

	Safety duplication of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
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Introduction & Purpose

The National Centre for Genetic Resources and Biotechnology (NACGRAB)'s genebank is in Ibadan and holds about 10,500 of accessions of over 40 crop species. As part of its strategy to secure and make the collection available in perpetuity, NACGRAB deposits duplicate samples of each unique accession in high-quality genebanks at two geographically distant locations. The primary safety back up for NACGRAB's collection is held at ICRISAT genebank located in Niamey. The secondary safety back up is deposited in the Svalbard Global Seed Vault (SGSV) on Spitsbergen Island in Norway (78° N, 15° E) as part of the global effort towards securing crop diversity in collaboration with the Crop Trust. Legal agreements (Annexes 1 and 2) have been made between NACGRAB and the recipients of the safety duplicates. These agreements describe the responsibilities, terms and conditions under which the materials are maintained.

The duplicate collections include both the material and its associated information. The deposits are made under the black box and safety duplication systems. The 'black-box' system is in which the repository genebank has no entitlement to the use and distribution of the germplasm while the safety duplication system is in which where the repository genebank can regenerate and make use of the germplasm. The deposited materials in 'black-box' system are legally under the administration of NACGRAB and the holding genebank does not have access to the materials without permission from the depositor. It is NACGRAB's responsibility to ensure that the deposited material is of high quality, and to use the base collection to regenerate the duplicate collections when they begin to lose viability.

The purpose of this SOP is to ensure the consistency and quality of the safety duplicates of NACGRAB's genetic resources in compliance with international standards.

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2. *Scope*

This SOP applies to the safety duplication of all crops conserved as seeds in NACGRAB's genebank and designated to the Multi-Lateral System (MLS) of the ITPGRFA. Accessions to be safety duplicated may be landraces, wild relatives, varieties and advanced cultivars. These materials are from farmers' fields or natural habitats in Africa and recently regenerated material from the field.

This SOP describes the following activities:

- Safety duplication review
- Withdrawal and seed processing
- Consignment preparation
- Post Consignment follow-up
- Data Management
- Schedules for the shipment of safety duplicates

Genetic resources that are duplicates from other collections or that are not considered as accessions (such as genetic stocks) are not safety duplicated.

Viability testing is described in detail in the standard operating procedure "Conservation of genetic resources at NACGRAB" (NACGRAB-CONS SOP-v1.2).

This SOP may be used to guide the safety duplication activities undertaken by staff and colleagues at other centers.

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3. *Definitions & Abbreviations*

The following definitions and key terms are pertinent to this SOP:

Accession	A distinct uniquely identifiable sample representing a cultivar, breeding line or a population, which is maintained in storage for conservation and use.
Active collection	A collection of germplasm maintained for ready distribution and conserved indefinitely. Users access material in active collections when their working collections prove inadequate for their particular purposes. Distribution is easier within a region than between regions for a variety of reasons (cost of shipment, plant health regulations, ease of interaction with users etc. It is conserved at 5-10°C and 15% RH. Seeds are sealed inside air-tight aluminium foil pouches after drying at 15% RH, 15°C and conserved at 5°C.
NACGRAB	National Centre for Genetic Resources and Biotechnology
Base collection	A collection of germplasm conserved for the long term, which is used to replenish the active collection as needed, but from which no distribution to users is done. Its physical proximity to users is not important, unlike active collections. However, proximity to the geographic origin of the crop might be an advantage because of the environmental suitability for regeneration
Breeder's line	Materials developed and fixed by a breeding method
Drying room	An air-conditioned room at 18°C and 15% RH used for seed drying
Genebank material	Materials imported by the genebank and introduced into the active collection for use and conservation
GRU	Genetic Resources Unit at NACGRAB
H&S	Health and Safety: the right of every employee to carry out his daily work in a safe environment
IPR	Intellectual Property Rights
ISGM	International Standards for Germplasm Management
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LTS	Long Term Storage: a cold room at -20°C and 15% RH for the conservation of the base collection
MTS	Medium Term Storage: a cold room at 5-10°C and 15% RH for the conservation of the active collection
NARS	National Agricultural Research System

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Safety back-up collection	A copy of a collection conserved off site as an insurance against loss
SGSV	Svalbard Global Seed Vault
SMTA	Standard Material Transfer Agreement
SOP	Standard Operating Procedure
TTT	Trueness to Type

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4. Procedure

4.1 **Safety duplication** is initiated when accessions are identified in the database as materials not yet duplicated at Location 1 or Svalbard (SGSV). Safety duplication is also initiated if there is loss of viability of seeds in the active or base collection if the current safety duplicate sample was taken from the same seed lot.

