Subspecific Features in Maintaining the Viability of Rice Seeds and the Productivity of Their Progeny after Long-Term Low-Temperature Storage of *Oryza Sativa* L Germplasm

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Abstract. To save genetic diversity of rice (*Oryza sativa* L.), accumulated in the collections of genebanks and scientific institutions, various methods and modes of seed storage are used. However, in the process of long-term low-temperature storage, there is a loss of seed viability among rice samples. With the aim of a differentiated approach to the choice of conditions, methods and terms of preservation of the gene pool of “Federal Scientific Rice Centre” (Krasnodar), the viability of seeds after operational and long-term storage was studied in 1252 samples of *indica* and *japonica* subspecies of 37 varieties. The influence of the temperature regime of seed storage for 3, 5, 10 and 35 years on the viability of the intraspecific diversity of rice is shown. In uncontrolled conditions for three years the seeds lost their germination by varieties by 20-68%, and at a temperature of +4.5° C by 5-13%. After 25 years of storage, the seeds of long-grain samples of the *indica* subspecies, as well as red-grain and awned forms, had the greatest viability. A clear relationship between grain size and its durability has not been identified. After 10 years of low-temperature storage, rice seeds give full-fledged progeny and retain plant productivity at the level of freshly harvested seeds. From the gene pool of the world rice collection, varieties with greater biological longevity of seeds were isolated.

1. Introduction

Rice production in the world is determined by the needs of the world's population, due to the fact that this crop is the staple food in many countries [1]. In total, almost 784 million tons of rice were produced in the world in 2019, and the leaders among the producing countries are China, India, Indonesia, Bangladesh and Vietnam. Scientists predict that by 2035 an additional 116 million tons of rice will be required to ensure food security due to the growing population of the planet. The priorities for the development of world rice cultivation include the improvement of varieties and technologies for their production. To avoid the negative consequences of climate change affecting the productivity of grain crops, it is necessary to involve genetic diversity resistant to external stressors in the breeding process [2]. In the 20th century, genetic resources (*ex-situ* collections) are considered by scientists as an insurance fund and a variety of forms for the future needs of agricultural production [3, 4]. Throughout the world in the 1960s – 70s, seed banks began to be organized and a "strategy for the conservation of plant biodiversity" appeared in the form of seeds under different regimes and times,
depending on the type of crop [3, 5]. Medium-term storage of seeds for several decades at a temperature of 5-10 °C and long-term low-temperature (-18 ± 3 °C) storage for many decades in genebanks are recognized by the international community as an effective way to extend the longevity of seeds and preserve genetic diversity. [6, 7]. The largest state-owned International Rice Collection Gene Bank is located at the International Rice Research Institute (IRRI, Philippines), which conserves more than 130 thousand samples of cultivated rice species and its wild relatives. IRRI scientists work to ensure long-term conservation of rice biodiversity as part of a global strategy for the conservation of genetic resources http://www.fao.org/plant-treaty/en/ [8].

It is known that during storage there is a decrease in seed germination due to biochemical aging processes [9], monitoring of their viability is important for safe preservation. The longevity of seeds, the effect of temperature and humidity on the germination of seeds of various crops were studied in details in 1970-1990 [5, 10]. Interest in this problem in the scientific community is not decreasing at the present time. This is evidenced by the bibliometric analysis of literature on the topic under consideration, carried out by the staff of the State Public Library for Science and Technology (Novosibirsk, Russia), where the abstract analytical database Scopus was used as a source of scientific publications, containing more than 200 thousand materials on genetics and molecular biology, biochemistry, as well as agricultural sciences. The selection of the array of publications was carried out by title, abstract, keywords: (TITLE-ABS-KEY (storage OR vitality) AND TITLE-ABS-KEY (rice OR oriza OR sativa) AND TITLE-ABS-KEY (conditions), as of 10.06.2020 The Scopus database included 1541 publications. The first article in the Scopus database in this area was published by Japanese researchers in 1948 and presented the results of studies of rice storage depending on thermal conditions and humidity. (Kondo, M., Y. Kasahara, and S. Akita. Storage Examinations on Rice under the Condition of High Temperature and Moisture // Japanese Journal of Crop Science, vol. 17, no. 1, 1948, pp. 25-27. SCOPUS, www.scopus.com, doi:10.1626/jcs.17.25.)

