

# **The Desiccation and Storage Protocol**

## **Revised June 2000**

**The following gives details on the procedures to be followed, and data to be recorded.**

### **1. Floral biology and ecology**

Existing information on floral biology and ecology should be recorded and additional supplementary data should be collected.

### **2. Seed source**

Complete Appendix A (Seed Source Description)

### **3. Seed collection**

3.1. Date

3.2. Harvest method.

3.2.1. Collection from trees. Note ripeness (e.g. colour, firmness).

3.2.2. Collection from the ground. Suspected recalcitrant seeds should be collected within 24 hours of shedding. Note whether collection was from bare ground or if ground cover was present.

3.2.3. Note other details, e.g. insect predation.

3.2.4. Tree numbers and selection.

Note the number of trees from which the seeds were collected. (Fill in Appendix B). Collect the same amount of fruits from at least 25 trees with an average appearance for that of the stand and producing a good crop. Bulk the fruits and mix them properly. (If the number of trees is lower than 25, the trial must be repeated later with a larger number of trees).

3.2.5. Fruit numbers. If possible, collect double the number of seeds required for the trial to allow for discarding infected seeds.

### **4. Details of fruit transport**

4.1. How were the seeds transported to the laboratory ?

4.2. Containers for transport

4.2.1. Nature and size

4.2.2. Would these containers maintain moisture content ? (this is important because the initial moisture content is determined only after transport).

4.3. Note minimum and maximum temperature during transport.

4.4. Duration of transport (Fill in Appendix B).

### **5. Fruit weight**

Before processing, measure fruit weight, average plus standard deviation. Measure 100 individual fruits (whenever possible). (Note that this is non-destructive and the seeds can be used for further studies).

### **6. Seed processing**

6.1. If processing of seeds is necessary (e.g. cleaning, depulping, extraction, removal of specific parts), describe the procedures.

6.2. Note the number of days between processing and the start of moisture content and desiccation trials in the lab.

6.3. Duration of processing (fill in Appendix B).

6.4. Take a sample of seed before processing for moisture content determination (8A) the seeds should be processed manually without water.

6.5. Avoid desiccation during processing.

6.6. Remove mechanically damaged, infected and infested seeds.

6.7. Soak the seeds for 10 minutes in a 1% solution of sodium hypochlorite (NaOCl)

6.8. Rinse and blot dry

## **7. Preparing for dispatch and desiccation**

7.1. Take samples for moisture content testing (8A), germination testing (9) and seed characteristics (10).

7.2. Immediately after sampling for moisture content determination, seeds for the replicating partner are packed and dispatched (7.3) and the rest of the seed lot is divided into portions for desiccation (11) and control storage (12), where after the individual portions are weighed.

7.3. Pack seeds for dispatch in plastic bags with a little vermiculite or similar material to absorb excess moisture. (Germination rate may be increased if the seed is exposed to free water). Bags must then be loosely sealed. For details see Appendix G.

## **8A. Initial moisture content testing**

8.1. Determine the moisture of whole seeds. The moisture content is determined before extraction on manually extracted seed (6.4) and another determination is made after processing. Use 25 seeds in 5 replicates of 5 seeds each. Cut large seeds into smaller pieces. Dry at 103°C for 17 hours. Express moisture content on a fresh weight basis.

8.2. Determine the variation in moisture content within the seed by measuring the moisture content of individual components, i.e. axis versus storage tissue and covering structures or other relevant parts.

Use 100 individual seeds. Weigh immediately after excising components (to avoid desiccation). In some cases the seed may be too small for separating, thus moisture content determination cannot be performed on the separate component parts.

## **8B. Subsequent moisture content testing (during/after desiccation)**

As in 8.1.

## **9. Seed characteristics**

9.1. Weight. Measure the weight of 100 individual seeds calculating the average and standard deviation. (Non-destructive)

9.2. Anatomy. Sketch and describe seed/fruit components, i.e. axis, cotyledons, endosperm, testa and pericarp.

9.3. Note the days elapsed between collection and these tests.

## **10. Initial germination capacity**

10.1 Note the number of days after collection that the germination test was initiated (Fill in Appendix B).

10.2 Describe the unit for testing (i.e. is the unit for testing the seed, part of the seed, or does it include parts of the fruit?).

10.3. Use a minimum of 4 replicates of 25 seeds.

10.4. Use an appropriate germination medium, moistened but not flooded. Sterilize medium by heating (e.g. expose to 130°C for 1 hour).

