1. Introduction & Purpose

NACGRAB's gene bank holds the collection of both cultivated species and crop wild relatives (CWR) in Nigeria and is composed of around 10,500 accessions made up of40cultivated crop species and 206CWR.

The collections conserved at the 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).

One of the main objectives of the gene bank is to insure the conservation of viable and sufficient quantity of germplasm collection with its original genetic integrity and diversity. The daily removal of seeds for distribution results in a reduction of seed quantity over time, newly acquired germplasm of foreign origin may have low viability or is insufficient for conservation. Therefore, gene bank accessions are regularly monitored for both viability and seed quantity in order to regenerate those which have low viability and small amounts of seeds.

Regeneration is the renewal of germplasm accessions by sowing and harvesting seeds that possess the same characteristics as the original sample. It is the most critical operation in gene bank management. Germplasm regeneration is costly and time consuming, it also involves the risk of losing accessions through poor adaptation or disease and pest damage, introducing admixture through contamination or error, and altering the genetic composition or the original accessions through conscious (human) or unconscious (natural) selection.

The purpose of this SOP is to ensure consistency in the regeneration of conserved cultivated genetic resources as well as CWR in order to maintain the genetic integrity of a given accession.

2. Scope

This SOP applies to the regeneration of genetic resources as seeds. It may be used to guide regeneration activities undertaken by NACGRAB staff on station and outside stations located in other regions. This SOP can also be made available to other research institutions as required.

This SOP applies to the regeneration of cultivated and wild germplasm accessions of different crops at NACGRAB's genebank and the following activities are described in this SOP:

- Selection of accessions and seed sample preparation
- Field preparation and layout

- Sowing and labelling
- Crop management
- Pollination control
- Monitoring accession identity
- Harvest and post-harvest seed processing

This SOP does not apply to the regeneration of genetic resources that do not belonged to cultivated and CWR species or Hybrid crops and genetically modified organisms (GMO).

3. Definitions & Abbreviations

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

Accession	A district uniquely identifiable sample of seeds representing a cultivar, breeding line or a population, which is maintained in storage for conservation and use.
CWR	Crop Wild Relatives
Cultivated crops	Arable crops, grown on farmers field
Genetic Resource	Genetic material of actual or potential value.
GMO	Genetically modified organisms
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
MTS	Medium-term Storage
STS	Short-term Storage

4. Procedure

4.1. The regeneration procedure is initiated for the following reasons:

- **Restoration of seed viability** (Quality threshold): Regeneration of all crop accession in the STS and MTS with viability below 85% are prioritized when identified during the periodical monitoring. According to FAO genebank standards for regeneration, "an accession will be regenerated when its viability has dropped below 85 percent of initial viability of the stored seed".
  CWR are prioritized for regeneration when viability is below 50%.
- **Seed increase** (Quantity threshold)–The genebank prioritize all crop seed for regeneration when the quantity of seeds in the STS is less than 1500 seeds.
  In view of this, regeneration shall be conducted when the quantity of seeds in the STS/MTS is very low or when the remaining seed quantity is less than what is needed for three sowings of a particular population of the accession.
- **Special requirement -** Special requirement for seed multiplication may arise for accessions that are often requested or with special traits that breeders and researchers frequently use (high yielding, pest and disease resistant accessions, genetic stocks, etc.) or accessions requiring safety duplication and repatriation. It also includes seed requests that need more than the standard amount the genebank normally distributes, i.e. 10 g.

4.2 Site of regeneration and planting season

The cultivated species are regenerated during the wet and dry (with irrigation) season when good quality seeds (free from disease and pest attack) can be produced.

Moreover, to ensure good quality and quantity harvest, due attention shall be paid to the environment in which the regeneration activity will be undertaken to avoid severe selection pressure on the accession.

Thus, materials to be regenerated are sent to most suitable agro-ecological environment (outside stations) closely related with the collecting site in order to minimize genetic drift as well as to produce the best quality of seed." and monitoring will be strictly carried out.

