Assessment of the quality of seeds formed *in situ* and *ex situ* as a mandatory element of maintaining seed banks of rare plants

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Abstract. The possibility of seed genetic banks creating is associated with the ability of many plants seeds (the so-called orthodox ones) to maintain germination ability for a long time, being in a dormant state. This opens up wide prospects for the formation of a reserve seeds fund of rare species that are threatened with extinction in natural communities. Botanical gardens in different countries of the world, including the Russian Federation, are working on the creation of such seed banks. To assess the quality of seeds in relation to agricultural crops, as well as natural flora species, radiography is used. It is this kind of non-damaging express assessment of the quality of seeds that turns out to be the most informative technique for seed material obtained in botanical gardens. The article presents the preliminary results of an X-ray study of the seeds quality of some Samara region rare plants - four species ofthe Iris genus - Iris aphylla L., I. halophila Pall., I. pumila L., I. sibirica L.

1 Introduction

Of the 345,777 species of higher plants, approximately 332,857 species are seed plants [1]. Of these, according to various authors, from 22% to 36–37% of species are threatened with extinction from natural habitats [2]. While in situ conservation of species and habitats is generally considered optimal, the Global Strategy for Plant Conservation recognizes the important complementary role of exsitu species conservation [3]. The international program of botanical gardens for the protection of biological diversity of the plant world determines the priority directions for the long-term preservation of plant gene pools *ex situ* [3]. First of all, these are: seed banks of rare and economically valuable species; field genebanks for the conservation of species whose seeds do not retain viability during storage (20% of the total world number of species), as well as species that reproduce vegetatively; seed banks and seed cryopreservation. The seed bank can be recognized as the most common, reliable and space-saving method of germplasm storing. The historical priority in such collection

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creation, thanks to the scientific feat of N.I. Vavilov, was been acquired by our country, belongs to cultivated plants varieties of various geographical origin and their wild relatives. This direction in the selection of seed material samples for conservation and use in breeding work was laid down in the formation of the collection of the All-Union Institute of Crop Production by N.I. Vavilov and his colleagues.

There are more than 1,750 traditional seed banks in the world today, most of them focusing on the conservation of crop species and their closest wild relatives germplasm; in others, priority is given to species of global or national economic importance (for example, fruit crops, tree species), or natural flora species that are threatened with extinction in nature. The Millennium Seed Bank (London, Kew) is in the leader in the number of stored samples, which is successfully developing the methodology of seed banks [4, 5].

The very possibility of creating genetic banks determines the ability of the seeds of many plants (the so-called orthodox ones) to maintain germination for a long time, being at rest. The limiting empirically determined shelf life of seeds in a limited number of species reaches thousands of years [6]. In this case, the seeds should be in conditions of low humidity (5-10%) and temperature (20° C). The procedure for handling stored material in a seed bank can be briefly described as follows. The incoming material is first assessed for quality, then brought to the required moisture content and stored in the form of packaged, coded samples. Samples are regularly tested for germination, and if it falls below the permissible level (approximately 85%), then the batch is renewed, germinating the stored seeds and getting a new harvest. This is the most difficult and costly part of the process. However, the need to obtain new generations of seeds, with a limited number of individuals, as a negative side is associated with the manifestations of inbreeding, which can lead to a decrease in the quality of seeds [7].

The formation of collections of local flora plants and introduced species in botanical gardens, as an obligatory part of the work, includes the collection of seeds of their own reproduction, their study and provision for exchange. This can be considered the moment of preliminary preparation of botanical gardens for the formation of their own genetic banks. The most important task of botanical gardens is the preservation of local florarare species in the collections and all possible assistance to their preservation in natural habitats. It is the seeds of these plant species that deserve attention in the first place as a source of material stored in genetic seed banks.

The experience of growing natural flora plants in the Botanical Garden of Samara University demonstrates the possibility of forming a collection of rare plant species of the Samara region and other regions, mainly in the form of population groups. The collection of the flora department includes more than 800 taxa, of which 183 are rare and Red Book ones, including 50 from the Red Data Book of the Samara Region (2nd edition) [8]. At the same time, for most taxa, population groups are represented by 10 or more specimens; for some species, the number has reached the level of 50 (*Glycyrrihza glabra* L. and others) and even 100 or more specimens (*Clematis integrifolia* L., *Iris aphylla* L., *I. halophila* Pall., *I. pumila* L. , *I. sibirica* L., *Paeonia tenuifolia* L., *Primula macrocalyx* Bunge, etc.). Some of the above species were successfully reintroduced by us into the natural biotopes of the Samara region during the implementation of the regional program for the conservation of biological diversity.

Over the past 10 years, 39 species of flowering plants included in the Red Book of the Samara Region have been preserved in culture in the form of permanently existing population groups, 24 of which have long been stable components of the collection fund. Over the past 10 years, their seeds of their own reproduction have been included in the lists of delectus every year and are offered to colleagues from domestic and foreign botanical gardens. These are plants of various rarity status, unequally widespread in the territory of the Samara region.

