



How to store seeds or prepare them for germination



Control of the Seed quality has a critical effect on the quality of the trees established

Food and Agriculture Organization of the United Nations (1985)

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1. Sociedade Chauá is a NGO working for the conservation of natural ecosystems and biodiversity in Paraná, Brazil: http://www.chaua.org.br/

Introduction

A good source of viable seed is a first step towards effective germination, growth and survival of threatened tree seedlings. After collecting seed from the wild there are a number of steps you should take to reduce losses of seed viability. These include extracting seeds from their fruits, assessing viability, storing seeds and/or applying pre-germination treatments. The purpose of this brief is to provide basic guidance on these processes to help maximise the effectiveness of seed collections for the conservation of threatened trees.

Who is this guidance for?

This brief is for individuals in conservation organizations (NGOs, forest departments, botanic gardens and protected-area managers) or anyone tasked with conservation and restoration of threatened tree species. Specialised training is not required, but some basic skills, listed on Page 2, should be present within your team.

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Before you start

This brief complements guidance provided by <u>GTC brief 5</u> on seed collection. Before you begin the task of preparing seed for storage or germination take the time to (1) research your target species; (2) ensure your team has the right skills and (3) set up an appropriate facility with the necessary equipment.

STEP 1: Know your target species in advance

Different tree species produce seeds with wide-ranging requirements for extraction, storage and germination treatment. The following provides a general guide for these processes but does not go into detail for individual species.

Therefore, it is essential to gather any information you can from the published literature on your target species. You should also contact botanic gardens, seed banks and herbaria or consult specialists. Two important questions to answer include:

- 1) Does your target species produce orthodox seeds (that can be stored for long periods of time) or recalcitrant seeds (that cannot be stored for long periods)?
- 2) Do the seeds from your target species exhibit dormancy, and if so, how is it broken?

In cases when you cannot find information on your target species, try researching closely related species that may have similar seed ecology.

STEP 2: Make sure your team has the right skills

No specific technical skills are required for basic seed processing and storage. However your team needs to be highly organised and should have experience with record keeping and data management.

For more technically difficult species (including those with recalcitrant seeds or those with orthodox but dormant seeds), you should have someone in your team with knowledge and experience in seed biology.

YOUR FACILITY	EQUIPMENT
 A small building to act as your seed laboratory Access to water Space for storage A workspace Access to electricity 	 Air tight containers Breathable containers Sieves for separating seeds from fleshy fruits Mortal and pestle for extracting seeds Knives for extracting or scarifying seeds Cement mixer - for extracting or scarifying high numbers of seed Wire mesh trays for drying fruits and seeds Kiln for drying fruits and seeds* A refrigerator for storing seeds Plastic containers for storing seeds Acids and chemicals for treating seeds - to be handled with extreme care!
* Don't worry if you can't afford this. For many species, basic drying of seeds and fruits can be achieved with minimal equipment. See Page 4 and 6 for guidance on drying.	

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STEP 3: Establish facilities and acquire equipment



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Seed extraction and cleaning

In many cases, seeds need to be removed from the fruit before they can be stored or used. Extraction procedures vary depending on the type of fruit. Below we highlight basic procedures for three types of fruit: fleshy, dry dehiscent (hard fruits that open to release their seeds upon maturity) and dry indehiscent (hard fruits that remain closed even when mature).

Extracting seeds from fleshy fruits

Fleshy fruits need to have their pulp removed immediately after collection to avoid fermentation and heating. In general, depulping can be achieved by a combination of soaking fruits in water and by applying pressure to the fruits with gentle abrasion.

Fruits with **small amounts of seed** and soft pulp can easily be macerated by hands.

When you are dealing with **larger amounts** of seed it may be more time efficient to macerate the fruits by rubbing them against a metal screen under a flow of water.

For fruits with **harder pulp** it might be necessary to first immerse them in water for 12 - 24 hours (or longer) until they are soft enough for abrasion or maceration.

Once depulped, the residual pulp can be separated from the seed by washing it through sieves or by hand. Alternatively, put the seeds in a bowl filled by slow running water and let the seed sink and the residuals rise to the surface.

The next step involves drying seed removed from the fleshy fruits, always in a shady and well-ventilated place, on canvas or on paper. Seeds should be stirred regularly to promote homogenous drying.

Example seed extraction methods for a fleshy fruit *Plinia trunciflora*



Mascerating fruit by hand



Washing seeds



Initiating the drying process





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Dry dehiscent fruits can be induced to release their seeds using one of these three methods:

Dry fruits under cover in a well-ventilated room. Spread fruits on a solid surface or, preferably, place them on trays with a wire mesh bottom to allow all-round air circulation. Fruits should be stirred regularly.

