Handling of Desiccation and Temperature Sensitve Tree Seeds

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HANDLING OF DESICCATION AND TEMPERATURE SENSITIVE TREE SEEDS

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Danida Forest Seed Centre (DFSC) is a Danish non-profit institute, which has been working with development and transfer of know-how in management of tree genetic resources since 1969. The development objective of DFSC is to contribute to improve the benefits of growing trees for the well-being of people in developing countries. The programme of DFSC is financed by the Danish Development Assistance (Danida).

CONTENTS

1. INTRODUCTION

The seed biology of most tropical tree species is today unknown. Since seed handling and storage requirements are very variable, lack of information is a hindrance for seed propagation of many important and potentially important tree species. DFSC has published two manuals for investigation of seed handling procedures and biology: Seed Handling Manual (Technical Note 54) and Laboratory Manual for Basic Tree Seed Studies (Technical Note 57). The present note is a supplement to these manuals providing particular information about seeds that are sensitive to drying and low temperatures, so-called recalcitrant and intermediate seeds.

The main problems with this type of seed are associated with seed quality and storage. The objective of seed procurement is to obtain seeds of high physiological quality. Without sufficient knowledge about how the seeds respond to various conditions, it can be very difficult to produce sound seeds and there will be a risk of damaging the seeds unintentionally during processing, particularly if they are very sensitive to certain conditions. Recalcitrant/intermediate seeds are sensitive to moisture loss and low temperatures, but vary a lot according to how much drying and which temperatures they tolerate; it is therefore important to know the critical and optimal moisture contents and temperatures for each species.

The possibility of storing seeds without reducing the number of living seeds is very important in seedling production of a species. If the seeds have to be sown within days of a collection, it is easy to envisage the problems that may arise at a seed centre, particularly if it is peak seed collection time and several species all have to be collected, extracted and sown immediately. Transport becomes a problem, and for some species collection time is not the most suitable sowing time. Commercial sale of seed with short viability is also difficult. There are therefore many advantages if the seed can be stored, and in practise it may determine whether a species is used or not. Although no methods have yet been found to extend the life period of very recalcitrant seeds to years, it is still worthwhile finding optimal storage conditions. Perhaps it is found that the species can be kept for a couple of months instead of a few weeks, and often species turn out not to be as recalcitrant as anticipated.

In this note, desiccation and temperature sensitivity are explained and examples of species with different seed storage physiology are given (in chapter 2); practical problems associated with handling of desiccation sensitive seeds are described together with advice concerning correct handling (in chapter 3). Finally, chapter 4 includes a simple research protocol for determination of optimal storage conditions.

2. PHYSIOLOGICAL BACKGROUND

2.1 Storage categories

Most agricultural crops have seeds that can be dried and stored at low temperatures for years without losing the ability to germinate. These seeds have been labelled: ‘orthodox seeds’, which reflects that they are considered the most usual and widespread type of seed. However, many tree species, particularly in the tropics, have seeds that do not follow the rules of the orthodox seeds: they are difficult to store because they do not tolerate drying, and have therefore been termed ‘recalcitrant seeds’. Other seeds do not seem to fit into either of these two categories, and are therefore called ‘intermediate seeds’. Some scientists believe that it is possible to catagorize all seeds into these three groups, others think that it is not possible to make well-defined categories; instead, the type of seed physiology should be found somewhere in a continuum from very orthodox seed to very recalcitrant seed. But from a practical point of view, the important point is that there are two factors that are very important for seed storage: **seed moisture content** and **storage temperature**. The current definitions on the three storage categories are given below, followed by examples of species that do or do not fit into the categories.

Orthodox seeds tolerate drying (desiccation) to low moisture contents, they also tolerate storage at low temperatures, and can generally be stored for very long periods. Examples of orthodox tree seeds are Pine and Acacia seeds.

Recalcitrant seeds are damaged by desiccation and tropical species may also be damaged by exposure to low temperatures. They are said to be desiccation and chilling sensitive.

Intermediate seeds are seeds that do not fit into the above two categories. They can be desiccated, although not to as low levels as orthodox seeds, and they are often chill sensitive.

Examples of species with non-orthodox storage physiology:

Hopea odorata is a typical tropical recalcitrant species. It is very desiccation sensitive and does not tolerate temperatures below 15°C.

Quercus robur fits the description of a typical temperate recalcitrant species. It is desiccation sensitive, but tolerates storage temperatures of -2°C.

Carica papaya seeds tolerate desiccation to 10% moisture content but are sensitive to low temperatures; they have therefore been categorized as intermediate seeds.

Cinnamomum cassia is desiccation sensitive but tolerates storage at 5°C.

Astronium graveolens is desiccation tolerant, but only tolerates low temperatures when the moisture content is below 10%.

Azadirachta indica (neem) has been very difficult to categorize. Studies of desiccation tolerance and storage condition have been made by many research groups, but with contradictory results, so the species has been classified as orthodox, intermediate as well as recalcitrant. This may partly be explained by differences in fruit maturity, as seeds from mature yellow fruits are more desiccation tolerant and store better than seeds from immature fruits. Recent research has demonstrated that imbibition injury after rehydration of desiccated seeds is a problem, not desiccation in itself. Furthermore, low temperatures do not pose a problem if the seed moisture content is below 10%. Finally, it should be kept in mind that genetic variation between provenances probably also plays a significant role.