The fund of its own seeds formed annually in the Botanical Garden, collectively obtained from the plants of the dendrology, flora, floriculture, greenhouse departments, offered in the delectus, is essentially the basis for creating a full-fledged seed bank. Its target storage units will be primarily seeds of rare plants of the local flora. The possibility of implementing plans to create a genetic seed bank is associated with the acquisition in 2020 of a complex of specialized laboratory equipment within the framework of the federal project "Development of advanced infrastructure for research and development in the Russian Federation" of the national project "Science at the end of 2020". includes a unique X-ray diagnostic unit for non-damaging transillumination of seeds PRDU, devices for assessing moisture, mass, studying the morphology of seeds, laboratory refrigerating chambers for their storage. The PRDU device, developed by native specialists, is successfully used not only to assess the quality of seeds of food crops, but also to study seeds of various plants by specialists in botanical gardens [9-11]. This report presents the preliminary results of the X-ray study of the seeds of some rare plants of the Samara region

2 Research methodology

The objects of the study were the seeds of four species of the genus Iris – *Iris aphylla* L., *I. halophila* Pall., *I. pumila* L., *I. sibirica* L., presented in the collection of rare plants of the flora department of the Botanical Garden of Samara University. They are characterized by unequal rarity for the Samara region. *I. halophila* is known from one habitat and is endangered; *I. aphylla* L. has about 7 habitats in two municipal districts of the region, being a rare species; *I. sibirica* L. is a rare species, found in 12 municipal districts; *I. pumila* L. is a recovering species, represented in most areas of the region.

Seed samples were taken from the reserve samples of the exchange fund, formed by the seed laboratory at the flora department of the Botanical Garden of Samara University. For one of the studied species - I. aphyllaL. we had seeds formed by plants in the nature monument Podvalskie Terraces (*in situ*) and in population groups of plants in the collection of the Botanical Garden (*ex situ*), for the other three species there were samples of seeds formed ex situ in different years.

Seeds were preliminarily sorted (rejection of feeble, defective, clearly empty seeds); in general, the samples were visually homogeneous.

The study of the internal structure of the seeds was carried out by the method of digital microfocus radiography. This method is included in international standards, primarily for assessing the infestation and damage of grain by pests [9, 10]. The PRDU unit has a protective chamber for X-ray imaging, a monoblock X-ray source RAP70M-0.1N-1, an X-ray image receiver based on a multifunctional portable flat-panel X-ray detector ViVIX-S for digital radiography. The control is carried out from a computerized console with the MicroCT-PRDU universal software for analyzing digital X-ray images of seeds. The installation can obtain images with a geometric magnification of 3.0 × [10]. The following parameters are adjusted in the MicroCT-PRDU software before the examination: anode voltage, exposure time. The range of variation of the anode voltage of our model is 30-50 kV, the anode current is 0.1 mA.

Seed samples were placed in the chamber of the device on plastic (PLA) plates, which made it possible to place seeds at different distances from the X-ray tube in the detector's sensitivity region. Individual samples, differing by years of reproduction, were placed on the plates in separate supports.

To study samples of different seeds, the following mode was chosen: the voltage applied to the tube was 40 kV, the exposure time was 2 seconds. Within 10 s, the image was displayed on the monitor screen to correct contrast, clarity, and subsequent analysis. After obtaining the image, the seeds were visually identified with the following negative signs: the absence of filling the integument with the seed, the presence of a cavity between the seed and the shell, insect damage, embryo defects, mechanical damage (cracks).

3 Preliminary results of x-ray study of the quality of seeds of some species of steppe flora

The use of radiography to assess the quality of seeds is today used in relation to agricultural crops, as well as species of natural flora preserved in seed banks. There is experience in using this method by specialists from domestic botanical gardens [11]. Our experience in using this method is relatively small, since a modern X-ray diagnostic unit of domestic production PRDU was acquired by Samara University only at the end of 2020. The initial testing of seeds and fruits of various sizes with it made it possible to determine the size of the irradiation field at different distances from the X-ray source (the position of the shelves inside the installation chamber or fixing the samples near the X-ray tube), which also corresponds to a different increase in the scanned objects. Experimentally, the modes of treatment of samples were selected that differed in the density of the shells and the content of the seeds.

Let us present the results of X-ray diffraction analysis of seed samples of four species of the genus Iris, which were formed by plants in culture (in population groups maintained in the collections of the Botanical Garden of Samara University). Each pair of images shows a photo of a seed (general view with a scale mark corresponding to 1 mm) and an image of a seed sample of this species, obtained by digital focal radiography (Fig. 1). For each object, images were examined in parallel - positive and negative, choosing the options optimal for further processing.

