1 INTRODUCTION

Agriculture depends on seeds, and the plant's ability to grow across a wide range of environmental conditions. During the last 10,000 years, plant genetic resources have evolved and may harbour allele combinations required for crop improvements in future. To safeguard valuable crop wild relatives, locally adapted landraces and varieties; *ex situ* gene banks were established at the beginning of the 20th century. Most accessions are maintained as seeds under low moisture contents and sub-zero temperatures. However, as any other material on Earth, seeds undergo ageing and loose viability during prolonged storage and, consequently, require frequent rejuvenation.

Orthodox, desiccation tolerant seeds are able to withstand extensive losses in water content and to survive adverse environmental conditions over long terms. The major factors determining seed viability and deterioration processes are the genotype, the environmental conditions during seed development and the storage conditions including relative humidity, temperature, gas composition and pressure. Most components, if not all, affect the water activity in the seed tissue and, consequently, biochemical and thermodynamic mechanisms.

The aim of the post-doctoral thesis is to elucidate differences in physiological, biochemical and genetic mechanisms of seed germination and deterioration in dependency of the seed storage conditions. Fundamental processes are compared in seeds of wheat, barley and oilseed rape genetic resources subjected to long-term ambient and cold storage and artificial ageing conditions using elevated temperatures, increased water contents and in some cases increased oxygen levels and atmospheric pressures. The relevance of artificial ageing for seed preservation and the relationship between seed germination, seed dormancy and seed longevity and the water content is discussed in conjunction with the genetic background and seed's morphology and chemical composition.

2 PROLOG

2.1 Maintenance of plant genetic resources in *ex situ* gene banks

2.1.1 The importance to collect plant genetic resources

Plant genetic resources are considered as a strategic resource at the heart of sustainable crop production (FAO, 2014). They include the sum of genes, gene combinations or genotypes which are available for the genetic improvement of crops (Gepts, 2006). Since the beginning of agriculture, selection of plants and seeds during sowing, growing, harvest and storage gave a rise of locally adapted varieties, so-called landraces, that reveal a specific variation of morphological and yield characteristics and quality traits (de Carvalho et al., 2013). At the mid of 19th century, the rediscovery of Gregor Mendel's work and the introduction of breeding schemes led to the development of high-yielding and more stress-tolerant varieties at the cost of local landraces (Damania, 2008).

At the end of the 19th century, the vanishing of plant genetic resources had already been recognized which motivated great plant explorers, i.e. Frank N. Meyer, Nicolai I. Vavilov, to initiate collection missions (Damania, 2008; Hummer and Hancock, 2015). At this time, plant genetic resources were generally considered as 'global public good' (Halewood et al., 2018). The legal transfer and exchange of plant material facilitated the establishment of eight international plant resources centres majorly located in the industrialized countries. Due to foundation of the 'Consultative Group on International Agricultural Research' (CGIAR) in 1972 and of the 'International Board for Plant Genetic Resources' (IBPGR) later renamed to 'Bioversity International' in 1975, the number of long-term genetic conservation centres increased to 33 in 1983 (Damania, 2008). The last survey of the 'Food and Agriculture Organization of the United Nations' (FAO) revealed more than 1,750 gene banks, nowadays (FAO, 2010). The *ex situ* approach, where plant genetic resources are maintained out of their natural environments, is supplemented by the *in situ* conservation. Here, species are protected in their natural habitats. In 2010, the FAO estimated a protected area of about 17.5 million km² (FAO, 2010). Due to the focus of this work on seed deterioration, *in situ* conservation is not discussed further.

