flour; 1. – high molecular weight – 2, 3 medium MW, 4 –low MW proteins

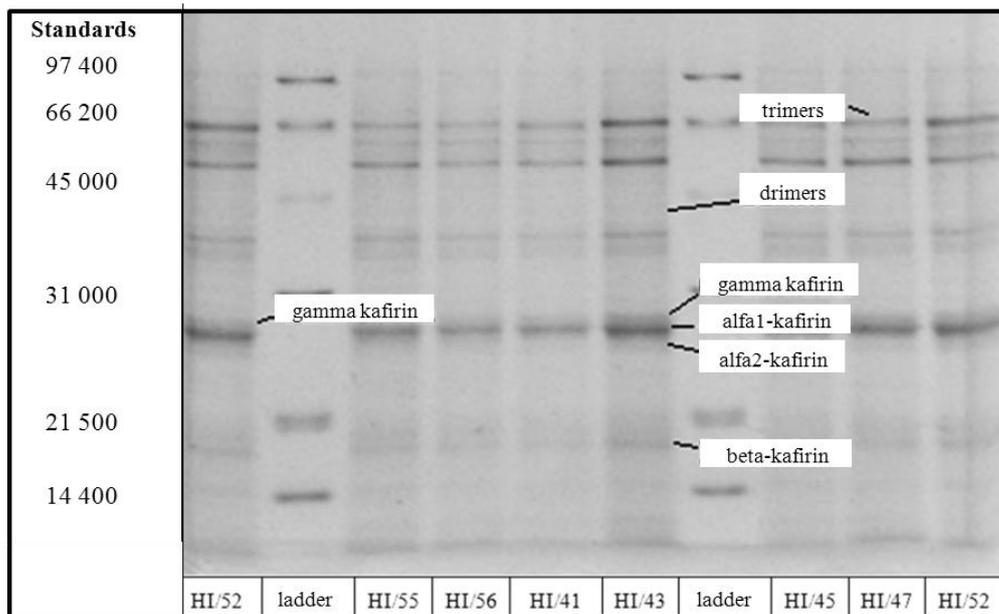


Figure 2 SDS-PAGE gel picture of proteins of 8 Hungarian sorghum cultivars

## **Experimental results on some alternative crops for Romanian agriculture**

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Climate changes and degradation of natural resources led to increased interest in plant species that are adapted to climate and soil conditions more difficult.

In many cases, these "alternative crops" called "neglected" or "under utilised" are the only ones that can survive harsh climatic conditions, unsuitable for other crops that can assure good yields. In this way, they would contribute significantly to biodiversity and to achieve more stable agro-ecosystems.

The main objective of the research was to study the biology, ecology and productivity of some alternative crops and their adaptability to soil and climatic conditions of the agricultural area in Southern Romania and to cultivation in the organic farming system.

The research was set up three field experiments, respectively: an experiment of 7 lentil genotypes; an experiment of five species of grain legumes (6 genotypes); an experiment with four species of oil crops (5 genotypes). Seeds came from organic crops from Romania and other European countries, and growing technology were in conformity for organic farming system.

Research carried out in 2009-2011 have shown the adaptability of species of legumes (lentils, field beans, chick peas, blackeyed peas, adzuki beans, fenugreek) and oil crops (safflower, pumpkin, flax oil and camelina) in terms of agricultural area in Southern Romania and possibility of these crops for cultivation in organic farming system, in order to diversify the range of crops and achieve correct rotation in which legumes are particularly important as ameliorative crops. However, the introduction and expansion in culture of these species may contribute to the diversification of food and animal feeding.

*Key words: alternative crops, organic agriculture, South Romania*

# Compositional, rheological and technological characterisation of hungarian millet and sorghum flour

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**Key words:** *sorghum, millet, composition, rheology, technology*

## Introduction

Although millet and sorghum production together come to less than 10 % of the total cereal production of the world, in certain parts of the globe they are among the most staple foods. The spread of the food-purpose production of these cereals are very uneven. In Africa these resistant crops are the primary protein and energy source of millions of people, while in developed countries they are cultivated mostly for animal feed. Nevertheless, in these countries pseudocereals gets increasing attention in the value-added food production because they are good sources of beneficial micro-nutrients like minerals, vitamins and antioxidants. Furthermore, they do not contain gluten, and so can be built in a gluten-free diet. As these grains are rarely studied and neglected in the food industry compared to conventional grains, it is expedient to reveal the nutritional and technological potential of them. The aim of the present study is examining neglected Hungarian millet and sorghum varieties in compositional, rheological and technological viewpoint.

## Material and methods

Whole grain and decorticated Hungarian millet and sorghum varieties and commercially available flours were investigated. To explore the nutritional values of millet and sorghum protein, ash, crude fat, crude fibre, dietary fibre content were determined. Protein composition was examined by SDS-PAGE, Lab-on-a-chip and HPLC techniques.

Methodology used for determination of functional characteristics of wheat was applied for millet and sorghum milled products. Experiments were carried out with Rapid Visco Analyser (RVA) and farinograph-type micro z-arm mixer. To reveal the composition of starch amylose/amylopectin ratio was determined.

To discover the technological properties of millet and sorghum flour end-product tests were carried out; bread and pasta products were prepared and characterized. For the rheological and technological measurements wheat-millet and wheat-sorghum blends were also brought under examination.

## Results and discussion

Compositional characteristics of millet and sorghum samples were determined. It was shown that both cereals possess considerably different values than commonly used cereals, e.g. wheat. It was also presented, that decortication process has remarkable effect on the compositional values. The extent and the direction of these changes are highly variant-dependent.

Protein characterization of millet and sorghum varieties is possible with the applied methods. Neither at millet nor at sorghum samples could varietal qualitative differences be found (Figure 1). SE-HPLC method showed quantitative differences among cultivars.

Dough forming capability of the examined samples was significantly different from that of wheat. Pure sorghum flour and some of the examined millet flours did not show any resistance against the stirring action of the farinograph-type instrument, in this way no curve

could be registered. In the case of certain samples unique farinographic behaviour was observed, of that the explanation requires a new approach.

Pasting characteristics of millet and sorghum flours differed significantly from that of wheat (Figure 2). Studying RVA curves of blend samples non-linear behaviour was observed.

End-product test of pseudocereal flours showed that 100% flour is not applicable for preparing standard bread (ICC 131) or pasta samples. Addition of even 30 % of millet and sorghum flour changed the end-product quality significantly (Figure 3).

