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Wheat Improvement

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Chapter 17
Conserving Wheat Genetic Resources

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Abstract Wheat genetic resources (WGR) are represented by wheat crop wild relatives (WCWR) and cultivated wheat varieties (landraces, old and modern cultivars). The conservation and accessibility of WGR are fundamental due to their: (1) importance for wheat breeding, (2) cultural value associated with traditional food products, (3) significance for biodiversity conservation, since some WCWR are endangered in their natural habitats. Two strategies are employed to conserve WGR: namely *in situ* and *ex situ* conservation. *In situ* conservation, i.e. the conservation of the diversity at the location where it is found, consists in genetic reserves for WCWR and on farm programs for landraces and old cultivars. *Ex situ* conservation of WGR consists in the storage of dry seeds at cold temperatures in germplasm banks. It is currently the most employed conservation strategy for WGR because it allows the long-term storage of many samples in relatively small spaces. Due to the great number of seed samples of WGR and associated passport data stored in genebanks, it is increasingly important for the management of *ex situ* collections to: (1) employ efficient database systems, (2) understand seed longevity of the seed accessions, (3) setup safety backups of the collections at external sites.

Keywords Germplasm banks · Genetic reserves · On farm conservation · Seed conservation · Seed viability · Wheat wild relatives

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17.1 Learning Objectives

- To know the principal categories of wheat genetic resources,
- To know the principles of *in situ* conservation of wheat genetic resources,
- To know the principles of *ex situ* seed conservation of wheat genetic resources in germplasm banks.

17.2 Introduction – Plant Genetic Resources (PGR) and their Conservation

Wheat domestication occurred 9000 to 12,000 BCE, resulting in cereal crops within the genus *Triticum*, two of which are among the most widely grown crops worldwide, namely bread wheat (*T. aestivum* subsp. *aestivum*) and durum wheat (*T. turgidum* subsp. *durum*). Wheat genetic resources are represented by several domesticated and wild taxa.

Overall, plant genetic resources for food and agriculture (PGRFA) are defined as "any genetic material of plant origin of actual or potential value for food and agriculture" [1]. Genetic diversity is the foundation for crop improvement and is an insurance against unforeseen threats to agricultural production such as plant pathogens and climate changes [2].

Wheat genetic resources can be grouped in the following biological/agronomic categories:

– Cultivated wheats: wheat species were gathered by ancient societies, gradually resulting in the domestication of several wheat crop taxa. Cultivated materials consist of:
  - Landraces (or primitive cultivars): "dynamic populations of a cultivated plant that have historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems" [3];
  - Old cultivars: sometimes known as obsolete cultivars, the term refers to cultivated varieties which have fallen into disuse;
  - Modern cultivated varieties (modern cultivars): agronomic varieties in current use and newly developed varieties;
  - Special stocks: such as advanced breeding lines (i.e. pre-released varieties developed by plant breeders), mapping populations, CRISPR-edited lines and cytogenetic stocks.
– Crop wild relatives (CWR): wild plant species that are genetically related to cultivated crops. CWR are not only the wild ancestors of the domesticated plant but also other more distantly related species.

Another category of PGR of significance are the neglected crops, also referred as underutilized or orphan crops: "crop species that have been ignored by science and development but are still being used in those areas where they are well adapted and competitive" [4]**.** An example is the einkorn (*Triticum monococcum* subsp. *monococcum*) currently cultivated by small-holder farmers in limited areas in Europe, Middle East and North Africa. In recent years, there is a renewed interest for einkorn, mainly due to its nutraceutical properties and adaptations to organic agriculture [5].

The aim of plant genetic resources conservation is to ensure that the maximum possible allelic genetic diversity, and therefore potential useful traits for breeding of a crop, is maintained and is available and accessible for utilization. Crop domestication and selection have favored preferred haplotypes and have reduced genetic diversity. The conservation of landraces and CWR is particularly important considering that in those plants is concentrated the bulk of genetic diversity and of potential useful traits within a crop genepool. The conservation of modern cultivar is also of great importance since breeders often wish to access "improved" or refined sources of PGR diversity. Conserving PGR is important not only in order to provide useful traits for crop improvement but also for cultural reasons, since many landraces and neglected crops are connected to local identities, especially through local foods and ceremonial products.

Two main strategies are employed for the conservation of PGR, namely *in situ* and *ex situ* conservation. *In situ* conservation, i.e. the conservation of the diversity in its natural habitat, means the designation, management and monitoring of a population at the location where it is currently found. On the other hand, the *ex situ* conservation, i.e. the conservation of a genetic resources outside its natural habitat, is intended as the sampling, transfer and storage of a sample of a population of a certain species away from the original location where it was collected. Several *ex situ* conservation strategies are employed for different crops e.g. *in vitro* storage, seed banking, field genebanks, DNA banks. Seed banking allows the storage of many seed accessions in relatively small spaces; seed collections are economically viable and can provide a good sample of the genetic diversity within the crop genepool, usually remaining viable for the long-term [6].

17.3 Wheat Genetic Resources (WGR)

17.3.1 Domesticated Wheats

Two species of wheat are widely cultivated, namely: the hexaploid *Triticum aestivum* (ABD genome) and the tetraploid *T. turgidum* (AB genome, Table 17.1). Both species include several subspecies (Table 17.1). As previously mentioned, einkorn (*Triticum monococcum* L. subsp. *monococcum*, A genome) is a locally cultivated, diploid wheat.