Over the past 25 years (Figure 1), the growth in the publication activity of researchers shows a steady interest in our topic. In terms of regional distribution, it can be stated that the largest number of authors of publications are affiliated in the United States (18.4 %), China (15.5 %), Japan (11.8 %) and India (10.5 %): the works of the authors of these countries occupy more than 56.8% of the total volume of documentary and informational flow of the studied scientific area. The growing interest in this topic is associated with the fact that the loss of viability of seeds of various plant species and the "genetic integrity" of samples even under optimal storage conditions [11, 12] in the world's genebanks poses a significant threat to food security [13]. It was noted that seeds have species [14,15] and genotypic differences in life expectancy [16], and their viability depends on the climatic conditions of growth and the timing of harvesting [17]. For long-term storage of seeds, the main parameters are temperature, humidity and the type of packaging material [6, 7, 18-21]. At the same time, scientists investigate not only changes in the germinating ability, but the preservation of the taste of rice at different temperatures and shelf life [20, 22-24]. To overcome dormancy of seeds of different species and improve their properties, Indian scientists from the "Indian Institute of Food Processing Technology" considered new technological methods (high pressure, ultrasound, ozone treatment, ultraviolet light, magnetic field, oxidizing water and activated plasma) [25]. Scientists have found that seeds and plants of the same species react differently to stresses from low-temperature storage, which can cause changes in architectonics and inhibit plant development, photosynthesis and energy metabolism [9, 16, 24].
When organizing long-term storage of seeds of the world's rice resources, it is important to ensure not only regeneration and germination in the field, but also the formation of a plant with the expected characteristics. Unfortunately, there is not enough available information in the literature on aspects of the varietal qualities of the progeny of plants (reproducers) of *ex-situ* collections grown from the seeds of *Oryza sativa* L.

Federal Scientific Rice Centre (FSC of Rice, Krasnodar, Russia) closely cooperates with the Federal Research Center VIR named after N.I. Vavilov (St. Petersburg, Russia) in the field of gene pool exchange and reproduction of rice samples after long-term storage in the genebank. FRC VIR stores more than 12 thousand rice samples, and in the collection of Federal Scientific Rice Centre there are more than 7.2 thousand specimens of *O. sativa* L from 42 countries. In this regard, it becomes necessary to predict changes in the seed qualities of the rice collection (taking into account the varietal and taxonomic characteristics of the samples) to optimize the conservation of the gene pool of this species. The aim of this study was to determine the differences in storage between rice varieties of different subspecies in terms of sowing qualities of seeds, longevity and elements of productivity of reproductive plants.

2. Materials and methods

Rice samples of two subspecies *indica* and *japonica* *O. sativa* L. of 37 varieties preserved in the collections of the Federal Scientific Rice Centre and the Federal Research Center VIR named after N.I. Vavilov were used as objects of research. Seed collection of "FSC of Rice" is preserved in two ways: 1) short-term in ambient conditions (+26 ± 2 °C) in glass containers for grain; 2) long-term low-temperature in refrigerating chambers in hermetically sealed moisture-proof foil bags at various temperature conditions.

