VOL. 7, NO. 11, NOVEMBER 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

GENOME CHANGES IN MUTANT LINES OF *Amaranthus* AS DETECTED BY MICROSATELLITE-DIRECTED PCR

Katarína Ražná, Jana Žiarovská and Mária Labajová
Faculty of Agrobiology and Food Resources, Department of Genetics and Plant Breeding, Tr. A. Hlinku, Nitra, Slovakia
E-Mail: jana.ziarovska@uniag.sk

ABSTRACT

Eight lines and two control genotypes of amaranth were characterized by unanchored and anchored microsatellite markers. The polymorphism of fragments length was evaluated for changes caused by γ-radiation in selected amaranth lines - *Amaranthus cruenthus* L. - genotype Ficha and Hybrid K-433 - the result of interspecific hybridization of species *A. hypochondriacus* x *A. hybridus*. Mutant lines were presented by the M8 generation of plants positively selected for weight of thousand seeds. The very specific changes of the mutant lines coding and noncoding regions were investigated for both the Ficha cultivar and K-433 hybrid. The obtained profiles sets confirm the active reaction of the lines to the gamma-radiance treatment. The molecular differences on length polymorphism of random microsatellite markers were observed and the changes of SPAR profiles of mutant lines when compared to control genotypes were confirmed. Primer Ama-AH 5 detected interspecific and primer Ama-AH 4 revealed intra - and interspecific polymorphisms, too. Primer Ama-AH 5 distinguished only the cultivar Ficha and hybrid K-433 accessions. Primer Ama-AH 4 created more polymorphic DNA profiles and has the ability to distinguish not only Ficha cultivar and hybrid K-433, but mutant accessions among themselves.

Keywords: *Amaranthus*, PCR markers, mutant lines, γ-radiation.

INTRODUCTION

The genus *Amaranthus* L. includes 60, according other authors 87 species and has a status of re-discovered plant with a potential of functional foodstuffs. Great interest is oriented on amaranth like pseudocereal, with nutritional value comparable or better like cereals, easily digestible starch, presence of cholesterol lowering fractions in seed oil, high carotene content in leaves, edible organic dyes, absence of gluten and absence of antinutritional factors like prolamins and gliadin. Compared with traditional crops, this pseudocereal is rich in protein with double the amount of essential amino acids than wheat grain protein (Bressani *et al*., 1992). The goals of general amaranth breeding programs define these problems: high rate of heterozygosity, low heritability for some important traits and disease resistance especially in the case of *A. caudatus*, L. Breeding goals on the nutrition level include proteins, fats, starch, vitamins and organic dyes content and composition. In the last decade properties and possible usage of amaranth grain evoke interest among scientists. Considering the agro-economic importance of amaranths, several studies on isozymes and various kinds of DNA markers have been performed to understand intra- and inter-specific genetic diversity and/or evolutionary relationships in amaranths (Lanoue *et al*., 1996, Chan and Sun, 1997; Sun *et al*., 1999; Pratt and Clark, 2001). Lee *et al*. (2008) reported the development and characterization of 12 microsatellite markers for *A. hypochondriacus* and their evaluation for cross-amplification in several wild species of the genus. The 12 loci were successfully amplified in 18 other amaranth species representing cultivated grain and vegetable species, their putative progenitors and wild species. Another approach based on the microsatellite is the use of unanchored primers directed toward micro satellites by using the SPAR (Single Primed Amplification Reaction) technique. It has, like the another random primers (e.g. RAPD) considerable appeal for surveying genomic variation because it is relatively inexpensive, utilizes arbitrary primers, and randomly samples a potentially large number of loci in a less complex pattern than other PCR-based markers (Das *et al*., 2001). Paran and Michelmore (1993) reported that RAPD products often contain repetitive DNA sequences. In addition to hybridization method radiation mutagenesis is employed like effective breeding tool. FAO/IAEA database listed more than 2300 radiation-modified plants including amaranth. Mutation technology was employed as a tool to create genetic variation in *Amarantus tricolor* in order to select lines with improved drought tolerance. The results of γ-radiance treatment were manifested in 1 mutant genotype with better grow vigor under the stress conditions and 2 mutant lines which retained more water in leaves under drought conditions compared to the wild type. Next research of mutant lines showed increased content of protein concentration. Also mutant lines were compared with wild type based on RAPD markers. From 19 arbitrary primers used, only two primer sets showed polymorphisms. The differences observed during the RAPD analyses of the two mutants as compared to the wild type, could be indicative of specific genomic areas possibly involved in drought tolerance (Kgang, 2008). Treatment by γ-radiation was used also for enhancing quality and quantity of amaranth grain of two selected genotypes: *Amaranthus cruentus* genotype 'Ficha' and hybrid K-433. Thanks to positive selection which was performed from 2nd to 8th mutant generation several putative mutant lines of *A. cruentus* and hybrid K-433

