JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THE EFFECT OF MICROSATELITTE CORE SEQUENCE IN THE DIFFERENTIATION OF *AMARANTHUS CRUENTUS* GENOTYPES

Matúš Kyseľ, Veronika Štefúnová, Milan Bežo, Jana Žiarovská*

Address(es):
Department of Genetics and Plant Breeding, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra; Tr. A. Hlinku 2; 94976, Nitra, SR.

*Corresponding author: xkysel@is.uniag.sk

doi: 10.15414/jmbfs.2018.7.6.607-610

ARTICLE INFO

Received 13. 3. 2018
Revised 11. 5. 2018
Accepted 16. 5. 2018
Published 1. 6. 2018

Regular article

OPEN ACCESS

ABSTRACT

A collection of *Amaranthus cruentus* accessions was analysed to compare the characteristics of different dinucleotide core sequences of microsatellites repeats. ISSR analysis was performed to obtain corresponding genetic fingerprints and based on them, to construct dendrograms for every individual primer used in the study. A total of eighteen accesions were analysed. Dinucleotide sequence of $(CA)_6$ repetition anchored by GG bases resulted in a total of 65 DNA fragments and 100-percent polymorphism. This marker has differ completely only 10 from all of the analysed genotypes. Dinucleotide sequence of $(CT)_8$ repetition anchored by AC bases has resulted in the amplification of totally 97 fragments and 84.62 % of polymorphism. Only seven accesions were different among themselves when regarding this microsatellite core sequence. Dinucleotide sequence of $(GA)_6$ repetition anchored by CC bases has resulted in the amplification of totally 171 DNA fragments and 100 % polymorphism. Not all the genotypes were separated based on this marker, two of them - Ames 5638 RRC 1139 and Ames 5648 RRC 1148 - possess the same profile of amplified ISSR fragments. The most distant genotype separated by all of the used primers was a landrace PI 511876 Huatle originated in Mexico.

Keywords: *Amaranthus cruentus* L., dinucleotide microsatellites, ISSR, core sequence

INTRODUCTION

Microsatellites, a unique untranscribed sequences through the entire genome defined by 4 parameters (motif, frequency and variability of motifs, form of interaction between single motifs and/or their copies and length of motifs and microsatellites). Their stability is a result of DNA recombination by unequal crossing-over or gene conversion while replication slippage costs losses or gains of a few copies and together affect a mutation rate decrease and improve sequence purity. Microsatellites or simple sequence repeats and short tandem repeats (SSR and STR) has no limits in genome appearence. Primarily, SSRs and STRs are parts of non-coding regions mostly AT-rich DNA, but they were found also in genes and pseudogenes which spread them by their own activity throughout the genome almost well as transponable elements.

Kalia et al. (2011) split microsatellites based on number of nucleotides per repeats into 6 classes: mononucleotide $(A)_n$, dinucleotide $(CA)_n$, trinucleotide $(CGT)_n$, tetranucleotide $(CAGA)_n$, pentanucleotide $(AAATT)_n$ and hexanucleotide $(CTTTAA)_n$ (n = number of variables) and at the same based on location of SSRs in the genome into nuclear (nuSSRs), chloroplastic (cpSSRs) and mitochondrial (mtSSRs). The most common motifs in plant and fungal genomes are poly(A)s, poly(T)s, $(AT)_n$, $(AC)_n$ motifs and $(GATA)_n$ sequences. In rice, sequences containing $(CGG/GCC)_n$ were most frequently identified, with the exception of $(GC/CG)_n$, $(AGT/TCA)_n$ and $(GACC/CTGG)_n$ (**Miah et al., 2013**). Among all 35 annotated EST-SSR markers assessed for cross transferability in the selected plants (*Datura metel* L. (86.45 %), *Datura innoxia* Mill. (81.29 %), *Withania coagulans* Stocks (96.77 %), *Withania somnifera* L. (98.06 %), *Capsicum annuum* L. (85.16 %), *Stevia rebaudiana* Bertoni. (34.84 %), *Eclipta alba* L. (49.68 %), *Citrullus colocynthis* L. (54.19 %), *Ocimum sanctum* L. (43.23 %), *Catharanthus roseus* L. (58.71 %), and *Moringa oleifera* Lam. (58.66 %)), tri-nucleotide repeats AAG/CTT, CCG/CGG, and AGC/CTG were highly abundant followed by tetra-nucleotide AAAT/ATTT and AAAG/CTTT, di-nucleotide AG/CT and AT/AT, and penta-nucleotide repeats AAAAC/GTTTT (**ul Haq et al., 2014**).