Although recalcitrance is predominant in some plant families such as Dipterocarpaceae, there seems to be no phytogenetic pattern and often two closely related species show very different storage behaviour.

The conclusion is that it is important to investigate each species with unknown storage physiology, before drying or exposing the seeds to low temperatures. Table 2.1 lists some characteristics that may help to give an idea about storage behaviour.

Table 2.1. Characteristics of orthodox versus recalcitrant/intermediate seed

Orthodox seed	**Recalcitrant and intermediate seed**
Desiccation tolerant	Desiccation sensitive
Tolerate low temperatures	Can be sensitive to temperatures below 15°C
Has low moisture content at shedding	Has relatively high moisture content at shedding
Includes dormant and non-dormant species	Usually no dormancy
Perennial, annual, woody and herbacious species	Mostly perennial and woody species
Found in all ecosystems	Often found in humid ecosystems (if very desiccation sensitive)
Usually small seeds	Often large, fleshy seeds

2.2 Desiccation sensitivity

The physiological background for desiccation intolerance is still unclear. Most likely, many physiological processes are involved. The ability to protect against damages occurring due to desiccation is probably most important, but the ability to repair damages also plays a role.

When a seed is dried, the volume of the seed is reduced and this results in mechanical stress. One way to re-arrange the seed to avoid damage may be to break up large vacuoles[1] into smaller vacuoles, or by filling them with insoluble reserve material. One hypothesis is therefore that there is a relationship between desiccation sensitivity and vacuolation and insoluble reserve accumulation. Dedifferentiation of intracellular structures, such as mitochondria, during maturation drying of orthodox seeds, with re-differentiation following imbibition before germination is another way to protect against desiccation damages which is not found in desiccation sensitive seeds. Ability to re-establish the cytoskeleton[2] after rehydration, maintenance of the integrity of the genetic material during dehydration and rehydration, the ability to switch off metabolism and use of antioxidant systems to control the free radicals generated during desiccation are other systems that may be involved in desiccation tolerance.

Furthermore, the role of putatively protective molecules such as LEA proteins[3], non-reducing sugars, amphipatic[4] molecules and oleosins[5] has been investigated, however without conclusive results.

Recent research indicates that drying rate affects the level to which recalcitrant seeds can be dried, since there seems to be less damage to the seeds if they are dried rapidly. These studies have mainly been made on excised embryos, since it is difficult to dry large seeds very fast; but for at least one recalcitrant species, fast drying of whole seeds using silica gel has been demonstrated to be better than slow air-drying. Drying rate is a factor that needs to be investigated further, because if the minimum safe moisture contents are dependent on drying rate, this needs to be specified in the recommendations for storage conditions of a species together with moisture content and temperature. Where required, fast and controlled drying of bulk quantities of seeds will be a technical challenge.

Desiccation tolerance increases during development (both for orthodox and recalcitrant seed). However, for some species with recalcitrant seeds, desiccation tolerance peaks before the seeds are shed. Desiccation tolerance also decreases during storage, probably because the seeds start to germinate.

2.3 Temperature sensitivity

Whereas it is logical that freezing injuries occur in seeds with high moisture contents at subzero temperatures, it is more difficult to explain why some species have seeds that are sensitive to temperatures, e.g. between 0 and 15°C. There is a theory about temperature dependent phase transitions in the membranes that lead to damage in certain temperature intervals, but it does not explain why some seeds are sensitive and others are not.

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[1] Vacuole: volume of water and dissolved materials surrounded by a membrane, often occupying 90% or more of a mature cell.

[2] Cytoskeleton: microtubules and microfilaments (protein structures) which organize the cytoplasm and nucleus in the cell.

[3] LEA: late embryogenic abundant proteins.

[4] Amphipatic molecules are hydrophilic at one end, and hydrophobic at the other end.

[5] Oleosin: protein that surrounds lipid droplets in plant cells. They have a hydrophobic part which interacts with the lipid, and an amphipatic part which interacts with the cytomatrix.

A clue to the behaviour of the seed can probably be found in the natural environment of the species. If the species is never exposed to low temperatures in the area of natural distribution, temperature sensitivity should be suspected.

2.4 Fungi

Storage of recalcitrant seeds at high humidity and often also high temperature provides very suitable conditions for micro-organisms. The actual role of fungi in storability of recalcitrant seeds is difficult to uncover. One of the problems is to distinguish between fungal infection of already dead material and fungal infection that leads or contributes to the death of the seed, but there is no doubt that fungi can accelerate deterioration of the seeds.