NACGRAB's experiment site is in Ibadan (7°23'N latitude and 3°53'E longitude), Badeggi (9°3'N latitude and 6°34'E longitude) and Kano (11°23'N latitude and 8°23'E longitude). These sites are often modified to accommodate irrigation facilities. Screenhouses are also used for the regeneration of accessions that have few seeds available (< 50 seeds) and for Nursery.

Regeneration site depends on the following factors:

(i) photoperiodism
(ii) Rainfall
(iii) Availability of irrigation
(iv) Security of sites

(v) Heat indexes
(vi) Low temperature
(vii) adaptation

4.3 Selection of accessions

Regeneration of accessions that have inadequate quality (low viability) take priority over accessions with inadequate number of seeds (inventory). Those in base collections or MTS take priority over accessions in active collections (STS).

4.4 Withdrawal of seed from sources

Depending on the percentage germination and the required amount of seeds, 130 – 200 seeds for self-pollinated species and 90 – 100 seeds for cross-pollinated species will be enough for a standard plot. Plot sizes vary depending on the planting distance, crop type, and growth behavior. Samples will be packaged from the STS frequently when the quantity and quality are reduced for pre-treatments as needed. However, samples are packed for regeneration from the MTS intermittently in 3-5 years during routine checks.
It is good to note that when seeds in the medium-term storage (MTS) collections losses viability, it shall be regenerated using seed of the initial sample. Also, when there is insufficient seeds stock in the short-term storage, seeds shall be regenerated from STS collections for up to three regeneration cycles before returning to the original medium-term storage sample.

True -to-type verification of seeds are done using digital seed file before planting.

4.5 Pre-planting treatments

Pre-treatment procedure is crop dependent at NACGRAB gene bank.

For dormant accessions such as *Corchorus spps, Abelmuscusspps, Oryzaspps*etc, a pre-treatment to overcome the dormancy is necessary to improve seed germination. The procedure applied at NACGRAB is the hot water treatment method. It consists of adjusting seeds to ambient temperature, then seeds are tied in small cloth bags, soaked water at 80°C for 10 minutes and air dried for 24 hours to break dormancy, followed by equilibration at ambient temperature for 24 hours before seeding.

For dormant accessions such as wild Vignaspps, African Yam Bean, wild vigna and pigeon pea scarification done to aid germination. The procedure involves slightly and carefully scratching off parts of the seed coat with rubber carpet, penknife, sandpaper or surgical blade before seeding.

4.6 Plot number assignment

Plot numbers are assigned to the accessions by randomization on excel sheet using the RAND formula(fig.1). The same plot number and accession number is written on label and used in the seeding, transplanting, and harvesting processes to minimize errors; keep a field map of the plot layout

in the field and in crop based folder. Consecutive numbers for plots grown are used on the station within the same crop season; never use the same plot number twice in one season.

S/N	Entry	RAND
1	NGB00228	0.781219
2	NGB00237	0.233439
3	NGB00205	0.526255
4	NGB00231	0.116989
5	NGB00209	0.483936
6	NGB00218	0.846278
7	NGB00651	0.522657
8	NGB00226	0.850502
9	NGB00193	0.552408
10	NGB00215	0.269898

S/N	Entry	RAND
4	NGB00231	0.116989
2	NGB00237	0.233439
10	NGB00215	0.269898
5	NGB00209	0.483936
7	NGB00651	0.522657
3	NGB00205	0.526255
9	NGB00193	0.552408
1	NGB00228	0.781219
6	NGB00218	0.846278
8	NGB00226	0.850502

Fig.1: Numbering field plots.