In the period 2015 - 2020 a series of experiments was carried out in the laboratory of the Institute “Collection of genetic resources of rice, vegetables and melons”. In the course of the experiments, the factors influencing the viability of rice seeds during storage at constant seed moisture were studied: shelf life, temperature regime, packaging method, genotypic characteristics, taxonomic belonging of the variety. The moisture content of seeds for short-term storage in uncontrolled conditions is 10 ± 0.2%, for long-term low-temperature seeds — orthodox rice seeds with a moisture content of 6 - 7 ± 0.2%. Storage options for seeds: 1 - 3 years at room temperature; 3, 5 years at a temperature of +4.5°C; 10 years – at *t* = +4.5°C and -4.5°C; 25 and 35 years – at *t* = -10 - 18°C. In 2018-2019 an experiment was carried out to study the effect of low-temperature stress on the productive properties of seed progeny plants with a storage period of 10 years.
In the experiment, we used lots of rice seeds (Oryza sativa L.), represented by a wide genetic diversity in morphological and biological traits. The material for monitoring the germination was a set of rice varieties of different ripeness groups from 25 countries of origin: 1100 samples of the world collection of reproductions of 1985–2005 after long-term storage in the genebank, 38 short-term storage varieties bred in "Federal Scientific Rice Centre" and 90 samples of 2010 reproduction after medium-term storage (t = +4.5°C and -4.5°C). The productivity of seeds progeny after 10-year storage was assessed in 24 varieties by biometric analysis of plants. The sowing quality of the seeds was checked in the laboratory according to the Russian standard (GOST R 52325-2005). The growth rate of rice seedlings during germination was determined on the seventh day in Petri dishes visually using a 9-point scale. Ten-day-old seedlings of rice samples were planted in May on a growing plot in 7-10-liter vessels filled with soil, the main application of fertilizers to the vessel before sowing. As the plants grew, the water layer was increased to 8 - 10 cm and maintained with daily watering until fully ripe. Harvesting in the vegetation experiment was carried out manually, as the grain ripened (September-October). When studying collection samples, we carried out counts and observations according to "Standard Evaluation System for rice" (INGER-IRRI, 1996) and "Classification of rice Oryza sativa L." according to Lyakhovkin (2005). The obtained data on seed viability, as well as data on the parameters of seedlings and plant productivity were analyzed using statistical analysis at a significance level of p ≤ 0.05.

3. Results and discussion
Subspecies of the genus O. sativa are divided into varieties, of which there are about 300 (H. Ikehashi, 2009, Amanda J., 2005). In Russia, the intraspecific taxonomic classification of rice according to Gushchin G.G. (1938) included 156 varieties, and in 2005, Lyakhovkin A.G. expanded the list of varieties to 284 [26].

The results of the assessment of genotypic features in the preservation of the viability of seeds in the germplasm of the rice collection by the methods and periods of storage are presented in Table 1. For a year of storage in uncontrolled room temperatures in glass jars, rice seeds lost laboratory germination by varieties by 1 - 38%, after two years it decreased to 52 - 97%.

Table 1. Influence of the term and method of storage on the seed germination of O. sativa L. germplasm.

| Term of storage (years) | Temperature regime (ºC) | Germination variation, min-max (%) |
|------------------------|-------------------------|-----------------------------------|
| 1                      | +26-28                  | 62-100                            |
| 3                      | +26-28                  | 32-80                             |
| 3                      | +4.5                    | 87-95                             |
| 5                      | +4.5                    | 65-95                             |
| 10                     | +4.5                    | 20-53                             |
| 10                     | -4.5                    | 85-99                             |
| 25                     | -10.0                   | 0-100                             |
| 35                     | -18.0                   | 0-80                              |

The conditions of low temperatures and storage of seeds in sealed foil bags during this period made it possible to keep seeds germination at the level of 87 – 95%. Already three years of storage made it possible to differentiate rice by the rate of seed aging associated with genotypic characteristics. Among the Russian varieties, the long-grain Izumrud, Avstral and Gagat, the red-grain Ryzhik, the short-grain Leader, the medium-grain Mavr and Kurchanka, as well as the aromatic variety Aromir, retained high germination (97 - 100%). Monitoring the germination of seeds of varieties of different groups of ripeness showed that the dynamics of a decrease in germination in early maturing forms is faster and their shoots form embryonic roots of shorter length. The interval of variation in germination
(X_{\text{min}} - X_{\text{max}}) in the varieties of the early-ripening group is 46 - 92%, while in the middle-ripening group – 67 - 97%, and in the late-ripening group – 54 - 95%.

There is some evidence in scientific publications that the large seeds of *O. japonica* and *O. glaberrima* have a shorter shelf life, while the purple pericarp and *indica* varieties have greater potential longevity [5]. In the set of varieties studied by us, no correlation between grain weight and germination was found, however, *japonica* varieties Soneta, Khazar, Atlant and Victoria, as well as *indica* varieties Avstral and Snezhinka, showed higher rates of seed viability in the year they formed a reduced weight of 1000 grains [17].

A detailed analysis of the data of monitoring the germination of rice germplasm with a storage period of seeds from 3 to 35 years at t = +4.5°C, -4.5°C, -10°C and -18°C confirms that the temperature regime and shelf life of seeds affect the preservation of their viability. In a number of samples, for 10 years of storage at t = +4.5°C, germination decreased to 20%, and at t = -4.5°C, the indicators remained from 85 to 99%. At the same time, the viability of rice seeds is differentiated at the population and varietal level, and no significant species and taxonomic differences were observed during a short storage period.