Table 17.1 Domesticated wheats. The more common domesticated subspecies of *T. aestivum* and *T. turgidum* are also presented

Taxonomic name	Common English Name	Genome(s)	Accessions conserved *ex situ*[a]
Triticum monococcum L. subsp. *monococcum*	Einkorn	A	6971
Triticum monococcum L. subsp. *sinskajae* (Filat. & Kurkiev) Valdés & H. Scholz	Naked einkorn	A	23
Triticum turgidum L.	Rivet wheat	AB	179,701
Triticum turgidum L. subsp. *dicoccon* Schrank (Thell.)	Emmer	AB	8793
Triticum turgidum L. subsp. *durum* (Desf.) van Slageren	Durum wheat	AB	149,485
Triticum turgidum L. subsp. *carthlicum* (Nevski) Á. Löve & D. Löve	Persian wheat	AB	1382
Triticum turgidum L. subsp. *polonicum* (L.) Thell.	Polish wheat	AB	766
Triticum turgidum L. subsp. *turanicum* (Jakubz.) Á. Löve & D. Löve	Khorasan wheat	AB	461
Triticum turgidum L. subsp. *turgidum*	Poulard wheat	AB	7171
Triticum timopheevii (Zhuk.) Zhuk. subsp. *timopheevii*	Chelta Zanduri	AG	189
Triticum aestivum L.		ABD	511,130
Triticum aestivum L. subsp. *aestivum*	Bread wheat	ABD	243,634
Triticum aestivum subsp. *compactum* (Host) Mac Key	Club wheat	ABD	1921
Triticum aestivum subsp. *macha* (Dekapr. & Menabde) Mac Key	Macha wheat	ABD	374
Triticum aestivum L. subsp. *spelta (L.) Thell.*	Spelt	ABD	7070
Triticum aestivum subsp. *sphaerococcum* (Percival) Mac Key	Indian wheat	ABD	684
Triticum zhukovskyi Menabde & Eritzjan	Zhukovsky's wheat	AAG	71

[a]Accessions conserved *ex situ* estimated using data from [7], FAO-WIEWS, USDA GRIN and data provided directly by CIMMYT. The number of accessions of *T. aestivum* and *T. turgidum* includes also the accessions of the different subspecies

Two additional species of wheat were cultivated in western Georgia but are probably currently extinct under cultivation and conserved only in germplasm banks: *T. timophevii* subsp. *timopheevii* (Chelta Zanduri or Timopheevi wheat, tetraploid, AG) and *T. zhukovskyi* (Zhukovsky's wheat, hexaploid, AGG, Table 17.1). The Zhukovsky's wheat was described in the 1960s growing in a restricted area of western Georgia. This hexaploid wheat is an allopolyploid, spontaneous hybrid between Timopheevi wheat (*T. timopheevii*) and einkorn (*T. monococcum*). Zhukovsky's wheat and the two parental species used to be cultivated together in a complex of domesticated wheats named *zanduri*.

Wheat landrace cultivation was endemic throughout the Mediterranean Basin, Europe, Near East, Ethiopia, Caucasus, China and Southern Asia, since time immemorial. Wheat landraces were subsequently diffused to Australia, South Africa and the Americas. For example, the Creole wheats descendant of Spanish wheats imported from the sixteenth century were cultivated in Mexico for four centuries by small-scale farmers. In many areas of the world those landraces were replaced since the twentieth century by modern, improved varieties.

Formal wheat breeding started in the eighteenth century, eventually resulting in a plethora of old and modern cultivars. Noteworthy examples of old cultivars of bread wheat are: 'Sherriff's Squarehead', selected in the end of the nineteenth century in Great Britain, 'Ardito' and 'Mentana' selected in Italy in the first decades of twentieth century, 'Marquis' selected in Canada at the beginning of twentieth century, the semi-dwarf cultivar 'Norin 10' selected in Japan in 1935 and the cultivar 'Bezostaya 1' selected in Russia in the1950s. Several old cultivars of durum wheat also exist, e.g. the renowned 'Senatore Cappelli' released in Italy in 1915. Today, many old cultivars figure in the pedigree of modern wheat varieties and are therefore of great priority for conservation (see Chap. 2 for a history of wheat breeding).

17.3.2 Wheat Crop Wild Relatives (WCWR)

A crop "genepool concept" was defined by Harlan and De Wet [8] based on formal taxonomy and genetic relatedness, determined by the crossing ability between related species. Three main categories are considered: Primary Gene Pool (GP-1) comprising the domesticated crop and its closed wild forms with which the crop can cross producing fertile hybrids; Secondary Gene Pool (GP-2) which includes less closely related species, from which gene flow, even if difficult, is still possible using conventional breeding techniques; Tertiary Gene Pool (GP-3) which includes species from which gene transfer to the crop is impossible without the use of "rather extreme or radical measures". The gene pool levels here presented are based on: "The Harlan and de Wet Crop Wild Relative Inventory" (see: https://www.cwrdiversity.org/checklist/). An additional gene pool level classification system is historically used in wheat based on chromosome pairing and recombination (see Sect. 16.4).

The primary gene pool (GP-1, Fig. 17.1) of wheat comprises, beside the aforementioned domesticated wheats (Table 17.1), also the four wild species of the genus *Triticum* (*sensu* van Slageren 1994 [9]) included in Table 17.2.

