Ex situ and *in vivo* conservation and utilization of crop wild relatives in Bulgarian National Genebank*

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Abstract. The protection of the crop wild relatives (CWRs) is prioritized goal in the world scale. The need of their effective conservation as a tool to reduce loss of biodiversity is underscored by the CBD, the ITPGRFA and the Global strategy for conservation of plant genetic resources. Bulgaria is one of the countries in the world possessing large distribution of crop wild relatives – more than 5000 plant species appeared as crop wild relatives. In the IPGR, Sadovo is situated the National genebank where are preserved more than 59187 accessions. In long term conservation, under the temperature of –18 °C, are preserved 18621 CWRs accessions from 26 plant families, 88 genera and 176 species. CWRs are also preserved *in vivo* in the Botanical garden of IPGR – Sadovo.

Key words: conservation, crop wild relatives, *ex situ*, *in situ*, *in vivo*

Introduction

Crop wild relatives (CWRs) are wild plant species closely related to crops, including wild ancestors. They have an indirect use as gene donors for crop improvement due to their relatively close genetic relationship to crops. They are an important socio-economic resource that offer novel genetic diversity required to maintain future food security (Maxted & al. 2006; Heywood & al. 2007; Kell & al. 2008). CWRs hold important plant genetic resources for food and agriculture (PGRFA) due to their potential to contribute beneficial traits to crops (Maxted & al. 2012). CWRs also contain greater genetic variation than modern crops as they have not been through the genetic bottleneck of domestication which tends to reduce diversity (Volbrecht & Sigmon 2005). They have valuable qualities and can be a starting material for selection of new varieties suitable for the changing climate conditions and to meet the new market demands (Maxted 2007).

Conservation of crop wild relatives is a priority worldwide. Convention on Biological Diversity, the International Treaty on Plant Genetic Resources (PGR) for food and agriculture, the Global Strategy for storing PGR emphasize the need for their effective conservation as a means of reducing the loss of biodiversity in global scale.

According to FAOs second State of the World PGRFA report the interest in and awareness of the importance of conserving CWR, both *ex situ* and *in situ*, and its use in crop improvement have increased substantially**.** From 50 000–60 000 CWRs worldwide (same genus as crop) 700 CWRs are considered as highest priority from a global perspective, being the species that comprise the primary and secondary gene pools of the world's most important food crops (http://biodiversity.europa.eu/topics/genetic-resources). Crop wild relatives are increasingly being recognized for their potential to contribute valuable traits to breeding programs (Feuillet & al. 2008;

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Guarino & Lobell 2011; Dempewolf & al. 2014). CWRs have provided breeders with genes for pest and disease resistance, abiotic stress tolerance, and quality traits in an ever increasing number of food crops, such as banana, barley, bean, cassava, chickpea, maize, lettuce, oat, potato, rice, sugar-cane, sunflower, tomato, wheat, and others (Hajjar & Hodgkin 2007; McCouch & al. 2007; Khoury & al. 2010, 2015a, b).

CWRs are vital genetic resources and in case of effective conservation and proper use can contribute to enhance food security and improve the ecosystem stability. These species, however, are seriously threatened by human activities and many of them are now rare, and some have even disappeared. According to the FAO, 75 % of plant genetic diversity has been lost over the past century (FAO 1998). The reason for these massive losses is an exact selection, i.e. dominance of the relatively small number of highly productive, but genetically similar varieties. Loss of genetic diversity in the world leads to the need to develop new varieties that respond to changes in climate. Hence, the great interest in the as source material for future selection. Therefore a more serious approach to their conservation is necessary.

Bulgaria is one of the world's countries with the highest prevalence of CWR. Our country falls into one of the Vavilov centers (Mediterranean) or the so-called centers of origin of cultivated plants (http://en.wikipedia.org/wiki/Center_of_origin).

CWRs that originate from the Mediterranean center, where our country is, comprise several major groups:

- Cereals and Legumes: durum wheat, emmer, Polish wheat, spelt, Mediterranean oats, sand oats, canarygrass, grass pea, pea, lupine
- Forage Plants: Egyptian clover, white clover, crimson clover, serradella
- Oil and Fiber Plants: flax, rape, black mustard, olive
- Vegetables: garden beet, cabbage, turnip, lettuce, asparagus, celery, chicory, parsnip, rhubarb,
- Ethereal Oil and Spice Plants: caraway, anise, thyme, peppermint, sage, hop.

