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Cereals and *Aegilops* genus biodiversity survey in the west Balkans: Erosion and preservation

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Abstract

The utilization of genetic diversity is very important, not only in breeding programs, but also in preservation of natural surroundings, heritage and high value food production. The useful part of total gene pool for some traits could be found in ancestors or wild relatives of wheat. Tetraploid and hexaploid wheat species of today, including cultivated forms, are the offspring of spontaneous interspecies crosses of *Triticum* sp. and *Aegilops* sp. Consequently, *Aegilops* genus has been considered as an important wheat relative and useful broadening genetic variability gene source. However, biodiversity erosion appears to be an existing problem in breeding programs of main agricultural plants, as well as, in preservation of our ecological surrounding. Preservation of that valuable genetic material, as well as, *in situ* and *ex situ* conservation is required. Summary results of six years expeditions conducted in order to collect samples and data of *Aegilops* sp. genetic variability, as well as landraces and local populations of cereals in Montenegro, are presented in this article. Significant genetic variability has been denoted and collected, but serious loss of genetic variability in cultivated cereals and spontaneous relatives has been in progress in last 15-20 years.

Key words: *Aegilops* sp., biodiversity, cereals, local population, wheat.

Introduction

The awareness of the importance of biodiversity preservation has been growing along with the intensification of agricultural production. Gene banks in some developed countries, that hold valuable genetic material, have a long tradition. In European Union a central gene bank AEGIS is organizing. *In situ* and *ex situ* preservation and conservation of biodiversity is an important task, beginning with inventorying and collecting genetic variability. However, that task requires a complex approach consisting of historical overview, data on cultural changes, economical conditions, agricultural development, society growing, local customs, climatic changes, soil condition etc. The part of biodiversity that covers wild relatives, landraces and local populations in main agricultural crops is especially endangered and eroded. In this article emphasis is on cereals that have long tradition of growing at Balkans and *Aegilops* genus that played an important role in wheat genomic changes.

Wheat (*Triticum* sp.) has been used as a food for about 10,000 years. The first traces of hexaploid wheat used as a diet were found in Knossos 7th to 6th millennium BC. Inhabitants of Knossos probably came there from Anatolia in the 7th millennium BC and established an agricultural society based on wheat and livestock rising. Charred remains of einkorn and barley grains from Early Neolithic period (Starchevo culture) had been found in the Balkan region.The first agricultural communities in Danubia were found 5300 BC. Neolithic culture on the Balkans had been formed by the migrating Neolithic population of Anatolia merging itself with the Mesolithic local population from the end of 7th millennium BC to the end of 6th millennium BC. Near Novi Sad town by the Beška village (position on the hill above the Danube River) in the hearth form the Early Iron Period charred einkorn and millet seeds were found (Fig. 1). Comparing these migrations both starting in Anatolia at the almost same period, but in different directions, as well as, later archeological findings, it could be concluded that wheat and some other cereal utilization for food and feed on the Balkans proceeds from the very dawn of civilization [10].

Figure 1. Einkorn (right) and millet (left) seed founded at Neolithic locality Kalakača near Beška village in Vojvodina, northern part of Serbia.

Along with the development of civilization the wheat handling had been more and more improved, not only in crop growing, but also in selection and breeding. Simple selection and archaic breeding had been gradually replaced by planned selection and sophisticated breeding. Second half and the end of XIX century in particular, was the period of wheat breeding remarkable progression. Consequently, wheat and cereal breeding and agricultural practice all the way through XX century had been

revolutionary developed resulting in new high yielding varieties and intensive agricultural production, pushing out from fields *T. vulgare* relatives and local populations.

The use of wheat in human dietary for a long time and the steady growth of human population, as well as, diminishing of agricultural productive soil urge a constant need for the improvement of wheat production. In order to achieve that, wheat-breeding programs are under permanent pressure to produce more and more superior varieties. That creates the narrowing of genetic variability and after a while the gene pool needs some refreshment, that is the point to rich for landraces and spontaneous relatives. These populations often posses a number of desirable genes, commonly for disease resistance, winter hardiness, drought tolerance, resistance to lodging etc.

