Viability of *Triticum* sp. seeds after 30 years long-term storage

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Abstract
The aim of the study was to assess the changes in the viability of wheat species stored for 30 years under long-term storage condition in National genebank of Bulgaria and discuss the opportunity for more appropriate monitoring. A total of 1955 wheat seed accessions from 7 plant species stored since 1980-1984 were evaluated. Based on the experimental data, seed storage characteristics - σ, P50% and P10% were estimated to predict seed storage life and regeneration needs. After 30 years of storage, the analyzed samples showed a slight decrease in germination above 10%. The frequency of odd results varied between 0 for *Triticum dicoccon* Shrank accessions to 0,419 for *Triticum durum* Desf. accessions. The σ values ranged from 41.67 years (for *Triticum dicoccon* Shrank) to 333.33 years (for *Triticum spelta* L. and *Triticum boeoticum* Boiss.). There were large differences between species, both in terms of the time required for viability to drop to 50% and the time required for seed viability to decrease by 10%. The calculated safe storage time (P10%) was the shortest for *Triticum dicoccon* Shrank (23.35 years) and the longest for *Triticum spelta* L. (311.87 years).

Key words: genebank; wheat; seed germination; seed longevity; seed viability

INTRODUCTION

Genebanks provide an excellent storage facility for the medium and long term *ex-situ* conservation of the plant germplasm (Probert et al., 2009). There are now more than 1 750 individual genebanks worldwide storing more than 7.4 million accessions (Börner et al., 2010; Rehman Arif, 2012; Fu et al., 2015). Globally, there are over 80 wheat germplasm collections, holding more than 800 000 accessions (Anonymous, 2007). The larger collections include that at CIMMYT-Mexico (>100 000 accessions), at the National crop genebank in the Institute of Crop Science (ICS), China and the USDA-NSGC, Aberdeen, Idaho (> 40 000 accessions) and the Vavilov Research Institute (VIR), Russian Federation, ICARDA, Syria and NBPGR, India (each holding approximately 30 000 accessions) (Anonymous, 2007). Wheat collection is also one of the largest in Europe. As presented in EURISCO web-site (*http://eurisco.ipk-gatersleben.de/apex/f?p=103:1*) in the European search catalogue are registered 86 839 accessions of *Triticum aestivum* L. and 14 171 accessions of *Triticum durum* Desf.

In Bulgaria wheat collections as genetic resources are maintained in the National genebank of Bulgaria in IPGR-Sadovo (12 847 accessions) (http://eurisco.ipk-gatersleben.de/apex/f?p=103:1), as main importance has both species: common and durum wheat (Desheva et al., 2013). The collection is maintained under long-term and medium-term storage condition. The total number of accessions stored under long-term storage is 11 510 accessions, 8 856 of which are from *Triticum aestivum* L., 1 998 from *Triticum durum* Desf., 176 from *Triticum sphaerococcum* Perciv., 121 from *Triticum monococcum* L., 75 from *Triticum turgidum* L., 65 from *Triticum dicoccon* Shrank, 54 from *Triticum spelta* L., 38 from *Triticum boeoticum* Boiss., and 227 accessions from other 32 species.

The maintenance of seed viability over long periods of time in seed genebanks is a key element in conservation of plant genetic resources (Fu et al.,

2015). The standards for long-term seed storage regime are well developed (FAO, 2014). Each seed lot stored in a suitable environment requires sampling to monitor seed viability over time in order to be able to regenerate the accession before substantial loss in viability has occurred; 85% is the minimum value before regeneration is required in order to avoid loss in genetic integrity (Ellis et al., 2017). Seed viability monitoring, usually through a germination test, is a key aspect of genebank management; a low viability result triggers the regeneration of an accession in order to ensure that the genetic diversity of the accession is conserved and available for distribution. Regular viability monitoring of large collections is costly in terms of seeds, labour and other resources (Hay & Whitehouse, 2017). Regeneration is also a costly genebank operation and may also negatively affect the genetic integrity of an accession through exposure to the potential influence of genetic drift, selection, contamination and human error. Therefore, seeds should be stored under conditions that maximize their longevity while keeping storage costs and logistics to a manageable level (Engels & Visser, 2003). Ho-Sun et al. (2013) noted that frequent regeneration may result in genetic erosion. Hay et al. (2015) point out those monitoring tests should be as infrequent as prudent management allows, and so estimates of longevity in long- and medium-term stores are required for the effective management of a genebank.

