

What cannot be frozen

Recalcitrant and clonal species, field genebanks, in vitro culture and cryopreservation

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Abstract

The seed bank is the backbone of ex situ conservation, but it presupposes that seed survives drying and cold. For a considerable part of the plant world this does not hold: around 8 per cent of species produce recalcitrant seed that dies when dried; among critically endangered species the figure is 36 per cent. Added to this are the clonally propagated crops — potato, cassava, banana, fruit trees — whose varieties cannot be kept true to type through seed. This paper classifies the four behaviour types, describes the three alternative routes of field genebank, in vitro culture and cryopreservation together with their failure modes, and derives a principle: every holding needs a main route and a conservation route independent of it.

1. Four behaviour types

Type	Behaviour	Examples
Orthodox	tolerates drying to a few per cent moisture and storage below freezing	wheat, barley, maize, bean, tomato, cabbage
Intermediate	tolerates drying only to a limit, cold-sensitive when dried	coffee, some citrus species, papaya
Recalcitrant	dies when dried	oak, horse chestnut, sweet chestnut, cocoa, avocado, mango
Clonal or seedless	variety maintained through tubers, cuttings or grafting	potato, cassava, sweet potato, yam, banana, fruit trees

The first three types describe the seed, the fourth the propagation. An apple variety produces orthodox seeds, but what grows from them is not the variety but a seedling with a new combination of traits. For conserving the variety, seed behaviour is therefore irrelevant; what counts is that it must be passed on clonally.

The classification is the first decision of any conservation effort, and it cannot be reversed: a recalcitrant lot dried for storage is lost. If the behaviour is unknown, the first step is to consult the Seed Information Database (now operated by the Society for Ecological Restoration, built by Kew) and, where there is no entry, to carry out a drying test on a subsample — never on the whole lot.

2. How large the gap is

Wyse and Dickie estimate the share of seed plants with desiccation-sensitive seed worldwide at around 8 per cent. That sounds marginal, but it is unevenly distributed. According to the analysis by Wyse, Dickie and Willis (2018):

Group	Share that cannot be secured in a seed bank
Critically endangered species	36 per cent
Vulnerable species	35 per cent
Endangered species	27 per cent
All tree species	one third
Canopy trees of tropical moist forests	about half

The gap therefore lies exactly where the need for conservation is greatest: among threatened species and among trees. In Central Europe this includes oak, horse chestnut and sweet chestnut; among food plants worldwide avocado, cocoa and mango.

3. Three alternative routes

3.1 Field genebank

The plants stand in the open, as trees, shrubs or perennial crops. For many woody species and clonal crops this is the only route that works immediately, and the material can be seen, checked and used at any time.

The field genebank, however, carries the full site risk: disease, pests, storms, drought, loss of land. It requires much space and staff, and a single pathogen can hit a collection built up over decades. A field genebank without a duplicate at a second site is a collection with built-in total loss.

3.2 In vitro culture

Shoot tips or small plantlets are kept sterile on culture medium, usually under conditions that slow growth. The material is disease-free, space-saving, protected from weather and easy to exchange.

The price is permanent labour: cultures must be subcultured regularly, and over long periods the risk of genetic change and contamination grows. In vitro culture is therefore a good working and exchange collection, but not long-term security.

3.3 Cryopreservation

Shoot tips, embryos or embryonic axes are stored in liquid nitrogen or nitrogen vapour at minus 196 degrees. At this temperature metabolism practically stops. Once established, storage is genetically stable, space-saving and needs little monitoring; the CGIAR genebanks describe it as a cost-effective alternative to labour- and cost-intensive in vitro maintenance and use it routinely for banana, potato, sweet potato, cassava and yam.

According to its own information, the International Potato Center cryopreserves more than 450 potato accessions a year using droplet vitrification of shoot tips and has applied strict quality standards since 2013: a thawed shoot tip must develop into a normal plant with apex, stem, leaves and roots, without intermediate callus formation.