There are only 30 % suitable primers/markers for analyses. So for future of these techniques is „transferability" the breakpoint. It requires precise sequences in related species for exchange microsatellites, because development a new ones is too much expensive. There already exist a similarity in flanking regions but only in few and very related species as triticale, oat or rye (**Yildirim et al., 2009**). SSRs arose from ESTs, called EST-SSRs are more consistant, significant and transferable into rice (**Cho et al., 2000**), barley (**Thiel et al. 2003**), sugarcane (**Cordeiro et al., 2001**), grape (**Scott et al., 2000**), tomato (**Areshchenkova and Ganal, 2002**), rye (**Hackauf and Wehling, 2002**), cereals (**Zhang et al., 2005**), leguminous and nonleguminous plants (**Gupta and Prasad, 2009**) and/or medical plants (**Mishra et al., 2001**). Up to 17 of 23 barley markers and 23 of 27 wheat markers were found at least in 1 another species (barley, wheat, rye and oat) (**Yildirim et al., 2009**).

Nowadays, we`re able to use microsatellites as DNA markers, especially long enough SSRs. If we`re going down along the scale of system`s features from (a) to (i): (a) marker system availability; (b) complexity of the technique and time investment; (c) estimated polymorphism levels within the study population; (d) quantity and quality of available DNA; (e) transferability between laboratories, populations, pedigrees and species; (f) the size and structure of the population to be studied; (g) availability of skilled workers and equipment; (h) cost per data-point and funding availability; (i) method of marker inheritance (e.g., dominant vs. codominant) and the type of genetic information needed in the population (**Staub et al., 1996**), we manage ourselves into conclusions only by the integration of our aim fitting in the area of taxonomic and phylogenetic studies and diversity and cultivar analysis that key features for our studies correspond with marker system inter-simple sequence repests (ISSR).

ISSRs do not require exact sequence information for each marker compared to SSRs. Also have no problem with a small number of potencial microsatellite loci to identify, polymerase slippage when analysing di-nucleotide repeats and non-homologous co-migrating fragments. ISSRs produce multilocus and highly polymorphic pattern per reaction, although, uninterpreted band profiles in terms of loci and alleles, frequently appearance of dominant alleles and non-homologous similar-sized fragments make serious limitation. In contrast, high level of polymophism, co-dominant inheritance and very high reproducibility of SSRs. technical simplicity and low expenses are much more effective objections. Additional, ISSRs use two approches, main approach is based on targeting multiple loci with knowledge of abundance of repeats in the genome which terminate them, alternative approach demand assistance radioactive labeled anchors (2-4 nucleotides) providing no primer slippage. **Xu and Sun (2001)** showed that ISSR markers have a vast potencial for generating a huge amounts of informative signs for phylogenetic analyses also as AFLP, and by the way, the unique advantage of ISSR is in ability to amplify various volume of DNA with qualitative profiles. **Xu and Sun (2001)** applied two ISSR primer combinations, 807/888 $((AG)_8T/BDB(CA)_7)$ and 811/891 $((GA)_8C/HVH(TG)_7$ from amaranth

with combination of 5 AFLP markers. This strategic step of choosing ISSRs help provide a strong support for a monophyletic origin of grain amaraths, and could be used also in searching of existing cultivars with potencial traits significant for marker-assisted breeding and selection.

Except these are 2 marker systems, simple repeats are used by another one system, REMAP (retrotransposon-microsatellite amplified polymorphism) when in combination of primer pairs is the one microsatellite and the second is LTR (long tandem repeats of arbitrary retrotransposon) (**Gupta et al., 1999**) which create very precise and specific matches in genomes. SSRs are superior to SNP markers because SSR markers can reveal more information per locus than biallelic SNP markers. This was a point of view of **Xu et al. (2013)** to release an information about more than 4 000 SSR markers have been developing and using in genetic mapping studies of wheat. E. g., Chinese spring wheat, at a density of 36.68 SSR/Mb is extremely low, even more with the reported numbers in the monocot species Brachypodium (191.3 SSR/Mb), sorghum (175.4 SSR/Mb), rice (363.3 SSR/Mb), Arabidopsis (418.6 SSR/Mb), Medicago (495.8 SSR/Mb), and Populus (667.9 SSR/ Mb) (**Han et al., 2015**).

The aim of the study was to analyse the resolution power of the microsatellite markers with different dinucleotide core sequences for the purpose of genetic variability description of *Amarantus cruentus* genotypes.

