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REVIEW

Use of plant genetic resources in crop improvement–example of Serbia

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Abstract Plant genetic resources are a link between agriculture, environment and trade, so their conservation requires cooperation from different sectors. The existing diversity in genetic resources is the foundation in breeding for new challenges or new markets in the future. The number of crop genetic resources in Serbia is obscure because there is no national inventory. It is thought that there are about 15,000 seed accessions and 3500 accessions of fruit trees and grape in collections of different national institutes and stakeholders. In the National Plant Gene Bank, there are more than 4000 accessions of nearly 250 plant species. Crops kept in ex situ collections are used in breeding programs and interspecific crosses, for selection and introduction of desired traits through pre-breeding programs. Serbia as a state participates in all national and international efforts for preservation, management and use of plant genetic resources. Additionally, Serbia has also established a set of national regulations related to plant genetic resources and their use in breeding. Since Serbia is one of Europe's most important crop producers, especially in maize (11% of EU-27 production), soya (35%), sunflowers (6%) and sugar beet (2.5%), this paper discusses certain issues and achievements in the use of plant genetic resources in cereal and oil crops improvement in Serbia, as well as national and international regulations affecting their exploitation.

Keywords Genetic resources · Plant breeding · Crops · Serbia · National legislation · International regulations

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Introduction

Plant genetic resources (PGR) have great potential in the context of climate change, organic agriculture, food diversity and stability of agricultural production systems, while the need for their conservation and sustainable use worldwide is growing and represent a huge challenge. Being a link between agriculture, environment and trade, their conservation requires cooperation from different sectors.

Plant genetic resources can be classified in two broad groups (Evenson et al. 1998). The first group consists of the genetic resources of cultivated species and encompasses landraces created by farmers over many years and selected for different environments. Wild relatives of the cultivated crops are also included in this group. Their value for plant breeding is well known, resulting in collection, evaluation and conservation of these genetic resources in ex situ gene bank conditions. Besides variability for agronomically important traits, the native landraces are a potential source of traits for increasing nutrient content and nutritional value of modern hybrids and varieties, in which the nutrient content is significantly reduced due to intensive breeding for increased yields. Furthermore, the development of best performing varieties utilizing the existing traditional gene pools is the only feasible and recommendable method for creation of genotypes for farming areas with unique geographical location and specific climatic conditions (Chandramohanan et al. 2016). However, in areas with intensive agricultural production, these traditional gene pools are under the threat of genetic erosion, as a result of rapid replacement of traditional varieties with hybrid and modern varieties (Saini et al. 2020). The second group consists of genetic resources of other plants (and even the species not belonging to the Plant kingdom). This group is gaining potential value in breeding by the development of biotechnology techniques and DNA transfer from ‘alien’ species into economically important crops.

Elite crop cultivars are bred from a genetically narrow germplasm and for high input agricultural production, making them less adapted to changes in the environment. Consequently, in the future, more attention should be paid to breeding for better adaptation with regard to climate change and predicted global warming (Miladinović et al. 2019). The contemporary challenge is to develop sustainable agriculture, as well as to increase utilization of genetic resources in breeding. Therefore, crop landraces and wild relatives are potentially very valuable resource for climate-smart traits and resistance to biotic and abiotic stresses but are underutilized in modern agriculture. Landraces are closer to elite cultivars than wild species and could be used as initial genetic pool for breeding.

Breeders usually have their own working collections, either in a cold storage or in the field, with adequate genetic variability for their needs. The existing diversity in genetic resources is a foundation in breeding for new challenges or new markets in the future. In this paper we present an overview of main crop genetic resources in Serbia, their availability and use in commercial breeding, as well as national and international regulations affecting their exploitation.

Plant genetic resources in crop improvement

The best link between crop genetic resources and breeding is the pre-breeding concept. It includes all activities related to evaluation, characterization and improvement of germplasm, which could be used as foundation material in breeding programmes (Nass and Paterniani 2000). This concept also encompasses identification of potential useful traits in non-adapted germplasm pool or even in adapted material, not exposed to any kind of selection. An additional advantage of pre-breeding programs is establishment of the so-called core collections. Core represents the genetic diversity of the species and its relatives with a minimum of repeatability (Brown 1989). The core collection is dynamic, meaning it is possible to introduce new genotypes, as well as to replace existing ones, depending on the needs of the breeding programs.

One of the basic problems of using crop genetic resources in breeding is that, besides the desired genes, a number of unwanted properties are introduced into the cultivated genotypes, which often leads to deterioration of agronomical important traits. In addition to this problem, the work on the practical application of the crop wild relatives in breeding is connected with series of difficulties, such as cross-incompatibility and introduction of undesired traits from wild relatives into cultivated germplasm (Miladinović et al. 2019).