Aegilops genus played an important role in wheat species development. Tetraploid and hexaploid wheats are natural amphidiploids offspring of wide crosses of diploid or tetraploid wheat with *Aegilops* sp. Spontaneous hybridization of diploid *Triticum urartu* and *Aegilops speltoides* led to tetraploid *Triticum dicoccum* with genomic constitution AABB. Further evolutionary step was *Triticum spelta* hexaploid with genomic constitution AABBDD, offspring of *Triticum dicoccoides* x *Aegilops squarrosa* [8].

Having in mind all the facts, the need of collecting, preserving and studying of spontaneous relatives is understandable and of great importance [1, 9]. Wild relatives and ancestors of remarkable genetic variability give a rich source and a great opportunity to create new and improved varieties, especially when the lack of desirable genes in cultivated forms is evident [15].

In a past twenty years several groups have collected a number of different species of *Aegilops* genus in the southern and littoral part parts of former Yugoslavia [3, 4, 13, 22, 23].

Wheat biodiversity at West Balkans: The very first forms of wheat that had been grown on the Balkans were einkorn wheat (*T. monococum* L.) and millet. Later on, emmer wheat (*T. dicoccum* Schübl.), spelta (*T. spelta* L.) and *T. turgidum* L., were grown in larger extent. In Early Middle Ages millet and barley were more important crops than cultivated wheat. Throughout the time cereal crops were grown as mixtures, rarely pure staying in production depending on habits, terrain configuration, economy, road network etc. By the end of 18th century, einkorn, emmer and other wheat species were substituted, in flat areas northern and western of Danube river, by local populations of hexaploid, or bread wheat (*Triticum aestivum* L.) [7]. At the end of 19th century these wheat species had been gradually replaced with local bread wheat populations mostly originated from Hungarian wheat, having local names from area to area. These populations were well adapted with good quality and small requirements. After the First World War landlords started to utilize wheat varieties from breeding programs, predominantly variety Bankut 1205, pushing out local populations. Local bread wheat populations under the local names could be found southern of Danube River up to mid 50's, when bred varieties took over. Nevertheless, einkorn had been grown as a cultivated crop in some small spots of hilly areas northern of Danube till the beginning of 30's of 20th century [10]. Einkorn and emmer wheat had been kept in production in some parts of Croatia, FYR Macedonia, Bosnia, and Herzegovina up to 1956 in extent of 10,000 - 15,000 ha, covered 25% with einkorn and 75% with emmer wheat [14]. Miladinović [11] in mid 50s collected samples from small populations of *T. dicoccum* and larger populations of *T. durum* and *T. vulgare* in Eastern Serbia (south of Danube and in the mountain area on Serbian-Romanian and Serbian-Bulgarian border). Up to the end of 50's local population of spring and winter wheat (*Triticum vulgare*) named Balca cel, Majerka, Starinka, Ranka, Belija, Jedrenka, Vidovača, Bugarka, Gru rošu peljag, Gru alb peljag, Dunavka, Rpjes alb, Orcan, Rapsalj, Domaća crvena, Gru dje primovar rošu tare, Gru primovar ku cepe, Gru primovar peljag, Dubočanka, Limac and Krupnik were grown in East Serbia. In the mountain regions of Rtanj, Miroč and Stara planina, at that time small populations of emmer wheat, found beside populations of *T. durum* and *T. vulgare,* were notified and collected. These populations named Krupnik and Limac were grown in villages of Babušnica, Miroč and Vrbovac [5, 6, 11]. These populations were grown in some villages under different local names.

In Mid and Southern Montenegro, Eastern Herzegovina, Southeastern Bosnia and Southwestern Serbia pure einkorn or mixed with emmer wheat had been keept in part of wheat production up to mid 70's [16]. In some isolated mostly highland areas emmer wheat was grown up to 10-15 years ago (Dimitrijević and Petrović, interview of locals in Montenegro 2005). This gradual substitution from einkorn, emmer wheat, via local populations of hexaploid wheat to bred wheat varieties has not been observed in Montenegro, where other species of *Triticum* genus were directly replaced with high yielding wheat varieties, some 20 years ago or producers stopped producing wheat. Montenegro appears to be particularly interesting area for biodiversity investigation as a historical trade crossroad, because of climate conditions, terrain configuration, traditionally oriented society etc. Up to nowadays there were a lot of biodiversity studies in that region [2, 20, 21]. Though, Montenegro with secluded in high mountains scattered villages, appeared to be the last stand for landraces and local populations, biodiversity erosion is a constant and evident process. Human activities have led to biodiversity endangerment in Montenegro, that is in accordance to situation in other parts of Europe [19].

