icrrd
ICRRD Journal
INTERNATIONAL CENTER FOR RESEARCH AND RESOURCES DEVELOPMENT
article
ISSN Number: 2773-5958, SSM Number: (1347468-T), doi.org/10.53272/icrrd, www.icrrd.com

Lifetime of Corn Seeds in Long-Term Storage in the National Genetic Bank of Albania

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Received: 20 March 2023
Revision: 29 April 2023
Accepted: 12 June 2023
Available Online: 15 June 2023
Published: 15 June 2023
Volume-4, Issue-2
Cite This: *ICRRD Journal,* 2023, 4(2), 137-148

ABSTRACT: Genetic banks manage seed collections for the conservation and utilization of plant genetic resources. AGB implements standards for genebank management recommending a temperature of -18 ± 3°C for long-term storage of the base collection and 5-10°C for medium-term storage of the active collection.The germination energy of the 12 maize accessions taken in the study has relatively low initial values. The values of germination energy, even after 20 years of seed storage, do not deviate much from the initial data (from 2000) and from those from 2010 (10 years of seed storage). The germination data for the three years of testing are somewhat small in the initial testing. The average capacity germination for all 12 accessions under study in the 2010 test is 4.2% lower than the initial test (year 2000), so there is a decrease in capacity germination. Whereas in the testing of 2020, i.e. after storing the seed for 20 years in the genetic bank, there was a decrease in capacity germination on average of 2.9%; that is, there was less decrease in capacity germination in the second 10 years of storage compared to the first 10 years. The reduction in germination is not to the same degree between accessions. Four pairs of correlations were found, three between energy germination indicators and one among capacity germination. The correlation coefficients are positive and related to the age of the seed.

Keywords: *accession, base collections, capacity germination, energy germination, long-term storage*

Introduction

Genetic banks manage seed collections for the conservation and utilization of plant genetic resources. To avoid frequent regeneration of accessions, which is laborious and costly and can cause loss of genetic integrity, gene banks aim to extend the life of the seed as much as possible (Engels and Visser 2003). Since most genetic banks were established in the 1960s/1970s and because seeds have a long shelf life under genetic bank storage conditions, it is still unknown how long seeds can be stored *ex situ* without significant loss of viability and what the optimal monitoring intervals might be (Walters,*et.al.* 2005, Hay,*et. al.*2013, VanTreuren, *et. al*. 2013, Ellis *et.al.*2017). Low seed moisture content and storage temperature are generally considered important criteria to maintain orthodox seed viability (Ellis and Roberts 1980a; 1980b), while anoxic (oxygen-free) conditions are also recommended to optimize seed longevity (Ellis and Hong 2007a;Groot *et.al.* 2015). Standards for genetic bank management recommend a temperature of -18 ± 3°C for long-term storage of the basic collection and 4-5°C for medium-term storage of the active collection (FAO, 2013).

The *exsitu* preservation of plant genetic resources in genetic seed banks plays an important role for food security for the future. Initial seed viability, seed moisture content and its interaction with relative air humidity and storage temperature have significant effects on seed longevity (Roberts, 1973). However, even in seeds stored under appropriate, optimal conditions for long-term storage, seed viability may decrease as a result of seed deterioration processes (Sastry,*et. al.* 2008). Seed viability initially declines slowly and then declines rapidly as seeds mature (Roberts and Ellis 1982). Therefore, it is important to know when this decline occurs so that accessions can regenerate in time to replace seeds with those that have high viability (Ho-Sun,*et. al.* 2013).

The Genetic Bank Standards for Plant Genetic Resources for Food and Agriculture (2014) recommended that the initial viability test be done as soon as possible, before the seeds are packaged and put into storage, and that subsequent tests be determined in certain intervals during storage. Viability monitoring test intervals may be set at one-third of the period expected for viability to decline to 85 percent of initial viability or lower, depending on species or specific accessions, but not more than 40 years. If this period of deterioration cannot be estimated, the interval is ten years for species expected to be long-lived and five years or less for species expected to be short-lived.

