International Workshop

Recent Advances in Neglected and Under-utilized Species Research

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WORKSHOP PROGRAMME

Oct. 18 (Sunday) Arrival day

Oct. 19 (Monday)

8.30 - 9.45 Registration of participants

9.45 -10.00 Opening ceremony: doc. RNDr. Ján Salaj, Dr.Sc. **(**director of IPGB SAS, Nitra, Slovakia)
Ing. Dagmar Janovská, PhD. (Crop Research Institute, Gene Bank, Praha - Ruzyně, Czech Republic)

Chairman of Plenary Sessions 10.00-12.20: Sezai Ercisli

1) Collection, conservation and evaluation of NUS genetic resources

10.00 - 10.20 Minor cereals in the Czech Gene Bank
JANOVSKÁ Dagmar

10.20 - 10.40 Current research on genetic resources management of neglected and under-utilized species in India
RANA Jai Chand, PHOGAT BS, LAL Hanuman

10.40 - 11.00 Coffee break

2) Cultivation of NUS and adaptation to environmental stresses

11.00 - 11.20 Evaluation on growth and seed yield of *Camelina sativa L.* varieties cultivated under controlled environment conditions
DĂNĂILĂ-GUIDEA Silvana-Mihaela, ROSU Ana, JURCOANE Ştefana, TOMA Radu, PODGOREANU Emanuela

3) Genetic improvement of NUS in terms of productivity and quality (different breeding strategies, advanced biotechnological approaches in propagation and breeding)

11.20 - 11.40 Characterization of a new amaranth variety developed through induced mutagenesis
HRICOVÁ Andrea, FEJÉR Jozef, LIBIAKOVÁ GABRIELA, GAJDOŠOVÁ Alena

11.40 - 12.00 Analysis of miRNA polymorphism during the selected developmental processes of flax
HLAVAČKOVÁ Lucia, NÔŽKOVÁ Janka, BRUTCH Nina, POROKHOVINOVA Elizaveta, SHELENGA Tatiana, BJELKOVÁ Marie, RAŽNÁ Katarína

12.00 - 12.20 Less-known small fruit species and their propagation using *in vitro* techniques
HUNKOVÁ Júlia, LIBIAKOVÁ Gabriela, GAJDOŠOVÁ Alena

12.20 - 14.00 Lunch

Chairman of Plenary Session 14.00-14.40: Andrea Hricová

4) Application of NUS in agricultural and environmental services (forage production, renewable energy sources, phytoremediation, etc.)

14.00 - 14.20 Comparative assesment for phytoextraction capability using different varieties of non- hyperaccumulator plant species *Cannabis sativa* L.
MACEČKOVÁ Barbora, BJELKOVÁ Marie, VĚTROVCOVÁ Martina, MOTYKA Oldřich, SEIDLEROVÁ Jana, KHEST Fili

14.20 - 14.40 Accumulation of heavy metals Pb and Cd by A*maranthus cruentus* L. plants
FEJÉR Jozef, PATLEVIČ Peter, PORUBSKÁ Jana

14.40 - 15.00 Coffee break

15.00 - 16.00 Poster Session

1) Starch variability in amaranth mutants induced by radiation mutagenesis.
ZÁHORSKÝ Michal, SOCHA Peter, GAŽO Ján, HRICOVÁ Andrea
2) Method suitability for isoenzymes determination in amaranth
MÚDRY Pavol, HRICOVÁ Andrea
3) *Smallanthus sonchifolius* (Poepp et Endtl) germplasm insight using retrotransposon based markers.
ŽIAROVSKÁ Jana, BOŠEĽOVÁ Danka, BEŽO Milan, FERNÁNDEZ Eloy C.
4) Comparision of 2-DE protein maps of poppy (*Papaver somniferum*).
KUŤKA HLOZÁKOVÁ Tímea, GREGOVÁ Edita, GÁLOVÁ Zdenka
5) Analysis of miRNA polymorphism during the selected developmental processes of flax.
HLAVAČKOVÁ Lucia, NÔŽKOVÁ Janka, BRUTCH Nina, POROKHOVINOVA Elizaveta, SHELENGA Tatiana, BJELKOVÁ Marie, RAŽNÁ Katarína

18.00 - Get-together party (in the entrance hall of the Institute of Plant Genetics and Biotechnology)

<u>Oct. 20 (Tuesday)</u>
Chairman of Plenary Session 9.00 – 11.00: Andrea Hricová

5) Contributing to food security, production of biologically active compounds for human health, processing

9.00 - 9.20 The role of African leafy vegetables in food and nutritional security.
GERRANO Abe S., van RENSBURG Willem Jansen, ADEBOLA Patrick O., NAIDO Krivashni, MAVENGAHAMA Sydney

9.20 - 9.40 Chemical composition and antioxidant characteristics of mung bean (V*igna mungo* (L.) Hepper) genotypes in Turkey.
ELKOCA Erdal

9.40 - 10.00 Chemical composition and antioxidant characteristics of wild and cultivated blackberries.
ERCISLI Sezai

10.00 - 10.20 Coffee break

10.20 -10.40 Essential oil bearing plants in Turkey and their characteristics.
KORDALI Saban

10.40-11.00 Selected nutrient analysis of common and tartary buckwheat genetic resources
SINKOVIČ Lovro, MEGLIČ Vladimir, NEČEMER Marijan, VIDRIH Rajko

11.00 - 11.10 Workshop closing

12.00 Lunch

CONTENT

MINOR CEREALS IN THE CZECH GENE BANK

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Key words – minor crops; genetic resources, gene bank

Introduction

There are at least 12 500 species of vascular plants in Europe, of which 3 500 are endemic. The Poaceae family, which includes all cereals, is represented by 880 species (IPGRI, FAO, 1996). However, only 82 species cover 90% of human energy (Prescott-Allen & Prescott-Allen, 1990). In worldwide, wheat, maize, sorghum and rice are considered as major cereals. Nevertheless, there are plenty of other species used in regional level as a food source, which are not planted in the wide scale. On the other hand, a lot of crops somewhere planting as a major crop might be growing as so-called minor crops in the other place. One of the typical examples is sorghum. It is the fifth most grown cereal in worldwide, however, in Europe it is the typical example of a minor crop (FAOStat, 2015). The huge number of species has been declined or disappeared from the cultivation practise because of the planting of major crops. In the last decade, a big effort has been targeting for the conservation of all plant genetic resources. The Plant genetic resource is defined as a material with current or potential value for food, agriculture or forestry (Brockhaus & Oetmann, 1996). One of the very suitable ways how to conserve plant genetic resources is under conditions of the gene banks. There are about 1 750 gene banks worldwide. All samples are stored in defined conditions according to the FAO standards. Genebanks help to conserve and make accessible and available the plant genetic resources for research and for breeding new varieties that meet the consumers' continually evolving needs and a changing climate (FAO, 2015). All accessions in the gene banks are evaluated and characterized mainly through the international descriptors. Essential information such as morphological and basic agronomic traits is obtained (Perry & Ayad, 1995).

In 2014 in the Czech Gene Bank 43 513 accessions of cereals, forage crops, vegetables, medicinal and spice crops etc. were stored. The structure of minor cereals is shown in Fig.1. This paper shows results of the evaluation of proso millet, foxtail millet and sorghum.

Material and Methods

All samples were sown in experimental fields of CRI in three successive years. 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 in breeding or in agricultural praxis.

Results

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

Qualitative data of sorghum

In the sorghum accessions waxy bloom were present in 86% slightly. 34% of inflorescences were semi-loose drooping, 28% loose dropping primary branches, 45% of glume colour was black. Shattering was in 85% very low. Grain colour was in 56% brown. Inflorescence exsertion was in 89% well exserted.

Qualitative data of foxtail millet

In case of foxtail millet the following traits stem colour, leaf colour, seed colour and shape and presence of awns were evaluated. From the evaluations were obtained following results: 85% of accessions had green stem colour, 95% green leaf colour, 75% yellow seed colour and globular seed shape. 89% of accessions have awns.

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, 80% grey green leaf colour, 42% light brown seed colour. The predominant seed shape was globular (49%).

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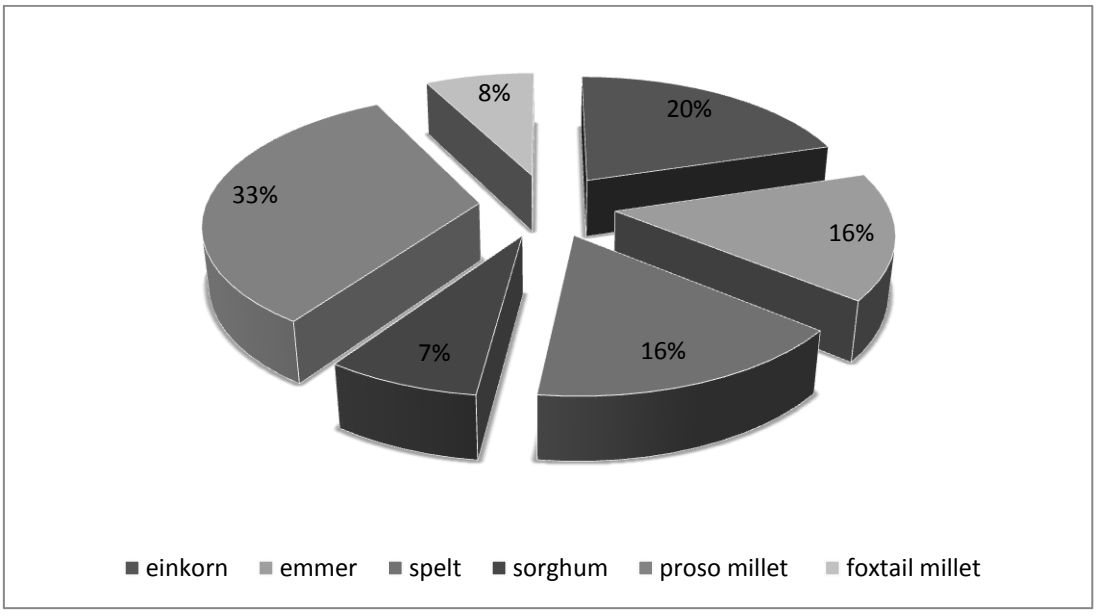

Figure 1 Structure of minor cereals in the Czech Gene Bank

Table 1 Quantitative data of sorghum

		Number of days from emergence to heading	Number of days from emergence to flowering	Length of the upper internodium (cm)	Length of inflorescence (cm)	Plant height (cm)	Number of days from emergence to maturity	WTS (g)	Yield per rows
Sorghum	MIN	54.00	63.00	37.33	17.00	41.67	109.00	8.07	21.00
	MAX	77.00	85.00	211.67	47.33	303.33	122.00	22.93	559.00
	Average	64.43	71.14	60.48	33.52	201.54	118.43	13.36	160.95
	SD	7.69	6.69	35.07	8.70	60.71	3.67	3.40	145.95

Table 2 Quantitative data of foxtail millet

		Number of days from emergence to heading	Length of inflorescence (cm)	Plant height (cm)	Number of days from emergence to maturity	WTS (g)	Yield per rows
Foxtail millet	MIN	54.00	8.00	71.67	112.00	1.89	27.00
	MAX	74.00	29.67	140.00	131.00	3.36	398.00
	Average	66.52	15.60	110.63	116.67	2.53	178.48
	SD	6.31	4.86	15.57	5.06	0.40	108.93

Table 3 Quantitative data of proso millet

		Plant height (cm)	Number of days from emergence to maturity	WTS (g)	Yield per rows	Number of days from emergence to heading	Length of inflorescence (cm)
Proso millet	MIN	82.67	89.00	4.78	13.00	47.00	22.33
	MAX	150.00	123.00	7.14	350.00	84.00	33.00
	Average	103.77	108.31	5.89	172.62	59.46	27.54
	SD	17.15	11.98	0.68	102.18	11.03	3.20

CURRENT RESEARCH ON GENETIC RESOURCES MANAGEMENT OF NEGLECTED AND UNDER-UTILIZED SPECIES IN INDIA

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Key words - Genetic, Resources, Neglected, Species, India

Introduction

Providing food and nutritional security to 9 billion people by 2030 would be a daunting task to governments in general and developing countries in particular. The current scenario of food and nutrition security favour this argument as nearly 1.2 billion people in the world do not have enough food to eat daily and a further 2 billion people are suffering from malnutrition (Azan-Ali, Battcock, 2002). Humans are deriving over 50% of the daily requirement of calories from just three crops - maize, wheat and rice while 150 crops are commercialized to a significant scale out of >7000 edible plant species known to be edible worldwide (Padulosi, 2013; Rana *et al*., 2011; Rana, Sharma, 2005). This recognises that humans have exploited the genetic diversity of few crops only, primarily as sources of food, and then improved their productivity and quality. While doing so large number of crops which had slightly unpleasant taste, appearance or anti-nutritional attributes could not find prime priority place among researchers, thus remained Neglected & Underutilized Species (NUS).

Why do we need research on NUS?

Humans being are facing and likely to face big challenge from newly emerging set of bodily diseases and disorders due to narrow food habits, changing life styles, coupled with climate change. This has compelled researchers and planners to look for new crops, which have inbuilt nutritional superiority and resilience to climate change (Rana *et al*., 2009; Nyadanu, Lowo, 2014). To meet these challenges, NUS have been considered to be the most potential candidate species to research upon. NUS grow more rapidly and intensely but are falling into disuse for variety of agronomic, genetic, economic and cultural reasons, not properly utilized, not competitive with other crops (Padulosi, 2013; Rana *et al*., 2000). The most distinct benefits and features of NUS are given below:

- Provide broad portfolio of crops for the diversification of agriculture and contingency planning
- Of local importance in consumption and production systems, and
- Responsive to poor management environment, thus useful for making better use marginal lands, and able to yield even under adverse conditions
- Store houses of immense nutritional, medicinal and industrial value, thus play greater role in the nutritional security and income generation for the world's poor
- Very high tolerance to biotic and abiotic stresses
- Longer storability under ordinary environments, hence serve as famine foods
- Most of them are short duration this fit into multiple cropping systems as catch-inter-crops
- Known to be resilient to Climate Change and other environmental adversities

Having so much richness, NUS has received scarce attention by national agricultural and biodiversity conservation policies, research and development (Padulosi *et al*., 2002; Vodouhe *et al*. 2011). Also, they are scarcely represented in *ex situ collections* and seed supply systems of public and private sectors.

