Fitogenetic Resources Conservation in Seed Banks – review

Conservação de Recursos Fitogenéticos em Bancos de Sementes – revisão

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A B S T R A C T

Plants are essential for the ecosystem proper functioning. They are a source of food for many species, a source of oxygen, and many Human expoitation like pharmaceutical industry and fibers. Their role in slowing down climate change and soil destruction is also very important. The possibility of losing some plant species is real, and there is a growing concern with the preservation of terrestrial flora. In this context, the conservation of germplasm through seed banks thus constitutes insurance against the extinction of plants in their natural habitat, and if a species is destroyed elsewhere, there will be seeds of that species for regrowth. A seed bank, is also, a source of controlled origin and quality material for scientific research.

Keywords: *ex situ* conservation, seeds conservation, viability tests.

R E S U M O

As plantas são essenciais para o bom funcionamento do ecossistema. São fonte de alimentação de muitas espécies, fonte de oxigénio, é possível retirar delas compostos para a indústria farmacêutica e fibras para confeção de vestuário, têm um papel muito importante na mitigação das alterações climáticas e na prevenção da destruição dos solos. A possibilidade de extinção algumas espécies vegetais é real, existindo uma crescente preocupação com a preservação da flora terrestre. Neste contexto, a conservação de germoplasma através de bancos de sementes, constitui assim, um seguro contra a extinção das plantas no seu habitat natural, e no caso de uma espécie ser destruída em todos os outros lugares, ainda existirão sementes dessa espécie para propagação. Um banco de sementes é também uma fonte de material de origem e qualidade controladas para investigação científica.

Palavras-chave: conservação *ex situ*, conservação de sementes, testes de viabilidade.

INTRODUCTION

The need to conserve seeds goes through the need to conserve plant genetic resources, in this case, *ex situ*, and started by the mid-twentieth century as a reaction to the rapid loss of agricultural biodiversity, mainly due to the replacement of landraces by improved varieties (Díez *et al*., 2018). The *ex situ* conservation aims to conserve vegetable collections outside their natural habitat and can occur in complete plants, plant propagules, seeds, pollen, tissues or cell cultures forms (Frankel *et al*., 1995). These processes allow the maintenance of germplasm, which is characterized by all the material capable of transmitting hereditary characters from one generation to another (Witt, 1985).

According to Draper *et al*. (2004) the conservation of genetic resources is a priority worldwide, given that a global change is facing, where ecosystems face great climatic changes, strong anthropogenic pressures, erosion and loss of genetic diversity. Something that is happening at a faster rate than the knowledge of existing species, and the role they play in maintaining ecosystems (Rosa, 2002). And the disappearance or impoverishment of ecosystems leads to the extinction of other species that depend on them, creating a cycle of destruction (Carapeto, 1998).

Biodiversity is, moreover, used as a way of measuring human impact on the planet, and is an indicator, par excellence, of the sustainability level of societies (Rosa, 2002). The flora being used as a privileged indicator of the conservation state of a given location (Silva, 2012).

There are considerable advantages in *ex situ* conserving genetic resources, namely through the establishment of seed banks, compared to other conservation methods, due to the ease of storage, space saving, and the capacity to maintain large quantities of samples at an economically viable cost (BGCI, 2019). Notions, which are in agreement with what Wyse Jackson and Akeroyd (1994) defend when stating that seeds are a practical way of conserving plant genetic diversity, due to the possibility of having small samples, of easy handling, which require little care maintenance and that can be preserved in the long term, remaining viable for a long time, for tens to hundreds of years.

However, it was already in the second half of the XX century, as referred in the beginning, and under the influence of the Food and Agriculture Organization of the United Nations (FAO), that initiatives of *ex situ* germplasm conservation began to appear. And it was with the creation of the Inernational Board of Plant Genetic Resources (IBPGR), in 1974, that numerous gene banks in the form of seeds were established, worldwide, in order to make them available for future revegetation programs, rehabilitation of population, or areas where genetic diversity has been reduced, if necessary (Draper *et al*., 2004).

It is important to subject the seeds to some processes (Hong and Ellis, 1996; Draper *et al*., 2004; ENSCONET, 2009; Silva, 2012) before their *ex situ* conservation, so that they can be preserved in the best possible way.

Considered that, this paper aims to review the: i) seed preparation for conservation; ii) seed conservation; iii) seed viability tests; and iv) seed dormancy to conserve plant genetic resources in *ex situ*.

