

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 1 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

1. *Introduction & Purpose*

The collections conserved at National Centre for Genetic Resources and Biotechnology (NACGRAB) are part of Multilateral System (MLS) and are kept and maintained in trust for humanity through an agreement with the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), under the auspices of the Food and Agriculture Organization of the United Nations (FAO). Its active and base collection are currently located at the NACGRAB's station in Ibadan, Oyo state, Nigeria (07° 23' 070" N, 003° 50' 399" E & 193m altitude).

The purpose of this SOP is to give detailed instructions to ensure consistency in conservation activities for all types of plant (arable, landraces and crop wild relative) representing the varying biodiversity in Nigeria, while demonstrating compliance with international genebank standards. The aim of NACGRAB's genebank is to conserve and maintain the genetic integrity of plant genetic resources (PGR) under conditions that meet and exceed recognized international standards based on current technologies and scientific knowledge and promote their sustainable use in Nigeria.

2. *Scope*

This SOP which is in line with the conservation guidelines, applies to the conservation of plant genetic resources (arable, landraces and crop wild relative) in NACGRAB and describes the following activities:

- Seed reception
- Seed processing (cleaning, moisture testing, drying, authentication, viability testing, health testing)
- Packaging
- Storage
- Monitoring of conserved germplasm

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 2 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

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.
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 equipped with a dehumidifier and utilized for seed drying
FAO	Food and Agriculture Organization of the United Nations
Genebank material	Materials imported by the genebank and introduced into the active collection for use and conservation
Genetic resource	Genetic material of potential or actual value
H&S	Health and Safety: the right of every employee to carry out his daily work in a safe environment
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
I&WE	Infrastructure and Work Environment
Short Term storage (STS)	A cold room at 5-10° C and 18% RH for the conservation of active collection
Medium Term storage (MTS)	A cold room at -5° C and 15% RH for the conservation of collection

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 3 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

Long Term storage	A cold room at -18° C and 15% RH for long term storage
Safety back-up collection	A copy of a collection conserved off site as an insurance against loss
SOP	Standard Operating Procedure
SGSV	Svalbard Global Seeds Vault
Temporary storeroom	Storage room for the conservation of breeder's lines at 25° C and 35% RH

4. Procedure

4.1. The conservation procedure for new accessions is initiated when the accession is released from post-entry quarantine, collection exploration or donation and all pertinent information is recorded in the inventory. This step marks the formal introduction of the accession into the collection and prompts the initiation of the following conservation activities:

- Seed reception
- Seed processing (cleaning, moisture testing, drying, authentication, viability testing, health testing)
- Packaging
- Storage
- Monitoring of conserved germplasm

Conservation activities for accessions within the collection may also be initiated if:

- 1) Materials are regenerated in the field or screen house
- 2) Monitoring systems in database trigger an alert notification indicating that a particular metric is below threshold value. Seed quantities are monitored via inventory alerts while viability is monitored via germination alerts. Alerts prompt the inclusion of an accession in a schedule.
- 3) Schedules indicate specific conservation tasks that are carried out at specified times. Schedules for the conservation of some crop germplasm are shown in Annex 1.
- 4) Genebank staff identify the need to engage in an extemporaneous conservation activity due to a specific request, new partnerships or projects, an emerging pathogen or the emergence of new technologies.

Seed Reception

4.2 Seed reception is initiated when new accessions or regenerated materials from the field or screen houses are transferred to the seed threshing area of the genebank. 40 - 120 seed lots are received and processed weekly at the genebank.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 4 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

4.3 Seed lots are received with the list of regenerated accession containing the following information:

- 1) Harvest date (mm/dd/yyyy)
- 2) Regeneration site
- 3) Geo-reference information of regeneration site (latitude, longitude and altitude)
- 4) Accession number
- 5) Accession name
- 6) Plot number
- 7) Staff in charge of regeneration

4.4 This list of regeneration is printed out and checked against the new harvested seedlots in the threshing area. Any mismatching accession is kept aside and investigated.

4.5 A batch code is assigned to the set of 60 seed lots to be processed. The batch code includes the current year and the serial number of reception of the batch (**yyyy-nn**); **yyyy** corresponding to the current year while **nn** represent the serial number. This batch code will follow the set of 60 - 100 seed lots during the seed processing steps to storage.

Seed processing

4.6 Accessions are threshed and winnowed manually and smoothly to remove the seeds from panicles, capsule or pods and discard unfilled seeds and light density materials. This task is done carefully to avoid breakage and loss or mix of labels of different accessions (Figs. 1 and 2).



Fig. 1. Manual seed threshing.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 5 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 2. Manual seed winnowing.

4.7 After threshing the following data are captured and registered in the database:

- 1) Entry date in the threshing area (mm/dd/yyyy)
- 2) Threshing date (mm/dd/yyyy)
- 3) Quantity of harvested seeds
- 4) Name of the staff in charge of threshing

4.8 An ordinary label which includes the plot number, harvest date, entry date in the threshing area, accession number and name is printed in ink.

4.9 The printed label and the harvest plot tag are placed inside the net bag and sent to the fumigation team.

Preparation of seeds file, verification and seed cleaning

Verification is the visual confirmation that physical characteristics of the newly harvested seed sample corresponds with those of previously harvested samples of the accession. Seed cleaning is the removal of debris, physical contamination, inert materials and damaged or infected seeds or seeds of other species. Verification and cleaning improve the quality of the samples and are done immediately before the fumigation.

4.10 Verification or authentication of seed lots is initiated before materials were sent to and retrieved from the fumigation.