Research work on NUS in India

Apart from some case studies, systematic research on NUS was started in India way back in 1982 with the execution of All India Coordinated Research Project on Underutilized and Under Exploited Plants (AICRP on UU & UEP, and now the project has been rechristened as All India Coordinated Research Net work on Potential Crops. The main aim of the project is find out new plant resources for food, fodder and industrial uses and conduct research on different areas such as genetic resources management, varietal development along with appropriate package of agronomic practices for their economic cultivation. Presently, research is being conducted on 15 crops (Table 1) at around 30 centres in different parts India (Anon, 2014).

Table 1 List of NUS crop species on which research is being in India

PSEUDOCEREALS	**OILSEEDS**
Grain Amaranth (*Amaranthus* spp.)	Perilla (*Perilla frutescens*)
Buckwheat (*Fagopyrum* spp.)	Simarouba (*Simarouba glauca*)
Chenopodium (*Chenopodium* spp.)	Tumba (*Citrullus colocynthis*)
Job's tear (*Coix lacryma*-jobi)	Jatropha (*Jatropha curcas*)
FOOD LEGUMES/ PULSES	Jojoba *(Simmondsia chinensis*)
Rice bean (*Vigna umbellate*)	**VEGETABLES**
Adzuki bean (*Vigna angularis)*	Kankoda (*Momordica dioica)*
Faba bean (*Vicia faba* L.)	Kalingada (*Citrullus lanatus*)
	Winged bean (*Psophocarpus tetragonolobus*)

The research work conducted over 25 years has led to the collection, evaluation and conservation of >10000 germplasm accessions of various crops. Of these, major share of germplasm belong to grain amaranth (5000), buckwheat (1200), rice bean (800) and faba bean (450). The germplasm has been extensively used for the development of 39 new varieties (Table 2) primarily through selection and partly through hybridisation.

Table 2 Varieties released under the All India Research Network on NUS

Crop/Varieties	**Characteristics**
Grain amaranth	Annapurna, GA-1, Suvarna, PRA-1, PRA-2, GA-2, PRA-3, Durga, BGA-2, VL Chua 44, GA-3, RMA- 4, RMA-7
Buckwheat	Himpriya , VL Ugal 7, PRB 9001, PRB 1, Himgiri, Sangla B-1
Chenopod	Himbathua
Rice Bean	RBL-1, PRR-1, PRR-2, RBL-6, RBL 35, RBL 50, BRS 1, VRB-3
Adzuki Bean	HPU51
Winged Bean	AKWB-1
Faba Bean	VH 82-1
Kalingada	Gujarat Karingada-1
Guayule	Arizona-1, HG-8
Tumba	RMT 59
Jojoba	EC-33198
Kankoda	Indira Kankoda
Jatropha	Chhatrapati

Besides, agronomic trials have been conducted and recommendations on dates of sowing, spacing, fertilizer applications including organic manures and weed control, water management, intercropping etc., have been published. Studies related to floral biology, pollination behavior, micro-propagation and application of molecular tools have also initiated. Biochemical profiling has helped in the identification of nutritionally superior lines and free from anti-nutritional factors. Two accessions in buckwheat (IC258233) for easy de-hulling and another in chenopod (IC258253) for brown seed colour, have been registered and unique genetic stocks.

Research issues and opportunities

NUS are essential to the livelihoods of millions of poor farmers throughout the world. While working in the field we observed that communities be it rural or urban are now more health conscious and therefore, looking for wider verity of nutritionally superior foods (Bisht *et al*., 2013; Rana *et al.*, 2012). Many crops such as buckwheat, quinoa, amaranths and small millets are in great demand and finding place in the food basket not only poor nut rich people largely in the form of value added products. Quinoa, which is a staple food of people living in the Andean region, is now popular among European and Western World and further spreading to Asia mainly due to its rich nutritional profile. Similarly the cultivation and consumption of grain amaranth and buckwheat is increasing India. The protein content and quality of pseudocereals is higher and much better than in cereal species (Padulosi *et al*., 1999; Dansi *et al*., 2012). Amino acid like Tryptophan, more particularly Lysine the limiting amino acid in cereals found to be present in high amount in pseudo-cereals. The high content of Arginine and Histidine, both essential for infants and children makes amaranth and quinoa significant and interesting for child nutrition. Examples from other parts of the world shows that vegetable rocket (collective name for the species *Eruca sativa, Diplotaxis tenuifolia* and *D. muralis*) has become a highly priced vegetable in Europe through innovative cultivation and commercial practices (Pimpini and Enzo, 1997), while it is among the cheapest vegetables in Egypt and a rich source of micronutrients for the poorer classes (Mohamedien, 1995).

Nevertheless, there is need to convert NUS into some modern high value commodity crops which is able to meet community's needs and also address their concerns. We need to explore how conservation and use can be combined to secure the resource base of such crops through linkages and collaborations, involving producers, consumers, the formal and informal sectors. Application of cross cutting technologies (e.g. molecular genetics and GIS) will certainly play their part in the process of developing conservation and use strategies. It is also mentioned that research on other NUS viz. Yams (*Dioscorea* spp.), taro (*Colocasia esculenta*), jackfruit (*Artocarpus heterophyllus*), noni (*Morinda citrifolia*), Annona (*Annona* spp.), coccinia (*Coccinia trilobata*), Moringa (*Moringa oleifera*), Bambara groundnut (*Vigna subterranea*), jack bean (*Canavalia ensiformis*), grasspea (*Lathyrus sativus*), and lablab (*Lablab purpureus*) etc., have been initiated by several public institutes and universities in India.

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EVALUATION ON GROWTH AND SEED YIELD OF *Camelina sativa* L. VARIETIES CULTIVATED UNDER CONTROLLED ENVIRONMENT CONDITIONS

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Introduction

The Kyoto Protocol (1997) set binding limits on emissions of greenhouse gases for industrialized countries. Under the new outlined global trends, the developed countries should make investments in green technologies, with which to reduce the greenhouse gas emissions (GHG) by 25-30% up to 2020. According to the European Fuel Quality Directive (FQD), a fuel must reduce GHG emissions by at least 35% compared to those of the fossil fuels by 2020, to count for the renewable energy target. An approach that receives much attention to alleviate the challenges of energy insecurity and the global warming due to GHG emissions, is turning from petroleum fuel to alternative sources of energy, such as liquid biofuels produced from renewable, biological sources (Putman, 1993a; Putman *et al.*, 2013). A biofuel is a viable alternative if it is sustainable, respectively if the evaluations based on Life Cycle Assessment studies regarding the flows of energy and materials through each pathway of fuel production, show a decreased level of GHG.

Currently, 98% of transportation fuels are derived from petroleum, with all the negative attributes in terms of fuel security and carbon emissions.

For the purpose of Biofuels Association of Romania (ABR), domestic production of biofuels must cover at least the requirements of urban road transport, but also ensure energy independence (fuel for farm machinery and transport) in rural areas. For this autonomy, by cultivating with energy crops only 0.1 million hectares of arable land of the 3 million hectares available will be sufficient. This alternative does not cause ethical, environmental, economic, etc. repercussions, but depends only on the capacity of decision makers to manage the food crisis and the prices of petroleum fuel for transport vehicles and for agricultural machinery.

The use of biofuels is an environmentally safe alternative, necessary to avoid the harm that may occur from the use of conventional petroleum-based products. However, for the production of biofuels several aspects of food security and natural resource availability should be taken into account. Thus, the agricultural products destined for food should not be used as feedstock for the production of biofuels.

This controversy, known as "food vs. fuel" dilemma appeared after the intensive use of maize crops and sugar cane for the production of bioethanol, a phenomenon that can affect the food needs of the population. Another aspect to be considered is the availability of land and water. To protect these finite natural resources, their exploitation should be done in a rational way. The first generation of bio- fuels were produced from sugarcane, starch, oils derived from agricultural products. They may not be sustainable because compete with agricultural products used for food.

Currently, the second generation biofuels derive from non-food feedstock, thus supplying a larger proportion of global fuel sustainably, affordably and with greater environmental benefits. Among the sources of second generation feedstocks for producing: lignocellulosic bioethanol and other advanced biofuels we may mention: residual non-food parts of current crops (stems, leaves, husks, etc); lignocellulosic energy non-food crops (switchgrass, *Miscanthus*, poplar, willow, etc.); industry waste (woodchips, sawdust, skins and pulp from fruit pressing); municipal solid waste, etc. (Bonjean *et al.*, 1999; Moraru *et al.*, 2013). The biodiesel of second generation is obtained by gasification of lignocellulosic feedstocks, from residues of the pulp/paper or forestry industry and from new energy crops such as *Jatropa, Salicornia bigelovii* and *Camelina sativa* (Downey, 1971; Pavlista *et al.*, 2012).

In Romania, C*amelina* is being investigated in several regions to determine the crop technology appropriate to the soil and climatic conditions of each zone (Dobre *et al.*, 2014a; Dobre *et al.*, 2014b; Toncea *et al.*, 2013; Dobre *et al.*, 2011). The rise of interest in *Camelina sativa* is due to the exquisite qualities of its seed oil: a unique fatty acid composition suitable for human and animal consumption and source of ”green energy”, by obtaining bio-based petroleum substitutes, especially biofuel for aircrafts (Budin & Breene,1995; Putman, 1993; Ciubota-Rosie *et al.*, 2013; Imbrea *et al.*, 2011, Robinson, 1987).

In the autumn of 2014 a greenhouse experiment was initiated by BIOTEHGEN (Romania) with *Camelina sativa* seeds derived from 20 selections (10-15 seeds/selection) sent by CCE (Camelina Company Espagna). Our aim was to determine the effects of genotype in controlled conditions on the growth, seed production and oil quality for each genotype of the 20 selections.

Material and Methods

The experiment was developed in the unit-greenhouse for automation research of the Center for quality research of the agro-food products (HORTINVEST), Bucharest. Characteristics of the compartment distributed to our experiment: 160 m², with the scope to obtain seedlings on culture benches. Facilities: heating, shielding, air-conditioning, electric set up, tide type irrigation, microaspersion, 4 assimilation lamps and an equipment of 2 LED panels with specific band lights in red and blue colour. Each of the two LED panels were made in the following sizes: 650mm x 350mm x 120mm, and insured with continuous illumination of 2000 lx each for 14 hours. The seeds were sown in11x11x11 cm square pots (1 seed/pot) placed in appropriate trays, at a density of 32 plants.m^{-2}. Inside the variants each pot was marked individually, in order to record the data for single seed descent.

Each pot was filled with 1 l substrate consisting of Kekkila DSM 2 W peat, which is a light ”breathable” peat (well milled) with addition of perlite, pre-fertilized with a base fertilizer (NPK 14-16-18), with the pH adjusted to 5,5-5,9. Before potting the substrate was sterilized by autoclaving at 100 ºC for 20 min. Prior to seeding the substrate was soaked up with as much water as it could take in. As a fertilizer, a complex chemical fertilizer “Azofoska” was chosen, that has the following characteristics: N:P:K 13,6-6,4-19,1 containing in addition small quantities of Mg, S, B, Fe, Mn, Mo, Zn. The photoperiod/temperature regime was of 16 h light, 24 ºC and 8 h dark, 20 ºC, light intensity of 4700 lux. In order to prevent fungal diseases a 0.1% solution of, a systemic fungicide (PREVICUR), was prepared and was applied when necessary.

Results and Discussion

We consider that the recorded data from this research may allow the identification of potential genotypes for further improving the performance and adaptability of *Camelina sativa,* including productivity, oil content and drought tolerance.

Though some plants reached the maturity stage after 80 days since seeding, we continued with watering added in trays every 3 days, up to full maturity of all *Camelina* plants. The data recorded for single seed descent of every genotype of the 20 *Camelina* selections had in view the following parameters: Plant height (cm); No. of branches/plant; Days to flowering; No. of pods/plant; Days to maturity for seeds; Biomass weight; Seed weight/plant; Total seed weight/biomas (%); Average seeds/pod; Number of seeds/plant; and weight of one thousand seeds.

In **conclusion**, the recorded data of our greenhouse experiment of seed multiplication of 20 *Camelina sativa* selections (270 genotypes in total), may allow the identification of potential genotypes for further improving the performance and adaptability of this crop, including productivity, oil content and drought tolerance. As regards the seed production 35 plants yielded more than 3 grams seeds/plant and 5 plants yielded more than 4 grams/plant.

Acknowledgment

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CHARACTERIZATION OF A NEW AMARANTH VARIETY DEVELOPED THROUGH INDUCED MUTAGENESIS

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The two selected mutant lines C26 and C82 with highly significant 1000-seed weight, generated through irradiation treatment of *Amaranthus cruentus* L. accession 'Ficha', were evaluated and compared to original non-treated control and variety of common knowledge 'Aztec'. The phenotypic traits and biochemical properties of the amaranth seeds were studied. The comparable values for crude protein content, albumins and globulins fractions and overall coefficient of nutritional quality was found in evaluated samples. However, C26 showed significantly lower concentration of celiac related prolamins and glutelins in comparison with the control sample. The significant differences in soluble oxalate content were observed among all analysed seed samples with average content of soluble oxalates in significantly lower levels in comparison with published data. Both of the mutagenesis-derived lines showed consistently superior performance of 1000-seed weight across two tested environments during multiyear evaluation. C82 showed seed weight advantage over control seeds ´Ficha´ and variety of common knowledge ´Aztec´ and was released as a new amaranth variety named Pribina in 2013.