GP-2 includes 22 species of the genus *Aegilops* and *Amblyopyrum muticum* (Table 17.3, Figs. 17.1 and 17.2). The geographic center of diversity, the areas where the most *Aegilops* grows in sympatry, is the Fertile Crescent, Turkey, the southern Caucasus, as well as the shores of the Aegean Sea. Spontaneous crosses between *Aegilops* species and cultivated wheats have been observed in several areas of the natural distribution of *Aegilops*. Those hybrids are classified in the genus x *Aegilotriticum* and are mostly sterile.

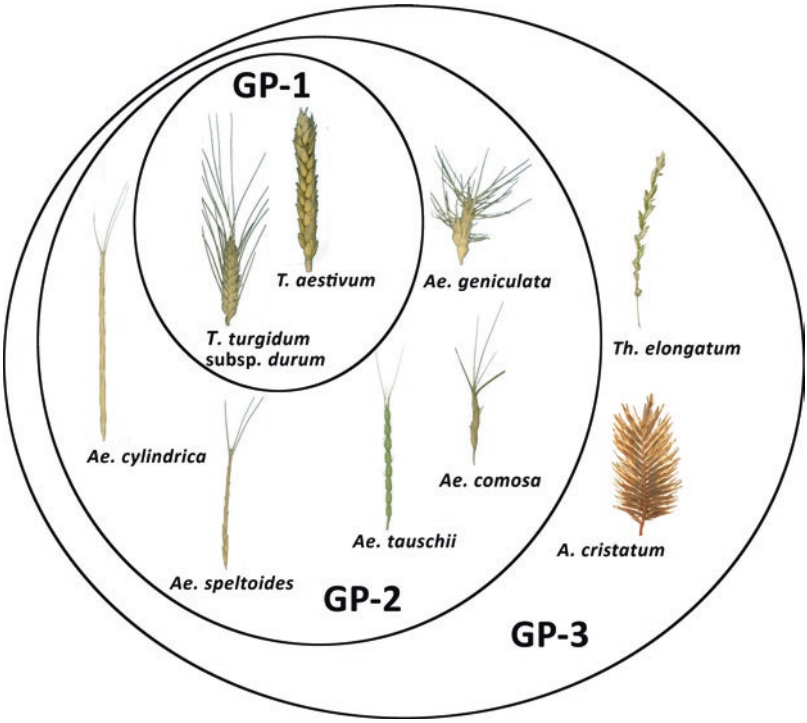

Fig. 17.1 Schematic representation of the genepool of wheat, only some species are shown

Table 17.2 Wild wheats of the genus *Triticum*

Taxonomic name	GP-1 ancestor of	Native to	Genome(s)	Accessions conserved *ex situ*[a]
Triticum monococcum L. subsp. *aegilopoides* (Link) Thell.	Einkorn	Near East, Western Asia, southern Balkans	A	5816
T. timopheevii (Zhuk.) Zhuk. subsp. *armeniacum* (Jakubz.) van Slageren	Timopheevi	Near East, southern Caucasus	AG	1849
T. turgidum L. subsp. *dicoccoides* (Körn. ex Asch. & Graebn.) Thell.	Emmer & tetraploid wheats	Near East	AB	11,535
T. urartu Tumanjan ex Gandilyan	Tetraploid wheats	Near East, southern Caucasus	A	2274

[a]Accessions conserved *ex situ* estimated using data from Genesys PGR, FAO-WIEWS, USDA GRIN and data provided directly by CIMMYT

Nevertheless, hybridization events between *Aegilops* and *Triticum* species were indeed involved in the process of evolution and domestication of tetraploid and

Table 17.3 The species of *Aegilops*, organized in the different sections in which is divided the genus, and *Amblyopyrum*. Data on the genome, ploidy and natural distribution are also provided

Section	Species name	Genome(s)	Ploidy	Distribution	Accessions conserved *ex situ*[a]
Aegilops	*Ae. umbellulata* Zhuk.	U	Diploid	Turkey, Fertile Crescent, Caucasus, Iran	794
	Ae. biuncialis Vis.	UM	Tetraploid	Mediterranean Basin, Fertile crescent, Caucasus, Russia, Ukraine	2505
	Ae. columnaris Zhuk.	UM	Tetraploid	Turkey, Crete, Fertile Crescent, Iran	509
	Ae. geniculata Roth	MU	Tetraploid	Mediterranean Basin, Caucasus, Turkey, Crimea	3218
	Ae. kotschyi Boiss.	SU	Tetraploid	Middle East, North Africa, Arabia, Central Asia	613
	Ae. neglecta Req. ex Bertol.	UM/UMN	Tetra/ Hexaploid	Mediterranean Basin, Crimea, Middle East, Turkmenistan	1818
	Ae. peregrina (Hack.) Maire & Weiller	SU	Tetraploid	Middle East, Greece, North Africa, Arabia	1642
	Ae. triuncialis L.	UC	Tetraploid	Mediterraean Basin, Crimea, Caucasus, Central Asia	6647
Comopyrum	*Ae. comosa* Sm.	M	Diploid	Southern Balkans, Cyprus, Turkey	423
	Ae. uniaristata Vis.	N	Diploid	Croatia, Greece, Albania, Italy, Turkey	79
Cylindropyron	*Ae. caudata* L.	C	Diploid	Aegean, Turkey, Fertile Crescent	701
	Ae. cylindrica Host	DC	Tetraploid	Eastern Europe, Middle East, Caucasus, Central Asia	3893