**Acknowledgment:** *This work is connected to the scientific program of the "Development of integrated agriculture production storage, processing and logistic system for sweet sorghum" project (TECH\_08\_A/2-2008-0401).*

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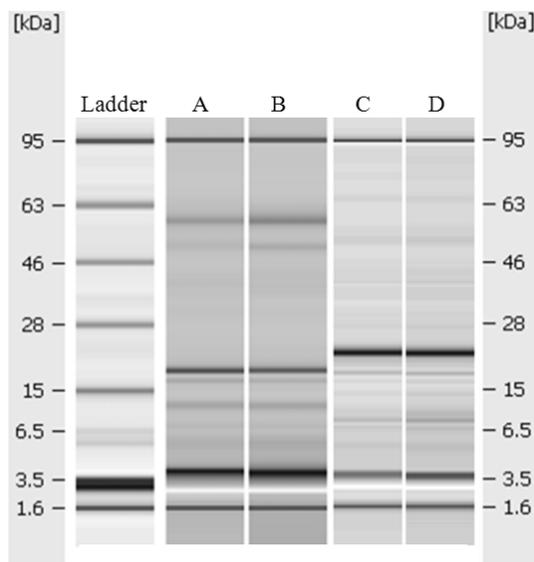


Figure 1. Lab-on-a-chip protein profile of two Hungarian millet (A, B) and two Hungarian sorghum C, D varieties

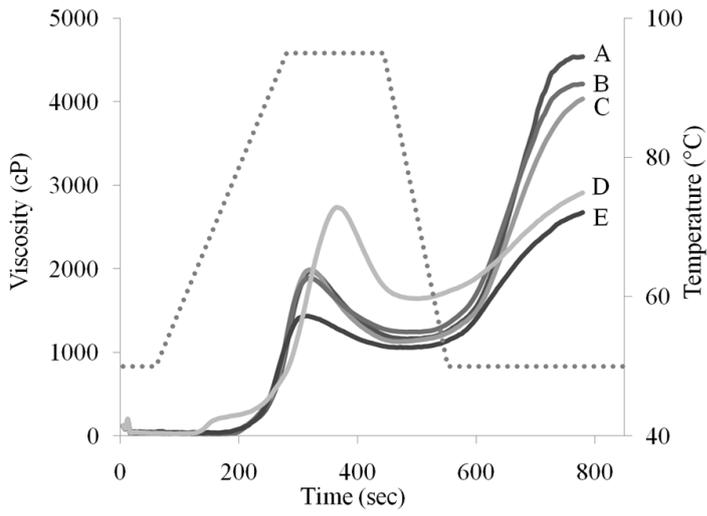


Figure 2. RVA curves of four millet flours (A, B, C, E) compared to the curve of normal wheat flour

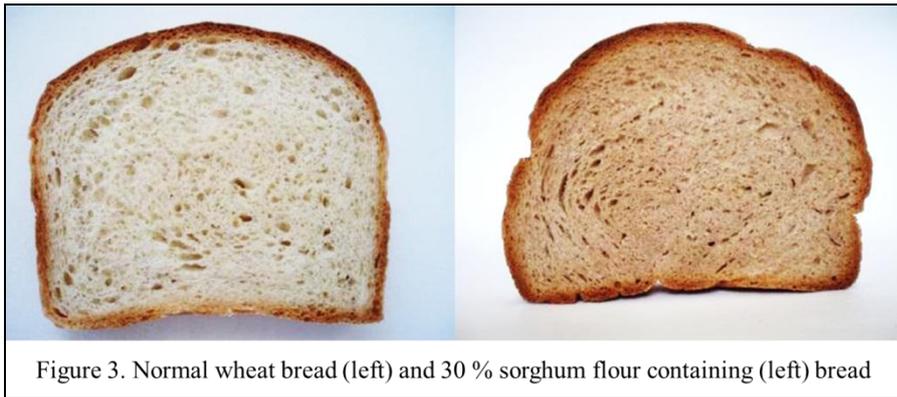


Figure 3. Normal wheat bread (left) and 30 % sorghum flour containing (left) bread

# Use of speciality and underutilised grain species and pseudocereals for gluten-free food production

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**Keywords:** *underutilised and specialty cereals, pseudocereals, gluten-free food, processing*

In all regions world-wide, cereals and cereal based foods are important staple foods and they are an important contribution to human nutrition and health. Cereals can and do contribute a significant amount of healthy food ingredients in the diet. Biological active constituents of cereals that promote beneficial physiological effects are especially dietary fibre, certain vitamins like folic acid and secondary plant metabolites like polyphenols.

In recent years the variety of used cereal species has become smaller and smaller. Worldwide, more than 86% of the total cereal production is maize (31.3%), wheat (27.7%) and rice (27%). Within Europe (EU-25) the production of wheat is even more dominant (47.2 %); production of barley is 21.5%, maize 18.6%, rice only 0.9% (FAOSTAT, 2010). These data show that the use of speciality and underutilised cereals, and pseudocereals is lower than 14%. For nutritional and agricultural reasons this low biodiversity is not very positive.

The minor produced cereal group comprises a large number of (true) cereal species like rye, oat, triticale, millet, sorghum and others, but also pseudocereals like amaranth, quinoa and buckwheat. Botanically, pseudocereals are assigned to the *Dicotyledonae* (unlike cereals, which are *Monocotyledonae*), but they all produce starch-rich seeds that can be used like cereals. All three pseudocereals show a valuable nutritional composition and interesting functional properties. For amaranth and quinoa the excellent protein composition with a high amount of essential amino acids and high physiological value has to be pointed out. As the starch granules of both plants are among the smallest known and the amylose content is very low, they show unique physicochemical properties. In contrast, buckwheat starch is high in amylose. Fatty acid pattern is good for all three pseudocereals, and mineral content is high.

It should also not be overseen, that among all cereals species (major cereals as well as underutilised ones), there are a lot of speciality varieties that offer certain functional qualities or health benefits. Examples are coloured species like purple or blue wheat, black, yellow or blue barley or black and red rice varieties, ancient species like emmer, einkorn wheat and spelt wheat.

Specialty or underutilised cereals like the pseudocereals show a different nutritional composition. These differences are found in the composition of secondary plant metabolites, dietary fibre, phenolics, amino acids, vitamins and minerals. Sometimes, their level of these functional components is higher. One example for this is the content of the vitamin folate. A comparison of total folate in pseudocereal and cereal species showed, that amaranth and quinoa possessed the highest total folate contents, which was about 5-10 times higher than in wheat species. In speciality wheat species like emmer and einkorn wheat it was about twice as much as in typical bread wheat.

Another interesting feature of these specialty cereals and pseudocereals is that many of them contain a different protein composition and often low amounts of prolamins, which are non-toxic to coeliac disease patients. Coeliac disease is a gastro-enteral disease and is triggered by the proteins of wheat, barley, rye and sometimes oat. The only treatment is a

strict lifelong adherence to a gluten-free diet. Additionally to coeliac disease an increasing number of persons is affected by gluten-sensitivity, and also these persons have to avoid gluten within their diet. Cereals which contain no toxic prolamins and thus are considered to be gluten-free are the species millet, sorghum, maize, rice, eventually oat and also the pseudocereals amaranth, quinoa and buckwheat.