4.11 The seed lots are placed on the well-lighted seed cleaning benches (Fig. 3).

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 6 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 3. Identity of accession is checked with the respective seed file



Fig. 4. Seed file

- 4.12. Seed characteristics are compared to the initial Seed lot from which regeneration seeds were taken to confirm seed size and variation within samples.
 - 4.12.1 If the newly harvested sample corresponds to initial seed lot and reference sample, the cleaning processes proceeds.
 - 4.12.2 If the newly harvested sample doesn't correspond to the initial seed lot sample, set aside nonmatching samples and compare them with additional samples of the same accession, if available. The new harvested sample is eliminated if it is not conformed to any previous sample. Genebank staff will inform the field team and possible sources of error are checked and corrected.
 - 4.12.3 Information on the identity checking is recorded in the database: Conformity (yes/no) and any comments.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 7 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

4.13 Weed seeds, inert materials, discolored, deformed, infected, soiled, immature or damaged seeds are removed from the seed lot and then discarded.

4.13.1 If the newly harvested sample corresponds to seed file or images in the reference photo book and reference sample, the cleaning processes proceeds.

4.13.2 If the newly harvested sample doesn't correspond to the seed file or images in the reference photo book and reference seed file, set aside nonmatching samples and compare them with additional samples of the same accession, if available. The new harvested sample is eliminated if it is not conformed to any previous sample. Genebank staff will inform the field team and possible sources of error are checked and corrected.

4.13.3 Information on the identity checking is recorded in the database: Conformity (yes/no) and any comments.

4.14 Healthy-looking seeds that correspond to the accession's color, size and shape are selected and returned in the tissue bag with its respective tags, forming the new clean seed lot.

4.15 Healthy-looking seeds that correspond to the accession's color, size and shape are selected and returned in the tissue bag with its respective tags, forming the clean seed lot.

4.16 Three sub-samples are prepared from the clean seed lot as follows:

- 1) 100 seeds for cultivated species and 20 seeds for wild are placed in paper envelope for viability testing;
- 2) 50 seeds of cultivated species and 10 seeds for wild species are placed in envelopes for phytosanitary testing. Samples for health test are transferred to NACGRAB seed health laboratory.
- 3) 12 seeds are placed in a small paper envelope for photographing if the accession has not been photographed previously.
- 4) The following data on cleaning and sub-sampling status is recorded in the database:

- Date of cleaning (mm/dd/yyyy)
- Withdrawal for viability testing (yes/no)
- Withdrawal for health testing (yes/no)
- Photography of seeds (yes/no)
- Any comment/observation
- Name of the staff in charge of cleaning

Seed counting and weighing

4.17 The whole clean seed lot is weighed using an analytical balance (Fig. 5).

4.18 100 seeds are counted manually and weighed with an analytical balance.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 8 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 5. Clean seed lot is weighed using an analytical balance

4.19 Data on total weight of clean seeds, 100-seed weight are recorded in the database and the total number of seeds in the seed lot is generated using the following formula:

$$\text{Number of seeds} = \frac{\text{Total weight of clean sample (g)} \times 100}{100 \text{ seed weight (g)}}$$

4.20 Based on the data on seed lot size (total weight of clean seeds, 100-seed weight and total number of seeds) seed lots are categorized into four groups:

- 1) **Category 1:** Accessions with sufficient seeds (≥ 300 g of seeds for cultivated species and ≥ 50 g for wild species). The amount of seeds is enough for storage both in active and base collection and to be duplicated for safety back up.
- 2) **Category 2:** Accessions with seed quantities between 150 and 300 g of seeds for cultivated and 20 and 50 g for wild species. The amount of seeds is sufficient for storage both in active and base collection but not for safety duplication. These accessions will be multiplied during the next cropping season and the harvested seeds will be processed for safety duplication.
- 3) **Category 3:** Accessions with seed quantities between 50 and 150 g for cultivated species and 5 and 20 g for wild species). Accessions of this category have to be stored only in active collection. These accessions will be multiplied during the next cropping season and the harvested seeds will be processed for introduction into long term store and safety duplication.
- 4) **Category 4:** Accessions with insufficient amount of seeds (< 50 g for cultivated species and < 5 g for wild species) are not conserved in active and base collection. They will be subjected to seed increase during the next cropping season and the harvested seeds will be processed for introduction into long term store and safety duplication.
- 5) The following data are captured and recorded in the database:
 - Date of weighting (mm/dd/yyyy),

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 9 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

- Total weight (g)
- 100-grain weight (g)
- Number of seeds
- Category (1-4)
- Name of the staff in charge of seed weighting

4.21 The initial moisture content (MC) prior the final drying is measured in order to evaluate the time needed to decrease the MC from the initial value to the optimal MC. Measurement of water activity (A_w) using a non-destructive equipment (DRAMINSKI)

(Fig 6):

4.21.1 A sample of 50-100 g of seeds is taken from tissue bag and poured into the tester.

4.21.2 The value of A_w expressed in percentage is displayed on the screen of the tester

4.21.3 The sample is returned back into the tissue bags

4.21.4 The measurement is repeated three times and the average is considered as the representative MC of the sample;

4.21.5 The values of MC of the 10 seed lots are recorded in the database

4.21.6 The following data are captured and recorded in the database:

- The Batch code (yyyy-nn)
- Randomly selected for MC measurement (yes/no)
- Initial MC value
- Date of measurement
- Name of the staff in charge of MC measurement



Fig. 6. The water activity (A_w) is measured with DRAMINSKI moisture meter

Preparation of sub-samples for storage

4.22 Based on the categorization information entered in the database, a list is generated of accessions with the information on their duplication status in MTS). Using this list, sub-samples are prepared as follows:

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 10 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

4.22.1 Clean seed lots of accessions belonging to category 1 are divided into 2 sub-samples (if not duplicated in MTS and/or safety back up):

- 150 g of cultivated and 30 g of wild seeds are withdrawn and placed in another tissue bag. This will be used for the preparation of seed samples for storage at MTS. The second sub-sample is composed of the remaining seeds in the clean seed lot. This will be processed for storage in active collection (STS).