3. HANDLING OF DESICCATION AND TEMPERATURE SENSITIVE SEED

3.1 Seed collection time

Collection at the optimal time is very important. Immature seeds are not as desiccation tolerant as mature seeds and generally do not store as well. On the other hand, the seed must not begin to deteriorate or germinate before collection. The best way to establish the optimal time of collection is to collect at different stages of maturity and compare germination, desiccation tolerance and storability. The maturity must be related to the appearance of the fruit: colour, hardness, release from the tree etc. However, if a seed centre has to procure many species, it will not be possible to make large trials for each collection of a "new" species, so it is recommended that an experienced seed officer follows fruit development in the field and decides when to collect the fruit. It is important that the maturity criteria are described and written down for later reference. If it turns out to be a problem to obtain high seed quality or to store the seeds, it may be relevant to investigate seed maturity (see table 3.1).

3.2 Collection method

The choice of collection method depends on the type of tree and fruit, accessibility, economy, quantity of fruit to be collected etc. Collection from the tree has the advantage that the fruits do not get into contact with the ground; but it is time consuming, and if the tree is tall professional tree climbers are needed and there is a risk of collecting immature fruits. Fruits collected from the ground are contaminated with microflora, and particularly pulpy fruits get damaged from falling to the ground. When seeds are collected from the ground, there is a risk of including overripe fruits; so preferably the ground should be cleared under the trees, if possible covered by clean tarpaulins, and fruits should be collected within a short time period.

3.3 Transport and temporary storage

Exposure of fruits and seeds to extreme temperatures and suffocation should always be avoided. If the seeds are desiccation sensitive (or suspected to be so), care should be taken to avoid drying. Since it is a balance between providing enough air and not desiccating the seeds, it is best to avoid long transport hours and temporary storage. Open, loosely covered containers should be used during transport. Gunny bags are good for pulpy fruits because the juice can run off and the bags allow some air exchange. Too large containers should not be used since it will lead to suffocation and elevated temperatures in the middle and bottom. Another reason to avoid temporary storage is that very recalcitrant seed may start sprouting. Some seeds are temperature sensitive at high moisture contents, so even though it may seem like a good idea to put small seed lots in the fridge, it should not be done until it has been established that the seeds tolerate low temperatures. This is of particular relevance in areas where low temperatures are never experienced naturally.

Temporary storage of the fruits in the field during collection, and surface drying of seeds after extraction in water should always take place in the shade.

3.4 Seed extraction

Seed extraction reduces bulk and facilitates germination. When the fruits are pulpy; it is important to extract the seeds fast to avoid quality problems due to fermentation. However, for some desiccation sensitive species it may be an advantage to keep a covering layer on the seed since it will reduce moisture loss.

Until it has been determined whether the seed tolerates drying, loss of moisture during extraction and cleaning should be avoided. Determine moisture content and test germination on seeds extracted manually from the fruits in the laboratory, before bulk processing. Make another moisture content determination and germination test after extraction. If moisture as well as viability have been lost during the process, the procedures must be revised.

Table 3.1. Research plan for investigation of seed storage behaviour

<table>
<tr><th>Step 1 →</th><th>Step 2 →</th><th>Step 3 →</th><th>Step 4</th></tr>
<tr><td rowspan="2">Fruit development is followed in the field

Maturity criteria are described

Fruits are collected and extracted

All steps are documented in a seed lot diary (See Technical Note 54)</td><td rowspan="2">Desiccation and storage trial is carried out</td><td>Good results are obtained</td><td>Repeat trial on another seed lot with relevant conditions

Test the laboratory results on bulk seed lots</td></tr>
<tr><td>Poor results are obtained</td><td>Make additional studies of e.g.:
seed maturity
or germination conditions
(see Technical Note 57)</td></tr>
</table>

3.5 Storage conditions

A research protocol for determination of optimal seed moisture content and temperature for storage is given in the next chapter. Moisture content and temperature are two of the main factors concerning seed storage, but it is important to keep in mind that there are other factors which also play a role: the initial quality and maturity of the seed, drying method, contamination with microflora, storage containers etc. Since it is not possible to initiate large research projects investigating seed biology of all relevant tree species, a more pragmatic approach must be adopted where an initial screening identifies the most urgent problems. Fortunately, many problems can be prevented by careful seed handling by experienced seed staff (see Seed Handling Manual, Technical Note 54, DFSC). Table figure 3.1 illustrates one way to investigate seed storage behaviour. If promising results are obtained in the first desiccation and storage trial, it is important to repeat (part of the trial) on other seed lots and test whether it is possible to obtain the same good results when handling a bulk seed lot. It may for instance be difficult to obtain the same drying rate for large bulks of seeds as was obtained in the laboratory with small seed samples mixed with silica-gel. If poor results are obtained, it is important to study the data in the seed lot diary that has followed the seed lot since collection. Was the initial germination high? If not, perhaps the seeds were not mature or were damaged during transport and temporary storage. It could also be a germination problem. Often additional moisture contents have to be tested to find the minimum safe moisture content. If fungi cause problems, surface sterilization or use of another type of container may help.

For very recalcitrant species it will not be possible to extend the storage period to anything like the ones of orthodox seeds. However, if storage can be prolonged from e.g. one week to a couple of months, much flexibility is gained at the seed centre. For some species it is also possible to avoid pre-sprouting by keeping the seeds at 15°C. Getting critical parameters identified is equally important, e.g. that some seeds must have a moisture content below 10°C before they are stored at 5°C.

