Available online at www.worldscientificnews.com

World Scientific News

WSN 69 (2017) 29-46 EISSN 2392-2192

Germinability of soybean seeds stored more than 30 years in the Bulgarian national seed genebank

Gergana Desheva*, Sofia Petrova, Manol Deshev

Department of Plant Genetic Resources, Institute of Plant Genetic Resources "Konstantin Malkov", 2 Druzhba Str., 4122 Sadovo, Bulgaria

*E-mail address: gergana_desheva@abv.bg

ABSTRACT

The maintenance of seed viability over long periods of time in genebanks is a key element in conservation of plant genetic resources. In this work, data obtained at the Bulgarian National seed Genebank from the routine task of monitoring seed viability from soybean were analyzed. Differences between initial, first and second germination test after 11 and 34 years of long-term storage were assessed among 182 accessions from *Glycine max* (L.) Merr. The samples were regenerated during 1978-1981 growing seasons. On the basis of experimental data, the seed storage characters - σ, P50% and P10% were determined allowing the prediction of seed storage life and the regeneration needs. Significant decrease in seed viability after 11 years of storage was not recorded in the investigated soybean accessions from the different regeneration years. When comparing the initial germination percentages with results from the second control test after 34 years of storage were found significant differences in the loss of seed viability among genotypes from different regeneration years. The frequency of odd results were most significant for genotypes regenerated in 1981 (0.691) in comparison with these from other regeneration seasons. The highest Ki value (2.09) was recorded for accessions from 1981 harvest year; where rate of seed deterioration (1/σ) was also highest (-0.044). The lowest values both for Ki and 1/σ were found for the groups of accessions harvested in 1978 and 1979. There was a wide variation between groups of accessions regenerated in different years in both the time taken for viability to fall to 50% and the time for seed viability reduction with 10%. The predicted mean safe storage time for *Glycine max* (L.) Merr. was 25.54 years. Total 76 accessions or 42,2% of analyzed accessions had significant decrease of germination after 34 years of storage and would need regeneration.

Keywords**:** genebank, soybean, seed germination, seed longevity, seed viability

1. INTRODUCTION

Soybean (*Glycine max*, family Fabaceae) is a species of legume native to East and South East Asia, widely grown for its edible bean which has numerous uses. It is described as wonder crop, golden crop, and the scene of life because of its high (38–45%) protein content as well as its high (approximately 20%) oil content [1]. Soybean is one of the most important legumes in the world, and its production dominates world oilseed followed by cotton seed, peanut and sunflower [2]. Soybean contains antioxidants and phytonutrients that have been linked with various health benefits. It is helpful for brain development because it contains 3% lecithine amino acid [3]. Soybean enriches the soil by fixing atmospheric nitrogen in symbiosis with bacteria for its own use with benefits to subsequent crops [4]. Various soybean products are available, including soy flour, soy protein, tofu, soy milk, soy sauce, and soybean oil. It is therefore important to maintain and to conserve existing soybean collections in genebanks in the most appropriate way possible to ensure its continued and sustainable use for both present and future generations.

Based on data from WIEWS (http://apps3.fao.org/wiews/wiews.jsp) there are more than 230,369 soybean accessions conserved as plant genetic resources (PGR) in the world. The largest collections are held in China (63,472 accessions), Brazil (23,085 accessions), USA (22,147 accessions), Taiwan, China (18,384 accessions), Korea (17,644 accessions), Japan (14,048 accessions) accessions) and India (13,670 accessions) accounting together 74.86% of the total accessions conserved worldwide. Russian Federation and Australia have collections of over 5,000 accessions. France, Côte d'Ivoire, Colombia and Indonesia have collections of over 3,000 accessions, while Germany, Mexico, Argentina, Zimbabwe and Thailand over 2,000 accessions. In Bulgaria soybean seeds as PGR are maintained in the National genebank in the IPGR-Sadovo. Totally 758 accessions from *Glycine max* (L.) Merr. are conserved under long term storage condition at subzero temperatures (−18 ºC) in hermetically closed containers (glass jars or three laminated aluminum foil packets).

The maintenance of seed viability over long periods of time in genebanks is a key element in conservation of plant genetic resources [5]. Standards aimed at maintaining seed viability have been developed and applied in genebank management procedures, including drying and storage under low seed moisture content and temperature [6,7]. Even in seeds stored under optimal conditions suitable for long-term storage, viability may decrease as a result of deterioration processes [8-10]. These deterioration processes include: an increase of the free radical content, changes in protein structure, depletion of food reserves, development of fat acidity, changes in enzymatic activity, membrane damage, chromosomal changes and an increase of respiration [11]. Seed viability and the rate of seed deterioration have been extensively investigated [12-14]. From a quantitative view point, seed deterioration can best be defined as an increased probability of death of an individual seed as deterioration proceeds [15]. Seed death is indicated by the failure to germinate and seed longevity is the period until seed death occurs [16-18]. Longevity of seed in storage is influenced by the seed quality as well as storage conditions. Storage of seeds with high initial quality will maximize accession longevity. Irrespective of initial seed quality, unfavourable storage conditions, particularly air temperature and air relative humidity, contribute to accelerating seed deterioration during storage. Hence, it is difficult to assess the effective storage period because the storability of the seed is a function of initial seed quality and the storage conditions, and may vary among different seed types [1,19]. However, seed longevity varies among species and genotypes, and stored seeds will lose their viability over time to a level at which seed regeneration is required [20-25]. Regeneration is a costly genebank operation and may also negatively affect the genetic integrity of an accession through exposure to the influence of genetic drift, selection, contamination and human error. Therefore, it is important to maximize seed longevity and keep operational costs and logistics manageable through monitoring of seed deterioration, an essential task for managing stored germplasm [26].

The objectives of this study were to assess the changes in the viability of soybean seeds stored for 34 years in the base collection of the National genebank of Bulgaria and to determine the risk of viability decreasing bellow acceptable levels after 34 years of storage and to discuss the opportunity for more appropriate monitoring.

2. MATERIALS AND METHODS

2. 1. Seed material

A total of 182 seed accessions from *Glycine max* (L.) Merr. stored since 1981–1990 in the National Genebank of Bulgaria were evaluated. All seed accessions were maintained as base collection under long-term storage conditions with low moisture contents (5±2%) in hermetically closed containers (glass jars or three laminated aluminium foil packets) at –18 °C.