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ANALYSIS OF miRNA POLYMORPHISM DURING THE SELECTED DEVELOPMENTAL PROCESSES OF FLAX

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Key words - microRNA, polymorphism, developmental processes, morphology, flax

MicroRNAs represent small non-coding RNAs that play important role in regulating gene expression at the post-transcriptional level. They are known to bind to the transcription factors involved in wide variety of biological and metabolic pathways. The synthesis of fatty acids is regulated by multigene family and depends on developmental stage of the plant. We were interested in the polymorphism of selected miRNA (miR156 and miR168) during the stages of flax seed formation (flower bud - flowering – boll development) as a reflection of the activity of specific miRNAs in these flax organs and tissues. As a target of miR156 in monocot and dicots have been identified squamosa promoter binding protein-like (SBP) transcripts which is involved in controlling flowering time and controls the transition from the juvenile to the adult vegetative phase. The highest expression of SBP target is in the flower and ovary of flax. On the other hand the highest expression of miR168b is exhibited in anthers and flowers. The miRNA polymorphism was evaluated on 9 flax genotypes and the data were supported by the morphology measurements on buds, flowers, petals and developing bolls.

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This research was supported by the project "ECOVA" (ECPUA - Excellent Centre of Protection and Use of Agrobiodiversity) under project no 26220120015 and by the project of Slovak Research and Development Agency APVV-0740-11 - Understanding of Plant Adaptation in the Radioactive Chernobyl Area.

LESS-KNOWN SMALL FRUIT SPECIES AND THEIR PROPAGATION USING *in vitro* TECHNIQUES

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Key words - *Amelanchier alnifolia*, *Lonicera kamtschatica*, micropropagation, iron source

Introduction

The *Amelanchier alnifolia* Nutt. (serviceberry, saskatoon berry) is a fruit bearing shrub native to the Canadian prairies and southern and northern territories of the United States. This fruit is potentially attractive because of his numerous advantages: frost-resistance, disease-resistance and decorative purposes (Ochmian *et al.,* 2013). But the most important reason for saskatoon growing is because of its high yields and fruit quality, which is very similar to blueberry. Ripe berries have dark-red, purple or black colour and contain high levels of vitamins C and B12. Besides vitamins they contain also significant amount of phenols, specifically anthocyanins, flavones and flavonoids. From minerals are the most important iron, potassium and magnesium (Mazza and Cottrell, 2008). Fruits can be eaten fresh, dried (usually with dry meat – pemmican) or be processed by production of syrups, marmalades, desserts or vines (Zatylny *et al*., 2005). *Lonicera kamschatica* (honeysuckle, sweetberry) is a deciduous shrub native to Russia and Japan. Similarly to saskatoon, it is frost- and disease-resistant and can be grown in lowlands as well as mountain regions (Sedlák and Paprštein, 2013). This species has edible fruits with elongated shape and dark-blue colour. They contain high amount of vitamin C (20–50 mg/100 g), vitamin B, anthocyanins and flavonoids. Several minerals (calcium, potassium, copper) are also present in elevated amounts (Plekhanova, 2000). Fruits can be eaten raw, or be processed to several products like jam, syrup or jelly. Berries can also be dried or frozen for later consumption. Honeysuckle is a first berry which is ripening already in the end of May (when cultivated in warmer regions) (Holubec *et al*., 2007). Both saskatoon and honeysuckle belong to the less-known small fruit species. Micropropagation as a method for obtaining significant amount of planting stock can be a first step for latter large-scale production of wider range of varieties.

Material and Methods

The aim of our work was to establish *in vitro* cultures of *Amelanchier alnifolia* var. *cusickii* and two cultivars of *Lonicera kamtschatica* 'Jugana' and 'Doč Velikana'. As the primary explants, dormant buds collected in October 2013 for *A. alnifolia* and nodal segments with one axillar or apical bud collected from actively growing shoots in March 2015 for *Lonicera kamschatica* were used. Sterilization was done as described: 1 min. in 70% ethanol and 5 min. in 0.1% $HgCl_2$ for *Amelanchier alnifolia*, and 1 min. in 70% ethanol and 5-7 min. in 30% solution of commercial product SAVO (contains

NaClO in concentration of 4.9 ml/100 ml) for *Lonicera kamtschatica* (5 min. for ´Doč Velikana´, 7 min. for ´Jugana´).

For *Amelanchier alnifolia* shoot initiation, MS medium (Murashige and Skoog, 1962) containing 8 $g.l^{-1}$ phytoagar and 30 $g.l^{-1}$ sucrose supplemented with 1 $mg.l^{-1}$ BAP was used. For shoot multiplication, BAP concentration was increased to 2 $mg.l^{-1}$ and auxin IBA was added in concentration 0.5 $mg.l^{-1}$. MS medium containing 1 $mg.l^{-1}$ IBA was used for rooting. Shoots obtained during multiplication stage were used for further experiments focused on shoot proliferation improvement. In the first experiment, several types of culture media (V1-V4) were chosen to analyze influence of medium type and growth regulators concentrations. V1 medium was MS 30 van der Salm (VDS) modification (Van der Salm *et al.*, 1994), V1-V3 were MS 30 media all supplemented with:

V1) 1 $mg.l^{-1}$ BAP + 1 $mg.l^{-1}$ IBA
V2) 0.5 $mg.l^{-1}$ BAP + 0.5 $mg.^{-1}$ IBA
V3) 1 $mg.l^{-1}$ BAP + 0.5 $mg.l^{-1}$ IBA + 0.1 $mg.l^{-1}$ GA_3
V4) 0.5 $mg.l^{-1}$ BAP + 0.5 $mg.l^{-1}$ NAA

For each medium type, 30 explants were analysed. After 1 month, the number of shoots for every variant was calculated. All results were statistically evaluated by analysis of variance (ANOVA).

For *Lonicera kamschatica* shoot initiation, MS medium (Murashige and Skoog, 1962) containing 8 $g.l^{-1}$ phytoagar and 30 $g.l^{-1}$ sucrose supplemented with growth regulators 2 $mg.l^{-1}$ BAP and 0.2 $mg.l^{-1}$ IAA was used. After 4 weeks, shoots were transferred to multiplication medium with 1 $mg.l^{-1}$ BAP and 0.2 $mg.l^{-1}$ IAA. After another 4 weeks, BAP concentration was increased to 2 $mg.l^{-1}$ and 36.7 $mg.l^{-1}$ FeNaEDTA was added to culture medium. All explants were incubated in growth chamber at 22 °C day/night temperature and 16h photoperiod.

Results and Discussion

In this study, *in vitro* cultures of *Amelanchier alnifolia* var. *cusickii* and two *Lonicera kamtschatica* cultivars ´Doč Velikana´ and ´Jugana´ were established. We have chosen different sterilization agents and time of sterilization for both species (Tab. 1). In case of *Amelanchier alnifolia*, we reported 32.04% infections and 67.96% healthy explants. From these, 33.01% of explants developed into shoots. In case of *Lonicera kamschatica* ´Jugana´, we reported 65% infections by sterilization with 30% SAVO solution for 7 minutes. From the rest, 5 explants developed into shoots, resulting in efficiency of 25%. By cv. ´Doč Velikana´, we have chosen only 5 min. sterilization compared to 'Jugana'. We have observed 41.18% infections, while the initiation was confirmed in 9 explants, thus resulting in efficiency 26.47%. Unlike 'Jugana', almost 33% of healthy explants did not develop into shoots. This could suggest strong genotype effect on shoot initiation. This observation has been reported before by Gawroński *et al*. (2013). Because of high contamination in 'Jugana´, we can conclude that 5 min. sterilization with 30% solution of SAVO is sufficient for successful *in vitro* cultures establishment. However, we have observed several problems during *Lonicera kamschatica* multiplication phase. After decrease of BAP concentration, the growth of shoots was retarded, the leaves manifested chlorosis and mild deformations. Therefore we had to increase BAP concentration to 2 $mg.l^{-1}$ and add FeNaEDTA, what resulted in improved

growth and vital, green shoots. These results are consistent with previously published articles from Dziedic (2008) and Karhu *et al*. (1997), who also reported positive influence of increased FeNaEDTA concentrations on shoot multiplication in *Lonicera kamschatica*. We are currently performing further experiments for determining multiplication ability for both cultivars.

In case of *Amelanchier alnifolia*, no visible problems were observed during initiation or shoot multiplication. Rooting was achieved on MS medium containing 1 $mg.l^{-1}$ IBA after 7 weeks of incubation with 35% efficiency. Subsequent acclimatization was efficient at 18%. Next, several types of culture media were chosen to analyze influence of medium type and growth regulators concentrations on shoot proliferation. The highest number of shoots, 298, was achieved with V3 variant containing 1 $mg.l^{-1}$ BAP, 0.1 $mg.l^{-1}$ IBA and 0.1 $mg.l^{-1}$ GA_3. On the other hand, V4 variant showed to be not suitable for shoot multiplication in *Amelanchier alnifolia*, with total of 106 shoots. However, NAA can be successfully used for breaking post-rooting dormancy, which commonly occurs in this species (Pruski *et al*., 1990). Statistical evaluation showed that V3 variant is significantly better for shoot proliferation compared to other variants (Tab. 2). We are currently performing further experiments focused on rooting of *in vitro* grown shoots. All obtained data can contribute to further optimization of *Amelanchier* spp. and *Lonicera* spp. micropropagation.

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Table 1 Results after sterilisation using different sterilization agents

Genotype	Sterilization agent	No. of explants (n)	Contaminated explants		Uncontamined explants that did not develope into shoots		Uncontaminated explants that did develope into shoots	
			n	%	n	%	n	%
A. alnifolia	5 min. 0.1% $HgCl_2$	103	33	32.04	36	34.95	34	33.01
L. kamtschatica ‘Jugana´	7 min. 30 % NaClO	20	13	65	2	10	5	25
L. kamtschatica ‘Doč Velikana´	5 min. 30% NaClO	34	14	41.18	11	32.36	9	26.47

Table 2 Influence of various medium types on shoot proliferation in *A. alnifolia* evaluated by ANOVA

Medium type	n	Shoot number/explant	Homogeneous groups		
V1	30	7.3000000		X	
V2	30	5.8666667		X	
V3	30	9.9333333			X
V4	30	3.5333333	X		

n = number of explants

COMPARATIVE ASSESSMENT FOR PHYTOEXTRACTION CAPABILITY USING DIFFERENT VARIETIES OF NON-HYPERACCUMULATOR PLANT SPECIES *Cannabis sativa* L.

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Key words - phytoextraction, non-hyperaccumulators, toxic metals, *Cannabis sativa* L.

Introduction

Hemp is the only general in the family *Cannabaceae.* There are several taxonomic views, but most famous is the division to three species *C. sativa, C. indica* and *C. ruderalis* (Clarke, 1999). Hemp represents a multipurpose crop and once the options industrial use are phytoremediation for relatively high uptake of heavy metals from the soil. First reports dealing with heavy metals uptake/accumulation by hemp were concentrated on the agrotechnological treatments (e.g. fertilization, liming) affecting heavy metal phyto-availability (Jurkowska *et al.*, 1990, 1992; Jasiewicz 1991) and toxic effect of metal elements on hemp plants (Gorlach and Gambus, 1992; Gorlach, 1994). Recently, several reports have seriously studied hemp phytoextraction potential of heavy metals (Löser *et al*., 2002; Linger *et al*., 2002; Angelova *et al*., 2004; Antonkiewicz *et al*., 2004; Kos and Leštan, 2004). The possibility of further industrial processing makes the flax/linseed and hemp economically interesting crops for farmers/operators of phytoextraction technology (Griga *et al*., 2003).

Materials and Methods

Experimental set up

The experiment was conducted at the agricultural research institute Agritec, Ltd. of Šumperk, located in the middle Europe in the North Moravia part of the Czech Republic at 49°58'21.213"N latitude, 16°58'0.341"E, longitude and 329 m above the sea level. Experiments were carried out in simulated natural conditions in pots setted in the land to a depth of 50 cm with contaminated soils sampled in insustrial brownfield. Following hemp varieties were used: ´Ferimon´, ´KC Dóra´, ´Futura´, ´Fedora´, ´Felina´, ´Beniko´, ´Tiborszallasi´, ´Tisza´, ´Epsilon´, ´Carmagnola´, ´Tygra´, ´CS´, ´Monoica´, ´Bialobrzeskie´, ´Uniko B´, ´Finola´.

Plant growth and harvesting

Plants were harvested after 90 days. Each uniform plant was rinsed in distilled water to remove soil particles and dust. One of grown plants - variety ´Carmagnola´ - was separated into leaves, stems, roots and seeds and was analysed by scanning electron microscope. Rest of the plants were kept in one piece and after were dried and analyzed by AAS. The results data were statistically analyzed by using the statistical package program Statistica, using analysis of correlation.

Analysis of plant mass

Atomic absorption spectrometry

The total concentration of metals in dry biomass/soil was determined by atomic absorption spectrometry AAS (SOLLAR M, Thermo Electron Spectroscopy Ltd, Cambridge UK) equipped with Zeeman and deuterium background correction, a graphite furnace GF95 and an auto-sampler. The digestion of plant materials was performed in a microwave oven operating system (Milestone, ETHOS D). Plant samples were digested using the optimatized microwave programs. Certified reference material NCS ZC73014 (GSB-7) tea (Beijing China) and CRM 9091 red clover ÚKZÚZ Brno were applied for quality assurance of analytical data

Energy dispersive X-ray spectroscopy

To obtain qualitative elemental information about heavy metals presence in plants tissues the Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX) was used. Dried samples – root, stem, leaves and seeds – were prepared by cutting into the small pieces and were placed to the SEM (QUANTA 450 FEG) and analysed. During microanalysis EDX all biological samples were analysed in the high-vacuum conditions and none of them was coated by metal layer.

Results and Discussion

Evaluation of plant growth

Amount of the final biomass of all grown *Cannabis sativa* L. varieties was quiet low. This fact appeared probably due to the high concentration of toxic metals in the soil what should be considered as inhibitive factor. The results in Table 1 show how many plants of each *Cannabis sativa* L. variety successfully grown. Also average length of each uniform plant is mentioned (Tab. 1). Futura variety has not been considerate due to low number of grown plants.