Since Serbia is one of Europe’s most important crop producers, especially in maize (11% of EU-27 production), soya (35%), sunflowers (6%) and sugar beet (2.5%) (Dajić Stevanović and Đođević Milošević 2018), certain issues and achievements in using PGR for cereal and oil crops improvement in Serbia are discussed below.

Cereals

According to estimaties, there are 8646 accessions of cereals in Serbia, including 1509 accessions of wheat, 447 accessions of barley, 260 accessions of oat, 46 accessions of rye, and 6384 accessions of maize. Out of this number, 2758 accessions (cultivars, breeds, landraces, and relatives) are grown on farm and in situ mostly within germplasm collections of state or private institutions (Dajić Stevanović and Đođević Milošević 2018).

Wheat plays very important role in global food security by providing 20% of the calories and proteins to the world's population. In the past few decades, a stagnation of the wheat yield in the world has been observed, while the demand for food is rising because of the growing human population. Therefore, there is an urgent necessity for increasing wheat production and this goal should be achieved from less land, with less water and fewer chemicals, according to sustainable development strategies. The best solution to meet these challenges is breeding wheat varieties with high and stable yield.

The identification of new genetic variability, both in breeding material and in different collections and wild relatives of wheat, is one of the basic prerequisites for a successful breeding work. Institute of Field and Vegetable Crops, Novi Sad (IFVCNS) has the largest ex situ collection of wheat in Serbia providing a solid background for successful wheat breeding programs. The organisation of wheat germplasm into three levels was proposed by Denčić and Kobiljski (2001) and has been kept utill now. The first level is the world collection (whole collection), comprising over 2,800 accessions, originating from more than 30 countries. Additionally, the collection contains 65 wheat ancestor and related species. For maintenance purposes, each year one third of the collection is sown and analysed for many agronomical important traits, enabling us to obtain valuable data and at the same time replenishing the seeds of the entire collection in three years. According to the evaluation results the genetic (core) collection (the second level) with more than 850 entries is extracted from the world collection and grouped into subunits according to the traits considered important for breeding. Part of the activities of the collection is currently conducted through the FAO project GRAINEFIT, dedicated to redesigning the exploitation of small grains genetic resources for increased sustainability of grain-value chain and improved farmers' livelihoods.

Maize displays how a different type of reproduction can affect utilization of genetic resources and wild relatives in crop improvement. Wheat and other small grain cereals are self-pollinated plants and their populations are genetically stable. Contrary, in maize as cross-pollinated plant, every plant is fertilized by a different one, making their population genetically heterogeneous and less stable. For example, if seed of different maize varieties, landraces and hybrids is used in the following year, progeny will be genetically divergent. Therefore, it is difficult to measure the contribution of landraces for germplasm improvement (Morris et al. 1999).

Although there are 135,000 maize accessions (FAO, 2010) in gene banks worldwide, most of them have not been properly evaluated for potential use in breeding. Double and three-way crosses in hybrid development doubled the yield in USA from 1930 to 1966, and by 1995 single crosses and breeding with better foundation material obtained triple the 1930 yields. Despite frequent warnings about the risk of genetic uniformity and the potential of exotic germplasm, globally only 5% of available maize germplasm is in commercial use (Carena et al. 2009). One of the world's largest maize collections is kept at the Maize Research Institute, Zemun Polje (MRI), consisting of more than 6,000 accessions, out of which 2,217 are local, domestic populations and the rest is material bred by the Institute (more than 600 hybrids) and material introduced from 40 countries. The database for 11,864 accessions has been completed, with MRI hosting the European Maize Database Collection (https://www.mrizp.rs/emdb/default.htm). Data of the complete maize collection has also been uploaded to EURISCO (5,475 accessions) (Dajić Stevanović and Đođević Milošević 2018).

The main goals of using genetic collections in wheat and maize breeding programs includes improved abiotic stress tolerance and increase of grain quality.

Abiotic stress tolerance

The high and stable production of the major crops for human consumption is one of the main priorities of contemporary agricultural research. Cereal production plays an important role in securing food stability threatened by climate change reflected in frequent and often unpredictable extreme weather events. Changes in temperature and rainfall distributions and patterns, may lead to occurrence of periods with extremely high or low temperatures, low relative humidity, strong insolation, droughts, floods and to a shift in the structure of prevailing plant pests and diseases of one region and emergence of new invasive species, which can ultimately endanger cereal production.