10.5. Germination temperature

10.5.1. For tropical seeds use a germination temperature of 25-30°C

10.5.2. If seeds are of a warm temperate origin, use 20-25°C.

10.5.3. If seeds are of a cold temperate origin, use 15-20°C.

10.5.4. Monitor temperature during germination.

10.6. Germinate at 8-12/16-12 hours light/dark (imitate natural conditions).

10.7. Germination assessment

10.7.1. Assess daily for quickly germinating species. For other species check at least weekly. Specify criterion used to score positive germination.

10.7.2. Assess seedling establishment. Score as positive when cotyledons have emerged (not for hypogeal germination) and first set of leaves show signs of normal development.

10.7.3. Score number of seedlings with abnormal appearance, and describe abnormality.

10.7.4. Continue the test until seeds either germinate or are rotten.

10.7.5. At the end of the test, non-germinating seeds are cut open to determine whether they are still in good condition or rotten preferably using the Tetrazolium test (optional). Note also whether any radicle extension has occurred within the seed.

10.7.6. Pre-sprouted seeds are counted, recorded and not included in trials.

10.7.7. In cases of polyembryony both the number of 'seeds' that have germinated with at least one seedling + the total number of seedlings are counted and recorded.

## 11. Desiccation sensitivity (Appendix C)

11.1. Desiccate seeds by mixing with an equal amount of silica gel and enclose in 3-6 (see table 1 below) containers (e.g. plastic bags), i.e. a separate bag for each target moisture content.

11.2 Place containers under ambient temperature (25-30°C). If ambient temperature is below or above this range, an incubator must be used.

11.3. Controls to determine if the time factor affects the results are placed in similar containers with vermiculite in place of the silica gel (see 12).

11.4. Additionally, less rapid methods of desiccation may be used.

11.5. Change the silica gel as required, and always in all containers at the same time.

11.6. Aerate seeds by mixing once or twice daily to avoid anoxia, as well as when weighing and/or changing silica gel.

11.7. Periodically monitor water loss by weighing seeds (sieve to remove silica gel) and note duration of drying. The frequency of this monitoring should be higher in the beginning.

11.8.1. Target moisture calculation

Initial mc (before processing) (%)	Target mc (%)
≤ 10	9, 6, 3
11-15	12, 9, 6, 3
16-20	15, 12, 9, 6, 3
21-25	20, 15, 9, 6, 3
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

Calculate corresponding target weight by using the following formula:

$$\text{Weight of seed (g) at TMC} = \frac{(100 - \text{MC after processing}) \times \text{initial seed weight (g)}}{(100 - \text{TMC})}$$

Example: 1500 g of seed at MC after processing of 50%; what is the weight when the seed is dried to a target moisture content of 30%?

$$\frac{(100 - 50) \times 1500 \text{ g}}{(100 - 30)} = 1071 \text{ g}$$

11.8.2. Calculate the target weight for the seed batch from each of the containers and take a sample for germination and moisture content testing when target weight is reached.

11.9 To prevent imbibition damage of seed samples at moisture contents below 15%, seeds should be humidified before they are germinated. Humidify seeds by placing above the water surface in a closed container at germination temperature for the time necessary for the seed weight to increase by 10-15%.

11.10. When very few seeds are available, a preliminary investigation may be performed by drying to 12% and 5% levels only.

11.11. If the relationship between moisture content and germination capacity reveals a critical moisture content at 15-20% or higher, the seed is considered recalcitrant.

11.12. Note the daily maximum and minimum temperatures during the drying period, possibly by using a thermohydrograph.

11.13. A small pilot sample can be started before the desiccation trial to test whether target and actual moisture contents correspond.

## **12. Control stock of seed during desiccation**

12.1. A control stock of seed is maintained at the initial moisture content to determine if the time factor affects the result (see 11.3.).

12.2. Sampling is done simultaneously with the seed undergoing desiccation.

12.3. Storage is therefore carried out in 6 or more containers under exactly the same conditions, except that the silica gel is not included. Avoid direct seed contact with bottom of container.

12.4. Aeration takes place simultaneously with that of seeds undergoing desiccation (see 11.6).

12.5. Note that moisture loss must be avoided during storage.

12.6. Note the number of seeds that are fungally contaminated, and the number that have germinated during storage.

## **13. Storage behaviour**

The desiccation trial (11.11) will determine whether or not the seed is recalcitrant. If the seed is recalcitrant refer to 16 if the seed is not recalcitrant refer to 14.