Dry fruits in the sun by spreading them on a canvas or in trays (preferably in a place with some shade and sufficient air circulation). Stir the fruits at least twice per day to ensure drying is homogenous. Take care to avoid exposing fruits to temperatures exceeding 35 – 40°C. At night, seeds should be brought inside or covered with tarpaulin.

Dry fruits with artificial heat provided by special kilns fitted with heat bulbs or fans to circulate hot air around trays of fruit. Air temperatures should not exceed 50°C to avoid killing seeds (although the temperature requirements of each species may vary slightly). Kiln drying is usually preceded by a period of air-drying.

Examples of a dry deshiscent fruit Lafoensi pacari, with extracted seeds (far right)



Dry indehiscent fruits must be broken mechanically to extract the seeds. Possible methods include:

- Opening fruits by hand
- Crushing in a mortar
- Using scissors, knife or machetes

Example of a dry indeshiscent fruit *Machaerium vestitum* with extracted seeds (far right)

- Beating with a metal rod
- Rolling over them with a drum
- Threshing fruits in a cement mixer



Regardless of the method used, take care to minimise damage to the precious seeds you are trying to extract from the disposable fruits.

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Assessing seed viability

After seeds are extracted from fruits, but before attempting storage or germination, assess the viability of your seeds.

You can use the results from your tests to (a) select the highest quality seed for subsequent germination or storage or (b) develop an estimate of viability from the seeds collected from different locations or individual mother trees.

If you aim to aim to assess viability for a particular species, location or tree, use a random sample of at least 100 seeds.

Percentage Viability

Number of healthy, developed, full seeds x 100

Total number of seeds in the sample

Below are three basic methods for assessing seed viability. Each test has limitations, but may act as useful guide when no protocols are available for your species.

VISUAL TEST: Inspect the consistency, shape, size and colour of the seeds.

GOOD SIGNS:

Plump, stiff seeds of expected colour and size for the species



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FLOATING TEST: Place seeds in water

GOOD SIGNS:

Seeds that sink



BAD SIGNS:

Evidence of insect predation or exudates.

Withered or brittle appearance with immature colours.

Example species *Lafoensia Pacari*

BAD SIGNS:

Seeds that float



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Example species **Drymis angistifolia**

CUTTING TEST*: Seeds are cut with a blade or knife in two or three sections (longitudinal, transversal and near the ends).

GOOD SIGNS:

Evidence of well-developed embryo and regular endosperm colour.



BAD SIGNS: Lack of embryo



Example species *Lafoensia Pacari*

*Cutting destroys individual seeds so should only be carried out on a small selection of seeds to provide a representative sample of viability.





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Storing seeds

Did you know?

If you don't plan to sow seeds immediately after processing, they can be stored to avoid loss of viability and to provide an ex-situ collection to be used for in-situ conservation purposes at a later date.

In general, storage can be prolonged by cold temperature, low moisture content and low oxygen level.

The storage capacity of seeds can be divided into two:

Orthodox seeds can be dried (down to moisture content levels as low as 5%), kept at low temperatures (0-5°C) and, under optimal conditions, can be stored for several years, or even decades, without losing their ability to germinate. **Recalcitrant** seeds, on the other hand, cannot survive at low levels of moisture content or at low temperatures and subsequently cannot be stored for long periods of time. Many tropical recalcitrant seeds can tolerate minimal drying but only up to moisture content levels of 20–35% and they generally do not survive temperatures lower than 12–15°C. Under the right conditions it is possible to store seeds from some recalcitrant species for a few days or, in some cases, up to several months.

Kew's Seed Information Database (http://data.kew.org/sid/) contains information on seed storage behaviour for more than 10,600 species.

Drying seeds for storage

Drying is the principal method for preparing seeds for storage. Seeds can be dried outside in sunny conditions (although not directly under the sun) or through artificial heating. Your aim is to reduce the moisture content of the seed to a level appropriate to your target species. Links to guidance provided by the Millennium Seed Bank on (a) how to measure content levels and (b) how to equilibrate seeds to specific moisture content are detailed on page 8.

Once dried, orthodox seeds should be stored in air-tight containers to avoid any re-gain of moisture. Potential containers include transparent plastic bags, glass jars and bottles or plastic or metal drums, barrels or jerry cans.

Recalcitrant seeds from some species can tolerate a small amount of drying but containers for recalcitrant seeds must allow some gaseous exchange. Potential containers include bags made of cotton, hessian, burlap fabric or polyethylene.

Containers with dried seeds should be held in storage units. A simple and relatively cheap option is to store seeds in a refrigerator or freezer or inside air-tight plastic barrels with natural desiccants such as rice or maize inside. A more expensive option is to place them in dry chambers, which allow you to control both humidity and temperature.