Most gluten-free foods found on the market today are produced from refined rice or maize flour and are of low nutritional quality, in particular in terms of dietary fibre, vitamins and minerals. The use of specialty cereals or pseudocereals would enhance the quality of gluten-free foods, one, due to their higher nutritional composition and second, due to the fact that these small cereal grains are mainly used as wholemeal flour.

The working groups of the Institute of Food Technology, Department of Food Sciences and Technology, University of Natural Resources and Life Sciences, Vienna, Austria and of the Department of Applied Biochemistry and Food Science, Budapest University of Technology and Economics, Hungary have performed many studies on chemical composition, physico-chemical, rheological and processing behaviour of a large number of speciality and underutilised cereals and pseudocereals. Gluten-free food processes and products investigated included extrusion cooking, drum drying, bread baking, pasta process, cookie production, popping of amaranth, cereal based drinks, distillation, oil separation and others. Raw materials used for these investigations were either used as single cereal component, or blended among each other. In both cases a lot of process adaptation was necessary. Examples for these mentioned studies will be presented in detail.

# Effect of conditions on the composition of amaranth phytomass

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## Abstract

In 2009 and 2010 was on to moderate the soil cultivated amaranth variety of Oscar Blanco family *Amaranthus caudatus*. We examined the effect of year on phytomass production and its composition (N, P, K, Ca, Mg, fiber, ash). In a wet year 2010 to create more phytomass (16.42 t.ha<sup>-1</sup> dry matter) with a higher composition of fabric, as in 2009 (15.84 t.ha<sup>-1</sup>). In a very strong correlation depending on the grade level was of N and P. The strong dependency of the K content and the ash. Strong indirect dependency between the fiber and ash from the elements N, Ca and Mg. In 2009, a higher potassium content in leaves and pulp in sheets and clusters. The content of other elements, fiber and ash were higher in 2010. Minimum grade of dependence conditions was observed in fiber content.

**Key words:** *Amaranthus*, yield, biomass, quality

## Introduction

The basic priorities of energy policy of the European Commission, called. Green Paper belong reducing energy demand, increase reliance on renewable energy sources, diversification of energy sources and increase international cooperation. Biomass in the form of plants is chemically conserved solar energy (Zacharda, 2009).

One of the possible crops grown for energy purposes is amaranth, which can be a substitute for conventional fuels. Amaranth (*Amaranthus*) are among the annual plants and uses of biomass for energy purposes depends largely on the physical and chemical properties of biomass.

When we think of amaranth phytomass biochemical conversion of methane fermentation (biogas) or thermochemical conversion of biomass heat (combustion). Pepich (2009) states that amaranth plants have a calorific value of straw 15.14 MJ.kg<sup>-1</sup>.

The aim of this work was to investigate the effects of year on of production capacity and the composition of amaranth phytomass.

## Materials and Methods

Experiment with the cultivation of amaranth was based on location, Barack (48 ° 20 'N., 18 ° 14' E) in 2009 and 2010. The site is located in a warm climate area of 180 m n. m. The land is moderate to slightly acidic reaction (pH 5.7), with a good stock of phosphorus (87 mg.kg<sup>-1</sup>), a good supply of potassium (265 mg.kg<sup>-1</sup>), very high in magnesium (350 mg.kg<sup>-1</sup>), humus content is 4.4%. A variety of amaranth: Oscar Blanco family from *Amaranthus caudatus*. Date of sowing: 5 5th 2009, 28 5th The 2010<sup>th</sup> Interline distance: 0.50 m. Quantities of the seeds: 200 000 seeds per hectare. Sampling for plant analysis took place on 9 9th 2009 and 12 10th The 2010<sup>th</sup>

## Results and Discussion

Conditions Temperature and consumptive water in the production years of 2009 and 2010 were very different. In 2009, the month of May precipitation formed only 65% of long-term normal, and in 2010 to 269% of normal (58 mm). In the month of July in which rainfall reached normal values, June, August and September were very wet. In July 2009 was normal, wet July and August and September is very dry. As the Peksová Kalinová (2011), amaranth is highly plastic species convenient tolerant to different extreme conditions of climate, weather

and soil conditions. This is also confirmed in the results of our experiment. In 2009 the dry phytomass yield  $15.84 \text{ t}\cdot\text{ha}^{-1}$  and in 2010 reached only by  $0.58 \text{ t}\cdot\text{ha}^{-1}$  more ( $16.42 \text{ t}\cdot\text{ha}^{-1}$ ). In both years, amaranth grown on soils with sufficient quality.

Yield formation is also affected by other factors. Yarn et. al. (2010) found effects of late sowing on phytomass production ranged from 39.34% to 79.91%, which is related to length of growing season amaranth. In wetter in 2010 to create more of amaranth phytomass. Pospíšil *et al.* (2006) found that the reaction year conditions also depends on the growing crop. Gimplinger *et al.* (2007) examined the density of vegetation and found that biomass production depend on the density of vegetation.

Before harvesting the crop, we sampled plants that were used on analysis we set the content of substances in phytomass. Green biomass is possible to use biogas as a dry solid fuel (Pekárová, 2010). Biomass, in addition to basic substances (carbon, hydrogen, oxygen) contains the elements that have a significant impact on the production of harmful substances in burning it. These include sulfur, chlorine and nitrogen. The elements that may impact on the environment. Potassium, sodium, calcium and magnesium are among the substances that indirectly affect the combustion process, the formation of harmful substances and the like.

Nutrient content in plants was studied in stems, leaves and clusters. The total biomass of the contents of all substances higher in 2010 (Table 1). Most affected by the nitrogen content expressed a correlation coefficient ( $r = 0.82 + +$ ) and phosphorus ( $r = 0.67 + +$ ). The difference in nitrogen content was  $8.34 \text{ mg}\cdot\text{kg}^{-1}$  at the plant in favor of 2010. In direct correlation depending on grade and content of the elements and ash content ( $r = + 0.4329$ ). Effect of year is at least reflected in fiber content. Its content was higher in 2010 only 0.16% compared with 2009. The strong dependence of the indirect correlation was found between fiber and mainly N, Ca and Mg (Table 2).

In some parts of the plants were significant differences of potassium content in leaves, which was higher in 2009 to  $0.91 \text{ g}\cdot\text{kg}^{-1}$  compared with 2010 ( $49.22 \text{ g}\cdot\text{kg}^{-1}$ ). More magnesium ( $6.17 \text{ g}\cdot\text{kg}^{-1}$ ) in 2009 in clusters than in 2010 ( $6.04 \text{ g}\cdot\text{kg}^{-1}$ ). Variability (2007) states that magnesium adversely affects the environment and life of plants.

Higher fiber content was in 2009 in the leaves. The difference in the letters is  $2.05 \text{ g}\cdot\text{kg}^{-1}$  clusters and  $5.20 \text{ g}\cdot\text{kg}^{-1}$  compared with year 2010.