Information about seed longevity of species is important for storage periods, reproduction cycles and germination test intervals (Nagel et al., 2010). The longevity of seed is different between species, but also between the genotypes within a species and depends on the storage conditions (Walters et al., 2005; Sallon et al., 2008; Nagel et al., 2009; Probert et al., 2009; Van Treuren et al., 2013; Nagel & Börner, 2010; Hay et al., 2015; Desheva, 2016). Understanding the differences in seed longevity in various species is therefore crucial to the effective management of seed conservation collections because it underpins the selection of viability re-test intervals, and hence regeneration or re-collection strategies (Probert et al. 2009; Groot et al., 2015).

The aim of this study was to assess the changes in the viability of wheat seeds stored for 30 years under long-term storage condition in National genebank of Bulgaria and discuss the opportunity for more appropriate monitoring.

MATERIAL AND METHODS

Seed material

A total of 1955 wheat seed accessions from 7 plant species (873 accessions from *Triticum aestivum* L., 1025 accessions from *Triticum durum* Desf., 21 *Triticum turgidum* L., 12 access. from *Triticum spelta* L., 8 access. from *Triticum boeoticum* Boiss., 8 access. from *Triticum dicoccon* Shrank and 8 access. from *Triticum monococcum* L.,) stored since 1980-1984 in the base collection under long-term storage conditions with low moisture contents (5±2%) in hermetically closed containers (glass jars or three-layer laminated foil packets (PET 12μm+Al 9μm+PE=113 μm) at –18°C in the National genebank of Bulgaria were evaluated.

Seed viability

Seed viability in the National genebank of Bulgaria was determinated on the basis of germination rate of accessions in storage. Seed germination is determined at regular intervals: immediately before storage and periodically every 10 years thereafter (i.e. 10 and 20 years respectively). The germination tests were carried out according to BDS 601-1985 (BDS, 1985). The recommendations for work in the gene banks (Ellis et al., 1985a; 1985b; Hanson, 1985) were also implemented. Seeds stored at –18°C for about 10, 20 and 30 years, respectively, were pre-conditioned before these were set to germinate: equilibration of seed containers at room temperature for 24 hours was followed by re-humidification of seeds, as reported previously (Stoyanova, 2001).

Seed moisture content

The moisture content of seed accessions, both before and after the time of storage was determined using oven methods of BDS (1985) for reduced working sample (3 g per accession).

Data analysis

The *Probit* analysis for modelling of data from seed storage experiments was used according to the models first described by Roberts (1973). It was based on a straight line relationship between viability and storage period. The slope of this line was the value of σ and the intercept was the (theoretical) initial viability of seeds K_i (Ellis & Roberts, 1980). The relationship used for calculation was:

$$\nu = K_i - p/\sigma,$$

where: v was the viability in *Probit* after p years in storage.

Seed longevity is described by storage constants P50 and P10 according to Ellis and Roberts (1980), where P50 is the time for viability to fall to 50% and P10 is the time for viability reduction of 10%.

The information for seed accessions in storage was maintained as ACCESS-database. The raw data files were used for statistical analysis by analysis of variance (ANOVA) and Paired-Samples T-test test.

RESULTS AND DISCUSSION

Seed viability is the ability of the embryo to germinate, and is affected by a number of different conditions. A variety of factors can affect seed viability such as the ability of the plant to produce viable seeds, predator and pathogen damage, and environmental conditions (Shaban, 2013; Solberg et al., 2020). The accurate assessment of seed viability in the period of long-term storage is important, as it provides a correct decision about regeneration of an accession, which is not only expensive but also risky from the point of loss in genetic diversity (Babić et al., 2015). Seed viability declines with period of storage. Therefore, it is necessary to assess viability periodically in order to detect loss in viability during storage (Hidayatun et al., 2017).

In the table 1 are presented the mean results from monitoring of seeds viability after 10 and 30 years long-term storage for 7 species of genera *Triticum* and 1955 accessions. Viability was monitored by conducting a germination test. The mean seed viability of all 7 crops at the beginning of the storage was high, ranging from 89% to 98.54%. The seeds of the species *Triticum aestivum* L. and *Triticum durum* Desf. indicated slightly decreasing germination activity after ten years of storage. The mean germination percentages of *Triticum dicoccon* Shrank, *Triticum monococcum* L., *Triticum boeoticum* Boiss. and *Triticum turgidum* L. after 10 years of storage increased between 2% and 8.5% in comparison with the mean initial germination values. The most considerable increase in germination percentage with 8.5%, after ten years storage at –18°C was found out at *Triticum monococcum* L. The increase in germination after storage relates to wheat post-harvest dormancy (Gao & Ayele, 2014; Tuttle et al., 2015). Statistical analysis showed significant differences ($p \leq 0.05$) between seed viability tests at the beginning of the storage and after 10 years of storage for accessions for *Triticum spelta* L. (Table 1).

