

Seed processing for IITA genebank

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26 Dingwall Road, Croydon CR9 3EE, UK

Within Nigeria:
PMB 5320, Oyo Road
Ibadan, Oyo State

ISBN 000 000 000 0

Printed in Nigeria by IITA

Correct citation:

Cover:

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1. Harvest

For each accession:

- Harvest pods by hand and keep in harvesting bags made of polyethylene, cloth, or paper with the corresponding tag number (Figure 1).
- **Minimum data capture:** Date and location of harvest. Record the data in the field book prior to keying in the computer and transferring in the regeneration table.
- **Important:** Harvesting data should be keyed in the computer within the week following harvest.
- **Future plan:** Use of bar code system will allow direct data capture.

2. Predrying

- 2.1 Transfer germplasm and keep in a clean glasshouse for predrying and temporary storage until further processing (Figure 2). Pods and seeds should remain in their harvesting bags.



Figure 1



Figure 2

- 2.2 Print the list of newly harvested accessions and match with the accessions brought in during the predrying stage.
- Set aside any mismatched accession and investigate the cause of mismatching.
- **Minimum data capture:** Date of entry in glasshouse

3. Threshing and purifying

- 3.1 Separate seeds mechanically from the pods and purify using threshing (Figures 3a, 3b, 3c) and blowing machines (Figures 4a, 4b, 4c), respectively.
- Alternatively, perform threshing and purification manually for small samples when threshing/cleaning equipment are not available). In the latter, gently beat harvesting bags containing the pods with a wooden stick. Empty each bag in an aluminum tray and separate seeds by hand.
- 3.2 Transfer seeds into processing bags with associated tags.



Figure 3



Figure 4

- 3.3 Secure each bag loosely and add an additional external label to each of them (Figure 5).
- **Minimum data capture:** Date of threshing

4. Fumigation

- 4.1 Fumigate seeds by batches of 100-150 accessions (Figure 6).

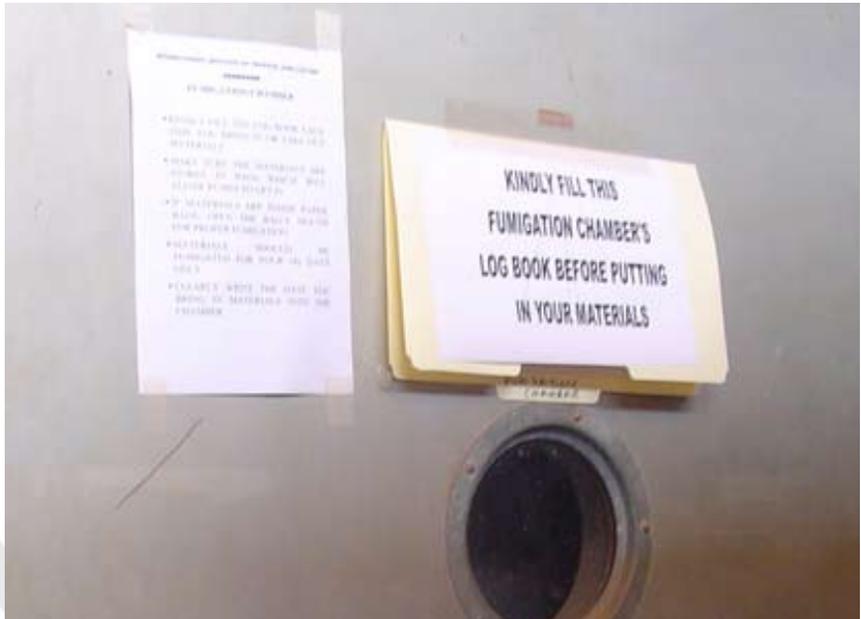


Figure 5



Figure 6

Batch details (crop, accession number, date of harvest) are provided to the seed bank manager once they enter the fumigation chamber. From there the seeds fall under the responsibility of the seed processing manager.

- 4.2 Fumigate using 57% aluminum phosphide (phostoxin) fumigation tablets.
- 4.3 Keep the fumigation chamber closed for 72 hours following treatment.
- **Minimum data capture:** Date of entry and exit from fumigation chamber.

5. Seed cleaning, purifying, and identity check

- 5.1 Empty each processing bag in an aluminum tray to eliminate any debris (broken seeds, inert materials, infected seeds).
- 5.2 For each accession, compare seeds with their respective seed file for conformity check (Figures 7a, 7b). Set aside any non-matching accession for matching and verification with other samples of the same accession maintained in the genebank.



Figure 7a



Figure 7b



Figure 8

- 5.3 Eliminate off type seeds with an overall proportion smaller than 10% (outcrosses) (Figure 8).