## **14. Determination of orthodox/intermediate behaviour**

If a seed shows evidence of reduced vigour at moisture contents 8-9% and above, it is probably not orthodox (assuming that it was fully mature when subjected to desiccation). The term "intermediate" has been used to cover all non-orthodox and non-recalcitrant storage responses. Note that sometimes confusing indications can be obtained e.g. chilling tolerant seeds that cannot be desiccated below 8-9%, or seeds that do not tolerate low temperatures, but may withstand relatively extreme desiccation in the short term.

14.1. Store samples hermetically at 3, 6, 9 and 12% moisture content at 20, 15, 5 and  $-20^{\circ}\text{C}$  in a factorial combination.

14.2. Samples for testing moisture content and germination capacity are drawn at 3, 6, 12, 18 and 24 months.

14.3. In the case of very little seed being available, store seed in a factorial combination at three moisture contents close to the lowest tolerated (e.g. 6, 9 and 12%) at 5 and  $15^{\circ}\text{C}$  and sample after 3, 6, and 12 months only.

14.4. Use sealed aluminium foil packages for storage. If these are not available, they will be supplied by the project.

14.5. Leave the seed package sealed at room temperature for one day prior to sampling, in order to avoid condensation on the cold seed. Rehydrate as described in 11.9 if moisture content is below 15%.

14.6. If results show an orthodox response to storage, the seed should be stored at the lowest moisture content tolerated at sub-zero temperatures to achieve the best longevity. In the event of a non-orthodox response refer to points 15 and 16 below.

## **15. Optimising storage conditions for intermediate seeds**

It is not possible to provide strict guidelines regarding intermediate seed, which will therefore depend on the results found in 14. Initiate additional storage trials using factorial combinations of moisture content and temperature in a narrow range around optimal values determined in 11 and 14.

## **16. Optimising storage conditions for recalcitrant seed**

16.1. Results from the first phase of the project have demonstrated that it is not possible to provide strict guidelines for recalcitrant seed either. Therefore, make additional storage trials using factorial combinations of moisture contents, in a narrow range around optimal values determined in 11, and  $5^{\circ}\text{C}$ ,  $15^{\circ}\text{C}$  and ambient ( $20-25^{\circ}\text{C}$ ).

16.2. Pack seeds in loosely folded plastic bags on top of some netting to avoid direct seed contact with the bottom of the bag where water may accumulate. The seeds should be positioned in the same way and not stacked on top of each other.

16.3. Draw samples for testing germination capacity and moisture content (simultaneous testing) on a regular basis according to your experience with seed storability, e.g. after 1, 3, 6, 9 and 12 months.

## **17. Supplementary trials**

The range of factors to test should not be limited by these recommended procedures as unexpected results are occasionally reported. The following are examples of what could be tested: removal of covering structures, alginate encapsulation, fungal dressing, controlled atmosphere storage.

# APPENDIX A. SEED SOURCE DESCRIPTION

## MINIMUM INFORMATION OF CANDIDATE SEED SOURCES

TENTATIVE REF. No: \_\_\_\_\_  
SPECIES Latin name: \_\_\_\_\_  
Local name: \_\_\_\_\_

OWNER/CONTACT PERSON: \_\_\_\_\_

REPORT AUTHOR: \_\_\_\_\_ DATE: \_\_\_\_\_

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Latitude: \_\_\_\_\_ Longitude: \_\_\_\_\_ Altitude: \_\_\_\_\_  
State/Province: \_\_\_\_\_ Forest Circle: \_\_\_\_\_  
Name of Forest: \_\_\_\_\_ Compart./Block: \_\_\_\_\_  
Detailed location: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
Map ref.: \_\_\_\_\_  
Seed zone/ecological zone \_\_\_\_\_

ACCESS ROAD DESCRIPTION: \_\_\_\_\_  
\_\_\_\_\_

NATURAL BOUNDARIES: \_\_\_\_\_  
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\_\_\_\_\_

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Total area: \_\_\_\_\_ Establishment year: \_\_\_\_\_  
Establishment method: Natural Planted/sown with seed from: \_\_\_\_\_ Unknown \_\_\_\_  
Associated important species: \_\_\_\_\_  
Present condition of stand (incl. uniformity of spacing or grouping of trees: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_ ::