After 30 years of storage mean germination percentages of *Triticum aestivum* L. and *Triticum durum* Desf. dropped between 1.73% and 2.61% in comparison with the mean initial germination values, respectively. Their standard deviations increased significantly from ±2.71 to ±4.80% and from ±2.90 to ±4.56%, respectively. A slow decline in mean seed viability of *Triticum spelta* L. and *Triticum boeoticum* Boiss. was also observed after the second control test presented at $p \geq 0.05$ level (Table 1). The mean germination values of *Triticum turgidum* L., *Triticum dicoccon* Shrank, and *Triticum monococcum* L. increased more than 3% as compared to the initial germination per-

Table 1. Monitored result of germination percentage (mean±SD) for 7 species of genera *Triticum* stored for 10 and 30 years under long-term storage condition in the National genebank of Bulgaria

Species	Number of accessions	Mean germination at beginning of storage±SD, %	Mean germination after 10 years of storage±SD, %	Mean germination after 30 years of storage±SD, %
Triticum aestivum L.	873	98.54±2.71	97.64±2.65	96.81±4.80*
Triticum durum Desf.	1025	98.49±2.90	98.16±2.22	95.88±4.56*
Triticum dicoccon Shrank	8	94.00±6.32	96.5±2.60	99.00±1.73*
Triticum monococcum L.	8	89.00±7.42	97.50±2.60*	96.00±2.0*
Triticum spelta L.	12	98.33±1.97	96.08±2.60*	97.67±2.56
Triticum boeoticum Boiss.	8	92.50±4.21	97.50±2.40*	92.30±6.14
Triticum turgidum L.	21	93.33±6.21	95.33±4.20	96.57±3.31*

SD-standard deviation, *$p \leq 0.05$

centages, respectively with 3.24%, 5% and 7%. This may be due, on the one hand, to wheat seed dormancy induced during storage and, on the other hand, to varying degrees of operator error as the personnel performing the germination tests changed over time (Desheva, 2016).

Predicting the frequency of control checks for different species and classification of them according to the safe period storage under the National genebank is essential in establishing a monitoring model by forming a "filter for endangered specimens" to predict the need for regeneration. This is also the guarantee for the predictability of conservation results in genebank for an extended period of time (Deshev et al., 2018).

In the table 2 are presented the grouping of accessions within species according to change in germination after 30 years of long-term storage The frequency of accessions with minimal increase of germination rate varied between 0.125 for *Triticum durum* Desf. and 0.750 for *Triticum monococcum* L. A total of 773 genotypes showed no change in germination after 30 years of storage in the genebank, representing 39.5% of the total analyzed sample. The frequency of total seed samples with decrease of germination value below 10% was 0.396. Decrease in germination value from 10 to 20% was recorded only for accessions from the following species- *Triticum aestivum* L., *Triticum durum* Desf. and *Triticum boeoticum* Boiss, as the frequencies were respectively 0.034, 0.075 and 0.125. Only the genotypes from bread and durum wheat showed a decrease in seed germination value more than 20% compared to the initial germination value, but the number of accessions was only 5, representing only 0.3% of the total analyzed samples.

Maximizing seed longevity is important for genebanks to efficiently manage their accessions, reducing the frequency of costly regeneration cycles and the loss of genetic integrity (Whitehouse et al., 2018). Seed longevity (time span during which seeds remain viable on dry storage) is affected by many factors, such as environmental conditions in storage like moisture content, temperatures and relative humidity, oxygen pressure, growing conditions, damage during harvest or seed extraction, post-harvest conditions or phenotypic/genetic factors, which accelerate seed deterioration and degradation leading to loss of seed viability (Godefroid et al., 2010; Sano et al. 2016; Kannababu et al., 2020; Yamasaki et al., 2020). The seed longevity varies among families, species, genotypes, seed lots, and even among individual seeds inside the same bag and depends on the storage conditions (Ho-Sun et al., 2013; Desheva, 2016; Desheva et al., 2017)

Based on the experimental data through *probit analysis* was determined the characteristics for prediction of storage under of conditions in the National Genebank. Seed longevity calculated as values for σ indicated that the seeds of some species can be preserved for more than 333 years (Table 3). For the investigated 7 species it ranged from 41.67 years

Table 2. Grouping of accessions within species according to change in germination after 30 years of long-term storage