4.7. Seeding and seedbed activities

The type of seed bed used is trays and plastic pots. The seed trays and plastic pots are filled with top soil. Trays have grids separation one row from another. Sometimes furrows are made using rulers and seeding rows. The number of rows and their length is determined by the number of accessions to be multiplied. Approximately, 10 g of seed of each accession is sowed on a row of 1.5 m length. The seedlings of each accession will be transplanted on 5 $m^{2.}$

Fig. 2a. Seeding on the planting tray

Fig. 2b. Seeding in the planting bag

- **Labeling and seeding**

Labels are placed at the start of each entry. The name and the plot number of each accession are written on the label. A small amount of fertilizer is applied, about 5 g/m2 of NPK (15-15-15). Seeds are sown evenly on the assigned rows for each accession. The seed bed in trays is covered with topsoil ('black soil'). Granular insecticide, e.g. carbofuran, is applied to control ants and crickets. This can also control nematodes. The seedlings will remain in these tray and plastic pots for approximately three weeks.

- **Water management**

The seed tray is flooded. To avoid mixing-up of seed samples, do not submerge the beds. Irrigation (wetting of trays) is done intermittently. The soil is maintained moist for 0 to 3 days after planting. Thereafter, irrigation is done in the morning and water is drained in the evening. This is more important during the cold period to avoid the seedlings suffering at night. At 8 to 15 days after planting, water level is maintained at 3–4 cm depth in plastic pots; 15 to 18 days after planting, water is drained to allow seedlings to establish their root systems.

- **Pulling and pre-pulling preparation**

The seed beds are flooded, ideally to 1–3 cm in the seedling trays. This will facilitate pulling and lifting of soil with the roots. Pull the seedlings carefully and put them in a tray. Each entry is put together with its tag in a tray. Note plant stand to decide what further action is needed on a particular accession, e.g. modifying plot size. After pulling, arrange the seedlings by ascending plot numbers in seedling trays.

4.8. Field preparation and layout

- **Field preparation**

Land preparation consists of two plowing and 1stage of harrowing. The final stage of land preparation is manual leveling.

- **Treatment**

To control weeds, herbicides are applied. Two products are mainly used:

- Gramoxone (or equivalent) at a rate of 2 or 3 L/ha, at least 3 days before transplanting;
- Diuron (or equivalent) at a rate of 3 L/ha after transplanting.

To control insects and nematodes, apply:

- Cypermethrine) at a rate of 2.5 L/ha; for flying insects and,
- Granular insecticide, e.g. carbofuran at a rate of 50–60 kg/ha for nematodes.

To control fungi, apply:

- Mancozeb at a rate of 2Kg/Ha for fungal leaf spot and leaf blight

- **Field Layout**

- Field layout plan depends on plot size and land dimension
- Field is marked with 0.75 – 1 m long wooden pegs.
- Plots consist of 4 rows of 4 m length, spaced 0.75 m apart
- Plots are separated by 1 – 1.5 m walk through space that also keeps the plants in different plots apart.
- Tags indicating the plot number and the corresponding accession number is placed in the field before planting
- Plots are tagged in a serpentine order
- Soft and hard copy of field layout plan is kept for reference

- **Transplanting/Re-planting**

Transplant three-week old seedlings using rulers and ropes as planting guides, with one seedling per hill. Collect excess seedlings of each entry and place them near the labels. More healthy replanting materials can be obtained if they are divided in smaller bundles and properly placed in plastic pots.
Transplant at least 100 seedlings per plot to produce between 2000 and 35 000 healthy and highly viable seeds. Each plot should have four 5 m long rows spaced 20 cm apart.
Transplant single seedlings 25cm apart, filling 21 hills per row. Leave 1m vacant between plots.

For the frequently requested accessions planting in larger plots is recommended.

Fig. 3. Transplanting

- **Direct seeding**

- Crops like sorghum, maize, cowpea, okra etc. planted directly using ruler and ropes as planting guides.
- Labeled envelopes containing seeds are placed on pre-tagged plots
- The labels on the envelops are checked against tags on plots for conformity
- Two (2) to three (3) seeds from the envelop are planted per drill and later tinned to 1 plant per drill 2 weeks after planting
- Collect excess seeds of each entry and place them near the labels.
- More healthy replanting materials can be obtained if they are returned to the STS if there is need for resupply.
- Planting should be made at least to maintain 100 stand of each crops per plot to produce between 2000 and 35000 healthy and highly viable seeds. Each plot should also have at least Four, 5 m long rows spaced 75 cm apart. This may be more depending on the spacing for the crop.