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Selected for: Timber Pulp Fuel Fruits Fodder Wood extractives Other extractives Wind breaks  
Other protection Other specify: \_\_\_\_\_  
Is stand superior or average for trait selected for: \_\_\_\_\_  
Approx. age: \_\_\_\_\_ Crown developm.: Dominant \_ Co-dominant \_ Suppressed \_  
Health: + +/- - Approx. height of dominant/co-dominant trees: \_\_\_\_\_m  
Remarks: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

DISTANCE TO NEAREST STAND OF SAME SPECIES: \_\_\_\_\_

SEED COLLECTION: Collection permit required: YES \_\_\_ BY \_\_\_\_\_ NO \_\_\_ UNKNOWN \_\_\_

Flowering period: \_\_\_\_\_ Fruiting period: \_\_\_\_\_

Percentage trees flowering/fruited: \_\_\_\_\_ %

Seed production capacity: \_\_\_\_\_

GENERAL REMARKS:

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## APPENDIX B

### Format for keeping track of seed - network on recalcitrant/intermediate seed:

(Filled in format should be forwarded with seed to replicating partner)

Name and institute of networker: \_\_\_\_\_

Species: \_\_\_\_\_ Seed source: \_\_\_\_\_

Date(s) of collection: \_\_\_\_\_ No. of trees collected from: \_\_\_\_\_

Kg of fruit collected per tree: \_\_\_\_\_ Total kg of fruits: \_\_\_\_\_ Total kg of seeds: \_\_\_\_\_

Date of transport to laboratory: \_\_\_\_\_

Means of transport (container, approximate temperature) and duration of transport: \_\_\_\_\_

Date of receipt at laboratory: \_\_\_\_\_

Date for determination of fruit weight (protocol step 5) \_\_\_\_\_

Date(s) for processing and method of processing (protocol step 6) \_\_\_\_\_

Date of sampling for testing seed weight: \_\_\_\_\_ Date of testing seed weight (protocol step 9) \_\_\_\_\_

Date of sampling for testing initial moisture content: \_\_\_\_\_

Date(s) of drying seed in oven for moisture content determination (protocol step 8) \_\_\_\_\_

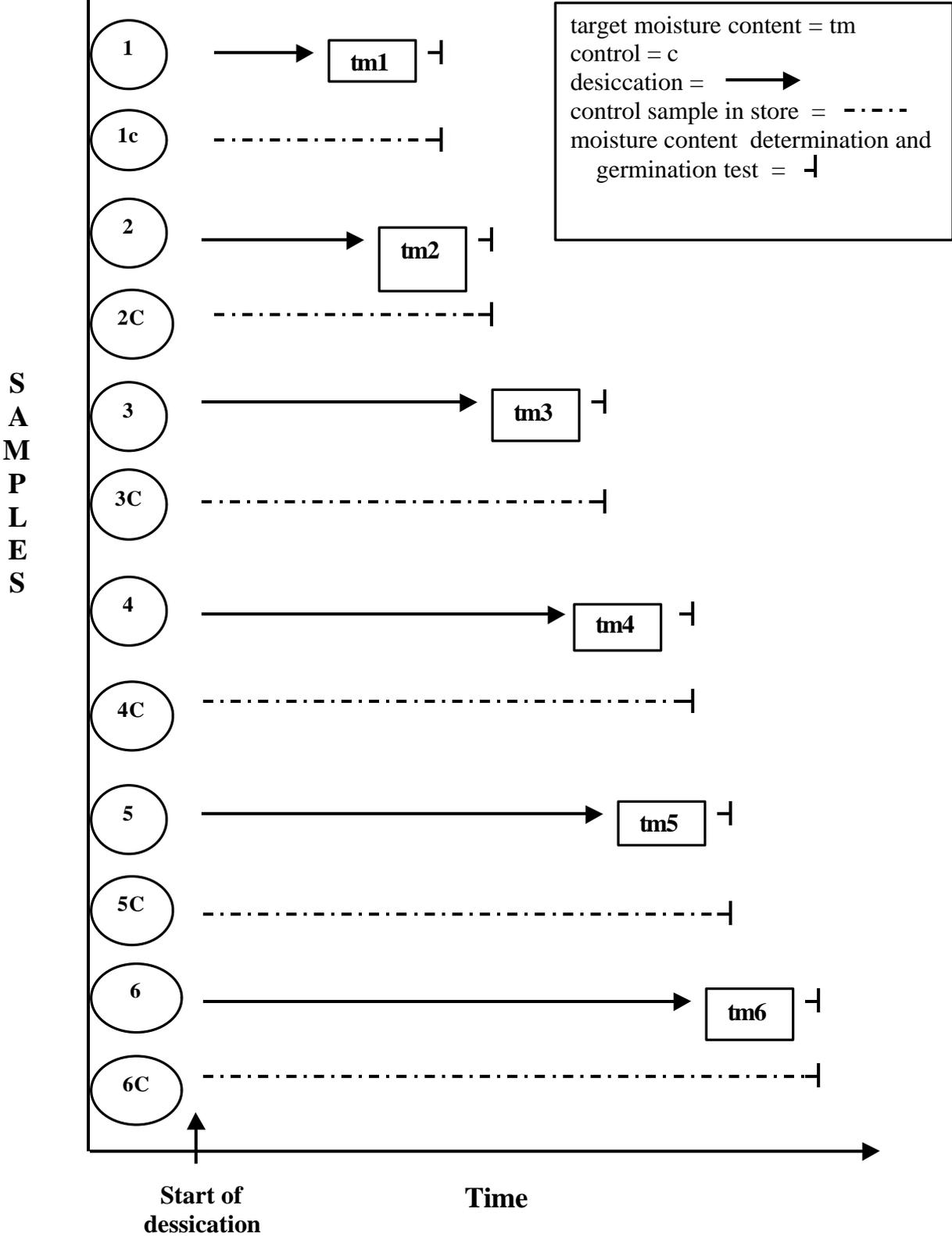
Date of sampling for testing initial germination capacity: \_\_\_\_\_