The aim of this review is to present the role and importance of the National Seed Genebank and the Botanical garden in the Institute of Plant Genetic Resources in *ex situ/in vivo* conservation of CWRs in Bulgaria.

Results and Discussion

The need to conserve CWRs taxa has been identified by policy-makers by including them in policy instruments, such as the European Strategy for Plant Conservation (Planta Europa 2008), the Global Strategy for Plant Conservation (CBD 2010a), CBD Strategic Plan for Biodiversity 2011–2020 (CBD 2010b), the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2001), ECPGR Concept for in situ conservation of crop wild relatives in Europe (Maxted & al. 2015).

Conservation of CWRs can be accomplished through a number of activities at national, regional and local level and coordination between them to obtain maximum results. However, it needs to first identify the CWRs taxa within a country and decide on efficient methods to conserve their genetic resources (Fitzgerald & al. 2012).

There are two primary techniques for CWRs conservation: *in situ*, primarily in natural habitats managed as genetic reserves and *ex situ* as seed in gene banks.

In situ conservation has been defined by the CBD (United Nations 1992) as "the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings where they have developed their distinctive properties". This method is often described as 'dynamic' (Frankel & al., 1995) conservation as species are subjected to changing environmental conditions, new pests and new diseases leading to evolutionary changes (Maxted & al. 1997; Phillips 2012).

Ex situ conservation is the protection of components of biological diversity outside their natural habitats which may be achieved by the following methods; seed storage, field gene bank, botanical gardens, *in vitro* storage, DNA storage and pollen storage (Maxted & al. 1997). *Ex situ* conservation not only complements *in situ* conservation, it makes a specific contribution through material being readily available and under direct control by the farmers and scientists (Frankel & al., 1995). According to Smith and Linington (1997) this technique is suitable for the majority of CWRs species because, the annual cost of maintenance may be as little as US$ 5 per year for a single accession.

Ex situ seed banks can be a relevant component in the functioning of Genetic Recourses as they provide a back-up of genetic diversity in case any catastrophe should occur.

The preservation of the plant biodiversity from the cultural and wild flora is the main priority in the research work of the Institute of Plant Genetic Resources "Konstantin Malkov". The institute is the National Coordinator of the National Programme for Plant Genetic Resources as a part of the European Programme for Plant Genetic Resources (ECPGR). The National Programme on Conservation and Utilization of Plant Genetic Resources and Agro-biodiversity is based on the applicable international documents, principles and methodologies that are stated in, e.g. the FAO Global Plan of Action, International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), Standard Material Transfer Agreement (SMTA), European Cooperative Programme for Plant Genetic Resources Networks (ECPGR), or generally in the Convention on Biological Diversity (CBD). It is also based on relevant recommendations of the international organizations, e.g. Bioversity International and Global Crop Diversity Trust. On the territory of the Institute is located the National Seed Genebank. It was built in 1984 and implemented a scientific program for long-term and medium-term storage of seed germplasm under controlled conditions, consistent with the standards of FAO. There are preserved 59 187 seed samples from 62 131 totally registered as plant germplasm in the country (http://eurisco.ecpgr.org). The crop groups of *ex situ* collections in the genebank include: cereals, grain legumes, oil and industrial, forage grasses, vegetables, ornamental and medicinal species. The collections provide the good opportunity of utilization based on existing large scale genetic diversity: wild species, local populations, primitive varieties, breeding materials and modern varieties with different origin (Stoyanova 2007).

Collection of CWRs in the National genebank is presented from 18 621 accessions, from 26 plant families, 88 genera and 176 species. The biggest part of them are species from *Poaceae, Fabaceae, Solanaceae, Linacea, Cucurbitacea, Asteraceae, Pedaliacae and Brassicaceae* botanical families (Fig. 1).

CWRs accessions with origin from Bulgaria are 5 531 (Table 1). They belong to 176 plant species. The collections of genus *Trifolium, Vicia* and *Aegilops* are presented from the highest diversity, respectively from the biggest number of species. Genus *Trifolium* consists of 18 spescies, *Vicia*-17 and *Aegilops*-8 species.

