

	Characterization of genetic resources at the National Centre for Genetic Resources and Biotechnology (NACGRAB).		
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	SOP Owners: Dr. Ibidun Adetiloye	SOP Approver: Dr. Sunday Aladele	

1. Introduction & Purpose

Germplasm characterization is the process of describing and recording of highly heritable traits that identify an accession in the collection. These heritable traits are measured at different growth stages of the plant's development and are usually performed during regeneration cycles.

The objectives of characterization are to 1) Provides essential information on the characters of accessions ensuring the maximum utilization of the germplasm collection to end users. 2) Enables an easy and quick discrimination among population or phenotypes to confirm “trueness to type” (ITT), 3) describe inter-and intra-accession diversity thus providing insight into the composition of the collection and its genetic diversity. 4) Helping identify possible gaps and duplicates; and retrieval of valuable germplasm for breeding programmes, and 5 Aid in the rationalization of management procedures. Characterization traits may be taxonomic, morphological, phenotypic, biochemical, nutritional, physiological and molecular. Characterization facilitates the use of the collection and allows for the detection of errors and misidentifications especially in the case of in-vitro germplasm that are prone somaclonal variation. Moreover, systematic characterization leads to improved maximum utilization of germplasm in the collection.

Characterization is an essential operation in genebank management. The genebank Standards for characterization section 4.5 (Genebank Standards, 2014) stated that:

4.5.1 Around 60 percent of accessions should be characterized within five to seven years of acquisition or during the first regeneration cycle. However, In NACGRAB genebank, characterization is carried out two to three years after acquisition or whenever there is sufficient seed and fund to conduct characterization.

4.5.2 Characterization should be based on standardized and calibrated measuring formats and characterization data follow internationally agreed descriptor lists and are made publicly available such as:

Descriptors for Crops, Bioversity International, 1993

Descriptors for vegetables, World Vegetable Center

NBPGR minimal descriptors

Descriptors for Crops/IITA accessions

Etc.

The main aim of drafting this SOP is to establish the identity of accessions and to discern genetic relationship among genotypes; and to ensure that the characterization of PGR conserved at National Centre for Genetic Resources and Biotechnology (NACGRAB) genebank is in consistency and harmony with the standard testing guidelines and internationally/Nationally accepted descriptors such

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as USAD, UPOV-TG, Bioversity International, NBPGR minimal descriptors etc and the Standard Evaluation System (SES) used by breeders.

2. Scope

This SOP applies to the morphological and agronomic characterization of crops conserved at the NACGRAB's genebank using selected traits from various crop species descriptors published by Bioversity International, World Vegetable Center etc.

This document does not cover molecular characterization and/or the evaluation of characters such as yield or responses to biotic and abiotic stresses.

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3. Definitions & Abbreviations

The following terms, definitions, abbreviations and acronyms are pertinent to this SOP.

Accession	A distinct uniquely identifiable sample of botanic seeds representing a cultivar, a breeding line or a population of a particular plant species, which is maintained in storage for conservation and use.
Characterization	The description of plant germplasm through recording the expression of highly heritable characters (not affected by the environment) ranging from morphological, physiological or agronomical features to seed proteins and oil or molecular markers.
Regeneration	The process that leads to the generation of a new seed-lot for a given accession with the intention to increase the stored seeds in the genebank and/or to increase the viability of the seeds equal to or above an agreed minimum level.
Plant genetic resources	The overall genetic diversity of the cultivated and wild plant species, which have actual or potential value and can contribute to the improvement of crops.
CGIAR	Consultative Group on International Agricultural Research
FAO	Food and Agriculture Organization of the United Nations
FIGS	Focused Identification of Germplasm Strategy, a trait targeted sub-setting approach within genebank's material
GCP	Generation Challenge Programme
ID_Number	Identification number provided by DONOR
NGB	NACGRAB's germplasm number. It is a unique identifying number that applies to each accession conserved in NACGRAB's genebank.
IPR	Intellectual Property Rights
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LTS	Long term storage for base collection and safety duplicates collections
MOS	A sample of seeds from the original seed lot or the one that have undergone the lowest number of regenerations since the material was acquired by the genebank, as recommended for storage as a base collection.
MTS	Medium term storage for active collection
Ne	Effective population size (N_e) is the size of an ideal population (i.e., one that meets all the Hardy-Weinberg assumptions) that would lose heterozygosity at a rate equal to that of the observed natural population.
NARI	National Agricultural Research Institutes
RH	Relative Humidity
SMTA	Standard Material Transfer Agreement
SOP	Standard Operating Procedure
SD	Safety duplicate collection
TTT	Trueness to Type

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4. Procedure

4.1 The characterization procedure: germplasm characterization covers the whole range of activities starting from the receipt of the new samples by the curator and planting the new samples for seed increase to characterization and documentation. It is initiated when genebank staff performs the yearly **review** of accessions in need of morphological characterization through the Genetic Resources Information Management System.