4.22.2 Clean seed lots of accessions belonging to category 2 are also divided in 2 sub-samples (if not yet duplicated in MTS) as follows:

- 1) 100 g of cultivated and 20 g of wild seeds are withdrawn and placed in another tissue bag for the preparation of seed sample for storage in MTS
- 2) The second sub-sample is composed of the remaining seeds in the clean seed lot. This will be processed for storage in active collection (STS).

4.22.3 Clean seed lots of accessions of category 3 and 4 are not divided.

4.22.4 At the end the preparation of sub-samples for storage, the following data are captured:

- Date of preparation of sub-samples (dd/mm/yyyy)
- Category (1-4)
- Withdrawal of seeds for MTS (yes/no)
- Withdrawal of seeds for LTS (yes/no)
- Withdrawal of seeds for SGSV (yes/no)
- Name of the staff in charge of sub-samples preparation

Seed drying and measurement of moisture content

4.23 The clean seed lots and sub-samples are transferred to the Seed Dryer for final drying before packing (Fig. 7). Final drying is one of the critical aspects in prolonging the seed viability. It consists in the reduction of the seed moisture content (MC) to 6% of moisture content (MC) which is the optimal MC level of safe for most seed storage. This optimal MC is estimated by the water activity (A_w) measured with DRAMINSKI water activity analyzer. Seed moisture content is an important factor that determines the rate at which seeds will deteriorate in genebanks and allows the prediction of the potential storage life of each seed sample.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 11 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 7. Cloth bags with seed samples are place in the seed dryer.

4.24 The seed lots are arranged serially on the shelves and turned upside down every 2-3 days. They are also shaken to allow homogeneous dehydration.

4.25 The moisture content of the samples placed in the seed dryer are monitored until it reaches optimal level of 6% MC or below, as follow:

4.25.1 The same 10 seed lots randomly selected at step 4.21 are identified in the drying room. On the day 1 (the first day in the drying room), the MC is measured using the same procedure described at steps 4.21.1 – 4.21.6;

4.25.2 On day 7 and 14, the moisture content of the samples is checked again.

4.25.2.1 If the moisture content of the selected samples is at or below the optimal MC of 6%, the batch of seed lot is considered dried and ready for packaging and storage.

4.25.2.2 If the moisture content of the selected samples is not at or below the optimal MC of 6%, the moisture content will be checked once every week until the MC reaches the optimal MC;

4.25.2.3 The data on moisture content are entered into the database.

4. 26 During the drying process, the following data are captured:

- Entry date in the drying room (dd/mm/yyyy)
- MC on day 1
- MC on day 7
- MC on day 14
- Any further measurement and the date
- Name of the staff in charge

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 12 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

Viability Testing

The purpose of viability testing is to assess seed quality and seedling vigor and to predict the performance of the seed and seedlings in the field. Viability testing at the genebank is conducted in “transparent plastics” germination testing. Germination in this procedure is defined as the emergence or development of essential plant structures from the seed embryo that would indicate normal plant development under favorable conditions in soil. 180 samples are processed weekly at the genebank.

4.27 The **“transparent plastic” germination test** is initiated when list of samples to be viability tested is provided from database and seed samples are sent to viability laboratory. Seed samples are tested for viability when they belong to one of the following categories:

- 1) Seed samples of new accessions are tested for viability within 30 days of receipt, prior to storage;
- 2) Newly harvested seed lots are tested for viability prior to storage;
- 3) Seed samples of conserved accessions in STS and viability tested more than 3 years ago;
- 4) Seed samples of conserved accessions in MTS and viability tested more than 10 years ago;
- 5) Requested seed samples in STS and viability tested unless within the previous year.

4.27.1 “Transparent plastic” germination tests are conducted using 100 representative seeds for cultivated species and 20 for wild of the sample. Two reps, each with 50 seeds for cultivated and 10 for wild species.

4.27.2 **Heat treatment** is firstly applied to crops that exhibits dormancy to break seed dormancy. After heat treatment, samples are transferred to viability laboratory for “transparent plastic” germination tests. Other dormancy breaking method such as scarification and hot water treatment are also used for some seeds.

4.27.3 The transparent plastics are cleaned with a solution of bleach (sodium hypochlorite).

4.27.4 The roll of tissue paper is folded in of a rectangle and the tissue papers are placed inside the transparent plastics (Fig. 8).

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 13 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 8 Tissue paper are folded in a rectangular shape and placed in transparent plastics

4.27.5 The list and labels of accessions are printed, labels are checked against the list and put inside transparent plastics.

4.27.6 100 representative seeds of cultivated species sample and 20 of wild sample are placed in transparent plastics, two reps, each with 50 seeds for cultivated and 10 for wild.

4.27.7 Seeds on tissue paper are moistened with 1% solution of Mancozeb. Mancozeb is a fungicide applied to avoid contamination of fungus.

4.27.8 Seeds in transparent plastics are arranged at regular intervals of 8 mm with a forceps (Fig. 9)

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 14 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 9. Seeds are arranged inside transparent plastics at regular interval of 8 mm

4.27.9 The transparent plastics are placed on shelves at room temperature.



Fig. 10. Transparent plastics on the shelves with 12/12 hours dark/light regime.

4.27.10 Transparent plastics are checked daily for moisture and rehydrated as needed (Fig. 11).

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 15 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 11. Transparent plastics daily checked for moisture and moistened if needed.