2. 2. Seed viability

Seed viability was detected on the basis of germination rate of accessions in storage. The seed germination was determined as following: just before the storage, after 11 years of storage and after 34 years of storage. The germination tests were carried out according to ISTA rules [27]. The recommendations for work in the gene banks were also implemented [28-30]. Seeds stored at –18 °C for about 11 and 34 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 described earlier [20].

2. 3. Seed moisture content

The moisture content of seed accessions, both before and after the time of storage, was determined using oven methods of ISTA [27] for reduced working sample (about 3 g per accession).

2. 4. Data analysis

The *Probit* analysis for modelling of data from seed storage experiments was used according to the models first described by Roberts [31]. 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 Ki [32]. The relationship used for calculation was:

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

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 [32], 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 using *IBM SPSS Statistics 19*.

3. RESULTS AND DISCUSSION

3. 1. Maintenance of seed germination depending on regeneration season and storage period

Maintenance of seed viability is a basic condition for successful preservation of germplasm as seeds [33]. The initial germinabilty of the seeds, seed moisture content and the storage temperature have significant influence on seed longevity in the germplasm banks [31]. Periodic testing of viability is crucial to operation of seedbanks because it permits the control of genetic erosion during storage [34]. The seed initial germination percentage in the National Genebank of Bulgaria should be above 80%, which is the lowest viability standard for seed accessions to be included in the long-term storage [20,25]. Good seed storage conditions maintain germplasm viability, but even under excellent conditions, viability declines with storage duration [21]. Seed viability declines slowly at first, and then rapidly as seeds age [35] accordingly, it is important to know when this decline occurs so that the accession can be regenerated by replacing exiting seeds with high-viability ones.

Soybean seed reaches its maximum potential for germination and vigour at physiological maturity. High seed moisture level increases seed mycoflora, which play an important role in deterioration of soybean seed quality and viability during storage [9]. Good climatic conditions during the post-maturation preharvest periods are vital to the quality of seeds at harvest time: dry weather accelerates the drying of seeds on the plant to moisture content that is favourable for handling while moist weather (with high relative humidity) delays drying of seeds on the plant, leading to seed deterioration even before harvest [36]. The germination potential is very short lived in soybean as compared to other oilseed crops and is often reduced prior to planting time [37]. According to Reuzeau and Cavalie [38], Trawatha et al. [39], and Balašević- Tubić et al. [40] in oil crops, such as soybean and sunflower, autooxidation of lipids and increase of the content of free fatty acids during storage period are the main reasons for rapid deterioration of the oil seed. Differential storability of various species was attributed to genetics, species and varietal characters [41].

In the present study, the viability after 11 and 34 years of storage were assessed among 182 soybean accessions regenerated between 1978-1981 years (Table 1).

The mean viability for different regeneration years varied from 90.03 to 98.29%. The highest mean seed viability was identified from regeneration carried out during the year 1981. The number of accessions regenerated during this season was also highest (93 accessions) compared to other seasons. The oldest seeds in the study represented by 28 accessions from 1978 regeneration had the lowest mean seed viability of 90.03%. In this year the low mean seed viability could be attributed largely due to a higher number of accessions (31% of accessions) in the unfavorable viability range of 80 to 84%. This shows that differences in soybean seed viability in the beginning of storage is largely due to the growing environmental conditions for the season in which the accessions were regenerated [33].

From the germination results obtained after 11 years of storage, means seed viability from 1978, 1979 and 1980 regeneration seasons showed slow increase in the germination percentages from initial values. After 34 years of storage, the means germination of soybean seeds declined from 90.03% to 86.28%, from 91.2% to 86.67% and from 91.11 to 82.43%, respectively. The increase in germination after storage relates to post-harvest dormancy [20,21, 42-47].

Significant differences were examined between means seed germinations at the beginning of storage and after a significant time of storage, in both first control test (with storage time of 11 years) and second control test (with storage time of 34 years) from regeneration carried out during the year 1981 (Table 1). However, it should be pointed out that as presented in a previous research, the rate of difference before and after the storage influenced the mean value of seed germination, and also had a stronger effect on the standard deviation [20,25].

The rate of variation between accessions was illustrated by differences of standard deviations calculated for the mean values of germination after storage (Table 1, Fig. 1). Differences were more significant in the cases with larger variations between minimum and maximum values and a considerable reduction in seed germinability [25]. As we noted above soybean seeds viability from 1981 regeneration season declined rapidly after 34 years of storage and its SD increased significantly from ±3.68% to ±17.48%, respectively (Table 1, Fig. 1).

Table 1. Changes in the seed viability values of soybean (*Glycine max* (L.) Merr.) depending on regeneration season and storage period in the National Gene bank of Bulgaria

Regeneration year	Number of accessions	Mean value of initial germination, % ±SD	Mean value of of germination after 11 years of storage, % ±SD	Mean value of germination after 34 years, % ±SD
1978	28	90.03±7.8	93.59±3.55	86.28±11.23
1979	15	91.2±5.89	94±3.09	86.67±9.88
1980	46	91.11±8.07	93.83±9.99	82.43±12.57***
1981	93	98.29±3.66	96.33±2.67***	75.44±17.48***
1978-1981	**182**	**94.69±6.49**	**95.07±3.19**	**79.73±15.56*** **

***P≤0.001; **P≤0.01; *P≤0.05; SD-standard deviation (%)

The effect of different regeneration seasons on germination percentage of soybean seed accessions stored for 11 and 34 years in the base collection at -18 C are presented from Table 2 to Table 5.

For determination of seed germination decline we used ‘conditions for compatibility’ of results as suggested by ISTA for statistical tolerance of data (ISTA, 1993). This approach is described earlier from Stoyanova [20, 48] and has been used by Chen et al. [45]. Different categories are defined using two statistical parameters, i.e. seed germination before storage

(SGI) and seed germination after storage (SGL). The conditions for compatibility of data within the accession categories are based on the level of seed viability decline: “no change”, “minimal change” and “significant decline” of seed viability. Accessions maintaining the same germination rate or a slightly higher level are classified in the category “conserved with no change”. Accessions with seed viability reduction of about 10% are described as showing “minimal change”. These two categories are considered as “successfully stored accessions“. Accessions with seed viability reduction of above 10% are described as “significant decline” [48].