In a context of climate change and soil and water resource degradation, it becomes increasingly important to reduce the need for high inputs, leading to more sustainable agricultural practices by selecting the best performing varieties in various deleterious environments, having both a higher yield, better quality and higher resource use efficiency in such stress-prone conditions. In order to help breeders in selecting such genotypes wheat genetic resources from the IFVCNS were intensively used and characterised for their responses to drought and other abiotic stresses during last two decades. From field experiments (Dodig et al. 2002; Denčić et al. 2012; Brdar-Jokanović 2017), in vitro screening studies (Kondić-Špika et al. 2008, 2009) to modern high-throughput shoot and root phenotyping platforms (Paul et al. 2019), different approaches and methodologies were used. The various parameters were investigated, with the results contributing to a better understanding of key features of shoot and root responses under different stress conditions. The studied genotypes reacted differently to applied stress conditions making it possible to select tolerant and sensitive cultivars. The results enabled the ranking of genotypes and providing valuable information about donor parents for better abiotic stress tolerance in wheat. Since the genotypes with good yield potential differed in their yield components, there is a potential for further improvement of yield under stress conditions by combining parents with good performance of different yield components under abiotic stress. Furthermore, by testing the extreme phenotypes of each trait we shall be able to establish more effectively the value of physiological and developmental traits as selection criteria for improved abiotic stress tolerance.

Also, molecular analyses were combined with phenotypic data in order to identify regions in a complex wheat genome which can contribute to better reaction of wheat genotypes to drought stress conditions (Dodig et al. 2010). Association mapping study was performed on a winter wheat core collection of 96 accessions with twenty-four agronomic traits evaluated over 3 years under fully irrigated, rain fed and drought conditions. The association of 46 SSR loci distributed throughout the wheat genome with yield and agronomic traits was analysed. A total of 26, 21 and 29 significant ($P < 0.001$) marker-trait associations (MTAs) were identified in irrigated, rain fed and drought treatments, respectively, with the marker effects ranging from 14.0 to 50.8%. Markers psp3200 (6DS) and gwm484 (2DS) were associated with many significant MTAs in each treatment and explained the greatest proportion of phenotypic variation for drought tolerance in wheat.

The IFVCNS the development of genotypes that perform well under drought conditions is one of the main focuses, not only in wheat, but also in maize breeding programmes at the IFVCNS. For this reason, grain yield performance and anthesis silk interval (ASI) were evaluated in field trials under drought stressed conditions (Mikić et al. 2016). The analysis clustered the genotypes into four groups: two expected clusters of inbred lines with short ASI and high yields and the inbred lines with large ASI and low yield, and two unexpected groups of maize lines, one with intermediate ASI and low yield and the other with large ASI and high yield. Three inbreds were selected for their potential drought tolerance, showing an above-average yield and small ASI in all environments. Association analysis indicated that three microsatellite markers (*bnlg1525, bnlg238* and *umc1025*) were significantly correlated with ASI and grain yield. The proportions of phenotypic variation explained by the markers (R^2 values) varied across environments and were higher for ASI then for grain yield. Eight alleles were selected for their favourable effect on yield increase and ASI decrease, indicating potential suitability of the makers for tolerant genotype selection.

Very important project for identification of drought tolerant maize accessions within whole MRI collection started in 2008. Field-trials were conducted in Egypt, at Sids Agricultural Research Station. Over 6,000 samples (accessions, elite inbred lines, commercial hybrids used as checks) were exposed to Manage Stress Environment (MSE) at sites without rainfalls during growing season. The whole collection was divided in five groups (extra early, early, medium, medium late and late maturity), and irrigation was applied up to two weeks before flowering. About 10% (672 samples) were selected based on of visual scoring: for stay green, ASI (anthesis-silking interval), bareness, seed set, grain filling, and total appearance of the plant. The chosen set of accessions was tested during the following season in Egypt, Serbia and Macedonia. Together with observed traits, ear, cob and kernel weight were measured on 53 selected samples. For further utilization in breeding programs, general combining ability and heterotic patterns were determined, and a mini core collection of 41 genotypes was created. The drought tolerant mini-core collection comprised 15 inbred lines, 13 local landraces, and 12 introduced populations (Anđelković and Ignjatović-Micić 2012). Their potential for improved nutritional quality of the grain was tested and the highest genetic gain with 5% of selection intensity could be achieved with introduced population for oil and protein content (Vančetović et al. 2014a). Six populations from drought tolerant mini-core collection that have universal combining ability (with BSSS, Lancaster and Iodent inbred testers) were tested for agronomic traits in diallel crosses, aiming to identify new heterotic pattern. Out of them, two exotic populations formed a new heterotic pattern, together with good combining ability with three well-known testers and are included in crosses with elite inbreds (Vančetovic et al. 2015).

Inbreds from the mini-core collection were studied for different physiological parameters and responses related to seedling performance under stress (Kravić et al. 2013) and for the identification of SNP markers (Assenov et al. 2013). Application of a new generation of molecular markers provides a better insight in genetic basis of valuable traits. In the last study unique SNP mutations in the MYB-like protein E1 (*MYBE1)* were found in five lines from drought tolerant mini-core collection. Recently, the same lines were phenotyped by automated high-throughput to evaluate their responses to water and nitrogen stress, as well as response to combination of those two stresses during vegetative growth (before flowering) in green house conditions (Dodig et al. 2019).