## Conclusion

Oscar Blanco variety of amaranth species of *Amaranthus caudatus*, grown in 2009 and 2010 in warm climate areas, responding to conditions years. In a wet year 2010 was create more phytomass, as in 2009. Conditions years significantly influenced mainly nitrogen and phosphorus. Among the vintage and potassium content, and ash was a direct correlation dependence. The strongest correlation between the elements was found in relation to nitrogen. The strong indirect correlation depending on the fiber element. In some parts of the plants were significant differences between the grades of potassium content in leaves and pulp in sheets and clusters in favor of 2009.

**Table 1** The average content of substances in plants amaranth varieties Oscar Blanco in 2009 and 2010 at the 100% dry matter

| Parts of plants   | Year | N                  | P    | K     | Ca    | Mg    | Pulp  | Ash   |
|-------------------|------|--------------------|------|-------|-------|-------|-------|-------|
|                   |      | g.kg <sup>-1</sup> |      |       |       |       | %     |       |
| Stalks            | 2009 | 7,37               | 2,46 | 40,02 | 8,77  | 4,77  | 31,51 | 8,71  |
|                   | 2010 | 10,29              | 3,04 | 48,83 | 16,39 | 6,60  | 39,24 | 14,66 |
|                   | x    | 8,83               | 2,75 | 44,42 | 12,58 | 5,69  | 35,37 | 11,68 |
| Leaves            | 2009 | 25,79              | 2,37 | 50,13 | 33,3  | 10,66 | 14,36 | 18,88 |
|                   | 2010 | 40,06              | 6,84 | 49,22 | 33,97 | 11,14 | 12,31 | 20,51 |
|                   | x    | 32,93              | 4,60 | 49,68 | 33,64 | 10,90 | 13,33 | 19,69 |
| Inflorescence     | 2009 | 28,91              | 4,26 | 50,88 | 14,12 | 6,17  | 26,17 | 11,69 |
|                   | 2010 | 36,75              | 7,52 | 51,47 | 15,23 | 6,04  | 20,97 | 14,51 |
|                   | x    | 32,83              | 5,89 | 51,17 | 14,68 | 6,11  | 23,57 | 13,10 |
| 2009              |      | 20,69              | 3,03 | 47,01 | 18,73 | 7,20  | 24,01 | 13,09 |
| 2010              |      | 29,03              | 5,80 | 49,84 | 21,86 | 7,93  | 24,17 | 16,56 |
| The total average |      | 24,86              | 4,41 | 48,42 | 20,30 | 7,56  | 24,09 | 14,83 |

**Table 2** Fabric composition of the amaranth plant parts Oscar Blanco variety expressed by a correlation coefficient (r)

| Factor | N                    | P                    | K                    | Ca                   | Mg                   | Pupl                  | Ash                   |
|--------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|-----------------------|
| Year   | 0,8257 <sup>++</sup> | 0,6723 <sup>++</sup> | 0,3660 <sup>+</sup>  | 0,1610               | 0,1501               | 0,0082                | 0,4329 <sup>+</sup>   |
| N      | -                    | 0,8257 <sup>++</sup> | 0,7044 <sup>++</sup> | 0,5476 <sup>++</sup> | 0,5378 <sup>++</sup> | -0,8163 <sup>++</sup> | 0,6198 <sup>++</sup>  |
| P      | -                    | -                    | 0,4899 <sup>+</sup>  | 0,1638               | 0,1537               | -0,4644 <sup>+</sup>  | 0,3472 <sup>+</sup>   |
| K      | -                    | -                    | -                    | 0,4335 <sup>+</sup>  | 0,4117 <sup>+</sup>  | -0,3967 <sup>+</sup>  | 0,5831 <sup>++</sup>  |
| Ca     | -                    | -                    | -                    | -                    | 0,9979 <sup>++</sup> | -0,7811 <sup>++</sup> | 0,9525 <sup>++</sup>  |
| Mg     | -                    | -                    | -                    | -                    | -                    | -0,7705 <sup>++</sup> | 0,9431 <sup>++</sup>  |
| Pulp   | -                    | -                    | -                    | -                    | -                    | -                     | -0,7069 <sup>++</sup> |

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# Cultivation technology the amaranth for phytomass energy

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## Abstract

When a sufficiently high dry matter production of 1 ha were, however, additional energy deposits currently used in the production of phytomass in fact the most effective (1:13,08). The crucial item of the energy deposits are nitrogenous fertilizers (110 kg.ha<sup>-1</sup>). Based on the intermediate results can be concluded that increased doses of nitrogen fertilizer significantly affect the number of leaves on the plant of Amaranth. Cultural forms of Amaranth (*Amaranthus* L.), its tolerance and adaptability to environmental conditions, production of phytomass and then a wide range of options for using it are remarkable models for the 21.st century plants.

**Key words:** *Amaranth, energy crops, phytomass, energy balance*

## Introduction

Energy crops should provide a sufficient number of high and stable yields of phytomass. One of these crops may also amaranth (*Amaranthus*). Estimates of direct and indirect input additional energy production technologies in different directions show that the highest energomaterálové inputs have been entered into the cultivation of *Amaranthus* phytomass production (Husky, Pospisil, 1999).

## Materials and Methods

The aim was to verify and refine the technology of cultivation of amaranth on energy use - a substrate for biogas production in the particular circumstances of an agricultural holding.

Crops amaranth are based on the years 2009-2011 on the site Baratsko 1 ha area. Area (12 km from Nitra north-east) in which the site is located, can be described as warm climates. The soil is moderate, with a neutral reaction, a sufficient supply of phosphorus, potassium, calcium and very high in magnesium.

We sowed the seed genotype *Amaranthus caudatus* Oscar Blanco variety imported from Peru. This variety has been recommended for the production of energy phytomass. The intention was to grow the crop amaranth as substrate for biogas plants.

## Results and Discussion

Based on the recommendations of especially foreign authors Aufhammer (1995), Lee *et al.* (1996) and other cultivation technologies in the cultivation of amaranth, but also from their practical experience in the cultivation of amaranth genotypes in cultural history (Vach, 1996), we paid attention to the selection of parcels of land and basic training. Cropping in the first experimental year was beet red, the next two years beet sugar.

Based on the three-year results we can conclude that the beet is suitable for cultural amaranth cropping and we recommend it despite the warning Jarošová *et al.* (1997). The amaranth is not suitable after sown plots, where the previous year were used herbicides with the active ingredient applied to amaranth.

The cultivation of amaranth for energy purposes in the future may not only decide the amount of phytomass, which is capable of producing, but especially the quality and economic aspects of production.