Seed longevity is defined as the viability of the seed after dry storage. During seed storage, the seeds deteriorate, lose germination energy and, therefore, become more sensitive to stresses during germination and, finally, die. The rate of this seed senescence depends on seed moisture content, storage temperature, and initial seed quality (Walters, 1998, Walters,*et. al.* 2005a). Seed longevity is a quantitative trait for which variation is present and occurs naturally among accessions (ThuPhuong, *et. al.*2012).

Therefore, the understanding of changes in seed longevity in different plant species is essential for the effective conservation management of seed collections, because it supports the determination of viability re-testing intervals, and therefore for regeneration or re-collection strategies (Probertet,*et.al.*2009; Groot,*et.al.*2015). All seed accessions are kept as basic collections under long-term storage conditions with low moisture content (5±2%) in hermetically sealed containers (in glass jars or in packages with three laminated aluminum foils) at -18°C.Genetic banks aim to create optimal seed storage conditions in order to delay seed aging as long as possible

Monitoring the viability of stored seed is extremely important (Murariu,1996) because, through monitoring, the loss of seed viability during long-term storage is detected, which helps to determine the regeneration period of the seed stored in the genetic bank. Since the viability of seeds stored in the genetic bank decreases slowly during storage, the decision for their necessary regeneration is based on the continuous monitoring of both the viability and theamountof seed in storage, in order to avoid the decrease of the amount of seed of each accession.

Seed viability is monitored by determining the capacity germination of accessions at predetermined time intervals. The interval between two viability checks depends on the expected seed life of the species (Hintum and Visser 2003).

Even under controlled storage conditions (i.e.low temperature and low seed moisture content), post-storage performance depends on the germinative energy status of the seed contingent (ISTA,2009). The use of hermetically sealed containers, desiccants and low temperatures improves storage as several physiological and biochemical processes and products are regulated during dry storage.

The relative effects of moisture content and storage temperature on seed longevity vary with species and with the structural and biochemical composition of seeds. A complete pattern of loss of viability can be understood based on seed moisture and storage temperature (Ellis,*et. al.*1982).

Genetic erosion of material held in genetic banks is considered an important problem at the international level (FAO, 1997). For this reason, it is highly recommended to monitor the main factors causing genetic erosion in *ex situ* collections to minimize the loss of genetic diversity. These factors include low quality of the original material, excessive drying of the seed before storage, increase in temperature or moisture content of the seed during storage, lack of regeneration, physiological changes in the seed during storage, and lack of detection of reduced germination caused by lack of viability monitoring (FAO,1997).

Seed quality is seed properties attributed to genetic quality, physical purity, seed health (disease status), viability, capacity germinating and moisture content (George,1999 Kerr, 2009 Bishaw, *et. al*. 2012). The effect of seed germination energy is very important for the useful value and seeds longevity (Sun,*et. al.* 2007;Milosevic,*et. al.* 2010).

Based on the above, we studied the impact of long-term corn seed storage on energy and capacity germination because corn is one of the main agricultural crops. After wheat, corn ranks second in the world in terms of cultivated area (Dunwell, J,M, 2014) in 2014, 183,319,737 ha of corn and 220,417,745 ha of wheat were planted in the world. A challenging problem is the long-term storage of corn seed in the genetic bank because, even in the best storage conditions, the viability, i.e. the capacity germination, decreases during the storage period.

The aim of this study was: to monitor the collections of corn seeds deposited in the National Genetic Bank of Albania, through the germination test and to know the viability of the seed, during its long-term storage, as well as to reduce the number of active seeds for each accession, except for the number of seeds taken for periodic testing.

MATERIAL AND METHODS

In the study, 12 maize accessions of local origin and accepted in the Genetic Bank in 2000 were taken, from 700 maize accessions stored in the base collection of the National Genetic Bank of Albania at -18°C, which account for 1.71 percent of corn accessions in storage, as follows:

1. AGB0713
2. AGB0715
3. AGB0717
4. AGB0718
5. AGB0720
6. AGB0722
7. AGB0724
8. AGB0725
9. AGB0728
10. AGB0730
11. AGB0734
12. AGB0739

Accessions were randomly selected for each seed storage period based on data files, registries and genetic bank databases with the aim of meeting the monitoring criteria. Samples of the selected accessions were first removed from the cold storage environments and the seed packets were placed briefly at room temperature with the aim of allowing the seeds to acclimate to the new environment and to avoid possible seed damage from sudden transit from storage temperature regime to room temperature environment.

