https://doi.org/10.61308/VMGZ9126

The seed storability of crops under medium-term storage condition in the genebank of Bulgaria

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Citation: Desheva, G., Velcheva, N., Neshev, V., Uzundzhalieva, K., & Masheva, V. (2026). The seed storability of crops under medium-term storage condition in the genebank of Bulgaria. *Bulgarian Journal of Crop Science, 63*(3) 84-89

Abstract: The aim of the study was to determine the seed storability of crops maintained under medium-term storage conditions at the genebank of Bulgaria, based on the changes in viability after 18-24 years of storage. 476 seed accessions from 10 plant species were analyzed. These accessions had been preserved within the genebank's exchange collection, maintained at a temperature of +5 ± 1°C and a relative humidity (RH) of 30-40%. Through the analysis of experimental findings, crucial storage parameters were calculated to enable the forecasting of seed shelf-life and the identification of optimal times for regeneration. The study revealed substantial disparities in the rate of viability loss, both between different species and within individual species. *Pisum sativum* L., *Phaseolus vulgare*L., *Lycopersicon esculentum* Mill., *Brassica juncea* (L.) Czernj et Cosson, and *Cicer arietinum*L. demonstrated minimal decline in viability. In contrast, *Vicia faba* L., *Sinapis alba* L., and *Cucurbita* sp. exhibited the most pronounced and statistically significant reductions in seed viability. The study highlights the critical importance of continuous viability monitoring, data-driven regeneration scheduling, and adherence to international best practices to ensure the long-term integrity and accessibility of Bulgaria's invaluable crop genetic heritage.

Key words: genebank, conservation, medium – term storage, seed storability, germination, seed viability

INTRODUCTION

Seed genebanks have assumed a pivotal function in ensuring the future security of global food supplies through the conservation of plant genetic resources. They are biorepositories responsible for the *ex situ* conservation of plant genetic resources over an extended period of time. The purpose of this storage is to provide material for research and plant breeding to improve food and feed production (Yamasaki et al., 2020).

For organizations housing genetic resources, a crucial goal is significantly extending the viability period of stored seeds. This strategy is essential for the efficient administration of their biological holdings, as it simultaneously minimizes the highly frequent and expensive requirements for regeneration cycles and prevents the degeneration of the genetic constitution (Whitehouse et al., 2018). Despite the fact that certain species demonstrate a greater capacity to endure in a genebank environment than others do, there is a paucity of information concerning the role of seed characteristics in their conservation (Gianella et al., 2022a).

Germinability of plant seeds has a significant impact on crop production. It is known that some crops have shorter storability, while other crops have longer storability. Seed longevity is influenced by the main storage parameters – temperature, seed moisture and initial viability on storage (Redden and Partington, 2019; Nadarajan et al., 2023). Most plant species, particularly a significant proportion of key agricultural crops, produce orthodox seeds that tolerate desicca-

tion and can be preserved under low-temperature conditions. Research demonstrates that decreasing seed moisture levels and lowering storage temperatures effectively extend the storability of these seeds (Fatima et al., 2023).

Maintaining adequate viability is imperative for the long-term viability of *ex situ* conserved seed collections. In order to address this, accessions are regenerated when viability falls below a predefined threshold. Viability is monitored by determining the germination ability of accessions at predetermined time intervals. Optimizing the frequency of germination tests is hindered by the paucity of data on seed storability, particularly for material maintained under genebank conditions. This is a key challenge in the context of *ex situ* conservation, where the viability of accessions is monitored by determining their germination ability at regular intervals. The goal is to ensure the sustainable management of these collections, which is paramount for the conservation of biodiversity (Wijnker et al., 2025). Therefore, the primary objective of this current investigation was to determine the seed storability of ten crops stored under medium-term storage conditions at the genebank of Bulgaria, based on the changes in viability after 18-24 years of storage.

MATERIAL AND METHODS

Study site and collection

This study was conducted using data from the National Genebank of Bulgaria, located at the Institute of Plant Genetic Resources (IPGR) in Sadovo. The genebank was established in 1984 and maintains a diverse collection of crop genetic resources, encompassing cereals, legumes, oilseeds, fodder crops, vegetables, medicinal and aromatic plants, and wild relatives, originating from Bulgaria and from different countries. The genebank operates both medium-term and long-term storage facilities.