were selected characterized by highly significantly increased weight of thousand seeds (Gajdošová, 2008).

The objective of the present study was to investigate the genetic diversity and relationships in nine mutant lines and two control genotypes of *Amaranthus* using SPAR approach, with emphasis on testing the hyphotese on the influence of gamma-radiation to the length polymorphism of random microsatellite markers in both coding and noncoding regions of the *Amaranthus* genome.

MATERIALS AND METHODS

Plant material

Seeds of *Amaranthus* mutant plants positively selected for the weight of the thousands seeds, including control genotypes, were obtained from Institute of Plant Genetics and Biotechnology - Slovak Academy of Sciences in Nitra, Slovakia (Table-1). Only the mutant lines with the statistically confirmed increase of weight of thousand seeds were chosen as asseccions for developing the SPAR method to identify them. On average, ten accessions (7 days plants) were studied for intra- and inter-specific comparison of mutant lines and control genotypes. Individuals are characterized by statistically significant increase of weight of thousand seeds.

Table-1. Species and accessions of *Amaranthus* studied.

Species	Accession	Type	WTS (g)*
Amaranthus cruentus	Ficha cultivar	Control A	-
	C 15/1	Mutant line	0, 969
	C 26/2		1, 000
	C 26/3		1, 014
	C 27/5		0, 978
	C 82/1		0, 975
	C 236/1		1, 086
A. hypochondriacus x *A. hybridus*	K-433 hybrid	Control B	-
	D 54/1	Mutant line	0, 896
	D 279/1		0, 804
	D 282/1		0, 811

*weight of thousand seeds in M5 positively selected generation (based on data from Gajdošová and Libiaková, 2002; Gajdošová *et al*., 2005)

SPAR and ISSR analysis

Total genomic DNA was extracted from fresh young leaves of ten individuals to represent each accession for the SPAR or ISSR assay. The extraction was done using the kit Invisorb™ Spin Plant Mini Kit following the instruction of manufacturer. Obtained DNA was quantified by fluorometer (Qubit ™). All the reactions were performed in a buffer solution 1 × PCR containing 100 mmol × dm^{-3} Tris-HCl (pH 8.8), 500 mmol × dm^{-3} KCl a 1.5 mmol × dm^{-3} $MgCl_2$ (Applichem), together with 0.08 mmol × dm^{-3} d NTP (InvitrogenTM) 750-1000 nmol × dm^{-3} primers (depended on the used primer), 1 U Taq polymerase (Applichem) and 50ηg of template DNA. Primers used for the reactions are listed in the Table-2.

Table-2. Nucleotid sequences and characterization of applied primers.

Primer name	Marker	SPAR profile*	Differentition ability *
Ama-AH 1	$(GTG)_3CC$	monomorphic	-
Ama-AH 2	$(GT)_6CC$	monomorphic	-
Ama-AH 3	$(CTG)_3GC$	polymorphic	inter-specific
Ama-AH 4	$(GATA)_2(GACA)_2$	polymorphic	inter-specific; intra-specific
Ama-AH 5	$(GACA)_4$	polymorphic	inter-specific; intra-specific

