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RAPD ANALYSIS OF GENETIC VARIATION IN NATURAL POPULATIONS OF AEGILOPS SP. FROM SOUTH ADRIATIC

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ABSTRACT

New challenges that food production is facing, requires novel approach in agricultural strategy. The scissors of growing demand for food and the limits of the Earth's resources are forcing plant breeders to run for the new borders, utilizing all the available genetic variation in order to create fruitful and economically sound cultivars. *Aegilops* sp. (*Poaceae*) is a potential source of genetic variation for wheat improvement. RAPD marker analysis was used in order to distinguish and evaluate different genotypes of *Aegilops* sp. population samples from the collection gathered during few years' expeditions in South Adriatic, along the coastal, littoral and the inland parts of Montenegro. Ten randomly amplified polymorphic DNA markers (RAPDs) were tested: OPA-05, OPA-08, OPB-06, OPA-02, OPA-07, OPA-25, OPB-07, OPB-18, OPC-06, OPC-10 to examine genetic structuring on 18 samples of 6 populations of different *Aegilops* sp. According to global AMOVA, 75% of total gene diversity was attributable mostly to diversity within population (Φ_{PT} =0.205 p=0.001), indicating that the groups of studied goat grass populations were seemingly to differing genetically. In contrast, 25% of the variation came from variation among populations. According to PCoA, the distribution of 18 goat grass accessions by Principal Coordinate Analysis shows 3 distinct groups. PCo axis 1, PCo axis 2, and PCo axis 3 account for 20.8%, 18.2% and 14.1% of the variation, respectively. The results showed that RAPD markers could be a convenient tool for investigating genetic variation and for detecting genetic structuring of populations. Genetic variability formed under natural selection was entrenched.

Keywords: *Aegilops, goat grass, RAPD, population, genetic diversity.*

INTRODUCTION

Humanity is facing the challenges of overpopulation, environmental erosion, and climate changes. Answering these challenges will be crucial to affect the very survival of the human species on Earth. As well as miners, we do not have the right to a mistake. The clock is ticking. The exact number of people on Earth on 6th of July, 2017, at 14:35 hours was 7,516.477.305, Land lost due to the erosion this year

up to this moment was 3,578.485 hectares. Ten minutes later human population rose to 7,516.478.889, and land erosion grew to 3,578.619 ha (worldometers, 2017). Humane species entered 20th Century with 1.7 billion souls, and left it with 6.1 billion. By the 2050, that number is going be 9.1 billion. Moreover, the ongoing processes are urbanization, environmental erosion including arable land degradation and climate change (Bongaarts, 2009; Myers *et al*., 2017). The previous century is going to be remembered by Green Revolution, the second agricultural revolution after the Neolithic one. Green Revolution enhanced average yield of the most important cultivars 3 to 5 times, however it has made primary food production highly dependent on fossil fuel, chemical plant protection, monoculture or nearly monoculture production organization. Consequences of agricultural industrialization are soil degradation, salinization mostly, environmental pollution, biodiversity loss, human diet narrowing, monoculture in food production. Furthermore, narrowing of genetic variability, genetic erosion, and species loss means the erosion of knowledge, as well. Loss of biological resources implies the loss of specific knowledge about them (Mooney, 2001). Agriculture has to meet the challenge in producing more food, adapt to climate change and adopt more sustainable and efficient mode of production. To achieve these goals, a novel genetic variability from wild relatives, local populations, old instinctively selected populations and varieties and other available genetic resources has to be utilized. Available agrobiodiversity is to be screened for usable genes, in order to broaden genetic variability suitable to meeting the sustainable food production requirements. Wheat (*Triticum* sp.) has been used as a food for about 10,000 years right from the "Neolithic Agricultural Revolution". With the rise of civilization the wheat growing, as well as, breeding had been improving. In our times, wheat is counted among the 'big three' cereal crops, with over 600 million tons being harvested annually. However, in order to meet new selection criteria in modern breeding programs for realization of wheat ideotype suitable for contemporary and future agricultural production requirements, genetic variability is to be broadened. Since, goat grass (*Aegilops* sp.) played an important role in wheat evolution, this genus possess a number of desirable genes to broadening wheat genetic variability, to withstand biotic and abiotic stress growth conditions and to satisfy growing demands for food (Shewry, 2009). Collecting genetic resources, gene bank establishing, genetic variability studying, by phenotyping and genotyping, using protein and molecular markers, are required in order to recognize and isolate desirable gene variation (Petrović and Dimitrijević, 2005; Dimitrijević *et al.*, 2011). The aim of this article is to investigate genetic variation of *Aegilops* sp. samples collected in Montenegro.