Seed accessions and storage conditions

A representative sample of 476 accessions was selected for this study, covering 10 plant species (*Vicia faba* L., *Cicer arietinum* L., *Phaseolus vulgaris* L., *Pisum sativum* L., *Lycopersicon esculentum* Miller, *Brassica juncea* (L.) Czern., *Helianthus annuus* L., *Cucurbita* sp., *Camelina sativa* (L.) Crantz, *Sinapis alba* L.). All selected accessions were stored under controlled medium-term conditions, maintained at a temperature of +5 ± 1°C and a relative humidity (RH) of 30-40%. Prior to storage, seeds were dried to a moisture content ranging from 5% to 8%, depending on the species, using dehumidified air at temperatures not exceeding 20°C. Seeds were sealed in glass jar to prevent moisture reabsorption and stored on shelving units within the medium-term vault.

Viability monitoring and data collection

Seed viability was monitored through standard germination tests conducted at intervals of 5 to 10 years, in accordance with genebank protocols. For this study, historical germination data spanning between 18 and 24 years were extracted from the genebank‘s database. For each accession, the following data points were collected:

✓ Initial germination percentage (after initial processing and drying, within one year of harvest).

✓ Subsequent germination percentages recorded at various time points during medium-term storage.

✓ Storage duration in years at each test interval.

Germination tests followed ISTA (International Seed Testing Association) rules and specific crop protocols (ISTA, 1993).

Data analysis

The collected data were analysed using descriptive statistics, followed by the Least Significant Difference (LSD) post-hoc comparison test. The *probit* analysis described by Roberts (1973) was applied to prediction of seed storage behaviour of individual species. These computations were performed using IBM SPSS Statistics software, version 22.

RESULTS AND DISCUSSIONS

The analysis of germination viability data after 10-14 and 18–24 years of storage demon-

strated varied longevity patterns across different crop types stored under medium-term conditions (Table 1). Pea, common bean, chickpea, mustard greens, tomatoes and sunflower had relatively stable storage after 10–14 years. The average rate of viability decline was estimated at less than 5%.. Pumpkin, faba bean, and camelina presented respectively 6.95%, 8.78%, and 26% reduction in viability. White mustard showed a slow increase in germination percentage compared to the initial values (with 1.59 %), which could be attributed to the occurrence of post-harvest dormancy. As posited by Yaniv et al. (1995) and Muhammad et al. (2023), the dormancy of white mustard can be impacted by various factors, including the characteristics of the seed coat, the prevailing environmental conditions at the time of maturation, and the nutritional availability in the post-harvest period.

A comparison of the initial germination rates with the results of the second control trial (18-24 years of storage) revealed the most significant reduction in germination rate for *Helianthus annuus* L. (with 19.72%), *Camelina sativa* (L.) Crantz (with 32.05%), *Cucurbita sp.* (with 48.63%), *Sinapis alba* L. (with 56.12%) and *Vicia faba* L. (with 60.94%). For the remaining 5 species, the decrease in germination was less than 10%. Lee et al. (2013) in testing the viability of 42 species stored for 10 years at 4°C and 30-40% relative humidity (RH) also found large differences in germination decline among vegetables, oilseeds, cereals and legumes. Complementing these observations, a subsequent study by Lu et al. (2018) examined over 18,000 accessions from 23 crop types after ten years of storage and indicated that a mere 0.5% of these accessions experienced a drop in germination to 70% or below.

The heterogeneity in post-storage germination performance across the accessions was quantified through the varying standard deviations associated with the coamputed average germination values. We determined that an expansive range between the maximum and minimum recorded germination percentages significantly amplified these standard deviations, resulting in a marked degradation of overall seed viability (Table 1). This relationship between broad variation and

Table 1. Variation in germination values of seeds of ten plant species during storage in the active collection of the National Gene Bank of Bulgaria