Species	TNA	Minimal increase of germination		Withouth change of germination		Decrease germination below 10%		Decrease germina-tion from 10 to 20%		Decrease germination above 20%	
		NA	F	NA	F	NA	F	NA	F	NA	F
T.aestivum L.	873	137	0.157	370	0.424	334	0.383	30	0.034	2	0.002
T. durum Desf.	1025	128	0.125	387	0.378	429	0.419	78	0.075	3	0.003
T. dicoccon Shrank	8	5	0.625	3	0.375						
T.monococcum L.	8	6	0.750	1	0.125	1	0.125				
T. spelta L.	12	3	0.250	5	0.417	4	0.333				
T. boeoticum Boiss	8	4	0.500	1	0.125	2	0.250	1	0.125		
T. turgidum L.	21	11	0.524	6	0.286	4	0.190				
Total	1955	294	0.15	773	0.395	774	0.396	109	0.056	5	0.003

TNA-total number of accessions, NA-number of accessions, F-frequencies

for *Triticum dicoccon* Shrank to 333.33 years for *Triticum spelta* L. and *Triticum boeoticum* Boiss. For *Triticum aestivum* L. and *Triticum turgidum* L. the determined seed longevity was 111.11 years. The seed longevity for *Triticum durum* Desf. and *Triticum monococcum* L. were calculated above 70 years, respectively 71,43 and 76.92. The time required to reduce germination to 50% (50%) ranged among species from 64.17 years for *Triticum dicoccon* Shrank to 666 years for *Triticum spelta* L. and *Triticum boeoticum* Boiss. The safe storage period is the time for minimum changes in seed viability, which is expressed by 10 % decrease from initial viability (P10%). According to the results indicated in Table 3, the safe storage period of the analyzed 7 species (P10%) ranged from 23.35 years for *Triticum dicoccon* Shrank to 311.87 years for *Triticum spelta* L. For *Triticum monococcum* L. it was 32.32 years, while for *Triticum turgidum* L. and *Triticum durum* Desf. it was 59.28 years and 69.12 years, respectively. For *Triticum aestivum* L. and *Triticum boeoticum* Boiss. the calculated values of P10% were 108.71 years and 168.31 years, respectively. According to the results presented in Table 3 we could be concluded that *Triticum dicoccon* Shrank should be monitored not later than 20 years from the beginning of storage and *Triticum monococcum* L. not later than 30 years. Although of many good preservation of the other species, the control checks should be not later than 40 years, as it is noted in the genebank standard (FAO, 2014). The results of this study will complement the storage control database and will be used to compile enriched models for planning the need for regeneration (Table 3).

CONCLUSION

The results obtained from the analyzed 1955 accessions from 7 plant species after 30 years long-term storage, indicated that there were no negative changes in seed viability that led to lethal results and loss of accessions. Only 114 accessions needed to be regenerated, which is about 5.83% of the total analyzed samples. The calculated seed longevity as σ values varied widely between species, respectively from 41.67 years for *Triticum dicoccon* Shrank to 333.33 years for *Triticum spelta* L. and *Triticum boeoticum* Boiss. There were also large differences between species, both in terms of the time required for viability to drop to 50% and the time required for seed viability to decrease by 10%. The calculated safe storage time (P10%) was the shortest for *Triticum dicoccon* Shrank (23.35 years) and the longest for *Triticum spelta* L. (311.87 years).

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Table 3. Seed longevity predicted after real long-term storage in National Gene bank of Bulgaria

Species	NA	MC, %	v=Ki-1/σ*p	σ, years	P50%, years	P10%, years
Triticum aestivum L.	873	7.13	v=2.139-0.009*x	111.11	237.56	108.71
Triticum durum Desf.	1025	7.30	v=2.191-0.014*x	71.43	156.43	69.12
Triticum dicoccon Shrank	8	7.20	v=1.541+0.024*x	41.67	64.17	23.35
Triticum monococcum L.	8	7.20	v=1.446+0.013*x	76.92	111.15	32.32
Triticum spelta L.	12	7.20	v=1.999-0.003*x	333.33	666.00	311.87
Triticum boeoticum Boiss.	8	7.18	v=1.653-0.003*x	333.33	550.67	168.31
Triticum turgidum L.	21	7.20	v=1.523+0.009*x	111.11	169.11	59.28

NA-number of accessions, MC-seed moisture content, % wet basis, v- viability in Probit after p years of storage in the genebank; K_i –*Probit* value of initial seed viability; 1/σ –measure of seed deterioration in storage; σ standard deviation of seed death in storage; $P_{10\%}$ –time in years for seed viability reduction with 10%; $P_{50\%}$ –seed half-life or measure of time to 50% seed viability in storage

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