MATERIAL AND METHODS

Samples of goat grass (*Aegilops* sp.) have been taken for genetic variability examination from an *ex situ* conservation gene bank consisting of 200 entries of landraces and wheat wild relatives (*Triticum* sp., *Hordeum* sp., *Aegilops* sp.), that had been established after six years of collecting expeditions, mainly in

Montenegro (Dimitrijević *et al*., 2011). *Aegilops* species classification is given after Kimber and Feldman (1987).

Table 1. Ten RAPD primers used for screening *Aegilops* sp. genotypes.

Primers	Sequence	Usability
OPA-02	5′-TGCCGAGCTG-3′	+
OPA-05	5′-AGGGGTCTTG-3′	-
OPA-07	5′-GAAACGGGTG-3′	+
OPA-08	5′-GTGACGTAGG-3′	-
OPA-25	5′-GACAGACAGA-3′	+
OPB-06	5′-TGCTCTGCCC-3′	-
OPB-07	5′-GGTGACGCAG-3′	+
OPB-18	5′-CCACAGCAGT-3′	+
OPC-06	5′-GAACGGACTC-3′	+
OPC-10	5′-TGTCTGGGTG-3′	+

A discontinuous genetic variation of *Aegilops* sp., was examined using ten Random Amplified Polymorphic DNA (RAPD) primers, where three did not yield any product (tab. 1). DNA extraction has been done using the method of Somma (2004). In order to test amplification profiles for polymorphism, readability and reproducibility, six decamer (10 nucleotides length) primers from ROTH®GmbH kits were tested. PCR was carried out in a 25-µL reaction volume containing 2.5 µL buffer; 0.2 mM of each dNTP; 0.5 µM of primer; 2 units of Taq polymerase (Fermentas) and 30 ng of DNA. Reactions were performed in Tpersonal PCR (Biometra) and Mastercycler ep gradient S (Eppendorf) thermocyclers with amplification profile: denaturation at 94°C for 4 min, followed by 40 cycles with 94°C for 2 min, 36°C for 1 min and 72°C for 2 min, with final elongation on 72°C for 10 min. PCR products were separated on 1.2% or 1.7% agarose gels containing 0.005% ethidium bromide and visualized under UV light. Each fragment amplified using RAPD primers was treated as binary unit character and scored "0" for absence and "1" for presence. Estimation of genetic variation was carried out by using the POPGENE and GenAlEx (Yeh and Boyle, 1997; Peakall and Smouse 2006, 2012, respectively).

RESULTS AND DISCUSSION

Variation of six goat grass (*Aegilops* sp.) populations was examined by RAPD molecular markers. Samples of these populations had been collected at three sites along the Adriatic coast, and, inland area of Montenegro around the town of Podgorica, during previous decade. All the tested samples of *Aegilops* sp., have a common U genome, and different ploidy level, and most of them, all except *Ae. kotschy* (SU), have genome M (tab. 2).

Table 2. *Aegilops* species, accession number, geographical location, elevation, ploidy level, genome constitution of goat grass samples examined in the experiment.