All accessions are maintained in the base collection of National genebank at the long term storage condition: at 3–7 % moisture (depending upon species) and at subzero temperatures (–18 °C) in hermetically closed containers (glass jars or three laminated aluminum foil packets). Under these conditions the plant germplasm could be preserved with minimal changes over decades or hundred and more years (Stoyanova 2002, 2003, 2005, 2007).

The Botanical garden is established in 2002 with a grant received from the Ministry of Envirommentand Waters under the National Plan for Biodiversity con-

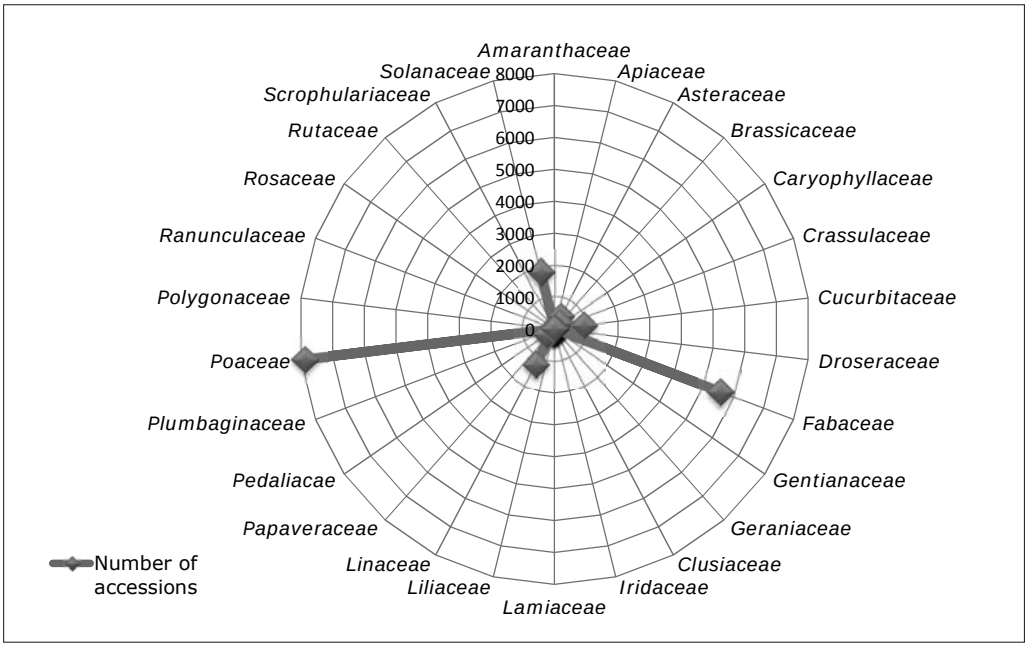

Fig. 1. Botanical families represented from CWRs accessions in the National genebank.

servation. The main activity is creation and maintenance of scientifically organized and documented collection of CWRs with Bulgarian origin for the study of plant genetic resources, their conservation and sustainable use.

As a result of the expeditions in the country and free exchange with botanical gardens until now the *in vivo* collection comprises 443 species, divided thematically as follows:

- Crop Wild Relatives – *Hordeum bulbosum, Secale rhodopaea, Vicia incisa, Sylibum marianum, Chenopodium bonus-henricus* etc.
- Demonstration collections, including old varieties, populations and forms from traditional and alternative crops – *Triticum spelta, Amygdalus nana, Vicia ervilia, Cynara scolymus etc.*
- Rare, endemic and protected species – *Haberlea rhodopensis, Tulipa rhodopaea, Anemone blanda, Alissoides bulgaricum, Iris reinchenbachii, Paeonia tenuifilia etc.*

In the botanical garden there are 238 species – CWRs from 37 plant families (Fig. 2).

Unfortunately, a large part of the biodiversity of CWRs in Bulgaria have not yet been studied, and many of them are potentially endangered or their natural distribution is severely limiting. So far, there was no national funding for this activity and studies are conducted with individual projects. The existing international coordination through the European Programme for Plant Genetic Resources (ECPGR) provides information on scientifically based approach for organizing conservation activities and utilization of plant species from the group of CWR.