Plant metals uptake

Qualitative EDX microanalysis results show presence of different toxic metals in all analysed tissues of the *Cannabis sativa* L. plants – root, stem, and seed. Leaves samples analysis results were not considered. Plant metals uptake was evaluated according to the results of two analyses – EDX microanalysis and AAS analysis. EDX results show presence of different toxic metals in particular plants tissues.vEDX microanalysis was run only for tissues of one hemp variety – Carmagnola. In the analysed samples of root barium (18.5% Wt) was founded. Copper (32,7% Wt) and zinc (20,7% Wt) were traced in the stem samples and also in the seeds samples in order: copper (16,8% Wt) and zinc (12,9% Wt). All sixteen *C. sativa* L. varieties were analysed by AAS analysis. In Figure 1 results of this analysis are presented. In dry plant biomass zinc > copper > lead > and cadmium was founded.

The results of AAS analysis show that individual varieties hemp has different variability in ability to accumulate heavy metals from contaminated soil. By mutual evaluation of accumulation of monitored heavy metals there was found middle correlative dependence between Fe and Zn (r = 0.54) and Fe and Cu (r = 0.74), see Tab. 3. Correlative dependence was monitored MaE Mg and Cu, Fe, Cd, Pb, Co, Mo.

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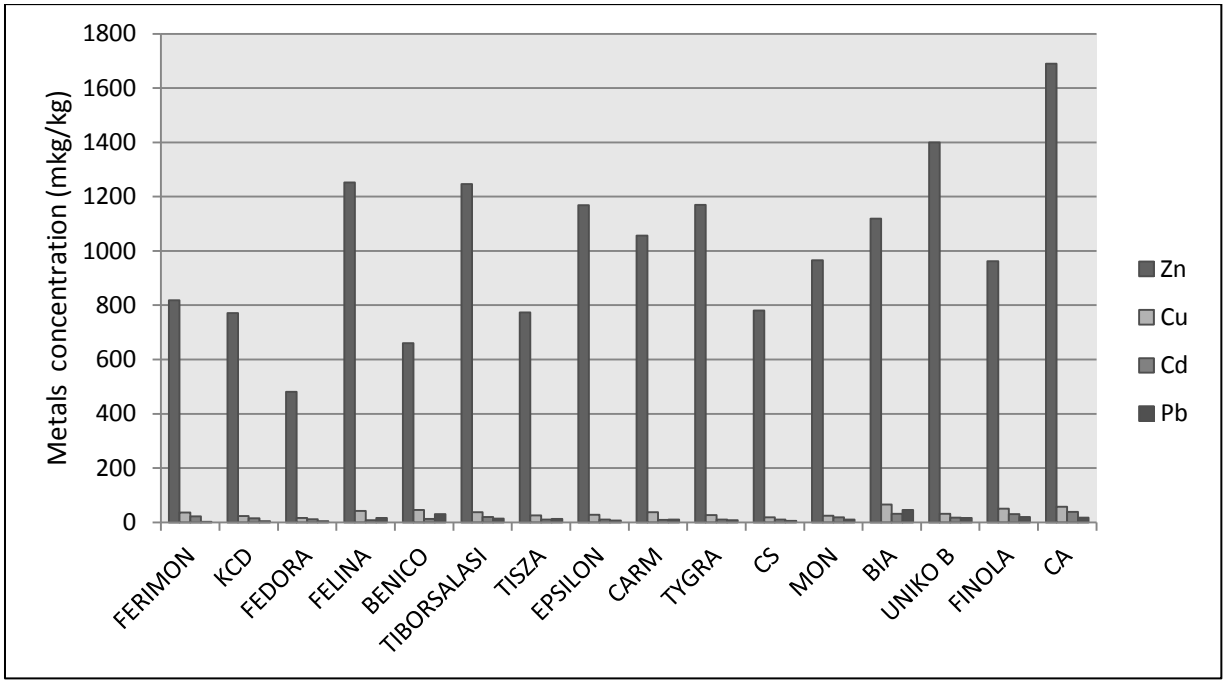

Figure 1 Metals concentration in each of *Cannabis sativa* L. variety

Table 1 Plant growth *Cannabis sativa* L. varieties

***C. sativa* L. varieties**								
	Bialobrzeskie	Futura	Epsilon	Felina	Tisla	Tygra	Uniko - B	KCD
Number of grown plants	8	2	26	7	4	12	13	21
Average shoot length (cm)	6,3	7,3	9,5	10,2	9,4	8,2	9,2	10,2
***C. sativa* L. varieties**								
	Fedora	Tiborszallasi	Finola	Ferimon	Monoica	CS	Beniko	Carmagnola
Number of grown plants	24	26	9	17	8	6	6	12
Average shoot length (cm)	10,5	10,1	10,7	9,8	8,8	11,1	9,2	10

Table 2 EDX microanalysis of different tissue samples

Spectrum (Wt %)														
Samples	C	O	Na	Mg	Si	P	S	Cl	K	Ca	Ba	Cu	Zn	Total
Root	51,2	20,2	0,67	0,28	0,43	0,46	3,91	1,22	2,53	0,71	18,5	x	x	100
Stem	35,2	11,5	x	x	x	x	x	x	x	x	x	32,7	20,7	100
Seed	59,9	9,7	x	x	x	0,31	0,19	x	0,24	x	x	16,8	12,9	100

Table 3 Correlation HMs between

	Cu	**Fe**	**Cd**	**Pb**	**Co**	**Mo**	**Mg**
Zn	0,34	0,54	0,14	0,2	0,02	-0,2	0,29
Cu		0,74	0,67	0,85	0,77	0,69	0,9
Fe			0,46	0,61	0,39	0,43	0,65
Cd				0,54	0,55	0,44	0,53
Pb					0,69	0,73	0,95
Co						0,68	0,75
Mo							0,7

ACCUMULATION OF HEAVY METALS Pb AND Cd BY *Amaranthus cruentus* L. PLANTS

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Keywords *- Amaranthus cruentus* L., Pb or Cd accumulation, root system, phytoremediation

Introduction

Many species of plants have been successful in absorbing heavy metals. There are the indications in the literature that *Amaranthus* species have the ability to accumulate heavy metals and radionuclides in their organs and they are also effective in degradation of some pesticides residues in the soil (Li *et al*., 2008). The aim of our study was to examine the accumulation of Pb and Cd in the *Amaranthus cruentus* L. plants - root system and stems with leaves after intoxication of Pb and Cd.

Material and Methods

For experiments, *Amaranthus cruentus* L. - new registered variety in the Slovak Republic under the name 'Pribina' was used. The plants were pre-cultivated in external environmental conditions and then whole plants with root system were collected, put into hydroponic containers and add to phyto chamber with standard setting conditions. Amaranth was contaminated with lead-once in different concentrations (0.1 M, 0.01 M, 0.001 M). The samples of plants were collected three times at weekly intervals after 7 days of cultivation with contaminated solution. The plants were dried and then the root and aboveground part (stems and leaves) were separated. The samples were analyzed by method atomic absorption spectrometry.

Results and Discussion

The content of accumulated Pb in the aboveground biomass varied depending on its concentration in the solution from 100.48 to 299.43 $\mu g.g^{-1}$ dry weight. The differences in amount of accumulated Pb among single solution concentrations were not statistically significant. The roots accumulated from 829.37 to 17 700.00 $\mu g.g^{-1}$ Pb dry weight. By analysis of variance, statistically significant differences in amount of accumulated Pb in roots in dependence on solution concentrations were found. Also, significantly higher accumulation of Pb was found in roots in comparison with aboveground biomass.

Accumulation of Cd was lower in comparison with Pb. Content of Cd in aboveground biomass reached 83.68 - 319.90 $\mu g.g^{-1}$ dry weight and in roots 102.69 - 232.81 $\mu g.g^{-1}$ dry weight. Statistical evaluation did not confirm significant differences in amount of accumulated Cd in dependence on its concentration in solution. Also, significant difference was not found in Cd accumulation between aboveground biomass and roots.

Phytoremediation is the direct use of living green plants for *in situ* removal of pollutants from the environment or for reduction of their concentrations to harmless levels (Salt *et al*., 1998). This study suggested that new registered variety of *Amaranthus cruentus* L. should be a potential hyper- accumulator of Pb in contaminated soil.

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Figure 1 Amaranthus plants in hydroponic containers containing Pb or Cd contaminated solution

STARCH VARIABILITY IN AMARANTH MUTANTS INDUCED BY RADIATION MUTAGENESIS

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Abstract
We have compared the weight of 1000 seeds, starch content and starch granule size in grains of two radiation-induced amaranth mutant genotypes 26 and 82 with a non-irradiated sample Ficha. Most stable performance of evaluated traits showed mutant genotype 82. Our obtained results are consistent with the results and data observed by other autors.

Keywords - amaranth, starch, starch granule, WTS, mutagenesis

Introduction

Over the last years there is a growing interest in crops and food products that are healthy and have medical and even curative effects on the human body. Neglected species such as amaranth (*Amaranthus spp.* L.), drawn attention especially for content of nutrients and for their exceptional dietary and therapeutic effects. Amaranth grain contains unusually low amonut of gluten protein and therefore amaranth grain can be used in gluten-free diet. It also contains other nutritionally important components e.g. content and composition of amino acids is almost comparable to animal proteins, thus amaranth could be a susbtitute of animal protein in the diet of vegetarians and vegans (Písařiková *et al*., 2005).

Starch is a major component of plant foods, is the most common carbohydrate and main calorie source. It is synthesized in the endosperm of seed as storage starch, or in the chloroplast during photosynthesis as a transition starch. Starch is the main component in grain *Amaranthus* seeds and contains two major types of biomacromolecules - amylose and amylopectin (Mikulová, 2008). Native starch granules usually contain 20-30% amylose and 70-80% of amylopectin. Varieties with an unusually high amylose content are used in the textile industry, in the production of adhesives, photographic film etc. The varieties with almost 100% amylopectin content (so called „waxy“ varieties) are used for the improvement of uniformity, stability, and texture in various food products. The clarity and viscous stability of amylopectin starch make it especially suitable for thickening fruit pies. It improves smoothness and creaminess of canned food and dairy products as well as freeze-thaw stability of frozen foods. It gives a more desirable texture and appearance to dry foods and mixes (Mikulová *et al*., 2008; Frigård *et al*., 2002).

Plant breeders create new species with different ratios of starch components. They regulate the physicochemical properties of the starch and this way also the functional properties of the bakery products.

The aim of the study was to analyze and compare the weight of 1000 seeds (WTS), starch content and starch granule size in the seeds of two amaranth (*Amaranthus cruentus* L.)

mutant genotypes 26 and 82, generated in the previous study by radiation mutagenesis (Gajdošová *et al*., 2007), and non-irradiated genotype Ficha.

Generally, content of nutritional and other biochemical parameters depend on the climate conditions in the year of cultivation. Therefore, we have analyzed the seeds collected from two growing seasons 2013–2014, grown at two different locations of Slovakia - Nitra and Prešov.

Materials and Methods

WTS (g) was calculated as the average of five independent measurements for each studied genotype and differences were evaluated using the Tukey test.

The percentage of total starch was done in triplicates and was performed by the Megazyme kit (AOAC Method 996.11, 76.13). The starch content were compared to standards with wavelength 510 nm.

The shape and starch granule size (μm) were observed in the amaranth seeds that reached the developmental phase of physiological maturity using a scanning electron microscope EVO LS 15 (Zeiss). The obtained microscopic micrograph were evaluated using the Axiovision Rel. 4.8.2. The average value was calculated from 200 measurements.

The obtained values of WTS, starch content and data from the starch granule size were compared to the original, non-irradiated genotype Ficha. Results were evaluated using statistical program Statistica 10 (StatSoft, Inc. 2011).

Results and Discussion

Evaluation of weight of thousand seeds

Generally, the highest and significantly different 1000-seed weight (0.97 g) we have observed in genotype 82 (already registered variety Pribina) grown in the year 2013 on location Nitra. The lowest value (0.83 g) was recorded for the control sample Ficha in the year 2014 on location Prešov ($p < 0.01$) (Tab. 1).

Mutant 26 has also a high, statistically significant WTS compared to non-irradiated control Ficha (0.93 g and 0.91 g, respectively) in both growing seasons at the same location Prešov.

Table 1 The stability performance over two growing seasons for 1000-seed weight in amaranth samples, grown in two different environments

Samples	2013		2014	
	Nitra	Prešov	Nitra	Prešov
Ficha (control)	0,86*	0,87*	0,84*	0,83*
mutant 26	0,89*	0,93**	0,89**	0,91**
mutant 82	0,97**	0,94**	0,94***	0,94***

(*) Significantly different (within each column) by Tukey test at the 0.01 probability level ($p<0.01$)

It is well known that the variability of the quantitative traits depends on both genetic and environmental factors. Yield parameters such as weight of thousands seeds, can be genotype property, but often depends on the climate conditions in the year of cultivation, culture system as well as agro-technical procedures (Vujacic *et al*., 2014). There are several studies that have previously dealt with the examination of this yield parameter in *Amaranthus cruentus* and *Amaranthus hypochondriacus* in conditions of Central Europe (Vujacic *et al*., 2014; Jamriška, 1996; Gimplinger *et al*., 2007). These authors present similar values of WTS as we obtained in our work for non-irradiated control sample Ficha.

Our two-year WTS analyses of amaranth genotypes, grown in two different locations of Slovakia, clearly showed that mutant 82 has ideal characteristics of a stable genotype with regard to important indicator of yield. Because of long-term significantly increased WTS this mutant was after successful completion of DUS trial registered as new large seeded amaranth variety Pribina in 2013.