MATERIALS AND METHODS

Eighteen genotypes of *Amaranthus cruentus* were used in the study (**Table 1**). The seeds of a world collection of *Amaranthus cruentus* were obtained from North Central Regional PI Station (NC 7), Iowa State University, Ames. The plant seedlings were cultivated under *in vitro* conditions on the basal **Murashige and Skoog (1962)** medium. Total genomic DNA was extracted according **Rogers and Bendich (1994)** protocol. Each of the accession was represented by a pool of five plants.

Primers used in ISSR reactions are listed in the **Table 2**. Amplification of ISSR profiles was assigned using the 1 U of polymerase, 20 mmol.dm^{-3} Tris-HCl (pH 8.0), 50 mmol.dm^{-3} KCl, 3 mmol.dm^{-3} $MgCl_2$, 0.2 mmol.dm^{-3} primer (all INVITROGEN™, Life Technologies (Thermo Fisher Scientific)), 0.1 mmol.dm^{-3} dNTP (PROMEGA Corp.) and 20 ng DNA in 25 ml PCR mixture. PCR amplification was performed in C1000 TOUCH™ THERMAL CYCLER BIO-RAD (Life Science Research) using the following cycle profile: 2 minutes at 94 °C followed by 45 cycles: 1 minute at 94 °C , 1 minute at 50 °C and 2 minutes at 72 °C with a final polymerization step 7 minutes at 72 °C.

Table 1 *Amaranthus cruentus* L. accessions used in the study

Number in analysis	Genotype	Origin	Type
1	Ames 1959 RRC 1	Ghana	Variety
2	Ames 5129 RRC 360	Nigeria	Unknown origin
3	Ames 21948	Papua New Guinea	Unknown origin
4	PI 566896 Komo	USA, Arizona	Landrace
5	PI 511719 Niqua	Guatemala	Cultivated material
6	PI 511876 Huatle	Mexico	Landrace
7	PI 527567 IZ 32	Burundi	Landrace
8	PI 604558 Mapes 821	Mexico	Landrace
9	PI 612169 Tibet Yellow	China	Cultivated material
10	Ames 5638 RRC 1139	Mexico, Puebla	Unknown origin
11	Ames 5648 RRC 1148	Mexico, Sonora	Unknown origin
12	PI 477913 RRC 1011	Mexico	Variety
13	Ames 5493 RRC 768	Mexico, Morelos	Unknown origin
14	Ames 5369 RRC 685	Kongo	Variety
15	Ames 5310 RRC 659	Mexico, Sonora	Landrace
16	CEN/IB/97/AMA/003	Nigeria, Oyo	Wild
17	Ames 2215 RRC 308	Mexico, Sonora	Landrace
18	PI 566897 Kerala Red	India, Kerala	Cultivated material

Footnotes: Alternative accessions of genotypes: 1 - RRC 78S-1, Yendi 15; 3 - DRL 1294; 4 - Ames 2259, Ames 5319, Cr071, GN 1101, LSK 113, RRC 537, RRC 80S-537; 5 - 87, Alegria, HH 87, Chang; 6 - 851109-05, CN351; 7 - CN374; 8 - Ames 22655; 9 - Ames 25450, Tibet; 10 - JMdC 13; 11 - Cr020, GN CO 2005; 12 - 81S-1011, Ames 2241, Cr048, LSK 481, PI 490661, RRC 104, RRC 78S-104; 13 - LFD/JMdC 138-2; 14 - Lenga-lenga; 15 – Alegria, Cr044, GN 1144, Guegui, PI 451711, Quequi, Weywi; 16 - Ames 25121; 17 - Ames 5320, GN 855, Guegui, RRC 538, RRC 78S-308, Wehwi; 18 - Ames 18058, Cr072, Red Spinach.
Komo; RRC 537; GN 1101; Ames 2259;
Ames 5319; RRC 80S-537
Komo; RRC 537; GN 1101; Ames 2259;
Ames 5319; RRC 80S-537
Komo; RRC 537; GN 1101; Ames 2259;
Ames 5319; RRC 80S-537
Komo; RRC 537; GN 1101; Ames 2259;
Ames 5319; RRC 80S-537
Komo; RRC 537; GN 1101; Ames 2259;
Ames 5319; RRC 80S-537
Komo; RRC 537; GN 1101; Ames 2259;
Ames 5319; RRC 80S-537

Amplified fragments were electrophoretically separated in 2 % AGE (3 : 1, AMRESCO E776-250G). Electrophoreograms were processed with documentation system G:BOX in GeneSnap program - Product version: 7.09 (SYNGENE) and GeneTools - Product version: 4.01 (SYNGENE). Pair-wise matrices were generated by Jaccard´s coefficient of similarity (**Jaccard, 1908**) for every couple of accessions using the SYNTAX softwer. Dendrograms were constructed under the UPGMA algorithm with the HIERCLUS module of SYNTAX to show a phenetic representation of genetic relationships as revealed by the dissimilarity coefficient.