Accession label	Population	Genome	Ploidy level	Latitude (N)	Longitude (E)	Altitude (m)
L-16/03	*Aegilops biuncialis*	UM	4x	42.463322	18.505762	84.9
L-28A/02	*Aegilops kotschy*	SU	4x	42.389494	18.664502	102.3
L-44/01	*Aegilops columnaris*	UM	4x	42.229633	18.909559	95.6
L-8/01	*Aegilops triaristata*[*]	UM(X)	4x/6x	42.444066	19.242864	41.6
L-17/01	*Aegilops ovata*[**]	MU	4x	42.411666	19.339257	78.8
L-10/01	*Aegilops umbellulata*	U	2x	42.382146	19.316676	57.0

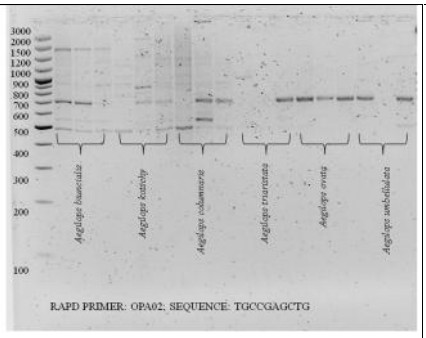
Remarks:
* A map of Montenegro shows exact collection site locations
[*] *Aegilops triaristata* (old name *Ae. recta*) apply to the hexaploid but they are indistinguishable from tetraploids of *Ae. neglecta* in the field. *Aegilops triaristata* is an illegitimate name. Currently used name is *Aegilops neglecta*.
[**] *Aegilops ovata*, which is an old name, is known by its current name *Aegilops geniculata*.
Source: http://herbarium.usu.edu/Triticeae/genomesaegilops.htm

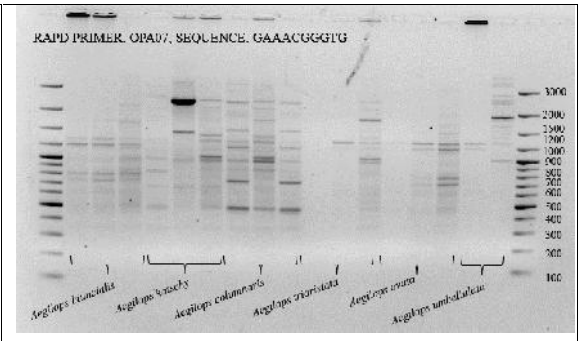
*Source: Google map.

Ae. umbellulata is considered as the main genetic source of genome U (Kihara and Yanashita, 1956; Kadosumi *et al*., 2005). According to the Kadosumi *et al*. (2005), U genome has at least three distinct sequences, and that could be the reason of different patterns obtained with the same RAPD primer for *Ae. umbellulata*. *Ae. comosa* (2x) is donor of genome M. A number of tetraploid *Aegilops* sp. obtained their genomes out of spontaneous hybridization of the diploids *Ae. comosa* (2n = 26= 14, MM) and *Ae. umbellulata* (2n = 26= 14, UU). However, genome M appeared to be even more polymorphic than U genome (Kihara and Yamashita, 1956; Molnár *et al*., 2013). Hence, a considerable variation that was denoted in products of RAPD markers in this study, even within products of the same primer, is in accordance to previously reported results. RAPD primers OPA02, OPA25, and particularly OPA07 that gave products in *Ae. umbellulata* are usable to mark

the presence of U genome in genetic constitution. A total of 61 bands were generated, ranging from 500 to 3000 bp. The high overall level of polymorphism was denoted. The highest number of polymorphic bands was obtained using primers OPA07, OPC 10, and OPA 02 (10), followed by OPB07 (9), and OPC06 (8), tab. 3.

Table 3. Random amplified polymorphic DNA profiles for six goatgrass species using RAPD primer OPA02 (on the left) and OPA07 (on the right). Below, number of RAPD markers generated by each one of the oligonucleotide primers that revealed polymorphism among examined goat grass samples. 1- unique band; 2- shared by two; 3- shared by three; 4- shared by four; 5- shared by five; 6- shared by six.