Another protocol on determination of storage behaviour is available from the IPGRI/DFSC Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest Tree Seeds (running from 1996-2002). The protocol will be updated during the project period and is available from DFSC homepage.

3.6 Direct seeding

Since recalcitrant seeds are designed to germinate immediately, it is worth considering using direct seeding for small as well as large scale propagation of important species with recalcitrant seed. More information about this subject can be found in DFSC Technical Note on direct seeding (in preparation).

4. DETERMINATION OF STORAGE PHYSIOLOGY AND OPTIMAL STORAGE CONDITIONS

4.1 Experiment protocol

In principle it is quite simple to set up an experiment to investigate desiccation tolerance and temperature sensitivity: the seeds are dried down to different levels of moisture content and stored at different temperatures for different lengths of time. But because three factors are involved, the experiment quickly gets very large. For instance five moisture contents x three temperatures x five durations = 75 treatments, which can be a lot to handle in the laboratory since there are four replicates per germination test and moisture content determination. Another thing is that quite a lot of seeds are needed for the trial. Fortunately, the trials will be spread over a longer period, but it has to be planned carefully. After one or two trials, the procedures will become routine.

A good starting point will be to study table 4.2. for an overview of the order of the tests to be made. Tables for recording of data have been provided with examples in this chapter, but empty tables for copying are found in appendix A.

The following equipment and material are needed for a trial with six moisture contents, two temperatures and four storage durations:

17000 seeds
7 flat trays/containers, e.g. size 20 x 30 x 10 (depending on the size of the seeds)
7 lids to the containers or plastic bags big enough to contain the trays/containers
Approx. 12 kg silica gel[6] (depending on seed size)
7 mosquito net bags, approx. double size of the containers
48 storage bags (should be able to contain 250 seeds), made of aluminium foil or thick plastic
Sealer
Water resistant pen to write on the storage bags
Storage chamber (or refrigerator) at 4-5°C
Storage chamber (or room with constant temperature) at 15°C

The moisture content of the seed can change during seed extraction. If the seeds are extracted in water, they may take up moisture, and if the fruits have to be dried to release the seeds, they may dry. Therefore it is best to make a moisture content determination on manually extracted seeds before bulk extraction of the seed lot. Sample 4 x 10 seeds and use table 4.3. This moisture content should be used to determine the target moisture contents (table 4.1).

[6] Silica gel is available in different qualities, a coloured (blue) and an uncoloured form. It is most convenient to use the type that changes colour during take up of water.

Table 4.1. Target moisture contents

Moisture content before extraction (%)	Target moisture content (%) (TMC)
≤ 10	9, 6, 3
11-15	12, 9, 6, 3
16-20	15, 12, 9, 6, 3
21-25	20, 15, 12, 9, 6
26-30	25, 20, 15, 12, 9, 6
31-35	30, 25, 20, 15, 10, 5
36-40	35, 30, 25, 20, 10, 5
41-45	40, 35, 30, 20, 10, 5
46-50	45, 40, 35, 25, 15, 8
51-55	50, 45, 40,35, 25, 10
56-60	55, 50, 45, 35, 25, 10
>60	60, 50, 40, 30, 20, 10

When all the seeds have been extracted, sample 40 seeds for moisture content determination and 200 seeds for germination testing (use tables 4.3 and 4.4). This moisture content (after extraction) should be used for calculation of target weights during desiccation.

Divide the remaining seeds into seven portions, 6 x 2500 seeds and 1 x 1500 seeds, weigh the six portions of 2500 seeds, note the weights in table 4.5 (initial weight) and mark the samples TMC 1, TMC 2, etc. The last portion is a control.

All seven samples are kept in loosely folded plastic bags at room temperature until the moisture content determination is ready next day. Use table 4.1 to determine what the target moisture contents should be (on basis of the moisture content before extraction) and note them in table 4.5.

Calculate target weights for each target moisture content (on basis of the moisture content after extraction) and note them in table 4.5.

Target weight:

Weight of seed (g) at TMC =

$$\frac{(100 - \text{moisture content after extraction})}{(100 - \text{TMC})} \times \text{initial seed weight (g)}$$

Put each 'Target moisture content (TMC) sample' into a mosquito net bag, which is marked with the TMC. Put a layer of silica gel in the bottom of the trays, place the seeds in one layer within the net bag on top of the silica gel, and cover the seeds with another layer of silica gel. Cover the tray and put it into a plastic bag. Every 10-20 minutes remove the seeds from the tray and weigh. The time and the weight are noted in table 4.5 and the seeds are placed between silica gel again. When the gel turns red, it should be substituted with fresh blue gel. The red gel is dried in the oven at 100°C, cooled down in a closed container, and used again.

The desiccation rate will vary for different species. Generally it will be fast in the beginning, and after a while it is not necessary to weigh the seeds very often.

At the end of each day the net bags with seeds are removed from the silica gel and kept in loosely folded plastic bags until the next day. Time for stop and start of desiccation should be noted in table 4.5.