Table 2 Sequences and characterization of ISSR primers used in the study

Primer	GC content (%)	Sequence length in bp
$(CA)_6GG$	57	14
$(CT)_8AC$	50	18
$(GA)_6CC$	57	14

Footnotes: All primers are 3´-anchored. Standard content of guanine-cytosine bases is 50-72 % and sequence length around 11-18 nucleotides.

RESULTS AND DISCUSSION

Different ISSR profiles were compared among collection of *Amaranthus cruentus* from the point of view of different characteristics of intra-specific variability of analysed accessions. Dinucleotide sequence of $(CA)_6$ repetition anchored by GG bases resulted in a total of 65 DNA fragments of 11 levels (**Figure 1**). None of the obtained level was monomophic what represents 100-percent polymorphism. The average count of amplified fragments was 3.61. The highest number of fragments (5) were obtained in the ISSR profile of genotype PI 566896 Komo and the lowes (3) was obtained for genotypes Ames 21948, PI 511876 Huatle, PI 527567 IZ 32, PI 604558 Mapes, Ames 5638 RRC 1139, Ames 5493 RRC 768, Ames 5310 RRC 659 and Ames 2215 RRC 308. The value of resolution power for this primer was Rp = 3,44. The lenght of the amplified ISSR fragments was in the range from 470 bp up to the 2375 bp. Constructed dendrogram based on the UPGMA has resulted in two basic branches where the genotypes PI 511719 Niqua and PI 511876 Huatle were the most distinct from all of the other accessions (**Figure 2**). In the case of this primer, a totally same profiles were obtained for three groups of genotypes. The first group has consisted from genotypes of Ames 5129 RRC 360 originated in Nigeria, Ames 5648 RRC 1148 originated in Mexico and Ames 5369 RRC 685 originated in Kongo. The second group comprises from genotypes originate in Mexico PI 604558 Mapes 821 and Ames 5493 RRC 768 and are connected to the first group at the level of the third cycling with average euclidian distance of 0.1. The third group of the same ISSR profiles is connected with the previous two at the level of average euclidian distance 0.306 and consisted from genotypes Ames 21948 originated in Papua New Guinea and two genotypes originated in Mexico - Ames 5638 RRC 1139 and Ames 5310 RRC 659. This marker has differ completely only 10 from all of the analysed *Amarantus cruentus* genotypes.

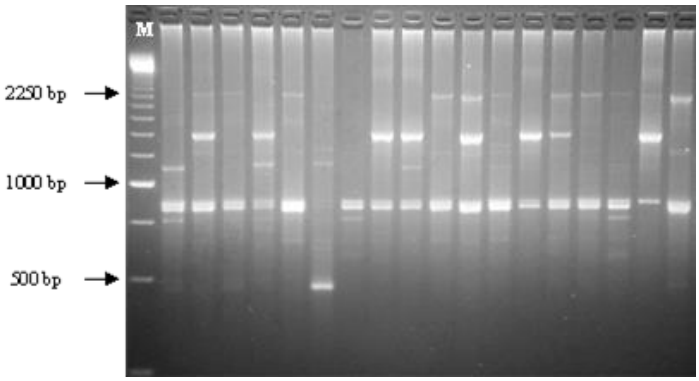

Figure 1 ISSR profiles of analysed *Amaranthus cruentus* accessions obtained by the microsatellite marker $(CA)_6GG$.

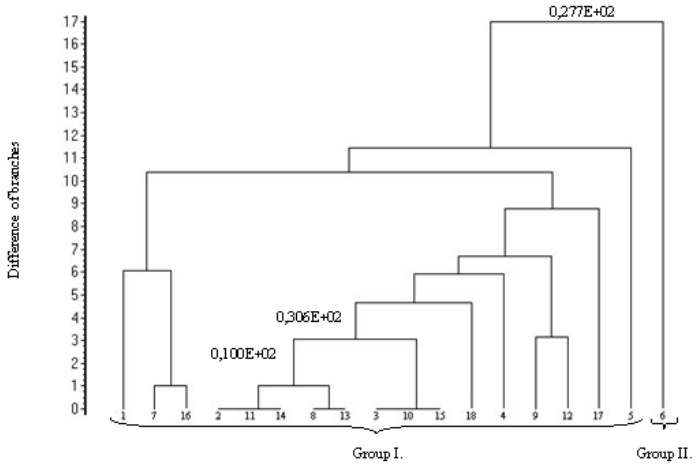

Figure 2 Dendrogram of analysed *Amaranthus cruentus* accessions constucted on the base of amplified ISSR profiles of $(CA)_6GG$ marker.

