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Assessment of seed viability of *Oryza sativa* L. accessions in long-term storage after more than a decade

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Abstract

The purpose of this research was to evaluate the seed viability of 16 *Oryza sativa* L. accessions including valuable endangered landraces, a breeder's line, and an advanced/improved cultivar after 13-14 years of long-term storage. Preservation of seed samples has been conducted *ex-situ* at low temperatures (-18°C). The germination test (germination energy and total germination) was carried out according to ISTA methods and Kameswara (2006). Furthermore, the following variables were examined: the germination index, root length, shoot length, root-to-shoot length ratio, total seedling length, and seed vigour index. The Pearson correlation coefficient was computed to assess the linear relationship between the vigour index and seedling length. All the sixteen accessions have had a high total germination rate, in the range of 89-100%, hence there is no need for their regeneration. The germination energy and total germination showed statistically significant differences for two rice accessions at P-0.05 and P-0.01. The seed showing a high vigour index produced seedlings with higher growth rates. The Pearson correlation of the vigour index and seedling length was found to be very highly positive and statistically significant ($r=0.99$, $p<0.001$). Based on the results obtained, it can be concluded that rice as a botanical species retains high seed viability during the long-term conservation, although there are individual differences between the accessions which are due to the genetic constitution of each genotype.

Key words: gene bank, *ex situ* storage, rice genotypes, vigour index, seedling growth.

Introduction

Rice is a principal food of nearly half of the world's population and amounts to more than 90% of crops grown in the developing countries, where food supply is an acute problem. A great deal of success has been achieved over the past 35 years. Rice production has more than doubled, from 257 million t to 596 million t in 1999. This phenomenal increase can be attributed to the large-scale adoption of improved rice varieties and technology developed at IRRI and by national rice improvement programmes. These gains have been made using science based on the Mendelian genetics and conventional breeding techniques (Padolina, 2001).

In the 20th century, scientists considered genetic resources (*ex-situ* collections) to be an insurance fund, and a variety of forms for the future needs of agricultural production (Patra et al., 2016; Peres, 2016). *Ex-situ* seed storage is important for preserving crop genetic resources. In addition, *Ex-situ* conservation is a safe and efficient way to conserve rice genetic resources and to make the germplasm readily available to breeders and other researchers. The storage quality of rice is usually affected by temperature, humidity, atmosphere, and storage time, so its physical, chemical, and physiological characteristics will undergo change during long-term storage (Park et al., 2012). Seeds can remain viable for extended periods of time if kept under conditions of low temperature and moisture content (Lu et al., 2009).

Gene bank management standards recommend a temperature between -18°C and ± 3°C for long-term storage of the base collection, and 5-10°C for medium-term storage of the active collection. Maintaining seed viability is a critical gene bank function. Viability monitoring test intervals should be set at one-third of the time predicted for viability to fall to 85 percent of initial viability or lower depending on the species or specific accessions. The viability threshold for regeneration or other management decisions such as recollection should be 85 percent or lower depending on the species (FAO, 2014). Regarding rice genetic resources, the critical level for regeneration is reached when the germination percentage becomes lower than 80% (Rabara et al., 2011).

Seed vigour is a key factor that determines seed quality. High-vigour seeds can ensure uniform emergence, robust crop, and good yields (Marcos, 2015; Zhang et al., 2018). Seedling viability is a complex agronomic trait with several indicators, such as germination energy, total germination percentage, and the germination index during a seed germination stage (Wang et al., 2010). High seed vigour, including rapid, uniform germination and vigorous seedling growth, is essential for the direct seeding of rice (Mahender et al., 2015). Seeds with rapid and uniform germination may significantly improve field emergence, lead to better suppression of weed growth, and produce high yields under various conditions (Foolad et al., 2007; Wang et al., 2010). High germination percentage and rapid germination are important to ensure good crop establishment in the field of direct-seeded rice (Pandey & Velasco, 2005). Varieties with rapid germination are required for early vigour, particularly when early season drought may affect grain yield and also in weedy fields (Kumar et al., 2009; Yaman et al., 2018).

Bláha (2019) reports that the seedling growth rates of roots and shoots during the vegetation period are continually adjusting to environmental conditions and the "genetic programme" of plant growth and development. For example, fertilization and irrigation can make important changes. In case of high values of this ratio, it is possible that more nutrients will be absorbed from the soil and this will help plants to increase the above-ground biomass, and probably also to increase plant's resistance to stress (drought conditions, low level of nutrients in the soil). A shoot-to-root ratio is genetically fixed, but can be modified by external conditions. The importance of the root-to-shoot ratio during vegetation is indisputable. This ratio is strongly associated with plant integrity during the growth and development of all vegetation periods (Bláha, 2019).