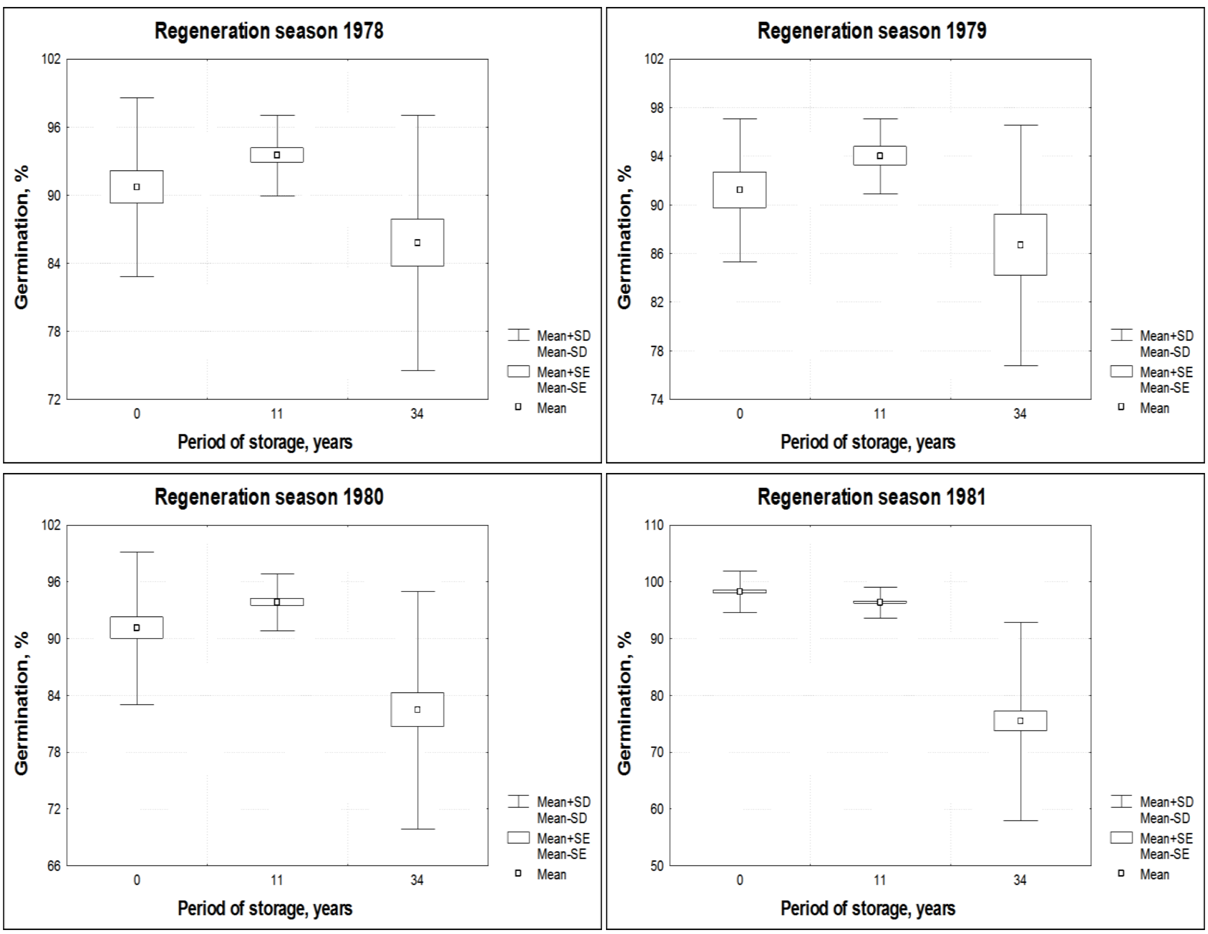

Figure 1. Box plots of mean germination, SD (standard deviation, %) and SE (standard error, %) of analysed 182 soybean accessions according to period of storage and regeneration season

The results obtained after 11 years and 34 years of storage of the soybean seed accessions from different regeneration seasons in the genebank showed changes in germination. Germination was significantly enhanced or decreased in some accessions and in the others remained the same. In the group regenerated in 1978, 28 seed accessions were evaluated (Table 2). Most of them preserved high seed quality after 11 years of storage. The frequency of seed accessions conserved without change and with minimal change in germination rate is 0.75 and 0.25 respectively. The frequency of seed sample saved withouth change varied from 0.867 to 0.609 for accessions from 1979 and 1980 regeneration seasons (Table 3 and Table 4). The number of accessions regenerated during 1981 season was the highest and the frequency of accessions with minimal change in germination rate was also higest (0.527) (Table 5). The frequency of samples with increase of germination rate after 11 years of storage was varied from 0.566 for the group from 1978 regenerated season to 0.129 for the samples regenerated in 1981 (Table 2-5). Significant decrease in seed viability after 11 years of storage was not recorded in the investigated soybean accessions from the different regeneration years (Table 2-5). This result is in the contrary to our earlier results where soybean seeds showed the largest reductions in viability after first (after 11years) and second retests (after 24 years). The largest range in seed germination rate observed in these two studies was due to differences in seed survival between genotypes induced both by pre-harvest conditions and by maintenance of seeds before and during storage. Concerning Glycine seeds the information in data files shows that some accessions are sent to the genebank 1- 2 years after harvest, which means these seeds were stored under ordinary conditions before maintenance in the genebank.

When comparing the initial germination percentages with results from the second retest after 34 years of storage were recorded significant differences in the loss of seed viability among genotypes from different regeneration years. The frequency of odd results were most significant for genotypes regenerated in 1981 (0.691) in comparison with these from other regeneration seasons. The number of accessions that increase the germination ability was also significantly less. In some genotypes, the second test exceeded the seed germination from the first test. As we noted before the cause for this was that in the past, the hard seeds in the germination test were described as 'non-germinating'. Some changes in the viability may have also been the results of different degrees of operator error, as the staff performing the germination test changed over time [25].

According to Lu et al. [49] and Benková and Žáková [36], the genetic characteristics of species and pre-storage environments are the main factors for seed viability decline. Adverse climate at the stages of seed ripening and harvesting, as well as the damage caused by seed extraction, drying and transportation after harvest could affect the rate of seed viability decline during storage [50].

