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Ways and solutions to preserve the gene pool of rare and endangered plants

Aigerim Yeginbay[1]*, Assilbek Burabaev[2], Roza Mamykova[3], Altunbek Burabaev[2], Dana Azhibayeva-Kupenova[2], Yelemanova Zhanar[1], Esimova Anar[1], Narymbayeva Zaure[1]

1. M. Auezov South Kazakhstan University, Tauke khan avenue, 5, Shymkent, Kazakhstan
2. South Kazakhstan Medical Academy, Shymkent, Kazakhstan
3. South Kazakhstan State Pedagogical University, Shymkent, Kazakhstan

** Corresponding author's Email: aigerim_eginbai@mail.ru*

ABSTRACT

The article provides an overview of methods of preserving the gene pool of rare plants in Kazakhstan. The topic is topical and the acuteness of the problem is constantly increasing as more and more plants are becoming extinct every year. The main reason for this extinction is human activity which causes direct or indirect damage to plants. Although there are many options for conserving the gene pool of plants, it is not yet possible to stop their extinction. This paper describes the methods used in Kazakhstan to preserve the gene pool of plants. An assessment of innovative biotechnology methods used by Kazakhstan and foreign scientists to preserve the gene pool of plants is given. The effectiveness and sufficiency of measures (including biotechnology) carried out in Kazakhstan for the conservation of rare plants are assessed, and recommendations in these areas are given. In general, a conclusion is made about the insufficiency of efforts to introduce advanced methods of protecting rare plant species in Kazakhstan.

Keywords: Gene pool, Conservation, Red List, plant, Biotechnology.
Article type: Review Article.

INTRODUCTION

The problem of preserving species diversity is now extremely acute. Human activities are global, affecting virtually all components of the biosphere and often destructive to nature. Even though there is no direct impact on nature, human activities affect nature indirectly. This may consist in the formation of "ozone holes", gradual "global warming", anthropogenic pollution, etc. (Abolhasani *et al.* 2021; Omidipour *et al.* 2021; Mirhashemi *et al.* 2021; Al- Abbasi *et al.* 2022). Such impacts significantly disrupt the growing conditions of plants, with the result that many species disappear even without the direct human impact on them. Direct impacts are to be understood as disturbance of habitats, such as deforestation, hydraulic construction, construction of buildings, roads and other facilities, uncontrolled collection of plants, and so on. Thus, direct impact should be understood as the direct removal of a plant from nature. Plants are now becoming extinct due to anthropogenic influences faster than they do naturally. Thus, over a period of two hundred and fifty years of observation, it has been noted that two or three plant species die out each year. Noteworthy, this is also true for Kazakhstan. Over the past few years, Kazakhstan has supposedly lost such species as *Gladiolus palustris* GAUDIN and *Daphne altaica* PALL. Notably, according to the Red Book of Kazakhstan published on 1 June 2005, there are over 600 rare plant species in Kazakhstan. All of them are classified in the Red Book of Kazakhstan, which makes finding a plant of interest much easier. List of some of them are as follows:

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1. Different species of the genus *Jatrachis* (clopod-bearing, fine-pointed, male, etc.) e.g. *Orchis* (*O. coriophora, O. punctulate, O. mascula, etc.*)
1. Stschapovia flagellaris Zinova
2. Osmunda japonica Thunb.
3. Exochorda serratifolia S. Moore
4. Globularia bisnagarica L.
5. Saffron of the valley, Crimean saffron and many other species. *Crocus vallicola Herb, C. tauricus* (Trautv.) Ruring, etc.

Thus, Kazakhstan is home to a great variety of rare plants which are included in the Kazakhstan Red Book. At the same time, every few years Kazakhstan loses at least one rare plant. The Kazakhstan Red Data Book of Plants has not been updated for over 16 years, which is an unacceptable fact. Are the measures sufficient and are the current methods of protecting the gene pool of rare plants in Kazakhstan adequate? Clearly, new innovative methods need to be introduced. Their evaluation makes this article relevant. Notably, one of the innovative methods is to employ biotechnology. They can be used to increase the efficiency of conservation of rare plant genetic resources. At the moment, many different biotechnology tools have been proposed and mastered and are being used in different countries. The subject of the paper is the gene pool of rare plants in Kazakhstan. The aims of the paper are to review the activities carried out in Kazakhstan to preserve the gene pool of rare plants; to assess the effectiveness and sufficiency of the activities carried out to preserve the gene pool of plants in Kazakhstan. The work is of theoretical significance, as various activities used in Kazakhstan to preserve the gene pool of plants are considered within the framework of a single article. So, special attention is paid to the most popular biotechnologies used in Kazakhstan and in other countries to preserve the gene pool of rare plants. The practical significance of the work lies in the assessment of the practical applicability of biotechnologies that are promising for implementation in the conservation of rare plant gene pools. In addition, the risks of using biotechnology for the conservation of rare plant genetic resources are assessed. This will make it possible to identify areas which should be primarily involved in further work on this issue.