The study was carried out in the laboratory of acceptance and processing of seed samples of the Albanian Genetic Bank, where the sample acceptance test was carried out under controlled laboratory conditions, temperature and light, as well as in the germinator, according to the standards defined by ISTA.

Analyzes performed and evaluation methods used in the study

In function of the purpose and objectives of the study, the evaluations were carried out in two periods of long-term storage of corn seed, in 2010, i.e. on 10-year-old seeds, and in 2020, which represents the 20-year age of the seed in storage.

Laboratory work was carried out for the following indicators:

1. Germinating energy testing
2. Capacity germination test
3. Determination of the moisture content in the seed.

The study is based on contemporary literature, methods and the work manual for genetic banks, as well as ISTA protocols and methods (ISTA, 2006) for seed germination tests. Using the germplasm monitoring standard, we performed germination energy and germination test of maize seed for 12 accessions. The first evaluation of these indicators was done in 2000, the year when corn samples were accepted in the Genetic Bank; the other two tests within the framework of monitoring were carried out in 2010 and in 2020, i.e.after 10 and 20 years of seed storage in the genetic bank.

The relevant protocols of the ISTA manual and the FAO/IPGRI genebank manual (FAO/IPGRI, 1994) were followed for seed germination testing. Since these accessions did not have significant amounts of seeds, the standard test for germination (4 replicates of 50 seeds) was used.

The protocols are presented as follows:

For corn, the method of placing the seeds on paper in the form of a roll was used. The paper was then labeled with the accession number and corresponding replicate; the test date of energy and capacity germinationin germinator.

Thenwas counting for each day and recording the germinated seeds, where for each repetition the number of germinated sprouts was taken into consideration, to then perform and interpret the results. The results obtained were calculated as a percentage of the total number of seeds for each test repetition. The percentage of germinated seeds for 4 days was evaluated for germinating energy and for 7 days for capacity germinating.

Then, the data obtained on energy and capacity germination were subjected to analysis of variance and correlation coefficients were calculated of discovering any dependence between the analyzed indicators. Correlative links were categorized according to these groups:

r = ± 0,3weak correlation,
r = ± 0,3to ± 0,5 middle correlative,
r = ± 0,5to ± 0,7 good correlation,
r = ± 0,7to ± 0,9 strong correlation

RESULTS AND DISCUSSION

The analysis of variance in the case of germination energy shows that the differences between the variants (accessions) are confirmed at the P≤0.01 level (table no. 1), while the differences between the repetitions (between the years of the study) are not confirmed.

According to the variance analysis data, of capacity germination, the differences between the accessions are confirmed, at the P≤0.05 levelbut, the differences between the years of the study (between repetitions) are also confirmed. Based on these statistical processing data, to interpret the data obtained from the energy and germination tests.

Table 1: Analysis of variance for genotypic differences in energy and germination test

Source of variance	(df)	ms (mean square)	
		Germination energy	Germination capacity
Variants (accessions)	11	576,4**	10,6*
Repetitions (years of study)	2	21,9	150,4**
Error	11	6,5	3,5
**P≤0.01; *P≤0.05			

From the data of the study on the germination energy of the 12 accessions of corn (Table 2) we notice that the initial values for this indicator (year 2000) are relatively low. Exclude accession no. 1 (AGB0713) which has the highest value (76.5%) and, separated from the other 11 accessions, the other accessions have low values which are included in the limits from 19.5% to accession no. 10 (AGB0730) in 28.5% to accession no. 3 (AGB0717).

The data after 10 years of seed storage in the genetic bank show that, again, accession AGB0713 had the highest value of germination energy (76.5%), which is at the same level as the initial value (year 2000), while the accessions of others had relatively low values, with minimum and maximum values of 23.0 and 30.5%, respectively. Even the data of 2020, i.e. after 20 years of seed storage, the germination energy values do not deviate much from the initial data (from 2000) and from those of 2010 (10 years of seed storage).This is based on the fact that differences between repetitions (between years of testing) are not confirmed (Table. 1)