When a target weight has been reached, one sample is taken for moisture content determination (40 seeds), another sample is taken for germination testing (200 seeds) and the rest of the seeds are divided into 8 samples for storage. Table 4.4 is used for the germination tests. Each storage sample should contain at least 250 seeds. Every time one of the samples reaches the target weight, samples should also be taken from the control for moisture content determination and germination testing. The purpose of this control is to determine whether it is the time taken to dry the seeds or the desiccation that is harmful.

Moisture contents and germination test results made on samples after desiccation + on controls are noted in table 4.6. The minimum safe moisture content is the lowest moisture content that did not result in any reduction in the germination percentage found for fresh seeds.

Use two storage temperatures: 4-5°C and 15-20°C. Seeds expected to lose viability very fast are stored for 2, 4, 6 and 8 weeks. Seeds expected to survive for a longer period are stored for 3, 6, 9 and 12 months. When the moisture content is below 20%, the storage bags should be sealed, when above 20%, the bags should be folded or loosely tied. Each bag should have the following information both inside and outside:

Species
Moisture content
Storage temperature
Date for start of storage
Date for end of storage

After storage, the moisture content is determined again and a germination test is made. The results are noted in table 4.7.

It is important to repeat the trial to confirm the results. Furthermore, the trial should ideally be made on a number of seed lots. The size of the follow-up trials will depend on the results. In some cases the number of moisture contents in the follow-up trials can be reduced, in other cases it may be relevant to have more storage durations etc.

Table 4.2. Trial procedures

Day 1	Day 2	Day 3, 4, 5….	After e.g. 2, 4, 6 and 8 weeks
Start moisture content determination (table 4.3) ↓ Start germination of control (table 4.4) ↓ Divide remaining seeds into seven portions and weigh them (table 4.5)	Determine moisture content ↓ Determine target moisture contents (use table 4.1) ↓ Calculate target weights (table 4.5) and start desiccation ↓ Weigh samples every 10-20 minutes until the rate of desiccation has slowed down (table 4.5). Substitute with fresh silica gel when needed.	Continue to follow the weight When a target weight has been obtained: start germination test and moisture content determination + germination test and moisture content determination of control (record results in table 4.6) ↓ Divide seeds into samples for storage, e.g.: TMC1, 5°C, 2 weeks TMC1, 5°C, 4 weeks TMC1, 5°C, 6 weeks TMC1, 5°C, 8 weeks TMC1, 15°C, 2 weeks TMC1, 15°C, 4 weeks TMC1, 15°C, 6 weeks TMC1, 15°C, 8 weeks Store samples	Remove samples from storage, make germination test and moisture content determination (record results in table 4.7)

Table 4.3. Moisture content determination

Replicate	A: Weight of container (g)	B: Weight of fresh seed + container (g)	C: Weight of dry seed + container (g)	D: Mc on fresh weight basis = (B-C)/(B-A) x 100%
1 (10 seeds)	A1: 77.052	B1: 84.563	C1: 83.994	D1: 7.576
2 (10 seeds)	A2: 77.282	B2: 85.002	C2: 84.433	D2: 7.371
3 (10 seeds)	A3: 77.456	B3: 85.121	C3: 84.559	D3: 7.332
4 (10 seeds)	A4: 77.383	B4: 84.085	C4: 83.590	D4: 7.386
Mean seed moisture content = (D1+D2+D3+D4)/4 = 7.42 %				

Divide large seeds into two-four pieces immediately before drying in the oven at 103°C for 17 hours. Keep the samples in a desiccator until they have cooled down and weigh them.

Table 4.4. Germination test

Date for initiation of tests: 28-5-1998			Medium: Vermiculite		
Temperature: 25°C			Light: 10 hours		
Treatment: Control					
Date	Days	Replicate 1	Replicate 2	Replicate 3	Replicate 4
1-6-98	4	0	0	0	0
5-6-98	8	29	4	36	8
8-6-98	11	33	9	40	22
12-6-98	15	34	10	40	24
Accumulated germination for each replicate		A1: 34	A2: 10	A3: 40	A4: 24
No of empty seeds		B1: 4	B2: 2	B3: 1	B4: 2
Accumulated germination % on basis of full seeds = A/(50-B) x 100%		C1: 74	C2: 21	C3: 82	C4: 50
Mean germination % on basis of full seeds = (C1+C2+C3+C4)/4					57 %
No of fresh seeds		0	0	0	4
No of insect infested seeds		0	14	3	2
No of dead seeds		12	24	6	18

Table 4.5. Desiccation

Moisture content before extraction: __21.3____% Moisture content after extraction: __23.0____%

		TMC 1	TMC 2	TMC 3	TMC 4	TMC 5	TMC 6
Initial weight (g)		121.781	121.779	121.787	121.791	121.788	
Target moisture content (TMC)		20	15	12	9	6	
Target weight (g)		117.214	110.317	106.564	103.049	99.763	
Date	Time						
22-5-99	10.50	121.781	121.779	121.791	121.791	121.788	
	11.20	119.044	-	-	-	-	
	11.50	116.914	-	-	-	-	
	12.50		114.093				
	13.50		111.971				
(stop)	14.40		110.388	109.422	109.262	109.306	
23-5-99 (start)	8.40			109.380	109.223	109.245	
	11.20			105.783	105.543	105.645	