Table 2. Data matrixes used for evaluation of soybean seeds (*Glycine max* (L.) Merr.) regenerated in 1978 and stored for 11 years and for 34 years in the Bulgarian genebank

SGI	SGL	Total number of accession	Freq.	SGI, %	Mean germination after 11 years of storage, %	Total number of accession	Freq.	SGI, %	Mean germination after 34 years of storage, %
		28	1			28	1		
>97	>97		0.000				0.000		
	95-97	1	0.036	100	95	2	0.071	100	96
	90-94	5	0.179	100	92.17±1.17	3	0.107	100	92.67±1.15
	85-89		0.000				0.000		
	80-84		0.000				0.000		
	<80		0.000			1***	0.036	100	64

95-97	>97		0.000				0.000		
	95-97	**2**	0.071	**96**	**96**	**1**	0.036	**96**	**96**
	90-94	4	0.143	96.25±0.50	92.75±0.96		0.000		
	85-89		0.000			1	0.036	96	88
	80-84		0.000			2***	0.071	96	80
	<80		0.000			2***	0.071	96.50±0.71	66±2.83
90-94	>97		0.000				0.000		
	95-97	**1+**	0.036	**92**	**96**	1+	0.036	92	96
	90-94	**5+**	0.179	91.6±0.89	92.20±1.10	**3**	0.107	**91.33±1.15**	**92.0**
	85-89		0.000			1	0.036	92	88
	80-84		0.000				0.000		
	<80		0.000			1	0.036	92	80
85-89	>97		0.000				0.000		
	95-97	**1+**	0.036	88	95		0.000		
	90-94		0.000				0.000		
	85-89		0.000				0.000		
	80-84		0.000			1	0.036	88	84
	<80		0.000				0.000		
80-84	>97	**3+**	0.107	**80**	**100**	1+	0.036	80	100
	95-97	**2+**	0.071	**80**	**96**	**3+**	0.107	**80**	**96**
	90-94	**3+**	0.107	**80**	**91.67±0.58**		0.000		
	85-89		0.000			**1+**	0.036	**80**	**88**
	80-84	1	0.036	84	82	**1+**	0.036	**80**	**84**
	<80		0.000			3***	0.107	81.33±2.31	69.33±2.31

The cases presented in bold (shaded cells) are evaluated as stored without change in the genebank.
The symbol (+) indicated the cases when the result after storage is better than germination at the beginning of storage.
SGI = initial seed germination
SGL = last seed germination (test after storage)
*** = odd results, when seed viability decline is significant

Table 3. Data matrixes used for evaluation of soybean seeds (*Glycine max* (L.) Merr.) regenerated in 1979 and stored for 11 years and for 34 years in the Bulgarian genebank

SGI	SGL	Total number of accession	Freq.	SGI, %	Mean germination after 11 years of storage, %	Total number of accession	Freq.	SGI, %	Mean germination after 34 years of storage, %
		15	**1**			**15**	**1**		
>97	>97		0.000				0.000		
	95-97		0.000				0.000		
	90-94	1	0.067	100	92		0.000		
	85-89		0.000				0.000		
	80-84		0.000			1***	0.067	100	84
	<80		0.000				0.000		
95-97	>97	1+	0.067	96	100		0.000		
	95-97	**2**	0.133	**96**	**96**	**1**	0.067	**96**	**96**

	90-94	1	0.067	96	92	1	0.067	96	92.0
	85-89		0.000				0.000		
	80-84		0.000			2***	0.133	96	82±2.83
	<80		0.000				0.000		
90-94	>97	1+	0.067	92	100		0.000		
	95-97		0.000			**3+**	0.200	**92**	**96**
	90-94	**5**	0.333	**92**	**91.6±0.55**	**1**	0.067	**92.0**	**92.0**
	85-89		0.000				0.000		
	80-84		0.000			1***	0.067	92.0	80
	<80		0.000			1***	0.067	92	68
85-89	>97		0.000				0.000		
	95-97	**1+**	0.067	**88**	**96**		0.000		
	90-94	**1+**	0.067	**84**	**92**	**1+**	0.067	**88**	**92.0**
	85-89		0.000				0.000		
	80-84		0.000				0.000		
	<80		0.000			1***	0.067	84	68
80-84	>97		0.000				0.000		
	95-97	**1+**	0.067	**80**	**96**	**1+**	0.067	**80**	**96**
	90-94	**1+**	0.067	**80**	**92**		0.000		
	85-89		0.000				0.000		
	80-84		0.000			**1**	0.067	**80**	**80**
	<80		0.000				0.000		

The cases presented in bold (shaded cells) are evaluated as stored without change in the genebank.
The symbol (+) indicated the cases when the result after storage is better than germination at the beginning of storage.
SGI = initial seed germination
SGL = last seed germination (test after storage)
*** = odd results, when seed viability decline is significant

Table 4. Data matrixes used for evaluation of soybean seeds (*Glycine max* (L.) Merr.) regenerated in 1980 and stored for 11 years and for 34 years in the Bulgarian genebank

SGI	SGL	Total number of accession	Freq.	SGI, %	Mean germination after 11 years of storage, %	Total number of accession	Freq.	SGI, %	Mean germination after 34 years of storage, %
		46	1			46	1		
>97	>97	**2**	0.043	100	99±1.41	**1**	0.022	**100**	**100**
	95-97	**5**	0.109	100	96	1	0.022	100	96
	90-94	8	0.174	100	91.63±0.74	4	0.087	100	92
	85-89		0.000			3***	0.065	100	88
	80-84		0.000			5***	0.109	100	80
	<80		0.000			1***	0.022	100	60
95-97	>97		0.000				0.000		
	95-97	**2**	0.043	**96**	**96**		0.000		
	90-94	3	0.065	95.67±0.58	92		0.000		
	85-89		0.000			1	0.022	96	88.0