SEED PREPARATION FOR CONSERVATION

The seed preparation for conservation involves some steps, namely, cleaning, drying, packaging and the conservation. But, this process is only feasible for orthodox seeds, which are characterized by survival to drying and freezing. With regard to recalcitrant seeds, which are characterized by susceptibility to dehydration and low temperatures, the challenge is greater. This is because its sensitivity to water losses makes it necessary to store it with a high moisture content (Chin *et al*., 1989; Berjak and Pammenter, 2003), which favors the attack of microorganisms and the possibility of germination during storage. Resorting to low temperatures could inhibit these problems, but they are not the solution, because recalcitrant seeds are also damaged by temperatures close to or below zero (Chin *et al*., 1989; Berjak and Pammenter, 2003). In these plants, germplasm must be conserved in ways other than through seed banks.

Cleaning and Drying

Cleaning consists of eliminating residual materials that often involve the seeds (eg. fruit pulp).

Drying is a process that consists of reducing the seed water content to minimum levels of metabolic activity, and thus avoiding their rapid deterioration, also avoiding the proliferation of fungi and other phytophagous agents that can alter the seeds quality (Draper *et al*., 2004). The seeds should be dried as quickly as possible to prevent them from deteriorating. The length of the drying period will depend on the seed size and its initial water content, the size of the sample and the degree of relative humidity of the drying environment (FAO/IPGRI, 1994). Ideally, the drying atmosphere should be in relatively low temperature and humidity conditions (≤15 ºC and 10 % RH) (Hong and Ellis, 1996).

Packaging

The packaging concerns the way in which the material is stored. The choice of the storage container depends on the characteristics of the seeds, the time to be preserved and the conservation conditions (Santos and Bettencourt, 2001). For this there is a wide range of containers with the most varied characteristics, from paper, aluminum envelopes, glass, plastic or metal bottles (Draper *et al*., 2004; Groot *et al*., 2015).

SEEDS CONSERVATION

Conservation is effectively the seeds storage. In this phase, it is intended to ensure that the seeds metabolic activity, and consequently, the processes involved in the loss of viability and vigor, are reduced (Draper *et al*., 2004).

There are two types of conservation as a function of time, short/medium term and long term. According to Santos and Bettencourt (2001), if the objective is to conserve seeds in the medium term, they can be kept between 0 ºC and 15 ºC with moisture contents between 3 to 7 %, with a viability of not less than 65 %. According to these authors, the seeds can still be preserved for 70 to 100 years, with a viability of not less than 85 %, if kept at temperatures between -10 ºC to -20 ºC with a water content between 3 to 7 %.

The problem associated with seed conservation has to do with its deterioration over time, which can result in its inability to generate new plants (Groot *et al*., 2015). Thus, it is important to adopt strategies that allow the long-term seeds viability to be prolonged, that is, to conserve them. Seeds conservation techniques have the objective of delaying the moment of seed death as much as possible (Gómez-Campo, 2002).

According to Cárdenas *et al*. (2004), the collections maintenance must be done in such a way that they undergo minimal changes in their genetic composition and, in turn, are available when requested.

In this sense, several studies point as fundamental the material conservation at low temperature and with low moisture contents (Desai *et al*., 1997; Santos and Bettencourt, 2001; Draper *et al*., 2004), being through the combination between these two factors that it is possible to prolong the seeds viability beyond their time of natural viability (Draper *et al*., 2004).

In studies based on thermodynamic principles and in the evaluation of the seeds vigor stored in the short term (some months) at 35 ºC, it was found that a moisture content in equilibrium with 19-27 % RH is ideal for the seeds longevity, and that drying the seeds with an RH between 10-12 % would be detrimental to their longevity, especially when stored at low temperatures (Vertucci and Ross, 1990, 1991, 1993; Vertucci *et al*., 1994). However, for airtight storage of 1000 days (about 2 years and 7 months) depending on the temperature, it has been suggested that the moisture content be reduced from an equilibrium with 19-27 % to an equilibrium with 15-20 % RH (Walters-Vertucci *et al*., 1996; Walters, 1998; Walters *et al*., 1998).

However, despite, traditionally, low humidity and low temperature being considered key factors, Groot *et al*. (2015) stated that they are not sufficient to prolong the seeds viability for considerable periods of time. These authors verified that it is evident that the deterioration of the seeds is predominantly

affected by oxidative processes, and as such, its storage in conditions of anoxia is important. Something that has been proven through a test by Groot *et al*. (2012), where seeds were subjected to oxygen under pressure, an artifice to recreate the effect of oxidation in the short term. And that is in agreement with what was described by Abdalla and Roberts (1968) who showed that the increase of oxygen levels during the seeds storage, resulted in more chromosomal irregularities during cell division, induced, apparently, by the cumulative effect of DNA oxidation during the storage period.