Important: Do not discard small seeds.

- 5.4 Return clean seeds back to processing bags with their tags (Figure 5).

Important: Close processing bags loosely to facilitate seed dehydration. Split seed lot into several bags when necessary and label accordingly.

- 5.5. For each batch, rank the accessions and transfer in ranked trays.

- **Minimum data capture:** Conformity (yes/no); Temporary location in tray.

*Keep outcrossed seeds in a common bag—do not discard them.

6. Seed drying

- 6.1 Transfer ranked trays to drying room (Relative humidity: 10-15; Temperature: 16-18 °C) (Figure 9).
- 6.2 Then transfer seed bags and arrange serially on dehydration shelves (Figure 5).
- 6.3. Once on shelves, regularly turn seed bags upside down every 2-3 days to allow homogeneous dehydration.
Important: Make sure bags are turned regularly (take record).
- **Minimum data capture:** Date of entry in dehydration room, accession location in drying room

7. Water monitoring during drying

Once a new batch is placed on the dehydration shelf, monitor the dehydration of the sample as follows:

- 7.1 Select one accession with a large number of seeds from each shelf.
- 7.2 Print the list of accession selected and use this list to monitor sample dehydration.
- 7.2 Determine initial water content for each accession selected on day 1 and regularly check samples (every 7 to 14 days depending



Figure 9

on crop and seed availability) until they reach optimal moisture content. Use a seed moisture reader to determine seed moisture content (Figure 10). Check water content gravimetrically once seeds have reached optimal water content (see below).

- **Minimum data capture:** water content of selected samples (see annex 1 for water content determination).

Optimal water content for storage

7% FWB (fresh weight basis) for cowpea, bambara groundnut, soybean, African yam bean, Wild Vigna, and other legumes

8% FWB for maize

Automatic water content determination

Requires a seed moisture reader (SINAR AGRIPO) – Use only for big samples as the average number of seeds needed for automatic reading is 485, 75, and 666 for cowpea, bambara groundnut, and soybean, respectively.

B Gravimetric water content determination

Take 20 to 50 seeds per sample (depending on availability)

1. Grind seeds in a grinder (Figure 11).
2. Transfer in pre-weighed and prelabeled aluminum cans (or aluminum foil) (Figure 12).
3. Weigh each container or foil containing the fresh matter.
4. Maintain at 80-105 °C until constant weight (8-17 hours depending on material) is reached.
5. Weigh the remaining dry matter (maintain sample in desiccators during weighing process to avoid sample rehydration—this may not be necessary during dry season).

Water content expression (% of fresh weight)

$WC = (\text{fresh weight} - \text{dry weight}) \times 100 / \text{fresh weight}$



Figure 10



Figure 11

8. Seed sorting for germination and sample water content check

Once random samples have reached optimal water content for storage, process seeds further

8.1 Take all the seed bags in the selected shelf out of the drying room.

8.2 From each batch, place 40 to 70 seeds in an envelope and label with accession number. Use these samples for water content and germination tests.

Important: Perform water content check shortly after seeds are packed in an envelope. Rehydration can occur fast especially during the raining season.

9. Water content determination for storage

Take 20 to 50 seeds out of the envelope and process as described below (same as 9).

Gravimetric water content determination

Grind 20 to 50 seeds (depending on availability).

Transfer in preweighed and prelabeled aluminum cans (or aluminum foil).

Weigh each container or foil containing the fresh matter.

Maintain at 80-105 °C until constant weight is reached (8-17 hours depending on material).

Weigh remaining dry matter (maintain sample in desiccators during weighing process to avoid sample rehydration—this may not be necessary during the dry season).

Water content expression (% of fresh weight)

WC = (fresh weight – dry weight) x 100 / fresh weight

Minimum data capture: Fresh weight before oven drying, dry weight after oven drying

10. Germination

Perform germination test in polyethylene boxes

10.1 Add a pinch of Mancozeb (anti-fungus) to each envelope containing the seeds.

Important: Wear a mask and gloves: Mancozeb is very toxic in powder form.

10.2 Cut and arrange seedburo K-22 germination paper sheets (also known as Kimpak or crepe paper) in the transparent polyethylene boxes (7 to 14 layers per box). Use round (110 x 45 mm) and

rectangular (170 x 110 x 50 mm) boxes for small and big seeds, respectively.

10.3 Add 20 to 30 ml of water to each box.

10.4 Eliminate excess water from germination boxes prior to autoclaving.

10.5 With the help of forceps, place 10 to 20 seeds in each autoclaved box (2 rows/lines) (Figures 13a, 13b).

Important: Wear a mask and gloves while placing the seeds in the box.