Dinucleotide sequence of $(CT)_8$ repetition anchored by AC bases has resulted in the amplification of totally 97 fragments distributed in 13 levels (**Figure 3**). Two of the obtained levels were monomorphic what represents 84.62 % of polymorphism. The average count of the amplified fragments per genotype was 5,39 with the maximum of 9 for PI 566896 Komo originated in Arizona and the minimum of 4 for genotypes Ames 5129 RRC 360 originated in Nigeria, PI 511719 Niqua-alegria-chang originated in Guatemala, PI 527567 IZ 32 originated in Burundi, Ames 5369 RRC 685 originated in Kongo, Ames 25121 CEN/IB/97/AMA/003 originated in Nigeria and PI 566897 Kerala Red originated in India. The resolution power of this primer has the value of 3.00. The lenght of the amplified ISSR fragments was in the range from 357 up to the 2000 bp. Constructed dendrogram based on the UPGMA has resulted again in two basic branches where the genotypes PI 566896 Komo and PI 511876 Huatle (the same situation as for the marker $(CA)_6GG$) were the most distinct from all of the other accessions. Here, the same ISSR profile was obtained for most of the analysed *Amarantus cruentus* accessions (11 from 18; **Figure 4**). They are distributed between two branches with the counts of six or five, respectively, genotypes. The first group of the genotypes with the same ISSR profile comprises from: Ames 5129 RRC 360, Ames 25121CEN/IB/97/AMA/003, PI 511719 Niqua-alegria-chang, PI 527567 IZ 32, Ames 5369 RRC 685 and PI 566897 Kerala Red. In the second group are genotypes all originated in Mexico: PI 604558 Mapes 821, Ames 5638 RRC 1139, PI 477913 RRC 1011, Ames 5493 RRC 768 and Ames 5310 RRC 659. From these genotypes, following has resulted as having the same ISSR profile with the primer $(CA)_6GG$, too: Ames 5638 RRC 1139 and Ames 5310 RRC 659. Only seven accesions were different among themselves when regarding this microsatellite core sequence with the Jaccard coefficient of dissimilarity range from 0.31 up to the 1.00 with the average value of 0.79. The most distant genotypes are Ames 21948 originated in Papua-New Guinea and PI511876 Huatle originated in Mexico.

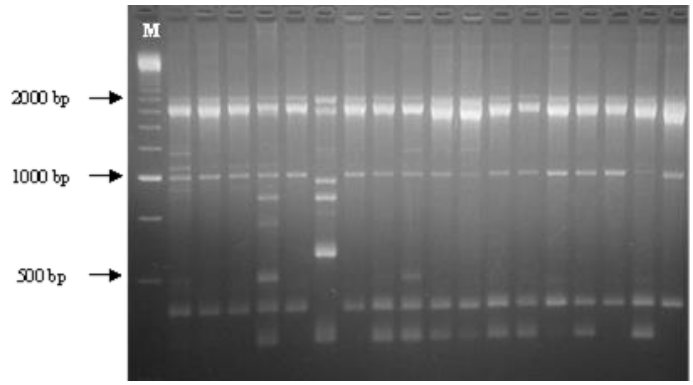

Figure 3 ISSR profiles of analysed *Amaranthus cruentus* accessions obtained by the microsatellite marker $(CT)_8AC$.

Dinucleotide sequence of $(GA)_6$ repetition anchored by CC bases has resulted in the amplification of totally 171 DNA fragments distributed in 21 levels (**Figure 5**). All of the levels were polymorphic. The average count of amplified fragments for a genotype was 9.5 with the maximum of 13 (genotype PI 612169 Tibet Yellow) and minimum of 4 (genotype PI 511876 Huatle). The resolution power of the primer has the value of 10.33. The length of the amplified fragments has renged from 500 up to the 1875 bp. Constructed dendrogram based on the UPGMA has resulted again in the most variable branchig when compering them to the previous cases (**Figure 6**, **Table 2**). Genotype PI 511876 Huatle was here, as well as in both of the primers used in the study the most distinct from all of the other accessions. Here again, not all the genotypes were separated based on the obtained ISSR profiles, but here only two of them (Ames 5638 RRC 1139 and Ames 5648 RRC 1148) possess the same profile of amplified fragments. All of the other accessions were distiguished among themselves. The values of Jaccard coefficient of dissimilarity ranged from the 0.15 up to the 1.00 with the average value of 0.6. The most distant genotypes were PI 511876 Huatle and Ames 5638 RRC 1139 as well as genotypes PI 511876 Huatle and Ames 5648 RRC 1148.