4.2 A preliminary list of accession to be safety duplicated is generated by the database manager. The target at NACGRAB is to have 100% of the collection safety duplicated at the two locations.

Safety duplication review

4.3 The **review** process to determine if accessions are to be safety duplicated is done by the genebank manager as approved by the curator. Accessions are available for safety duplication when the following conditions are met:

- Legal aspects: the legal and IP status allows for safety duplication
- Quantity: inventory is checked to determine if the amount of seeds conserved in LTS or newly harvested is enough to withdraw 30-40 g seeds.
- Viability: there is $\geq 90\%$ germination. For accessions with strong dormancy such as wild relatives, $\geq 40\%$ germination is allowed.

4.3.1 If all accessions on the preliminary list meet the above conditions, the safety duplication procedure is continued.

4.3.2 If accessions or new harvests have insufficient amount of seeds, they are withdrawn from the list and added to the regeneration list for the upcoming planting season.

4.3.3. If accessions or new harvests have $< 90\%$, they are withdrawn from the list and added to the regeneration list for the upcoming planting season. If accessions or newly harvested materials have not been tested for viability, they are tested following the viability procedure described in the SOP for conservation (NACGRAB-CONS SOP-v1.2). Results are uploaded to the database and conditions in step 4.3 are re-evaluated.

Identity and Genetic Integrity:

4.4 To ensure that the genetic composition of the safety duplicated material is identical to the originally acquired material, we make sure that seeds in LTS are regenerated and after harvesting, the seeds prepared for safety duplicate are authenticated against the corresponding samples in LTS.

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Withdrawal and seed processing

4.5 An updated list of accessions to be safety duplicated is generated by the data manager and sent to the research assistant. The list includes the following information:

- 1) Accession number
- 2) Accession name
- 3) Full scientific name
- 4) Date of regeneration (month and year)
- 5) Country of origin
- 6) Number of seeds to be sent to Location 1 and SGSV
- 7) Viability of seed lot

4.6 **Seed withdrawal.** Seed samples of 30-40g are withdrawn from LTS or from newly harvested samples after cleaning and drying. The equilibrium relative humidity (eRH) of the seeds for a random sample of the accessions is checked using the Rotronic water activity instrument. Specify the moisture content needed for safety duplicates.

4.7 **Seed treatment.** All seed lots in net or tissue bags are placed in a plastic barrel and fumigated using 57% aluminum phosphide (Phostoxin®) tablets as a precautionary measure to prevent insect infestations (Fig. 1). The barrel is hermetically sealed for 5 days.



Fig. 1. Seed fumigation of newly harvested accessions.

4.8 Accessions are re-evaluated for the following criteria:

4.8.1 Seed quantity:

The standard quantity of orthodox seeds for safety duplication depends on the genetic composition, the breeding system of plants and the number of seeds (the sample size should be sufficient to conduct at least three consecutive regenerations with low probability of losing a large number of alleles) The FAO 2014 Genebank Standard specifies that the minimum sample size for allogamous and heterogeneous accessions is 500 seeds and the minimum for genetically uniform accessions is 300 seeds. Other standards for safety duplication and long-term storage of seeds are described in the

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International Board for Plant Genetic Resources (IBPGR) document, Advisory Consultation on Genebank and International Standards for Germplasm Management (ISGM) Framework for Seed Collection.

- Taking into account the criteria above, the safety duplicate of accessions should contain 300 seeds for inbred/uniform materials and 500 seeds for outbreeders/diverse material, if possible.

4.9 Two barcode labels per accession are printed by the database manager. The bar codes are printed in the following way: the list of accessions is exported in Excel format from the database to a computer hosting the label printing software. The information is printed on thermal transfer labels and includes the NACGRAB logo, accession name, accession number, country of origin and the barcode. The Desk label printer for aluminum foil bags used in long-term storage is Zebra ZT400 Series ZT411 Industrial Label Printer and the recommended type and label size:

- Label size: 1.3 x 3.75 inch
- Material: Synthetic polypropylene
- Glue type: Permanent

4.10 **Seed packing.** To avoid the reabsorption of moisture, the packing is carried out in the drying room at 15% RH, 15°C. The packing process for each accession is done in three steps.

4.11 The trilaminate aluminum foil envelope is labeled by placing one of the labels inside the aluminum foil envelope and by pasting the second label on the outside of the foil.