However, negative effects on the seeds viability of anoxia with relatively high humidity levels have been recorded (Robert and Ellis, 1989). This is because, with moisture, the seeds are metabolically active and the lack of oxygen results in asphyxia and anaerobic respiration, accompanied by the production of toxic acetaldehyde and ethanol (Groot *et al*., 2015). However, in studies with dry seeds, Barzali *et al*. (2005), González-Benito *et al*. (2011) and Schwember and Bradford (2011), found neutral or positive results in the seeds longevity stored in the absence of oxygen.

Even so, seed deterioration can also occur if they are excessively dry (Groot *et al*., 2015), but according to Walters and Engels (1988), the reduction in the longevity of seeds preserved in ultra-dry environments, occurs in the presence of oxygen. Something that was also observed by Steiner and Ruckenbauer (1995), Hong *et al*. (2005) and González-Benito *et al*. (2011), who report that they did not register declines in the quality of ultra-dry seeds stored hermetically, therefore, in an oxygen-free environment or with limited oxygen levels. And Bass and Stanwood (1978), Ellis *et al*. (1993, 1996) and Steiner and Ruckenbauer (1995) even found that, at room temperature, ultra-dry storage is more advantageous, compared to conventional dry storage (hermetically).

This concept of "ultra-dry" began to be used by the International Board for Plant Genetic Resources (IBPGR), in view of the results of studies that quantified seed longevity according to a wide range of humidity levels, some of them quite low (± 1%), in the storage of these (Hong *et al*., 2005).

The seeds are so susceptible to oxidation, that in the food industry it is quite common for seeds (nuts, almonds, pumpkin, sunflower, ...) to be sold in airtight packaging (impermeable to oxygen) and/or with a modified atmosphere, to avoid the development of a "rancid" flavor that is caused by lipid oxidation, because the seeds contain polyunsaturated fatty acids (in varying contents, depending on the seeds) that are very susceptible to oxidation (Allen and Hamilton, 1994).

The effect of oxygen damage on seed conservation is increasingly accepted (Rajjou and Debeaujon, 2008), as shown by studies by Gane (1948), Shrestha *et al*. (1985) and Barzali *et al*. (2005), where they compared the seeds longevity conserved under different atmospheres and/or their absence. However, there are those who have obtained contrary results, such as those of Bass and Stanwood (1978), who did not find significant differences in the germination of *Sorghum bicolor* (L.) Moench seeds stored with atmospheric air, nitrogen, carbon dioxide, helium, argon or vacuum.

González-Benito *et al*. (2011) suggest that the contradiction between results is due to the length of the storage/conservation period, and the possible improvements in seed storage under controlled/modified atmosphere conditions are not reflected in the medium term, but in the long term.

Despite the wide range of containers where seeds can be stored, these containers may differ in terms of oxygen permeability, while some impermeable ones may include considerable amounts of oxygen. Therefore, even when airtight containers are used, the amount of oxygen included must be limited or reduced, there are several methods that can be used to create anoxic conditions or decrease the oxygen concentration in storage (Groot *et al*., 2015), namely:

i) *Modified atmosphere* - this methodology consists of replacing the natural atmosphere in the container, with another more appropriate for the product preservation. In other words, the atmosphere is replaced by a gas or a mixture of gases that better protect the product, slowing down the product's deterioration process, resulting not only from its normal metabolism, but also from the action of anaerobic organisms.

ii) *Vacuum conservation* - consists of extracting air from the storage packaging, therefore, in the absence of atmosphere.

iii) *Inclusion of oxygen absorbers* - the oxygen levels inside the seed storage containers, can also be reduced by the inclusion of oxygen absorbers, whose active ingredient is iron powder.

The airtightness of the containers is such a sensitive issue that according to Baccheta *et al*. (2008), for the long-term conservation/storage of collections of rare and/or endemic species, glass ampoules obtained from folded and flame-sealed glass tubes are recommended. However, for collections that are intended to be handled relatively frequently, containers are needed that can be opened and closed easily. In this context, Groot *et al*. (2015) studied six containers types, and found that glass bottles with a rubber ring between the lid (also glass) and the bottle, as well as glass bottles with a twisted metal lid lined on the inside with a layer of flexible "plastisol", are the ones that offer greater impermeability to oxygen. This is in contrast to plastic containers and glass bottles with plastic lids, which are permeable to oxygen to varying degrees. These authors also warn that when using plastic containers or with plastic closures, the type of plastic must be carefully considered. Although all plastics have a degree of oxygen permeability, the level can vary widely between types of plastic. For example, Massey (2003) states that the permeability of polypropylene is 50 times higher than that of polyethylene terephthalate.