Table 4.5. continued. Desiccation

		TMC 1	TMC 2	TMC 3	TMC 4	TMC 5	TMC 6
Target weight (g)					103.049	99.763	
Date	Time						
	13.20				104.401	_	
	14.20				103.803	_	
(stop)	15.20					103.106	
24-5-99	8.40					103.197	
	12.40					102.564	
(stop)	15.20					102.234	
25-5-99	8.40					100.023	
	12.40					99.894	

Table 4.6. Results after desiccation

Target moisture content	Actual moisture content	Germination %
Fresh	48.50	99
40	42.48	85
Control a	50.27	98
35	38.41	84
Control b	49.28	100
30	32.89	74
Control c	47.87	98
20	20.94	0
Control d	46.52	99
10	9.27	0
Control e	45.94	100
5	6.44	0
Control f	46.34	100

Table 4.7. Results of the storage trial

Target moisture content	Actual moisture content before storage	Storage temperature	Duration of storage (weeks)	Moisture content after storage	Germination %
Fresh	50	5°C	2	49.7	0
			4	47.9	0
			6	43.8	0
			8	35.5	0
		20°C	2	49.5	95
			4	45.4	16
			6	41.2	26
			8	32.7	0
45%	44.8	5°C	2	42.3	0
			4	41.7	0
			6	39.8	0
			8	32.6	0
		20°C	2	42.2	90
			4	40.7	45
			6	37.9	28
			8	24.7	0
40 %					

Table 4.7. continued. Results of the storage trial

Target moisture content	Actual moisture content be-fore storage	Storage temperature	Duration of storage	Moisture content after storage	Germination %
35%					
30%					
20%					

4.2 Interpretation of results and follow-up trials

It is a good idea to make a graph with the results from the desiccation trial and figures 4.1 and 4.2 show results from two different trials. In both cases there is no significant decrease in germination of the controls during the desiccation, so the effect seen on the dried seeds must be due to the reduction in moisture content. In the first example (fig. 4.1), even small reductions in moisture content lead to a loss in germination percentage, but for approximately 30-40% moisture content, the germination is still high. The seeds die between 21 and 33% moisture content, so to be able to establish more precisely what the minimum moisture content is, this interval should be studied in the next trial. The target moisture contents for the next trial could e.g. be 18, 21, 24, 27, 30 and 33%.

In the other example (figure 4.2), the seeds die between 14 and 31% moisture content. In this case relevant target moisture contents could be 15, 18, 21, 24, 27 and 30%.

Please note that minimum safe moisture content may not always be the optimal moisture content for long term storage, therefore it is important also to include a range of moisture contents in the second storage trial.

Changes in moisture content during storage may affect the results, e.g. if the seeds dry below the critical moisture content.