Wheat germplasm of Romanian origin utilization in East Serbia: There is a notable introduction of wheat germplasm from Romania, brought to East Serbia due to transmigrations, or local populations having Romanian names grown by local inhabitants (Fig. 2). Commonly grown in small areas these wheat materials had been domesticated and became well adapted to the local environment. The common names of these populations were oftenly descriptions of spike or seed color, as well as, vernalization habit. For example, Romanian stockmen from Transilvania settled in village Glogovica, Salaš County grew wheat local population named Gru dje primovar rošu tare (*Triticum durum* var. *hordeiforme*), that is "hard red spring wheat" in English translation (point 5, Fig. 2). Beside mentioned, wheat local population with distinctive descriptive names of Romanian origin had been grown at different locations from the town of Zaječar to the town of Kladovo (East Serbia). Miladinović [11]collected samples of wheat local populations: Balca cel (*Tr. vulgare* var. *erythrospermum*), winter form, village Kobišnica (point 1); Gru rošu peljag (*Tr. vulgare* var. *milturum*), winter form, and Gru primovar ku cepe (*Tr. vulgare* var. *erythrospermum*), spring form, village Zlot, (point 2); Gru alb peljag (*Tr. vulgare* var. *albidum*), winter form, village Brestovac (point 3); R'pjes alb (*Tr. durum* var. *aegyptiacum*) winter form, village Vajuga (point 4); Gru primovar peljag (*Tr. vulgare* var.

Figure 2. Mountain region of East Serbia kept local wheat population in production up to the beginning of 60s. A part of these local populations having Romanian common names were recorded through 1956 -1960 and reported by Miladinović [11]. The position of villages where local wheat populations with the names of undoubtedly Romanian origin were recorded is given in the map.

lutescens), spring form, village Podgorac (point 6). All the names are given by the pronunciation according to Miladinović [11]. There is no clear evidence of the origin or pathways for the most of the above mention wheat germplasm. Serbian economic emigration before the First World War brought probably from Iaşi wheat variety locally called "Rumunska crvenka" (Romanian red) - *Tr. vulgare* var. *sardoum* that had started to spread rapidly at the time and samples were still preserved at the Field Experimental Station in Zaječar (point 7) at the beginning of 60's (Fig. 2).

Pilot investigations conducted by M. Dimitrijević, and S. Petrović in fall 2004 covering a north part of East Serbia (Milanovac-Kladovo-Negotin-Majdanpek) indicated a significant loss of wheat biodiversity in respect of local populations reported some fourty years earlier.

Previous investigations of Aegilops sp. at Balkans: In 1986., G. Kimber (University of Missouri, Columbia, USA), M. Dimitrijević (Faculty of Agriculture, University of Novi Sad, Serbia) and Lj. Pavićević (Academy of Sciences, Podgorica, Montenegro), as a guide and consultant in studies in Montenegro, conducted an expedition covering part of Montenegro inland and coastal area, and a coastal and inland of today Croatia, up to the Neretva River. The aim of the investigation was to observe and collect *Aegilops* spp. in South Adriatic region. Several species of *Aegilops* genus were found. In Montenegro samples of *A. biuncialis*, *A. triaristata*, *A. ovata*, *A. triuncialis* and *A. uniaristata* were gathered. *Aegilops uniaristata* was observed at one locality, only. That was a small field by the local road in Zeta valley near the town of Podgorica, at the locality Martinići (Fig. 3.).

In Croatia (broader Dubrovnik region) up to River Neretva mouth, and in Herzegovina, up to the town of Mostar, *A. biuncialis*, *A. triaristata*, *A. ovata*, *A. triuncialis* and *A. columnaris* were found. Expedition consisting of Shoji Ohta and Yoshihiko Furuta from Kyoto and Gifu University (Japan) was conducted in 1991. That expedition was planned more ambitiously, covering vast part of former Yugoslavia and having a broader collecting goal. The results of that expedition were given in detail in several publications of the authors [13]. The samples of *Triticum monococcum, T. dicoccum, T. durum, T. turgidum, T. aestivum*, as well as, *T. beoticum*, *A. biuncialis*, *A. triaristata*, *A. ovata*, *A. triuncialis, A. comosa, A. cylindrica* and *A. uniaristata* were collected.