In this research, the attention is focused on rice genetic resources in the Republic of North Macedonia, part of the seed collection of the Gene Bank hosted in the Institute of Agriculture in Skopje. The national rice collection originates from the breeding collection of the former Rice Institute in the city of Kochani (nowadays part of the Institute of Agriculture in Skopje), established in the early seventies (Atanasova et al., 2015). The lack of capacities for mid-term and long-term conservation during the first decades caused high loss risks for certain materials as well as high costs necessary for frequent regeneration of the rice germplasm. According to Andov et al. (2012), rice seeds are not suitable for short-term conservation. Stored in paper bags at room temperature, they can maintain their viability (germination energy and total germination over 80%) within two years at the most. Later on, germination drops depending on the genotype and growing conditions of seed production. Therefore, capacities for long-term conservation at -18°C, built in the Institute during 2004 and 2005, have provided secure preservation of plant genetic resources including the rice collection. Using 183 rice accessions stored in the active (2-4°C) and base (-10°C until 1993, then -20°C) collections of the T. T. Chang Genetic Resources Centre, Hay et al. (2012) reported the germination ability of "control" seed samples which was determined after they had been stored for 20.5-30.5 years. The germination of seeds stored in the base collection was generally high (> 70%), whereas it was more variable for seeds stored in the active collection. The samples with lower viability after storage in the active collection were likely to have lower viability after storage in the base collection.

The aim of this study was to estimate the viability and seedling properties of several rice seed accessions after 13-14 years of long-term *ex-situ* conservation.

Material and Methods

In this study, sixteen rice accessions from the base collection were used as a material for examination. They belong to the national rice collection within the Gene Bank at the Institute of Agriculture in Skopje, derived from the breeding collection of the former Rice Institute in Kochani (nowadays part of the Institute of Agriculture in Skopje, Republic of North Macedonia).

Tab. 1 Basic rice accessions data examined in the study

Accession №	Accession name	Acquisition date	Country of origin	Biological status
MKD00086	Biser – 2	2006----	MKD	breeder's line
MKD00129	Kochanski	2006----	MKD	advanced/improved cultivar
MKD00140	Makedonija	2006----	MKD	breeder's line
MKD00143	Mesen Blatec	2006----	MKD	traditional cultivar/landrace
MKD00146	Montesa	2006----	MKD	advanced/improved cultivar
MKD00147	Monticelli	2006----	ITA	advanced/improved cultivar
MKD00148	Monticelli x M-101	2006----	MKD	breeder's line
MKD00149	M-101	2006----	USA	unknown
MKD00150	M-101 x Monticelli	2006----	MKD	breeder's line
MKD00152	Nada 115	2006----	MKD	advanced/improved cultivar
MKD00159	N-51	2006----	MKD	traditional cultivar/landrace
MKD00161	N-69	2006----	MKD	traditional cultivar/landrace
MKD00167	Osogovka	2007----	MKD	advanced/improved cultivar
MKD00176	Prima Riska	2007----	MKD	advanced/improved cultivar
MKD00202	R-76/6	2007----	ITA	unknown
MKD00207	San Andrea	2007----	ITA	advanced/improved cultivar

Table 1 presents some essential data about this material. Most of the related accessions (12) are of Macedonian origin, while three are Italian and one is from the United States. Diverse material was used regarding the biological status of the accessions - seven of them are advanced cultivars, four breeder's lines, and three landraces; the biological status for two accessions is unknown. The applied method of conservation was *ex-situ* - long-term. The conservation procedures were carried out during 2006 and 2007. Paddy rice seeds were packed in double-layered Al-bags, sealed, and maintained in vertical freezers at -18℃. This testing is actually the first monitoring of their viability after around 13-14 years of conservation.

The analyses were carried out in the accredited Laboratory for Testing of Agricultural Plant Seeds at the Institute of Agriculture - Ss. Cyril and Methodius University in Skopje. The germination test (germination energy and total germination) was carried out according to ISTA methods and Kameswara (2006). In addition, the following parameters were evaluated: the germination index, root length, shoot length, root-to-shoot length ratio, total seedling (root + shoot) length, and seed vigour index.