In short, to prolong the longevity of seed samples *ex situ* conserved, they should be stored in a cool and dry environment, with no availability or with limited availability of oxygen, as soon as possible after collection and drying (Groot *et al*., 2015).

However, it is essential that the stored seeds have the capacity to germinate and be used whenever necessary, as such, it is important to carry out viability controls periodically (Draper *et al*., 2004).

VIABILITY TESTS

Viability tests are tests to determine the potential of the seed sample to produce normal and healthy seedlings. There are several methods to analyze seed viability but the most common are the germination tests, and for that it is enough to place the seeds in favorable environmental conditions to the germination, namely, humidity, light, temperature and oxygen, whose needs vary from species to species (Baskin and Baskin, 2001; Maciel, 1994).Viability tests are carried out as a way of determining whether the seed are alive or not (Gosling, 2003).

According to the International Seed Testing Association (ISTA, 2006), there are several ways to determine the seeds viability, namely:

i) *Germination tests* - consists of placing the seeds in favorable conditions of humidity, light, temperature and oxygen, and a viable seed that does not present dormancy will germinate in these conditions. According to Garcia and Villamil (2001), the germinative capacity of a seed lot is a direct indicator of its viability.

ii) *Cut or excision test* - consists of cutting the integument and opening the seeds to observe their interior aspect and the embryo degree development. This is, however, a methodology that already requires experience to interpret what is being observed.

iii) *Tetrazolium staining test* - consists of the red staining of the embryo's living cells, by reducing the tetrazolium salt. Neto *et al*. (1998) explain that the principles of this test are based on the enzymes activity that catalyze respiratory reactions in mitochondria during glycolysis and the Krebs cycle. When the seed is immersed in the colorless tetrazolium solution (2,3,5-triphenyl tetrazolium chloride), a tetrazolium salt reduction reaction occurs in living tissues, which results in the formation of a red, stable and non-diffusible compound. The color resulting from the reaction is a positive indication of the seed viability, through the detection of cellular respiration. Non-viable tissues do not react, and as such, do not stain.

iv) *X-Ray Test* - consists of subjecting the seeds to the effect of X-rays, and allows detecting empty, malformed and damaged seeds.

The samples must be regenerated when the viability is less than 85 % (Gómez-Campo, 2009).

It is important to know the characteristics of the plant species and its seeds before submitting them to viability tests, at least with regard to the germination test, because the seeds may have some degree of dormancy, which can interfere with the success of the test.

SEEDS DORMANCY

When performing germination tests, despite being placed in favorable conditions and being viable, some seeds do not germinate because they are dormant.

The seed dormancy term applies, therefore, to the condition of viable seeds that do not germinate despite being provided with suitable conditions. This is a phenomenon that results from the adaptation of species to the environmental conditions in which they reproduce, being a resource used by plants/seeds to germinate at the appropriate time for their development, and which aims at the perpetuation of the species (Vieira and Fernandes, 1997; Nazari *et al*., 2014; Shu *et al*., 2016; Brito, 2020).

There are several types of dormancy that can be caused by several factors, so it is recommended to read the work of Baskin and Baskin (2004), who suggest a modified version of the Russian physiologist Marianna G. Nikolaeva for the seed dormancy classification.

In some cases it may be necessary to subject the seeds to pretreatments to break dormancy and facilitate germination. In this context, thermal, physical, chemical and osmotic methods have been applied for this purpose (Sozzi and Chiesa, 1995; Ren and Tao, 2004; Sadeghi and Rasouli, 2012). Technological solutions for breaking seed dormancy have also been suggested, such as "ultrasonic waves" and "magnetic water" (Yaldagard *et al*., 2008; Fateh *et al*., 2012; Nazari *et al*., 2014).

FINAL CONSIDERATIONS

A seed bank allows the seeds preservation and conservation in general, having a marked importance when dealing with species at risk of extinction.

However, the problem of ensuring the viability of the material is faced, and due to the considerable variety of types of seeds, the standardization of processes. Hence the need to establish the temperature, humidity, aeration and light control parameters necessary for their conservation and storage, thus the bank's management and management protocols (Villota *et al*., 2018).

Another important feature is that a seed bank must be a deposit of high density of viable seeds, capable of being used in the species maintenance or (re) establishment after natural and/or anthropogenic disturbances. It is difficult to define concrete numbers, but the ideal is to maintain a reasonable seeds amount depending on the species dynamics.

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