Date of initiating test of initial germination capacity (protocol step 10) \_\_\_\_\_

Date of packaging sample for dispatch: \_\_\_\_\_ Date of dispatch of sample: \_\_\_\_\_

Date for initiation of desiccation trial: \_\_\_\_\_

**Appendix C – Diagram of Desiccation Trial**



## Appendix D – Calculation of Seed Consumption

Minimum quantities of seed needed for the trials (not including replication)

<b>Trial</b>	<b>No. of seeds needed</b>
Initial tests	250  2 moisture content (m.c.) determinations (manual extraction and after processing) + weight determination and m.c. determination of seed components + 1 germination test
Desiccation trial	1500  12 samples x (1 germination test + 2 m.c. determinations)
A. Storage trial (14)	12,000 (2,700)  80 or 18 treatments x (1 germination test + 2 m.c. determinations)
B. Storage trial (16) e.g.	9,000  e.g. 4 mc x 3 temp x 5 durations  60 treatments x (1 germination test + 2 m.c. determinations)
Moisture content determination	5 x 5
Germination test	4 x 25

## Appendix E – Guidelines for Replication

### Guidelines for replication

<b>Collecting partner (c.p)</b>	<b>Replicating partner (r.p)</b>
Keeps r.p. informed about expected collection time	Obtains any relevant import permits in advance and informs c.p. of any special arrangements (e.g. use of agent)
Informs r.p. when seeds are collected	Confirms that the laboratory is ready to receive the seeds
Packs and sends the seeds according to the Guidelines in Appendix G. 'Format for Keeping track of seed' (appendix B) and 'Information for replicating partner (appendix E) are filled in and enclosed with the seed.	
	Acknowledges receipt of the seeds
<p>Keep each other informed about the results and discuss problems that may arise.</p> <p>Prepare article together when the trials are completed. C.p. is first author.</p>	

**Appendix F – Information for the replicating partner**

Fill in the table below and send with the seeds to the replicating partner

<b>Initial moisture content</b>	
Germination temperature	
Germination medium	
Drying temperature	
Target moisture content	
Storage temperature	
Storage durations	

Other important information:

## APPENDIX G. SEED DISPATCH AND PACKAGING PROCEDURES. (STEP 10)

*In order to reach the goal of this network it is vital that the utmost care is taken when packing and dispatching seed. The procedures described below should therefore be followed carefully.*

SEED MUST BE SHIPPED AS SOON AS POSSIBLE AFTER COLLECTION!!!