**Table 1** Cultivation technology used on the farm in 2011 Baratsko

| <b>Technological operation</b>           | <b>Description of technology</b>   | <b>The cost per 1 ha in €</b>    |
|--|--|----------------------------------|
| Primary tillage                          | 0.25 m, 5-rotary mouldboard plough Lansberg  | 66.00                            |
| Adjustment after ploughing               | Cultivator Lemken-Smaragd.   | 20.00                            |
| Fertilization NPK 15-15-15               | dose 200 kg ha <sup>-1</sup><br>spreader   | 78.00<br>8.30                    |
| Sidedress during vegetation LAD (N 27 %) | dose 200 kg ha <sup>-1</sup><br>spreader   | 54.00<br>8.30                    |
| Dragging soil before sowing              | Skid 6 m.  | 14.00                            |
| Seed bed preparation - harrowing         | combined harrow 6 m.   | 10.30                            |
| Sowing (original seed from Peru)         | Quantities of the seeds 1.5–2 kg ha <sup>-1</sup> , the provide about 200 thousand individuals per ha, sowing depth 1.5 cm, 50 cm, optimum 220 plants per square m. Precision drills Schmotzer 3 m | 26.00                            |
| Weed regulation                          | Spraying of pre emergent herbicide Dominator 2.5 L ha <sup>-1</sup> + application Cosmic 2.5 L ha <sup>-1</sup> x 6.30 € = 15.75 €.  | 15.75                            |
| Spraying                                 | Sprayer Schmotzer.   | 9.80                             |
| Weeding                                  | Knife - weeder shot 12 m.  | 15.00                            |
| Weed control                             | Spraying of post emergent herbicide Targa Super 1,2 L.ha <sup>-1</sup> + application.  | 29.40                            |
| Spraying                                 | Sprayer Schmotzer.   | 12.00                            |
| Weeding                                  | Knife - weeder – 12 m.   | 15.00                            |
| Hoeing                                   | Hand cultivation in row  | 70.00                            |
| Rent for land and property tax           | Rent for land  | 66.50                            |
| Harvest                                  | Cutter - Claas Jaguar 4,5 m  | 90.0                             |
| Removal of material                      | Tatra 815 with large extension per hour  | 33.0                             |
| Silaging                                 | Compression of chop into plastic tubs 1 hour<br>10 €   | 60.0                             |
| Harvest (effect gigantism)               | 118,4 t ha <sup>-1</sup> in green, 24% solids, 28.4 t ha <sup>-1</sup> dry matter  | ∑ 701.30<br>without<br>subsidies |
|  | The price of € 5 per tonne is a production value amaranth 592 € z 1 ha   |                                  |

Experiments show on the reserves provision of mechanization, post harvest treatment and storage of seed and phytomass. We must not forget the economic and marketing aspects, which are indispensable for the success of this idea to market.

Cultural forms of amaranth (*Amaranthus* L.) for its tolerance and adaptability to environmental conditions, production of phytomass and consequently a wide range of possibilities of its use, however, represent a promising plant for the 21 century.

For this purpose is realized project in cooperation with Darwell al. with r. a. Bratislava Project Agency: VMSP - 0063-09: "The use of renewable biomass for energy purposes."

### Conclusions

Based on previous research results and knowledge of manufacturing experience, we can summarize the advantages and problems in the cultivation of amaranth for energy:

1. Pay attention to the selection of suitable land (not sloping, area with mrlíkmi and weed species of amaranth).
2. Perform precise preparation soil for the autumn (plowing of the settlement).
3. Implement timely and quality soil preparation (to ensure suitable conditions for destruction of weed).
4. Apply a total herbicide before sowing (effective and economical method of controlling weeds).
5. The shallow depth of sowing at the optimum time (soil temperature 10° C, small seed good contact with the soil).
6. The take advantage of the mechanical treatment of vegetation in the vegetation (for lack of herbicidal treatments, or in dry years of vegetation ).

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## Effects of popping on amaranth seed nutrients

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### Abstract

The seeds of amaranth can be popped under suitable heating. The traditional popping method is to heat the seeds manually on a hot plate, although a problem arises because of nutritional value loss through overheating. We developed continuous popping methods based on hot-air heating. This study investigated the effects of heat treatment during seed popping on B-group vitamins and minerals. Results show that the B-group vitamin and mineral contents were not lost when using this method.

**Key words:** *Amaranthus hypochondriacus, popping seeds, B-group vitamins, minerals*

### Introduction

The genus *Amaranthus* has received considerable attention in many countries because of the great amounts of proteins, minerals, and vitamins in its seeds Alvarez-Jubete *et al.* (2009, 2011).

Amaranth seeds can be popped by rapid evaporation of moisture within the seeds, accompanied by starch gelatinization, when heated. The popped seeds, which have a soft texture, taste like a nutty-flavored popcorn. Popping is a simple means to process amaranth seeds for use as food.

As for popping procedures, heating on a hotplate has been used traditionally, although some problems with the product can occur: low recovery of popped seeds because of inhomogeneity of heat efficiency and browning or carbonation by overheating. These shortcomings can also decrease the nutritional value, such as by loss of lysine because of the amino-carbonyl reaction Tovar *et al.* (1989), Gamel *et al.* (2005).

Iyota and his co-workers have developed a continuous processing system for popping amaranth seeds using hot air Inoue *et al.* (2009). Effects of the new system on the popping quality of seeds, such as their volume expansion ratio and yield, were examined. This study is intended to investigate the effects of heat treatment by the processing system on the B-group vitamins (riboflavin, niacin, pantothenic acid, vitamin B<sub>6</sub>, biotin and folate) and minerals.

## **Materials and Methods**

*Amaranthus hypochondriacus* K-343 seeds (from the USA; Shinkyo Sangyo Co., Ltd., Japan) were used.

### **Sample preparations**

The raw seeds were heated and popped using fluidized-bed continuous processing system Inoue *et al.* (2009) with hot air under the temperature of 260 °C. A schematic of the system is presented in Fig. 1. Raw and popped seed samples were ground using an ultracentrifugal mill (ZM200; Retsch GmbH and Co. KG) and were stored at -20°C before analysis.

### **Moisture determination**

Moisture contents were measured according to the AOAC Official method analysis (drying 130°C for 3 hr).

### **Vitamin analysis**

Riboflavin was analyzed by HPLC with a spectrofluorimetric detector. Niacin, Vitamin B<sub>6</sub> and biotin were analyzed using the microbiological assay method of AOAC Official methods analysis. Pantothenic acid and folate were analyzed using enzyme extraction in combination with microbiological assay Gonthier (1998); Hyun and Tamura (2005).

### **Mineral analysis**

Samples were digested using HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in a microwave digestion system (Multiwave 3000; Anton Paar GmbH). Analyses were conducted using an ICP-MS (XSERIES 2; Thermo Fisher Scientific Inc.) with standard conditions for each element as described by the instrument manufacturer.

## **Results and Discussion**

### **Vitamins**

Table 1 shows effects of the popping treatment on the B-group vitamins of amaranth. The contents of riboflavin, folate, vitamin B<sub>6</sub>, and biotin, but not niacin and pantothenic acid, in raw seeds were similar to data referred from the Food Standard Table in Japan 2010. No significant difference was found between the vitamin contents of popped and raw seeds.