Grain quality

Wheat breeders pay special attention to technological quality of wheat in order to provide the population with baking products of the highest possible quality. Work on wheat quality improvement is challenging and complicated by the fact that it is affected by many factors, particularly genotype, environment, their interactions and furthermore by harvesting method, postharvest treatment and storage (Hruškova and Famera 2003; Hruškova et al. 2004). Breeders are expected to develop cultivars which will meet the requirements of growers, processors and consumers and which will grow successfully under various environmental conditions. In response to different environmental conditions, wheat has developed a number of mechanisms that involve molecular and cellular changes depending on the genotype.

The effects of the environmental factors and genotype on the protein component and other wheat quality parameters were examined in different collections of wheat genetic resources during more than 60 years long breeding period in Serbia (Mišić and Mikić 1976; Đurić et al. 2010; Kondić-Špika et al. 2019). In the study of Denčić et al. (2007) the contribution of 50-year breeding process to the advancement of wheat technological quality was assessed. Changes of quality were analysed in the varieties from different breeding periods. The results indicated no progress in the 1000-kernel weight and wet gluten content, while increasing trends were registered for test weight, flour yield, protein content and bread yield. In a similar study of Hristov et al. (2009) the following annual changes in genetic potentials were found: protein content—−0.03%, wet gluten—−0.14%, farinograph absorption—0.04%, dough energy—1.53 cm^2, loaf volume—2.94 ml, and baking score—0.07. Although there has been a negative trend for some wheat quality traits over decades of breeding, it could not be concluded that new varieties have lower quality compared to old ones. Quite often the situation is even reversed and there is a number of modern wheat cultivars with very good bread making quality. The possible reason is that decreases in the protein and wet gluten contents were compensated by an improvement in protein quality.

The increase in gluten structure stability and appropriate combinations of high molecular weight glutenin subunits have contributed to the improvement of other quality indicators. This assumption was confirmed in the study of Hristov et al. (2013), in which a total of 168 winter wheat cultivars developed at IFVCNS in the period 1987–2008 were analysed for high molecular weight glutenin subunits (HMWGS) composition. Twelve different alleles and nineteen different GS combinations were determined. The most frequent combinations of GS were 2*, 7 + 9, 5 + 10, but also several rare GS with positive effect (13 + 16 and 15 + 16) were determined.

Development of high-yielding maize hybrids was a primary goal in breeding, while qualitative traits of grain was of less importance, except in rural areas where heirloom varieties were used for human consumption. Pigmented maize grain, mainly preserved in old landraces and varieties, is very rich in flavonoids (e.g. anthocyanins), phenolic compounds, and carotenoids that have numerous health benefits for humans. Maize is gluten-free, so there is an increased interest for maize grain consumption in celiac disease patients, and in food production research. In the investigation of the contents of total phenolics, flavonoids, anthocyanins, β-carotene, and lutein in whole kernels of 10 different coloured maize genotypes, the anthocyanin contents were highest in pop-corn landraces with dark red and light blue colour, belonging to gene bank collection in MRI (Žilić et al. 2012). Pop-corn populations were used as donors for increasing antioxidants in maize grain in ZP hybrids by method simulating Top-cross system (Vančetović et al. 2014b). Flour of blue standard maize, together with the flour of blue popping corn and dark-red popping corn (derived from landraces from MRI gene bank), was used to prepare cookies. The aim was to investigate the effect of baking and dough formulations on phenolic compounds, antioxidant capacity and colour of cookies made from anthocyanin-rich maize flour (Žilić et al. 2016). Utilization of pop-corn landraces from the gene bank collection resulted in four red grain ZP maize hybrids, one blue popcorn, and one red popcorn released by Executive Agency of Variety Testing Field Inspection and Seed Control Republic of Serbia. ZP maize hybrids with blue grain are under official testing and will be released in the coming years.

Oil crops

Majority of oil crops grown in Serbia are registered domestic cultivars (rapeseed—9, soybean—21, sunflower—47, castor bean—4) (Dajić Stevanović and Đođević Milošević 2018). Those cultivars were mostly created at Institute of Field and Vegetable Crops, Novi Sad (IFVCNS) by using extensive collections of *Brassica* and *Helianthus* species.