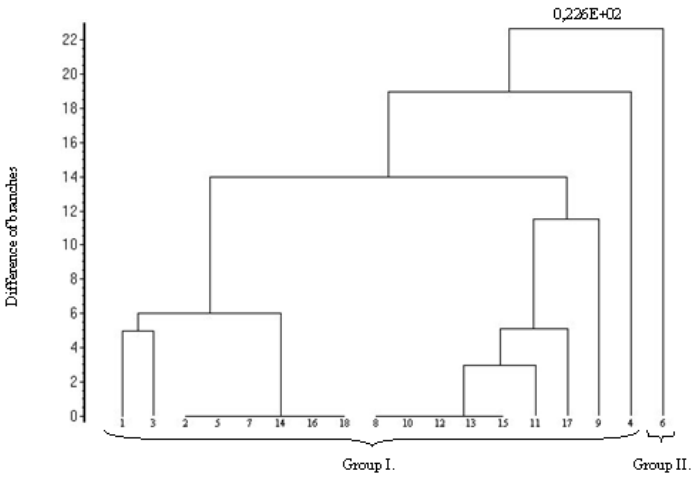

Figure 4 Dendrogram of analysed *Amaranthus cruentus* accessions constucted on the base of amplified ISSR profiles of $(CT)_8AC$ marker.

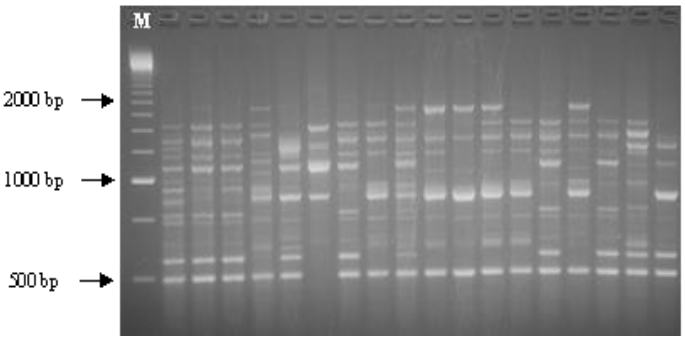

Figure 5 ISSR profiles of analysed *Amaranhtus cruentus* accessions obtained by the microsatellite marker $(GA)_6CC$.

Amplification of microsatellite primed markers is reported to be highly variable in different studies and types of used primers. Generated loci very often correlated to the number of core bases in repeat units. **Blair et al. (1999)** has reported that the number of amplified fragments by GA or GT repeats is different and correlate with the diversity of these repeats in rice (**Blair et al., 1999**).

The repeated motif of GA-CA rich regions in the amaranth genome has been reported by **Lee et al. (2008)**. The authors reported in total twelve polymorphic microsatellite markers for different Amaranthus species and using the SSR markers they have found the average of microsatellites alleles ranged from 1.2 up to the 2.9 for the individual repetitions. In the study of **Ray and Roy (2007)**, dinucleotide repeats with the core sequences of CA and AC resulted in a sucessfull fingerprints patterns, however when the CA repeat was anchored by G or A, no amplification was obtained. In our study, an amplification profile was obtained, but when comparing it to the others, the lowest value of the primer resolution power was generated.

Microsatellites are widely reported to a good marker to determinate the variability between related subjects (**Vivodík et al., 2017**; **Joshi et al. 2007**) where the microsatellite based markers were used to distinguish parental lines and hybrids in maize, bajra, rice, sunflower and sorghum hybrids. The ISSR technique was previously used succesfully for distinguishing of *Amaranthus cruentus* mutant lines where a different levels of differentiation ability was obtained for individual microsatellite core sequences (**Ražná et al., 2012**). The primers with the GACA and GATA-GACA core sequence reveals inter-species DNA polymorphism to the different *Amaranthus* species as well as the ability to find DNA polymorphism among the mutant lines of the *Amaranthus cruentus.*