4.27.11 Germination count and classification of normal vs abnormal seedlings is taken after 7 days of incubation.

The transparent plastics are removed to report the results. Normal seedlings, abnormal seedlings and un-germinated seeds in each transparent plastic are observed. Seedlings are considered normal when they show the potential for continued development into satisfactory plants when grown in good quality soil and favourable conditions of moisture, temperature and light. To be classified as normal, a seedling must be an intact seedling which has all essential structures complete, in proportion and healthy: (i) well-developed root system with secondary adventitious roots, and (ii) a green coleoptile which elongates and becomes the first leaf and later, the second leaf appear. The second leaf is a complete leaf. It is differentiated into a leaf blade and a leaf sheath as described in ISTA rules, 1999). Abnormal seedlings don't show the potential to develop into normal plant when grown in good quality soil and under favourable conditions.

Seedling is classified abnormal when it is a damaged, deformed, diseased or decayed seedling with lack or abnormal of the essential structures of plant as described in ISTA rules, 1999. Un-germinated seeds are hard, diseased or dead seeds which fail to germinate by the end of the test period when tested under good conditions. After the germination test, to ensure that the non-germinated seeds are dead, the non-germinated seeds are hulled and cut off in the middle and tested again for germination.

If the seeds tested are still not germinated, a new sample of seed of the same accession is obtained, dormancy is broken (by heat treatment at 45C for 5 days) and the sample is viability tested.

Abnormal seedlings and un-germinated seeds are not included in the calculation of the germination percentage.

4.27.12 the number of replicates, number of seeds per replicate, number of normal seedlings, number of abnormal seedlings and un-germinated seeds are counted and entered into the data base.

The formula used to calculate germination is the following:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings} \times 100}{\text{Number of seeds tested}}$$

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 16 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

4.27.13 Accessions with germination rate $< 85\%$ are earmarked for regeneration.

4.27.14. Accession with germination rate $\geq 85\%$ are processed for storage and will be monitored periodically.

4.27.15 the Un-germinated seeds are manually checked to know whether they are hard or dead seeds.

4.27.15.1 When the seeds are dead, the viability test result is confirmed.

4.27.15.2 When the seeds are hard and fresh, the seeds could be dormant. So they are hulled and cut off in the middle and tested again for viability.

4.27.15.3 A sample of this accession with hard seeds is withdrawn and applied to heat treatment to break the dormancy as explained in section 4.27.2. Then the seeds are hulled and cut off in the middle prior to conduct the germination test.

4.27.15.4 The results of the two germination tests are analyzed. When the germination rate of the test conducted in 4.27.15.2 is higher than the first test conducted, we can conclude that the seeds were dormant.

4.28 **Viability monitoring** consist of periodically testing the viability of accessions under medium term and long-term storages to ensure they do not lose their capacity to produce viable plants when needed. Conserved accessions are monitored for viability by conducting “transparent plastics” germination tests.

4.28.1 **Viability of seed samples conserved in STS** is periodically monitored at 5 years intervals after the initial viability test prior storage (year 0, 5, 10, 15, etc.) following the procedure of “transparent plastics” germination described at steps 4.27.1 – 4.27.13.

4.28.1.1 If the germination test at periodic intervals is $\geq 85\%$, the viability is considered acceptable, and the sample remains in STS for an additional 3 years period before being re-tested.

4.28.1.2 If the germination test at periodic intervals is $< 85\%$, the accession is added to the list of accessions to be regenerated (regeneration schedule) and a sample of 5 g of seeds of the accession is prepared for field planting. The seed sample will be replaced by newly harvested seeds obtained after the regeneration procedure.

4.28.2 **Viability of seed samples conserved in MTS** is periodically monitored at 5 years intervals after the initial viability test prior storage and also as required during monitoring and evaluation.

4.28.2.1 The viability monitoring package prepared as described in the steps 4.35.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 17 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

4.28.2.2 These accessions are tested for viability by “transparent plastics” germination tests following the procedure described at step 4.27.

4.28.2.3 The results of the germination test is analyzed and recorded in the database.

4.29 Results from “transparent plastics” germination tests are saved in desktop application system and analyzed.

4.29.1 If at least $\frac{1}{3}$ of accessions has a germination rate $< 85\%$, the batch of these accessions are earmarked for regeneration.

4.29.2 If at least $\frac{3}{4}$ of accessions has a germination rate $\geq 85\%$ the batch of these accessions are processed for storage and will be monitored periodically.

4.30 Once seed quantity, moisture content, germination rates and sanitary health results on the batch of 60 accessions are uploaded to the computer, the seed packaging team is informed.

Packaging

Packing and labelling materials used at the genebank are water and humidity proofs, withstand freezing and are suitable for long-term use.

4.31 Once results have been uploaded to the database from seed processing, viability and phytosanitary labs, the information for each accession is reviewed to confirm that seed quantity, moisture content, germination rates and phytosanitary results meet the established requirements for medium term and long-term storage.

4.32 Sample to be conserved in Short Term Storage (STS) and Medium-Term Storage (MTS) must meet the following conditions:

- 1) It belongs to category 1, 2 and 3;
- 2) The germination rate is $\geq 85\%$;
- 3) Health testing result acceptable (free from pathogens).

4.32.1 If the samples don't belong to category 1, 2 and 3 (sample of category 4), the seed quantity is considered insufficient, and the sample are put in paper envelope and kept in temporary storage. The sample is regenerated during the next cropping season.

4.32.2 If the germination rate is not $< 85\%$ the initial viability is considered unacceptable, and the accession is added to the list for regeneration. However, the sample will be packed stored in STS. This sample will be replaced later by the new one obtained from the next regeneration.

4.32.3 If the health status is unacceptable (presence of pathogens), the sample is considered for treatment. The cleaned sample is sent back to genebank and packaging process is initiated.