The limits are equally clear. Cryopreservation requires knowledge of stress physiology, developed infrastructure and trained staff; protocols are species-specific and still lacking for many species. The nitrogen supply must be uninterrupted. For recalcitrant species, cryopreservation of embryos or embryonic axes is possible but far less tested than for clonal crops.

4. Comparison

Route	Suitability	Effort	Failure risk	Failure mode
Seed bank	orthodox	low	low	equipment, regeneration backlog
Field genebank	recalcitrant, clonal	high	high	site, disease, weather
In vitro culture	clonal, partly recalcitrant	high	medium	contamination, genetic drift, staff
Cryopreservation	clonal, partly recalcitrant and intermediate	medium once established	low	nitrogen supply, missing protocols
In situ and on farm	all	medium	high	land loss, change of use, climate
Botanic garden	wild species, woody plants	medium	high	site, narrow genetic base

Effort and risk are comparative classifications between the routes, not cost figures. A reliable cost calculation depends on species, protocol and site; the literature refers to separate feasibility studies for this. Published individual figures are given in section 4.1.

4.1 What the routes cost

The following cost figures come from individual institutions and rest on different price bases. They indicate orders of magnitude, not transferable rates.

Route	Value	Basis	Source
Seed bank, storage	under 1.50 US dollars per accession and year (maize 2.16)	CGIAR genebanks, US dollars of 2000, storage only	Koo, Pardey and Wright 2003
Seed bank, in perpetuity with monitoring and regeneration	present value of around 10 to 141 US dollars per accession (wheat 10, maize 141)	CGIAR, US dollars of 1996, present value in perpetuity	Smale 2019
In vitro collection	11.98 US dollars per accession and year	cassava at CIAT, US dollars of 2000	Koo, Pardey and Wright 2003
Field genebank	labour costs of 38.26 euros per accession and year, around 81 per cent of total costs	garlic at IPK Gatersleben	Keller et al. 2013
Cryopreservation, establishment	around 320 to 430 euros per accession (garlic); 480 US dollars (potato, CIP)	IPK Gatersleben; CIP at 500 accessions stored per year	Keller et al. 2013; Major, undated

Route	Value	Basis	Source
Cryopreservation, maintenance	20.88 euros per accession and year (garlic, 100 accessions); around 2 US dollars (CIP)	IPK Gatersleben; CIP	Keller et al. 2013; Major, undated
Cryo instead of field: recovery of establishment costs	after 8 to 13 years	garlic at IPK Gatersleben, depending on starting material	Keller et al. 2013

The present value for seed bank conservation in perpetuity is listed as partially verified in the data layer; it is available as reproduced from Koo, Pardey and Wright (2004) in Smale (2019). The other figures are verified. The *Conservation Pathway Guide* now also shows this cost table.

Around 400,000 accessions are held worldwide in field genebanks (Panis, Nagel and Van den houwe 2020, reporting a study by the Alliance of Bioversity International and CIAT, CIP and the Crop Trust; figures from before 2020). No global figure for in vitro and cryo collections was found.

5. The principle: two routes

A simple principle follows from the comparison: **every holding needs a main route and a conservation route independent of it**. The main route serves use or adaptation, the conservation route long-term preservation. A holding on only one route shares that route's entire failure risk.

Typical combinations:

Material	Main route	Conservation route
Orthodox seed	seed bank	duplicate at a second site, Seed Vault
Clonal crop	field genebank	in vitro culture, cryopreservation
Recalcitrant species	field genebank or in situ stand	cryopreservation of embryos where a protocol exists
Intermediate seed	field genebank	cryopreservation
Unknown behaviour	as a precaution like recalcitrant	like recalcitrant until the behaviour is clarified

For orthodox seed, the main and the conservation route can be the same method; what is independent is then the site. For all other types a second method is needed, because the only available main route — usually the field genebank — carries the highest failure risk.

The tool *Conservation Pathway Guide* derives both routes from seed behaviour, available equipment and objective, and marks where a conservation route can only be reached through a partner institution.