* reported in this paper

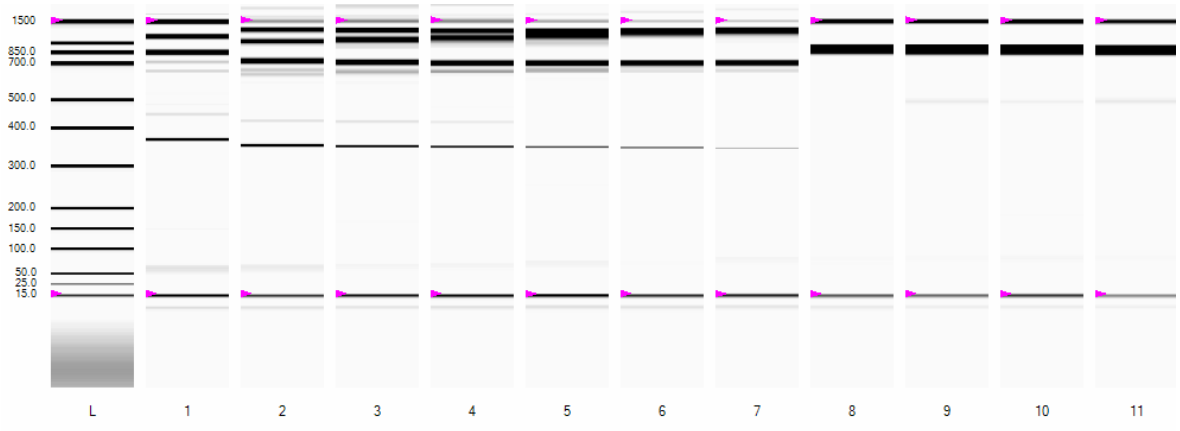
PCR amplification was performed in C1000 Thermal Cycler Biorad using the following cycle profile: 1 cycle at 95°C for 3 min followed by 29 - 32 (depending on marker) cycles at 95°C for 15 sec, 49.5°C for 40 sec, and 72°C for 2 min; and the reaction was terminated with a 7 min DNA extension stepat 72°C. Amplified fragments were electrophoretically separated in agarose gel (Applichem) together with 1 × TBE and Gel Red 10 000 × (Biotium). To determine the profile changes of the multiplicated fragment the control plants were used. Electrophoreograms were processed with documentation system G:Box in GeneSnap program - Product version: 7.09 (Syngene) and GeneTools - Product version: 4.01 (Syngene).

Data analysis

On the basis of DNA fragments separated according to the size by electrophoresis, the matrix of presence and positions of DNA fragments was prepared. Pair-wise dissimilarity matrices were generated by Jaccard´s coefficient of similarity (Jaccard, 1908) by using the SYNTAX software format. A dendrogram was constructed by using the unweigted pair group method with arithmetic average (UPGMA) with the HIERCLUS module of SYNTAX to show a phenetic representation of genetic relationships as revealed by the dissimilarity coefficient.

RESULTS

Different profiles were found among mutant lines and compared them to control genotypes, too. The accessions were analyzed using microsatellite markers of which two produced reproducible polymorphic banding patterns (Table-2). Ama-AH 3 gives a very specific profile for Ficha accessions consisted from 5 clearly separated monomorphic fragments. The identification of K-433 hybrid is performed by the only fragment with a length of 840 bp. Very clearly differentiation among *Amaranthus cruentus* and hybrid lines based on K-433 obtained by this marker is visible on the Figure-1. The ISSR profile is monomorphic among the species but not between them. Only the one abundant fragment is visible in case of hybrid K-433 and its mutant lines and three strong and visible fragments are in the ISSR profile of genotype Ficha and its mutant lines.



L - Marker; Ficha accession: 1- C 15/1, 2 - C26/2, 3- C26/3, 4- C27/5, 5- C82/1, 6- C236/1, 7- Control A; K-433 hybrid accessions: 8- D54/1, 9- D279/1, 10- D282/1, 11- Control B

Figure-1. Electrophoreogram of tested Amarant accessions using the primer Ama-AH 3.

Primers Ama-AH4 and Ama- AH5 give polymorphic profiles. A total of 11-13 band levels for the primer Ama-AH 5 and 14-16 for the primer Ama-AH 4 were obtained. Only the cultivar Ficha and hybrid K-433 accessions can be distinguished with primer Ama-AH 5 without the ability to reveal the polymorphism among their mutant lines (Figure-2).