Within the framework of the cooperation agreement, from 2008 to 2020, the institute received 4108 samples from the VIR genebanks after long-term low-temperature storage for the restoration of germination and reproduction, 81.1% of the samples were reproduced, the rest turned out to be unviable. In an experiment with a shelf life of over 25 years, 250 samples of the *indica* Kato subspecies and 848 forms of the *japonica* Kato subspecies of the VIR base collection were studied. After long-term low-temperature storage, the germination rate by varieties varied from 0 to 100% (table 2). The share of samples with a critical value of germination (from 0 to 30%) of the total number studied was 78%. Significant differences in germination between subspecies were revealed: in *indica Kato*, the proportion of viable samples was 96.1%, and in *japonica Kato* – 76.9%. With an increase in the storage period of seeds to 35 years, rice genotypes with germination up to 10% – 28%, from 51 to 70% – only 2%, more than 70% – 16.7%, the rest with lost germination.

### Table 2. Variations in germination and proportion of viable seeds in samples of various botanical varieties of the world collection of *O. sativa* L. after long-term low-temperature storage.

| Botanical variety of samples | Number of samples (species) | Interval of seeds germination (min-max, %) | Proportion of viable samples (%) | Note |
|-----------------------------|-----------------------------|------------------------------------------|---------------------------------|------|
| **Subspecie indica Kato** (varieties with long and slender grain type) |                |                                          |                                |      |
| gilanica Gust.              | 92                          | 2-90                                     | 92                             |      |
| mutica Vav.                 | 45                          | 6-98                                     | 99                             |      |
| fortuna Gust.               | 14                          | 4-70                                     | 98                             |      |
| ceylonica Gust.             | 8                           | 10-67                                    | 100                            |      |
| brevieristata Vav.          | 12                          | 0-81                                     | 77                             | semi-awned |
| maldehica Gust.             | 16                          | 28-84                                    | 100                            | awned |
| aristata Vav.               | 15                          | 5-68                                     | 98                             | awned |
| ratoonica Gust.             | 3                           | 12-70                                    | 100                            | awned |
| philippensis Gust.          | 23                          | 5-90                                     | 99                             | red-grain |
| sarica Gust.                | 9                           | 13-82                                    | 100                            | red-grain |
| dicolorata Gust.            | 6                           | 0-50                                     | 90                             |      |
| rubriculata Piac.           | 7                           | 18-62                                    | 100                            |      |
| Mean value of the group     | 8,1-73,8                    |                                          | 96.1                           |      |
| **Subspecie japonica Kato** (short- and medium-grain varieties) |                |                                          |                                |      |
| italic Alef.                | 193                         | 0-70                                     | 41                             |      |
| ochracea Bat.               | 11                          | 4-51                                     | 98                             |      |
| cinnamomea Bat.             | 22                          | 0-60                                     | 80                             |      |
| nigro-apiculata Gust.       | 144                         | 0-89                                     | 48                             |      |
| anandica Gust.              | 14                          | 0-64                                     | 53                             |      |
On the example of studying a set of varieties of the world collection from different growing places, it was revealed that the seeds of long-grain *indica* samples, as well as red-grain and awned forms of varieties: *pyrocarpa*, *sundensis*, *aristata*, *mutica*, *maldehica*, *philippines*, *caucasica*, *vulgaris*, *rubrapiculata*, *sordida* varieties: it was revealed that the seeds of long-grain samples of different subspecies were selected with high rates of seed viability, the passport data of the studied varieties showed that the seeds of genotypes from regions with a hot climate had the greatest viability. Analysis of the passport data of the studied varieties showed that the seeds of genotypes from regions with a hot climate were resistant to long-term low-temperature storage: Azerbaijan, India, Afghanistan, Pakistan, Nepal, Uruguay, Peru, Mali, Senegal.