Family	Species	Number of accessions	Initial seed germination ±SD, %	Storage period to the first control test, year	Mean value of germination in the first control test ±SD, %	Storage period from first to second control test, year	Mean value of germination in the second control test ±SD, %	Total storage period, year
Fabaceae	*Vicia faba* L.	107	94.95±7.51	10.93	86.17±9.54**	10.47	34.01±24.57***	21,4
Fabaceae	*Pisum sativum* L.	22	96.72±5.43	12.27	95.10±6.28	8.73	92.36±7.18*	21
Fabaceae	*Phaseolus vulgaris* L.	44	91.82±9.52	12.93	88.00±8.41	8.52	82.54±18.08***	21,45
Fabaceae	*Cicer arietinum* L.	60	95.80±5.72	13.8	95.20±6.45	9.80	90.43±18.81***	23,6
Solanaceae	*Lycopersicon esculentum* Mill.	39	90.67±9.05	11.54	88.62±7.87	8.58	81.74±15.70**	20,12
Brassicaceae	*Brassica juncea* (L.) Czern.	38	97.52±4.49	12.35	94.11±6.08	9.76	92.32±5.98*	22,11
Brassicaceae	*Sinapis alba* L.	38	89.57±10.50	12.00	91.16±5.59	6.00	33.45±25.66***	18,00
Brassicaceae	*Camelina sativa* (L.) Crantz	20	92.40±8.09	12.00	66.4±38.98***	9.35	60.35±37.72***	21,35
Cucurbitaceae	*Cucurbita* sp.	20	96.00±5.67	11.79	89.05±7.16**	8.84	47.37±41.77***	20,63
Asteraceae	*Helianthus annuus* L.	88	94.86±4.77	9.95	92.89±6.52	9.55	75.14±29.93***	19,50
Total/Average		476		11.96		8.96		20.92

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, SD-standard deviation*

subsequent viability decline aligns consistently with findings reported in our prior research (Desheva, 2016; Desheva et al., 2017) (Table 1).

From a pragmatic standpoint, it is imperative to ascertain the optimal frequency of control tests within the genebank (Redden and Partington, 2019). It is recognised that seed storability is a complex trait that depends on many factors, such as environmental conditions in storage, growing conditions or phenotypic/genetic factors. It varies among species and populations, and within different seed morphs produced by the same plant (Gianella et al., 2022b; Pirredda et al., 2023; Tan et al., 2025).

The results of the probit storability assessment for 10 plant species under medium-term storage conditions involved the estimation of several key parameters related to seed viability and deterioration (Table 2). Among the species examined, *Brassica juncea* (L.) Czern. exhibited the most robust initial seed viability (Ki=1.929). Following closely were *Cucurbita* sp. (1.899), *Pisum sativum* L. (1.857), and *Vicia faba* L. (1.820). Conversely, *Vicia faba* L. demonstrated the most rapid rate of seed deterioration during storage. In contrast, *Pisum sativum* L. and *Cicer arietinum* L. experienced the slowest deterioration rates, both recording a 1/σ of -0.019. The standard deviation of seed death (σ) showed considerable variation, with *Vicia faba* L. having the shortest duration for seed death events (8.93 years), while *Pisum sativum* L. and *Cicer arietinum* L. displayed the longest durations (52.63 years). Significant inter-species differences were also observed in the time it took for seed viability to halve. The values ranged substantially, from a relatively short 16.24 years for *Vicia faba* L. to a considerably longer 97.68 years for *Pisum sativum* L. (Table 2).

The concept of a „safe storage period" refers to the timeframe during which seed viability experiences minimal decline. This is operationally defined as a 10% reduction in the initial viability, denoted as P10%. Our analysis revealed that it ranged from a minimum of 6.38 years for *Vicia faba* L. to a maximum of 38.81 years for *Pisum sativum* L. The differences observed within the legume group, highlight the genetic and biochemical di-

Table 2. Seed longevity of ten plant species predicted after real medium-term storage in the National genebank of Bulgaria

Family	Species	Number of accessions	Storage period, year	Ki	1/σ	σ	P50%	P10%
Fabaceae	*Vicia faba* L.	107	21.4	1.820	-0.112	8.93	16.24	6.38
Fabaceae	*Pisum sativum* L.	22	21.00	1.857	-0.019	52.63	97.68	38.81
Fabaceae	*Phaseolus vulgaris* L.	44	21.45	1.408	-0.021	47.62	52.71	23.38
Fabaceae	*Cicer arietinum* L.	60	23.60	1.795	-0.019	52.63	94.42	36.64
Solanaceae	*Lycopersicon esculentum* Mill.	39	20.12	1.360	-0.020	50.00	67.70	23.28
Brassicaceae	*Brassica juncea* (L.) Czern.	38	22.11	1.929	-0.024	41.67	80.33	32.82
Brassicaceae	*Sinapis alba* L.	38	18.00	1.520	-0.079	12.66	19.22	6.86
Brassicaceae	*Camelina sativa* (L.) Crantz	20	21.35	1.309	-0.056	17.86	23.36	8.03
Cucurbitaceae	*Cucurbita* sp.	20	20.63	1.899	-0.086	11.63	22.07	8.91
Asteraceae	*Helianthus annuus* L.	88	19.50	1.749	-0.050	20.00	34.96	13.39
Mean			20.92	1.66	-0.05	31.56	50.87	19.85
Std. Error of Mean			0.478	0.08	0.01	5.94	10.16	4.06
Std. Deviation			1.51	0.24	0.03	18.79	32.12	12.85
Variance			2.29	0.06	0.00	352.88	1031.40	165.02