The main methods of preserving the gene pool of rare plants in Kazakhstan

The key methods currently in use for the conservation of the gene pool of rare plants in Kazakhstan are presented in the strategy for the conservation of rare and endangered animal and plant species in Kazakhstan until 2030 [9]. The formation of such a strategy is an important and effective step towards the conservation of the plant gene pool. The existence of such a strategy means the introduction of state control over the problem of preserving the gene pool of rare plants. So, we study this document and present the main methods.

Principles of a plant genetic pool conservation strategy

The strategy is based on principles. Using a principle, ways of preserving the gene pool are shaped. Principles:
1. The species principle;
2. The population principle;
3. The organismic principle.

The species principle. It is based on preserving a sufficient number of individuals of each plant species as well as its range. It is important to maintain the spatial and genetic population structure of all species, the genetic diversity in each population and the life forms of each species, e.g. ecologic. In order to implement this principle, each rare plant population is monitored, with an obligatory check on the status of rare plants. An important measure is to preserve and restore the area where the rare plant grows under normal conditions. The area may be designated a "special conservation area" to avoid damage to the natural habitat of the plant. Noteworthy, the method of recreating lost populations is also used to implement this principle. When implementing the principle, it is important to remember the prerequisite for preserving a rare plant species - the need to preserve the population structure, since each individual population has unique adaptations that allow the plant to adapt to non-standard environmental conditions. Local populations, intraspecific forms and subspecies all have such adaptations. If the natural conditions are disturbed or the isolation from other species is insufficient, the plant population may die or the unique adaptations will disappear naturally. Thus, the isolation of different populations is important, since the

spatial and genetic structure characteristic of each individual plant population will be altered, with negative consequences, if the isolation is disrupted. Note that the following items could be negative:
1. The formation of excessive isolation;
2. Complete elimination of insulation;
3. Mixing populations is artificial.

The population principle. This principle involves the application of methods similar to the species principle, however, has some special features. For example, according to this principle, the number of plants and their natural habitat should be maintained and restored in sufficient quantities to form a sustainable existence. At the same time, the health of the plants is monitored in an amount that is optimal for the entire population. Within each population, the formation of diverse groups of plants within the population is monitored, along with the formation of specificity within each population. Notably, by implementing this principle, consideration is given to maintain diversity within a population in association with several parameters, such as age. This principle differs in the application of different methods for the conservation of the gene pool both in the natural habitat and in the artificially created environment, whereas by employing the species principle, only the conservation of the plants gene pool growing in the natural environment can be realized. The methods of conserving plant populations growing under natural conditions do not differ significantly from the methods used in implementing the species principle. In this case, the status of plants is also monitored. Measures are taken to conserve their populations, the natural habitat of plants is restored, special protected areas are formed, and so on. However, there are significant differences. For example, a population may be reproduced artificially. In this case, it is obligatory to carry out measures, which do not allow the plants of the population to die due to technical works over, e.g., creation of engineering infrastructure. The need to assist the plants in case of emergencies is also noted. The need for measures to eliminate the uncontrolled spread of alien (invasive) plant species in the natural environment is also suggested. At the same time it is important to prevent the introduction of genetically-modified objects into the natural environment of plants, which can crossbreed with plants in the protected population and disrupt its genetic structure. In implementing this principle, many measures are aimed at eliminating factors that negatively affect the state of the plants. If the population is artificially established and introduced into the natural habitat, re-acclimatization activities are carried out. In such a case, genetic "health improvement" measures are important. Also, according to this principle, relocation of some populations to new, suitable habitats should be used in the case of exposure to negative factors. Notably, such negative factors can be both natural, e.g. flooding of the area by a water body in the case of a natural increase in water level, and anthropogenic, e.g. hydro-construction, construction of infrastructure facilities etc. The conservation of the plant gene pool using this principle under artificial conditions involves the formation of conditions for the conservation of the population within botanical gardens and other conservation sites. When conserving a population, it is important to consider the number of plants in the population. If the number of plants in a population declines, there is the possibility of an accidental extinction of the species or population. A reduction in the number of individuals leads to a drop in the genetically diverse individuals in the population itself. A minimum number of individuals in the population should be formed by means of various measures, which should only increase in the future. The number of individuals in the population should be above the minimum values in the long term. Note that there is no consensus on the minimum number of individuals in a population. All critical and minimum thresholds for the number of individuals in a population are calculated individually, taking into account many influencing factors:
1. Biological characteristics of this plant species,
2. The level of differentiation within a population,
3. The rate of increase in the number of individuals in a population,
4. Specifics of intraspecific interbreeding in a population,
5. Specific features of the existence of individuals in the population and so on.
Using this principle, it is important to form the closest possible natural habitat for the population in full and over a long period of time. It has already been proven that the gene pool will only be fully conserved over the long term in an environment that is historically characteristic of the population. In the presence of conditions that differ from the natural environment, the population transforms itself by modifying and adapting to other conditions. The described population principle forms the basis of the methods and parameters which are used to adopt a strategy for the conservation of the gene pool of rare plants.