(continued)

Table 17.3 (continued)

Section	Species name	Genome(s)	Ploidy	Distribution	Accessions conserved *ex situ*[a]
Sitopsis	*Ae. bicornis* (Forssk.) Jaub. & Spach	S^b	Diploid	Cyprus, North Africa, Middle East	505
	Ae. longissima Schweinf. & Muschl.	S^l	Diploid	Egypt, Israel/ Palestine, Jordan	1779
	Ae. sharonensis Eig	S^{sh}	Diploid	Israel/Palestine, Lebanon	2546
	Ae. searsii Feldman & Kislev ex K. Hammer	S^s	Diploid	Israel/Palestine, Syria, Jordan, and Lebanon	519
	Ae. speltoides Tausch	S	Diploid	Fertile crescent, Turkey, Southeastern Europe	3369
Vertebrata	*Ae. tauschii* Coss.	D	Diploid	Caspian seashores, Caucasus, Central Asia, China	7186
	Ae. crassa Boiss.	DM/DDM	Tetra/ Hexaploid	Middle East, Central Asia	608
	Ae. vavilovii (Zhuk.) Chennav.	DMS	Hexaploid	Middle East	345
	Ae. ventricosa Tausch	DN	Tetraploid	Mediterranean Basin, North Africa	486
	Ae. juvenalis (Thell.) Eig	DMU	Hexaploid	Central Asia, Azerbaijan, Fertile Crescent	132
Genus *Amblyopyrum*	*Amblyopyrum muticum* (Boiss.) Eig	T	Diploid	Turkey, Armenia	181

[a]Accessions conserved *ex situ* estimated using data from Genesys PGR, FAO-WIEWS, USDA GRIN and data provided directly by CIMMYT

hexaploid wheats. The wild tetraploid wheats (i.e. *T. turgidum* subsp. *dicoccoides* and *T. timopheevi* subsp. *armeniacum*) resulted from hybridization events that occurred a few hundred thousand years ago between *T. urartu* and an unknown species of the genus *Aegilops*, probably similar to the only existing outcrossing species of this genus, *Ae. speltoides*. Hexaploid wheats belonging to *T. aestivum* do not have a single wild progenitor. This crop arose from hybridization events that occurred probably 8000 BCE in the coastal areas of the Caspian Sea, between the domesticated *T. turgidum* susbsp. *dicoccon* and the wild species *Ae. tauschii* (Fig. 17.3).

Wild species of *Triticum* and *Aegilops* have significantly contributed to wheat improvement, especially in terms of biotic resistances, as well as for grain yield and

Fig. 17.2 Examples of Wheat Crop Wild Relatives (WCWR): (**a**) *T. turgidum* subsp. *dicoccoides* at CIMMYT screenhouse (Texcoco, Mexico); (**b**) *Ae. biuncialis*, wild population at Santeramo in Colle (Italy); (**c**) *Ae. geniculata* (left) and *Ae. ventricosa* (right) growing together in Garda (Italy); (**d**) *Ae. tauschii* at CIMMYT screenhouse (Texcoco, Mexico); (**e**) x-ray scan of a spikelet of *Ae. biuncialis*, a dimorphic pair of seeds can be noticed in the basal fertile spikelet; (**f**) x-ray scan of a spike of *Ae. cylindrica*, in some of the spikelets composing the spike a pair of dimorphic seeds can be noticed

abiotic stress tolerance [11]. The genetic diversity of species belonging to the GP-1 and GP-2 can be exploited to generate Synthetic Wheat Hexaploid (SWH) and chromosomal translocation introgressions. The most common SWH are produced by hybridizing durum wheat with *Ae. tauschii*, as the latter is a huge source of diversity, being adapted to a variety of environments in different subspecies and morphological varieties (see Chap. 18).

The GP-3 of wheat includes grass species of the genera *Agropyron*, *Elymus*, *Leymus* and *Thinopyrum* (Fig. 17.1). Those species have been hybridized with cultivated wheat as genetic sources for disease resistance, salinity tolerance, and other traits. Given the sexual barrier between cultivated wheat species and their tertiary gene pool, to transfer traits from GP-3 species both physical and genetic methods (causing random chromosome breaks and promoting recombination) have been used, namely: spontaneous translocations, *in vitro* cultures, irradiation, and induced homologous recombination [12] (see Chap. 18).