Complementary conservation (i.e., conservation using both *in situ* and *ex situ* techniques) is perhaps more suitable now in the face of climate change, shifting ecosystems and habitat loss than ever before (Maxted & Kell 2015). A very essential step towards conservation of bulgarian CWRs is establishment of Natianal Strategy for their conservation.

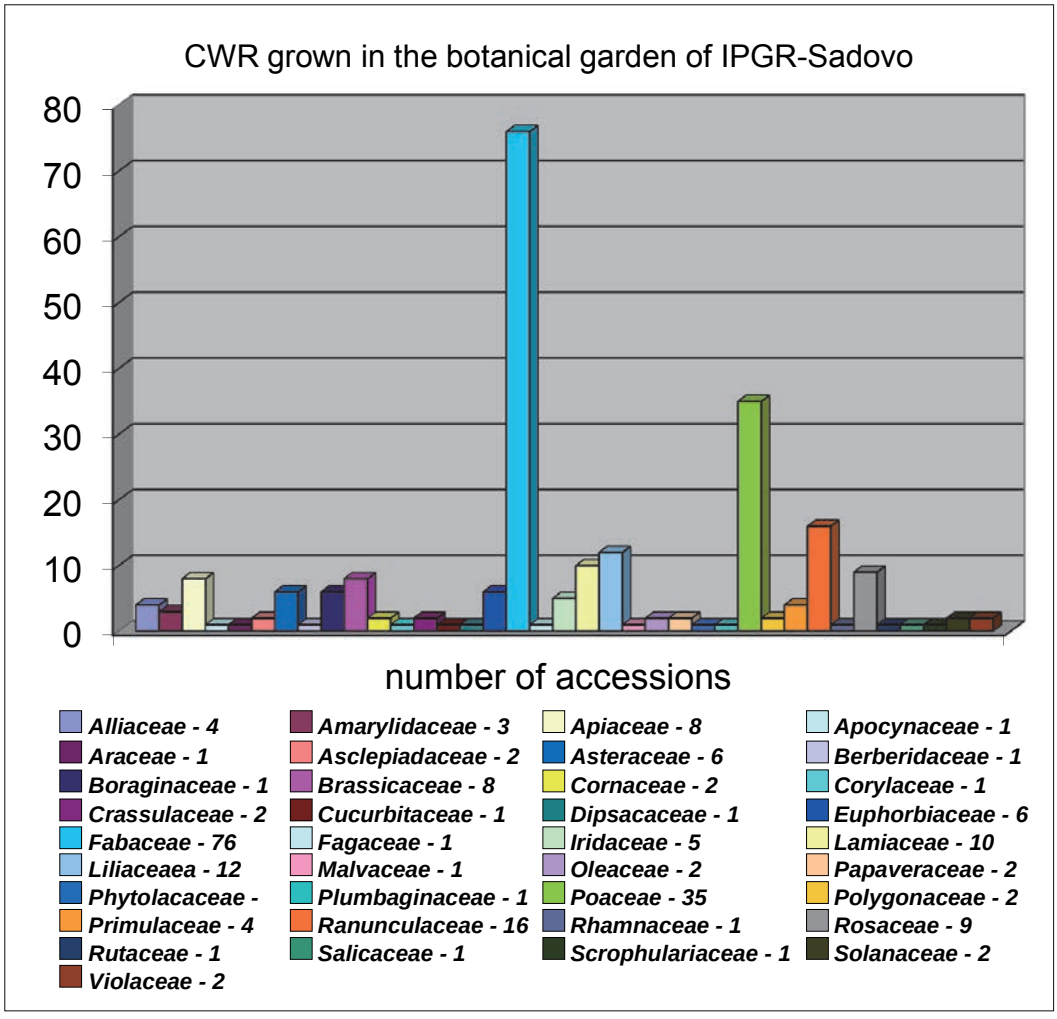

Fig. 2. CWRs grown in the botanical garden in the Institute of Plant Genetic Resources-Sadovo.

Table 1. List of Bulgarian CWRs accessions maintain in the base collection of National Genebank of Bulgaria.