One of the largest sunflower collections in the world and the only one in Serbia is kept at IFVCNS, consisting of over 7,000 sunflower inbred lines developed from different genetic sources and used in breeding, as well as 27 wild *Helianthus* species (https://www.ifvcns.rs/kolekcija-divljeg-suncokreta/wild.html) (Škorić et al. 2006; Jocić et al. 2012a; Atlagić and Terzić 2014; Mladenović et al. 2017; Hladni et al. 2017; Dimitrijević and Horn 2018; Terzić et al. 2020). The long history of sunflower breeding has resulted in almost 700 registered hybrids so far, alone or in collaboration with other companies and countries. Researchers from IFVCNS participated in seven collection explorations in the United States during the 1980s and 1991 (Marek 2016). The use of wild relatives in sunflower breeding has a very long tradition and has increased genetic variability. The most commonly used method for the introduction of traits from wild relatives into cultivated sunflower is targeted crossing, which involves finding the genes of interest in a particular wild relative and transferring them into a cultivated sunflower (Kaya et al. 2012; Miladinović et al. 2019). So far, significant results have been achieved using wild sunflower relatives in IFVCNS sunflower breeding programme: discovery of cytoplasmic male sterility and restoration fertility which enabled the introduction of hybrids into sunflower production, discovery of many sources of resistance to diseases and broomrape, herbicide tolerance, improvement of agronomic and morphological traits. Particular success was the discovery of resistance to diseases such as *Phomopsis* (Škorić 1985, 2016), downy mildew (Jocić et al. 2012b), *Sclerotinia* white rot (Vasić et al. 2004), charcoal rot (Tančić et al. 2012), *Phoma* black stem (Dedić 2012) and broomrape (Terzić et al. 2010; Hladni et al. 2009; Cvejić et al. 2012).

IFVCNS is the only research institute in Serbia dealing with the cultivation of oil crops from the *Brassicaceae* family. Breeding work on *Brassica* species from IFVCNS collection resulted in the introduction and spread of cultivation of black and white mustards and false flax (*Camelina sativa* (L.) Crantz) previously rarely grown in Serbia. Rapeseed collection consists of 11 varieties and 20 lines of spring rapeseed and 56 varieties and 980 lines of winter rapeseed. All genotypes from the IFVCNS collection have been phenotypically and cytogenetically characterized, with particular reference to their phenology, morphology of the flowers, pollen properties and the number of chromosomes (Atlagić et al. 2010), the resistance / sensitivity to diseases and pests, as well as seed quality (oil, protein content, fatty acid and tocopherol composition), and significant genetic variations were found. Because commercial rapeseed breeders are directly involved in the evaluation and selection of the material, the collection is a valuable resource for a more detailed characterization of other traits that are of importance for breeding (Marjanović Jeromela et al. 2016). The constant and systematic use of this collection with the application of conventional methods of breeding, followed by various molecular-genetic methods, is an effective tool in the development of IFVCNS varieties and hybrids adapted to changing environmental conditions and market demands. Genetic resources are used to develop productive genotypes as renewable raw materials for the production of food and non-food oils, especially in low-input systems and marginal soils, suitable for conventional and organic farming systems.

Stem canker resistance

Stem canker, caused by *Diaporthe/Phomopsis helianthi* Muntanola-Cvetković Mihaljčević et Petrov, is one of the most widely distributed and most damaging diseases of sunflower. Its large-scale occurrence was first registered in Serbia and Romania in 1980, when it caused substantial economic damage to sunflower production. Soon afterwards, it was observed in most sunflower-growing countries in Europe. In the year of *Phomopsis* epidemic 1981, IFVCNS collection was extensively screened and only four lines out of the entire breeding material (around 5,000 inbred lines) demonstrated high tolerance to stem canker. Two of these lines were interspecific crosses of cultivated sunflower and an *H. tuberosus* accession from the IFVCNS collection, one originated from a local population from Morocco, and the fourth one was derived from the cross *H. argophyllus* × Armavirski 9345 (Miladinović et al. 2019). The first *Phomopsis* tolerant hybrids in the world, NS-H-43, NS-H-44 and NS-H-45, were developed from these lines.

Since the population of this pathogen is stable and occasional strong attacks of *Phomopsis* in some regions of sunflower production are the result of favourable weather conditions for its development, and not changes in the pathogen population (Jocić et al. 2004), breeders have been able to use the same resistance sources throughout the years. *Phomopsis* resistance is incorporated in all IFVCNS breeding material and NS commercial hybrids.

False flax introduction

False flax or camelina (*Camelina sativa* (L.) Crantz.) is an oil seed plant in the *Brassicaceae* family, which has become especially interesting in recent years due to its diverse use and modest agro-ecological requirements for cultivation. Within the framework of the 2016–2017 Serbian-Austrian bilateral project “Introduction of camelina—sustainable oil species of the future”, genotypes were exchanged between the Universität für Bodenkultur, Vienna and IFVCNS, in order to enrich the genetic collection established at IFVCNS 20 years ago (Marjanović Jeromela et al. 2007). The joint collection has been phenotypically characterized to gain greater insight into this germplasm as a potential source of genes and genotypes useful for breeding (Mladenov et al. 2017; Marjanović Jeromela et al. 2018a). For this purpose, accessions from collection were tested in different agro-ecological conditions, and their oil and protein content and composition were determined (Marjanović Jeromela et al. 2018b). All these activities resulted in release of the first false flax varieties in Serbia—NS Zlatka and NS Slatka, reintroducing this almost forgotten oil crop into production in Serbia.