Storage methods will vary from species to species. Take accurate records of your successes and failures to help your team optimise its storage procedures over time.

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Preparing seeds for germination

Before attempting to sow your seeds, go back to your research and consider whether they require any additional treatment to enable germination to take place.

Many species exhibit a period of **dormancy** that needs to be broken before seeds are able to germinate. Dormancy exists as a mechanism to help seeds synchronise timing of germination with the climatic and environmental conditions that will favour survival and growth. It is controlled by either **endogenous factors** (i.e. a property of the embryo) or **exogenous factors** (i.e. a property of the seed coat).

In natural systems, dormancy is broken by changes in temperature, light, fire, frost, drought or by being passed through the gut of animal. Under artificial conditions, **stratification** or other pre-sowing treatments may be required in order to mimic this process and ensure a rapid and uniform seedling establishment.

Overcoming endogenous dormancy

A process known as stratification may be used to mimic particular seasons that seeds have to go through to trigger germination. For some dryland species, seeds must be exposed to a **warm stratification**, of temperatures as high as 50°C. Other species require **cold stratification**, kept for several weeks at temperatures lower than 5°C in order to mimic winter seasons.

Other options include surgically removing seed tissue close to the embryo, thereby removing a physical mechanism inhibiting seedling growth. This method requires an understanding of seed structure to avoid damage.

Overcoming exogenous dormancy

Removing of part of the seed coat (known as **scarification**) aids water uptake and can help break dormancy. For small samples of seed, press seed against an abrasive surface, or carefully cut part of the seed coat with a knife, sandpaper or a file (take particular care to avoid damaging the embryo). For large amounts of seeds, place them in a concrete mixer with coarse sand and stones.

Other methods used for overcoming exogenous dormancy include:

- Soaking seeds in hot water up to a temperature of 90°C
- Drying seeds in a heat oven
- Soaking seeds in chemicals such as hydrogen peroxid sulfuric, hydrochloric or nitric acid.
 Great care, from skilled workers, is needed in the handling of these substances.
- Mimicking natural fire by applying temperatures of over 100°C alongside smoke

As strategies to overcome dormancy involve risking a loss of seed viability, test your chosen methods on a small batch of your seed. Refer back to your research on your species to help you decide whether, and if so how, you should carry out pre-sowing treatments for your species.

When your seeds are ready for germination see <u>GTC Brief 7</u> for guidance on how to germinate seed and care for young seedlings.

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Selected references and further guidance

References and further guidance on some of the methods described in this brief are provided below.

Guidance on seed handling:

Aguiar, I.B., Piña-Rodrigues, F.C.M. and Figliolia, M.B. (1993). Sementes Florestais Tropicais. *Brasília: Associação Brasileira de Tecnologia de Sementes - Comitê Técnico de Sementes Florestais*, p.349.

Auer, C.G. and Graça, M.E.C. (1995). Método para seleção de mudas de canela-sassafrás a partir de fragmentos florestais remanescentes. *Boletim de Pesquisa Florestal,* Colombo, n. 30/31, p. 75-77.

Schmidt, L. (2002). Guide to Handling of Tropical and Subtropical Forest Seed. Danida Forest Seed Centre, p.511.

Willian, R.L. (1985). A guide to forest seed handling. FAO Forestry Paper 20/2, p.379.

Guidance on drying seeds

Millennium Seedbank Project – Technical Information Sheet 05 – Measuring seed moisture status using a hygrometer: http://bit.ly/gtc_ref_6a1

Millennium Seedbank Project – Technical Information Sheet 07 – Low-cost monitors of seed moisture status: http://bit.ly/gtc_ref_6b1

Millennium Seedbank Project – Technical Information Sheet 08 - Small-scale seed drying methods: http://bit.ly/gtc_ref_6c1

Millennium Seedbank Project – Technical Information Sheet 09 - Equilibrating seeds to specific moisture levels: http://bit.ly/gtc_ref_6d1

Guidance on seed storage behaviour

Kew seed information database: http://bit.ly/gtc_ref_6e

Millennium Seedbank Project – Technical Information Sheet 10-Identifying desiccation-sensitive seeds: http://bit.ly/gtc_ref_6f1

Guidance on overcoming seed dormancy

Willan R.L. (1987). A Guide to Forest Seed Handling, with Special Reference to the Tropics. Rome, Food and Agriculture Organization of the United Nations. **Available at: http://bit.ly/gtc_ref_4d**

Guidance on assessing seed viability

USDA Forest Service's Woody Plant Manual - Chapter 5 - Seed testing: http://bit.ly/gtc_ref_6h

For more information, or to download the other briefs in this series, visit www.globaltrees.org/resources/practical-guidance

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