### **Minerals**

Tables 2 and 3 show the effects of popping treatments on minerals of amaranth seeds. The Mg, K, Ca, Fe, Cu, and Mo contents in raw seeds were similar to those appearing in the Food Standard Table in Japan 2010, although Na, Cr, and Se were higher and Mn and Zn were lower.

Konishi *et al.* (1998) reported that P, K, and Mg are localized in embryonic tissues and Ca was present in seed coats and the boundary between the perisperm and embryo of amaranth seeds. In this study, these elements in seeds were not lost during popping; suggesting that all seed tissues might be recovered by this system.

Evaluation by comparison with the dietary reference intake in Japan shows that amaranth

seeds are a good source of the elements most commonly lacking in the Japanese diet: Mg, Ca, Fe, Zn, and Cu.

### **Conclusion**

We developed a continuous processing system based on hot-air heating for popping amaranth seeds. Heat treatment during popping did not affect the contents of the B-group vitamins or minerals in amaranth seeds. This continuous processing system is apparently useful for processing amaranth for commercial applications.

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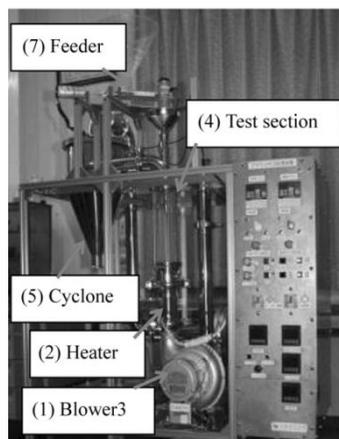
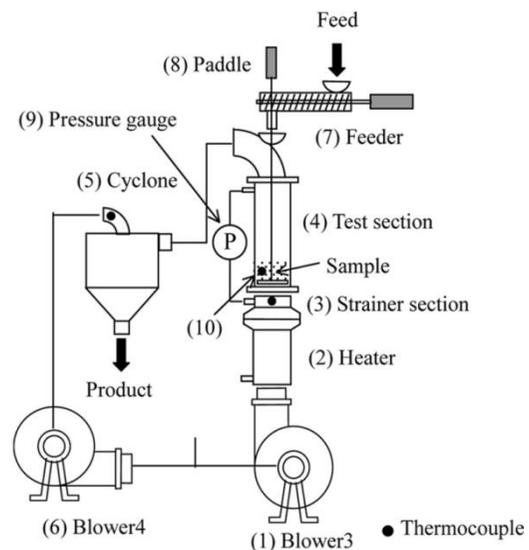


FIG. 1 Continuous processing system for popping amaranth seeds

Table 1 Effect of popping treatment on B-group vitamin contents in amaranth seeds

| Vitamins         | Raw        | Popped seeds | Reference <sup>*1</sup> |
|------------------|------------|--------------|-------------------------|
| Riboflavin       | 147 ± 9    | 140 ± 2      | 162                     |
| Niacin           | 3230 ± 340 | 3080 ± 230   | 1160                    |
| Folate           | 152 ± 16   | 137 ± 8      | 150                     |
| B <sub>6</sub>   | 454 ± 47   | 408 ± 54     | 670                     |
| Biotin           | 24.8 ± 3.7 | 25.2 ± 2.9   | 18.8                    |
| Pantothenic acid | 1150 ± 60  | 991 ± 78     | 1950                    |

Data presented as µg/100 g dry-weight basis ± standard deviation (*n* = 3).

<sup>\*1</sup> Referred from Standard Table of Food Composition in Japan 2010.

Table 2 Effect of popping treatment on macro elements in amaranth seeds

| Elements | Raw       | Popped seeds | Reference <sup>*1</sup> |
|----------|-----------|--------------|-------------------------|
| Na       | 1.9 ± 0.4 | 2.2 ± 0.3    | 1.2                     |
| Mg       | 308 ± 4   | 331 ± 9      | 310                     |
| P        | 779 ± 36  | 1022 ± 9     | 620                     |
| K        | 632 ± 10  | 674 ± 16     | 690                     |
| Ca       | 234 ± 6   | 243 ± 11     | 190                     |

Data presented as mg/100 g dry-weight basis ± standard deviation (*n* = 3).

<sup>\*1</sup> Referred from Standard Table of Food Composition in Japan 2010.

Table 3 Effect of popping treatment on microelements in amaranth seeds

| Elements | Raw         | Popped seeds | Reference <sup>*1</sup> |
|----------|-------------|--------------|-------------------------|
| Cr       | 31.5 ± 1.5  | 65.2 ± 7.7   | 8                       |
| Mn       | 3510 ± 41   | 3780 ± 147   | 7200                    |
| Fe       | 10700 ± 112 | 11400 ± 84   | 10900                   |
| Co       | 11.7 ± 0.1  | 15.7 ± 4.2   | -                       |
| Cu       | 752 ± 28    | 807 ± 3      | 1060                    |
| Zn       | 3200 ± 36   | 3290 ± 252   | 6700                    |
| Se       | 40.4 ± 3.0  | 39.1 ± 0.5   | 15                      |
| Mo       | 59.6 ± 8.2  | 69.2 ± 7.3   | 68                      |

Data presented as µg/100 g dry-weight basis ± standard deviation (*n* = 3).

<sup>\*1</sup> Referred from Standard Table of Food Composition in Japan 2010.

# Production and utilization of amaranth in Slovakia

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## **Abstract**

Since 1994, was on the experimental basis of the Faculty of Agrobiology and Food Resources, Slovak Agricultural University in Nitra, founded polyfaktoriálne field experiments with amaranth (*Amaranthus* L.) with aim pursue certain articles of agricultural engineering in relation to grain yield in maize production area.

Habana - Illes (2001) indicate the current and prospective possibilities of using amaranth: pseudocereals, 2 energy and technical plant, 3 animal feed, 4th source of natural dyes, 5 source of protein, oil, starch and dietary fiber, 6 vegetables, 7 ornamental plant, 8 plant with healing properties. The most commonly grown amaranth genetic resources in Slovakia may use different directions, depending on seed production, respectively. economically usable parts.

The seeds and leaves contain vitamins and minerals, can be used as a vegetable or like fruit. This results in extremely wide range of applications. The leaves can be used to produce vitamins, protein extracts, dyes and pharmaceuticals and the like. The from seeds can be prepared soup, various drinks, porridge, popcorn, rub, pancakes, many different products in confectionery and bakery products. Stalk, vegetative phytomass can be fed to livestock or used for energy as fuel, or as raw material for industry (Jamriška, 2001).