There were four repetitions of 50 seeds for each of the 16 rice accessions, which were placed in Petri dishes using paper filter towels wetted with distilled water. The Petri dishes with seed samples were incubated in a germination cabinet at 25oC for 5 days to examine the germination energy and for 14 days to examine the total germination. The seeds were considered to have germinated when the sprout length reached half of the seed length or above. The average values for germination energy and total germination of all 4 repetitions were calculated and are shown in 100%. Just before the germination process, the seeds were disinfected with a 1% solution of sodium hypochlorite.

Germination energy is the ratio between the number of germinated rice seeds on the fifth day and the number of seeds on the tray, multiplied by 100. Total germination is calculated as the ratio between the number of germinated rice seeds on the fourteenth day and the number of seeds on the tray, multiplied by 100. The root length and shoot length of 40 randomly selected seedlings in each of the variants developed in dark conditions were measured using a ruler at the end of the experiment (day 14). After determining the root length and shoot length, the seed vigour index was calculated by using the method suggested by Abdul-Baki and Anderson (1973): Seed vigour index = Germination (%) × Seedling total length (mm). The data obtained from the examined variables were statistically analyzed. They were tabulated in Microsoft Excel using One-way analysis of variance (ANOVA) and separation of means was performed using the LSD test at 0.05 and 0.01 significance level. To determine the intensity of the linear relationship between the vigour index and seedling length, the Pearson coefficient was used and calculated according to the formula (Mead et al., 1993):

$$r = \frac{n\Sigma xy - \Sigma x \Sigma y}{\sqrt{n\Sigma x^2 - (\Sigma x)^2} * \sqrt{n\Sigma y^2 - (\Sigma y)^2}}$$

r - Pearson coefficient
x - values of the x-variable
y - values of the y-variable
n - number of observations

Values of *r* range from -1 for a perfectly inverse, or negative, relationship to 1 for a perfectly positive correlation. Values at, or close to, zero indicate no linear relationship or a very weak correlation.

Results and Discussion

Germination energy and total germination

Most of the rice accessions maintained their high germination energy during the studied period (Table 2). Eleven out of sixteen accessions showed values above or around 95%, six accessions in the range of 97-99.5%, and three in the range of 98-99.5% for germination energy. In Montesa and Monticheli x M-101, the germination energy rate significantly dropped to lower values of 79.5-87.5%. The interval of variation (Xmin - Xmax) of the germination energy in the genotypes of the middle early-ripening group (Kochanski, Nada-115, Osogovka, Biser-2) was 2-10, while in the middle late-ripening group (Montesa, N-51, N-69, Prima Riska) it amounted to 2-18. The maximum total germination percentage (100%) was observed for Mesen Blatec followed by San Andrea (99.5%) and N-69 (99.5%), while the least germination percentage (89%) was found for Monticelli x M-101. In thirteen out of sixteen genotypes, total germination was 97-100%. In the other accessions it was in the range of 89-96.5%. Korotenko et al. reported similar results for germination (97-100%) in rice genotypes stored low temperatures after 10 years. The values for germination energy and total germination for Montesa and Monticelli x M-101 were found to be statistically significant ($P>0.05$ and $P>0.01$) in comparison to all other accessions. Rice seeds retain high viability during long-term storage, but there are individual differences between the accessions which are due to their genetic constitution.

Tab. 2 Germination energy and total germination percentages of 16 *Oryza sativa* L. accessions

Accession	Years of storage	Germination energy				Total germination			
		Mean (%)	Min	max	IV	Mean (%)	Min	max	IV
Kochanski	14	94.00	92	96	4	97.00	96	98	2
Makedonija	14	97.50	96	98	2	98.50	98	100	2
Mesen Blatec	14	99.50	98	100	2	100.00	100	100	0
Montesa**	14	79.50	70	88	18	92.50	86	96	10
Monticelli	14	95.00	92	98	6	97.00	96	98	2
Monticheli x M-101**	14	87.50	86	88	2	89.00	88	90	2
M-101	14	95.50	92	98	6	97.50	96	98	2
M-101 x Monticelli	14	96.50	94	100	6	98.00	96	100	4
Nada-115	14	97.50	96	100	4	99.00	98	100	2
N-51	14	97.00	94	100	6	98.50	98	100	2
N-69	14	99.00	98	100	2	99.50	98	100	2
Osogovka	13	98.50	98	100	2	99.00	98	100	2
Prima Riska	13	96.50	94	100	6	96.50	94	100	6
Biser-2	14	96.00	90	100	10	97.50	96	100	4
R-76/6	13	93.00	88	96	8	97.00	94	98	4
San Andrea	13	94.50	88	98	10	99.50	98	100	2
$LSD_{0.05}$		4.10*				2.42*			
$LSD_{0.01}$		5.47**				3.23**			

Note: min - Minimum; max - Maximum; IV - Interval of variation.

