

Implementation of the Programme for Conservation of Plant Genetic Resources in the Republic of Srpska from 2009 to 2012

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Abstract

The Programme for Conservation of Plant Genetic Resources in the Republic of Srpska was established in 2008. The main objective of the Programme is effective management of plant genetic resources through carrying out of continuous field inventories and collection, evaluation, exchange and conservation of germplasm. The Genetic Resources Institute, University of Banja Luka was appointed as a expert unit for coordination and implementation of the Programme. In the period from 2009 to 2011, the inventory was made for part of the area of the Republic of Srpska. An innovative approach was adopted for conservation of plant genetic resources by means of long-term seed preservation, *in vitro* conservation, morphological and molecular characterisation, as well as regular database updates. Contacts were established with producers for the purpose of on farm protection of local ecotypes and populations. An *ex situ* collection was established in the Botanic Garden for plant species that can not be conserved in the form of seeds. By the end of 2011, the Gene Bank had reached its full operation with 455 accessions in long-term storage (-18°C), around 150 accessions in the working collection and 100 accessions in the field collection. With its 91 accessions, the Genetic Resources Institute is part of a European web-based catalogue of inventories of plant genetic resources (EURISCO). Having adopted the Programme, the Republic of Srpska has not only fulfilled one of the world's peremptory obligations to conserve biodiversity of agricultural crops, but also a moral obligation to future generations.

Key words: inventory, collection, characterisation, working groups, *ex-situ* and *in situ* conservation, EURISCO Catalogue.

Introduction

Genetic resources have lately been drawing more and more attention in terms of collecting, conserving, evaluating and usage. The scientific community is increasingly warning about genetic erosion of the overall germplasm. It has become clear that biodiversity is a foundation of our existence on earth therefore it is necessary to preserve the biological heritage both for the purpose of saving it from contingencies and disappearance of some species and making the resources available in the future.

Bosnia and Herzegovina, including the Republic of Srpska, has rich biological diversity (*Bosnia and Herzegovina – Land of Diversity*, 2008). Owing to people's migrations, trade and exchange, species and varieties grown by various cultures reached many different areas, but only those that had adjusted best to local conditions and provided satisfactory yields were used for further reproduction. Spontaneous crossing and natural selection under the impact of the environment led to creation of a number of valuable spontaneous ecotypes and new varieties in our region.

Diversity of plant and animal species ensures raw materials for the production of food, medical preparations and remedies as well as all other types of products that people need to survive. Biological diversity is a source of genetic material that enables development of new and enhancement of plant varieties for agricultural production.

As a result of increasing commercialisation of plant production, loss of traditional peasant households and migrations from rural areas, the genetic stock and biological diversity in the Republic of Srpska have been under the extreme jeopardy of vanishing completely.

Instruments for ensuring conservation of biodiversity in agriculture and food production include various international treaties, global action plans and others that play a significant role in the regulation of use, transfer, protection, management and trade in regards with biological diversity at the regional, national and international level.

The Convention on Biological Diversity of the United Nations recognises that signatory countries have sovereign rights over their resources and biodiversity. The signatory countries' obligations have been set out in article 6 of the Convention, which states that all member countries should, in accordance with their potential and capacities, among other things, set up national strategies, plans or programmes for protection and sustainable use of biological diversity. The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) entered into force in 2004. It represents a comprehensive treaty for sustainable agriculture and food security and whose objectives are the conservation and sustainable use of plant genetic resources for food and agriculture (PGRFA) and the fair and equitable sharing of benefits derived from their use.

The National Assembly of the Republic of Srpska adopted the Programme for Conservation of Plant Genetic Resources in the Republic of Srpska (hereinafter referred to as Programme) in June 2008 ("The Official Gazette of the RS" 59/08). It was set out in the Programme that the Genetics Resources Institute within the University of Banjaluka was to be established as a coordinating institution for the

implementation of the Programme. Having established this Programme that sets up the conservation and sustainable use of plant genetic resources, the Republic of Srpska is so far a unique example in the region when it comes to legal basis for the activities related to genetic resources.

Materials and methods

This paper presents the results of the implementation of the Programme in the period after it was laid down, i.e. from 2009 until the end 2011.

The main objective of the Programme is effective management of PGR through carrying out continuous field inventories and collection, evaluation of collected accessions, but also exchange and conservation of collected germplasm. The University of Banja Luka founded the Genetic Resources Institute in January 2009 while the Contract on the implementation of the Programme was signed with the University of Banja Luka in June 2009. The Plant Genetic Resources Committee, established in December 2008, laid down the Action Plan for the period 2009 - 2015.