10.6 Optional: In the case of wild *Vigna* species or other crop species that exhibit dormancy, mechanically scarify seeds prior to germination. One way to do this is to puncture the seed coat with a scalpel.



Figures 13a



Figures 13b

- 10.7 Write the accession number on a small piece of paper and place in the germination box.
- 10.8. Tightly close boxes and transfer into a germination room (25-30 °C, natural light).
- 10.9 Take the first germination record 6 days after seed setting, then every 48 h until germination rate reaches a plateau (11 days maximum) (Figure 14). Discard contaminated seeds.
Open boxes after 9 days to allow full seedling development
- 10.10 Record seedling vigor/normality at day 11 (Figure 15).
- Important: Germination room temperature is monitored with a temperature logger** (Figure 16).



Figure 14



Figure 15



Figure 16

Minimum data capture: Number of germinated seeds, number of contaminated/discarded seeds, and number of vigorous seedlings (development of epicotyls). Record data first on paper (see Table 1 below), and then transfer final germination rate and vigor data to the computer.

Table 1: Germination record table

Minimum and maximum temperature in germination room:

Date of germination test:

Seed batch:

Operator name:

| Accession no. | No. seed set for germination | No. seed germinated at day | | | | Final % germination | No. of normal seedlings |
|---------------|------------------------------|----------------------------|---|----|----|---------------------|-------------------------|
| | | 6 | 8 | 10 | 12 | | |
| | | | | | | | |

11. Seed counting and weighing

This step takes place once seeds have reached minimal water content and germination % is known.

11.1 Weigh the total seed lot with an electronic balance.

11.2 Sort 100 seeds with a counting machine (Figure 17).

11.3 Weigh.

Minimum data capture: Total weight, 100-seed weight.



Figure 17

12. Packaging

Pack seeds using the priority below:

Medium-term store (5 °C) in plastic box (Figure 18).

Long-term store (-20 °C) under vacuum in laminated foil (Figure 19).

Safe duplication (sample to be transferred to host bank) under vacuum in laminated foil.

Svalbard (sample to be transferred to Svalbard) under vacuum in laminated foil

Important: Prior to packaging for safe duplication or Svalbard, check whether or not the accession has already been sent. If yes, there is no need for duplication.

Important: Prior to packaging, make sure that all seeds harvested from the same accessions are ready to be packed (merge samples harvested during the same season even if processed in different batches).



Figure 18



Figure 19

12.1 Seed repartition

12.1.1 Seed samples containing more than 1,000 seeds with 50% germination minimum are packed as follows:

Medium term: 250 minimum

Long term: 250

Safe duplication: 250 (if not yet duplicated)

Svalbard: 250 (if not yet in Svalbard)

12.1.2 Seed samples containing between 500 and 999 seeds with 50% germination minimum are packed as follows:

Medium term: At least 250

Long term: 250

Safe duplication: 250 (if not yet duplicated)

Seed samples containing less than 500 seeds

Medium term: 250 minimum unless sample is less than 250

Long term: 100 seeds (minimum) to 250 (maximum)

12.1.4 Repartition of accessions with germination rate below 50% are treated on a case by case basis

12.2 Once packed, bar code each container (Figures 20a, 20b). In addition, print the following data and include in the storage container:

Crop name, Accession number, Number of seeds, Harvest date, Germination, Water content.



Figure 20 a



Figure 20 b

13. Storage

Store seeds temporarily at 5 °C (Figure 21) until packaging of all the accessions of the same crop harvested in the same season is completed. Allot a location for each accession in the store.

14. Sample sorting for distribution after storage

Perform seed request sorting as follows:

14.1 Print a list showing accession number, quantity of seeds requested, and location in the store.

14.2 Sort selected accessions out of the store.

Important: Maintain samples stored at –20 °C overnight at 5 °C prior to bringing them at ambient temperature.

14.3 Count the exact number of seed requested using a seed counter and pack in a small paper envelope (Figure 22). Prelabel each envelope either by hand or with a bar code sticker. The label must clearly show the name of the crop and the accession number.



Figure 21



Figure 22

15. Shipment

Send sample/s out for shipping once the import permit and transfer agreement are cleared (see distribution process).

15.1 Pack the paper envelopes containing requested accessions in a carton box or envelop with the list of germplasm requested.

15.2 Bring the box to the secretary of the genebank where the packed accessions will be matched with the initial request list.

15.3 Once all the data match, add the material transfer agreement and import permit (where necessary) to the shipment box and ship via express mail.

Minimum data capture: Distribution data (see distribution process)