The aim of this article is to inform scientific and general public about the state of biodiversity, distribution and collection results in cereals and *Aegilops* genus at the West Balkans, especially in Montenegro, as the northern border of goat grass (*Aegilops*) area, according to previous results, as well as, personal extensive six years investigations of the authors conducted in the last decade. The further goal is to guide eventual following expeditions that are to investigate biodiversity in cereals and wild relatives in Balkan

Figure 3. The photo on the left shows locality Martinići (Montenegro) the only *A. uniaristata* population found in 1986. The photo on the right shows the same locality in 2003, with no trace of *A. uniaristata*.

area. Finally, to stress the importance of preservation of that valuable genetic material, as well as, *in situ* and *ex situ* conservation that is required due to ongoing rapid biodiversity erosion.

Materials and Methods

The samples of *Aegilops* genus were collected from localities, previously visualy screened, photographed, audio described as well as in writing and positioned using GPS (Global Positioning System Garmin 12 Channel Receiver Personal Navigator). The state of populations was recorded with the emphasis to number of plants, maturity and pureness (one species or mixed population). Each species was represented with minimum ten spikes collected per locality. In some cases, whole plants were taken. The data and general information of state of biodiversity in cereals were gathered interviewing locals, and comparing data with the situation observed in the field. The samples of cereals were collected from the local producers in amount of about 500 g. For each sample short description was made, species, local name, the source where the producer obtained that seed from, name of the producer, area description, village and field description, the exact position of the field etc.

Results and Discussion

Extensive field research in Montenegro and pilot investigations in Eastern Serbia that have been conducted in a six years period starting in 2001, revealed vast endangerment and loss of cereal gene fond. Einkorn, emmer, turgidum or durum wheat, as well as, local *vulgare* wheat populations had been lost in large extent, not only by substitution with high yielding wheat varieties, but also because changing wheat for more profitable or easier agricultural production or different property assignment from agricultural to construction land (Fig. 4).

Although genetic variability of wheat suffered greatly, there are still some families up in high mountains at altitudes higher than 1000 m and quite isolated because of reduced "road" network, continuing self-sufficient production of inherited wheat seed. In somewhat larger extent that goes for rye, barley and white varietal corn. Samples of these populations were collected in Northern Montenegro from western to central part of that region (Fig. 5).

Inherited populations of spring two-row barley, rye, spring wheat, and white varietal corn are still grown particularly at Durmitor mountain in North Montenegro (altitudes 1250 - 1550 m). In village Palež (long. 43.18264°N, lat. 19.14040°E, alt. 1431m) two-row barley has local name "Bušket". Inherited cereal populations are still grown at Golija mountain (NW Montenegro), Kričak mountain (N Monteengro) (Fig. 6).

Emmer wheat that was grown up to mid 70's in NW Montenegro on Ljubišnja mountain has been replaced by triticale. In Southern Montenegro, above the Adriatic coast (about 700 m of altitude, village Lunje, 42.05°N and 19.20°E) the very last traces of the local wheat population named Grblja or Grbljanka (presumable *Triticum turgidum* L.) were collected [4, 17] (Fig. 7).

In the past times, that local population has been extensively grown along the Montenegro Adriatic Coast from Boka Kotorska Bay to Ulcinj. The common name Grblja (Grbljanka) has been given after region Grbalj, the valley between Budva and Kotor. The samples were collected in 2003 from the last remain grower of that particular wheat.