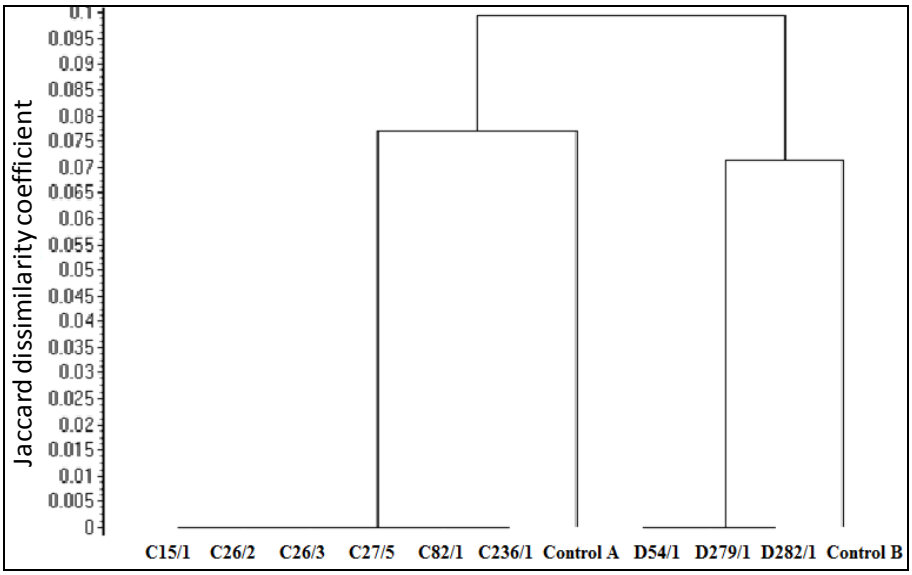
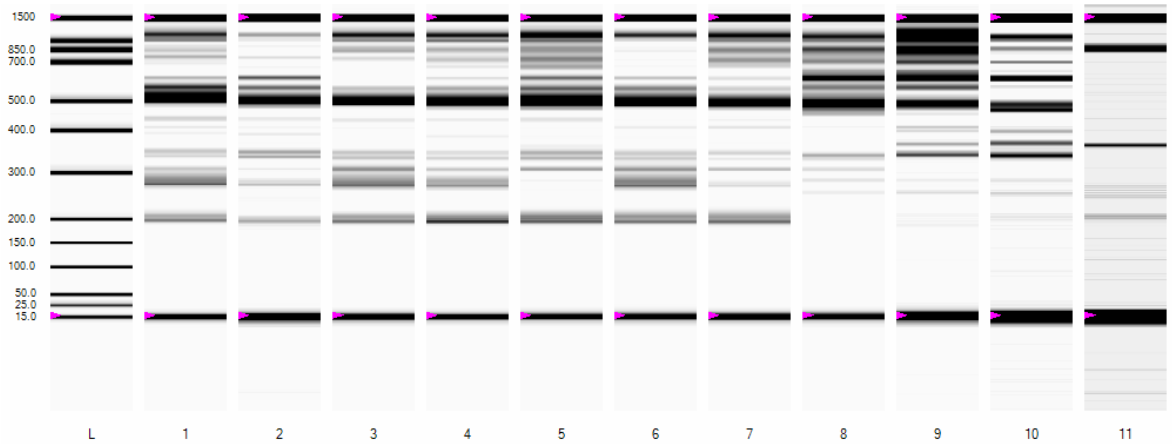


Figure-2. Dendrogram of Ficha accessions for Ama-AH 5 primer.

Primer Ama-AH 4 DNA fragments profile was more polymorphic compared to Ama-AH 5 and the primer has the ability to distinguish not only Ficha cultivar and hybrid K-433, but mutant accessions among themselves, too (Figure-3).



L - Marker; Ficha accession: 1- C 15/1, 2 - C26/2, 3- C26/3, 4- C27/5, 5- C82/1, 6- C236/1, 7- Control A; K-433 hybrid accessions: 8- D54/1, 9- D279/1, 10- D282/1, 11- Control B

Figure-3. Electrophoreogram of tested Amarant accessions using the primer Ama-AH 4.

Coding and noncoding length polymorphism based on microsatellites evaluated by Ama-AH 4 SPAR primer shows in the Ficha accessions dendrogram variability of tested mutans plants corresponding to the fact that they all are the offsprings and the only difference is the treatment of gamma-rays and the control genotype without this treatment.