When industrialized countries claimed the international recognition of the 'intellectual property protection for living material', a debate around commercialisation and protection of ge-

netic resources was pushed forward by developing countries (Halewood et al., 2018). Therefore, the 'Convention on Biological Diversity' (CBD) was established in 1993 (CBD, 2018a) and adopted the 'Global Strategy for Plant Conservation'. The strategy includes, i.e. target 8 that aims to keep 75 % of threatened plant species in *ex situ* collections and to make use of 20 % for restoration programs. Target 9 describes the goal to conserve 70 % of the genetic diversity of crops and major socio-economically valuable plant species (CBD, 2018b). The continuing discussions about the recognition of sovereign rights to regulate access to genetic resources and benefit sharing agreements resulted in the development of the 'Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization' (Nagoya Protocol) which came into force in 2014.

Meanwhile, in 2004, the 'International Treaty on Plant Genetic Resources for Food and Agricultur' created a multilateral system of access and benefit-sharing for contracting parties and international organisations. Furthermore, access to the genetic diversity of 64 crops (Annex I) for the purposes of conservation, research, training and plant breeding was enabled and commercial users are obligated to make financial payments to an international benefit-sharing fund (Halewood et al., 2018). The first payment to the 'Treaty's Benefit-sharing Fund' was made by Nunhems Netherland in July 2018 (Nunhems Netherlands, 2018).

2.1.2 Long-term storage and utilization of *ex situ* collections

Since the 16th century, botanical gardens have collected and preserved a variation of more than 80,000 plant species in about 2,500 collections. *Ex situ* gene banks have been established since the mid of the 20th century. They preserve more than 7.4 million accessions in about 1,750 facilities and focus on the maintenance of genetic diversity of crop species and their wild relatives (FAO, 2010). About 45 % of the accessions are cereals, i.e. wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and *Sorghum* (L.) Moench, followed by food legumes (15 %), forages (9 %) and vegetables (7 %). A quarter of the material are landraces and wild species, whereby major parts originated in North and South America, Europe and South Asia. It is estimated that only 1.9 to 2.2 million of the 7.4 million accessions are unique (FAO, 2010). Among the 10 largest centres for plant conservation are those held by the CGIAR, i.e. the 'Centro Internacional de Mejoramiento de Maíz y Trigo' (CIMMYT) and the 'International Centre for Agricultural Research in the Dry Areas' (ICARDA) (Table 1). In 2008, the first and only global germplasm conservation facility, the 'Svalbard

Global Seed Vault' (SGSV) built by the Norwegian government and operated by the 'Global Crop Diversity Trust' and NordGen (FAO, 2010) opened and houses about 1,061,909 accessions currently (SGSV, 2018).

Table 1. The ten biggest *ex situ* gene banks storing crop genetic resources. Based on FAO (2010) and updated in * June 2018 based on IPK (2018) and personal communication with Chinese gene bank manager and *[2] September 2018 based on SGSV (2018).

	Gene bank	Location	Genus	Species	Accessions	SGSV
1	NPGS	USA	2,128	11,815	508,994	30,868
2	ICGR-CAAS	China	-	2,386*	470,295*	-
3	NBPGR	India	723	1,495	366,333	-
4	VIR	Russia	256	2,025	322,238	945
5	NIAS	Japan	341	1,409	243,463	
6	CIMMYT	Mexico	12	48	173,571	80,492
7	IPK	Germany	756	3,127*	150,751*	48,655*
8	ICARDA	Lebanon*	86	570	154,000*	62,834
9	ICRISAT	India	16	180	118,882	20,003
10	IRRI	Philippines	11	23	109,161	4,008
	SGSV	Norway	>664	5,979*[2]	1,061,909*[2]	-

CIMMYT, Centro Internacional de Mejoramiento de Maíz y Trigo (Mexico); ICARDA, International Centre for Agricultural Research in the Dry Areas (Lebanon); ICGR-CAAS, Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics (India); IPK, Leibniz Institute of Plant Genetics and Crop Plant Research (Germany); IRRI, International Rice Research Institute (Philippines); NBPGR, National Bureau of Plant Genetic Resources (India); NIAS, National Institute of Agrobiological Sciences (Japan) NPGS, National Plant Germplasm System (United States of America); SGSV, Svalbard Global Seed Vault; VIR, N.I. Vavilov all-Russian Scientific Research Institute of Plant Industry (Russian Federation)