The organism principle. This principle is used to form measures that focus solely on the conservation of the gene pool of individuals. By this principle, it is possible not only to conserve, but also to reproduce an entire population of several individuals. Notably, in this case the measures relate exclusively to the conservation of individuals and their gene pool under artificial conditions. The main activities are carried out in botanical gardens, where individual plants are competently maintained and subsequently bred in sufficient numbers. Noteworthy, in some cases the principle is implemented through measures to preserve only genetic material, such as embryos, taking place in a genetic bank, where stores such embryos in low temperature conditions. Since the embryos and seeds stored are single specimens, this principle makes it possible to form only a specific population. The genes in such a population will be non-diverse, derived only from a limited pool of available genes in the gene bank. All this leads to the impossibility of preserving natural genetic processes in long-term breeding of plants in botanical gardens. As a result, genetic diversity within a population will be reduced. This principle is used as a key principle only when it is completely impossible to use other reserves that would help to maintain the population in the natural or artificial environment. These exceptional cases are as follows: the population as such is almost completely extinct; the population is in danger of extinction (with such a high probability that it cannot be guaranteed to remain in its natural environment); and cases where hybridization of plants in a population would result in the loss of a "pure", natural gene pool. Thus, when identifying the principles to be used in the conservation of each individual rare plant population, preference should be given to principles that prioritize the conservation of the gene pool in its natural habitat. The key reason is that the ability to fully conserve rare plant populations in the long term is only created in a natural environment in which the natural mechanisms of micro-evolution are implemented.

Activities to conserve rare plant populations

The combination of measures selected for each individual population of a rare plant species forms the conservation program for that species. The artificial environment is formed only in a few special cases:

1. Failure to eliminate or reduce the impact of negative factors on the population at present;
2. There is a critical threshold in the number of individuals, which makes the possibility of accidental extinction extremely high;
3. The genetic structure within the population is significantly disrupted, leading to a reduction in the viability of individuals, as well as the formation of uncharacteristic species parameters in individuals within the population;
4. The self-regenerating mechanisms of the population are disrupted, leading to the need to reproduce the population under artificial conditions.

If the population is restored in an artificial environment, measures are implemented in advance to promote the natural habitat of the plants. If such a habitat is available, the effects of various negative factors on this habitat are reduced. Measures are not taken only if the population cannot be re-established in the near future in a natural environment, or if the objective is to preserve the plant genome artificially. This is often necessary to preserve plant genes for scientific or educational purposes. These genes can also be used in the future to 'restore' lost or endangered species.

Regardless of the principle chosen, if scientists are to maintain the population in its natural environment, the following measures are necessary:

1. Formation of the fight against the illegal use of natural resources;
2. Legislative regulation of legal methods of using natural resources for various purposes: cultural development, science, recreational purposes;
3. Implementation of an environmental impact assessment for all economic projects that will affect or already affect the natural habitat of plants. The expertise is also carried out if the economic activity affects the number of individuals in the population.