4.2 Selecting accessions for characterization

The criteria for selecting an accession for characterization are the following:

4.2.1 All newly registered accessions are by default included in the characterization schedule.

4.2.2 Registered accessions with incomplete information recorded during the previous planting season (due to photo-period response, disease infection and other factors like inclement weather) will also be included.

4.3 Number of entries to be characterized.

The number of entries selected for characterization is dependent on the number of staff with technical-know-how, the land/space available for planting and the type of material to be characterized. The number of entries/accessions characterized yearly at NACGRAB is more or less 500 accessions. The type of material is carefully considered in deciding the number of entries to be included; Self-pollinated species are more easily managed than cross pollinated species. Cross pollinated species requires pollination control. The following steps should be followed:

4.3.1 Coordinate with and requests the data management team to provide a list of materials or accessions that need characterization information following the criteria for selection.

4.3.2 The materials are selected, and a list is generated. This list will be used to request for planting materials from the seed distribution team.

3.10 Optimal times for data collection

Characterization at NACGRAB is conducted during the wet season (June to November). Since wet season starts when day length is longer, entries that are photo-period sensitive entries will not be forced to flower early as what is happening when planted in the dry season where shorter day length can make late maturing photoperiod sensitive entry almost flower at the same time with early maturing entries. During the wet season we can distinguish the photo-period sensitive from those that are non-sensitive to photoperiod.

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Randomized Complete block design (RCBD) is usually used with at least two replications. One or two improved/popular varieties are usually planted within plots or as borders so as to serve as control/checks.

Observations are recorded on ten randomly chosen plant within the plot and pests and diseases are controlled by applying appropriate chemicals.

Morphological and agronomic characters of plants are best scored at different growth stages of the crop to capture the best expression of the trait (with the exception of seedling height which is measured before transplanting (5-leaf stage ~20 Days after sowing (DAS)). NACGRAB genebank deals with multi-crop. However, for the purpose of this SOP, COWPEA will only be mentioned because it is the most important germplasm/crop in NACGRAB genebank.

Characterization information is obtained at four developmental stages:

3.10.1 Vegetative- the stage of crop plant growth from seedling to maximum vegetative stage before the expected time of flowering. Observation of the traits at this stage can be done to all entries.

Plant growth habit

- 1 - Prostrate: plant completely flat on ground: branches spread for several meters
- 2 – Semi-prostrate: Main stem up to 20cm or more above ground; branches spread 1-4m
- 3 - Intermediate: most lower branches touch the ground
- 4 - Semi-Erect: branches tend to be perpendicular to main stem but do not touch ground
- 5 - Erect: branches less acute than above
- 6 - Acute erect: branches form acute angle with main stem
- 7 – Climbing

Twining tendency

- 1 – None
- 2 - Moderate
- 3 - Pronounced

Growth vigor

- 1 - Vigorous - Height < 37.5cm and Width < 75cm
- 2 - Intermediate - Height > 37.5cm or Width > 75cm
- 3 - Vigorous - Height > 37.5cm and Width > 75cm
- 4 - Very Vigorous - Height > 50cm and Width > 1m

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Determinacy

- 1 - Indeterminate main stem
- 2 - Determinate main stem

No of main branches

No of main stem nodes

Peduncle length

- 1 - Short - average length < 5cm
- 2 - Long - Average length > 5cm

Raceme position

- 1 - In all layers of canopy
- 2 - In upper leaf layers
- 3 - Above Foliage

Terminal leaflet shape

- 1 - Sub-hastate
- 2 - Sub-Globose
- 3 - Hastate (Lanceolate)
- 4 – Globose

Terminal leaflet length (cm)

Terminal leaflet width (cm)

3.10.2 Early reproductive- the stage from 50% flowering to onset of fruit/seed/pod development. Observation of the traits at this stage is done after flowering.

Days to first flowering: Days from planting to the notice of first flowering

Days to first ripe pod: Days from planting to first ripe pod

Pattern of pigmentation on plant

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- 1 - Moderate on base and tip of petioles
- 2 – Extensive
- 3 - None or Very pale
- 4 - Almost Solid

Pattern of pigmentation on flower

- 1 - Completely Pigmented
- 2 - Wing & Standard petal has pigmented margin
- 3 - Wing pigmented petal has light V-shape pattern
- 4 – None
- 5 - Wing has pigmented upper margin; standard petal pigmented
- 6 - Wing is pigmented ; Petal is lightly pigmented
- 7 - Wing is pigmented; Petal is lightly pigmented

Pattern of pigmentation on green pod

- 1 – None
- 2 - Pigmented tip
- 3 - Uniform pigment
- 4 - Splashes of Pigment
- 5 - Pigmented Sutures
- 6 - Pigmented valves, green sutures

3.10.3 **Late Reproductive**- the stage from fruiting/seeding/podding to ripening or early maturity stage.