Table 2: Germinating energy data for 2000, 2010 and 2020 test years

No.	**Accessions**	**Germination energy according to test years**			**Average and classification**
		2000	**2010**	**2020**	
1	AGB0713	76,5	76,5	68,0	**73,7a**
2	AGB0715	27,0	25,5	29,5	**27,3hk**
3	AGB0717	28,5	27,0	30,0	**28,5hk**
4	AGB0718	26,0	26,0	28,0	**26,7hk**
5	AGB0720	26,0	24,0	26,0	**25,3il**
6	AGB0722	24,0	25,0	25,0	**24,7il**
7	AGB0724	25,0	23,0	27,0	**25,0il**
8	AGB0725	28,0	30,5	30,5	**29,7hk**
9	AGB0728	22,5	24,0	28,5	**25,0il**
10	AGB0730	19,5	23,0	30,0	**24,2il**
11	AGB0734	22,5	24,0	29,0	**25,2il**
12	AGB0739	24,0	23,0	27,0	**24,7il**
Average		**29,1**	**29,3**	**31,5**	**29,8**
D_{01} = 5,9; D_{05} = 4,3;Cv = 45,5					

The germination data for the three years of testing give an interesting picture where the differences between the accessions, although proven at the P≤0.05 level, are somewhat small in the initial testing where the extreme data between them differ by 3.0%. In the test after 10 years of seed storage (year 2010), the differences between the minimum and maximum values are 8.5%, while those of the test after 20 years of seed storage (year 2020) differ by 11.0%.

Differences between test years stand out in the germination data. Thus, for example, the average capacity germination for all 12 accessions under study in the 2010 test is 4.2% lower than the initial test (year 2000), so there is a decrease in capacity germination. Whereas in the testing of 2020, i.e. after storing the seed for 20 years in the genetic bank, there was a decrease in capacity germination on average of 2.9%; that is, there was less decrease in capacity germination in the second decadeof storage compared to the first 10 years, but 7.1% compared to the initial values (of 2000).

Of interest is the fact that the decrease in capacity germination is not at the same level among the accessions (Table 3). Thus, for example, the capacity germination in 2010 decreased from 1.5% in accessions AGB0717 and AGB0728 to 10.0% in accession AGB0724 compared to the initial test (year 2000). In the second 10 years of seed storage (year 2020) the capacity germination has decreased from 0.5% in accession AGB0717 to 7.0 % in accessions AGB0717 and AGB0739 compared to the first 10 years of storage (year 2010). Even so, the exception was accession AGB0725, which has maintained the capacity germination of the 2010 test, that is, it has not shown a loss of capacity germination during the second 10 years of seed storage (Table 3).

Accession no. 7 “AGB0724” had the highest rate of loss of capacity germination after 10 years of storage at -18°C (10.0 %) but a low rate of loss of capacity germination after 20 years of storage (1.0 %). Accessions “AGB0722” and “AGB0739” had the highest rate of loss of capacity germination in the second 10 years of storage (year 2020) with 7.0% each, which had an average loss during the first 10 years of storage, respectively 4.5% and 3.5%. 4 accessions (AGB0720, AGB0722, AGB0728 and AGB0739) had the greatest loss of capacity germination during the second 10 years of storage, compared to the first 10 years of storage, 7 other accessions had the greatest loss of capacity germination during the first 10 years of storage compared to the second 10 years.

Table3: Germination data for 2000, 2010 dhe 2020 test years

No.	Accessions	Germination capacity according to test years					Average and classification
		2000	2010	2000-2010	2020	2010-2020 / 2000-2020	
1	AGB0713	90,0	86,0	4,0	84,0	2,0	**86,7a**
2	AGB0715	88,0	84,5	3,5	82,0	2,5	**84,4ab**
3	AGB0717	89,5	88,0	1,5	87,5	0,5	**88,3a**
4	AGB0718	89,5	83,5	6,0	81,0	2,5	**84,7ab**
5	AGB0720	90,0	88,0	2,0	82,5	5,5	**86,8a**
6	AGB0722	89,5	85,0	4,5	78,0	7,0	**84,2ab**
7	AGB0724	89,5	79,5	10,0	78,5	1,0	**82,5b**
8	AGB0725	87,0	80,5	6,5	80,5	0,0	**82,7b**
9	AGB0728	87,5	86,0	1,5	84,0	2,0	**85,8a**
10	AGB0730	87,5	84,5	3,0	83,5	1,0	**85,2ab**
11	AGB0734	90,0	86,5	4,0	82,5	3,5	**86,2a**