Protected areas are often formed to protect rare plants. It has been proven that the creation of such an area is highly effective in protecting rare plants from the negative impact of people on rare species. Nowadays, the formation of protected areas is the main measure for the conservation of rare plants. Many such areas are formed solely for the purpose of creating special conditions that preserve the population of a single rare plant. The high efficiency of using protected areas is due to the distribution of the plants only over a limited area. As a result, the population can fully exploit a given area, leading to its conservation with sufficient numbers of individuals. Notably, sometimes the plants cannot cover the whole protected area at once. In this case, only a part of the protected area

is set aside for the purpose of conserving a particular rare plant species. There are already natural protected areas that are home to rare plants. The key distinction of such an area is limiting the use of natural resources. For instance, there are forests with special protected areas. Sometimes they are marked by plaques, e.g. with an inscription like this or similar "Rare plant species forest". Such areas are highly effective in preserving rare plant species. Noteworthy, the maximum beneficial effect can be achieved through the formation of a network of protected areas with different protection regimes. In order to create a direct network, so-called "ecological corridors" are formed. The ecological network created should be structured in such a way that the conservation of different plants in both space and time is taken into account. Gene pool conservation measures are particularly important in areas where there is intense anthropogenic activity. Often all that is needed for a rare plant species is to restore, and subsequently maintain, areas with a natural environment that has been altered by human activity. A common measure is the artificial reproduction of an existing population in nature. In this process, reproductive material is obtained from an existing plant. A similar plant is then artificially produced, which is then grown in an artificial environment with full control of the most vulnerable growth stages of the plant. The grown plants are then moved to their natural habitat, where their further growth will take place. Another technique is the artificial reproduction of rare plants that have a disturbed reproduction mechanism. Notably, the disadvantage of such measures is the disruption of the natural mechanism that forms the genetic structure of the entire population. However, this reduces the genetic diversity of the population. Thus, artificial reproduction should be used as rarely as possible, only in exceptional cases if it is impossible to preserve the gene pool under natural conditions.

The next activity, which is also often used to preserve the gene pool of rare plants, is reintroduction. Reintroduction is to be understood as recreating rare plant species in their natural environment, in which the plant had previously become extinct. Reintroduction is also called reclamation. There are several types of reintroduction:

1. Reintroduction through the relocation of individuals from other natural populations;
2. Reintroduction through the relocation of artificially created individuals, such as those obtained in botanical gardens.

The effectiveness of this measure can be increased by the formation of special protected areas. If in such an area the requirements of the natural environment, the specificity of the genetic structure, and the effects of the action on the whole ecosystem are taken into account, reintroduction will be successful and highly effective. To prevent rare plant species from dying due to careless human activities, such as during any repair work, technological and organizational protection measures are implemented. For example, if a power line is being repaired, the site is fenced off and rare plants in the immediate vicinity of the line are moved to a safe distance. Similar measures are taken in case of emergencies. Natural and man-made emergencies are also taken into account. For example, if the area is expected to flood due to a river bursting its banks, rare plants are moved to a safe distance. It is also possible to fence them off from the water. Similar measures are taken when, for example, a dam break is signaled.

In addition, measures are taken to prevent the uncontrolled spread of plant species that may harm rare protected species. In this case, a set of the following activities are carried out:

1. Identifying the pathways of pest plants;
2. Identifying individuals of plant pests;
3. Control the development of pest plants;
4. Implementation of a measure to prevent the emergence of hybrids;
5. Implementing measures to eliminate the negative effects of pest plants;
6. Formulating predictions about the effects of pest plants, assessing the future possibility of similar situations recurring.

A number of important measures should be aimed at preventing the introduction of genetically modified organisms into the natural environment. Such measures are important, since the genetically-modified organisms negatively affect populations of rare plants. The main ecological risks associated with the emergence of genetically modified plants are

1. Possible infection of genetically modified objects;
2. The pathogenicity of genetically modified organisms;
3. Competition, from genetically modified organisms;
4. The possibility of alien genes being introduced by genetically modified objects into the genome of rare plant species.

The implementation of this precautionary principle is set out in some important international and national documents on the conservation of rare plant species. These include, for example, the Rio Declaration [15]. Note that this is only an example of such a document, of which there are many in practice. In some cases, individual rare species or individuals are kept and bred exclusively in artificial habitats. Botanical gardens are the mainstay of plant conservation. There, the maintenance and breeding methods of a particular rare species or specimen are substantially improved. This can be realised through:

1. Exchanging plants from different botanical gardens;
2. Exchanging genetic material between different botanical gardens;
3. Formation of genealogy books;
4. Special selection of the most suitable reproductive couples.

The latter method is particularly effective, since it minimizes the negative effects of inbreeding.

The implementation of such measures is necessary in the following cases:

1. The need to form a 'reserve' is not uncommon when a population is in critical condition:
2. Building up a stock of individuals to quickly re-establish a population of a rare plant species in the natural environment if it has disappeared in nature;
3. To meet the demand for certain plant species (e.g. for cultivation in private gardens) that cannot be covered through the removal of significant numbers of individuals from nature.

The measures for introducing rare species into culture are also applied. This is done when a rare species is important for human livelihoods and its removal from nature in significant quantities is not possible.