The international 'Genebank Standards for Plant Genetic Resources for Food and Agriculture' provide the guidelines the maintenance of plant genetic diversity. In 2014, the standards were renewed and aspects on the legal status, physical security, identity of accessions, monitoring of viability and genetic integrity were updated. In general, it was agreed to dry orthodox seeds between 5 °C and 20 °C and 10 % and 25 % relative humidity (RH) and initial germination should exceed 85 % for most cultivated crop. Seeds in active collections are used for distribution and are held between 5 °C and 10 °C and 15 % RH for about 30 years. Seeds in long-term base collections should maintain high seed quality for more than 30 years. Therefore, seeds are packed in airtight containers and stored between -20 °C and -15 °C at different sites. Some repositories can only provide short-term storage conditions. Here, the minimum requirements are that high seed quality should be preserved at stable temperatures of maximum 25 °C for up to eight years. However, at any case, an active viability monitoring program should ensure that viability is tested before it drops below 85 % of the initial viability (FAO, 2014).

To capture the genetic variability of gene bank collections, molecular marker assisted germplasm curation offers a powerful tool for germplasm managers, basic researchers, and plant breeders. In the past, molecular information generated in studies to investigate the diversity, domestication, evolution and phylogeny of plant genetic resources have not sufficiently used (Kilian and Graner, 2012; McCouch et al., 2012). Therefore, FAIR principles of Findability, Accessibility, Interoperability and Reusability (Wilkinson et al., 2016) and an improved meta data management system is acquired to facilitate data integration and the potential for data sharing (Halewood et al., 2018). To promote the networking of high quality data repositories, the 'Global Information System' of the 'International Treaty on Plant Genetic Resources for Food and Agriculture' plans to link existing information systems by using 'Digital Object Identifiers' (DOIs) (Alercia et al., 2018). The major goals are to take advantage of the ongoing revolution in the exploration, manipulation and synthesis of biological systems, to increase the efficiency and effectiveness of conservation, trait discovery and utilization of plant genetic resources in gene banks (Halewood et al., 2018).

However, the security status of the collections should be prioritized and range ahead utilization and exploration of the genetic diversity. The need for a backup system of gene banks have been exemplified by ICARDA. Due to the civil war in Syria, the institute was forced to leave the collections in Aleppo and move to the Lebanon. Fortunately, most accessions were backed up in Svalbard and in total, 38,073 seed samples have been delivered to Morocco and Lebanon since 2015. The ancient varieties, majorly seeds of wheat, barley, lentil (*Lens culinaris* Medikus), chickpea (*Cicer arietinum* L.), wild cereals and pulses were used to re-establish an active collection (Crop Trust, 2015) which comprises about 154,000 accessions currently (ICARDA, 2016). In general, many *ex situ* collections are still maintained under suboptimal conditions which have negative consequences on the viability status (FAO, 2010). Low seed viability involves the necessary of rejuvenation. Seed regeneration in gene banks is mostly limited to small field plots which increase the potential to create population bottlenecks and to loose genetic diversity (Parzies et al., 2000). Therefore, the maintenance of a high quality seed collection and a sufficient control of seed deterioration should be prioritized for gene bank collections.

2.1.3 Wheat genetic resources

Wheat (*Triticum aestivum* L. and *Triticum durum* Desf.) is among the 'big three' cereal crops and adapted to a wide range of temperate environments (Shewry, 2009). After maize and rice, 750 million tonnes of wheat seeds are produced annually on more than 220 million ha (www.fao.org/faostat). In world's gene banks, 856,000 wheat accessions are maintained whereby about 13 % of the accessions are stored at CIMMYT in Mexico, 7 % at the 'National Plant Germplasm System' (NPGS) in the USA, 5 % at the 'Institute of Crop Germplasm Resources - Chinese Academy of Agricultural Sciences' (ICGR-CAAS) in China and 4 % at the 'National Bureau of Plant Genetic Resources' (NBPGR) in India in 2010 (FAO, 2010). In Gatersleben, at the 'Leibniz-Institute of Plant Genetics and Crop Plant Research' (IPK), 28,206 accessions have been preserved in 2018 (IPK, 2018).