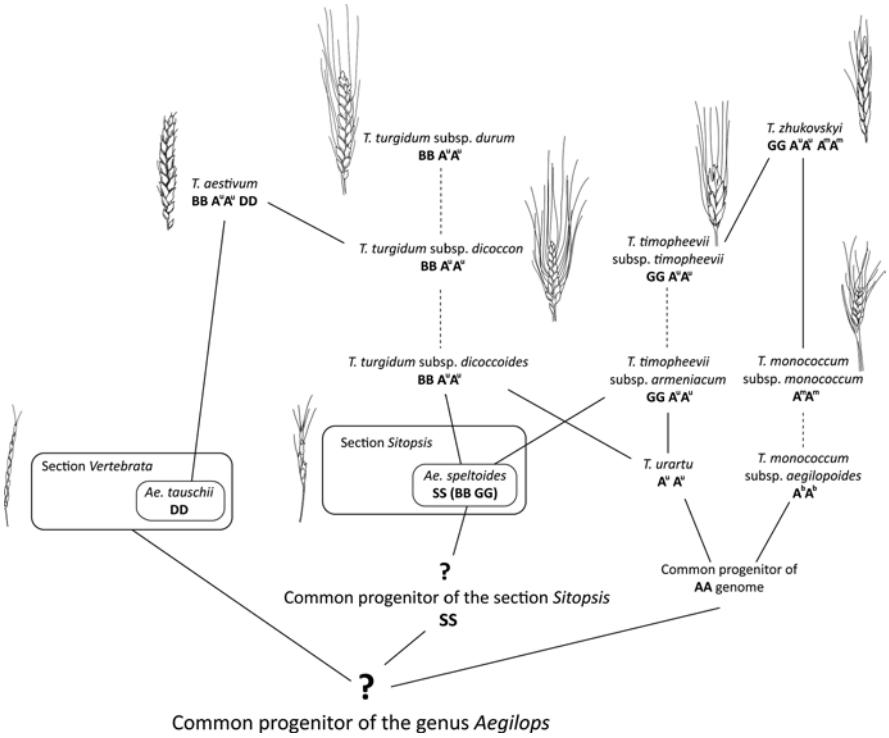

Fig. 17.3 Schematic representation of wheat evolution and domestication. Solid line represents spontaneous events of speciation and hybridization. Dashed line indicates human selection events. (Redrawn with permission from [10] by Marco Canella, Padua, Italy)

17.4 Wheat Genetic Resources Conservation

17.4.1 In situ Conservation

Some wheat wild relatives are considered endangered by the International Union for Conservation of Nature (IUCN) at global level and therefore their conservation is considered priority: i.e. *Amblyopyrum muticum* (EN-endangered), *Aegilops sharonensis* (VU-vulnerable), *Agropyron dasyanthum* (EN) and *Agropyron cimmericum* (EN). Other species, even if labeled as of "least concern" are showing populations declines in their natural habitats (e.g. *Aegilops longissima*). At continental level some species are recognized as endangered, e.g. in Europe *Ae. tauschii* is considered EN and *Ae. bicornis* is VU [13]. Considering the importance of wheat wild relatives for wheat breeding, it is also important to guarantee the conservation of species and populations that are not threatened but that have a great impact on wheat improvement as carriers of useful traits.

In this context, the implementation of *in situ* conservation strategies for wheat wild relatives is necessary. Indeed, even if *ex situ* conservation of genetic resources is easy and cost effective, *in situ* conservation has the advantage of allowing species to evolve in their original place and to retain a higher genetic diversity compared to seed bank accessions.

Maxted et al. [14] and Phillips et al. [15] identified regional diversity hot spots of *Aegilops* in which conservation reserves should be established: Syria and north Lebanon, central Israel, north-west Turkey, the Hatay region of Turkey, Turkmenistan and south France.

In Table 17.4 are listed the existing in situ reserves that conserve wild wheats.

In situ conservatories for crop wild relatives are also called genetic reserves and are generally located where protected areas have been established to conserve also other aspects of biodiversity, and so the additional resource requirements to conserve wild wheats may be minimal. Nevertheless, some specific actions are suggested to enhance the conservation of those species, for example: (I) reduce over-grazing, (II) decrease fire frequency and intensity, (III) reduce use of herbicides and pesticide (e.g. on field margins and roadsides), (IV) perform systematic monitoring of threatened populations, (V) carry out population reinforcement measures of the threatened populations, using seeds of the same populations conserved in genebanks [16]. National parks, military reserves, mountainous and controlled pastoral areas are often ideal locations for *in situ* reserves. Climate change will probably decrease, in the next few decades, the range of many wild wheats in core areas of WCWR diversity such as: North Africa, Middle East and southern Europe [17]. This underlines the importance of protecting populations of WCWR and of complementing *in situ* reserves with *ex situ* conservation to prevent the loss of many of these populations.

The *in situ* conservation of landraces and old cultivars is known as on-farm conservation, defined as: "the management of genetic diversity of locally developed crop varieties by farmers within their own agricultural systems" [18]. While in the abovementioned genetic reserves wild populations of WCWR are conserved in their natural habitats, on-farm conservation consists in the cultivation by farmers of

Table 17.4 *In situ* reserves for wheat and other cereals genetic resources conservation

Reserve name	Country	Taxa
Erebuni	Armenia	Wild wheats (*T. urartu, Triticum monococcum* subsp. *aegilopoides* and *T. timopheevii* subsp. *armeniacum*), goatgrasses (*Aegilops* spp.); also conserving: Vavilov's rye (*Secale vavilovii*), wild barley (*Hordeum* spp.)
Ammiad Project	Israel	*Triticum* spp. (also conserving *Hordeum* spp.)
Ham	Lebanon	*Triticum* spp. (also conserving *Hordeum* spp.)
Wadi Sweid	Lebanon	*Ae. biuncialis*, *Ae. geniculata*, *Ae. triuncialis*, *T. urartu*
Sale-Rsheida	Syria	*T. dicoccoides* (also conserving *Hordeum* spp.)
Ceylanpinar State Farm	Turkey	*Triticum* spp., *Aegilops* spp., (also conserving *Avena* spp. and *Hordeum* spp.)

locally developed, domesticated wheat varieties (landraces and/or old cultivars) to prevent their genetic erosion and eventual extinction. Strengthen value chains and therefore market opportunities for these varieties is likely the best incentive to promote their on-farm conservation by farmers.