4.12 The seeds are dispensed into the trilaminate aluminum foils which are of these dimensions:

- 321/04 Mate Standard foil pouches 90x120mm (external) (+/-2mm) 321/04 pouches
- 321/04 Mate Standard foil pouches 130x130mm (external) (+/-2mm) 321/04 pouches
- 321/04 Mate Standard foil pouches 130x260mm (external) (+/-2mm) 321/04 pouches

4.13 The envelopes are sealed with a Hulme Martin heat sealing machine.

Consignment preparation

4.14 The following documentation is assembled for inclusion inside the container:

- 4.14.1 **Inventory list:** The list of accessions to be packed in each container is generated. The list is printed and placed inside the corresponding container. This list includes the following information:

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- Batch number
- Crate number
- Accession number
- Accession name
- Full scientific name or species
- Germination percentage
- Date of last regeneration (month and year)
- Seed lot identification number
- Country of origin
- Number of seeds

4.14.2 **Import permit:** NACGRAB contacts the managers of the repository genebanks, sends the inventory list and requests import permits.

4.14.2.1 If the repository genebank is [Location 1], the import permit is sent to the genebank together with any other documentation required for seed import. An acknowledgment of receipt is sent to [Location 1] and the consignment is shipped when duplicates and paperwork are ready for shipment.

4.14.2.2 If the repository is SGSV, the import permit is sent to the genebank and the date of the next opening of the vault is specified. An acknowledgment of receipt is sent to SGSV and the shipment is prepared for the specified date.

4.14.3 **Phytosanitary certificate:** The genebank submits a **phytosanitary certificate request form** to National Agricultural Quarantine Services (NAQS). The form includes the following information:

- Repository genebank's name,
- Full address,
- Recipient country,
- List of material to be shipped,
- Import permit

4.15 Based on the documents above, the NAQS issues a phytosanitary certificate. The phytosanitary certificate and the stamped list of germplasm are sent to the genebank.

4.16 **Packing the containers.** A large plastic sheet is placed inside the container as a barrier against moisture. The containers are moisture-proof and eliminate the fluctuation of relative humidity in changing environments.

4.17 The aluminum foil envelopes are verified against the final safety duplicate list.

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4.18 The envelopes are inserted directly into plastic containers in consecutive order with the labeled side on top (Fig. 2).



Fig. 2. Accessions are packed in plastic containers for safety duplication.

4.19 The containers are visually checked to ensure that the aluminum foils are well arranged inside the container.

4.20 Copies of the following documents are placed in a paper envelope:

For both safety duplicate locations:

- List of accessions
- Phytosanitary certificate issued by [National Institution]
- Import permit

4.21 The paper envelope with all documentation is placed on top of the aluminum envelopes.

4.22 The plastic sheet is folded over the envelopes and secured with tape.

4.23 Each container is assigned a consecutive identification number (also called box number). The box numbers are printed on sticky labels and one label is affixed in the interior of the container on top of the plastic sheet (as a means of identifying the container).

4.24 The container is closed and box labels are placed on the exterior of the container on five sides (Fig. 3).

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Fig. 3. Box labels are placed on 5 of the container's sides.

4.25 The following **original documents** are placed in a paper envelope and taped on the outside of container having the smallest identification number:

- List of accessions
- Phytosanitary certificate issued by [National Institution]
- Import permit
-

4.26 The containers are sealed with packing tape (Fig. 4).



Fig. 4. Containers are sealed with packing tape.

4.27 All containers are consolidated and stored temporarily in the medium-term storage facility (5°C) awaiting the shipment process.

4.28 Additional documents are required for shipment. The following documents are assembled and provided to the courier:

- Commercial invoice/customs declaration,
- Completed courier waybill,
- Copy of the import permit,
- Copy of official phytosanitary certificate

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4.29 The repository genebank is informed by email of the shipping arrangements and the probable date of arrival of the consignment.

4.30 The consignment is shipped by the fastest way possible, usually via international courier. The consignment is picked up at the institute by the courier and shipped to the repository genebank.

4.31 Relevant information on the shipment such as tracking number, scanned waybill, import permit, and phytosanitary certificate are sent to the repository genebank via e-mail by genebank staff.

Post-Consignment follow-up

4.32 Staff track the delivery of the consignment online using the shipment's tracking number. When the consignment is delivered, the proof of delivery is printed and filed (Fig. 5).

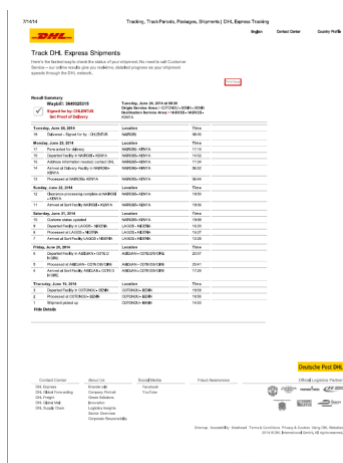


Fig. 5. Once the shipment arrives at the repository genebank, the proof of delivery is printed and filed.