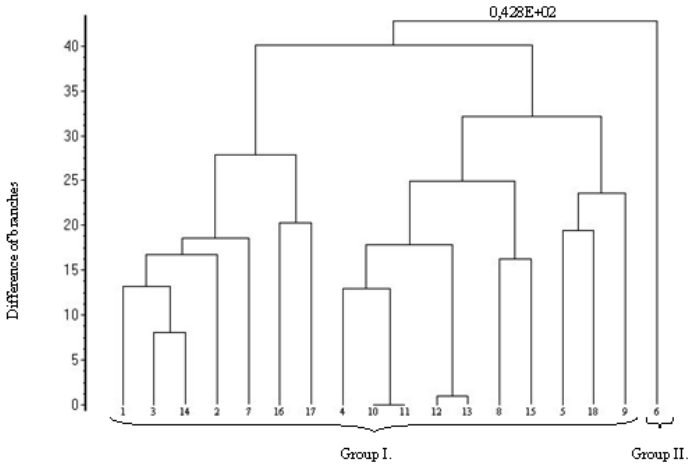

Figure 6 Dendrogram of analysed *Amaranthus cruentus* accessions constucted on the base of amplified ISSR profiles of $(GA)_6CC$ marker.

CONCLUSION

Different ISSR profiles that were obtained for *Amaranthus cruentus* based on the specific microsatellites repeats were evaluates for the ability of the corresponding primers to distinguish the accessions of the analysed collection. Resolving pover of dinucleotide repeats seems to be core sequence dependend. All of the markers used in the study has generated a high polymorphism but none of analysed microsatellite core sequences was suitable for the distinguishing of all of the collection accessions. All of the used ISSR primers has determined the landrace PI 511876 Huatle originated in Mexico as the most distinct when comparing them to the other genotypes from the analysed accessions.

Acknowledgments: This work was supported by AgroBioTech Research Centre built in accordance with the project Building „AgroBioTech" Research Centre ITMS 26220220180 and by the VEGA project 2/0041/16 Molecular methods in breeding of naturally gluten free amaranth.

REFERENCES

Areshchenkova, T., & Ganal, M. W. (2002). Comparative analysis of polymorphism and chromosomal location of tomato microsatellite markers isolated from different sources. *TAG Theoretical and Applied Genetics*, *104*(2), 229-235. URL: https://doi.org/10.1007/s00122-001-0775-2.

Blair, M. W., Panaud, O., & McCouch, S. R. (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *TAG Theoretical and Applied Genetics*, *98*(5), 780-792. URL: https://doi.org/10.1007/s001220051135.

Cho, Y. G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., Mccouch, S. R., ... & Cartinhour, S. (2000). Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, *100*(5), 713-722. URL: https://doi.org/10.1007/s001220051343.

Cordeiro, G. M., Casu, R., McIntyre, C. L., Manners, J. M., & Henry, R. J. (2001). Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. *Plant science*, *160*(6), 1115-1123. URL: https://doi.org/10.1016/S0168-9452(01)00365-X.

Gupta, P. K., Varshney, R. K., Sharma, P. C., & Ramesh, B. (1999). Molecular markers and their applications in wheat breeding. *Plant breeding*, *118*(5), 369-390. URL: http://dx.doi.org/10.1046/j.1439-0523.1999.00401.x.

Gupta, S., & Prasad, M. (2009). Development and characterization of genic SSR markers in Medicago truncatula and their transferability in leguminous and non-leguminous species. *Genome*, *52*(9), 761-771. URL: https://doi.org/10.1139/G09-051.

Hackauf, B., & Wehling, P. (2002). Identification of microsatellite polymorphisms in an expressed portion of the rye genome. *Plant Breeding*, *121*(1), 17-25. URL: https://doi.org/10.1046/j.1439-0523.2002.00649.x.

Jaccard, P. (1908). Nouvelles rechearches sur la distribution florale. *Bulletin de la Société vaudise des sciences naturelles*, 44, 223-270. URL: https://doi.org/10.5169/seals-268384.

Jain, R., Sharma, M., Kachhwaha, S., & Kothari, S. L. (2014). Identification and characterization of microsatellites in expressed sequence tags and their cross transferability in different plants. *International journal of genomics*, *2014*, pp. 12 URL: http://dx.doi.org/10.1155/2014/863948.

Joshi-Saha, A., & Gopalakrishna, T. (2007). Inter Simple Sequence Repeat (ISSR) markers for detecting radiation induced polymorphisms and its application as genetic marker system in *Sesbania rostrata* (Bremek. and Obrem.). *Plant Mutation Reports*, *3*(1), 41-45. URL: http://www-pub.iaea.org/MTCD/publications/PDF/Newsletters/PMR-01-03.pdf.