Species	*Aegilops biuncialis*							*Aegilops kotschy*							*Aegilops columnaris*							*Aegilops triaristata*							*Aegilops ovata*							*Aegilops umbellulata*						
Primers	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18	OPA02	OPA25	OPB07	OPA07	OPC10	OPC06	OPB18
Molecular Size (bp)																																									
500	4							4			2			2	4		1	2	3	2	2					3	2						3			4						
600	3							2		2	2				3		2		3	2						3	2	2					3	2								
700	6			3			5	6					2	5	6		1	3	3		5	6				3		5	6				3	3	5	6						
800		4		3							3					4		3	3							3			4					3			4					
900		1						1		4	4						4	4	3							3					4	4	3								
1000				5			5	1	5	1	5		3	5		5		5	3	3	5	1	5			5	3	3	5		5			3		5		5			5		
1200				6					2	2	6						2	6	3				2			6	3						6	3			6					
1500	3		2				5	1			5			5	3	2		5	3	2			2			5	3	2	5				2	5	3		5			5		5
2000	1							3	2	3	5		3	4			3	5	2	3	4		2	3	5		3	4					5	2	4					5		
3000							4	1		1			3	4						3						2	3	4	1					2	4							

Number and percentage of polymorphic loci, effective number of alleles, expected heterozygosity and Shannon's information index were used to estimate genetic variation. *Ae. columnaris*, *Ae. triaristata*, *and Ae. kotschy* had the highest values for exhibiting the highest level of variation, whereas varieties *Ae. biuncialis* and *Ae. umbellulata* exhibited the lowest. *Ae. columnaris* had the highest effective number of alleles (1.368), as well as, expected heterozygosity (0.213), and Shannon's information index (0.316). The lowest values of all three parameters, leading to the low diversity had *Ae. biuncialis* (1.080, 0.050, and 0.077, respectively) and *Ae. umbellulata* (1.087, 0.055, and 0.085, respectively), tab. 4.

Six *Aegilops* sp. population under study were grouped in two clusters. The first consisting of *Ae. columnaris*, solely, and the second consisted of all the others. This clustering partly correlates with the different estimates of genetic variation obtained for the samples, since *Ae. columnaris* expressed the highest divergence in this study. The same goes for *Ae. kotschy*, at the next level of grouping (fig. 1).

Table 4. Genetic variation out of 61 bands in *Aegilops* sp. using RAPD markers in experiment

Species	P (No.)	P (%)	Ne±σ	He±σ	I±σ
Aegilops biuncialis	9	14.75	1.080±0.212	0.050±0.126	0.077±0.190
Aegilops kotschy	30	49.18	1.305±0.360	0.181±0.197	0.271±0.287
Aegilops columnaris	34	55.74	1.368±0.384	0.213±0.204	0.316±0.294
Aegilops triaristata	33	54.10	1.240±0.233	0.165±0.155	0.261±0.244
Aegilops ovata	24	39.34	1.315±0.422	0.170±0.220	0.245±0.312
Aegilops umbellulata	10	16.39	1.087±0.217	0.055±0.130	0.085±0.196

P (No.): number of polymorphic loci; P (%): the percentage of polymorphic loci; Ne: effective number of alleles; He: expected heterozygosity; I: Shannon's information index; σ:standard deviation

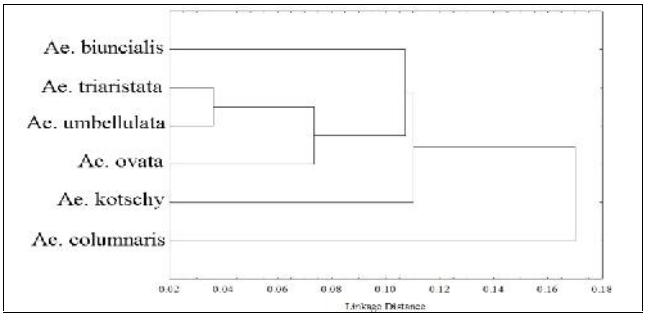
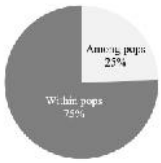


Figure 1. Dendrogram of genetic relationships among 6 populations of *Aegilops sp.* based on RAPD primers

AMOVA partitioned 75% of the total variation within samples of goat grass populations in study, and 25% among samples. This corresponds to previously reported results since all the populations in study share the genome U, and most of them the genome M, as well. However, RAPD primers that were used, gave different DNA patterns within populations, resulting in high level of polymorphism, that contributed greatly by its AMOVA sum of squares to total variation that appeared in the experiment (tab. 5).