12	AGB0739	87,0	83,5	3,5	76,5	7,0	**82,3b**
Average		**88,8**	**84,6**	**4,2**	**81,7**	**2,9 / 7,1**	**85,0**
D_{01} = 4,3; D_{05} = 3,1; Cv = 4,4							

From the data of the correlation coefficients between the tested indicators, we note that a total of 4 pairs of correlations were found, three correlations between indicators of germination energy and one correlation between indicators of capacity germination (Table 4). All four correlation coefficients are positive and related to seed age; thus, for example, the correlation between 2010 and 2000 germination energy and the correlation between 2020 germination energy and 2010 germination energy have coefficient r_{11}=0.99** and r_{22}=0.99** which is estimated strong connection.

Similarly, the relationship between the germinating energy of 2020 and that of 2000 is a strong relationship, which is expressed by the coefficient r_{21}=0.98**. Even the correlation in the capacity germination, that between the capacity germination of 2020 and that of 2010, is a good correlation, which is expressed by the coefficient r_{55}=0.64*. Of interest would be the relationship between germination power and moisture content in the seed, but this relationship has not been proven.

Table 4: Correlation coefficients of indicators in the study of the impact of long-term storage on corn seed viability

	Germination energy in 2000 yr	Germination energy in 2010 yr	Germination energy in 2020 yr	Capacity germination in 2000 yr	Capacity germination in 2010 yr	Capacity germination in 2020 yr
Germination energy in 2010 yr	0,99**					
Germination energy in 2020 yr	0,98**	0,99**				
Capacity germination in 2000 yr	0,34	0,29	0,26			
Capacity germination in 2010 yr	0,16	0,15	0,16	0,38		
Capacity germination in 2020 yr	0,25	0,26	0,32	0,26	0,64*	
Seed moisture in 2000 yr	-0,15	-0,05	0,02	-0,22	-0,39	0,06

DISCUSSION

Maize seed samples kept in the genetic bank in their initial testing showed relatively low level of germination energy, except accession no. 1 “AGB0713”, which has the highest value of germination energy (76.5 %) and is clearly separated from other accessions. But, on the other hand, the germination energy values of all accessions in the study almost remain stable in their values even in the tests performed after 10 years and after 20 years of storage at -18°C. These observed results cannot be due to the influence of the seed storage environment (table no. 2).

We think that the conditions of seed production for each accession, post-harvest procedures and the degree to which the seeds are exposed to oxygen are important factors that are embedded in the seed and affect their formatting which is expressed as genetic factors, so their behavior is and becomes a genotypic characteristic. This because the accessions taken in the study have experienced different management procedures before they entered in the collection which is supported by the fact that the maize accessions in the study, although they are of country origin, come from different local origins of their cultivation.

The fact that the accessions in the study more or less preserved their initial values even after 10 and 20 years stored in the genetic bank is argument that the behavior of the seeds of these accessions is characteristic of their genotype, therefore they preserve the characteristic features of each genotype.

As for the capacity germination, the data of the study given in tab.3, show that the differences between the accessions are somewhat small in the initial test where the extreme data differ by 3.0%, but also the capacity germination values are relatively low, reaching up to 87%, which may be due to the conditions of cultivation and the procedures followed after harvesting or the duration of exposure of the seed to oxygen (the duration of stay in open environment conditions) because, as far as is known, atmospheric oxygen reduces the life of the seed.

Despite this, the deterioration of the seed against the storage conditions is of interest, i.e. the loss of the seed's viability, which is assessed through the capacity germination as a result of the duration of the seed's storage in the genetic bag (storage at -18°C), which also represents the degree of aging of the seed, but that is the aim of the study. In testing after 10 years of seed storage (year 2010), capacity germination has decreased by an average of 4.2%., whereas in the test after 20 years of seed storage (year 2020), the capacity germination decreased by 2.9% compared to the 2010 test, or a 7.1% decrease in the germination power compared to the initial test (year 2000).

Based on the data of the study, there was less decrease in capacity germination in the second 10 years of storage compared to the first 10 years. Based on these data, we judge that the corn seeds did not suffer from storage at -18°C.