	80-84		0.000			3***	0.065	95.67±0.58	81.33±2.31
	<80		0.000			1***	0.022	96	72
90-94	>97	**2+**	0.043	**91.99±1.41**	**99±1.41**		0.000		
	95-97	**1+**	0.022	**90**	**95**	**1+**	0.022	**92**	**96**
	90-94	5	0.109	92±1.41	91.8±0.45		0.000		
	85-89		0.000			1	0.022	90	88
	80-84		0.000			3	0.065	91.33±2.31	81.33±2.31
	<80		0.000			3***	0.065	92	60±10.58
85-89	>97		0.000				0.000		
	95-97	**2+**	0.043	**88**	**96**	**1+**	0.022	**88**	**96**
	90-94	**2+**	0.043	88	92	**1+**	0.022	**88**	**92**
	85-89		0.000				0.000		
	80-84		0.000			2	0.043	88	80
	<80		0.000				0.000		
80-84	>97	**2+**	0.043	**80**	**99±1.41**		0.000		
	95-97	**4+**	0.087	**81±2.0**	**96**	**3+**	0.065	**80**	**96**
	90-94	**6+**	0.130	**80.67±1.63**	**91.83±0.41**	**1+**	0.022	**80**	**92**
	85-89	**2+**	0.043	**80**	**88**	**2+**	0.043	**82±2.83**	**88**
	80-84		0.000			**6+**	0.130	**80.66±1.63**	**82±2.19**
	<80		0.000			2***	0.043	80	48±11.31

The cases presented in bold (shaded cells) are evaluated as stored without change in the genebank.
The symbol (+) indicated the cases when the result after storage is better than germination at the beginning of storage.
SGI = initial seed germination
SGL = last seed germination (test after storage)
*** = odd results, when seed viability decline is significant

Table 5. Data matrixes used for evaluation of soybean seeds (*Glycine max* (L.) Merr.) regenerated in 1981 and stored for 11 years and for 34 years in the Bulgarian genebank

SGI	SGL	Total number of accession	Freq.	SGI, %	Mean germination after 11 years of storage, %	Total number of accession	Freq.	SGI, %	Mean germination after 34 years of storage, %
		93	1			93	1		
>97	>97	**24**	0.258	**100**	**99.33±0.96**	**1**	0.011	**100**	**100**
	95-97	26	0.280	100	96.04±0.20	2	0.022	100	96
	90-94	20	0.215	100	93.25±1.12	12	0.129	100	92
	85-89		0.000			5***	0.054	100	88
	80-84		0.000			21***	0.226	100	81.52±1.99
	<80		0.000			29***	0.312	100	58.34±14.20
95-97	>97	**4+**	0.043	**96**	**98.5±1**		0.000		
	95-97	**5**	0.054	**96**	**95.80±0.45**	**2**	0.022	**96**	**96**
	90-94	3	0.032	96	92.33±1.53	3	0.032	96	92.0
	85-89		0.000			3+	0.032	96	98.0
	80-84		0.000			2***	0.022	96	84
	<80		0.000			2***	0.022	96	38±2.83

90-94	>97	**1+**	0.011	**92**	**100**		0.000		
	95-97	**2+**	0.022	**92**	**96**	**1+**	0.011	**92**	**96**
	90-94	**3**	0.032	**92**	**92.33±1.53**		0.000		
	85-89		0.000				0.000		
	80-84		0.000			3	0.032	92.0	81.33±2.31
	<80		0.000			2***	0.022	92	52±5.66
85-89	>97	**2+**	0.022	**88**	**100**		0.000		
	95-97		0.000				0.000		
	90-94	**1+**	0.011	**88**	**94**		0.000		
	85-89		0.000			**1**	0.011	**88**	**84**
	80-84		0.000				0.000		
	<80		0.000			2***	0.022	88	62±2.83
80-84	>97	**2+**	0.022	**84**	**100**		0.000		
	95-97		0.000				0.000		
	90-94		0.000				0.000		
	85-89		0.000			**1+**	0.011	**84**	**88**
	80-84		0.000				0.000		
	<80		0.000			1***	0.011	84	60

The cases presented in bold (shaded cells) are evaluated as stored without change in the genebank.
The symbol (+) indicated the cases when the result after storage is better than germination at the beginning of storage.
SGI = initial seed germination
SGL = last seed germination (test after storage)
*** = odd results, when seed viability decline is significant

3. 2. Monitoring of seed viability and longevity

In storage, soybean seed rapidly loses its viability [51-53]. Seed deteriorated during storage is one of the basic reasons for low productivity in soybean. Changes that occur in seed during ageing are significant in terms of seed quality among other things, also implies seed longevity [40, 54]. Different periods of seed storage, as well as ageing conditions adversely affected the seed vigor [55]. The variation in speed of seed deterioration of soybean varieties is a genetic character [56]. Soybean genotypes differ in their ability to maintain seed longevity. The longevity of seeds in storage is influenced by four major factors - genetics, quality of the seed at the time of storage, moisture content of seed or ambient RH and temperature of storage environment [57].

Seed species that possess high oil content do not store well compared to those that contain less oil [58]. Nagel and Börner [59] concluded that oil content seemed to influence longevity under open storage conditions and further analysis will be necessary to come to a solid conclusion. In other site Probert et al. [23] reported that seed oil content was not correlated with P50%, but that seed longevity was related to seed

structure and climate of origin.

The chemical composition of oilseeds causes specific processes to occur during storage. The seeds rich in lipids have limited longevity due to their specific chemical composition [60]. Oil seeds are very sensitive to the harsh environmental conditions. It is hypothesized that their oil content readily oxidize, which deteriorate the seed health in storage [61]. Association of SSR markers with seed longevity in soybean has been demonstrated by Singh et al. [62]. In total, four (Satt538, Satt285, Satt600 and Satt434) independent SSR markers were declared to be significantly associated with seed longevity. Singh et al. [63] reported that Satt538 and Satt434 were also associated with seed coat permeability in soybean.

Seed longevity is defined as seed viability after seed dry storage (storability) and, therefore, describes the total seed life span [64]. This storability period includes both the dormant and nondormant states. During seed storage, seeds deteriorate, lose vigor, and, as a result, become more sensitive to stresses during germination, and ultimately die. The rate of this aging depends on the seed moisture content, temperature, and initial seed quality [13]. 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 [21-25,59,65].

Results from assessment of *probit* longevity for soybean seeds from different regeneration years stored 34 years in the real condition in National genebank of Bulgaria are presented in Table 6. The measure of seed longevity in this study is based on the σ value (standard deviation of seed death in storage), defining the period during which the percentage viability is reduced by one *Probit* as described by Hong et al. [66]. According to Ellis and Roberts [32], the life span of a seed-lot, the time until all the seeds have lost viability, depends on the value of σ and on the proportion of the seeds which are viable at the start of the storage, Ki (in *Probit*). In our study the highest Ki value (2.09) was recorded for accessions from 1981 harvest year; where rate of seed deterioration (1/σ) was also highest (-0.044). The lowest values both for Ki and 1/σ were found for the groups of accessions harvested in 1978 and 1979. The σ values varied from 22.73 years for seeds regenerated in 1981 to 111.11 years for accessions regenerated in 1978 and 1979 years. The mean σ value for all accessions included in this study was 38.46 years, respectively. This value is near to value that we received in our earlier investigation [25].