Use of modern breeding techniques (MBTs)

The advancements in developing MBTs can potentially increase the utilization of genetic resources in breeding by simplifying the incorporation of valuable traits in new varieties. These techniques include the application of molecular and omics-scale technologies for gene discovery, and advanced techniques to transfer genes of interest from wild plant species to cultivated crops (Zhang et al. 2016). These techniques could be useful for gene transfer from very diverse species, as well as study of different mechanisms affecting the traits of interest that could further be used in breeding new cultivars.

Different molecular markers have been used to identify variability and duplicate accessions in the MRI maize collection (Ignjatović-Micić et al. 2003; 2008@@; Anđelković et al. 2018). Furthermore, specifically-designed primers were used to identify functional single nucleotide polymorphisms (SNPs) in the *MYB-like protein E1* (*MYBE1*) gene of tolerant and sensitive inbred lines from the MRI gene bank (Assenov et al. 2013), for identification of functional markers related to drought stress tolerance and their further use for genomic improvement of MRI selection programs. The sequence alignment revealed several SNPs, one of which was unique to the drought tolerant MRI inbreds T3, T5, T6, T7, and T8.

Within the IFVCNS sunflower breeding program, marker-assisted selection is routinely used for introduction and analysis of the presence of the Pl_6 and Pl_{arg} genes, conferring resistance to downy mildew race 730, predominantly present in Serbia, and resistance to all known races of this pathogen, respectively (Panković et al. 2007; Jocić et al. 2010; Imerovski et al. 2014). Hence, all NS commercial hybrids possess one or both of these genes. Marker-assisted selection has also successfully been used for detection of *cms* and *Rfo* genes in rapeseed hybrid breeding at IFVCNS, leading to creation of the first commercial hybrid NS Ras (Dimitrijević et al. 2015). Ongoing research comprises screening and identification of markers for broomrape resistance in inbred line collection, as well as material developed by interspecific crosses using accessions from IFVCNS wild sunflower collection (Imerovski et al. 2013; 2016; 2019).

There are no reports of the application of genetic engineering for the improvement of the existing and introduction of new traits in maize and rapeseed in Serbia. The same stands for sunflower, where only attempts were reported to introduce *Sclerotinia* white mold resistance from wild germplasm into cultivated sunflower via protoplast fusion (Taški-Ajduković et al. 2006; 2008). The Law on Genetically Modified Organisms (GMOs) from 2009 is probably one of the reasons for this situation, as the Law prohibits commercial cultivation and placing GMOs and products of GMOs on the market. Although there is no ban on researching GMOs, the ban on commercial use as well as concerns regarding risk assessment procedures (Tarasjev 2012), the risk of transgene escape and possible impact on crop wild relatives and landraces (Cantamutto and Poverene 2007), as well as effective coexistence with other crops (Miladinović and Miladinović 2012; 2014) deemed such research less interesting for breeders.

Institutional framework

Conservation, management and utilization of PGR is decentralized in Serbia, and the responsibility is shared among the National Gene Bank (NGB) and several agricultural institutes and other organizations dealing with different aspects of PGR (maintenance of collections, breeding programs, regeneration, multiplication, characterization, etc.) (Dajić Stevanović and Đođević Milošević 2018). A review of conservation status of PGR collections both kept in NGB and other institutions is given by Dajić Stevanović and Đođević Milošević (2018).

Serbia has participated in the European Cooperative Program for Plant Genetic Resources (ECPGR) since 1980 and regularly sends passport data to the European Search Catalogue for Plant Genetic Resources (EURISCO). The Memorandum of Understanding on participation in the European Gene Bank Integrated System (AEGIS) has been signed by the Serbian Minister of Agriculture. Serbia has also established the National Information Sharing Mechanism (NISM), a partnership among stakeholders that contribute to the conservation and sustainable use of PGRFA in the Republic of Serbia. It is also an information network for monitoring activities carried out to implement the Global Plan of Action (GPA) on PGRFA in the Republic of Serbia. NISM database will serve as a working instrument for the National Plant Genetic Resources Committee and for national institutions for drafting strategies and plans. The NISM uses an information system including a database and a search engine, which includes the inventory of national PGRFA-related institutions, experts, publications, laws, and projects, as well as of cultivated varieties. The Mechanism operates through contribution of national institutions, coordinated by the Ministry of Agriculture, Forestry and Water Management. Access to genetic resources from the Serbian research institutions is regulated by specific mechanisms of these institutions. External (international) material exchange exists mostly between plant breeding institutions on a bilateral basis. However, the most frequent form of exchange is personal exchange of the selected material by breeders. Such an exchange lacks a centralized record keeping and control system (Dajić Stevanović and Đođević Milošević 2018).