The great genetic diversity of bread wheat (*Triticum aestivum* L.) has been developed as part of the 'Neolithic Revolution' over the past 10,000 years. During domestication, the tetraploid cultivated emmer (AABB) hybridized with unrelated wild grass *Aegilops tauschii* Coss. (DD) and formed the allohexaploid wheat (2n=6x=42) with three sub-genomes, A, B, D. The A genomes of tetra- and hexaploid wheat are closely related to the A genomes of wild and cultivated einkorn and the B genomes are derived from the Sitopsis section of *Aegilops*. Here, *Aegilops speltoides* Tausch is the closest species (Shewry, 2009). Just recently, the 'International Wheat Genome Sequencing Consortium' achieved to annotate a reference sequence in the form of 21 chromosome-like sequence assemblies that is giving an access to 107,891 high-confidence genes (IWGSC, 2018).

The wheat caryopsis (Figure 1), for simplicity termed seed, comprises a large starchy endosperm, the embryo and the pericarp fused with the seed coat (Xiong et al., 2013). The endosperm cells contain 60 % to 70 % starch and 10 % to 15 % storage protein which form the gluten protein fraction. The unique composition of wheat seeds determines its functional properties among milling efficiency, bread making and nutritional value. During domestication, wheat changed from a hulled to the free-threshing naked form (Shewry, 2009).

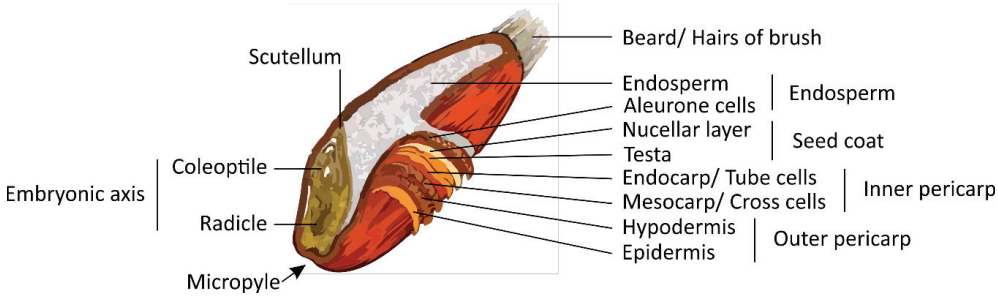

Figure 1. Wheat caryopsis including major structures. Based on Rathjen et al. (2009).

2.1.4 Barley genetic resources

Barley (*Hordeum vulgare* L.) has been important since the dawn of agriculture and was among the earliest domesticated crops (Mayer et al., 2012). Barley can be grown over a wide range of environmental conditions, from 70°N in Norway to 46°S in Chile (Grando and Gormez Macpherson, 2005). About 141 million tonnes of seeds are produced annually for food, feed and the malting industry (www.fao.org/faostat). The genetic variation is presented by 466,531 accessions, whereby 23 % of the material is classified as landraces. About 9 % of the collections is hold by 'Plant Gene Resources of Canada' (PGRC), 6 % by the NPGS in the USA, 6 % by the 'Embrapa Recursos Genéticos e Biotecnologia' (CENARGEN) in Brazil, 6 % by ICARDA. About 23,600 accessions, 5 % of the total collection, are preserved at IPK in Germany (IPK, 2018).