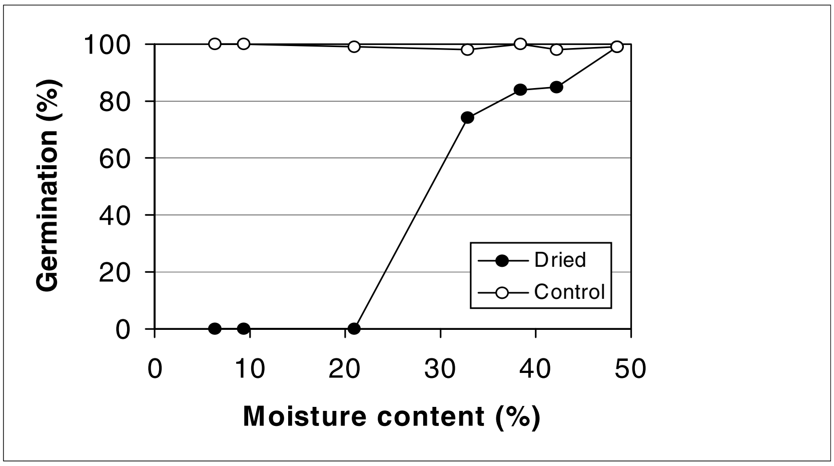

Figure 4.1. Example of results from a desiccation trial

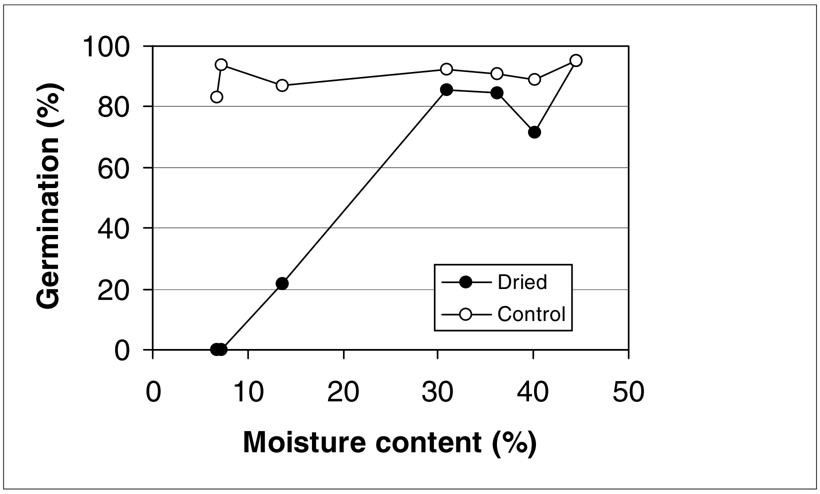

Figure 4.2. Example of results from a desiccation trial

4.3 Some general advice

One should not always expect to obtain moisture contents very close to the target moisture contents. If the moisture contents are very different from the target moisture contents, there is of course good reason to check whether the procedures have been followed correctly; but it is more important to obtain an evenly distributed range of moisture contents than the exact target moisture contents.

It should be ensured that the staff who carry out the trial have been properly informed about the procedures, and know e.g. the purpose of the controls. Often a lot of people will be involved at one point or another in such a trial, and it is therefore important that everybody is informed, including those who are collecting the fruits and extracting the seeds. Everybody should also be informed about the results of the trials. This will keep up the motivation among the staff to actively participate in developing seed handling methods.

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APPENDIX A

This appendix contains tables for data recording during desiccation and storage trials. Please note that a number of copies should be made of some of the tables: determination of moisture content after desiccation, determination of moisture content after storage, germination test, and results of the storage trial.

Laboratory report – Desiccation and storage trial

Species	
Seed lot no	
Seed collection date	
Seed extraction method	
Moisture content before extraction	
Moisture content after extraction	
Target moisture contents	
Initial germination percentage	
Obtained moisture contents after desiccation	
Minimum safe moisture content (of tested moisture contents)	
Storage temperatures tested	
Storage durations tested	
Best storage conditions (of tested combinations)	
Recommendations for follow-up trial	
Signed by responsible scientist	

Seed lot:___________

Determination of moisture content <u>before</u> extraction

Date:__________

Replicate	A: Weight of container (g)	B: Weight of fresh seed + container (g)	C: Weight of dry seed + container (g)	D: Mc on fresh weight basis = (B-C)/(B-A) x 100%
1 (10 seeds)	A1:	B1:	C1:	D1:
2 (10 seeds)	A2:	B2:	C2:	D2:
3 (10 seeds)	A3:	B3:	C3:	D3:
4 (10 seeds)	A4:	B4:	C4:	D4:
Mean seed moisture content = (D1+D2+D3+D4)/4 = %				

Determination of moisture content <u>after</u> extraction

Date:__________

Replicate	A: Weight of container (g)	B: Weight of fresh seed + container (g)	C: Weight of dry seed + container (g)	D: Mc on fresh weight basis = (B-C)/(B-A) x 100%
1 (10 seeds)	A1:	B1:	C1:	D1:
2 (10 seeds)	A2:	B2:	C2:	D2:
3 (10 seeds)	A3:	B3:	C3:	D3:
4 (10 seeds)	A4:	B4:	C4:	D4:
Mean seed moisture content = (D1+D2+D3+D4)/4 = %				

Seed lot:___________

Determination of moisture content <u>after</u> desiccation

Target moisture content:__________ Date:__________

Replicate	A: Weight of container (g)	B: Weight of fresh seed + container (g)	C: Weight of dry seed + container (g)	D: Mc on fresh weight basis = (B-C)/(B-A) x 100%
1 (10 seeds)	A1:	B1:	C1:	D1:
2 (10 seeds)	A2:	B2:	C2:	D2:
3 (10 seeds)	A3:	B3:	C3:	D3:
4 (10 seeds)	A4:	B4:	C4:	D4:
Mean seed moisture content = (D1+D2+D3+D4)/4 = %				

Determination of moisture content <u>after</u> desiccation

Target moisture content:__________ Date:__________

Replicate	A: Weight of container (g)	B: Weight of fresh seed + container (g)	C: Weight of dry seed + container (g)	D: Mc on fresh weight basis = (B-C)/(B-A) x 100%
1 (10 seeds)	A1:	B1:	C1:	D1:
2 (10 seeds)	A2:	B2:	C2:	D2:
3 (10 seeds)	A3:	B3:	C3:	D3:
4 (10 seeds)	A4:	B4:	C4:	D4:
Mean seed moisture content = (D1+D2+D3+D4)/4 = %				

Seed lot:___________

Determination of moisture content <u>after</u> storage

Moisture content before storage:__________ Storage period:_________

Storage temperature: Date:__________

Replicate	A: Weight of container (g)	B: Weight of fresh seed + container (g)	C: Weight of dry seed + container (g)	D: Mc on fresh weight basis = (B-C)/(B-A) x 100%
1 (10 seeds)	A1:	B1:	C1:	D1:
2 (10 seeds)	A2:	B2:	C2:	D2:
3 (10 seeds)	A3:	B3:	C3:	D3:
4 (10 seeds)	A4:	B4:	C4:	D4:
Mean seed moisture content = (D1+D2+D3+D4)/4 = %				