Ki – Probit value of initial seed viability; 1/σ – measure of seed deterioration in storage; σ – standard deviation of seed death in storage; P10% – time in years for seed viability reduction with 10%; P50% – seed half-life or measure of time to 50% seed viability in storage

versity even within closely related crop families. This suggests that distinct regeneration schedules might be warranted for different legume species. Notably, oilseed crops exhibited shorter safe storage durations. Specifically, *Sinapis alba* L. lasted 6.86 years, *Camelina sativa* (L.) Crantz for 8.03 years, and *Helianthus annuus* L. for 13.39 years. *Cucurbita* sp. also fell into this category with a safe storage period of 8.91 years. In contrast, other studied crops displayed medium average safe storage periods. These included *Lycopersicon esculentum* Mill. at 23.28 years, *Phaseolus vulgaris* L. at 23.38 years, *Pisum sativum* L. at 38.81 years, *Cicer arietinum* L. at 36.64 years, and *Brassica juncea* (L.) Czern. at 32.82 years.

The findings from this comprehensive analysis of seed storability in the genebank of Bulgaria provide valuable insights into the efficacy of medium-term storage conditions and underscore the importance of continuous monitoring for effective *ex situ* conservation. The general trend of gradual viability decline is consistent with well-established principles of orthodox seed deterioration (Roberts, 1973; Bewley et al., 2012), and the observed differences among crop groups align with previous research on species-specific storage behaviour Ellis et al., 2018, 2019; Yamasaki et al., 2020; Gianella et al., 2022b).

The practical implications of these findings for our genebank are significant. Firstly, the study validates the effectiveness of the current medium-term storage conditions for maintaining the viability of a large proportion of the collection for several decades. Secondly, it provides empirical evidence to support the prioritization of monitoring and regeneration efforts. Species identified as short-lived (*Sinapis alba* L., *Camelina sativa* (L.) Crantz, *Vicia faba* L., *Cucurbita* sp. and *Helianthus annuus* L.) should be subjected to more frequent viability tests (e.g., every 5-10 years) and scheduled for regeneration proactively, potentially before they reach the 85% threshold, to minimize the risk of genetic erosion and reduce regeneration costs associated with very low viability. Conversely, long-lived species can be safely tested at longer intervals (*Lycopersicon esculentum* Mill., and *Phaseolus vulgaris* L., no later than 20 years, while *Pisum sativum* L., *Cicer arietinum* L., *Brassica juncea* (L.) Czern - no later than 30-35 years), optimising resource allocation.

Future research could focus on controlled deterioration tests to predict longevity more accurately, investigate the molecular mechanisms underlying the observed species-specific differences, and explore the impact of specific pre-storage treatments or packaging materials on longevity. Furthermore, a deeper dive into specific Bulgarian landraces to understand their unique storage behaviours would enrich the conservation strategy. The integration of seed health testing and vigour tests alongside germination percentages could also provide a more holistic understanding of seed quality.

CONCLUSION

The genebank of Bulgaria at IPGR Sadovo successfully maintains the viability of a diverse collection of crop genetic resources under medium-term storage conditions for an extended period. This research has demonstrated that while many orthodox crop seeds, particularly pea, common bean, chickpea, tomatoes, and mustard greens, exhibit excellent longevity, while certain crops, notably sunflower, camelina, pumpkin, white mustard and faba bean, show a significantly faster decline in viability.

These findings are crucial for informing and refining the genebank‘s management practices. A differentiated approach, with more frequent assessments and proactive regeneration for short-lived accessions, will be critical to prevent the loss of irreplaceable genetic diversity. This study underscores the invaluable role of national genebanks in safeguarding agricultural biodiversity and highlights the continuous need for research and adaptive management to ensure the long-term integrity and accessibility of these vital genetic resources for future generations.

Acknowledgments

This research work was carried out under the project IS-PGR-SADOVO “Intelligent system for

managing Bulgarian plant gene pool, conserved in the genebank of IPGR-Sadovo" with the financial support of the Ministry of Education and Science, Contract number KP-06-H86/9/09.12.2024 of Bulgarian National Science Fund.

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Received: March, 07, 2026; *Approved:* May, 05, 2026; *Published:* June, 2026