4.33 Packaging is initiated when all requirements above are met.

4.33.1 Labels contain the following information:

- 1) Accession number;

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 18 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

- 2) Accession name;
- 3) Country of origin;
- 4) Species;
- 5) Seed lot identification number;
- 6) Crop year;
- 7) Position code in STS/MTS.

4.34 The packing process for each accession is done in three steps:

4.34.1 One label is handwritten outside (towards the middle) while the second hand written label is placed inside of the plastic jars for the samples to be conserved in STS and for the laminate aluminum foil envelope for MTS.

4.34.2 Depending on the species and the location, an amount of seed is prepared by weight and dispensed into the containers in plastic jars or aluminum envelopes. Three (3) seeds packets are prepared for each accession as follows:

- 1) 100 grams (approximately 4,000 seeds) for cultivated species and at least 20 g for wild, are dispensed into the aluminum envelope (165 mm x 130 mm) for medium-term storage (10% RH and -5 °C).
- 2) The remaining seeds are poured into an airtight plastic jar of a capacity of 800 grams for distribution and stored at NACGRAB in Ibadan, Nigeria, at 18% RH and 5-10°C (Short Term Storage). For frequently requested accessions, large quantity of seeds is packed in 2 or 3 plastic jars.

4.34.3 Plastic jars are closed with lid while aluminum pouches are sealed with vacuum sealer, AUDIONVAC heat sealers (Fig. 16).



	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 19 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

Fig. 16. Aluminum envelopes are sealed with AUDIONVAC heat sealers

Storage

4.35 Storage of plastic jars to STS: Seeds are conserved in the active collection or Short Term Storage (STS) for distribution to breeders and other users. The STS is equipped with iron and polish wood shelving system.

- 4.35.1 Plastic jars are put on the tray and are checked against the final list of accessions packed in the drying room.
- 4.35.2 The tray is transported to the STS and plastic jars are placed on the shelves (fig 17). The location of each plastic jar is identified by rank number, shelf number, position code on the shelf, sample identification number, accession number and name.



Fig. 17. Plastic jars are arranged on shelves in STS

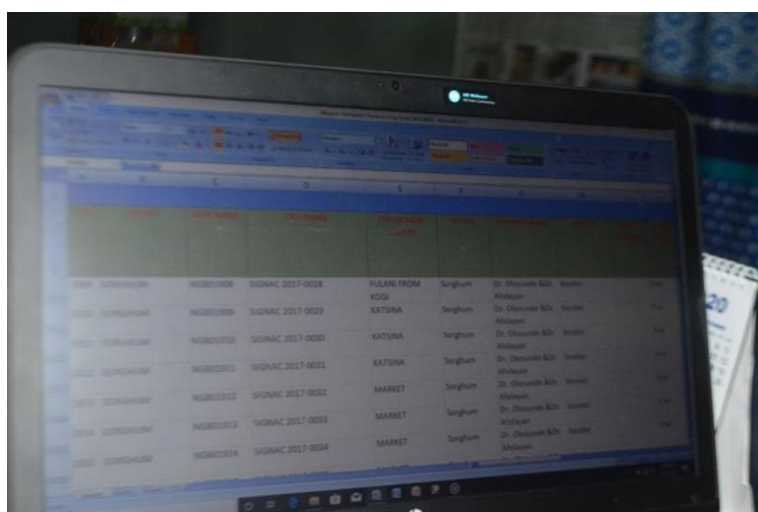
- 4.33.3 Information on the plastic jars is updated in the database

4.36 Storage of accession in MTS at NACGRAB Seed genebank in Ibadan, Nigeria: The MTS is located at the NACGRAB headquarters at the Moor plantation, Ibadan where the STS is also based.

- 4.36.1 The materials for the MTS is sealed into the aluminum foil envelopes and arranged in plastic trays (50 cm x 25 cm x 6 cm)
- 4.36.2 **Inventory list:** The final list of accessions is generated, printed and placed inside the corresponding crate (Fig 18). This list includes the following information:
 - Batch number
 - Crate number
 - Accession number
 - Accession name

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 20 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

- Full scientific name or species
- Germination rate (%)
- Date of last regeneration (month and year)
- Seed lot identification number
- Country of origin
- Number of seed (4,000 seeds)
- Weight of seedlot



Accession Number	Date	Location	Name
NACGRAB-0001	2017-0028	FULANI FROM KOSO	Dr. Olosunde B.O.
NACGRAB-0002	2017-0029	KATUNA	Dr. Olosunde B.O.
NACGRAB-0003	2017-0030	KATUNA	Dr. Olosunde B.O.
NACGRAB-0004	2017-0031	KATUNA	Dr. Olosunde B.O.
NACGRAB-0005	2017-0032	MARKET	Dr. Olosunde B.O.
NACGRAB-0006	2017-0033	MARKET	Dr. Olosunde B.O.
NACGRAB-0007	2017-0034	MARKET	Dr. Olosunde B.O.

Fig. 18. Inventory list

4.37 Separate containers for **viability monitoring** are prepared in the following way:

4.37.1 A list of accessions representing a subset of the consignment is prepared by the database manager. The list contains 5% of the accessions and represents genetically diverse germplasm.

4.37.2 The viability monitoring container is labeled consecutively with MTS plastic crate. An additional label that reads “Samples to be used for viability monitoring” is placed on the container(s) with instructions that indicate its purpose for periodic retrieval and viability monitoring.

4.38 The inventory list is uploaded in the database.

4.39 **Samples preparation for safety duplications.**

4.39.1 Prior to the packaging of aluminum foil envelopes in plastic crates (60 cm x 40 cm x 25 cm), each crate is assigned a consecutive identification number (also called box number). Then the aluminum foils envelopes are divided into crates, 175 aluminum envelopes per crate.