Table 6. Seed longevity of *Glycine max* (L.) Merr. predicted after real long-term storage in National Gene bank of Bulgaria

Regeneration season	**Number of accesions**	**Period of storage, years**	**Moisture content, %wb**	**Ki**	**1/σ**	**σ, years**	**P_{50}%**	**P_{10}%**
1978	28	34±0.00	6.27±0.75	1.44	-0.009	111.11	159.55	56.00
1979	15	34±0.00	6.27±0.75	1.47	-0.009	111.11	163.22	57.78
1980	46	33.91±0.28	5.88±0.52	1.491	-0.015	66.67	99.33	35.40
1981	93	33.01±0.10	5.84±0.38	2.176	-0.044	22.73	49.43	22.18
1978-1981	182	33.47±0.50	5.95±0.50	1.744	-0.026	38.46	67.04	25.54

Ki – *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

Longevity can also be described by determining the time for seed viability reduction of 10% ($P_{10\%}$) and the time taken for viability to fall to 50% ($P_{50\%}$). Many researchers reported different rate of variation in P50% between families, genera, species, cultivars and wild types of crops [21,23,25,67]. In this study there was a wide variation between groups of accessions regenerated in different years in both the time taken for viability to fall to 50% and the time for seed viability reduction with 10%. P50% ranged from 49.43 years to 163.22 years. The calculated value was the lowest for the group regenerated in 1981 (49.43 years) and the higest for the group harvested in 1979. The safe storage time (P_{10}%) varied between 22.18 and 57.78 years depending on regeneration years (Table 6). The predicted mean safe storage time for *Glycine max* (L.) Merr. was 25.54 years. Comparing this result with these received for *Glycine max* (L.) Merr. after 10 years [20] and 24 years of long term storage in the National genebank of Bulgaria [25] we could be concluded that soybean seeds should be monitored not later than 20 years from the beginning of storage. Moreover, seed viability monitoring should be done individually, and should not be monitored selectively because there was a large variation of viability among accessions even when supplied by the same unit in the same year.

3. 3. Regeneration requirements

According to the Genebank Standards for Plant Genetic Resources for Food and Agriculture [7] regeneration should be carried out when the viability drops below 85 percent of the initial viability. Based on this standard and the results obtained from the monitoring tests after 34 years of storage we could be concluded that the number of samples that need to be regenerated are the following: 5 accessions from regeneration season 1978, 2 accessions from 1979 year, 12 from 1980 year and 57 accessions harvested in 1981 year. Total 76 accessions or 42,2% of analyzed accessions had significant decrease of germination after 34 years of storage and would need regeneration.

4. CONCLUSIONS

The results obtained from the monitoring tests of 182 soybean accessions indicate that there were no significant differences in the loss of seed viability among accessions after 11 years of storage and significant decrease in seed viability after 34 years of storage in compare with initial viability. Differences in soybean seed viability are attributed largely to the growing environmental conditions of the season in which the accessions were regenerated. However, viability differs between genotypes within the species. Seed longevity calculated as σ values varied from 22.73 years for seeds regenerated in 1981 to 111.11 years for accessions regenerated in 1978 and 1979 years.There was also a wide variation between groups of accessions regenerated in different years in both the time taken for viability to fall to 50% and the time for seed viability reduction with 10%. The predicted mean safe storage time for *Glycine max* (L.) Merr. was 25.54 years. Soybean seeds should be monitored not later than 20 years from the beginning of storage. Moreover, seed viability monitoring should be done individually, and should not be monitored selectively because there was a large variation of viability among accessions even when supplied by the same unit in the same year. Accessions with germination under critical value should be regenerated. The presented results are a useful tool for the monitoring of gene bank storage and the prediction of regeneration needs in the National Gene bank of Bulgaria.

Acknowledgments

This work was financially supported by the Agricultural Academy of Bulgaria. We would like to acknowledge Prof. Siyka Stoyanova for her long standing work as a head of the National Gene bank of Bulgaria, as well as to all the staff members who have been involved in the seed storage processing and germination testing at the National Gene bank of Bulgaria since 1978.

References

[1] J. Singh, S. Paroha, R. P. Mishra. Effect of Storage on Germination and Viability of Soybean (*Glycine max*) and Niger (*Guizotia abyssinica*) Seeds. *International Journal of Current Microbiology and Applied Sciences* 5 (7) (2016) 484-491.

[2] A. Odoba, N. I. Odiaka, A. U. Gbanguba, M. Bashiru. Germination characteristics of twenty varieties of soybean (*Glycine max* (L.) Merr) stored for seven months. *Scientia Agriculturae* 13 (3) (2016) 151-155.

[3] P. I. Akubor, M. U. Ukwuru. Functional properties and biscuit making potential of soybean and cassava flour blends. *Plant Foods for Human Nutrition* 58(3) (2005) 1-12.

[4] P. Gobinath, P. Pavadai. Effect of gamma rays on morphology, growth, yield and biochemical analysis in soybean (Glycine max (L.) Merr.). *World Scientific News* 23 (2015) 1-12.

[5] Y. B. Fu, Z. Ahmed, A. Diederichsen. Towards a better monitoring of seed ageing under *ex situ* seed conservation. *Conserv. Physiol.* 3 (2015) 1-16

[6] FAO, Genebank Standards. 1994. Food and Agriculture Organization of the United Nations, Rome, International Plant Genetic Resources Institute, Rome, 1994, Available at ftp://ftp.fao.org/docrep/fao/meeting/015/aj680e.pdf.

[7] C. Walters. Understanding the mechanisms and kinetics of seed aging. *Seed Science Research* 8 (1998) 223-244.

[8] FAO, Genebank standards for plant genetic resources for food and agriculture, Rome, 2014, Available at http://www.fao.org/3/a-i3704e.pdf.

[9] V. R. Shelar. Strategies to Improve the Seed Quality and Storability of Soybean – A Review, Seed Technology Research Unit (NSP), Mathtme Phule Krighi Vidyprth, Rahuri 413-722, India, *Agricultural Review* 28 (3) (2007) 188-196.