On-farm conservation of wheat landraces and old cultivars is being put in place to enhance conservation as well as revival of those entities in several areas of the world. In particular, in some regions (e.g. East Shewa, Ethiopia; Emilia-Romagna, Italy; New England, USA; Czechia) wheat landraces are being rediscovered and re-introduced in cultivation often starting from *ex situ* collections.

17.4.2 Ex situ Conservation

Seed banking is currently considered as the most suitable *ex situ* conservation strategy for plants, like wheat, with orthodox seeds, i.e. seeds that can tolerate drying to low moisture content and subsequent freezing. The Commission on Genetic Resources for Food and Agriculture of the FAO proposed a series of standards for *ex situ* conservation of PGRFA that are currently followed by many international genebanks [19].

Ex situ seed conservation in genebanks can be divided into seven main activities: acquisition, seed drying, seed storage, viability monitoring, regeneration, characterization and distribution.

17.4.2.1 Acquisition

Materials can be acquired either from genebanks or from research or breeding programs. Wild relatives or landraces can be collected in the wild or obtained from farmers, respectively. When collecting populations of wild relatives in their natural habitat, it is important not to exceed the 20% of total seeds available in the sampled population not to affect the natural recruitment of natural populations.

Materials must be acquired legally, in accordance with local, national and international regulations. Materials must be described with Multi-crop Passport Descriptor data [20] and characterization data. A seed sample and its related passport data is defined as a seed accession.

17.4.2.2 Drying

Seed drying is one of the most crucial steps in seed conservation. High seed moisture content detrimentally affects seed storage viability. Seeds are dried to equilibrium in controlled environments (‘drying rooms’) with a temperature of 5–20 °C and 10–25% of relative humidity. Seed moisture content is regularly monitored until the seeds reach equilibrium, i.e. the moisture content of the seeds is in equilibrium

with the relative humidity of the surrounding air. Wheat seeds are conserved in genebanks when they reach a moisture content between 5% and 8%. It is fundamental that, after the drying phase, seeds are stored in airtight containers to maintain the low moisture content. In some national and regional seed banks, equilibrium drying in drying rooms is not possible due to lack of infrastructure or capacity. In those cases, desiccants such as silica gel or zeolite beads can be used for seed drying [21].

17.4.2.3 Seed Storage

High temperatures also detrimentally affect seed longevity in storage. For long-term conservation, it is recommended to store dried seed accessions at a temperature of −18 ± 3 °C. In addition to the long-term ('base' collection), some banks have duplicate samples in an active short-medium term collection stored at a temperature range between −5 and 10 °C. Seed conserved in this 'active' collection are generally employed for regeneration, distribution and characterization, not to decrease the stocks conserved in the base collection.

It is important that seed accessions conserved in a germplasm bank are safety duplicated, e.g. the same accession is stored at other locations to provide an insurance against loss of material. Many genebanks duplicate their accessions at the Svalbard Global Seed Vault, located in the Artic Island of Spitsbergen, a seedbank that currently holds more than one million (with a capacity of 4.5 millions) of store duplicates (backups) of seed samples from the world's crop collections [22].

17.4.2.4 Viability Monitoring

Initial and regular seed viability testing is required to evaluate the quality of a seed lot. Seed germination is generally tested using standard protocols [23] with light and temperature-controlled incubators, using agar or filter paper as the germination medium. International standards recommend that initial germination percentage should exceed 85% for crop seed accessions stored for conservation purposes. As some specific wild relatives' accessions do not reach this threshold a lower viability can be accepted. The International Seed Testing Association (ISTA) suggests that the most suitable temperature to test wheat seed germination is 20 °C [23], while some *Aegilops* species were demonstrated to reach a higher germination when incubated at alternating temperature (e.g. 20/10 ° C) [24]. The germination of some wheat wild relatives can also be elicited by after-ripening, a period of dry storage during which seeds lose dormancy (i.e. the inability of viable seeds to germinate under optimal environmental conditions).

Many wheat wild relatives species show seed heteromorphism, defined as the production, within a spike, of two or more seed types that differ in morphological and/or eco-physiological traits. Indeed, within the genera *Aegilops* and *Triticum*, a dimorphic pair of seeds is often present in each of the spikelets composing the spike, with one seed being larger and brighter-colored than the other (Fig. 17.2). In

the field, larger seeds germinate few weeks after dispersal, while the smaller ones remain dormant for several months due to the presence of a germination inhibitor in the glume. Due to this complex germination strategy, seeds of wild wheats need to be extracted from the spikelets and manually dehulled prior to the germination testing. Seed heteromorphism has implications also in longevity and conservation: it has been observed that smaller seeds of several *Aegilops* and wild *Triticum* species are longer-lived than their larger paired seeds when subjected to artificial ageing, having a greater endowment of antioxidant compounds, these being possibly involved in protection against ageing-related oxidative stress. Preliminary results revealed that smaller seeds of wild wheats are longer-lived also in *ex situ* conservation within genebanks [25].