Evaluation of starch content

It is assumed that the WTS, as a quantitative trait, can be increased at the expense of the quality attributes of the seeds, such as starch. Analysis of starch in the seeds of our mutants showing long-term increased 100-seed weight, is therefore very important. The amaranth seeds contain from 48 to 69 % of starch (Grobelnik Mlakar *et al*., 2009). According to literature, majority of amaranth varieties have generally less amylose and high content of amylopectin [waxy type, (Mikulová *et al*,. 2005)].

In seeds of our samples, content of starch varied between 42.65% and 46.55% (Fig. 1), which is consistent with results and data observed by other autors. The lowest starch content (42.65%) was found in seeds of mutant genotype 26 which was grown in 2014 on locality Nitra, while the highest content (46.55%) was observed in mutant genotype 82 grown in 2013 on the same location. Based on our results we can conclude that the content of starch in evaluated seeds were not reduced at the expense of quantitative properties represented by WTS.

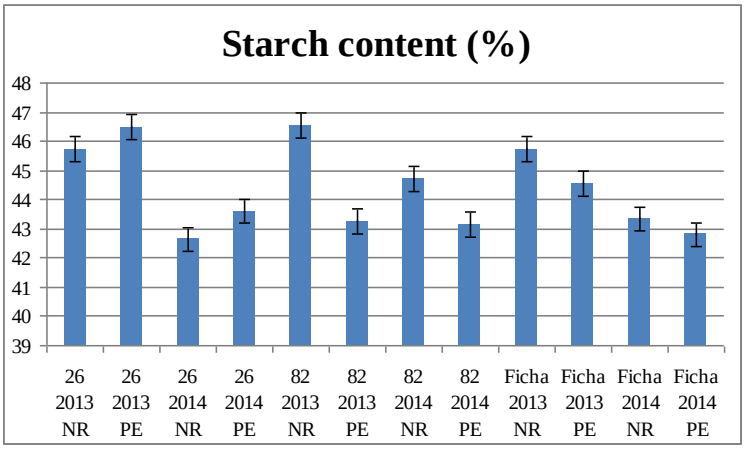

Figure 1 Total starch content in seeds of amaranth grown on two localities in Slovakia between 2013 and 2014

Starch granule size

As reported by Grobelnik Mlakar (2009) amaranth starch granules are extremely small (0.8 - 2.5 μm) compared to the size of e.g. rice (3 - 8 μm), wheat (3 - 34 μm), or maize (5 - 25 μm). Kong *et al*,. (2009) determined the size of the starch granules in a set of fifteen amaranth genotypes from 1.05 to 1.32 μm. Choi *et al*. (2004) reported that starch granules in Korean amaranths have polygonal shape and ranged from 0.8 to 2.0 μm.

The average size of starch granules in seeds of our amaranth samples ranged from 2.14 to 2.69 μm (Fig. 2). The highest average size we have observed in seeds of genotype 26 grown in the year 2014 on Nitra location. The smallest starch grains were observed in the same genotype but grown in the year 2013 also on location Nitra. This difference in the genotype 26 could be due to different climate conditions in 2013 and 2014. These results are similar with two older reports (Wolf *et al*., 1950; MacMaster *et al*., 1955) showing size of starch granules from 1 to 3.5 μm.

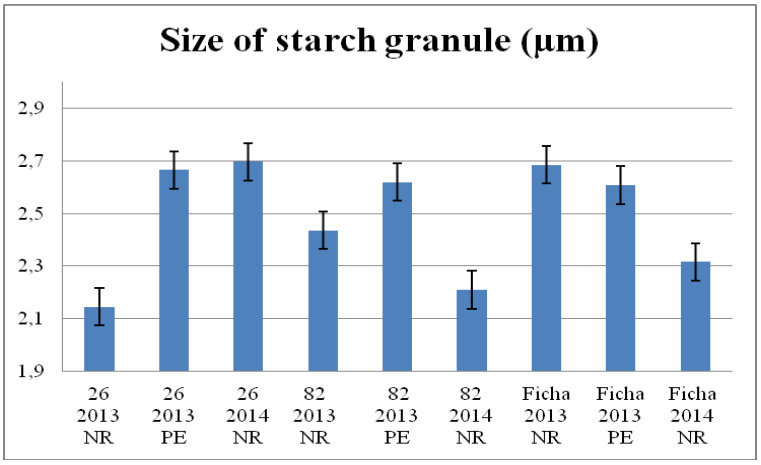

Figure 2 Size of starch granules in amaranth seeds grown on two research areas in Slovakia in 2013-2014. Samples of habitat through 2014 were not available, so measurements are not indicated.

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METHOD SUITABILITY FOR ISOENZYMES DETERMINATION IN AMARANTH

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Keywords - *Amaranthus* sp., isoenzymes, horizontal starch gel electrophoresis, isozymograms

Introduction

During the last five decades much work was done concerning enzyme polymorphism or plant enzyme multiplicity. Most of the work was focused on identification of plant enzyme polymorphism diversity for purposes of population genetics, selection and breeding. In this manner the plant species of high economical importance, such as maize, soybeen or sunflower, were intensively studied. This effort led to the creation of standardized analytical methodologies (e.g. Stuber *et al.*, 1988; Greneche and Giraud, 1989; Bourgoin-Greneche & Lallemand, 1993), which enabled unification and cooperation in analyzing and genetic interpretation of enzyme polymorphism. In spite of the considerable progress in enzyme polymorphism analysis in above mentioned species, amaranths belong to plant species which have not been so extensively studied. It is perhaps due to lover economic importance of this minor and underutilised crop or low allozymic variation among amaranth populations documented by research. Jain *et al*. (1980) presented a summary of studies based on morphological and allozyme markers and reported remarkable contrast between the high level of morphological polymorphism and almost monomorphic allozyme loci. The low allozyme variation in total 52 amaranth populations and two cultivars were described by Yudina *et al*. (2005). Genetic diversity and relationships within/among crop species and wild relatives of amaranth were generated by means of complementary approach using information from both isozymes and RAPD analyses (Chan & Sun, 1997). On the contrary, a high degree of polymorphism was found within and between populations of amaranth even in neighbouring populations, as characterized by different alleles or isoenzymes (Jacobsen & Mujica, 2003).

The main objective of the present study was to devote attention to testing the commonly used method reported by Stuber *et al*. (1988), slightly modified for enzyme multiplicity analysis for amaranths and to testing feasibility of chosen analysed organ weight, dimensions of Whatman No. 2 wicks and different volumes of extract buffer.

Materials and Methods

Plant material

Genotype Ficha of *Amaranthus cruentus* L. and mutant line D 279 of hybrid K-433 (*Amaranthus hypochondriacus* x *Amaranthus hybridus* L.) were selected for these experiments.

In the first experiment, dry seeds of different weight (10, 50, 100, 150, 200 mg), as well as three dimensions of Whatman No. 2 wicks (11 × 1.5, 11 × 2.0 and 11 × 3.0 mm) were tested.

In the second experiment, samples of seedlings after 1, 3 and 6 days of germination in the same weight as dry seeds have been used. The dimension of Whatman No. 2 wicks was uniform in the second experiment – 11 × 1.5 mm. For comparing mobility of zones of enzyme activities of amaranth with mobility of zones of maize coleoptile section enzyme activities,

extract from coleoptile section after five days cultivation of maize single-cross hybrid grain under the same cultivation conditions have been used. Plant extracts were obtained by crashing of samples using glass stick in mortar on ice with addition of several grains of pure sand (only for dry seeds of amaranth).

Electrophoresis

The standard technique of horizontal starch gel electrophoresis was used (Stuber *et al*., 1988). This method 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.), ß-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). Starch gels consisted of 77.31 g of hydrolyzed potato starch for electrophoresis, 15 g of sucrose and 600 ml of gel buffer (Stuber *et al*., 1988). The extracts were inserted into gels by means of paper wicks (Whatman No. 2) approximately 3 cm from the cathodic end. An electrophoretic separation was running in refrigerator at 4°C. After separation gels were cut horizontally into several thin slices (appr. 1.2 mm thick) and placed into boxes for staining of zones of enzymatic activity in buffer systems.

Results and Discussion

The main role for successful analysis and correct interpretation of enzyme polymorphism plays complete fingerprints clearly distinguishing each of their bands or spots. There exist many factors affecting he readability of isozymograms, such as cultivation through homogenization and extraction of biological material (chosen individual seed or seedling as sample, organ or tissue, ontogenetic stage, type of extraction buffer), starch gel preparing, immersion of wicks soaked by extract (Whatman No. 2 or 3 and wick dimensions), electrophoresis (constant voltage, power or current, time in hours), slicing of gel (thickness of gel plate) and gel staining (staining buffers and composition of staining solutions). From this point of view, the most important factor seems to be enzyme activity in analysed extract volume. Because of absence of the universal methodology for enzyme polymorphism analysis for the majority of plant species, there can be some problems when comparing results obtained in different laboratories. According to our and other laboratories experimental experiences, slightly modified methodology of horizontal starch gel electrophoresis published by Stuber et al. (1988) and Bourgoin-Greneche et al. (1998) for coleoptile section of maize seedling can be successfully utilised for study of enzyme multiplicity in pea, soybeen, grass pea, chickpea seeds and cotyledons of sunflower (Bourgoin-Greneche and Lallemand 1993; Múdry and Juráček 1998, 1999a,b; Múdry et al. 1998), coleoptiles, leaves and roots of maize seedlings, leaf and root tissues regenerated from anther culture of maize (Uváčková et al. 2008) or leaf tissues of sugar beet (Engelhardt and Múdry 1999; Engelhardt and Bežo 2000).

The effects of studied factors on the electrophoretic phenotypes and results of the influence of some modifications of experimental conditions on amaranth isozymograms and convenience of methodology published by Stuber *et al*., (1988) for enzyme polymorphism analysis of amaranths are summarized in Table 1.

All enzymes which were chosen for experiment belong to the most frequently studied enzymes with plant genotyping in genetics, breeding and seed improvement. Our results led us to the following conclusions: a) differences between amaranth seed and seedling fingerprint qualities are low but more practical for quick testing were 3 days cultivated seedlings; b) effect of Whatman No. 2 wick dimensions on quality of isozymograms was low. It is known that increasing width of wicks decreases one gel sample capacity; c) extract concentration number 5 (100/200 μl mg^{-1}) was the best for dry seed and seedling samples.

According to isozymograms (data not shown) was clear, that tested methodology is suitable for analyses of MDH, ADH, PGI, PGD, IDH, PGM and ACP multiplicity in amaranth species.

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Table 1 Effect of cultivation and amaranth seedling extract concentrations on quality of isozymograms

Genotype	*Amarantus cruentus* L. "Ficha"															*A. hypochondriacus* L. *x A. hybridus* L. "D 279"													
Cultivation (days)	1 day					3 days					6 days					1 day					3 days					6 days				
Number of sample / Enzyme	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
ACP	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
ADH	+++	+++	+++	+++	+++	++	++	++	++	++	+	+	+	+	+	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	++	++	++
CAT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GLU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++	++	++	++	++	++	++	++	++	+++	+++
GOT	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	-	-	-	-	-	+	+	++	++	++
IDH	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+
MDH	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
PGI	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
PGD	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
PGM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Abrevations:
+++ very strong band (spot) intensity
++ good band (spot) intensity
+ weak band (spot) intensity
- very weak nearly no band (spot) intensity
1, 2, 3, 4 and 5 number of sample weights in mg versus extract solution volumes in μ l (10/10; 50/50; 100/100; 150/100 and 200/100 mg μ l^{-1}, respectively)

Smallanthus sonchifolius (Poepp et Endtl) GERMPLASM INSIGHT USING RETROTRANSPOSON BASED MARKERS

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Keywords - iPBS markers, yacon, retrotransposon, germplasm

Introduction

Yacon (*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson), a native plant of the Andes, belongs to the family *Asteraceae* and is a traditional crop of the original population of Peru used in traditional medicine. Yacon has been neglected and underutilized crop for Europe. The facts that this crop has antidiabetic, nutritious and fertility enhancing properties change the view and increase economic value of this crop in European conditions. *Smallantus sonchifolius*; is a perennial herb that accumulates sugars in its parenchyma that possess some diet benefits (Milella *et al*., 2005). In South America, Bolivia, Brazil and Argentina, yacon roots and leaves are commonly consumed by people suffering from diabetes or various digestive or renal disorders and this ethnobotanical use was confirmed by recent scientific research (Aybar *et al.*, 2001; Simonovska *et al*., 2003). Genetic variability of yacon has been maintained *in situ* to date, almost by small farmes. Gene banks are located in Bolivia. Ecuador and Peru (Svobodová *et al*., 2013).

Here, the iPBS strategy was applied for *Smallanthus sonchifolius*, (Poepp. et Endl.) accessions characterization and discrimination based on the first results of the testing of retrotransposon repetitive sequences based universal markers, that are present in yacon genome (Žiarovská *et al*., 2013). Retrotransposons are applied widely for plant germplasm evaluation (Trebichalský *et al*., 2013; Guo *et al*., 2014) and are sucessfull even when limited or no information about the plant genomes are known (Mehmood *et al*., 2013; Duan *et al*., 2015) The method, that can be applied directly without any previous knowledge about the genome is iPBS developed by Kalendar *et al*. (2010).

Material and Methods

In total, 15 accessions of different provenience (8 from Peru; 5 from Equador and 1 from Bolivia) were evaluated. All of them were provided by the Institut of Tropics and Subtropics of the Czech university of Life Sciences, where it has been acquired since 1993 from different parts of the world. The plant material for DNA extraction was obtained from plants cultivated under the field conditions at the experimental base of the Department of the Genetics and Plant Breeding. For the purpose of molecular analyses only the young leaves without insect or another damages were chosen.