In addition to the influence of the duration of seed storage in general, which is characteristic of the species, our data show differences in the values of germination also between cultivars (among accessions). Of interest is the fact that the decrease in capacity germination is not at the same level among the accessions (table no. 3). Thus, for example, the capacity germination in 2010 decreased from 1.5% in accessions AGB0717 and AGB0728 to 10.0% in accession AGB0724 compared to the initial test (year 2000). In the second 10 years of seed storage (year 2020) the capacity germination has decreased from 0.5% in accession AGB0717to 7.0% in accessions AGB0717 and AGB0739 compared to the first 10 years of storage (year 2010). Even so, the exception was accession AGB0725, which has maintained the capacity germination of the 2010 test, that is, it has not shown a loss of capacity germination during the second 10 years of seed storage (table no. 3).

Accession no. 7 "AGB0724" had the highest rate of loss of capacity germination after 10 years of storage at -18°C (10.0 %) but a low rate of loss of capacity germination after 20 years of storage (1.0 %). Accessions "AGB0722" and "AGB0739" had the highest rate of loss of capacity germination in the second 10 years of storage (year 2020) with 7.0% each, which had an average loss during the first 10 years of storage, respectively 4.5% and 3.5 %.4 accessions (AGB0720, AGB0722, AGB0728 and AGB0739) had the greatest loss of capacity germination during the second 10 years of storage, compared to the first 10 years of storage, 7 other accessions had the greatest loss of capacity germination during the first 10 years of storage compared to the second 10 years.

These different values show the different degree of seed aging of different accessions. All these data may have been due to the unintentional change of the characteristics of the material produced in different years but also in different environmental conditions in which they were grown, conditions which are fixed in the genotype of the cultivar (accession) accepted and stored in the genetic bank. But the possible causes of variation in seed senescence between years of regeneration could not be proven from the accession data in this study.

From the data of the correlation coefficients between the tested indicators (table no. 4) we judge that they are also related to the age of the seed; thus, for example, the correlation between 2010 yrand 2000yr seed energy and the correlation between 2020yr seed energy and 2010 yrseed energy have coefficient r_{11}=0.99** and r_{22}=0.99** which is estimated strong connection. Similarly, the relationship between the germinating energy of 2020yr and that of 2000yr is a strong relationship, which is expressed by the coefficient r_{21}=0.98**. Even the correlation in the capacity germination, that between the capacity germination of 2020yr and that of 2010yr, is a good correlation, which is expressed by the coefficient r_{55}=0.64*. All this shows that the age of the seed is not characteristic of the species but of the genotype (characteristic of the cultivar expressed through the accession in the study). But, in the conditions of our study, we do not know the conditions of seed production for each accession, that is, we do not know the degree of exposure of the seed of each accession to oxygen, which affects the life of the seed.

This argument is also supported by other studies (Ellis and Hong 2007a Groot et al, 2015). The initial moisture content of the seed has no proven contribution to the life of the seed because the limits of its content were not sensitive, they were in the range of 5.8 to 7.0%, that is, with a difference of 1.2%, which that cannot be expressed in the form of influence on the characteristics of the seed according to different genotypes (accessions). However, we do not think that the moisture content of the seed does not affect the longevity of the seed but, from the data of our study, this is not proven because the percentages of moisture in the seed are not sufficiently differentiated between the accessions in the study.

CONCLUSIONS

Based on the results obtained from the monitoring tests of the capacity germination of corn seeds stored in the conditions of the National Genetic Bank of Albania, we draw the following conclusions.

- The capacity germination of corn seeds stored under the conditions of the Genetic Bank is lost more in the first 10 years of storage, compared to the second 10 years;
- Loss of maize seed germination also varies between genotypes (among accessions)
- The capacity germination of corn accessions does not maintain the same rate in different periods of long-term storage;
- Maize seed can keep the capacity germination unchanged in different periods of long-term storage, depending on the genotype;
- The results obtained from the monitoring tests show that the storage conditions of corn seeds in the Genetic Bank at -18° are suitable for long-term storage of corn seeds;

- The results presented by this study are a useful for monitoring the conservation of maize seeds in the genetic bank and help to avoid the frequent regeneration of accessions, which is laborious and costly and can cause loss of genetic integrity.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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