The National Gene Bank (NGB) that keeps PGR collection was established in 2015, as a consequence of Article 41 of the Food Safety Law. It is a part of the Directorate of National Reference Laboratories of the Ministry of Agriculture, Forestry and Water Management of Republic of Serbia. NGB stores over 4000 accessions, mainly of local and autochthonous populations, as well as heirloom varieties (Dajić Stevanović and Đođević Milošević 2018), and performs activities related to the preparation of protocols, keeping a collection of seeds and plant material, registration of seed samples, and their storage and maintenance, reproduction and regeneration of samples, sharing samples with other gene banks, creation of national program for plant genetic resources, realization of international (funding) projects, development of a database on plant genetic resources, cooperation with scientific research institutions, and carrying out other tasks in this area.

The Plant Gene Bank of Serbia started to operate with the financial and technical support of two international projects. During the implementation of the project „South -Eastern Europe Development Network for Plant Genetic Resources—SEEDNet ", financed by the Swedish International Development Agency (Sida) (2004–2011), equipment was procured and gene banks in Southeast European countries were formed. In 2015, the National Plant Gene Bank of Serbia started to work officially. The Technical Cooperation Project (FAO TCP) "Support to the Development of the National Program for the Conservation of Plant Genetic Resources in Serbia" (2011–2013) significantly contributed to the establishment of a national program in Serbia. The enormous significance of plant genetic resources for food and agriculture (PGRFA) is also recognized by numerous strategies and programs of the State, such as: Agriculture and Rural Development Strategy, Biodiversity Strategy, Nature Protection Strategy, and others.

Legal framework

Serbia participates in all national and international bodies for preservation, management and use of plant genetic resources. Different international regulations regarding agriculture, biodiversity, as well as intellectual property are mutually compatible and lead to sustainable and effective exploitation of plant genetic resources. An overview of Serbia's regulations related to maintenance and use of plant genetic resources in breeding is given in Fig. 1.

In most countries in our region and Europe, the work on plant genetic resources conservation and sustainable use is carried out through national programs (Mladenović Drinić and Savić Ivanov 2017). During the regional SEEDNet project, several participating partners adopted National PGRFA Conservation Programs: Albania, Croatia, Montenegro, Republic of Srpska and Slovenia and are successfully implementing those programs. In Serbia, the final draft of the Law on PGRFA Management was prepared by the expert group (2016). This Law regulates management of plant genetic resources for food and agriculture of the Republic of Serbia; basic goals and principles of conservation, sustainable use and access to plant genetic resources; law enforcement entities; measures and procedures for the conservation and sustainable use of plant genetic resources; a unique database of plant genetic resources; establishment of the National Plant Gene Bank; formation of the Expert Council on Plant Genetic Resources; adoption of the National Program; financing; oversight of law enforcement; and penal provisions. The document will regulate the access and exchange of PGRFA, as all countries are mutually dependent on plant genetic resources.

The process of signing international treaties related to PGR by the government was slowed down because the breeders were concerned that their interests could be jeopardized by accepting documents on access and benefit-sharing (ABS). Nowadays, after many years of negotiations with stakeholders, our country is a signatory of all the important international documents dealing with the PGRFA (CBD, FAO ITPGRFA, Nagoya protocol). Most countries in our region are members of the FAO ITPGRFA with the exception of Bosnia and Herzegovina and North Macedonia. FAO ITPGRFA was signed by the Serbian Government in 2001 and ratified in Parliament in 2013. The Nagoya protocol has not yet been implemented in Serbia, as it was ratified only recently (2018) by the Ministry for Environmental Protection and the national legislation related to Nagoya are planned to be prepared in the near future, with the cooperation of the two ministries (Agriculture and Environmental Protection). From