**Key words:** *seed, phytomass, roughage, energy purposes*

## **Cultivation of amaranth for seeds**

Amaranth for seed we can grow in our of corn and beet field. The genotypes with shorter vegetation in potato field. It can grow in the extensive (organic product) as intense conditions. The on seed yield was affected most weather conditions (yield from 0.75 to 5.9 t.ha<sup>-1</sup>). Especially important is the sum of temperatures from sowing to emergence. The best date sowing was during the first decade of May, but is very important temperature of the soil. Sowing in mid-June is too late. Most suitable for the sowing is distance 0.125 m. Critical period of weed emergence is the creation of the ninth pair of leaves. Vegetation with narrower medziriadkami was also more resistant to weeds. Weed infestation reduced the maximum proportion of leaves and seed yield (significantly less than for maize). Plant amaranth are most sensitive to weed emergence from the ninth pair of leaves. All varieties responded to the decline in crop weed infestation, burinový A. retroflexus however is not (Jamriška, 2001).

## **The cultivation of amaranth as a forage or for green vegetables**

The current research shows that it is amaranth with a high content of mineral elements and low in fiber. In terms of quality of food has been shown that especially in the mineral content amaranth surpasses even seed alfalfa. In particular, young plants are characterized by high content of B - carotene, iron, calcium and vitamins. When his crop as forage obtained the following results: Amaranth *Amaranthus mantegazzianus* grown in succession to winter triticale provide aboveground phytomass yield 6.77 t.ha<sup>-1</sup>, with the average yield of dry matter 8.36 t.ha<sup>-1</sup>. *Amaranthus hypochondriacus* had a higher average content of crude protein and fat. Both species are characterized by high content of mineral elements and low in fiber. The contents of P, Mg and K amaranth exceeded alfalfa in the same conditions. The amaranth had a lower average content of crude protein, calcium and fiber (Gregorová, 2001).

### **Amaranth grown for energy purposes**

Besides the production of grain or forage is another very important way is using the for energy purposes. The high calorific value combustion amaranth phytomass (14-21 GJ.t<sup>-1</sup>) it almost predetermines the cultivation on energy purposes. Estimates of direct and indirect input additional energy production technologies in different directions show that the highest energomateriálové inputs were inserted into the cultivation of amaranth. The crucial item for energy deposits are nitrogenous fertilizers (110 kg.ha<sup>-1</sup>) necessary for to obtain sufficient quantities of phytomass. At sufficiently high dry matter production of 1 ha were however additional energy deposits used precisely in this direction (1:13,08). To confirm these facts have been established specifically experiments with intensive nitrogen fertilization of amaranth (0 to 120 kg.N.ha<sup>-1</sup>). Based on the intermediate results can be concluded that higher doses of nitrogen fertilization significantly affect the number of leaves on the plant amaranth. Plants retain a relatively high number of leaves in the vegetation, but mainly due to higher doses of fertilizer and the end of vegetation. Solids leaves during vegetation increases, high fertilization significantly affected the physiological parameters of plants. Leaf area index is highest in the variant with a single dose of nitrogen fertilizer (Illés *et al.*, 2001).

### **Economics aspects of cultivation of amaranth**

When quantifying the economic effects of the cultivation of amaranth is necessary know, how use the final product. The economics of production láskavca is vary. In the economy growing amaranth is not a significant difference. In fact, the amount of profit depends on the conditions of sales. The positive economic effect is from 2 tonnes of seed yield per hectare. The increase in yield per unit area is subject to increasing values of inputs to production. The increased costs relate mainly to post-harvest treatment, fertilizer and other costs. The second ton of the manufacturer paid the cost of sales and ensure a profit. The analysis of costs in relation to the price of amaranth showed that the success will depend on the in to mastering technology and especially the manner of implementation. As seems to be very efficient production of seeds for food production in particular cereal products used for routine and dietary nutrition. Production of seed for feed purposes is problematic in terms of economy, mainly due to very low but also variable hectare yields (Boreková, 2001).

The high content of useful energy product, adequate fertility and its stability in aridnejších climatic conditions, amaranth has increased resistance to adverse factors, the efficient use of nutrients, water and solar energy. These are the positives of the crop.

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## Options energy use of the seeds of *Amaranthus*

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### **Abstract**

In research aimed to verification of amaranth cultivation technology for seed production and phytomass analysis of seeds for starch content were also carried out. The starch content plays an important role in use of amaranth phytomass for energy production primarily as a substrate for biogas production. Starch as a polysaccharide is a expeditious and concentrated source of energy for metanogenic bacteria.

Analysis of dry seeds of amaranth, we determine the starch contents of 62.3 to 69.9% . An interesting fact is that the seeds of amaranth produced for seed purposes have a higher starch content than seeds obtained from nutrition production areas.

The highest starch content of 69.9% was determined in seeds of amaranth grown in Peru.

**Key words:** *amaranth, seeds, starch*

### **Introduction**

Amaranth can be classified in terms of content substances into several groups of crops (cereals, legumes, forage crops, vegetables, medicinal and ornamental plants). Carbohydrates amaranth are balanced cereals, but their digestibility is higher. The seeds and leaves contain vitamins and minerals, can be used as food, vegetables, krmovinu and energy crop. This results in extremely wide range of use.

Comprehensive research process amaranth biomass is not yet given sufficient attention. Perspective is particularly to produce clean biogas (Jelínek, 2006).

It's kind of crops with high hydrostabilitou and relatively good resistance to pests and diseases.

Amaranth for seed are to be planted corn field and beet field, genotypes with shorter vegetation period in potato field. Bad are difficult, cold soils, and land with residues of herbicides against amaranth. Improper slope lands, also at risk of erosion. Preference should be easy to medium hard soil. It can be grown in intensive and extensive conditions.

The seed yield was most affected weather conditions (yield from 0.75 to 5.9 t.ha<sup>-1</sup>). Especially important is the sum of temperatures from sowing to emergence. Surest sowing was during the first decade of May, but the critical temperature of the soil. Sowing in mid-June is too late. Most suitable for the sowing Interline distance 0.125 m.

Vegetation with narrower medziriadkami was also more resistant to weeds. Weed infestation reduced the maximum proportion of leaves and seed yield (significantly less than for maize). Plant amaranth are most sensitive to weed emergence from the ninth pair of leaves. All varieties responded to the decline in crop weed infestation (Jamriška, 2001).

### **The chemical composition of phytomass amaranth**

The starch content of amaranth seeds is the range from 53% to 65%. On average, amaranth seeds contain 60% starch. The fiber content of the seed is from 2.89% to 6.94%, an average of 4.4%.

Amaranth seed contains about 3% of the minerals in the dry state. The ash content in seeds of four species of amaranth was around of 4.5%. Amaranth is different from other cereals and pseudocereals especially high potassium, calcium, magnesium and iron. The fat content of amaranth seeds is in the range of 4-10%.

Amaranth contains no gluten, a has positive quality of fat content (51% linoleic acid), starch, amaranth is five times more easily digestible than corn, amaranth seed has a higher content of minerals and vitamins as our cereals.