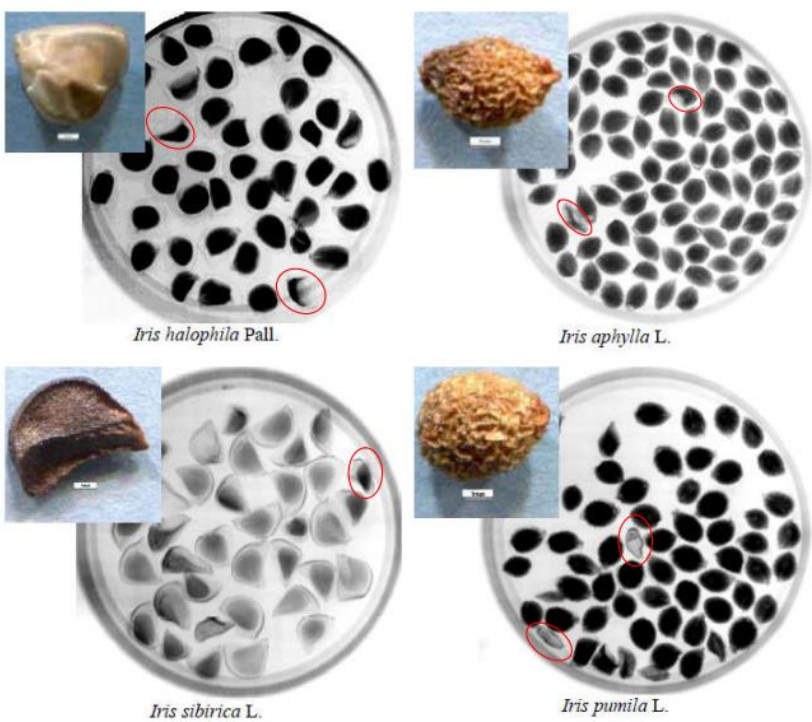

Fig.1. Photo of seeds and samples of results of microfocus X-ray examination of seed samples using the PRDU installation. Examples of poor quality seeds are marked with ovals

With a similar appearance of seeds, for which samples were previously sortered by visual criteria, the images obtained using X-ray imaging made it possible to reveal the presence of seeds with damaged, underdeveloped seeds that have critical content defects. Otherwise, such seeds can be identified either by opening them or by laboratory germination, but with a small number of seeds, both of these methods are unacceptable, each seed is valuable. It is the non-damaging express assessment of the quality of seeds in this case that turns out to be the most informative method for the material obtained in botanical gardens [11], which are subsequently used for exchange or preservation in genetic banks.

In the presented photos (Fig. 2), we noted examples of identified defective seeds. Subsequently, the calculation of the total number of seeds and the number of defective ones found among them in the replicate samples in the images made it possible to calculate the quality index of seeds of various irises. For one of the species, as a comparison, we were able to use seed material formed in natural conditions (*I. aphylla*).

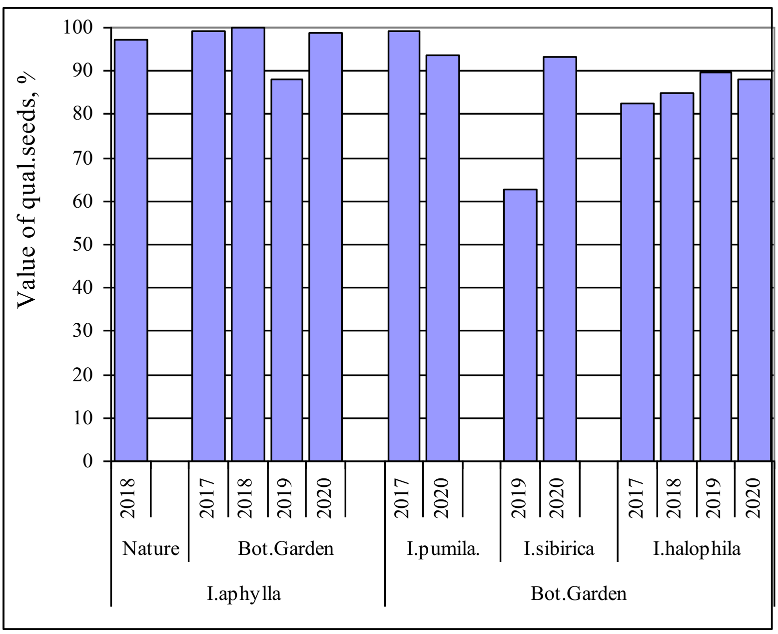

Fig.2. Quality of seeds formed by plants of the Iris genus in different years

It can be noted that for all studied species, the quality of stored seeds turned out to be rather high (in most samples, more than 80% were completed, without signs of damage). Only for one of the accessions (*I. sibirica*, 2019), the proportion of unfulfilled seeds exceeded 30%. This shows that in some years seeds of this species may require more careful rejection of low-quality material. It is also worth noting that I.halophila, which is the rarest in the region, forms seeds with a high degree of fulfillment - up to 90%. This gives good prospects for using seeds of this species as storage units in a seed bank, as well as preparing material for creating new reserve populations in culture and reintroduction into natural communities.

4 Conclusions

Thus, for the seeds of 4 species of the genus Iris, rare plants of the natural flora of the Samara region, the possibility of a non-damaging quality assessment using X-ray has been confirmed. The application of this method will be a mandatory step in the preparation of seeds for their further preservation in the regional seed bank.

The acknowledgements should be typed in 9-point Times, without title.

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