The Programme activities are being carried out within the activities of 6 working groups: Cereal and Maize Working Group, Fruit and *Vitis* Working Group, Vegetables Working Group, Industrial Plants Working Group, Forages Working Group and Medicinal and Aromatic Plants Working Group. Apart from the Institute staff, associates from a number of institutions take part in the activities of working groups: Faculty of Agriculture in Banja Luka, Agricultural Institute of the Republic of Srpska, The Agency for the Provision of Consultancy Services in Agriculture, the Centre for Rural Development of the City of Banja Luka and representatives of municipal departments dealing with agriculture as well as associations of citizens and nature lovers.

The conservation and use of plant genetic resources is continuously evolving. It is widely accepted that conservation can be performed *in situ* and *ex situ* (Engels and Visser, 2003).

In situ conservation not only safeguards genetic variability of plant species, but it also enriches this variability by means of mutation, recombination and selection. Thus, this form of conservation enables continuous flow of the evolution process. The conservation of an area where populations of a particular species exist naturally and where they have developed their distinctive features is the main requirement for conservation of biological diversity. Protected areas have been highly recommended as the instruments for *in situ* conservation (Engels J.M.M., Visser, L. 2003).

Ex situ conservation implies sampling, transfer and storage of target taxon populations in the collection area whereas *in situ* conservation includes marking, management and monitoring of target taxons where they have been located. A key difference is reflected in the dynamic nature of *in situ* conservation in comparison to the static nature of *ex situ* conservation. The use of only one of these methods can not facilitate conservation of genetic diversity of any one of the plant species. Wherever possible, it is necessary to use the combination of the two aforementioned conservation methods. *Ex situ conservation* refers to conservation of the components of biological

diversity outside of their natural habitats (*The Convention on Biological Diversity*, 1992). It is the process of protecting an endangered plant and animal species outside of its natural habitat, e.g. by removing part of the population from a threatened habitat and placing it in a new location. Depending on the techniques used, *ex situ* conservation can take various forms, but the ones mostly used include: seed gene banks, field gene banks and *in vitro* conservation.

Results and discussion

In the period from 2009 until the end of 2011, significant results were accomplished at the republic, regional and international level as regards the implementation of the Programme.

An innovative approach to conservation of plant genetic resources by means of balanced application of both *in situ* and *ex situ* strategies of conservation is one of the most crucial outcomes resulting from the implementation of the Programme activities. From 2009 until 2011, the Institute was entirely equipped for conservation of plant genetic resources whereas the gene bank became fully operational in 2010 for long-term conservation of germplasm, which implies long-term conservation of the collected material at – 18°C.

Taking part in drafting of the Second Report on the State of the World's Plant Genetic Resources is an important result at the international level. The Second Report on the State of the World's Plant Genetic Resources¹ was published in 2009 by the Commission for Plant Genetic Resources within Food and Agriculture Organization of the United Nations (FAO UN, 2009). It is worth mentioning that the Republic of Srpska, that is BiH, joined ECPGR – The European Cooperative Programme for Plant Genetic Resources². During this period of the Programme activities, 91 accessions of the Gene Bank of the Republic of Srpska were registered in the European Plant Genetic Resources Search Catalogue (EURISCO).

Making inventories. The first step and a main prerequisite towards conservation of PGR is to carry out field inventories. In the work conducted in regards with the Programme implementation so far, inventories were made for considerable part of the Republic of Srpska, resulting in 747 inventoried accessions (287 cereal and maize accessions, 89 forage accessions, 227 vegetables accessions, 58 accessions of medicinal and aromatic plants, 8 accessions of industrial plants and 78 fruit and vine accessions).

Conservation of plant genetic resources. The conservation of plant genetic resources within the Programme takes place in the form of *in situ* and *ex situ* conservation. One of the priority activities of the Programme for Conservation of Plant Genetic Resources in the Republic of Srpska is to establish a network of protected areas where endangered plant species would be conserved in their natural environment. In the previous period, two such initiatives started with the aim to declare protected

¹ www.fao.org/agriculture/crops/core-themes/theme/seeds-pgr/sow/en/#cr

² <http://www.ecpgr.cgiar.org/>

areas for conservation of genetic resources in both dynamic and developing state. The first initiative, unfortunately unfruitful although the study and harmonisation of the field state was completed, referred to the initiation of procedures to declare “Bukovica” locality as a protected natural area. The second initiative is reflected in the initiation of a procedure to protect “Univerzitetski grad” (“University Town”) Park Complex together with other facilities covering 21 ha³. During 2010, the Genetic Resources Institute started analysing the state of the park in “University Town” Complex. Initial activities referred to evaluation of ligneous species in “University Town” Park. Positions of all trees were noted down and represented in a map. In May 2012, “University Town” Complex was protected on the basis of the Law on Nature Protection and categorised as – *Resource Management Area*.