Barley is a diploid (2n=2x=14), inbreeding plant that has considered as a model plant for cereal genetics. *Hordeum vulgare* ssp. s*pontaneum* is the immediate ancestor (Pankin and von Korff, 2017). During domestication, a great diversity in morphological forms evolved ranging between spring, winter, 6-row, 2-row, awned, awnless, hooded, hulled and naked, feed, malting and food type barleys (Fan, 2017). In 2012, the International Barley Genome Sequencing Consortium presented an ordered physical, genetic and functional sequence resource including a physical map of 4.98 Gb and 26,159 high confidence genes (Mayer et al., 2012). This achievement is supplemented by map-based reference sequence of the barley genome including the first comprehensively ordered assembly of the pericentromeric regions of a Triticeae genome (Mascher et al., 2017) and the first barley Pan-genome which will be published shortly.

Comparable with wheat, barley produces a caryopsis (Figure 2), for simplicity termed seed. Interestingly, only barley varieties show a strong hull-caryopsis adhesion in which the hull (outer lemma and inner palea) is firmly adherent to the pericarp epidermis at maturity. Few accessions are of a free-threshing variant called naked (hulless) barley (Taketa et al., 2008). About 70 % of the barley seed consists of starch, whereby amylose content can vary between 0 % and 100 %. The protein content of the starch ranges between 0.07 % and 0.3 % and the amount of free and bound lipids are estimated to between 0.05 and 0.85 %. Due to functional components such as tocochromanols and β-glucan, naked barley seeds are preferred for human food (Fan, 2017).

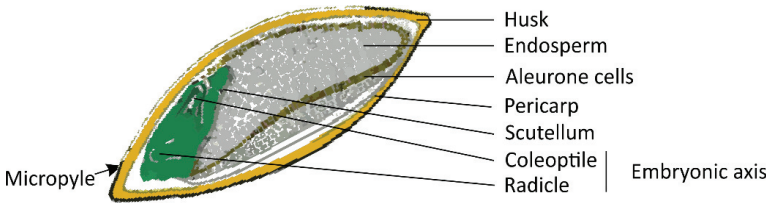

Figure 2. Barley caryopsis including major structures. Based on Fox (2010).

2.1.5 Oil seed rape genetic resources

About 16 % of the global oil production, produced by 69 million tonnes of seeds, is contributed by oilseed rape (*Brassica napus* L.) (www.fao.org/faostat). Only 25,566 oilseed rape accessions are kept in gene banks, whereby the ICGR-CAAS in China and the NBPGR in India maintain 16 % and 10 % of the collection, respectively (FAO, 2010). The IPK preserves 1,205 accessions at the satellite collections North on the Island Poel, Germany (IPK, 2018).

The oilseed rape genome (AACC, 4n=2x=38) comprises about 1.2 Gb and has been formed about 7,500 years ago (Chalhoub et al., 2014). The short domestication history is assumed to be the reason for the limited genetic variability. Oilseed rape derived from a small number of hybridization events between the diploid progenitors *Brassica rapa* L. and *Brassica oleracea* L. which contributed the A and C genomes, respectively (Bancroft et al., 2011). Since the Middle Ages, oil seed rape cultivation have began in Europe and led to the selection of valuable agronomic traits such as those for oil biosynthesis, glucosinolate contents, disease resistance and flowering time (Chalhoub et al., 2014). Canadian oil, low acid (Canola) also known as low erucic acid-type oil seed rape, derived from the selection of plants having an improved seed

composition. Those seeds are characterized by high yield, low erucic acid and glucosinolate content and are predominantly used for culinary purpose (Wang et al., 2017). High erucic acid-types are used in the industry (Woodfield et al., 2017). Recent releases of the oilseed rape genome (Chalhoub et al., 2014; Schmutzer et al., 2015; Bayer et al., 2017) enabled first predictions of gene content. However, due to problems of mis-annotations, it is still a challenging task (Bayer et al., 2017).

The oilseed rape embryo comprises a central embryonic axis embraced by two cotyledons (Figure 3). The whole embryo is encased by a liquid endosperm, a cellular aleurone layer, and a seed coat. When the embryo imbibes and germination is initiated, the two cotyledons fold in toward the embryonic axis, one staying outermost while the other becomes restricted to the inner part of the seed. During seed development seeds accumulate about 45 % oil by dry weight (DW) which consists of 62 % oleic acid (18:1), 22 % linoleic acid (18:2) and 10 % α-linolenic acid (18:3) (Woodfield et al., 2017). In addition, the inner integuments produce significant amounts of flavonoids assuming a function as protective substance (Moise et al., 2005).