Seed germination of stored accessions must be tested at regular intervals (e.g. every 10-15 years) to understand the loss of viability in storage and to plan recollection or schedule regeneration activities. Walters et al. [26] found that the p_{50} (i.e. the time for seed viability to fall by 50%) for wheat seed accessions conserved in genebank conditions was 54 years. When the viability of an accessions falls below the 85% of the initial, regeneration or recollection activities need to be carried out in order to maintain available an accession with a high viability.

17.4.2.5 Regeneration

Seed multiplication is required when seed germination drops below 85% of the initial value, or when the quantity of seeds has been depleted due to frequent use of the accession. A sufficient number of seeds needs to be used for regeneration activities in order to maintain the genetic variability within the accessions. Commonly used approach is to employ between 7 and 10 g of seeds (approximately 140 to 250 seeds) for regeneration of wheat varieties. 100–130 plants should be regenerated for each accessions of wheat wild relatives. As wild wheats are considered as possible noxious weeds outside their native range, accessions belonging to those taxa are regenerated in controlled environments (i.e. screenhouses).

17.4.2.6 Characterization

A detailed description of different important traits is fundamental to ensure the maximum usability of the accessions by plant breeders. The characterization stage is often carried out during regeneration when several morphological, phenological and agronomical descriptors are assessed, also in order to confirm accessions' trueness to type. Regarding wheat genetic resources, these descriptors can be grouped as follows:

1. Seed traits, comprising morphological traits (e.g. germination, color, size, weight, vitreousness, number of shriveled seeds) but also grain quality (e.g. protein content and suitability for food processing) and agronomical traits (e.g. preharvest sprouting).

2. Spike morphology, with a characterization of the awns, glumes and spikelets.
3. Plant morphology, considering traits such as: plant height, young plant habit (e.g. upright or prostrate), straw color, leaf pubescence and tillering capacity.
4. Phenological traits, such as growth classes, i.e. classifying if an accession is a spring, winter or intermediate wheat. Inflorescence traits are also considered, e.g. days to flowering and daylength sensitivity (i.e. extent to which long days hasten flowering).
5. Stress susceptibility, considering the effects on plant growth of abiotic stresses (e.g. cold/high temperatures, drought, salinity) as well as biotic ones in terms of fungi (e.g. rust, powdery mildew, glume blotch, eye spot), pests (e.g. nematodes, hessian fly) and viruses (e.g. barley yellow dwarf virus).

Beside the morphological and agronomical traits, physiological and molecular descriptors are often employed to achieve the most reliable and complete characterization of wheat germplasm collections: this allows to evaluate trueness-to-type, to understand and organize the diversity of large germplasm collections and to mine collections for useful traits for breeding.

Some of the most used molecular techniques in wheat genotyping are:

- Studies based on restriction fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP).
- Use of wheat microsatellites (WMS), simple sequence repeats (SSR), commonly known as microsatellites, have been shown to be very useful markers for trueness-to-type evaluation in wheat germplasm, being highly polymorphic both in cultivated and wild species. SSR can be genomic or 'expressed sequence tag' (EST-SSR), the latter having the advantage of possessing good generality between species.
- DArTseq genotyping, in-depth and robust technique to estimate genetic diversity among germplasm accessions. Single nucleotide polymorphisms (SNPs) detected through DArTseq can be investigated by assessing their allelic effects (i.e., genome wide association study, GWAS) and subsequently exploited for breeding.

17.4.2.7 Distribution

Germplasm distribution consists in the shipment of a sample of a seed accession conserved in a genebank in response to a request from a germplasm user. The accessibility of PGR accessions is strictly linked with the existence and updating of information databases, where the users can search the different conserved accession and linked passport data and order seed samples of the accessions they are interested in. The major database of PGR accessions conserved worldwide is Genesys PGR (https://www.genesys-pgr.org/). It brings together four million accessions located in over 450 genebank around the globe and allows the users to quickly search for and request germplasm accessions. Distribution is a fundamental activity for genebanks,

involving a great number of accessions, for example the genebank of the International Maize and Wheat Improvement Center (CIMMYT, Mexico) sends worldwide, on average, more than nine thousand seed samples of WGR in more than 100 shipments annually, those seed samples are employed by the users mainly for research activities, breeding and direct cultivation.

Acquisition and distribution of germplasm across borders must follow international rules on phytosanitary certification and adhere to international treaties and conventions. Two main international treaties regulate the access and share of PGR: the Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). The CBD of 1992 has three main aims: (1) the conservation of biological diversity; (2) the sustainable use of the components of biological diversity; (3) the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity, also known as the Nagoya Protocol on Access and Benefit Sharing is a 2010 supplementary agreement to the CBD, it is an international agreement which aims at sharing the benefits arising from the utilization of genetic resources in a fair and equitable way. The ITPGRFA, adopted in 2001, aims at promoting the conservation of plant genetic resources and protecting farmers' rights to access and have fair and equitable sharing of benefits arising from the use of PGR. ITPGFRA established a multilateral system to exchange plant germplasm of a pool of 64 species of crops (Annex I species), through a Standard Material Transfer Agreement (SMTA). The SMTA is a private contract with standard terms and conditions that ensures that the relevant provisions of the ITPGRFA are followed by providers and recipients of material of plant genetic resources.