Kalia, R. K., Rai, M. K., Kalia, S., Singh, R., & Dhawan, A. K. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, *177*(3), 309-334. URL: https://doi.org/10.1007/s10681-010-0286-9.

Lee, J. R., Hong, G. Y., Dixit, A., Chung, J. W., Ma, K. H., Lee, J. H., ... & Park, Y. J. (2008). Characterization of microsatellite loci developed for *Amaranthus hypochondriacus* and their cross-amplifications in wild species. *Conservation genetics*, *9*(1), 243-246. URL: https://doi.org/10.1007/s10592-007-9323-1.

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Islam, K. N., & Latif, M. A. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International journal of molecular sciences*, *14*(11), 22499-22528. URL: http://dx.doi.org/10.3390/ijms141122499.

Mishra, R. K., Gangadhar, B. H., Yu, J. W., Kim, D. H., & Park, S. W. (2011). Development and Characterization of EST Based SSR Markers in Madagascar Periwinkle (*'Catharanthus roseus*) and Their Transferability in Other Medicinal Plants. *Plant Omics*, *4*(3), 154. URL: http://search.informit.com.au/documentSummary;dn=027944752311186;res=IELHSS.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3), 473-497. URL: https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Ray, T., & Roy, S. C. (2008). 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*(3), 338-347. URL: https://doi.org/10.1093/jhered/esn102.

Ražná, K., Žiarovská, J., & Labajová, M. (2012). Genome changes in mutant lines of *Amaranthus* as detected by microsatellite-directed PCR. *ARPN Journal of Agricultural and Biological Science*, *7*(11), 877-884.

Rogers, S. O., & Bendich, A. J. (1994). Extraction of total cellular DNA from plants, algae and fungi. In *Plant molecular biology manual* (pp. 183-190). Springer Netherlands. URL: https://doi.org/10.1007/978-94-011-0511-8_12.

Scott, K. D., Eggler, P., Seaton, G., Rossetto, M., Ablett, E. M., Lee, L. S., & Henry, R. J. (2000). Analysis of SSRs derived from grape ESTs. *TAG Theoretical and Applied Genetics*, *100*(5), 723-726. URL: https://doi.org/10.1007/s001220051344.

Staub, J. E., Serquen, F. C., & Gupta, M. (1996). Genetic markers, map construction, and their application in plant breeding. *HortScience*, *31*(5), 729-741.

Thiel, T., Michalek, W., Varshney, R., & Graner, A. (2003). Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *TAG Theoretical and Applied Genetics*, *106*(3), 411-422. URL: https://doi.org/10.1007/s00122-002-1031-0.

Vivodík, M., Gálová, Z., Balážová, Ž., & Petrovičová, L. (2017). Genetic variation of european maize genotypes (*Zea mays*, L.) detected using SSR markers. *Potravinarstvo: Slovak Journal of Food Sciences*, *11*(1), 126-131. URL: https://dx.doi.org/10.5219/697.

Xu, F., & Sun, M. (2001). Comparative analysis of phylogenetic relationships of grain amaranths and their wild relatives (*Amaranthus*; *Amaranthaceae*) using internal transcribed spacer, amplified fragment length polymorphism, and double-primer fluorescent intersimple sequence repeat markers. *Molecular Phylogenetics and Evolution*, *21*(3), 372-387. URL: https://doi.org/10.1006/mpev.2001.1016.

Xu, J., Liu, L., Xu, Y., Chen, C., Rong, T., Ali, F., ... & Cao, M. (2013). Development and characterization of simple sequence repeat markers providing genome-wide coverage and high resolution in maize. *DNA research*, *20*(5), 497-509. URL: https://doi.org/10.1093/dnares/dst026.

Yıldırım, A., Kandemir, N., Sönmezoğlu, Ö. A., & Güleç, T. E. (2009). Transferability of microsatellite markers among cool season cereals. *Biotechnology & Biotechnological Equipment*, *23*(3), 1299-1302. URL: http://dx.doi.org/10.1080/13102818.2009.10817657.

Zhang, L. Y., Bernard, M., Leroy, P., Feuillet, C., & Sourdille, P. (2005). High transferability of bread wheat EST-derived SSRs to other cereals. *Theoretical and Applied Genetics*, *111*(4), 677-687. URL: https://doi.org/10.1007/s00122-005-2041-5.