Seed is shipped at the moisture content it has at shedding, i.e. at a very high moisture content. Therefore seed will be respiring actively and may lose moisture during transport. Seed is shipped in plastic bags in order to avoid moisture loss, but, due to the ongoing respiration there is a risk of suffocating the seed. Therefore, do not seal the plastic bag, just bind a piece of string loosely around the top, allowing a small passage of air. Do not put more than 0.5-1 kg of seed in each bag. The plastic bags are packed in a cardboard box or similar, allow air between the bags, fill in with some flamingo foam or cotton wool to protect from mechanical damage and to allow air passage between the bags. Each plastic bag should carry a tag inside as well as outside containing the completed format in Appendix B. Write in large print on the cardboard box something like: 'For research purposes only, keep at temperatures above 15°C'.

- Seed source name should be the name found in Work plan I.
- Phytosanitary certificates are required for all seedlots to all countries.
- Import requirements and contact addresses for some of the networkers are found in the table below. As soon as possible you should determine the requirements (e.g. import permit required?) and contact addresses for your area.
- Receiving institutes should always be notified by fax about: Date and time of arrival, flight number, name, phone, fax, address of delivering agent, air way bill (AWB) number or delivery note number.
- The shipping agent should guarantee temperatures above 15°C during transport. Make sure that seed is placed in the compartment of the plane for potted plants and animals, this compartment is heated. In other compartments temperatures may drop well below 0°C. (Fruit/seed should never be placed in the refrigerator, since temperatures below 15°C may not be tolerated).
- Based on collecting period as stated in work plan 1, receiving networkers should request import permits in advance (if import permits are required), as they will probably not be given notice of arrival of seed in sufficient time to get the import permit.
- When the seed is received, the trials should be initiated immediately (see the Screening Protocol). After receipt notify the sender and DFSC, that seed has been received.

## APPENDIX H - 4 DATA SHEETS

### DATA Sheet 1 (of 2) for INITIAL TESTING and DESICCATION TRIAL

Species: \_\_\_\_\_

Seed source: \_\_\_\_\_

Seed collection date(s): \_\_\_\_\_

Kg fruit collected: \_\_\_\_\_ Number of trees: \_\_\_\_\_

Kg seed extracted: \_\_\_\_\_

Date of dispatch to replicating partner(s): \_\_\_\_\_

Replicating partner (1): \_\_\_\_\_

Replicating partner (2): \_\_\_\_\_

Kg seed dispatched: Partner 1: \_\_\_\_\_ Partner 2: \_\_\_\_\_

Initial moisture content (manual extraction): \_\_\_\_\_

Moisture content after processing: \_\_\_\_\_

<b>Component</b>	<b>Moisture content / %</b>
Whole fruit	
Whole seed	
Seed coat	
Embryo	
Storage tissue	

**DATA Sheet 2 (of 2) for INITIAL TESTING and DESICCATION TRIAL**

Seed weight: \_\_\_\_\_

Germination conditions

Number of replicates: \_\_\_\_\_ Number of seeds per replicate: \_\_\_\_\_

Temperature: \_\_\_\_\_ Medium (paper/sand..): \_\_\_\_\_

Initial germination percentage: \_\_\_\_\_

Drying temperature/°C: \_\_\_\_\_

Target moisture content/%	Time to reach target moisture content/- hours*	Actual moisture content/%	Germination/%
Fresh	-----		
Fresh	-----		
Fresh	-----		
Fresh	-----		
Fresh	-----		

\* "Effective drying time" excluding temporary storage without silica gel, e.g. over night.

**DATA Sheet 1 (of 2) for STORAGE TRIALS**

Species: \_\_\_\_\_

Seed source: \_\_\_\_\_

Seed collection date: \_\_\_\_\_

Collecting partner: \_\_\_\_\_

Replicating partner: \_\_\_\_\_

Date of initiation of storage trial: \_\_\_\_\_

**Germination conditions**

Number of replicates: \_\_\_\_\_

Number of seeds per replicate: \_\_\_\_\_

Temperature: \_\_\_\_\_

Medium (paper/sand..): \_\_\_\_\_

Comments: \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
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\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**DATA Sheet 2 (of 2) for STORAGE TRIALS**

Storage temp. °C	Storage duration	M.c. after Storage	Germination test results after storage (total = 100%)				
			Germinated seeds %	Dead seeds %	Empty seeds %	Fresh un-germinated seeds %	Pre-sprouted seeds %