№	Family	Genus	Species	NAT	INT	CULT	NACC
1	*Amaranthaceae*	*Amaranthus*	*retroflexus*	A	I		1
2	*Apiaceae*	*Anethum*	*graveolens*	AN			3
3	*Apiaceae*	*Carum*	*carvi*	N			1
4	*Apiaceae*	*Coriandrum*	*sativum*	N			3
5	*Apiaceae*	*Daucus*	*carota*	N			1
6	*Apiaceae*	*Foeniculum*	*vulgare*	N			8
7	*Apiaceae*	*Opopanax*	*chironium*	N			1
8	*Apiaceae*	*Pimpinella*	*anisum*	A	I		2
9	*Asteraceae*	*Achillea*	*thracica*	N			1
10	*Asteraceae*	*Artemisia*	*annua*	N			1
11	*Asteraceae*	*Helianthus*	*annuus*	A		C	70
12	*Asteraceae*	*Lactuca*	*serriola*	N			2
13	*Brassicaceae*	*Brassica*	*juncea*		I		8
14	*Brassicaceae*	*Brassica*	*nigra*	N			1
15	*Brassicaceae*	*Brassica*	*rapa*	S			3
16	*Brassicaceae*	*Camelina*	*microcarpa*	N			1
17	*Brassicaceae*	*Camelina*	*sativa*	N			13
18	*Brassicaceae*	*Eruca*	*vesicaria*	N			1
19	*Brassicaceae*	*Isatis*	*tinctoria*	N			1
20	*Brassicaceae*	*Lepidium*	*sativum*	AND		C	1
21	*Brassicaceae*	*Matthiola*	*odoratissima*	N			4
22	*Brassicaceae*	*Sinapis*	*alba*	N			14
23	*Caryophyllaceae*	*Arenaria*	*rigida*	N			1
24	*Caryophyllaceae*	*Dianthus*	*nardiformis*	N			1
25	*Caryophyllaceae*	*Gypsophila*	*paniculata*	N			2
26	*Caryophyllaceae*	*Silene*	*caliacrae*	N			1
27	*Cucurbitaceae*	*Cucumis*	*melo*	A		C	21
28	*Cucurbitaceae*	*Cucumis*	*sativus*	A		C	357
29	*Cucurbitaceae*	*Cucurbita*	*maxima*	A		C	6
30	*Cucurbitaceae*	*Cucurbita*	*moschata*	A		C	5
31	*Cucurbitaceae*	*Cucurbita*	*pepo*	A		C	12
32	*Fabaceae*	*Arachis*	*hypogaea*	A		C	88
33	*Fabaceae*	*Astragalus*	*centralpinus*	N			1
34	*Fabaceae*	*Astragalus*	*dasyanthus*	N			2
35	*Fabaceae*	*Astragalus*	*physocalyx*	N			1
36	*Fabaceae*	*Lathyrus*	*annuus*	N			1
37	*Fabaceae*	*Lathyrus*	*pratensis*	N			1
38	*Fabaceae*	*Lathyrus*	*sativus*	A	ID	C	60
39	*Fabaceae*	*Lathyrus*	*cicera*	N			8
40	*Fabaceae*	*Lathyrus*	*pancicii*	N			1

Table 1. Continuation.