Seedling growth

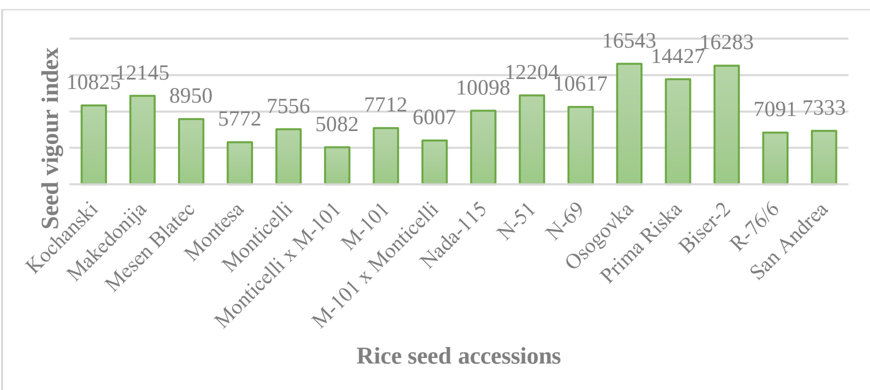
The results for seedling growth are shown in Table 4. The maximum root length (9.06 cm) was found for Biser-2 as well as for Osogovka (8.58 cm). The highest coefficient of variation was recorded for the root length of Monticelli, Monticelli x M-101, and Kochanski. The longest shoot was measured for Osogovka (8.13 cm) followed by Biser-2 (7.64 cm), and Mesen Blatec (7.57 cm). Saha et al. (2017) presented similar results for 21-day-old seedlings of two rice varieties, IR64 and Nayanmani, so the control root length was around 9.57-10.8 cm and 6.95-7.17 cm for the shoot length. The shortest root length (1.38 cm) was found for Mesen Blatec followed by Montesa (1.48 cm). The breeder's line Monticelli x M-101 revealed minimum values for root length (1.81 cm) and also minimum values for shoot length (3.90 cm). There were differences in the root-to-shoot ratio between the varieties, so the maximum was in the range of 1.06-1.19 for Osogovka and Biser-2, while in Mesen Blatec and Montesa, the root-to-shoot ratio apparently dropped to lower values of 0.18-0.31. Fifteen out of sixteen accessions showed high variability (>20%) for root length, while significant variability for the shoot length was found in Prima Riska (27.05%) and Montesa (24.91%). The shoot length variability for all of the other accessions was moderately expressed (10.91-17.80%).

Tab. 3 Root length (cm), shoot length (cm), and root-to-shoot length ratio of 16 *Oryza sativa* L. accessions

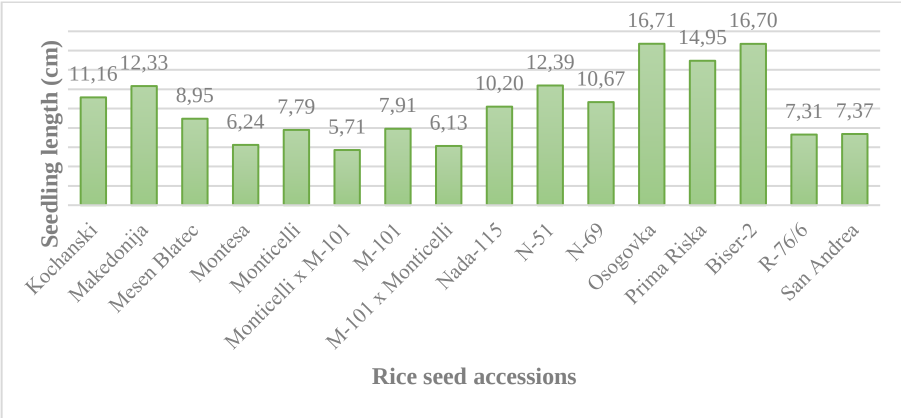
Accession	Root length			Shoot length			Shoot to root ratio
	Mean (cm) ± SE	CV (%)	SD	Mean (cm) ± SE	CV (%)	SD	
Kochanski	5.25±0.28	33.38	1.75	5.91±0.16	17.05	1.01	0.89
Makedonija	6.13±0.25	25.42	1.56	6.2±0.15	14.79	0.92	0.99
Mesen Blatec	1.38±0.06	25.00	0.35	7.57±0.22	17.80	1.35	0.18
Montesa	1.48±0.06	25.16	0.37	4.76±0.19	24.91	1.19	0.31
Monticelli	2.69±0.15	33.99	0.91	5.10±0.14	17.29	0.88	0.53
Monticelli x M-101	1.81±0.10	33.45	0.61	3.9±0.09	14.50	0.56	0.46
M-101	3.19±0.10	19.69	0.63	4.72±0.10	13.85	0.65	0.68
M-101 x Monticelli	1.66±0.06	21.99	0.37	4.47±0.09	12.94	0.58	0.37
Nada-115	4.77±0.17	22.30	1.06	5.43±0.09	10.91	0.59	0.88
N-51	6.38±0.31	30.60	1.95	6.01±0.15	15.15	0.91	1.06
N-69	5.36±0.30	34.39	1.84	5.31±0.15	17.61	0.94	1.01
Osogovka	8.58±0.32	23.57	2.02	8.13±0.19	14.58	1.19	1.06
Prima Riska	7.84±0.30	23.58	1.85	7.11±0.31	27.05	1.92	1.10
Biser-2	9.06±0.31	21.11	1.91	7.64±0.18	15.03	1.15	1.19
R-76/6	2.46±0.11	26.78	0.66	4.85±0.11	13.90	0.67	0.51
San Andrea	2.67±0.10	22.43	0.60	4.70±0.12	16.59	0.78	0.57