Total genomic DNA was extracted followed the procedure of Friar (2005). The integrity of gDNA was checked electrophoreotically in 1% agarose gel and its quantity was setting by Nanodrop Nanophotometer™.

iPBS primers that was tested previously (Žiarovská *et al*., 2013) were used for the yacon germplasm characterization following the optimized PCR conditions: PCR reactions were performed in a 15 µl reaction mixture with MyTaq™ Mix with 20 ng of DNA and 300 nM of iPBS primers. Amplification was performed in BIO-RAD

C1000™ Thermal Cycler under following conditions of gradient of the annealing temperature: 95°C 4 min (95 °C 1 min; 52 - 62 °C 1 min; 72 °C 2 min) 35x; 72 °C 10 min.

Results and Discussion

In spite of advancements in yacon morphological characterizations, the genetic diversity of the crop in molecular terms is still unknown. Yacon belongs to the organisms where only a very limited information about the genome sequences are known. That is why for the reliable detection of molecular markers only universal and sequence non-specific methods like RAPD (Random Amplified Polymorphic DNA), ISSR (Inter-Simple Sequence Repeat) or AFLP (Amplified Fragment Length Polymorphism) can be used. As yacon is still not characterized on the DNA sequence level, mainly non-specific methods like RAPD (Random Amplified Polymorphic DNA), ISSR (Inter-Simple Sequence Repeat) or AFLP (Amplified Fragment Length Polymorphism) were utilized for yacon germplasm evaluation. All of them were used for yacon sucessfully and present a substantial part of yacon molecular data actually available (Mansilla *at al*., 2006; Milella *et al*., 2011; Svobodová *et al*., 2013).

When RAPD and ISSR methods are used, the problems about reproducibility, low level of polymorphism and inter laboratory cross analyses need to be overcome. But all of them are still used as a start point for species with no or only a few informations about the sequences. All of them were tested for yacon germplasm evaluation and present a substantial part of yacon molecular data actually available (Milella *et al*., 2011; Svobodová *et al*., 2011). Here, using a set of universal primers that anneal to the conserved regions of retrotransposons, iPBS polymorphism of DNA was analysed for *Smallanthus sonchifolius*, (Poepp. et Endl.). For each of the used primers, UPGMA dendrogram was constructed based on the binary matrix that corresponds to the presence or absence of the separated PCR products in the agarose gels (Fig. 1). Five of the primers used in this study provide no specific banding pattern that dicriminate the accesions according some pattern. For two of the used primers, Peru accessions were separated in dendrograms from the Equador and Bolivia accessions (Tab. 1; Fig. 1). The UPGMA dendrogram showed a colse relationship among the analysed yacon accession, what is in concordance with is vegetative manner of reproduction.

Table 1 Primers used for iPBS fingerprinting and dendrogram construction for analysed accessions

Primer name	Grouping pattern
1846	unspecific
1880	unspecific
2078	provenience specific
1845	unspecific
1875	unspecific
1886	provenience specific
2080	unspecific

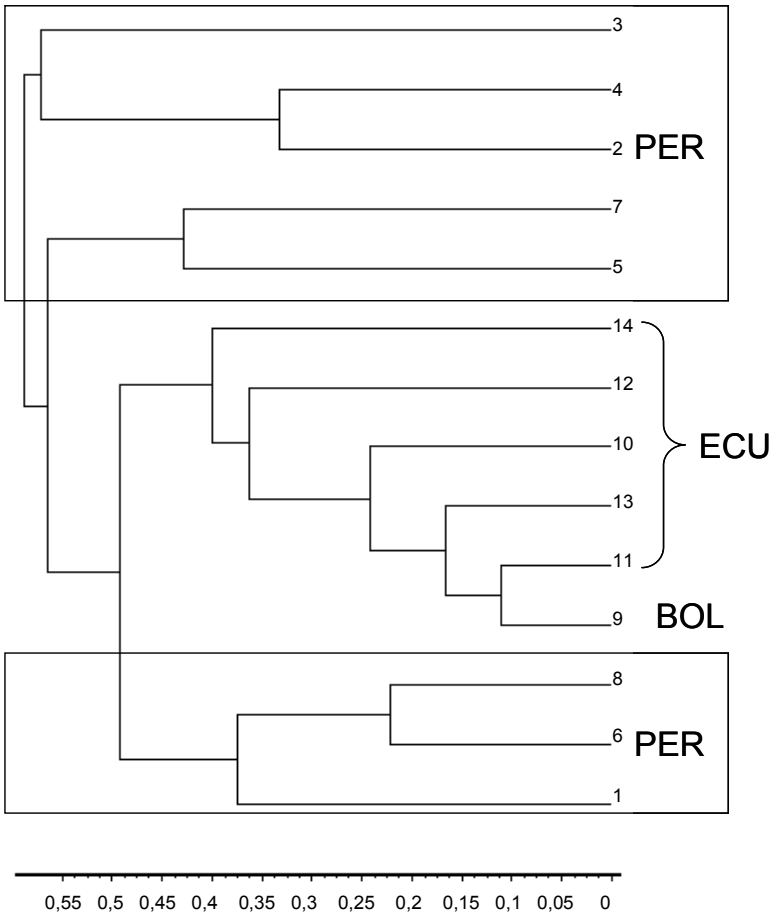

Figure 1 Dendrogram of yacon accession constructed by UPGMA for the iPBS primer 1886

The number of amplified fragment levels obtained by individual primers ranged from 10 up to the 17 and the polymorphism ranged from 80 up to the 100%. This result pointed the potenial of iPBS markers for the purpose of yacon germplasm discrimination and characterization, espec ialy for the reason of its vegetative reproduction and different ploidity level. Svobodová *et al*. (2013) reported only 80.3% of polymorphic band for the ISSR markers when applied for the yacon.

Mapping and analysing the genetic diversity of underutilized species is still an inevitable part for developing the conservation strategy of gene pools. Gathering of new information about neglected and underutilized crops has worldwide importance - to identify constraints in and possible solutions to the use of the crops, to identify possible untapped genetic diversity for breeding and crop improvement programs and to detect existing gaps in available conservation and use approaches (Herman and Heller, 1997).

The banding patterns obtained in iPBS depend on the relative abundance of differerent retrotransposon families as well as on their distribution with respect to one another (Kalendar *et al*., 2010) and up to now was applied in many plant species (Belogrudova *et al*., 2012; Trebichalský *et al*., 2013).

Here, the first insight into the yacon fingerprints patterns based on iPBS markers was performed with the result of a potential o distinguish the provenience of this underutilized specie.

Acknowledgment
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COMPARISION OF 2-DE PROTEIN MAPS OF POPPY (*Papaver somniferum*)

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Key words - 2-DE, protein maps, *Papaver somniferum*

Introduction

Papaver somniferum, the opium poppy, is the species of plant from which opium and poppy seeds are derived. The opium poppy is the only species of *Papaveraceae* that is an agricultural crop grown on a large scale. The opium poppy is the source of two food ingredients: poppy seed and poppyseed´s oil. The seeds contain very low levels of opiates, and the oil extracted from them contains even less. Both the oil and the seed residue also have commercial uses. In our country, opium poppy belongs to traditional crops of Slovak agriculture, even if in recent years a growing areas are very low (± 1000 ha) (Havrlentová *et al.*, 2014).

Two-dimensional gel electrophoresis (2-DE) is one of the most powerful and common tools for separation and fractionation of complex protein mixture extracted from tissues, cells, and other biological specimens. It is an orthogonal technique that allows separation of thousands of proteins in one gel and in a two tandem electrophoretic steps where a major proportion of proteins can be resolved for further analysis (Weiss and Görg, 2007; Vensel *et al.*, 2014).

The objective of our work was to prepare and compare two-dimensional gel electrophoresis protein maps of the opium poppy varieties originated from Slovakia and Hungaria.

Material and Methods

Plant material

Seeds from seven Slovak registrated (´Albín´, ´Bergam´, ´Gerlach´, ´Manor´, ´Malsar´, ´Maratón´, ´Opál´) and one Hungarian registrated (´Buddha´) varieties of the opium poppy were obtained from GeneBank of Slovak Republic in Piešťany.

Sample preparation

The first protocol used for extraction of the proteins was using phenol followed methanolic ammonium acetate precipitation (Hurkman and Tanaka, 2001). Plant tissue (100 mg) was homogenized well in the extraction buffer (0.1 M Tris-HCl pH 8.8, 10 mM EDTA, 0.4% 2 – mercaptoethanol, 0.9 M sucrose) and the same volume of 0.4 M phenol buffer pH 8.8 was added. This mixture was shaken vigorously for 30 min at 4 ºC and then centrifuged at 5000g for 10 min at 4 ºC. The upper phenol phase containing the proteins was collected very carefully. Ammonium acetate (0.1 M) was added five times to the volume of the phenol phase. Sample was mixed well and kept for precipitation overnight at - 20ºC. Next day, the mixture was centrifuged at 5000 g for 20 min at 4ºC, the supernatant was discarded and precipitates were washed in 0.8 M acetone twice and once in 0.7 M ethanol.

Two – dimensional gel electrophoresis

The samples dissolved in lysis buffer were taken such that their concentration reached to 0.1-2.5 $mg.ml^{-1}$ for 2-DE. This concentration of the sample was dissolved in rehydration buffer (8 M urea, 2 % CHAPS, 5 mM DTT, 0,2 % 3/10 ampholyte, 0,001 % Bromophenol

blue). This buffer was stored in small aliquots as per requirement at – 20 ºC. The last two ingredients (DTT and ampholyte) were added fresh to the rehydration buffer just before use. A total of 175 µl of rehydration buffer containing the sample was evenly distributed in the rehydration strip holder. The ReadyStrip™ IPG Strip 7 cm (pH 3-10, Bio-Rad) was placed on it and this assembly was allowed to rehydrate passively overnight. Current of 50 mA strip as applied. The focusing conditions were: step 1-500 V, step 2-1000 V, step 3-4000 V, step 4-8000 V. The focused strips were first reduced in equilibration buffer (6 M urea, 50 mMTris-HCl pH 8.8, 30 % glycerol and 2 % SDS) containing 50 mg DTT (added just prior to use) for 15 min on a gel rocker at room temperature. The reduced strips were then alkylated by adding fresh 1 g Iodoacetamide (IAA) at similar conditions. The reduced and alkylated strips were washed with 1x SDS buffer. These strips were then loaded onto 10 % SDS-PAGE without any stacking gel. This assembly was sealed using 1 % agarose sealing buffer. The gels were run, stained and destained just as for 1-D electrophoresis. The gels were scanned using GS-800™ Calibrated Imaging Densitometer (Bio-Rad) and analyzed using Delta2D program (Decodon).

Results and Discussion

Protein separation is a core part of proteomics analysis and two-dimensional gel electrophoresis is a basic and fundamental procedure to separate each protein from protein complexes. 2-DE with immobilized pH gradients (IPGs) combined with protein identification by mass spectrometry (MS) is currently the workhorse for proteome analysis. Two-dimensional gel electrophoresis has frequently been used to characterize the diversity of protein components. The first dimensional involves isoelectric focusing, in which proteins are fractionated across a specific pH range using commercially available pH gradient strips. The second-dimension fractionation resolves the proteins on the basis of molecular mass, using sodium dodecyl sulfate polyakrylamide gel electrophoresis (SDS-PAGE) (Skylas *et al.*, 2000).The aim of our study was to evaluate the electrophoretic profiles of storage proteins of the opium poppy seeds, which were obtained by two-dimensional electrophoresis.

Eight varieties of the opium poppy, originated from Slovakia and Hungary were evaluated for the number of detected spots (storage proteins) using 2-D electrophoresis (Tab. 1). The highest number of detected proteins was in the variety ´Buddha´ (342) and the lowest one in the variety ´Bergam´ (266). Likewise, the highest total volume of all detected spots was detected in the variety ´Buddha´ (2027) with the boundary relative volume of divided spots 1.0 % and the lowest one in the variety ´Bergam´ (1699 with the boundary relative volume of divided spots 0.5 %).

Our results also showed that many proteins of the opium poppy were focused over pH 3-10 and between 3.5-116 kDa (Fig. 1). The most abundant proteins were observed in the basic region of the gel. The huge cluster of proteins was identified in pH6-8 and between 10-116 kDa. This cluster was contained in each eight analyzed varieties. The most important difference between Hungarian highmorphine-basedcontent variety ´Buddha´ (Fig. 1a) and the Slovakian varieties represented by variety ´Malsar´ (Fig. 1b) was the cluster of proteins over pH 4-6 and between 100-200 kDa, what probably represented different origin of varieties.