4.39.2 **Inventory list:** The final list of accessions is generated, printed and placed inside the corresponding crate (Fig 18). This list includes the following information:

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 21 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

- Batch number
- Crate number
- Accession number
- Accession name
- Full scientific name or species
- Germination rate (%)
- Date of last regeneration (month and year)
- Seed lot identification number
- Country of origin
- Number of seed (4,000 seeds)

4.39.3 Import permit: NACGRAB seed genebank informs the repository genebank (e.g Svalbard genebank and Millenium Genebank) of his intention to send a batch of seeds for conservation in LTS. The repository genebank for safety duplicate informs NACGRAB seed genebank on is acceptance to receive the materials and the preparation of the consignment is initiated.

4.39.4 NACGRAB seed unit request an import permit from repository genebank. The GRU staff provide the following information to the repository genebank.

- The repository genebank's name
- The provider:
- list of material to be imported

4.39.5 Based on the information above, the import permit issued. The import permit is scanned and sent to NACGRAB seed unit via email.

4.39.6 **Phytosanitary certificate:** NACGRAB seed unit submits a phytosanitary certificate request form to National Agricultural plant Quarantine Service (NAQS), Nigeria (Fig. 19). The form includes the following information:

- repository genebank's name,
- full address,
- recipient country,
- list of material to be shipped,
- import permit

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 22 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	



Fig. 19. Nigeria Agricultural Quarantine Services, Ibadan. Nigeria (NAQS). Inspects seed and issues phytosanitary certificate

- 4.39.7 Based on the documents above, the NAQS issues a phytosanitary certificate. The phytosanitary certificate and the stamped list of germplasm are delivered to NACGRAB
- 4.39.8 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
- 4.39.9 The aluminum foil envelopes are verified against the final inventory list
- 4.39.10 The envelopes are inserted directly into plastic crates, 175 envelopes per crate with the labeled side on top (Fig. 20).

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 23 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

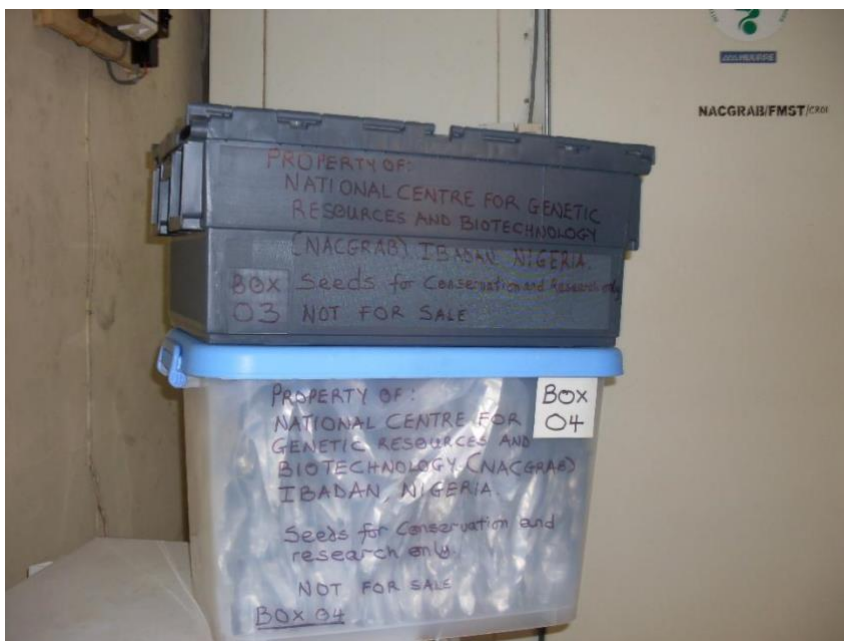


Fig. 20. Accessions are packed in plastic crate for LTS.

4.39.11 The plastic crates are visually checked to ensure that the aluminum foils are well arranged inside the crate.

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

- Inventory list (Fig. 19)
- Phytosanitary certificate issued by NAQS (Fig. 21)
- Import permit issued by repository country phytosanitary services

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 24 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

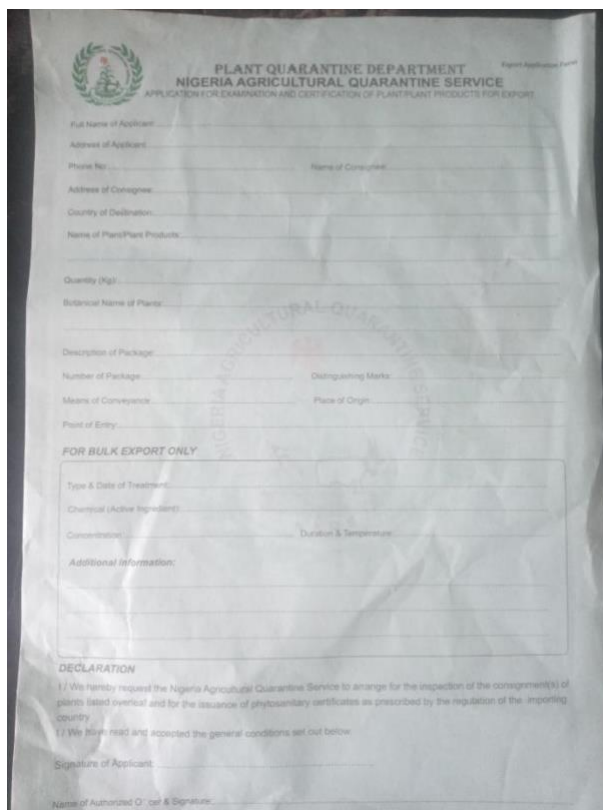


Fig. 21. Phytosanitary certificate form as prerequisite to issuing of phytosanitary certificate by NAQS

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

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

4.39.15 The crate numbers are printed on sticky labels and one label is affixed in the interior of the crate on top of the plastic sheet (as a means of identifying the container).