A special form of *in situ* conservation, becoming more important lately, is on farm conservation. This type of conservation is of interest primarily in regards with field crops, having in mind that on farm conservation is to protect endangered plant species in their natural habitats and that it takes into consideration social and cultural factors such as traditional knowledge. *In situ* – on farm conservation includes maintenance of traditional cultivar crops or ecotypes and traditional growing systems (Hodgkin at all, 1993; Jarvis, 1999). In the course of activities undertaken so far for the purpose of Programme implementation, contacts were made with producers who are interested in this form of conservation of autochthonous field crops and growing systems. Most interest for on farm conservation came from vegetable, fruit and vine producers at the following locations: Gomiljani, municipality of Trebinje; Karanovac, municipality of Petrovo; Bančići, municipality of Ljubinje; Prusci, municipality of Novi Grad; Velika Bukovica, municipality of Doboje and Nožičko, municipality of Srbac.

A seed gene bank serves for *ex-situ* conservation of plant genetic resources in the form of seeds, by drying of the seeds until they reach low moisture level and storing them at low temperatures without losing vitality. Most key crops being used as food are tolerant to seed drying until reaching low moisture level, thus being suitable for seed type of conservation. Currently, there are more than 1,500 gene banks and germplasm collections across the world, maintaining estimated 6,100,000 accessions (*The State of Food and Agriculture*, 1996). Conservation of the genetic diversity in the form of seeds is the most studied widely used and most suitable method of *ex situ* conservation (Hamilton, M.B, 1994). The seed gene bank of the Republic of Srpska became fully operational during 2010 when all necessary equipment was purchased for the preparation of seeds and long-term conservation (seed counter, germinator, phytotron, bag sealer, moisture meter, drying chamber, refrigerators, freezers and chambers for long-term storage of seeds). Internationally established FAO/IPGRI standards for seed handling in gene banks are the guidelines that the Gene Bank of the Republic of Srpska also abides by in its procedures with the aim to conserve seeds in the best possible condition prior to storage and to maintain high germination before entering the collection. Conservation of plant material in the seed form requires

³ http://www.vladars.net/sr-SP-Cyrl/Vlada/Ministarstva/mgr/PAO/Pages/Javni_uvid.aspx

everyday laboratory work. Newly-arrived accessions are being prepared for storage at low temperatures through processes such as seed cleaning, seed germination tests (according to ISTA rules⁴), drying to the optimal moisture level (3-7%) in drying chambers at low temperatures and at low moisture level in the chamber (-25°C and 10-15% relative moisture), then seed packing in three-layered aluminium bags and storing in freezers at -18°C. The accessions already in long-term storage are monitored daily. By the end of 2011, 455 accessions had been prepared for long-term conservation. The active, base and safety collections were formed in the Gene Bank of the Republic of Srpska, with the plan to move the safety collection somewhere else in the future, that is, to another gene bank. As a rule, minimum 1,000 seed grains are conserved for each accession, but in the case of poor germination and a small number of collected seed grains, if possible, recollection is recommended, or multiplication.

Multiplication of cereal and maize seeds is carried out on the fields of the Agricultural Institute of the Republic of Srpska; multiplication of vegetables seeds in cooperation with the Agency for the Provision of Consultancy Services in Agriculture whereas multiplication of forage crops seeds is performed on the fields of the Centre for Rural Development of the City of Banja Luka. Multiplication of the varieties with vegetative propagation (fruits, vine, potato and vegetable species reproducing in this way) implies multiplication of the collected material being conserved either in field collections or *in vitro* conditions. These activities are carried out at the Genetic Resources Institute.

Field gene banks as a form of *ex-situ* conservation are used for plant species that can not be conserved in the form of seeds. This primarily refers to those species that do not produce seeds and that reproduce vegetatively. They also include tropical and subtropical species, that is, plant species with high moisture seeds that can not tolerate drying well. In field gene banks, the plant genetic resources are kept as live plants that undergo continuous growth and require continuous maintenance. The plant material conserved in this way is easily available for evaluation, characterisation and generally for research of any kind. On the other hand, field gene banks are more expensive to maintain since they require more labour and more space for the maintenance of a collection. Part of the field gene bank of the Republic of Srpska has been situated in the Botanic Garden within the Campus of the University of Banja Luka since 2010. So far, the field gene bank contains autochthonous fruit varieties, with total of 62 trees of 31 autochthonous apple and pear varieties. 36 trees of 18 native apple varieties have been planted including: (*Malus × domestica* Borkh.): “Zelenika”, “Senabija”, “Bobovec”, “Kanada”, “Srebrenjača”, “Cvjetača”, “Ovčiji Nos”, “Lederka”, “Plemka”, “Batulenska” (“Staklara”), “Kanjske”, “Habikusa”, “Srebrenicka”, “Sarenika”, “Funtaca”, “Sadicka”, “Eriflana” and “Slatka Zelenika”. In addition, 26 trees of native pear varieties (*Pyrus communis* L.) have been planted, too: “Medenka”, “Huseinbegovaca”, “Stambolka”, “Urumenka”, “Avranska”, “Izmirska”, “Batva”, “Dugalica”, “Lederica”, “Karamut”, “Lubenicarka”, “Ranka”, “Kantarusa”.