Table 5. Analysis of molecular variance (AMOVA) of 6 *Aegilops sp.*, based on RAPD primers

Source of varition	Degree of freedom	Sum of squares	Mean squares	Est. var.	%	Percentages of Molecular Variance
Among populations	5	76.833	15.367	2.530	25%	
Within populations	12	93.333	7.778	7.778	75%	
Total	17	170.167		10.307	100%	

CONCLUSIONS

The RAPD marker analysis could be of use in getting the general insight in genetic variation of samples of *Aegilops* sp. RAPD primers used in this experiment gave a considerable variation, not only among, but also within the examined goat grass population of six different species. The nature of this variation is complex consisting on variation in RAPD primers that were utilized, and on genetic variation of the natural populations sampled in their natural habitats in Montenegro. This genetic variation appears as a consequence of inter- and intra-species hybridization, as well as, diversity in genetic constitution of U and M genomes. The complex nature of variation illustrates Principal Coordinates Analysis (PCoA), which had to be omitted due to six pages limit, which carried out three significant axes of variation that contributed 21% (PCoA1), 18% (PCoA2) and 14% (PCoA3) to total denoted variation in the experiment. Though, some RAPD markers could be used in following the presence of particular genomes, more detailed and more expensive investigations are needed to get further information.

REFERENCES

Bongaarts, J. (2009). Human population growth and the demographic transition. Phil. Trans. R. Soc. B, 364, 2985–2990.

Dimitrijević M., Petrović S., Cîmpeanu C., Bucur, D., and Belić, M, (2011). Cereals and *Aegilops* genus biodiversity survey in the west Balkans: Erosion and preservation. Journal of Food, Agriculture & Environment – JFAE , Vol. 9, Issue 3&4, pp. 219-225, 2011.

Kadosumi, S., Kawahara T., Sasanuma, T. (2005). Multiple origins of U genome in two UM genome tetraploid Aegilops species, Ae. columnaris and Ae. triaristata, revealed based on the polymorphism of a genome-specific PCR fragment. Genes & Genetic Systems, Vol. 80, No. 2, pp. 105-111.

Kihara H. and Yamashita K. (1956). Wheat and its Relatives ", Wheat Information Service No.4, p.16.

Kimber, G., and Feldman, M. (1987). Wild wheat. An introduction, Special Report 353. College of Agriculture, Univ. of Missouri, Columbia, p. 145.

Molnár, I ., Šimková, H., Leverington-Waite, M. , Goram, R. , Cseh, A. , Vrána, J. , Farkas, A., Doležel, J. , Molná r-Láng, M., Simon Griffiths, S. (2013). Syntenic Relationships between the U and M Genomes of Aegilops, Wheat and the Model Species Brachypodium and Rice as Revealed by COS Markers. PLoS ONE, 8(8), pp. 1-12.

Mooney, P.R. (2001). The ETC Century – Erosion, technological transformation and corporate concentration in the 21st century. Dag Hammarskjöld Foundation, Uppsala, and Rural Advancement Foundation International, Winnipeg, p. 128.

Myers, S.S., Smith, M.R., Guth, S., Golden, C.D., Vaitla, B., Mueller, N.D., Dangour, A.D., and Huybers, P. (2017). Climate Change and Global Food Systems: Potential Impacts on Food Security and Undernutrition. Annual Review of Public Health, Vol. 38, pp. 259-277.

Peakall, R., and Smouse P.E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6, 288-295.

Peakall, R., and Smouse P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28, 2537-2539.

Petrović, S. and Dimitrijević, M. (2005). Biodiversity of Aegilops Genera in Montenegro. Contemporary Agriculture 3-4, pp. 460-463.

Shewry, P.R. (2009). Wheat. Journal of Experimental Botany, Vol. 60, No. 6, pp. 1537–1553.

Somma, M., (2004). Extraction and Purification of DNA. In: Querci M, Jermini M, van den Eade G, editors. The Analysis of Food Samples for the Presence of Genetically Modified Organisms. European Commision, Joint Research Centre; Ispra, Italy. Special publication 1.03.114 ed.; Chapter 4, 1-18.

Yeh, F.C. and Boyle, T.J.B. (1997). Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129: 157.

Worldometers (2017). Current World Population. http://www.worldometers.info/