Though the detailed analysis of collected material is still to be finished, according to the situation witnessed in the field and

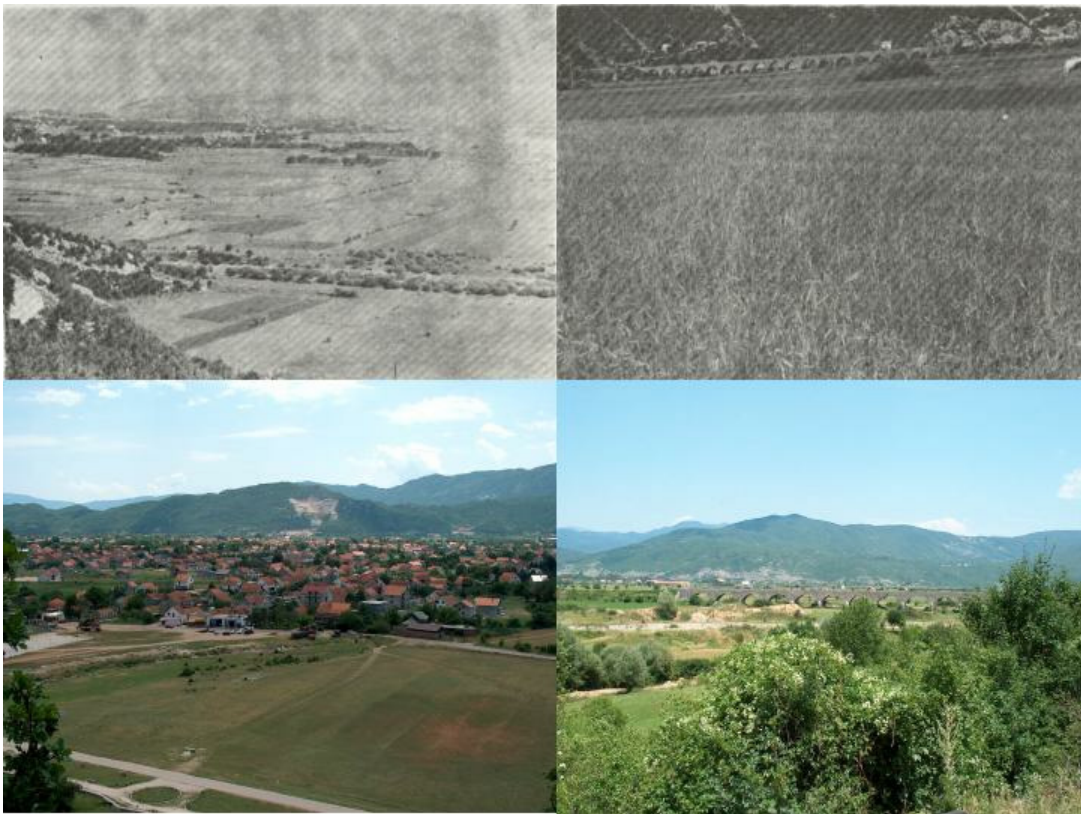

Figure 4. The scenery of Nikšić (Nikshich) field, near the town of Nikšić in Montenegro. Photographs in the upper row were taken in mid 70 by Pavićević, showing fields of emmer wheat. Photographs in the lower row were taken in 2003, by the authors from the same position showing construction land and abandon field.

Figure 5. A part of the samples collected in Montenegro through six years period, consisting of cereals, mainly. The unique sample of local population Grbljanka (*T. turgidum*,), the first sample on the left in the top row.

Figure 6. Wheat field in village Zabrdje (long. 43.13366°N, lat. 18.76251°E, alt. 1381m) at Golija mountain. Seed inherited from grandfather in a self sufficient production (on the left). Village Gradina (long. 43.10027°N, lat. 19.29078°E, alt. 1232m) at Kričak Mountain. Inherited seed of barley, rye and buckwheat still in sowing (on the right).

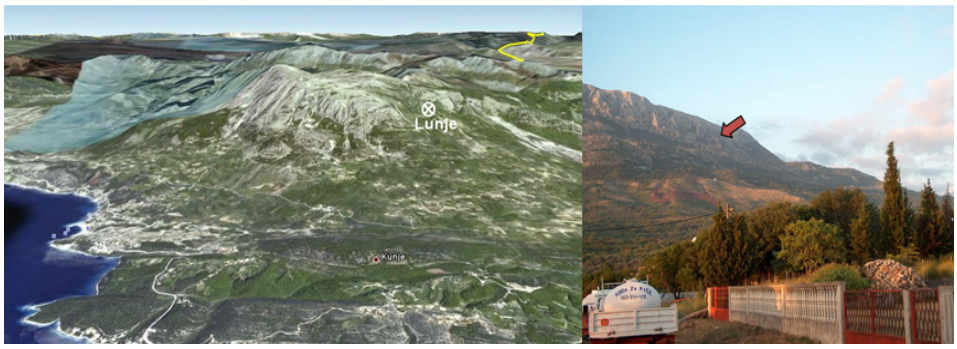

Figure 7. The last trace of Grbljanka (*T. turgidum*, preliminary established), collected in 2003., under the top of Rumija mountain. Google Earth (4.3.7284.3916 beta) relief map (on the left), and the position of the village Lunje, viewed from the coastal village Dobre Vode (arrow marked), on the right.