№	Family	Genus	Species	NAT	INT	CULT	NACC
41	*Fabaceae*	*Lens*	*culinaris*	A	D	C	63
42	*Fabaceae*	*Lotus*	*corniculatus*	N			
43	*Fabaceae*	*Lupinus*	*albus*	N^2A	I		7
44	*Fabaceae*	*Medicago*	*orbicularis*	N			1
45	*Fabaceae*	*Medicago*	*polymorpha*	N			3
46	*Fabaceae*	*Medicago*	*sativa*	NA	D	C	68
47	*Fabaceae*	*Onobrychis*	*viciifolia*	AN	I	C	12
48	*Fabaceae*	*Phaseolus*	*vulgaris*	A		C	337
49	*Fabaceae*	*Pisum*	*sativum*	NA	I	C	329
50	*Fabaceae*	*Trifolium*	*alexandrinum*	AN		C	10
51	*Fabaceae*	*Trifolium*	*angustifolium*	N			1
52	*Fabaceae*	*Trifolium*	*balansae*	N			1
53	*Fabaceae*	*Trifolium*	*campestre*	N			3
54	*Fabaceae*	*Trifolium*	*cherleri*	N			3
55	*Fabaceae*	*Trifolium*	*constantinopolitanum*	N			1
56	*Fabaceae*	*Trifolium*	*echinatum*	N			3
57	*Fabaceae*	*Trifolium*	*fragiferum*	N			1
58	*Fabaceae*	*Trifolium*	*glomeratum*	N			1
59	*Fabaceae*	*Trifolium*	*hirtum*	N			3
60	*Fabaceae*	*Trifolium*	*hybridum*	N			3
61	*Fabaceae*	*Trifolium*	*incarnatum*	N			1
62	*Fabaceae*	*Trifolium*	*lappaceum*	N			2
63	*Fabaceae*	*Trifolium*	*medium*	N			1
64	*Fabaceae*	*Trifolium*	*pratense*	N			20
65	*Fabaceae*	*Trifolium*	*repens*	N			35
66	*Fabaceae*	*Trifolium*	*resupinatum*	N			3
67	*Fabaceae*	*Trifolium*	*squarrosum*	N			1
68	*Fabaceae*	*Vicia*	*cassubica*	N			2
69	*Fabaceae*	*Vicia*	*cracca*	N			1
70	*Fabaceae*	*Vicia*	*dalmatica*	N			1
71	*Fabaceae*	*Vicia*	*ervila*	NA	I	C	18
72	*Fabaceae*	*Vicia*	*faba*	A	ID	C	107
73	*Fabaceae*	*Vicia*	*grandiflora*	N			1
74	*Fabaceae*	*Vicia*	*hirsuta*	N			1
75	*Fabaceae*	*Vicia*	*hybrida*	N			1
76	*Fabaceae*	*Vicia*	*lutea*	N			2
77	*Fabaceae*	*Vicia*	*melanops*	N			1
78	*Fabaceae*	*Vicia*	*narbonensis*	N			12
79	*Fabaceae*	*Vicia*	*peregrina*	N			2
80	*Fabaceae*	*Vicia*	*sativa*	NA	I	C	277

Table 1. Continuation.

№	Family	Genus	Species	NAT	INT	CULT	NACC
81	*Fabaceae*	*Vicia*	*sepium*	N			2
82	*Fabaceae*	*Vicia*	*varia*	N			1
83	*Fabaceae*	*Vicia*	*villosa*	N			38
84	*Fabaceae*	*Vigna*	*unguiculata*	A		C	5
85	*Fabaceae*	*Cicer*	*arietinum*	A	I	C	32
86	*Gentianaceae*	*Gentiana*	*lutea*	N			6
87	*Geraniaceae*	*Erodium*	*absinthoides*	N			2
88	*Iridaceae*	*Crocus*	*tomasinianus*	N			1
89	*Lamiaceae*	*Hyssopus*	*officinalis*	N			2
90	*Lamiaceae*	*Lallemantia*	*iberica*	A		C	3
91	*Lamiaceae*	*Salvia*	*officinalis*	AN	D	C	1
92	*Lamiaceae*	*Salvia*	*sclarea*	N			3
93	*Lamiaceae*	*Satureja*	*hortensis*	A	I	C	6
94	*Lamiaceae*	*Sideritis*	*scardica*	N			2
95	*Liliaceae*	*Allium*	*angulosum*	N			2
96	*Linaceae*	*Linum*	*usitatissimum*	A		C	71
97	*Papaveraceae*	*Papaver*	*somniferum*		I		20
98	*Pedaliacae*	*Sesamum*	*indicum*	A		C	233
99	*Plumbaginaceae*	*Goniolimon*	*besseranum*				1
100	*Plumbaginaceae*	*Goniolimon*	*collinum*				1
101	*Plumbaginaceae*	*Goniolimon*	*dalmaticum*				1
102	*Plumbaginaceae*	*Goniolimon*	*tataricum*				1
103	*Plumbaginaceae*	*Limonium*	*asterotrichum*	N			1
104	*Plumbaginaceae*	*Limonium*	*bulgaricum*	N			1
105	*Plumbaginaceae*	*Limonium*	*gmelinii*	N			1
106	*Plumbaginaceae*	*Limonium*	*latifolium*	N			1
107	*Plumbaginaceae*	*Limonium*	*meyeri*	N			1
108	*Plumbaginaceae*	*Limonium*	*vulgare*	N			1
109	*Poaceae*	*Aegilops*	*biuncialis*	N			36
110	*Poaceae*	*Aegilops*	*caudata*	N			4
111	*Poaceae*	*Aegilops*	*comosa*				2
112	*Poaceae*	*Aegilops*	*cylindrica*	N			26
113	*Poaceae*	*Aegilops*	*geniculata*	N			7
114	*Poaceae*	*Aegilops*	*neglecta*	N			12
115	*Poaceae*	*Aegilops*	*triuncialis*	N			93
116	*Poaceae*	*Aegilops*	*umbellulata*	N			2
117	*Poaceae*	*Agropyron*	*cristatum*	N			10
118	*Poaceae*	*Agropyron*	*pectinatum*	N			13
119	*Poaceae*	*Arrhenatherum*	*elatius*	N			5
120	*Poaceae*	*Avena*	*fatua*	N			2