4.39.16 The container is closed and crate labels are placed on the exterior of the container on five sides

4.39.17 **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 NAQS
- import permit

4.39.18 The containers are sealed with packing tape (Fig. 22).

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 25 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

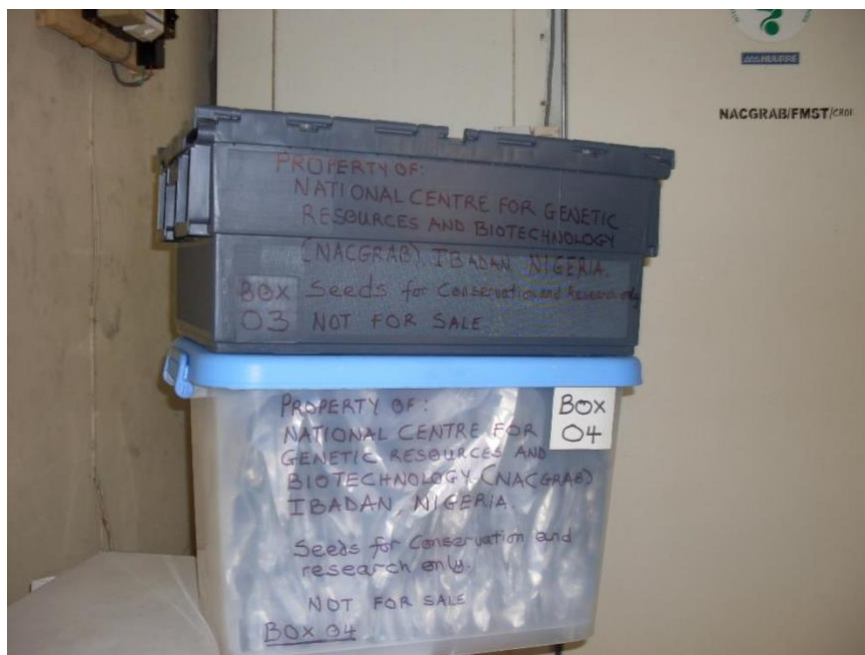


Fig. 22. Containers are sealed with packing tape.

4.40 Separate containers for **viability monitoring** are prepared in the following way:

4.40.1 A list of accessions representing a subset of the consignment is prepared by the database manager. The list contains 5% of the accessions and represents genetically diverse germplasm.

4.40.2 Seed samples are prepared in the same way as described in steps 4.14-4.23.

4.40.3 Samples for viability monitoring are packed separately in a container in the same way as described in steps 4.26-4.27.

4.40.4 The viability monitoring container is labeled consecutively with LTS plastic crate. An additional label that reads “Samples to be used for viability monitoring” is placed on the container(s) with instructions that indicate its purpose for periodic retrieval and viability monitoring.

4.41 The shipment to LTS is processed when at when at least 5 crates (875 seed samples) are ready.

4.41.1 Transport arrangement to repository genebank. A NACGRAB seed unit Staff request an authorization local travel to DHL courier services Ibadan, Nigeria office and request a vehicle and a driver. When approved, the crates including the package for viability monitoring are transported by road to repository genebank.

4.41.2 Once arrived, the crates are stored in the acclimation room at 5°C and 15% RH. Three days later, they are moved to the LTS at -18°C. They are arranged inside the repository LTS.

4.41.3 The inventory list is uploaded in the database.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 26 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

4.42 **Inventory monitoring** consist of the regular checking of the quantity or the existing stocks regarding the number or weight of seed sample conserved in STS. Seed weight is monitored each time seeds are removed from STS for whatever purpose mainly for distribution and planting.

4.42.1 The withdrawn amount of seeds is weighted and packed in envelope and the remaining seeds in the plastic jar is also weighted.

4.42.2 The actual weight is recorded in the database and the plastic jar is returned back in STS at its same position.

4.42.3 For cultivated species, when the actual weight is < 100 g, the accession is earmarked for regeneration; when the actual weight is ≥ 100 g the accession is still conserved in STS and will be monitored periodically.

4.42.4 For wild species, when the actual weight is < 20 g, the accession is earmarked for regeneration; when the actual weight is ≥ 20 g the accession is still conserved in STS and will be monitored periodically.

4.43 Activities in the conservation procedure are cyclical in nature and may be carried out when monitoring alerts, scheduling systems or specific needs arise and prompt particular conservation activities. One cycle of the conservation procedure is considered finalized when: 1) samples are placed in long term storage and 2) all pertinent information has been entered into the genebank database. The conservation procedure for an accession is conducted on a cycle of no more than 50 years.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 27 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

5. *Staff Training and Competency*

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

- Adam Olosunde, Assitant Director Seed Genebank and Head Seed Genebank Unit.
Contact: olosundam@yahoo.com
- Olutoye Fashola, Oversight/Monitoring of Genebank.
Contact: sholapearl@yahoo.com
- Mary Adiat-Adegoke, Agric Suprintendent, oversight of the seed distribution procedure.
Contact maryadiat18@gmail.com;
- Adetoro Babatunde, Agric Suprintendent, oversight of the seed testing and packaging.
Contact torosangokunle@gmail.com;
- Genebank Staff undergo yearly training and periodic competency testing. The complete training, competency schedule and succession plans are available at National Centre for Genetic Resources and Biotechnology.
- Staffs are trained on all necessary precautions when working in MTS, STS and drying areas. The time allowed to stay in the areas (low temp and dry room conditions) are specified, PPE are available at the entrance of the MTS to manage the effect of low temperature. Also, the various compartment and rooms allow for prevention of cold/heat shock when moving from outside environments to inside conditions and vice versa. Genebank staffs are not allowed to work alone.
- Staffs are trained in health and safety measures with periodic evaluation of staff knowledge on requirements and recommendations.