preliminary results compared to previous extensive research conducted by Pavićević during late 60s and 70's, it could be stated that rich genetic variability preserved in Montenegro as a result of terrain configuration, producers traditional habits and reduced road network in last 10-15 years has been rapidly reduced to traces and that traces are in danger of extinction.

Aegilops genus, a very close wheat relative, has vast genetic variability in Mediterranean region [3, 8, 18]. Opposite to situation in cereals, considerable variability of *Aegilops* genus has been noted and collected (about 200 samples) in inland and littoral Montenegro. During several years (2000-2006), extensive investigations of *Aegilops* sp. had been done, not only of collecting samples, but also of following the changes populations during consecutive years.

Within that period of inventory, samples of *A. ovata* L., *A. triaristata* Willd, *A. kotschyi* Boiss., *A. biuncialis* Vis., *A. cyilindrica* Host., *A. columnaris* and *A. umbellulata* has been gathered. That material is to be further genetically analysed and preserved. The changes in *Aegilops* population structure in Montenegro area was observed and ought to be monitored periodically, since *A. cyilindrica* has been found at one locality in one year, only by the road from Danilovgrad to Nikšić above Zagorak village (42.586°N, 19.045°E), and *A. kotschy* has been spreading in a past six years, to the south along the coast, from one locality by the Sutorina River, at the exit of Herceg Novi Town toward the border to Croatia at 42° 27' N of latitude and 18° 29' E of longitude (Fig. 8).

Species of *Aegilops* genus, that has been denoted and collected, commonly settled sunny slopes and terrain, mostly on shallow, poor land base, near the road, being exposed to air and soil pollution. In our investigations the presence of *Aegilops* sp. populations at the localities with higher heavy metal content could indicate more tolerant genotypes, having in mind possible utilization of that biodiversity in wheat breeding programmes for improving abiotic stress tolerance that is in accordance to Monneveux *et al.* [12]. During six years period the process of *Aegilops* locality destruction has been noted, especially in coastal area by construction work in developing tourism.

In the inland part of Montenegro, the first population of *Aegilops* genus (*A. ovata*) appeares about 4 km before Bioče village (42.543°N, 19.336°E, about 120 m alt.), about 12 km north from Podgorica, the Capital of Montenegro. The highest position where representatives of *Aegilops* genus were found was at the old King Nicolas road from Mateševo to Podgorica, after Bolesestre village (42.572°N, 19.394°E, 588 m alt.), where samples of *A. ovata* were collected. The general position of that locality is at the canyon exit of river Morača, by the road to Podgorica. At collection sites near villages Tološi, Mareza and Lazarev Krst, between Podgorica, and the town of Danilovgrad (42.49°N, 19.18°E; 42.49°N, 19.16°E and 42.52°N, 19.10°E, respectively) *A. triaristata* and *A. ovata* were collected.