Acknowledgment

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Table 1 Comparison of quantitative parameters of 2-DE protein maps

Genotype	N	V	V_{mix} (%)
Albín	291	1923	0.7
Bergam	266	1699	0.5
Gerlach	268	1720	0,5
Manor	292	1966	0.7
Malsar	293	1975	0.7
Maraton	287	1804	0.6
Opál	312	2008	0.85
Buddha	342	2027	1.0
x±σ	293,86±24,35	1890,25±130,69	0,69±0,17

N - the number of detected spots
V - the total volume of all detected spots
V_{mix} - boundary relative volume of divided spots

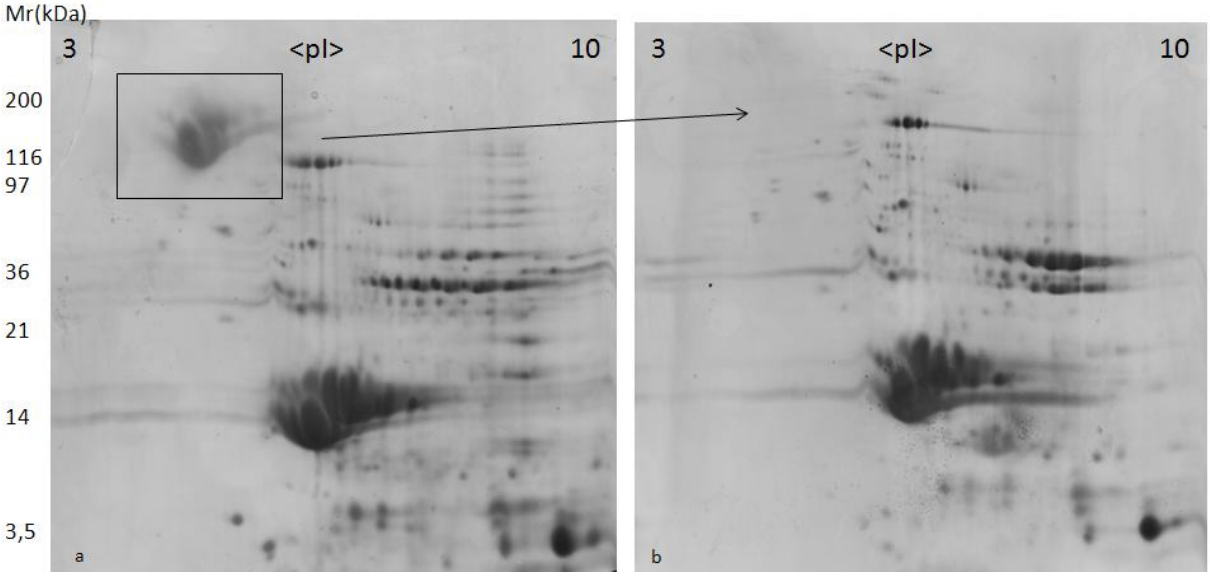

Figure1 Protein maps of the opium poppy: a – cv. ´Buddha´, b – cv. ´Malsar´

THE ROLE OF AFRICAN LEAFY VEGETABLES IN FOOD AND NUTRITIONAL SECURITY

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Keywords - Food security, indigenous vegetables, medicinal value, nutritional value

A wide diversity of indigenous leafy vegetable crops are grown and consumed in South Africa. The cultivated species of these vegetables and wild relatives are mostly grown by resource poor farmers for home consumption as well as income generation. The indigenous vegetables are main sources of vitamin A and C, iron, calcium, potassium and essential amino acids, lysine which makes them an important food sources to people who are nutritionally vulnerable. Despite this, the traditional vegetable crops are underutilized and no special attention has been given for their improvement and promotion. Therefore, this paper provides a general summary of the use of indigenous leafy vegetable crops and their impact and contribution to food security, nutritional value, local and national economy of South Africa.

CHEMICAL COMPOSITION AND ANTIOXIDANT CHARACTERISTICS OF MUNG BEAN (*Vigna mungo* (L.) Hepper) GENOTYPES IN TURKEY

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Key words - Mung bean, composition, under utilized plant

Introduction

Mungbean is one of the most important short season summer-growing legumes grown widely throughout the tropics and subtropics (Thomas *et al*., 2004), being well suited to different cropping systems. It constitutes important cereal-based diets to many people in India, Pakistan, Thailand, Indonesia, the Philippines, and China (Jansen *et al*., 1996). Mung bean is one of the most important neglected field crops in Turkey. More recently there is an increasing interest in Turkey to this plant due to its important human health properties.

Material and Methods

In this study seeds of 23 genotypes of mung bean (*Vigna mungo* L. Hepper) were investigated for proximate composition, antioxidant potential, fatty acids, tocopherols, and minerals profiles.

Results and Discussion

Methanol-extracted seed oil content of the investigated genotypes of mung beans ranged from 1.36% to 1.72%. Mung bean seeds were found to be a rich source of protein (18.22% to 33.42%). The mung bean seeds contained linoleic acid in the highest amount, followed by palmitic, oleic, linolenic, and stearic acids. The seeds were found to be a rich source of tocopherols (alpha, gamma, delta). Methanolic extracts of the seeds of the mung bean cultivars exhibited a good antioxidant activity and total phenolic content. The obtained results revealed that there were the big variation among genotypes in terms of composition and suggesting that they have potential source of essential fatty acids, antioxidants, and protein, all of which are linked with positive health benefits.

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CHEMICAL COMPOSITION AND ANTIOXIDANT CHARACTERISTICS OF WILD AND CULTIVATED BLACKBERRIES

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Key words - *Rubus*, blackberry, antioxidants, neglected plants

Introduction

In this study, some selected physico-chemical properties such as fruit weight, fruit length, fruit width, total soluble solids (TSS), titratable acidity, TSS/acidity ratio, pH, total phenolic content, antioxidant activity and free radical scavenging capacity of 9 cultivated and 16 selected wild blackberry (*Rubus fruticosus* L.) genotypes grown in Turkey were investigated.

Material and Methods

The total phenolic content, antioxidant activity and free radical-scavenging capacity of blackberry cultivars and genotypes were determined by using Folin-Ciocalteu, β-carotene bleaching and DPPH radical assays.

Results and Discussion

The results showed that, average fruit weight and fruit dimensions were higher in cultivated blackberries than wild materials. However, TSS, acidity and pH values were higher in wild materials. The total phenolic contents of blackberry cultivars and wild genotypes were in a range of 584 (cv.´Bartin´) to 788 (cv.´Chester´) mg/100 g and 610 (Genotype R2) to 1455 mg/100 g (Genotype R16), expressed as gallic acid equivalents (GAE), on a fresh weight basis. Antioxidant activity of cultivated and wild growing blackberry fruits was found between 72.15 (cv.´Arapaho´)-89.75% (cv.´Bursa3´) and 59.85 (R1)-87.42% (R10), respectively. The antioxidant activity of standard BHA was 85.07%. Different cultivars grown in same location consistently showed differences in antioxidant capacity. The results of this study outlines that the wild blackberry fruits tested are good sources of natural antioxidants compared to cultivated ones.

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ESSENTIAL OIL BEARING PLANTS IN TURKEY AND THEIR CHARACTERISTICS

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Key words - Essential oil, underutilized plants, germplasm, hydro distillation

Introduction

Turkey is designated within the seven countries that are known internationally for existence of medical and aromatic plants. The country is not only the gene-center for numerous plants but it also accommodates important endemic species. Today more than 9000 species are identified in Turkey's flora. About 1000 of these species are medical and spices plants. Essential oils used in many branches of industry such as cosmetic, food, chemical and medical industry are produced from medical and aromatic plants. Essential oils being purely natural material are valuable and indispensable raw materials of odors and tastes.

Material and Methods

The essential oils obtained from various plant species belongs to genera: *Origanum, Satureja,* and *Thymus* collected in Turkey. Those plants are known underutilized plants. The air-dried plant material was hydro-distilled according to the standard method described in the European Pharmacopoeia.

Results and Discussion

We determined main components of all mentioned *Origanum* species are carvacrol, thymol, p-cymene and y-terpinene. Carvacrol rich essential oils are of special commercial interest as ingredients in animal feed and for the preservation of food, because of their high potency as antibacterials and antifungal agents. *Thymus* contains thymol as major constituent. Carvacrol and/ or thymol were detected as main components in all taxa of *Satureja* growing in Turkey.

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SELECTED NUTRIENT ANALYSIS OF COMMON AND TARTARY BUCKWHEAT GENETIC RESOURCES

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Key words - ash; elements; fatty acids; fibre; proteins; X-ray fluorescence

Introduction

The genus *Fagopyrum* (family *Polygonaceae*) includes several different species, among which common buckwheat (*Fagopyrum esculentum* Möench) and tartary buckwheat (*Fagopyrum tartaricum* L. Gaerth) are mainly cultivated worldwide and used for foods. Consumption of the grains of common and tartary buckwheat, as part of an everyday diet, has increased over the past few years due to the number of health-beneficial properties (Bonafaccia *et al*., 2003). It is well established that both buckwheat types represent a rich source of high quality proteins, with a balanced amino-acid composition, dietary fibre, retrograded starch, high quality lipids, vitamins, essential minerals and antioxidants (Pongrac *et al*., 2010). Additionally, both buckwheats are gluten-free, and thus provide an important alternative nutritious food for people with celiac disease (Giménez-Bastida *et al*., 2015). The aim of the present study was to determine the chemical composition (crude proteins, crude fibre, crude ash, crude oils and fats), fatty acids composition and elemental content to get an overview of those nutrients in tartary and common buckwheat genetic resources from Slovenian plant gene bank.

Material and Methods

Eight common (*Fagopyrum esculentum* Moench) and eleven tartary buckwheat (*Fagopyrum tartaricum* L. Gaerth) genetic resources provided from Slovenian plant gene bank (Table 1) were grown in the experimental fields of the Infrastructure Centre Jablje, Agricultural Institute of Slovenia, Slovenia (304 m above sea level; 46.151°N 14.562°E). The mature grains were collected in September 2014. The dried grains, containing on average 12.8 % of moisture for common and 11.5 % of moisture for tartary buckwheat samples, were milled with a laboratory mill (Retsch ZM 200). The methods used for analysis of buckwheat samples were methods used for analysis of animal feed, either for raw components or compound feed. Moisture was determined by heating the samples at 103 °C for 4 hours (EC 152/2009 App. III A). Crude proteins were analysed using method ISO 5983:2, using factor 6,25; modified method ISO 6865 using FiberCap was used for the determination of crude fibre, for crude ash ISO 5984 was used, and crude oils and fats were analysed with petroleum ether extraction (152/2009 App. III H).

Fatty acid composition was determined using gas chromatography of fatty acid methyl esters (FAMEs). In the analytical procedure NaOH and BF_3 in methanol were used for transesterification (IUPAC method 2.301) and heptadecanoic acid (Sigma H 3500) as internal standard for quantification of fatty acids (IUPAC method 2.302). The solution of FAMEs was quantified on a gas chromatograph (Agilent 6890N, USA) with flame ionization detector.

Separation was carried out on column SPB PUFA (30m×0.25mm×0.2µm column; SUPELCO). Identification of fatty acids was carried out using a reference standard mixture of methyl esters of higher fatty acids (Lipid standard Sigma 189-19). The content of the fatty acids was expressed as mg/100 g dry weight and as the mass ratio of all of the fatty acids analysed.

The multi-element analysis was performed non-destructively using EDXRF spectroscopy. Pellets made from 0.5 g to 1.0 g of powdered sample material were prepared using a pellet die and a hydraulic press. The pellets were then analysed using an energy dispersive X-ray spectrometer composed of XR-100 SDD silicon drift detector (Amptek), PX5 digital pulse processor (Amptek) and lap top based digital acquisition software (DPP MCA, Amptek). For the excitation the disc source of Fe-55 was applied. The analysis of complex X-ray spectra was performed using the AXIL (Nečemer *et al*., 2008) spectral analysis program. Quantification was then performed using the Quantitative Analysis of Environmental Samples software (Nečemer *et al*., 2011). The estimated uncertainty of the analysis was 5 %. The data are expressed as $\mu g.g^{-1}$ DW (dry weight).

Results and Discussion

An average chemical composition of different common and tartary buckwheat genetic resources is presented in Fig. 1. All results are calculated to % dry weight. The average crude proteins content was 14.1 % dry weight for common and 12.2 % dry weight for tartary grains, while the crude fibre represented 16.6 % dry weight in common and 18.1 % dry weight in tartary buckwheat grains, respectively. Common buckwheat grains contained more crude proteins (+ 1.9 %) and less crude fibre (– 1.5 %) compared to tartary buckwheat grains. The crude ash content was on average 1 % higher in tartary buckwheat grains. Tartary buckwheat grains contained on average 0.2 % more crude oils and fats than common buckwheat grains.

The total amounts of the all fatty acids as the mg/100 g dry weight of the both types of buckwheat grains are reported in Fig. 2. The total fatty acid content varied considerably, from 1643 mg to 2525 mg/100 g dry weight. The data show differences among the samples and the type of the buckwheat. We identified and quantified seven fatty acids: saturated myristic (C14:0), palmitic (C16:0), stearic (C18:0) and arachidic (C20:0); and unsaturated oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3). Prevailing fatty acid in both buckwheat grains (data not shown) is linoleic acid (40.7 %), followed by oleic (35.6 %), palmitic (16.1 %), α-linolenic (3.2 %), arachidic (2.3 %), stearic (1.9 %) and myristic acid (0.3 %).

Eight different elements were obtained in this study of 19 common and tartary buckwheat samples, and these elements are Si, K, P, Al, S, Ca, Cl and Ti. Their concentrations are presented in Table 2. The highest levels among these elements was seen for K (from 4720 to 6480 $\mu g.g^{-1}$ dry weight), followed by P (from 3750 to 5380 $\mu g.g^{-1}$ dry weight) and Si (from 636 to 10400 $\mu g.g^{-1}$ dry weight), which varied the most among all elements. The less abundant elements in buckwheat grains were Ca (with an average 747 $\mu g.g^{-1}$ dry weight), Cl (with an average 150 $\mu g.g^{-1}$ dry weight) and Ti (with an average 51 $\mu g.g^{-1}$ dry weight).