Table-3. Intraspecific similarity (% Jaccard´s coefficient) among mutant lines of Ficha genotype based on Ama-AH 4 marker results.

Genotype	C 15/1	C 26/2	C 26/3	C 27/5	C 82/1	C 236/1	Control A
C 15/1	-						
C 26/2	57,9	-					
C 26/3	68,4	64,7	-				
C 27/5	65,0	61,1	72,2	-			
C 82/1	55,0	58,8	61,1	57,9	-		
C 236/1	52,6	56,3	58,8	55,6	52,9	-	
Control A	67,5	73,3	86,7	81,3	68,8	66,7	-

Based on the fragmet length polymorphism data, the Jaccard similarity coefficient was 63, 93% for the Ficha accessions and 86, 23 for the K-433 accessions. Variability of genetic coefficient values (Table-3) and dendrogram clusters corresponds to the analysed intergenic space of Ficha mutant lines compared to Control A as well as mutant hybrid K-433 lines compared to Control B (Table-4).

The Jaccard similarity coefficient was in all cases higher than 50% what shows the high level of the DNA fragments of teh same lenght in their intergenic space. As the most similar to the Control A was the mutant line C 26/3.

Table-4. Intraspecific similarity (% Jaccard´s coefficient) among mutant lines of K-433 hybrid based on Ama-AH 4 marker results.

Genotype	D54/1	D279/1	D282/1	Control B
D54/1	-			
D279/1	86,7	-		
D282/1	92,9	81,3	-	
Control B	86,7	76,5	93,3	-

Using Ama-AH 4 primer for hybrid K-433 accessions the results corresponds to those for Ficha. The Jaccard similarity coefficient is in the range of 76, 5 - 100% and DNA fragment profile was polymorphic in only 4 band levels. Dendrogram based of Ama-AH 4 primer PCR results shows very high intergenic SPAR profile similarity of C 282/1 and Control B genotypes.

DISCUSSIONS

Nowadays, molecular markers are used in a wide range of analyses (Faltusová, Kučera, Ovesná, 2011; Smýkal *et al*., 2011, Balážová *et al*., 2007, Heldák *et al*., 2007), so the potential for mutation radiance caused DNA changes is predictable. Mutagenesis is widely used method in plant breeding. Breeders use mutagenesis to improve characters and properties of plants and to increase genetic polymorphism within plant genomes. Mutagenesis experiments were realized by Morita *et al*. (2009) and Agrawal *et al*. (2001) with rice, Ramesh *et al*. (2001) and Zayats (2001) with barley, Das *et al*. (2001) with potato, but also by Kgang (2008) and Gajdošová *et al*. (2002, 2005) with *Amaranthus*. Plant genome reaction on γ-radiation was similar in the case of amaranth, poppy and fenugreek and the expression of mutagenesis has lead to the incremenent of seed yield. Furthermore, in the case of poppy seed and fenugreek the content of morphine and diosgenin were increased, too. However, there was not found any considerable change in biochemical seed composition due to γ-radiation in amaranth (Floria and Ichim, 2006a; Floria and Ichim, 2006b; Gajdošová *et al*., 2005, 2008). As the results of presented study shows, the polymorphism changes caused by γ-radiation in selected amaranth lines can be detected using PCR technique. Nowadays, different molecular methods are used to distinguish DNA polymorphisms of inter- and intra-species variability between plants. These techniques lead to mapping the whole spectrum from individuals to populations. Moreover, mutation breeding produces population of genotypes, which need to be sorted according genetic diversity. Ray, Roy (2009) determined genetic diversity and relationships among *Amaranthus* species by RAPD and SCAR markers. RAPD primers yielded a total of 262 amplicons, of which 96.94% were polymorphic. A mean similarity coefficient among all the *Amaranthus* species was 0.56. Ranade *et al*. (1997) evaluated inter- and intra-species variability present in the genus *Amaranthus* at the molecular level by RAPD molecular markers. To determine the polymorphism between individual samples, presented technique was shown as a suitable tool for the determination of the variability between related subjects (Souframanien 2002; Štefúnová and Bežo 2003; Joshi *et al*., 2004; Joshi *et al*., 2007) where the microsatellite based markers were used to distinguish parental lines and hybrids in rice, bajra, sorghum and sunflower hybrids. Distinguishing ability of SPAR primers used in this study was similar to results mentioned above. The primer Ama-AH 5 reveals inter-species DNA polymorphism between cultivar Ficha and hybrid K-433 without the ability to find DNA polymorphism among their mutant lines. DNA profiles were more polymorphic when comparing to the Ama-AH 4 primer. Ama-AH 4 primer provides the ability to observe inter-specific and intra-specific polymorphism, too. Consequently, Ama-AH 4 primer distinguished