Seeds are planted 20 cm between hills to ensure 21 hills per row and a total of 105 plants per plot.

For the frequently requested accessions planting in larger plots is recommended.

Fig. 4. Direct seeding

- **Fertilizer application**
  Fertilizer can be applied at recommended type and dosage as required. Application of fertilizers can be in split doses to ensure effective plant utilization.
  - As basal: 10-18-18 NPK at 150 kg/ha 75 kg TSP/ha + 75 kg urea/ha
  - First top-dressing: 50 kg/ha 14 days after sowing
  - Second top-dressing: 50 kg urea/ha 45 days after sowing
  - Third top-dressing: 50 kg urea/ha 60–70 days after sowing

- **Irrigation**

In seed multiplication plots, overhead sprinkler irrigation is done and the field should have a good irrigation system and excellent drainage to allow good water control. During land preparation, the water level should be maintained to keep the soil soft prior to transplanting. Irrigation is done intermittently throughout the cropping season so as not to submerge all the materials. Maintain 3–5 cm level of water during the early stage of crop to control early growth of weeds.

- **Pest and disease control**

Germplasm health unit should be contacted immediately when any disease occurs. Routine visits by the plant health unit are carried out with 3-5 visit during growing period of each regenerated crop. Visits are targeted toward early vegetative stage, reproductive stage and at harvest. For crops like cowpea known to be more prone to insect attack, visits are more regular.
In the case of insect attack, Chemical control using Cyparmetrin + Lamda-Cyhalothrin, applied at 70mls in 15 L Knapsack spray can. In severe case of insecticides Dimethoate© may be applied at 70mls in 15 L Knapsack spray. Mancozeb is used to control fungal infections.

- **Roguing**

Roguing is one of the most important activities during seed multiplication. Before pulling and after replanting in the field, rogue-out crop plants growing off the row. These are assumed to be "dropseeds" or volunteer seeds. At the late vegetative stage, obvious off types are removed. Re-identify the plants by comparing the seeds with the seed file. This is to ensure genetic identity and uniformity of the seed. Note that this should be done several times as the flowering period varies among lines. In addition, final purification should be done during seed processing.

- **Data collection**

A minimum data set is collected during seed multiplication such as plant height, duration, panicles/m2, flower color, grain color, grain shape, apex color, plant architecture and reaction to diseases and soil problems using a list of descriptors developed by Bioversity International (Bioversity International *et al.*, 2007), world vegetable centre or any international research centre that has mandate for the crop.

Maintenance of genetic integrity

Cross pollination should be controlled and genetic integrity maintained for each accession by using appropriate methods that include:

- **Selfing:** Use of pollination bags to cover. In cross pollinating species,100 or more plants are used while 25 to 50 plants are used in self-pollinating species in order to maximize the seed yield and avoid loses of allele. This is to ensure genetic identity and uniformity of the seed. Also, final purification should be done during seed processing. Date of covering is written on the paper bags to ensure timely removal.
- **Selfing and crossing:** Inflorescence is covered using transparent paper bags before stigma is visible. Pollen from dehiscent anthers of another plant in the accession is collected and the envelop used to cover (pollinate) emerged receptive stigma of covered plant used as the female. The pollen donor is re-bagged for selfing. Dates of covering and selfing/crossing are written on the paper bags to ensure timely removal.
- **Cluster bagging:** Involves covering one panicle each from 2 to 4 adjacent plants in a row with one parchment paper bag before stigma emergence. This ensures cross-pollination among diverse plants of the accessions thereby reducing in-breeding depression while maintaining the gene pool of the accession. Date of covering is written on the paper bags to ensure timely removal.
- **Sibbing:** This is similar to crossing except that pollens are collected from anthers of more than one panicle and used to pollinate the receptive stigmas of one female panicle. Date of pollination is marked on the bags
- **Use of protective nets:** Crop accessions are planted in cages of nets and avoid insect pollination.
- **Isolation:** different accessions of a crop can be planted in isolation of long distances where it is safe to believe their pollens will not get to each other. Isolation may also be by time or season of planting.
- **Intercropping:** Here, accession of one crop species is planted alternately with accession of another crop species. For instance, cowpea with crawling tendrils will be separated by alternating each accession with okra plants.