⁴ <http://www.seedtest.org/en/home.html>

Another fruit variety collection is being set up in municipality of Cajnice and a field collection vineyard in the area of municipality of Trebinje.

In vitro conservation of plant genetic resources has become a complementary approach to conventional conservation methods. *In vitro* conservation is used to maintain the plant material for short, middle and long term in small space and under controlled sterile conditions. When material is conserved in this way, it is easily available for use and exchange is simplified. The Genetic Resources Institute has established a conservation methodology by using *in vitro* techniques in its gene bank. An *in vitro* lab work protocol was developed and, during 2010, procedures for *in vitro* conservation of garlic and onion were set up and working. Further, there have been experiments as regards vine conservation, prior to which multiplication *in vitro* takes place.

Development of molecular techniques. The elementary role of collecting and conserving plant genetic resources in the world is collection and safeguarding of vital genotype samples of various plant species. Introduction of molecular techniques and molecular markers is of importance and interest in maintenance and management of modern gene banks (Ivanović, V; Konstantinov, K. 2000). The Genetic Resources Institute has set up a protocol for molecular characterisation using RAPD method comprising five stages: DNA extraction, quantification of extracted DNA, RAPD primers polymerase chain reaction, electrophoresis and visualisation and analysis of the results. Characterisation of rye and some native varieties of pear has been done. However, the objective is to ensure conditions for characterisation using microsatellites.

Documentation. Collection forms and passport descriptors comprise main information noted down when inventorying and collecting accessions for a gene bank. These data are entered into a database each time an accession is conserved for a long-term period when each is assigned a unique accession number. An internal database has been created following the model of European Plant Genetic Resources Search Catalogue (EURISCO catalogue) so as to register accessions in the European catalogue more easily. By the end of 2011, 91 accessions of the Genetic Resources Institute were registered in EURISCO (<http://eurisco.ecpgr.org/>). Along with collection forms and passport descriptors, traditional knowledge and customs relating to inventoried accessions are also collected and recorded. Furthermore, recipes for preparation of dishes, beverages and tea are noted as well as specifics of traditional production and use of accessions. These data are also part of the internal database.

Conclusion

The Programme for Conservation of the Plant Genetic Resources in the Republic of Srpska was implemented according to the plan for 2009-2011 resulting in significant achievements in terms of inventorying, collecting and conserving the plant material.

During 2011, the Institute became fully operational technically and was equipped for carrying out all activities in regards with the Programme implementation.

So far, the Institute has an equipped laboratory for the analysis and preparation of seeds for short-term, mid-term and long-term conservation of seed collections, facilities for long-term conservation of seed collections, laboratory for *in vitro* multiplication and maintenance of the plant material, laboratory for morphological and molecular characterisation.

In order to carry out the Programme activities, an innovative approach is applied aiming at optimal use of complementary methods and all available techniques in conservation of plant genetic resources.

Taking into consideration that genetic resources are part and parcel of human heritage, the Republic of Srpska is obliged to keep on collecting, describing and maintaining valuable genetic resources for the generations to come and for their needs, but also to make them available to everyone through well-developed documentation and information systems.

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Реализација Програма очувања биљних генетичких ресурса Републике Српске у периоду 2009 – 2012. године

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Сажетак

Програм очувања биљних генетичких ресурса Републике Српске донешен је 2008. године. Основни циљ Програма је ефикасно управљање биљним генетичким ресурсима кроз перманентну инвентаризацију терена и колекционисање, евалуацију, размјену и конзервацију гермплазме. За имплементацију и координацију спровођења Програма задужен је Институт за генетичке ресурсе Универзитета у Бањој Луци. У периоду 2009. до 2012. године извршена је инвентаризација дијела Републике Српске, усвојен је иновативан приступ очувања БГР кроз дугорочно чување сјемена, *in vitro* конзервацију, морфолошку и молекуларну карактеризацију, те редовно ажурирање базе података. Успостављени су контакти са произвођачима у циљу *on farm* заштите локалних екотипова и популација. За биљне врсте које се не могу чувати у форми сјемена успостављена је *ex situ* колекција у Ботаничкој башти. Банка гена је крајем 2011. године постигла оперативност са 455 принова на дугорочном чувању (-18 °C), око 150 принова у радној колекцији и 100 принова у пољској колекцији. У Европском електронском каталогу инвентара о биљним генетичким ресурсима (EURISCO) пријављена је 91 принова Института за генетичке ресурсе.

Кључне ријечи: инвентаризација, колекционисање, карактеризација, радне групе, *ex situ* конзервација, EURISCO каталог.

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