17.4.3 Wheat Genetic Resources Collections Worldwide

Since the end of nineteenth century, researchers highlighted the importance for breeding of the conservation and availability of landraces and crop wild relatives, especially witnessing the risk of genetic erosion of landraces due to their substitution with high-yielding improved varieties. The present concept of a genebank, as a facility for the long-term conservation of PGR, was first concretized, at the beginning of twentieth century, at the N. I. Vavilov Institute of Plant Industry in Saint Petersburg by its director R. Regel and especially its successor N.I. Vavilov, who personally focused a significant part of his research activity in collecting, conserving and studying wheat genetic resources. After the World War II, many genebanks were established in several country of the world to conserve and keep available wheat genetic resources and prevent the loss of landraces [27].

Currently, according to FAO (2010), there are more than eight hundred and fifty thousand accessions of wheat and wheat wild relative stored worldwide in genebanks. Accordingly, in our dataset there are 784,753 accessions of the genera

Table 17.5 The ten largest wheat genebanks (by number of accessions) worldwide

Institution code	Institution name	Country	Number of accessions
AUS 165	AGG	Australia	48,065
CHN001	ICGR-CAAS	China	43,039
IND001	NBPGR	India	32,154
ITA436	IBBR-CNR	Italy	32,751
LBN002	ICARDA	Lebanon	47,152
JPN183	NARO	Japan	37,907
MAR088	CRRA	Morocco	42,191
MEX002	CIMMYT	Mexico	141,759
RUS001	N.I. Vavilov Research Institute of Plant Industry	Russia	41,679
USA029	NSGC: USDA-ARS	USA	62,119

Data extracted from Genesys PGR [7], WIEWS and USDA databases and FAO [28]

Triticum and *Aegilops* recorded in the databases: Genesys PGR, FAO-WIEWS and USDA-GRIN (when the same accession is recorded in more than one of these databases, it is counted only once). Considering individual genebanks, CIMMYT holds the greatest number of accessions worldwide (with more than 140 thousand accessions) followed by the National Small Grains Germplasm Research Facility, USDA-ARS (USA) and the Australian Grain Genebank (Table 17.5).

However, it is difficult to estimate the number of unique accessions conserved *ex situ* as in many cases information about duplication is not recorded in passport data, although it is possible to do it. A study genotyping a sample of accessions of *Ae. tauschii* from 3 genebanks found that over 50% of the accessions in the sample were redundant [29].

To assess the representativeness of the diversity of the germplasm conserved *ex situ*, as opposed to the one existing (or that existed) in cultivation or in the wild, different approaches have been used, considering: the total size of collections, taxonomic coverage (number of genera and species), and ecogeographic coverage. A recent gap analysis conducted by the CGIAR Genebank Platform divided the diversity within the wheat genepool in hierarchical clusters (https://www.genesys-pgr.org/c/wheat) based on literature and experts' opinion, and estimated the number of accessions conserved *ex situ* for each group. This methodology was originally suggested by Van Treuren et al. 2009 to assess the composition of a germplasm collection. The results of this analysis suggested that in *ex situ* there are gaps of Durum wheat landraces from arid areas of Mali, Chad, Niger, Sudan, Libya, and Mauritania as well as *T. aestivum* subsp. *tibeticum* and *T. aestivum* subsp. *yunnanense* from China. Several gaps were also found in the coverage of the geographical distribution of wild and domesticated emmer.

When dealing with very large seed collections, in order to increase the accessibility of the conserved material, it is useful to cluster the accessions in core collections, grouping accessions with similar characteristics in terms of e.g. taxonomy, distribution, breeding history, characterization data.

Given the importance of wheat for agriculture worldwide, the seed conservation of wheat genetic resources is important not only for international and national genebanks but also for much smaller institutions, like community seed banks (CSB): i.e. small-scale local organizations that conserve seeds of landraces and wild useful plants on a medium-term basis and serve the needs of local communities [30]. For example, wheat accessions are conserved in CSB in Guatemala, Palestine and India.

17.5 Key Concepts

- Genetic resources of wheat are represented by: (1) WCWR, (2) landraces, (3) old cultivars, (4) modern cultivars and (5) special stocks.
- *In situ* conservation is the conservation of the diversity at the location where it is found, it consists in genetic reserves for WCWR and on farm programs for landraces and old cultivars. This conservation strategy allows genetic resources to evolve in their original area of distribution under selection by farmers and environmental factors and to retain a higher genetic diversity compared to seed bank accessions.
- *Ex situ* conservation of WGR consists in the storage of dry seeds at cold temperatures in germplasm banks. It is currently the most employed conservation strategy for WGR because it allows the long-term storage of many samples in relatively small spaces.

17.6 Conclusions

- To enhance the conservation of WGR it will be increasingly important to complement *ex situ* long-term conservation of seed accessions within genebanks with *in situ* conservation strategies both as genetic reserves for wheat wild relatives and on farm programs for landraces.
- To increase the usability of WGR collections, genebanks need to provide users with the most complete possible passport data, integrating information about collecting sites and phenotypic characterization with novel molecular data.
- Due to this increasing amount of passport information, genebanks need to invest in database systems that can efficiently store and keep available these data.
- Due to the increasing age of historical genebanks and therefore the storage time of many wheat seed accessions, the number of accessions that needs regeneration is going to increase. For this reason, is fundamental to characterize seed longevity of wheat genetic resources to prioritize accessions for viability monitoring and regeneration and avoid losses of germplasm.
- Safety duplication of seed accessions of WGR in external sites is a top-priority for genebanks in order to reduce the risk of losing the collections.

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