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Table 1 List of common and tartary buckwheat genetic resources used for analyses

Buckwheat type	Genetic resource name	Sample number
Common	Darja (a)	CB1
	Darja (b)	CB2
	2012	CB3
	Čebelica	CB4
	KIS SVNDOL 2007/41	CB5
	Dolenjska siva (S)	CB6
	SVNOR 2010-14	CB7
	Bamby	CB8
Tartary	KIS VRHS	TB1
	26	TB2
	96	TB3
	61	TB4
	66	TB5
	156	TB6
	115	TB7
	116	TB8
	65	TB9
	213	TB10
	29	TB11

Table 2 Multi-element (mineral) composition of different common and tartary buckwheat grains

Element	Concentration (µg/g dry weight)							
Sample	Si	K	P	Al	S	Ca	Cl	Ti
CB1	8680	5310	4260	3500	1130	587	116	134
CB2	3490	6460	4520	1190	1020	517	121	58
CB3	3520	6040	5320	2100	1090	681	160	46
CB4	3580	5530	4890	1320	1260	655	114	57
CB5	2500	4980	4640	1220	1280	1030	106	39
CB6	1110	5720	4760	436	1070	677	127	9
CB7	1610	5280	4390	459	946	556	119	23
CB8	3450	5770	4780	1450	1100	642	124	65
TB1	9920	5460	3750	3170	1030	606	155	140
TB2	10400	6480	4260	4420	794	675	117	139
TB3	6280	5440	4330	3020	1270	582	144	112
TB4	2790	5900	5380	1310	1660	678	168	27
TB5	2010	5990	4740	1450	1430	484	160	21
TB6	636	5130	4490	734	1330	687	155	6
TB7	2090	5920	5010	828	1380	1150	265	29
TB8	858	5290	4480	816	1400	855	205	13
TB9	1114	4720	4570	779	1580	1190	133	14
TB10	699	5450	3980	405	1030	861	195	11
TB11	3030	5140	4110	1260	1190	1080	164	28

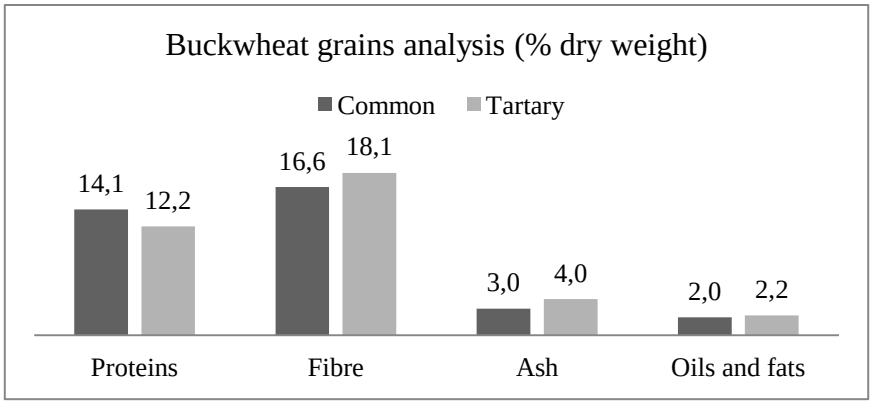

Figure 1 The average chemical composition of common and tartary buckwheat grain samples

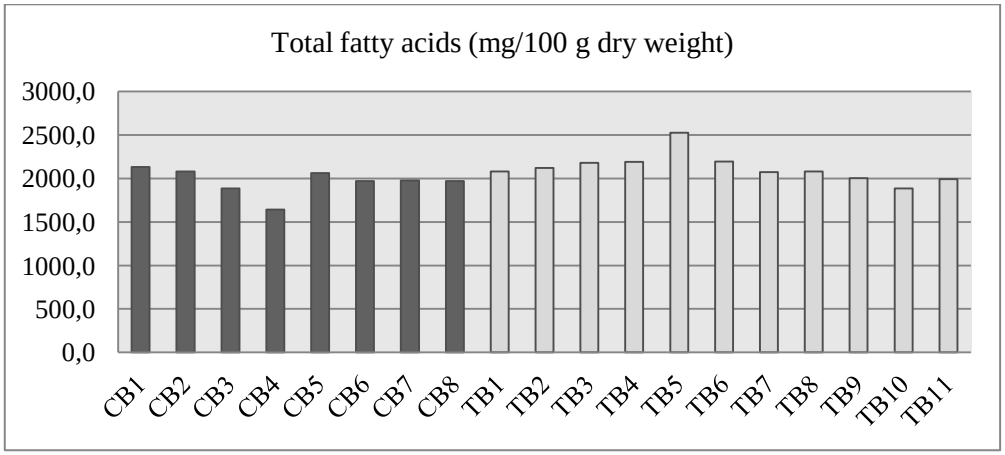

Figure 2 Total fatty acids content of common and tartary buckwheat grain samples

RAPID SCREENING FOR DETERMINATION OF HEAVY METAL-SENSITIVE PLANTS BY PLANT COMET ASSAY

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Key words - heavy metals, sunflower, *Verbesina encelioides*, alkaline comet assay

Introduction

Zinc (Zn) and lead (Pb) are heavy metals from contaminated environment. They are one of the main components found in waste waters of many industrial and mining productions. The accumulation in the soils can become dangerous to plants, causing many genotoxic effects (Pourraut *et al*., 2011). Zinc is essential microelement for normal plant growth, for root and stem elongation (Tao *et al*., 2014), but it is toxic for plants at high concentrations. Also, genetic variations in sensitivity to Zn toxicity have been marked in plants (Dong *et al*., 2006). Lead that usually occurs in uncontaminated soils, at high concentrations reduced strongly the plant growth, root elongation, seed germination, seedling development, transpiration, chlorophyll production and cell division (Aras *et al*., 2012).

The present study is a part of a sunflower research program for the production and evaluation of new intergeneric hybrids for transferring desirable traits from wild relatives to cultivated sunflower lines. In previous studies, a new sunflower hybrid *HA-Verbenc* with compact plant architecture, high drought tolerance and antioxidant activity was developed by wide hybridization between *H. annuus* L. and *V. encelioides* [(Cav.) Bentham & Hooker fil. ex Gray], (Vassilevska-Ivanova *et al*., 2013). Extending the previous analyses on the total antioxidant capacity and morphological characteristics of the *HA-Verbenc* line, here we have performed an alkaline plant comet assay to detect changes occurring in its DNA profile following lead (Pb) and zinc (Zn) treatment at selected concentrations.

Material and Methods

Plant material and treatment

Seeds of cultivated sunflower (*Helianthus annuus* L.) cv 1114, wild *Verbesina encelioides* (pollen source) and an advanced hybrid line *H. annuus* x *V. encelioides* (*HA-Verbenc*) were included in the current study. The line was developed in the Institute of Plant Physiology and Genetics, BAS, Sofia following conventional hybridizing methods (Vassilevska-Ivanova *et al*., 2013).

Three different concentrations of heavy metals (Pb and Zn) were applied for seed treatment: low, (50 mM), medium, (100 mM) and high, (150 mM). The lengths were measured at 5 days old seedlings. Morphological variations of seedlings produced under different concentrations were investigated. The highest concentrations of both heavy metals were selected for comet assay experiments.

Preparation of nuclei and alkaline plant comet assay

The procedure for nuclei isolation was basically as described by Van`t Hoff (1975) with minor modifications. The alkaline comet assay was carried out following the method described by Georgieva and Stoilov (2008). The first layer was made using 0.5% normal melting agarose and dried at room temperature. An aliquot of 40 µl of 0.5% low melting agarose mixed with 40 µl of the nuclei suspension was used to make the second layer. Each

drop was covered with a 22 x 32-mm coverslip and solidified on ice. Nuclei were subsequently lysed in lysis buffer (2.5 M NaCl, 10 mM Trizma base, 100 mM EDTA, 1% N-laurylsarcosin, pH 10, 10% DMSO, 1% Triton X-100, added freshly) at 4°C in dark for 1 hour. After lysis, slides were subjected to unwinding phase in the electrophoretic buffer (300 mM NaOH, 1 mM EDTA, pH> 13.0) for 15 minutes. Electrophoresis was conducted in the same buffer at 300 mA and 1 V/cm. Slides were neutralized using neutralizing buffer (0.4 M Tris, pH 7.5) for 15 minutes. After dehydration and drying, the DNA was stained with solution of the fluorescent dye acridine orange (10 $\mu g.ml^{-1}$).

Comet capture, image and statistical analysis

The comet`s measurements were examined in nuclei at 25X magnification using a fluorescent microscope (Zeiss Jenamed-2) coupled with a digital camera (Samsung Digimax V50) equipped with appropriate filters (510 nm excitation filter). More than fifty nuclei per slide were analyzed with 2 slides per treatment. All experiments were carried out in duplicate to take into account possible variations between different nuclei preparations. Results are expressed in terms of the percentage of DNA migrated from the comet head to the tail region (% DNA in tail).

Statistical analysis was performed using SigmaSTAT (Systat Software, Inc.). Considering that the data were not in line with the requirements for the application of parametric tests, differences between treatments were tested using Kruskal-Wallis test followed by Duncan test. Three levels were considered significant: $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results and Discussion

The genotoxicity of both heavy metals (Pb and Zn) was studied on the parental lines and *HA-Verbenc* hybrid line. The results showed a dose-dependent effect on coleoptiles and radicle lengths. The radicle length was more affected than the coleoptiles length and the root growth was more sensitive than shoots at all Pb-/Zn- concentrations. Zn had a stronger inhibitory effect on growth and morphogenesis than Pb (Figure 1).

The genotoxic effect of the heavy metals on the higher plants is connected with block in the active site and conformational changes in enzymes, which may cause oxidative damage by generating reactive oxygen species (ROS) (Aras *et al*., 2012). The relationship between ROS, DNA strand-breaks and chromosome aberrations were established (Pourrut *et al*., 2011). The comet assay is one of the newest methods used to identify DNA oxidative damage in plant species as it is capable to determine DNA strand breaks in individual plant nuclei. Applying this method, Gichner *et al*. (2008) were the first who demonstrated dose-dependent heavy metal (Pb)-induced DNA damage in tobacco plants. In the current study, both tested heavy metals (Zn and Pb) showed significantly different genotoxic effects on the all three investigated sunflower genotypes (Figure 2 A, B). The nuclei from control variants of cultivated sunflower *H. annuus* cv 1114 and the hybrid line produced the minimum DNA migration and were no significantly different in contrast with *V. encelioides* control. DNA strand breaks in *H. annuus* cv 1114 and in hybrid line exposed to 150 mM Zn were higher as compared to these treated with Pb at the same concentration. It might be supposed that these two genotypes possess similar sensitivity to zinc. An opposite effect was found in the wild parental species *V. encelioides*.

In conclusion, both heavy metals Pb and Zn induced changes in the genomic DNA-profiles of *HA-Verbenc* hybrid line and in its parents *H. annuus* and *V. encelioides*. The present study extends the current knowledge on the wide hybridization in sunflower, particularly on cross *H. annuus* x *V. encelioides*. Our data put in evidence that the alkaline plant comet assay is an appropriate method for detection the genotoxicity of environmental pollutants in plant model systems.

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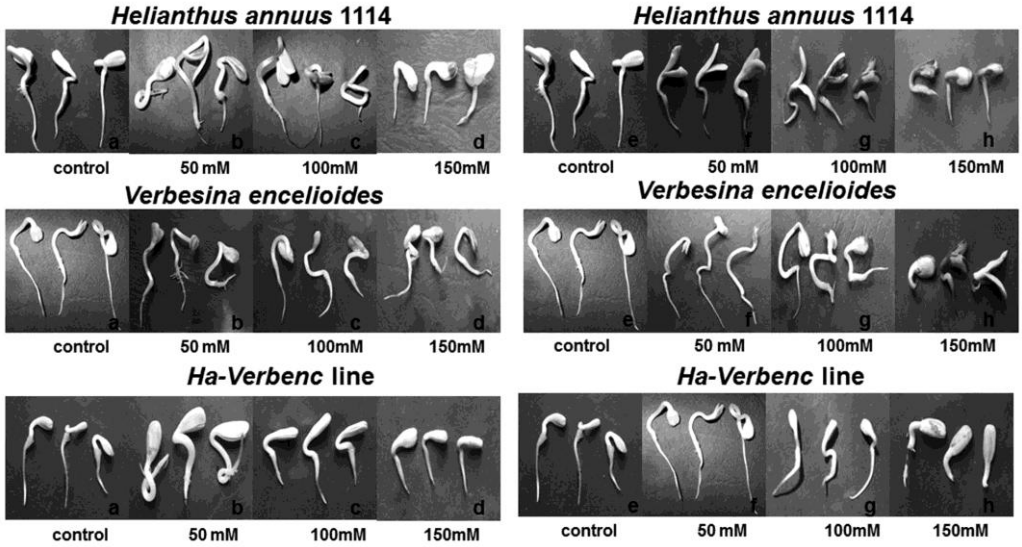

Figure 1 Five days old seedlings grown in Petri plates treated with different concentrations of heavy metals: a - control (Pb); b - 50 mM (Pb); c - 100 mM (Pb); d - 150 mM (Pb); e - control (Zn); f - 50 mM (Zn); g - 100 mM (Zn); h - 150 mM (Zn).

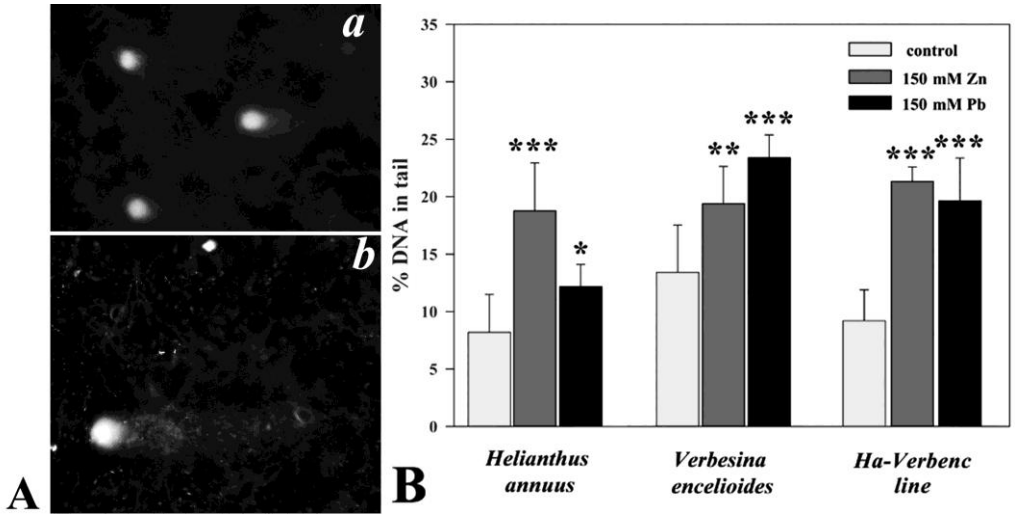

Figure 2 A. Typical DNA comets from *HA-Verbenc* hybrid line after treatment with Pb (a) and Zn (b).

B. Effect of zinc and lead-induced DNA strand-breakage in cultivated *H. annuus*, wild species *V. enceloides* and their hybrid line (*HA-Verbenc*). Each value represents the mean ± SD at least two separate experiments. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) (Kruskal-Wallis test followed by Duncan's test).

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