cultivar Ficha and hybrid K-433 and also there was found variability among mutants. In comparison, the examination of genetic diversity and relationships between cultivated and wild *Amaranthus* species using isozyme and RAPD markers high levels of genetic diversity were found between species, but genetic uniformity was observed among selected genotypes (Chan, Sun, 1997).

Successful generation of randomly amplification primed micro satellite markers, and the number of fragments produced, is highly variable across studies and primers. The number of generated loci correlated to the number of bases in repeat units, but the number of bands produced by GA vs GT repeats differed and correlated with the known diversity of these micro satellites in rice (Blair *et al*., 1999). Competition during PCR may influence fingerprints profiles. This was demonstrated by two-primer experiments in which some bands identified by the primers individually were lost when the primers were combined (Huang and Sun, 2000; Liu and Wendel, 2001). Results of Lee *et al*. (2008) demonstrate wide potential applicability of these markers for the study of intra- and inter-specific genetic diversity as well as evolutionary relationships among cultivated and wild amaranths. The 12 loci were successfully amplified in all the tested species demonstrating wide potential applicability of these markers for the study of intra- and inter-specific genetic diversity as well as evolutionary relationships among cultivated and wild amaranths, which may be of considerable value for the conservation and use of amaranth genetic resources. The repeated motif of GA-CA rich regions in the amaranth genome has reported Lee *et al*. (2008), too. Kgang (2008) used mutation to create genetic variation in *Amaranthus tricolor* with improved drought tolerance. Mutant lines were compared with wild type based on RAPD markers. From 19 primers used, only two primers showed polymorphisms between the amaranth wild type and the two mutant lines. Joshi-Sacha and Gopalakrishna (2007) detected the polymorphism between a radiation induced Sesbania rostrata mutant and the parental genotype using three different marker systems. Of the 200 RAPD primers used, only 3% produced a polymorphism between the mutant and the parental genotype, whereas 12.5% of the AFLP primers and 15.7% of the SPAR primers produced polymorphisms. Similarly, SPAR method was used by Nolah *et al*. (2010) to find genetic diversity of A. *pumilus* populations. Genetic variation was detected among and within *A. pumilus* populations, according to the author's statement found genetic variability was low. Due to its desirable characteristics in plant breeding trials, genetic variation within *A. pumilus* was also compared to variation of grain varieties *A. hypochondriacus*, L. and *A. cruentus*, L. From the results follows, that genetic diversity within *A. pumilus* was lower than either grain species. The observed genetic variability provides information about population and its background. Accordingly, similarity of mutant population in comparison to untreated control, from which mutant population comes from, is high. Jaccard similarity coefficient reveals 74% average similarity between mutant population and untreated control of Ficha cultivar and 86% average similarity between K-433 mutant population and untreated control. The same collection of amaranth mutant lines was tested for possible change in biochemical composition by Hricová *et al*. (2011). Author's states that nutritional value of selected mutant lines in comparison with untreated controls remain unchanged. The highest result for nutritional value was observed in mutant line C82/1, which was 17% higher than control average and 22% higher than mutant lines average. Accordingly, γ-radiance has no big influence on studied intergenic and intragenic space of DNA. Further research should be done to find, which mechanism cause such visible change like is the change of weight of thousand seeds. The average of Jaccard similarity coefficient to the Control A untreatment genotype is 74, 05% with the level of the average of the same coefficient among the Ficha mutant lines of 59, 87% what indicates the effect of gamma-radiation on the changes that were realized under this abiotic stress factor. The average of Jaccard similarity coefficient to the Control B is 85, 5% and the average among hybrid K-433 mutant lines is 86, 97%, what means no significant difference in this group. This may be a result of a hybrid origin of tested accessions and better hybrid seed mass formation vigor, what affects both, coding and non-coding regions, too.