[10] V. R. Shelar, R. S. Shaikh, A. S. Nikam. Soybean seed quality during Storage: A Review, *Agric. Rev.* 29 (2) (2008) 125-131.

[11] O. T. Isaac, E. A. Seweh, S. Apuri, B. K. Banful, S. Amoah. Effect of storage periods on seed quality characteristics of three soybean (*Glycine max* (L) Merrill) varieties. *International Journal of Scientific Research in Science, Engineering and Technology*, 2 (4) (2016a) 823-831.

[12] M. Kundu, J. Kachari. Desication sensitivity and recalcitrant behavior of seeds *Aquilariaagallocha* Roxb. *Seed Science Technology* 28 (2000) 755-760.

[13] C. Walters, L. M. Hill, L. J. Wheeler. Dying while dry: kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology*, 45 (2005) 751-758.

[14] A. A. Sajo, V. T. Tame. Effects of storage materials and environmental conditions on time of germination of soybean (*Glycine max* (L.) Merril) seed in Yola, Nigeria. *Acta Horticulture* 1012, 1 (2012) 71-76.

[15] S. Tang, D. M. Tekrony, D. B. Egli, P. L. Cornelius. An alternative model to predict corn seed deterioration during storage. *Crop Sci.* 40 (2000) 463-470.

[16] F. R. Hay, A. Mead, K. Manger, F. J. Wilson. One-step analysis of seed storage data and the longevity of Arabidopsis thaliana seeds. *Journal of Experimental Botany* 54 (2003) 993-1011.

[17] A. F., Mollah, M. M. Haque, S. M. M., Ali, A. T. M. M. Alam, A. B. Siddique, M. G. Mostofa. Quality evaluation of Jute seeds collected from different sources. *Journal of Biological Sciences* 2(7) (2002) 477-480.

[18] M. Sacande, J. Buitink, F. A. Hoekstra. A study of water relations in Neem (*Azadiractaindica*) seed that is characterized by complex storage behavior. *Journal of Experimental Botany* 51(344) (2000) 635-643.

[19] L. G. Heatherly, R. W. Elmore, Managing inputs for peak production. In: Soybeans: Improvement, Production and Uses, Eds- Boerma H.R., Specht, J.E. 3rd Edition, Agronomy N-16, ASA, CSSA, SSSA, Madison, Wisconsin, USA, (2004) 451-536.

[20] S. D. Stoyanova. Ex situ conservation in the Bulgarian genebank: I. Effect of storage. *PGR Newsletter* 128 (2001) 68-76.

[21] C. Walters, L. M. Wheeler, J. M. Grotenhuis. Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research* 15 (2005) 1-20.

[22] M. Nagel, H. Vogel, S. Landjeva, G. Buck-Sorlin, U. Lohwasser, U. Scholz, A. Börner. Seed conservation in ex situ genebanks – genetic studies on longevity in barley. *Euphytica* 170 (2009) 5-14.

[23] R. J. Probert, M. I. Daws, F. R. Hay. Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Annals of Botany* 104 (2009) 57-69.

[24] R. van Treuren, E. C. de Groot, J.L. van Hintum. Preservation of seed viability during 25 years of storage under standard genebank conditions. *Genetic Resources Crop Evaluation* 60 (2013) 1407-1421.

[25] G. Desheva. The longevity of crop seeds stored under long-term condition in the national gene bank of Bulgaria. *Agriculture (Poľnohospodárstvo)* 62(3) (2016) 90-100.

[26] J. M. M. Engels, L. Visser. A guide to effective management of germplasm collections. International Plant Genetic Resources Institute Handbook for Genebanks No 6, International Plant Genetic Resources Institute, Rome, Italy, (2003).

[27] ISTA, International rules for seed testing. Seed Science and Technology 21 (Supplement), (1993) 1-288.

[28] R. H. Ellis, T. D. Hong, E. H. Roberts, Handbooks for genebanks 2, Handbook of seed technology for genebanks Vol. I: Principles and methodology, Rome, Italy: International Boards for Plant Genetic Resources, (1985) pp 210.

[29] R. H. Ellis, T. D. Hong, E. H. Roberts, Handbooks for genebanks 3, Handbook of seed technology for genebanks, Vol. II: Compendium of specific germination information and test recommendations. Rome, Italy: International Boards for Plant Genetic Resources, (1985) pp 456.

[30] J. Hanson, Practical Manuals for Genebanks, IBPGR, Rome (1985), pp. 115.

[31] E. H. Roberts. Predicting the storage life of seeds. *Seed Science and Technology*, 1 (1973) 499-514.

[32] R. H. Ellis, E. H. Roberts. Improved equations for the prediction of seed longevity. *Annals of Botany* 45 (1980) 13-30.

[33] D. V. S. S. R Sastry, H. D. Upadhyaya, C. L. L. Gowda. Seed viability of active collections in ex-situ genebanks: an analysis of sorghum germplasm conserved at ICRISAT genebank. *Journal of SAT Agricultural Research* 6 (2008) 1-8.

[34] H. K. Parzies, W. Spoor, R. A. Ennos. Genetic diversity of barley landrace accessions (*Hordeum vulgare ssp. vulgare*) conserved for different lengths of time in ex situ gene banks. *Heredity* 84 (2000) 476-486.

[35] E. H. Roberts, R. H. Ellis. Physiological, ultra structural and metabolic aspects of seed viability, In A. A. Khan, (Ed) The Physiology and biochemistry of seed development dormancy and germination, Amsterdam, Elsevier Biomedical Press, (1982) 465-485.

[36] M. Benková, M. Žáková. Seeds germinability of selected species after five and ten years storage at different temperatures. *Agriculture (Poľnohospodárstvo)* 55 (2) (2009) 119-124.

[37] S. K. Agha, Z. H. Malik, M. Hatam, G. H. Jamro. Emergence of healthy seedlings of soybean as influenced by seed storage containers. *Pakistan Journal of Biological Sciences* 7 (1) (2004) 42-44.

[38] C. Reuzeau, G. Cavalie. Activities of free radical processing enzymes in dry sunfl ower seeds. *New Phytol.* 130 (1995) 59-66.

[39] S. E. Trawatha, D. M. TeKrony, D. F. Hildebrand. Relationship on soybean quality to fatty acid and C6-aldehyde levels during storage. *Crop Sci.* 35 (1995) 1415-1422.