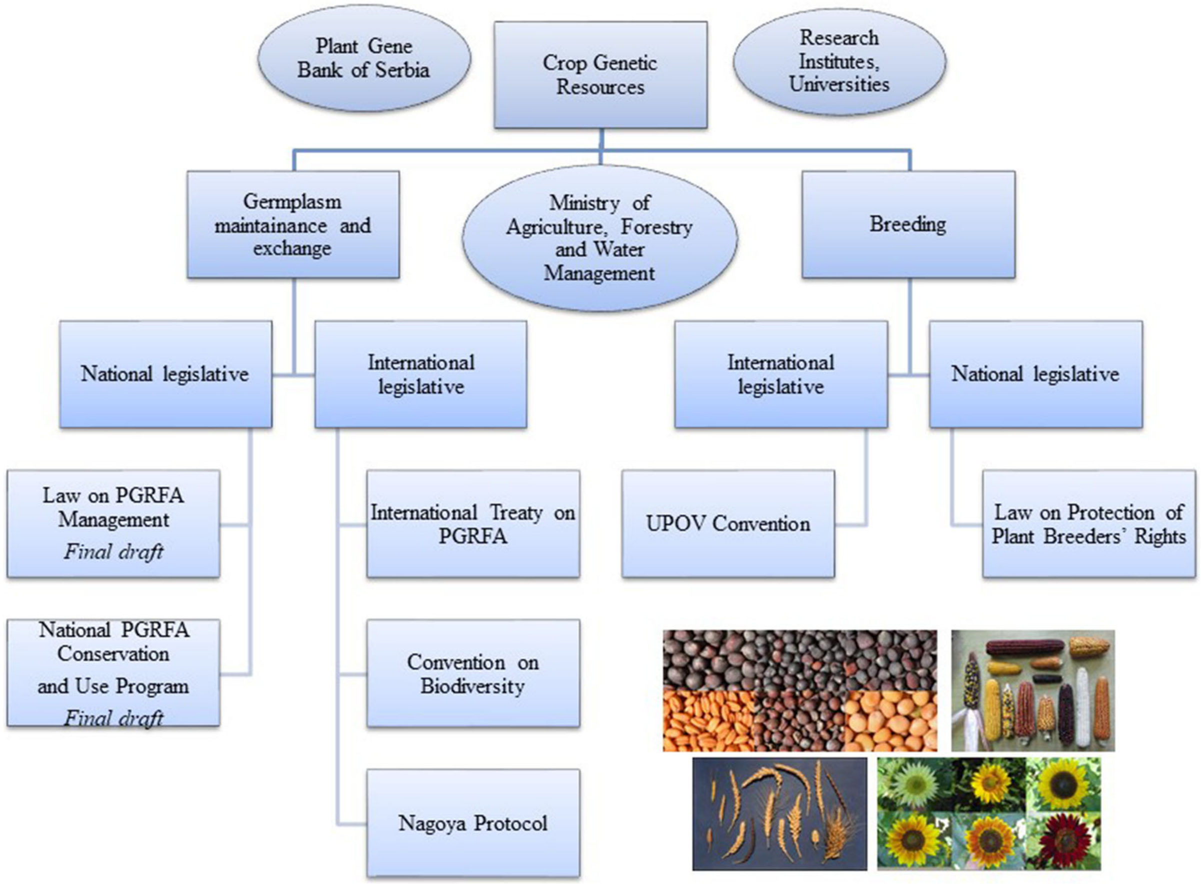

Fig. 1 Overview of Serbia's regulatory system related to plant genetic resources and their use in breeding. PGRFA–Plant Genetic Resources for Food and Agriculture; UPOV—International Union for the Protection of New Varieties of Plants

2014 to 2019, some of the neighbouring countries became members to Nagoya Protocol from (Albania, Croatia, Bulgaria, Hungary and Romania) and some have not signed or ratified it yet (Bosnia and Herzegovina, North Macedonia, Montenegro and Slovenia). The implementation of Nagoya Protocol is currently under debate in many countries, so there is a need to gain more experience in its implementation that could be shared among countries from the region, to avoid potential complications for both breeding research and practical breeding (Louwaars 2018).

By signing the related international agreements, Serbia has become an equal member of the world PGR community together with all other signatory parties and is also working on harmonizing its national legislation with the international.

Future prospects

In order to achieve further progress in crop breeding, it is necessary to increase genetic variability, especially when considering climate change, human population growth and changes in the food production system (Miladinović et al. 2019). Future importance of germplasm collections will be connected to mapping of valuable genes, especially in heirloom varieties and wild progenitors. The advances in genotyping and biotechnology tools in recent years are making genetic resources and their variation more accessible for breeders. High-throughput genotyping and phenotyping data of gene bank accessions make them more useful. New breeding tools may also facilitate applications for efficient crop improvement through the removal of deleterious mutations or enrichment of cultivated sequences with alleles from wild relatives or landraces that confer different resistances or improve yield and quality.

Researchers in Serbia have a long-standing history of maintaining and using crop genetic resources in breeding. The results of these breeding efforts are crop varieties, which have been released in Serbia and abroad. The existence of national Plant Gene Bank facilitated these efforts, which could be even more successful after completion of plant genetic resources inventory and adequate system for electronic data management. However, the most significant portion of crop breeding is carried out in the public research institutes that are granted the plant breeders' rights, and where most of the crop varieties are not protected as intellectual property, thus making them unprepared for full implementation of international treaties, signed by Serbia, regarding use of plant genetic resources in breeding. This could be overcome by more extensive activities of the research institutes in the protection of the intellectual property, supported by adequate measures from the state.

As for new breeding techniques, the lack of regulations and uncertainty about possible applications, along with the ban on the commercial use of GMOs makes breeders less motivated to implement these techniques. The researchers have knowledge and resources to apply these techniques for introduction of new traits from wild relatives and landraces into crops, but for the time being this research is generally put on hold, waiting for the change of public perception and regulations regarding GMOs, as well as new regulations and proper assessments of new breeding methods, such as gene editing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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