CONCLUSIONS

There is possibility to study the genome of mutant amaranth plants by molecular markers to distinguish γ-radiation induced changes on the molecular level when selected markers gain DNA unique profiles of concrete genotype and allows studying genetic similarity and dissimilarity among tested genotypes. *Amaranthus* accessions of control genotypes and mutant lines when analysed by microsatellite markers produced reproducible polymorphic banding patterns and with a potential to distinguish both, the species specifity and mutant lines specifity, too.

ACKNOWLEDGEMENTS

Biological material was kindly provided by Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Nitra. This project was created by realization VEGA 2/0109/09 - Exploitation of genomics and proteomics approaches in characterization of amaranth mutant lines and KEGA 001SPU-4/2012 Plant Genetic technologies.

REFERENCES

Agrawal P.K., Sidhu G.S. and Gosal S.S. 2001. Induction of bacterial blight resistance in elite Indian rice cultivars usány gamma-rays and ethyl methanesulfonate. Mut Breed New. 45: 17-19.

Balážová Ž., Vivodík, M., Gálová, Z. 2007. Determination of genetic relationships among wheat genotypes by

microsatellite markers. In: Acta Biochimica Polonica. vol. 54, suppl. 1, 2007, p. 45.
Blair M.W., Panaud O. and McCrouch S.R. 1999. Intersimple sequence repeats (ISSR) amplification for analysis of micro satellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor Appl. Gen. 98: 780-792.

Bressani R., Sánchez-Maroquín A. and Morales E. 1992. Chemical composition of grain amaranth cultivars and effects of processing on their nutritional quality. Food Rev. Int. 8: 23-49.

Chan K.F. and Sun M. 1997. Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species *Amaranthus*. Theor Appl. Gen. 95: 865-873.

Das A., Gosal S.S., Sidhu J.S. and Dhaliwal H.S. 2001. In vitro mutagenesis and production of agronomically useful potato variants. Mut Breed New. 45, 44.

Faltusová Z., Kučera L. and Ovesná J. 2011. Genetic diversity of *Brassica oleracea* var. capitata gene bank accessions assessed by AFLP. Electronic Journal of Biotechnology, North America, 14 9 05.

Floria A. F. and Ichim M.C. 2006a. High Yielding Opium Poppy (*Papaver somniferum* L.) Mutant Lines. Plant Mut Reports. 1: 28.

Floria A. F. and Ichim M.C. 2006b. Valuable Fenugreek (*Trigonella foenum-graecum* L.) Mutants Induced by Gamma Rays and Alkylating Agents. Plant Mut Reports. 1: 30-31.

Gajdošová A. and Libiaková G. 2002. Šľachtiteľský program pre kultúrne druhy láskavca. Nové poznatky z genetiky a šľachtenia poľnohospodárskych rastlín. Piešťany: VÚRV, ISBN 80-88790-25-5.

Gajdošová A., Libiaková G., Ostrolucká M. G. and Fejér J. 2005. Aplikácia radiačnej mutagenézy v šľachtení láskavca. Nové poznatky z genetiky a šľachtenia poľnohospodárskych rastlín. Piešťany: VÚRV, ISBN 80-88790-43-3.

Gajdošová A., Libiaková G., Ostrolucká M. G. and Fejér J. 2008. Mutation breeding inselected *Amaranthus* spp. Amaranth - Plant for the Future. Nitra: SAV. pp. 93-94.

Heldák J., Bežo M., Štefúnová V. and Galliková A. 2007: Selection of DNA markers for detection of extreme resistance to potato virus Y in tetraploid potato (*Solanum tuberosum* L.) F1 progenies. Czech J. Genet. Plant Breed. 43(2007): 125-134.

Hricová A., Kečkešová M., Gálová Z., Libiaková G. and Gajdošová A. 2011. Skúmanie zmien profilu bielkovín v semenách láskavca podrobených radiačnej mutagenéze. Chemické listy. 105: 542-545.

Huang J.C. and Sun M. 2000. Fluorescein PAGE analysis of micro satellite-primed PCR: a fast and efficient approach for genomic fingerprinting. Bio Techniques. 28: 1069-1072.