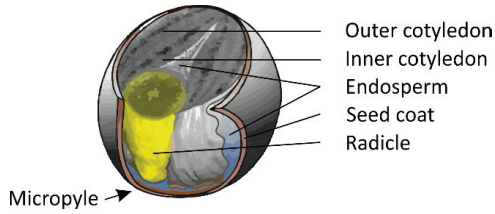

Figure 3. Oilseed rape seed including major structures. Based on Munz et al. (2017).

2.2 Survival in the dry state

Life on Earth fundamentally depends on water. Over 1 billion years ago, plant terrestrialization was only possible by the transition of plants to live on lands including adaptation to high-stress environments (Vries and Archibald, 2018). Land plants, formally called embryophytes (Adl et al., 2005), have developed important features to survive fluctuating environments (Delwiche and Cooper, 2015). Most important ones were the acquisition of desiccation tolerance in parallel with the acquisition of dormancy. Dormant organisms can reduce metabolic functions to a minimum under unfavourable conditions and resume their activities when the conditions become supportive to life (Lubzens et al., 2010; Costa et al., 2016). The outstanding features of desiccation tolerance are the ability to survive loss of more than 90 % of cellular water

(≤0.1 g H_2O g^{-1} DW) (Leprince et al., 2017), the rapidity of rehydration and recovery, the tolerance against multiple cycles of dehydration and rehydration, the stability of the dry tissue and its longevity in the dry state (Gaff and Oliver, 2013).

Vegetative desiccation tolerance has been first developed in bryophytes (Oliver et al., 2000) and is widespread in less complex clades such as algae and lichens; but rare in larger and more complex groups of vascular land plants (Oliver et al., 2000; Hilhorst et al., 2018). It is assumed that genes evolved for cellular protection and repair had been recruited for other tolerance mechanisms. However, genes were conserved in seeds and re-evolved multiple times in the history of flowering plants (angiosperms) (Oliver et al., 2000). About 135 angiosperm species display a certain degree of desiccation tolerance in the vegetative tissue. Most commonly, their reproductive organs, seed embryos and pollen (Gaff and Oliver, 2013), are desiccation tolerant and seeds of most of them are considered as orthodox (Table 2). About 95 % of the spermatophytes, angiosperms and gymnosperms, produce orthodox seeds with the ability to survive long-term storage. In contrast, about 3 % have 'recalcitrant' seeds. These seeds are desiccation- and chilling sensitive and have short life spans. About 1 % is categorized as 'intermediate' of variable degrees of desiccation and cold tolerances (Kew, 2018).

Table 2. The numbers of angiosperm and gymnosperm species with desiccation tolerant or desiccation-sensitive seeds. Seed types are categorised according to the Royal Botanic Gardens Kew Seed Information Database (Kew, 2018).

Seed type	Number of species	% of the total species
Orthodox	18,858	76.2
Presumably orthodox	4,587	18.5
Total orthodox seeds	**23,445**	**94.7**
Intermediate	73	0.3
Presumably intermediate	77	0.3
Total intermediate seeds	**150**	**0.6**
Recalcitrant	345	1.4
Presumably recalcitrant	275	1.1
Total recalcitrant seeds	**620**	**2.5**
Uncertain	540	2.2

The extraordinary stability of orthodox seeds to resist prolonged storage have been fascinated researchers over centuries. Around 370 BC, Theophrastus of Lesbos, the father of Botany, recognized the importance of the dry state for seeds and that seeds differ in their germinability according to the place in which they are stored (Leprince and Buitink, 2015). In the 19th century, in parallel the American William Beal and the Austrian Friedrich Haberlandt initiated