Innovative methods for preserving the gene pool of rare plants

At First, it should be noted that Kazakhstan already uses such an innovative biotechnological method as preserving the gene pool of individual plants at very low temperatures (in cryocapsules). This method is used for many plant species, for which banks of genetic materials preserved at low temperatures are formed. Using this biotechnology in practice is expected to increase in the future due to its rather high efficiency, relative simplicity and relatively low cost. Parthenogenetic, androgenetic and gynogenetic specimens are obtained through this technology. In some cases, gonads are also transplanted and interspecies chimeras are formed from different embryos. As a result, it is possible to recreate a viable plant individual. The main reason for using this method is the low numbers of plants, which makes it impossible to obtain mixed-sex individuals at the same time. Often genetic material is conserved in order to restore populations and species that have been lost. Such material is also used to build up genetic diversity in populations where it has been significantly disrupted. However, cryopreservation is less common at the moment In foreign countries, the use of biotechnology for gene pool conservation is more developed. A few of the most common biotechnologies are as follows:

1. Formation of genetic banks using cryo-freezing (see above);
2. The introduction of various biotechnological methods that simplify breeding.

In this case, genetic engineering as well as genetic transformation should be mentioned in general. By their help, it is possible to obtain the necessary properties (parameters) in a rare plant species;

1. Clonal micropropagation of plants. The method of somatic embryogenesis should be highlighted here. This method allows rare plants to be propagated quickly, resulting in high quality planting material;
2. Molecular marking. It makes selection more efficient, as does genetic passporting, as well as seed certification.

Some states are building entire collections of seeds and plants that are produced artificially. Cryobanks operate with high efficiency. Some states have designated collections of seeds and plants as national treasures, which are strictly protected. For example, in the United States (Oregon) a plant bank has been established which contains over five hundred thousand seeds and plants of economic importance. Around ten thousand have the status of rare or endangered plants. Among the economically useful plants in the collection, there are: pear, gooseberry, blueberry, blueberry and many others. In the middle of European collections, the German collection can be noted, which includes about seven hundred plant specimens. Also worth mentioning are the French, Romanian, Italian, Spanish, Belgian and Polish collections, which also retain many plant species. The Japanese and Indian collections can also be mentioned. Notably, in each of the developed countries, using biotechnology is increasing and the methods used are becoming more sophisticated. At the same time, the list of developed countries is gradually expanding. In 2008, for example, the UN pointed to the Republic of Palau's own plant collection. Kazakhstan is gradually developing its own plant collection. A domestic collection appeared as early as 1978. The basis for the

collection was established at the K. A. Timiryazev Institute of Plant Physiology. It is still being developed by this institute. Overall, however, we have to conclude that innovative technologies (in particular, based on biotechnology), by which the gene pool of plants is preserved, are underdeveloped in Kazakhstan. Nowadays, only one biotechnical method is effectively used: preservation of the gene pool of individual plants at low temperatures. It is necessary to adopt the positive experience of colleagues and implement all the above biotechnologies in practice in Kazakhstan.

Noteworthy, there are also some risks in using biotechnology to preserve the gene pool artificially:

1. Danger of preserved seeds or plants that may contain pathogenic microflora, e.g. latent bacteria;
2. Danger of changes in the parameters of the sample to be preserved during cryopreservation. For example, the viability of a sample can be significantly reduced if the temperature of liquid nitrogen ranges from -96 to -130 ºC.

When introducing cryo-technology and other biotechnologies into the practice of domestic science, these risks should be taken into account. In other words, it is necessary to develop biotechnologies that will prevent negative scenarios of such risks.

CONCLUSION

To summarize the work on the conservation of the gene pool of rare plants in Kazakhstan, the following measures should be noted. Our scientists have created a strategy which contains three key principles for the conservation of the gene pool of plants and animals. Each principle includes various specific measures.

Based on the chosen principle, a set of necessary measures to conserve and increase the gene pool of plants is formed. In this case, the measure can refer to breeding in the natural environment and in the artificial environment. In contrast to other countries where emphasis is placed on the application of biotechnology, in Kazakhstan the priority is given to the breeding of plants under natural conditions. At the moment, Kazakhstan is lagging behind in the application of biotechnology for the conservation of the plant gene pool. Currently, only cryobanks and cryopreservation are relatively common in our country. However, Kazakhstan has a significant potential for biotechnology development. Every year the scientists conduct research in these fields, the leading universities of the country annually graduate new specialists who may make a breakthrough in this direction. Thus, our state will be able to make up for this lag if such research becomes a priority in science.

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