Jaccard P. 1908. Nouvelles researches sur la distribution florale. Bulletin de la Société vaudise des sciences naturelles. 44: 223-270.

Joshi-Saha A. and Gopalakrishna T. 2007. Inter Simple Sequence Repeat Markers for Detecting Radiation Induced Polymorphisms and its Application as Genetic Marker System in Sesbania rostrata (Bremek. and Obrem). Plants Mut. Reports. 1: 41-45.

Joshi A., Souframanien J. A., Manjaya J., Gopalakrishna T. and D'Souza S.F. 2004. Utilization of inter simple sequence repeat markers in distinguishing parental lines and hybrids in selected crop plants. The Indian Journal of Genetics and Plant Breeding. 64: 181-184.

Kgang I.E., Emmenes L., Laloo N., Kunert K., Matole N. and Schluter U. 2008. Characterization of drought tolerant A. tricolor mutant plants. Amaranth - Plant for the Future. Nitra: SAV. pp. 66-69.

Lanoue K.Z., Wolf P.G., Browning S. and Hood E.E. 1996. Phylogenetic analysis of restriction-site variation in wild and cultivated *Amaranthus* species (Amaranthaceae). Theor Appl Genet. 93: 722-732.

Lee J.R., Hong G.Y., Dixit A. *et al*. 2008. Characterization of micro satellite loci developed for *Amaranthus hypochondriacus* and their cross-amplifications in wild species. Conerv Genet. 9: 243-246.

Liu B. and Wendel J.F. 2001. Intersimple sequence repeats (ISSR) polymorphisms as a genetic marker system in cotton. Molecular Ecology Notes. 1: 205-208.

Morita R., Kusaba M., Ilda S., Yamaguchi H., Nishio T. and Nishimura M. 2009. Molecular characterization of mutations induced by gamma irradiation in rice. Genes Geget Syst. 84: 361-370.

Nolah C., Noyes A., Bennett A., Hunter R. and Hunterl K. L. 2010. Inter Simple Sequence Repeats (SPAR) Reveal Genetic Variation among Mid-Atlantic Populations of Threatened *Amaranthus pumilus* and Phylogenetic Relationships. Castanea. 75: 506-516.

Paran I. and Michelmore R.W. 1993. Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. Theor Appl. Genet. 85: 985-993.

Pratt D.B. and Clark L.G. 2001. *Amaranthus rudis* and *A. tuberculatus* - one species or two? Journal of the Torrey Botanical Society. 128: 282-296.

Ramesh B.B., Prasad K. and Singh V.P. 2001. Semidvarf, high yielding and high protein mutant in barley. Mutation beeding newsletter. 45, 26.

Ranade S.A., Kumar A., Goswami M., Farooqui N. and Sane P.V. 1997. Genome analysis of amaranths: Determination of inter- and intra-species variations. Jour Bioscie. 22: 457-464.

Ray T. and Roy S. C. 2009. Genetic Diversity of Amaranthus Species from the Indo-Gangetic Plains Revealed by RAPD Analysis Leading to the Development of Ecotype-Specific SCAR Marker. Journal of heredity. 100: 338-347.

Smýkal P., Bačová-Kerteszová N., Kalendar R., Corander J., Schulman A.H. and Pavelek M. 2011. Genetic diversity of cultivated flax (*Linum usitatissimum* L.) germplasm assessed by retrotransposon-based markers. Theor Appl. Genetics 122: 1385-1397.

Souframanien J., Pawar S.E. and Rucha A.G. 2002. Genetic variation in gamma ray induced mutations in blackgram as revealed by RAPD and SPAR markers. Ind Jour Genet Plant Breed. 62: 291-295.

Štefúnová V., Bežo M. 2003. Genetic diversity analysis of amaranth (*Amaranthus cruentus*) germplasm collection by RAPD. Biologia. 58: 53-58.

Sun M., Chen H. and Leung F.C. 1999. Low-cot sequences for fingerprinting analysis of germplasm diversity and relationships in Amaranthus. Theo Appl. Genet. 99: 464-472.

Zaytas O.M. 2001. Winter barley mutants created in the Ukraine. Mut Breed Newsll. 45, 27.