Note: CV (%) - Coefficient of variation (%); SE - Standard error; SD - Standard deviation.

The seedling growth rate is the result of seed vigour. High seed vigour ensures good quality seed and higher productivity. Early seedling growth parameters indicate seed vigour in rice (Barik et al., 2022). The increased vigour index of rice seeds observed with the increasing length of seedlings has been presented in Graphs 1 and 2.



Graph 1 The seed vigour index of 16 *Oryza sativa* L. accessions



Graph 2 Seedling length (cm) of 16 *Oryza sativa* L. accessions

The Pearson correlation of vigour index and seedling length (Table 4) were found to be very highly positive and statistically significant ($r=0.99$, $p<0.001$). This shows that an increase in the vigour index can lead to a higher seedling length.

Tab. 4 The Pearson correlation between the vigour index and seedling length

Correlations		Seedling length
Vigour index	Pearson Correlation	,999**
	Sig. (2-tailed)	,000
	N	16
** Correlation is significant at the 0.01 level (2-tailed).		

Conclusion

After having been stored for 13-14 years at -18°C, all the analyzed accessions have maintained high seed viability, with total germination of over 89%, which means that the long-term method is suitable for the conservation of rice genetic resources. At this moment, there is no need for regeneration. The seedlings with higher growth rates have originated from the accessions with the high vigour index Although rice as a botanical species retains high viability for a long time, there are individual differences between accessions which are due to the genetic constitution of each accession.

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Процјена одрживости сјемена у дуготрајно ускладиштеним узорцима *Oryza sativa* L. након више од деценије

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Сажетак

Сврха овог истраживања је била да се процијени одрживост сјемена 16 узорака Oryza sativa L. укључујући вриједне угрожене сорте, оплемењивачку линију и напредну/побољшану сорту након 13-14 година дуготрајног складиштења. Чување узорака сјемена је вршено ex-situ на ниским температурама (-18°C). Испитивање клијавости (енергија клијања и укупна клијавост) спроведено је према ISTA методама и Kameswara (2006). Поред тога, испитиване су сљедеће варијабле: индекс клијања, дужина коријена, дужина изданка, однос дужине коријена и изданка, укупна дужина клијања и индекс бујности сјемена. Пирсонов коефицијент корелације је израчунат да би се процијенио линеарни однос између индекса виталности и дужине садница. Свих шеснаест узорака имају високу укупну клијавост, у распону од 89-100%, тако да нема потребе за њиховом регенерацијом. Енергија клијања и укупна клијавост су показале статистички значајне разлике за две врсте пиринча, за P-0,05 и P-0,01. Сјеме које је показало висок индекс виталности, дало је саднице са већим стопама раста. Пирсонова корелација индекса виталности и дужине семена је веома позитивна и статистички значајна ($r=0,99$, $p<0,001$). Из добијених резултата може се закључити да пиринач као ботаничка врста задржава високу виталност сјемена током дуготрајног чувања, али постоје индивидуалне разлике међу узорцима које су посљедица генетске конституције сваког генотипа.

Кључне ријечи: генбанка, ex situ складиштење, сорте пиринча, вигор индекс, раст садница.

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