Table 1. Continuation.

№	Family	Genus	Species	NAT	INT	CULT	NACC
121	*Poaceae*	*Avena*	*sativa*			C	138
122	*Poaceae*	*Brachypodium*	*pinnatum*	N			3
123	*Poaceae*	*Dactylis*	*glomerata*	N			160
124	*Poaceae*	*Festuca*	*rubra*	N			10
125	*Poaceae*	*Festuca*	*valesiaca*	N			1
126	*Poaceae*	*Lolium*	*multiflorum*	N			5
127	*Poaceae*	*Lolium*	*perenne*	N			70
128	*Poaceae*	*Panicum*	*miliaceum*		I		81
129	*Poaceae*	*Phleum*	*phleoides*	N			1
130	*Poaceae*	*Phleum*	*pratense*	N			2
131	*Poaceae*	*Poa*	*pratensis*	N			2
132	*Poaceae*	*Sorghum*	*bicolor*				9
133	*Poaceae*	*Triticum*	*baeoticum*	N			18
134	*Poaceae*	*Triticum*	*monococcum*				36
135	*Poaceae*	*Zea*	*mays*				1302
136	*Polygonaceae*	*Fagopyrum*	*esculentum*	A	I	C	2
137	*Ranunculaceae*	*Aquilegia*	*aurea*	N			1
138	*Ranunculaceae*	*Nigella*	*damascena*	N			1
139	*Rosaceae*	*Alchemilla*	*achtarowii*	N			1
140	*Rosaceae*	*Alchemilla*	*mollis*	N			1
141	*Rutaceae*	*Ruta*	*graveolens*	N			1
142	*Scrophulariaceae*	*Verbascum*	*anisophyllum*	N			1
143	*Scrophulariaceae*	*Verbascum*	*nobile*	N			1
144	*Scrophulariaceae*	*Verbascum*	*roripifolium*	N			1
145	*Scrophulariaceae*	*Verbascum*	*tzar-borisii*	N			2
146	*Solanaceae*	*Atropa*	*bella-donna*	N			1
147	*Solanaceae*	*Capsicum*	*annuum*			C	485
148	*Solanaceae*	*Lycopersicon*	*esculentum*			C	275
149	*Solanaceae*	*Nicotiana*	*tabacum*			C	206
150	*Solanaceae*	*Solanum*	*melongena*			C	13
	22	**78**	**176**				**5531**

List of abreviations:

Codes for recording Native Status (NAT): **N** – Native; **S** – Assumed to be native; **D** – Doubtfully native; **E** – Formerly native (extinct); **A** – Not native; **F** – Recorded as native in error;

Codes for recording Introduced Status (INT): **I** – Introduced; **S** – Assumed to be introduced; **D** – Doubtfully introduced; **E** – Formerly introduced (Extinct); **A** – Not introduced; **F** – Recorded as introduced in error;

Codes for recording cultivated status (CULT): **C** – Cultivated; **S** – Assumed to be cultivated; **D** – Doubtfully cultivated; **E** – Formerly cultivated (extinct); **A** – Not cultivated; **F** – Recorded as cultivated in error;

NACC – Number of Bulgarian CWRs accessions, maintained in the National Genebank.

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