Fig. 5. Bagging sorghum panicles to control pollination

➢ **Harvesting**

1. Harvesting methods:

- After removing all the off-types, field labels are checked against bag labels prior to initiating the harvest.
- Plants are harvested individually and manually in a bag with corresponding labels to prevent mechanical injuries to the seeds.
- If plots are machine-harvested, the machinery is inspected and thoroughly cleaned prior to harvesting the next plot.
- Harvested material is placed in bags made from a porous fabric to allow good air circulation and drying.

2. Physiological maturity

- Seeds are harvested when the maximum number of seeds are ripe and before natural dispersal occurs. If there are differences in maturity between plants within the accession, plants are harvested individually in a staggered manner.

3. Genetic composition

- To ensure that the genetic composition of the accession is maintained, an equal number of seeds is harvested from the largest possible number of maternal plants to prevent a larger or smaller contribution by certain plants.

4.9 Postharvest activities

➢ **First Drying**

In order to increase the longevity of freshly harvested seeds, the first drying is done under sun on concrete slab (at about 30°C).

Fig.6. Drying after Harvesting

- **Threshing**

Threshing involves the detachment of seeds from pods/panicle; it is done manually. Treading by feet, flail-threshing, and beating on tubs, threshing boards, mats or racks are the most common methods of manual threshing. It should be done smoothly in order to avoid breakage.

Fig.7. Threshing by beating and sorting

- **Blowing**

Blow the harvest using a winnower for large quantities to separate unfilled grains and light density materials. For small quantities, do it manually.

Fig.8. Blowing the harvested seed manually

- **Drying**

After blowing, transfer the materials to net bag and place them in an automated dryer with a temperature of 15°C and 15% relative humidity (RH).
After blowing, transfer the materials to net bags and place them in a drying room with a temperature of 15°C and 15% relative humidity (RH).

4.10. Updating the master file

After drying the following data is updated in the database:

- Date of planting (yyyy-mm-dd)
- Harvest date
- Regeneration site
- Latitude (DD)
- Longitude (DD)
- Altitude (m)
- Accession number
- Accession name
- Plot number
- Purpose of regeneration
- Staff Regeneration
- Arrival Date
- Batch code (YYYY-nn)
- Temporary ID

All data collected shall be verified and validated prior to uploading it to the genebank's database by the SOP owner and contributors.

The regeneration procedure is completed and finalized when:

i. all plots are harvested and,
ii. all data are validated by the genebank regeneration SOP approver
iii. all pertinent information has been entered into the genebank database.

5. Staff training and Competency

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

- Afolayan Gloria,Assisstant Director Research, Seed Genebank Unit.
  Contact: ogo246@yahoo.com
- Aladele Sunday, Director/CEO NACGRAB.
  Contact: saladele6083@gmail.com

Genebank staff undergo yearly training and periodic competency testing. The complete **training, competency schedule** and **succession plans** are available at the Institute's 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.

Breese, E.L. 1989. Regeneration and multiplication of germplasm resources in seed genebanks: the scientific background. Rome, IBPGR.