Determination of moisture content <u>after</u> storage

Moisture content before storage:__________ Storage period:_________

Storage temperature: Date:__________

Replicate	A: Weight of container (g)	B: Weight of fresh seed + container (g)	C: Weight of dry seed + container (g)	D: Mc on fresh weight basis = (B-C)/(B-A) x 100%
1 (10 seeds)	A1:	B1:	C1:	D1:
2 (10 seeds)	A2:	B2:	C2:	D2:
3 (10 seeds)	A3:	B3:	C3:	D3:
4 (10 seeds)	A4:	B4:	C4:	D4:
Mean seed moisture content = (D1+D2+D3+D4)/4 = %				

Seed lot:___________

Germination test

Date for initiation of tests:			Medium:		
Temperature:			Light:		
Treatment:					
Date	Days	Replicate 1	Replicate 2	Replicate 3	Replicate 4
Accumulated germination for each replicate		A1:	A2:	A3:	A4:
No of empty seeds		B1:	B2:	B3:	B4:
Accumulated germination % on basis of full seeds = A/(50-B) x 100%		C1:	C2:	C3:	C4:
Mean germination % on basis of full seeds = (C1+C2+C3+C4)/4					
No of fresh seeds					
No of insect infested seeds					
No of dead seeds					

Seed lot:___________

Desiccation

Moisture content before extraction: _______% Moisture content after extraction: _______%

		TMC 1	TMC 2	TMC 3	TMC 4	TMC 5	TMC 6
Initial weight (g)							
Target moisture content (TMC)							
Target weight (g)							
Date	Time						

Seed lot:___________

Desiccation, continued

		TMC 1	TMC 2	TMC 3	TMC 4	TMC 5	TMC 6
Target weight (g)							
Date	Time						

Seed lot:___________

Results after desiccation

Target moisture content	Actual moisture content	Germination %
Fresh		
Control a		
Control b		
Control c		
Control d		
Control e		
Control f		

Seed lot:___________

Results of the storage trial

Target moisture content	Actual moisture content before storage	Storage temperature	Duration of storage	Moisture content after storage	Germination %

These studies revealed that Jaman seeds are sensitive to desiccation tolerance and their storage behaviour may be recalcitrant. Key words: Jaman, desiccation tolerance, EC, germination, viability. INTRODUCTION. Seeds are classified in two categories: orthodox and recalcitrant, on the basis of their storage behaviour (Roberts, 1973). Recalcitrant seeds are characterized by their nature of desiccation sensitivity (Pammenter and Berjak, 1999). Jaman seeds have high moisture content and therefore remain sensitive to desiccation. The limited storage potential of recalcitrant seeds is a big problem ...Â Desiccation and low temperature (-196Â°C) tolerance of Citrus limon seed. Seed Sci. & Technol. 7: 407-10. Seed deterioration is an undesirable and detrimental attribute of agriculture. This process is a separate event from seed development and germination. Losses in seed quality occur during field weathering, harvesting and storage. Deterioration caused by field weathering is directly related to seed exposure to adverse conditions. Seeds are highly susceptible to damage and mechanical injury during post-harvest handling. Several environmental factors contribute to seed deterioration and these conditions make very difficult to maintain viability during storage. Seed quality depends upon initial see... Manual of Seed Handling have the opportunity to attend courses on seed conservation and genebank management. Using the manual and its module, genebank staff can learn on their own about the different tasks of the genebank and also have a quick reference to essential genebank procedures.Â Several important tropical and subtropical tree species produce seeds that do not survive desiccation and cannot tolerate low temperatures, and which are therefore not easy to store; these are known as recalcitrant seeds. Techniques exist for storing some recalcitrant seeds, but the seeds are usually short-lived and each species requires its own method. Based on the desiccation sensitivity, seeds are classified as orthodox and recalcitrant (Roberts, 1973). Around 37,500 species of seeded plants in the world are desiccation sensitive and belong to the group â€˜recalcitrantsâ€™. They are generally seen in humid tropics where there is sufficient rainfall (Pritchard et al., 2004). However, temperature associated climate change causes severe threat to these species (IUCN, 2015).Â These seeds are highly sensitive to desiccation and cannot withstand Critical Water Content (CRC) below 20 percent (Pammenter and Berjak, 2000). Hence the most viable solution is to conserve them under lowest temperature they can tolerate and to provide conditions that will not permit water loss. This is possible by in vitro techniques. Resistance to desiccation and brief exposure to high temperatures are found in a number of other invertebrates. Various groups of small, planktonic crustaceans such as Copepoda possess at least one dormant form during their ontogenesis, which could be the egg, a larval stage or even the adults (reviewed in 62).Â For instance, anhydrobiotic juveniles of the seed gall nematode Anguina agrostis survived a 5 minute exposure to 155 Â°C 73 .Â To test the effect of desiccation time on the survival of specimens subsequently exposed to extreme temperatures, we firstly compare the specimens that were desiccated for 1 hour and then heated to 70 Â°C with the specimens that were desiccated for 24 hours and then heated to 70 Â°C. Each of these two groups consists of 5 replicates each with 20.