4.33 An **acknowledgement receipt** is requested from the repository genebank to confirm arrival of the shipment.

Data management

4.34 Box numbers and shipment date are updated in the database.

4.35 Information on the final accession list is uploaded to the database

4.36 The inventory list, the consignment documents and the proof of delivery are uploaded to the genebank database by the database manager. In most cases, we ask for the requirements of the repository genebanks which are provided. The data are inputted on Microsoft excel spreadsheet and both the electronic and hard copies are sent. The onus of inputting data of safely duplicated

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materials in their own database lies in the repository genebanks. Some of the data that always accompany our safety duplicate materials are:

- FAO WIEWS institute code
- Deposit box number
- Collection name
- Accession number
- Full scientific name
- Country of collection or source
- Number of seeds in the sample
- Regeneration month and year
- Viability (%)

4.37 The **safety duplication** procedure is completed and finalized when:

- 1) The genebank receives an official confirmation by the repository genebank that the shipment has arrived to its destination and,
- 2) All pertinent information has been entered into the genebank database

4.38 Quality Management System: We have not had a case of renewing/replacing safety duplicated materials that have declined in viability in the repository genebanks. However, if such cases arise and the repository genebanks contacted us, we will send fresh samples that are of adequate viability. Meanwhile, the clause of replacing safety duplicated materials is noted and will be included in our memorandum of understanding in our future dealings with repository genebanks.

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Schedule for the shipment of safety duplicates (*taiilor this table to show how your genebank plans to achieve 100% safety duplications in the coming years*)

4.38 Table 1 below outlines the yearly safety duplication schedules for the period 2022-2030. As of 2022, 367 (3.5%) of NACGRAB's genetic resources are stored in [location 1] and 399 (3.8%) accessions are stored in SGSV. Table 1 also shows the estimated number of accessions that will be sent to the primary and secondary locations during 2022-2030. It is estimated that by 2030? the entirety of the costed collection will be duplicated at the two locations.

Table 1. Number of accessions conserved at NACGRAB and safety duplicated at two levels for the period 2022-2030.					
	Total No. of accessions in the costed collection	Primary safety duplicate		Secondary safety duplicate	
Year		No. of accessions duplicated at Location 1	% of the collection at Location 1	No. of accessions duplicated at SGSV	% of the collection at SGSV
2022	10,500	500	4.76	0	0
2023	10,500	500	4.76	500	4.76
2024	10,500	500	4.76	500	4.76
2025		1000	9.52	1000	9.52
2026		1000	9.52	1000	9.52
2027		1000	9.52	1000	9.52
2028		1000	9.52	1000	9.52
2029		1000	9.52	1000	9.52
2030		1000	9.52	1000	9.52

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5. *Staff Training and Competency*

Training and competency requirements to perform this SOP have been met by the following staff:

- Mayowa R. Olubiyi- Chief Scientific Officer; mayoraph@yahoo.com
- Dr. Adam Olosunde- Asst. Director and Genebank manager; olosundam@yahoo.com
- Ms. Mary Adiat-Adegoke; maryadiat18@gmail.com
- Ms. Adetoro Babatunde; adetorob27@gmail.com

Genebank Staff undergo yearly training and periodic competency testing. The complete training, competency schedule and succession plans are available at [NACGRAB] Human Resource Office.

6. *References*

FAO. 2014. Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rev. ed. Rome

FAO. 2022. Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation in field genebanks. Commission on Genetic Resources for Food and Agriculture. Rome.

Global Public Goods Phase 2 (GPG2). Principles and strategy for safety duplication of plant genetic resources for food and agriculture. 30 pages

International Board for Plant Genetic Resources (IBPGR) document

International Standards for Germplasm Management (ISGM) Framework for Seed Collection

NACGRAB 2020. NACGRAB Genebank operation manual. 30 pages, not yet accessible.

NACGRAB 2020. Seed processing for distribution. Poster available at NACGRAB Genebank.

NACGRAB 2020. Flowchart for germplasm management. Poster available at NACGRAB Genebank.

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7. *Revision History and frequency*

Effective Date of the SOP	Version #	Description	Reviewed By
19/09/2022	1.0	Original SOP	Sunday Adadele
06/01/2023	1.1	Audited SOP	Janny van Beem
15/01/2024	1.2	Edited SOP	Janny van Beem

Revision frequency: the review of this SOP is done on a yearly basis and is part of the staff's work plan and performance evaluation process.

8. *Citation, Copyright, Access agreement*

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NACGRAB has an explicit [access agreement](http://www.NACGRAB.org) (www.NACGRAB.org) that defines the terms of use of the information.

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Annex 1. Contractual agreement between Centers for first level safety duplication (*to be inserted*)

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Annex 2. Contractual agreement between Center and SGSV

To be inserted

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Annex 3. Safety duplication flowchart