7. Revision History and frequency

Effective Date of the SOP	Version #	Description	Reviewed By
25/05/2021	1.0	Original SOP	Gloria Afolayan
04/08/2021	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 and Access Agreements

Citation: NACGRAB, 2024, *Regeneration of genetic resources at NACGRAB, SOP NACGRAB. NACGRAB-REG-v.1.2.* Contributors: Sunday Aladele, Adam Olosunde, Olutoye Fashola

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

Appendix 1: List of data

Parameters	Abbreviation	Data type	Descriptions	Scale
Heading Date (days)	**Date_80%CSE**	quantitative		
ligule shape	**Form_Lig)**	quantitative		0=absent (no ligule) 1=truncate 2=Acute to acuminate 3=2-cleft
Ligule lenght (mm)	**Long_Lig**	quantitative	Measured on five samples from base of collar to the tip of the ligule of the penultimate leaf	
Leaf lenght (cm)	**Long_F**	quantitative	Measure the length of the leaf below the flag leaf on the tiller main of the ligule at the top of the leaf blade of 5 samples.	
Leaf width (cm)	**Larg_F**	quantitative	Measure the width of the widest part of the leaf below the flag leaf on the main tiller of 5 samples.	
Plant height (cm)		quantitative	Count tillers with panicles and those without panicles on 5 samples	
Total tillers number	Till_num	quantitative	Measured from ground level to the top of the longest panicle (aristation Excluded) 5 samples.	
Fertile tillers number	Till_Fnum	quantitative	Count only the tillers with panicles on 5 samples	
Maturity date	**Date_Mat**	quantitative	Maturity is reached when 80% of the grains on a panicle are completely walls	
Harvesting date	**Harv_Date**	quantitative		

Appendix 2: Standards for regeneration (2014)

SELECTED REFERENCES

Breese, E.L. 1989. *Regeneration and multiplication of germplasm resources in seed genebanks: the scientific background.* Rome, IBPGR.

Crossa, J. 1995. Sample size and effective population size in seed regeneration of monecious species. *In* J.M.M. Engels & R. Rao, eds. *Regeneration of seed crops and their wild relatives*, pp. 140–143. Proceedings of a consultation meeting, 4–7 December 1995. Hyderabad, India, ICRISAT, and Rome, IPGRI.

Dulloo, M.E., Hanson, J., Jorge, M.A. & Thormann, I. 2008. Regeneration guidelines: general guiding principles. *In* M.E. Dulloo, I. Thormann, M.A. Jorge & J. Hanson, eds. *Crop specific regeneration guidelines.* [CD-Rom], Rome, SGRP-CGIAR.

Engels, J.M.M. & Rao, R., eds. 1995. *Regeneration of seed crops and their wild relatives*, pp. 140–143. Proceedings of a consultation meeting, 4–7 December 1995. Hyderabad, India, ICRISAT, and Rome, IPGRI.

Engels, J.M.M. & Visser, L., eds. 2003. *A guide to effective management of germplasm collections.* Handbooks for Genebanks No. 6. Rome, IPGRI.

Johnson, R.C., Bradley, V.L., & Evans, M.A. 2002. Effective population size during grass germplasm seed regeneration. *Crop Science*, 42: 286–290.

Johnson, R.C., Bradley, V.L., & Evans, M.A. 2004. Inflorescence sampling improves effective population size of grasses. *Crop Science*, 44: 1450–1455.

Lawrence, L. 2002. A comprehensive collection and regeneration strategy for *ex situ* conservation. *Genetic resources and crop evolution*, 49(2): 199–209.

Lehmann, C.O. & Mansfeld, R. 1957. Zur Technik der Sortimentserhaltung. *Kulturpflanze*, 5: 108–138.

Rao, N.K., Hanson, J., Dulloo, M.E., Ghosh, K., Nowell, D. & Larinde, M. 2006. *Manual of seed handling in genebanks.* Handbooks for Genebanks No. 8. Rome, Bioversity International.

Sackville Hamilton, N.R. & Chorlton, K.H. 1997. *Regeneration of accessions in seed collections: a decision guide.* J. Engels, ed. Handbooks for Genebanks No. 5. Rome, IPGRI.

SGRP-CGIAR. Crop Genebank Knowledge Base (available at: http://cropgenebank.sgrp.cgiar.org).