In our studies, we compared the productive properties of plants of 24 varieties grown from seeds with a shelf life of 1 year under uncontrolled conditions and after 10 years of storage $t = -4.5^\circ C$. At this stage, samples of different subspecies were selected with high rates of seed viability, the comparative characteristics of 8 varieties are shown in Table 3. There were no significant differences in the progenies from seeds stored in contrasting conditions, according to the passage of the vegetation phases by plants, the duration of phenophases differed in 1-3 days. Almost all samples showed higher growth rates of seedlings from seeds after short-term storage. In most of the studied varieties grown after cold stress, an increase in the spikelets sterility was noted. Reproductive plants from seeds after low-temperature storage retain all economically valuable traits and biological properties inherent in fresh seeds of the studied samples.

Table 3. Results of evaluation of productivity elements and vegetative traits of plants of reproduction of rice varieties during seed storage in contrasting conditions, (mean value for 2018-2019).

| Name of variety (A) | Way of storage (B) | Shoot length (cm) | Panicle length (cm) | Total number of spikelets (pcs) | Grain sterility (%) | Panicle density (pcs/cm) | Mass of grain from the panicle (g) | Mass of 1000 grains (g) |
|---------------------|-------------------|------------------|---------------------|-----------------------------|-------------------|-------------------------|----------------------------------|-----------------------|
| Belozerny           | uncontrolled      | 100,3            | 14,2                | 101,0                       | 10,8              | 7,1                     | 2,43                             | 27,5                  |
| Antei               | uncontrolled      | 102,1            | 15,1                | 131,9                       | 25,2              | 8,7                     | 2,68                             | 26,4                  |
|                     | refrigerator      | 105,0            | 17,6                | 71,9                        | 28,2              | 4,1                     | 1,70                             | 33,2                  |
| DON 1737            | uncontrolled      | 103,2            | 19,5                | 89,3                        | 13,4              | 4,5                     | 2,31                             | 31,4                  |
|                     | refrigerator      | 98,3             | 15,3                | 106,6                       | 31,8              | 6,9                     | 2,11                             | 34,3                  |
| VNIIR1016           | uncontrolled      | 86,4             | 15,1                | 88,0                        | 30,6              | 5,8                     | 1,92                             | 32,1                  |
|                     | refrigerator      | 114,2            | 16,4                | 127,9                       | 20,1              | 7,7                     | 2,95                             | 27,9                  |
|                     | 0                 | 117,1            | 17,1                | 168,6                       | 13,9              | 9,8                     | 4,08                             | 27,1                  |
O. sativa indica Kato

|                | uncontrolled | refrigerator |
|----------------|--------------|--------------|
| Indus          | 79.0         | 21.8         |
| Maya           | 93.2         | 22.5         |
| Hankayskiy     | 86.2         | 28.5         |
| Mars           | 92.0         | 30.8         |

Differences in storage methods were observed: low-temperature storage, due to a decrease in seed respiration, made it possible to preserve mass of 1000 grains and their high productive capacity in all studied samples, except for the Don 1737 and Mars. However, much remains to be learned about the reproduction of these two subspecies after a longer seed storage period.

4. Conclusion

When stored under uncontrolled conditions, the rate of seed aging is lower in rice varieties with a longer growing season. The short storage period of seeds did not reveal taxonomic differences and subspecies features of rice varieties in maintaining germination. To ensure the viability of the seeds of O. sativa L., which are in long-term low-temperature storage, it is necessary to take into account that japonica varieties are less durable than indica. The research results make it possible to recommend a differentiated approach to the choice of the method and timing of the safe preservation of rice gene pool seeds with different bioecological properties.

For further fundamental research, varieties of the world collection of rice with a high seed preservation index were selected: Krihana Ruda (France); Insen (Spain); Bala, Ratna, Tamuma, Vaigai, Convery, Budgi (India); DD-48, IR 485-214-1, Tongil, Shao-nam (Philippines); Santi, Khursu, Kangni 27 (Pakistan); Bical selection (Afghanistan); Gajar gual, Badmase (Bangladesh); Gladio (Italy); Etym (Azerbaijan); Isaribi (Japan); Chaltyk Champa (Iran); Rajado (Portugal); Vegold (USA); 5718 RS-32 and 5739 IR 11 (Uruguay); Sharm, Dolinin, Belozerny, Kabanets 575, Zeravshanka 2586-1, Favorite, Kumir, Polevik, Yuzhanin, Apollon, Atlant, Avstral, Kulon, Razdolny, Mutant 355 (Russia).

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