6. What this means for small organisations

Conservation associations, networks and community seedbanks rarely have a tissue culture laboratory and almost never access to liquid nitrogen. For them the principle means in practice:

1. **Orthodox seed:** store it yourselves and give a duplicate to a genebank.
2. **Clonal varieties:** keep them at at least two widely separated sites — two orchards, two farms — and, for the most threatened varieties, find an institution with in vitro or cryo capacity as a partner.

3. **Recalcitrant species:** do not store them, but maintain them as stands in the open, distributed, with documented origin, and work with botanic gardens and forest administrations.
4. **Unknown behaviour:** never test on the whole lot.

Figures

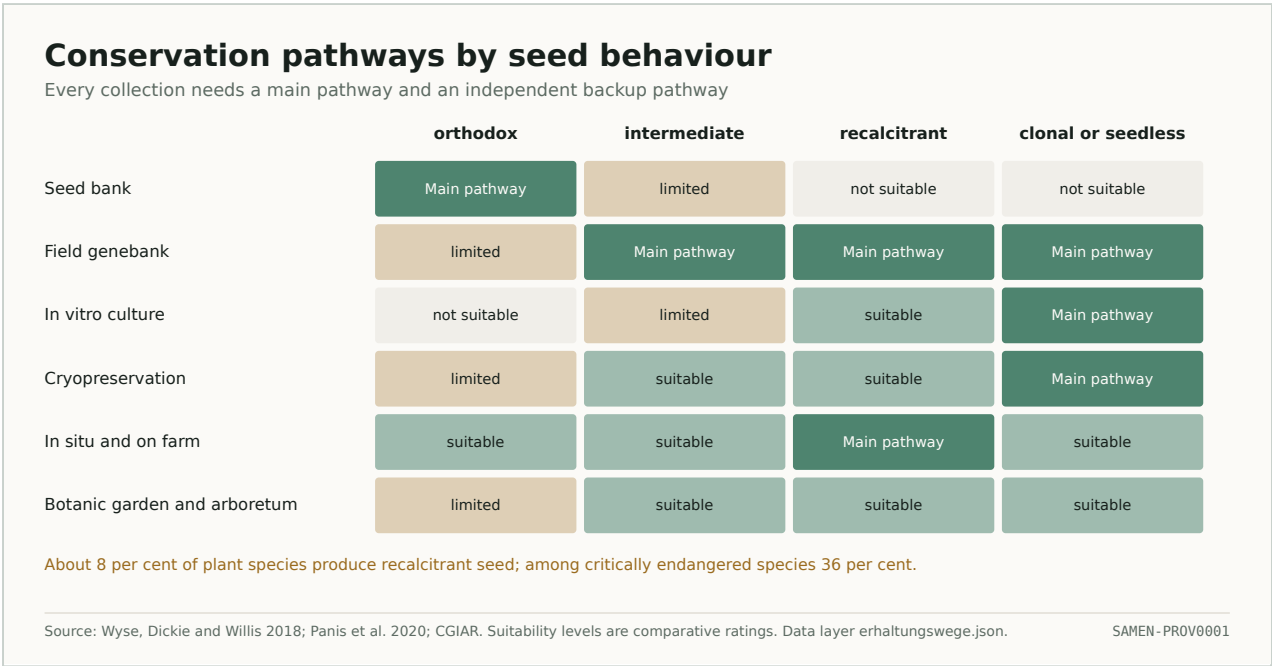


Figure 1. Conservation pathways by seed behaviour. Source: data layer of the strand, see figure footer.

Limits of this paper

The paper classifies conservation routes; it is not a procedural guide for tissue culture or cryopreservation. For that, the FAO practical guides to the genebank standards (cryopreservation; species producing non-orthodox seeds, both 2025) and the protocols of the respective institutions should be consulted. The cost figures in section 4.1 are published individual values on different price bases; they do not replace a cost calculation of one's own that could be transferred. The suitability levels in the data layer are comparative classifications. For field genebanks a global order of magnitude from before 2020 is available; for in vitro and cryo collections no global figure was found.

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