In the coastal area genetic variability of *Aegilops* genus is greater. At the localities covering west bank of Boka Kotorska Bay from village Bijela (42.45°N, 18.65°E) to village Njivice (42.45°N, 18.50°E), by the border to Croatia *A. ovata*, *A. triaristata* and *A. kotschy* were collected. Two types of *A. kotchy* were denoted, one with white, and the other with red awns. Collection sites covering Luštica peninsula, from village Radovići (42, 398°N, 18.670°E) to crossroad town Tivat to town Budva (42.378°N, 18.749°E) shelter large populations of *Aegilops* sp., holding almost all biodiversity of goat grass denoted throughout Montenegro. Populations of *A. ovata*, *A. triaristata*, *A. biuncialis* and *A. kotschy* are partly pure, consisting of one species and partly mixed on the skeletal terra rossa soil. The narrow area along Adriatic coast and highways E65 and E851 that goes from Bigova (42.36°N, 18.70°E), via Budva and Petrovac, to Bar (42.10°N, 19.10°E), harbours large populations mostly of *A. ovata* and *A. triaristata*, on skeletal calcocambisol and redish-brown soil. Mountain area between Bar and Virpazar (42.26°N, 19.08°E), and the south bank of Skadar lake up to villages Ostros (42.09°N, 19.31°E, 232 m alt.) and Arbneš (42.08°N, 19.34°E, 379 m alt.), all the way to Albanian border is populated with *A. ovata*, *A. triaristata* and *A. kotschy*. Samples of *A. ovata* and large population of well developed specimens *Ae. triaristata* with redish-violet owns were found around the village of Bolevići (42.22°N, 19.10°E, about 200 m alt.). The triangular area between Rumija mountain, Albanian border and Adriatic coast appears to be home of three species, *A. ovata*, *A. triaristata* and *A. biuncialis* (large populations). *A. kotschy* was found at one locality (42.07°N, 19.16°E), only, above Dobre Vode village. *A. columnaris* was collected at two areas along the Montenegro Adriatic coast. First area is going from Budva Town southern exit (village Boreti) to St. Stefan, and second area is village Dobre Vode on the South to Bar Port. *A. umbellulata* was found at one site in coastal part, at the very top of Luštica Peninsula, village Rose, and at one site in inland part by the road from Podgorica to Albanian border, near village Tuzi.

The results of six years field research of *Aegilops* genus diversity in inland and coastal part of Montenegro revealed six species of

Figure 8. The locality by the road Podgorica - Nikšić, where *Aegilops cylindrica* was found (photo on the right). Sutorina locality, where *Aegilops kotschyi* was first time spotted and collected in 2003 (photo on the left corner).

Aegilops. *A. biuncialis*, *A. ovata* and *A. triaristata* were found, and that is in accordance to previous results. Comparing genetic variability that has been found in previous investigations in 1986, and 1991, to our results, the lack of *A. triuncialis*, *A. uniaristata* (found in 1986 and 1991) and *A. comosa* (found in 1991, only) could be observed. However, *A. cylindrica* was collected, which is in agreement with results of 1991 expedition, but not observed in 1986. Species *A. umbelullata* and *A. kotschy* were not spotted in previous expeditions. *A. kotschy* was found for the first time in 2003, near Herceg Novi, spreading to the south along the coast, in following years. *A. umbelullata* was collected in 2001 in Luštica Peninsula and Tuzi area, just to the East from Podgorica the Capital of Montenegro. Both species were not observed in 1986 and 1991. Analyzing previous stated one has to keep in mind a certain differences that occur in names used for *Aegilops* species. For example, *A. triuncialis* in above statements is in some articles named *A. triuncialis* var. *triuncialis*, while *A. triuncialis* var. *kotschyi* sometimes stands for *A. kotschy*. The same could be stated for *A. ovata* that is named as *A. geniculata*, as well.

The names for *Aegilops* sp., used in the article rely on names given at: http://herbarium.usu.edu/triticeae/genomesaegilops.htm

Conclusions

Genetic variability of cereals, genus *Triticum* in particular, having in mind its use as a food, archeological findings, the existence of broad spontaneous relatives variability and the growth of ancient civilizations, as well as, trade roots in and out of Mediterranean and its hinterland lead to potentially rich genetic variability of wheat and other cereals in South-Eastern Europe and south parts and flat areas suitable for agriculture of Balkans in the past. The question is what could still be found, and what has been preserved? Though, significant biodiversity erosion took its share, there are still some isolated areas where local populations of cereals could be found. The situation with wild wheat relatives is somewhat better, but needs to be monitored. Up to now the collection and preservation of that valuable genetic material has been done by some enthusiastic researchers. The present time requires well organized and systematically work of experienced and well trained researchers, who know not only what to search and collect, but also how to organize and conduct an expedition, as well as, how to contact and approach local inhabitants. The other part of the story is analyzing and preserving collected material that requires technically well equipped facilities. The importance of that plant material in this modern time of gene manipulation makes worthy every effort, particularly in developing countries were that gene fond commonly could still be collected and safely conserved in modern gene banks.

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