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.

NACGRAB 2020. NACGRAB Genebank handbook. 20 pages.

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 28 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

7. *Revision History and frequency*

Effective Date of the SOP	Version #	Description	Reviewed By
09/09/2020	1.0	Original SOP	Sunday Aladele
11/01/2021	1.1	Audited SOP	Janny van Beem
15/01/2024	1.2	Edited SOPs	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 Agreements)*

Citation: NACGRAB, 2024, Conservation of plant genetic resources, (NACGRAB-SOP-CON-v1.2). Contributors: Sunday Aladele, Adam Olosunde, Olutoye Fashola, Mary Adiat-Adegoke, Adetoro Babatunde, Uthman Tolani

© NACGRAB, 2024

NACGRAB has an explicit access agreement (www.nacgrab.ng.gov) that defines the terms of use of the information

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 29 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

Annex 1: Schedules for the conservation of germplasm at NACGRAB

Procedure	Frequency	Number of accessions processed	Start date	End date
Viability Testing	weekly	200	Day 1: date of test	Day 7: date of counting
Regeneration	annual	1,000	November	May
Characterization	annual	250	April	November
Introduction to LTS	Annual	1,000	July	December

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 30 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

Annex 2. Sample preparation by procedure

Sample preparation for:	Number of seed prepared	Seed source/provenance	Packing material	Pack size	Number of packs prepared	Storage conditions (if applicable)	Comments
MTS	4,000	original or subsequent most-original seed sample	Aluminium pouche	165 x 130 mm	1	MTS (-18°C)	1 sample per accession
STS	40,000	regenerated sample	Aluminium pouche	210 x 300 mm	1	STS (+ 2 °C)	1 sample per accession
Phytosanitary testing	400	regenerated sample	paper bag	90 x 90 mm	1	Drying and packaging room (+15°C, 15%RH)	1 sample per regenerated sample
Viability testing	100	MTS, STS or generated sample	paper bag	90 x 90 mm	1	Drying and packaging room (+15°C, 15%RH)	1 sample per regenerated sample or accession
Distribution	400	STS	paper bag	90 x 90 mm	1	Drying and packaging room (+15°C, 15%RH)	based on request
Safety Duplication	400	STS	Aluminium pouche	130 x 90 mm	1	Drying and packaging room (+15°C, 15%RH)	1 sample per accession
Regeneration	400	STS	paper bag	90 x 90 mm	1	Drying and packaging room (+15°C, 15%RH)	1 sample per accession
Voucher/Reference sample	50	original or subsequent most-original seed sample	paper bag	90 x 90 mm	1	MTS (-18°C)	1 sample per accession

	Conservation of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB)		
	SOP Number: NACGRAB CONS SOP v1.2	Version: 1.2	Page 31 of 31
	SOP Owner: Adam Olosunde	SOP Approver: Sunday Aladele	

Annex 3: Summary table: Number of Crop/species conserved at NACGRAB (November, 2019)

Crop	Genus	Species	No of Accession
Amaranth	<i>Amaranthus</i>	<i>hybridus</i>	252
Celosia	<i>Celosia</i>	<i>argentina</i>	248
Corchorus	<i>Corchorus</i>	<i>olitorius</i>	118
Okra	<i>Abelmoscus</i>	<i>callei</i>	506
Pepper	<i>Capsicum</i>	<i>species</i>	214
Tomato	<i>Lycopersicon</i>	<i>species</i>	144
Sorghum	<i>Sorghum</i>	<i>bicolor</i>	1808
Onion	<i>Allium</i>	<i>cepa</i>	9
African spinach	<i>Basella</i>	<i>alba</i>	12
Garden egg	<i>Solanum</i>	<i>species</i>	114
African Apple	<i>Chrysophyllum</i>	<i>albidum</i>	3
Basil	<i>Occimum</i>	<i>basilicum</i>	6
Sesame	<i>Sesamum</i>	<i>indicum</i>	12
Saint leaf	<i>Occimum</i>	<i>graticinum</i>	3
Pumpkin	<i>Cucurbita</i>	<i>species</i>	27
Roselle	<i>Hibiscus</i>	<i>species</i>	12
Rice	<i>Oryza</i>	<i>species</i>	492
Pearl Millet	<i>Pennisetum</i>	<i>species</i>	1467
Wheat	<i>Triticum</i>	<i>aestivum</i>	212
Groundnut	<i>Arachis</i>	<i>hypogea</i>	368
African yam bean	<i>Sphenostylis</i>	<i>stenocarpa</i>	100
Melon	<i>Cucumis</i>	<i>species</i>	444
Castor seed	<i>Ricinus</i>	<i>communis</i>	3
Bambara nut	<i>Vigna</i>	<i>subterranea</i>	42
Maize	<i>Zea</i>	<i>mays</i>	1375
Soybean	<i>Glycine</i>	<i>max</i>	424
Butter bean			92
Cabbage	<i>Brassica</i>	<i>oleracea</i>	3
Pigeon pea	<i>Cajanus</i>	<i>cajan</i>	50
Cotton	<i>Gossypium</i>	<i>hirsutum</i>	12
Phaseolus	<i>Phaseolus</i>	<i>species</i>	52
Sword bean	<i>canavalia</i>	<i>gladiata</i>	3
Wild vigna	<i>Wild vigna</i>	<i>species</i>	1062
Cucumber	<i>Cucumis</i>	<i>sativus</i>	6
Cowpea	<i>Vigna</i>	<i>unguiculata</i>	810
Acha	<i>Digitaria</i>	<i>exilis</i>	110
Total			10,615