In terms of transformation processes of organic matter and maintain soil fertility, it is important not only quantity but also quality of the carbonaceous material resources. The largest share of the scheduled substances in crop residues amaranth plant hemicellulose was 26.97% (with a tolerance of + - 6.72%). Cellulose accounted for 26.0% of (+ -8.53%), lignin 12.47% (-2.66%). Starch, which was mainly in the husk accounted for 5.73% of (+ -2.14%).

## Materials and Methods

In the research, production technologies, the verification of amaranth seed production and phytomass were also carried out analyzes of seeds and seed collected on starch content. The starch content plays an important role in the energy utilization of amaranth phytomass mainly as a substrate for biogas production. Starch is a polysaccharide as expeditious a concentrated energy source for methanogens bacteria. Some researchers examine clean gasification seeds amaranth .

## Results and Discussion

Based on analysis of samples and seeds of amaranth starch content was determined in individual samples (Table 1)

**Tabl. 1** The starch content in pulp of amaranth

| Seed amaranth                    | Locality                                | The starch content in dry matter (%) |
|----------------------------------|---|--------------------------------------|
| <b>Amaranth on the seed</b>      | Production area, Nitra                  | 63,0                                 |
| <b>Amaranth on the phytomass</b> | Seed varieties – Oskar Blanco, Peru     | 69,9                                 |
| <b>Amaranth on the feed</b>      | Seed varieties – Elbrus, Czech Republic | 66,5                                 |
| <b>Amaranth on energy</b>        | Seed varieties - Koliňany               | 62,3                                 |

An analysis of dry seeds of amaranth on starch content was determined from 62.3 to 69.9 %. Our results correspond with the published literature sources that the starch content in dry seeds of amaranth is from 53% to 65%. The highest starch content was determined in the seeds of amaranth grown under conditions of Peru.

Starches from amaranth population is degraded compared with starch obtained by aqueous extraction much faster. The difference is caused heat treatment pop. Methanogenesis amaranth starch is slow (Jelínek, 2006).

## Conclusion

An analysis of dry seeds of amaranth was found starch content from 62.3 to 69.9%. Our results correspond with the published literature sources that the starch content in dry seeds of amaranth is from 53% to 65%.

It is interesting that the seeds of amaranth on the seeds have a higher starch content than seeds from production areas on the consumption.

The highest starch content of 69.9% was set in amaranth seeds grown in the agroclimatic conditions of Peru.

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## **Influence of genotype and interlinear spacing on yield of amaranth seeds (*Amaranthus* spp.) in beet and potato growing areas**

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Genus *Amaranthus* contains species characterized by high variability and good adaptability to different climatic conditions. In Slovakia, amaranth can be generally cultivated mainly in corn and beet growing areas. Amaranth genotypes with shorter growing season can be grown also in these potato areas which are characterized by warmer climate. Certain orientation in this direction can give area of *Amaranthus retroflexus* occurrence. In amaranth cultivation aimed at seed production besides of genotype also interlinear spacing is important factor. In conditions of Slovakia the spacing 200, 250, 375 or 350 mm was found to be suitable for different genotypes. In this paper, influence of interlinear spacings (125 and 375 mm) and two different localities (Holzhausen, Germany - beet growing area and Ľubietová, Slovakia - potato growing area) on yield of amaranth seeds in two amaranth genotypes (K343 and K432) was searched during three-years field experiments. Besides of seed yield also density of plant growth and weight of thousand seeds (WTS) were evaluated. The highest source of variation showed to be climatic conditions. Comparing genotypes, the higher seed yield (2.91 t.ha<sup>-1</sup>) was achieved in genotyp K432. Interlinear spacing has influence on evaluated trait, whereupon the higher seed yield (2.85 t.ha<sup>-1</sup>) was recorded at 125 mm interlinear spacing. By comparison of two localities it was found, that higher seed yield (3.00 t.ha<sup>-1</sup>) was achieved on the locality Ľubietová. Density of plant growth was the most influenced by temperature and moisture conditions during seed germination. Reducing of interlinear spacing has no influence on WTS. The obtained results confirmed that hybrids K 343 and K 432 are suitable for cultivation in conditions of Slovakia what is in agreement with the results of other authors which recommend these cultivars for cultivation in the area of Middle Europe in relation to their stabile seed yields and very effective exploitation of nitrogenous fertilization.

## **Insect pests associated with *Amaranthus spp.* and their natural enemies in Ibadan, Nigeria**

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The insect fauna of *Amaranthus spp.* was assessed using the sweep net and aspirator. The objective of the study was to provide basic information on the insect pest complex of amaranth and their natural enemies. The insect pest and the damage they caused on amaranth showed that the vegetable has many potentially destructive pests. A total of 84 species of insects belonging to 8 orders and 24 families were found. The insect orders in order of abundance were coleopterans (36.90%) lepidopterans (29.79%), heteropterans (15.48%), while the dermapterans and the dipterans (1.19%) each. Among the natural enemies the heteropterans were the most abundant (66.67%) belonging to the reduviidae. The hymenopteran parasitoids (22.22%) were all ichneumonids. The only coleopteran and dipteran natural enemies were *Cheilomenes lunata lunata* Fabricius and *Stomorhina apta* Curran respectively. The major leaf eating larvae were *Hymenia recurvalis* Fabricius, *Psara bipunctalis* Fabricius and *Psara palpalis* Hampson all from pyralid. The major stem borers were *Baris circumscutellata* Hustache, *Gasteroclisus rhomboidalis* Boheman, *Leucogrammus paykulli* Boheman, *Lixus camerunus* Kolbe, *Hypolixus nubilosus* Boheman and *Hadromerus sagittarius* Olivier all curculionid. The major grains sucking bugs were the coreid *Cletomorpha unifasciata* Blote and *Cletus fuscescens* Walker and the pentatomid *Aspavia armigera* Fabricius.

## **Diversity of *Amaranthus* species as revealed by phenotypic and RAPD analysis**

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Assessment of genetic diversity helps in the identification of diverse parental combinations to create segregating progenies with maximum genetic variability and facilitates introgression of desirable genes from diverse germplasm. Twenty nine grain amaranth accessions were characterized based on phenotypic and molecular (Random Amplified Polymorphic DNA - RAPD) markers. Phenotypic characterization involved evaluating 10 morphological and 17 nutritional traits. For molecular characterization, out of the 40 RAPD primers screened only 16 were polymorphic and so were used. Multivariate analysis showed that the first four principal components contributed 57.5% of the variability observed in phenotypic traits. Cluster analysis grouped the accessions into five clusters that displayed a wide range of diversity for most of the traits. RAPD analyses revealed high level of polymorphism; yielding a total of 193 fragments of which 157 were polymorphic (81%) with an average of 12.06 loci per primer and mean polymorphic information content of 0.872. Genetic similarity coefficient ranged from 0.61 to 0.88, dendrogram grouped accessions into nine clusters with population of the same species clustering together. RAPD marker proved to be better for assessing genetic diversity than phenotypic marker.

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