[40] S. Balašević-Tubić, Đ. Malenčić, M. Tatić, J. Miladinović. Influence of aging process on biochemical changes in sunflower seed. *Helia* 28 (42) (2005) 107-114.

[41] M. B. Kurdikeri, M. N. Merwade. Channaveeraswamy, Maintenance of viability in different crop species under ambient storage. *Seed Res.* 28 (2000) 109-110.

[42] M. Ruiz, I. Martin, C. Cuadra. Cereal seed viability after 10 years of storage in active and base germplasm collections. *Field Crops Research*, 64 (1999) 229-236.

[43] L. O. Copeland, M. B. McDonald. Principles of seed science and technology, 4th. Massachusetts, USA: *Kluwer Academic Publishers*, (2001) 190-230.

[44] [S. Stoyanova. Conservation of peanut germplasm in the Bulgarian Genebank, *Arachis* genetic resources in Europe, *Ad hoc* meeting, 15-16 November 2002, Plovdiv, Bulgaria, International Plant Genetic Resources Institute, Rome, Italy (2004) 11-16.

[45] X. Chen, X. Lu, C. Cui, H. Chen, H. Wang. Monitoring of sorghum (*Sorghum vulgare*) seed viability after a decade of storage in the National Genebank of China. *PGR Newsletter* 136 (2003) 54-57.

[46] F. Pérez-García, C. Gómez-Campo, R. H. Ellis. Successful long-term ultra-dry storage of seed of 15 species of Brassicaceae in a genebank: variation in ability to germinate over 40 years and dormancy. *Seed Science and Technology* 37(3) (2009) 640-649.

[47] D. G. F Walsh, S. Waldren, J. R. Martin. Monitoring seed viability of fifteen species after storage in the Irish threatened plant genebank. *Biology and Environment: Proceedings of The Royal Irish Academy* 103B (2) (2003) 59-67.

[48] S. Stoyanova. Effect of seed drying on seed viability under long-term storage conditions in the Bulgarian National Genebank. *Agricultural Sciences* 2(3) (2010) 33-38.

[49] X. Lu, X. Chen, C. Cui. Germination ability of seeds of 23 crop plant species after a decade of storage in the National Gene Bank of China. *Plant Genet Resources Newsletter* 139 (2004) 42-46.

[50] K. G. Sai Babu, S. H. Husaiai, R. B. Muralimonan. Effect of moisture and container on the storability of paddy seed under ambient conditions of Hyderabad. *Seed Research* 11 (1) (1983) 71-73.

[51] V. R. Shelar, N. Shaikh. Soybean seed quality during storage. *Seed Technology research, Agricultural Review* 29 (2) (2002) 125-131.

[52] P. Pratt, P. Bolin, C. Godsey, (Eds.). Soybean Production Guide, Oklahoma Cooperative Extension Service, Division of Agricultural Sciences and Natural Resources, Oklahoma State University, (2009) 12-114.

[53] N. Khaliliaqdam, A. Soltani, N. Latifi, F. G. Far. Quantitative response of soybean seed under controlled conditions. *American –Eurasian J. Agric. and Environ. Sci*. 12(2) (2012) 224-230.

[54] H. Kandel, (Ed.). Soybean Production. North Dakota State University, Fargo, Norht Dakota (2010) 128-142.

[55] M. Tatic, S. Balesevic- Tubic, V. Dordevic, V. Miklic, M. Vujakovic, V. Dukic. Vigor of sunflower and soybean ageing seed. *Helia* 35(56) (2012) 119-126.

[56] Đ. Malenčić, M. Popović, J. Miladinović. Stress tolerance parameters in different genotypes of soybean. *Biology Plantarum* 46 (2003) 141-143.

[57] P. C. Gupta. Viability of stored soybeans seeds in India. *Seed Res.* 4 (1) (1976) 32-39.

[58] L. O. Copeland, B. M. McDonald. Seed viability testing. In principles of seed science and technology (3rdEd), Chapman and Hall, New York, (1995) 111-126.

[59] M. Nagell, M. A. R. Arif, M. Rosenhauer, A. Börner. Longevity of seeds – intraspecific differences in the Gatersleben genebank collections. *Tagung der Vereinigung der Pflanzenzüchter und Saatgutkauflfleute Österreichs* (2010) 179-182.

[60] S. Balešević-Tubić, M. Tatić, V. Đorđević, Z. Nikolić, V. Đukić. Seed viability of oil crops depending on storage conditions. *Helia* 33(52) (2010) 153-160.

[61] M. Kausar, T. Mahmood, S. M. A. Basra, M. Arshad. Invigoration of low vigor sunflower hybrids by seed priming. *Int. J. Agric. Biol.* 11 (2009) 521-528.

[62] R. K. Singh, R. K. Raipuria, V. S. Bhatia, A. Rani, Pushpendra, S. M. Husain, D. Chauhan, G. S. Chauhan, T. Mohopatra. SSR markers associated with seed longevity in soybean. *Seed Science Technology* 36 (2008) 162-167.

[63] R. K. Singh, R. K. Raipuria, V. S. Bhatia, A. Rani, Pushpendra, S. M. Husain, C. T. Satyavathi, G. S. Chauhan, T. Mohapatra. Identification of SSR markers associated with seed coat permeability and electrolyte leaching in soybean. *Physiology and Molecular Biology of Plants* 14 (2008b) 173-177.

[64] L. Rajjou, I. Debeaujon. Seed longevity: survival and maintenance of high germination ability of dry seeds. *Crop Research Biology* 331 (2008) 796-805.

[65] L. Ho-Sun, J. Young-Ah, L. Young-Yi, L. Sok-Young, K. Yeon-Gyu. Comparison of seed viability among 42 species stored in a genebank. *Korean Journal Crop Science* 58(4) (2013) 432-438.

[66] T. D. Hong, S. Linington, R. H. Ellis. Appendix 1. List of viability constants for use in the viability equation of Ellis and Roberts (1980a) and predicted seed storage longevity. In T. D. Hong, S. Linington, R. H. Ellis, (Eds) Compendium of information on seed storage behavior, vol. 2, I–Z, Kew : The Royal Gardens, Kew, (1998) 881-882.

[67] R. H. Ellis, T. D. Hong. Quantitative response of the longevity of seed of twelve crops to temperature and moisture in hermetic storage. *Seed Science and Technology* 35 (2007) 432-444.

(Received 22 February 2017; accepted 07 March 2017)