No of pods/peduncle

- 1 - 2 to 3
- 2 - Less than 2
- 3 - 4 or more

Pod attachment to peduncle

- 1 - Angle of 30° to 90°
- 2 – Erect
- 3 – Pendant

Pod shape

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- 1 – Curved
- 2 – Straight
- 3 – Coiled

Pod length (mm)

Pod width (mm)

No of locules/pod

3.10.4 **Post-harvest** – the stage in which observations are made on fruits/pods/seeds collected at a mature stage at the laboratory. Seed samples are authenticated by comparing with the remnant seeds or the seedfile before characterization.

Eye color

- 1 - T10: Tan or buff
- 2 - R40: red
- 3 - S11: Speckling on tan
- 4 - T20: dark tan
- 5 - B30: Solid black
- 6 - M60: solid brown over tan
- 7 - B45: black with light spots of red
- 8 - W25: white body: tan eye
- 9 - T60: brown
- 10 - T41: light orange tan
- 11 - W23: white body: mottled eye
- 12 - M11: brown mottling on tan
- 13 - W21: white body: black eye
- 14 - X51: black spots with mottling on tan
- 15 - W45: cream body: tan eye
- 16 - M12: brown mottling on orange
- 17 - R10: maroon
- 18 - T57: reddish brown
- 19 - W43: cream body: mottled eye
- 20 - S63: Speckling on brown with clear areas

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- 21 - W41: cream body: black eye
- 22 - T44: medium orange tan
- 23 - B10: Purple
- 24 - B20: Solid black: gray body
- 25 - R70: pink
- 26 - W40: cream body: used only with A10 eye
- 27 - W65: brown splash on body: tan eye
- 28 - T16: buff with blue tinge
- 29 - W20: white body: used only with A10 eye
- 30 - W61: brown splash on body: black eye
- 31 - B71: blue covering tan
- 32 - V11: Speckled with mottling on tan
- 33 - S15: Speckling on red
- 34 - S61: Speckling on tan with clear areas: as though speckles had erased with random strokes
- 35 - X71: blue covering mottled on tan
- 36 - M65: solid brown over red
- 37 - X81: blue covering solid brown
- 38 - W71: gray body: black eye
- 39 - V71: solid brown with speckling on tan
- 40 - M15: brown mottling on red
- 41 - S62: Speckling on orange with clear areas
- 42 - B51: black spots with speckling on tan
- 43 - V15: Speckled with mottling on red
- 44 - S65: Speckling on red with clear areas
- 45 - W60: brown splash on body: used only with A10 eye
- 46 - W22: white body: red eye
- 47 - W42: cream body: red eye
- 48 - W63: brown splash on body: mottled eye
- 49 - B75: blue covering red
- 50 - X55: black spots with mottling on red
- 51 - X61: black spots on solid brown
- 52 - W81: gray body with brown splash: black eye
- 53 - V61: Speckled with clear areas and mottling on buff
- 54 - V75: solid brown with speckling on red
- 55 - X75: blue covering mottled on red

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- 56 - V62: Speckled with clear areas and mottling on orange
- 57 - W70: gray body: used only with A10 eye
- 58 - B56: black spots on red
- 59 - W62: brown splash on body: red eye
- 60 - W80: gray body with brown splash: used only with A10 eye
- 61 - B41: black with light spots of tan

Testa texture

- 1 - Smooth
- 2 - Rough
- 3 - I: Intermediate
- 4 - Wrinkled
- 5 - Split
- 6 - S-W: Smooth & Wrinkled
- 7 - L: Loose
- 8 - S/W: Smooth Eye; Wrinkled Body
- 9 - I-W: Intermediate & Wrinkled
- 10 - P-W: Split & Wrinkled
- 11 - R-W: Rough & Wrinkled

Seed size

- 1 - 810mg/10 seeds
- 2 - 1400mg/10 seeds
- 3 - 472mg/10 seeds
- 4 - 2150mg/10 seeds
- 5 - 48mg/10 seeds
- 6 - 189mg/10 seeds
- 7 - 2880mg/10 seeds
- 8 - 117mg/10 seeds
- 9 - 3370mg/10 seeds

Seed length (mm)

- 1 - Short - slightly longer than it is wide
- 2 - Very Short - Length equal to Width
- 3 - Intermediate - twice as long as wide
- 4 - Long - thrice as long as wide

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5 - Very Long - at least four times as long as wide

Seed thickness (mm)

- 1 - Intermediate - thickness slightly less than the width
- 2 - Flat - thickness two-thirds the width
- 3 - Thick - thickness equal to width
- 4 - Very Flat - thickness half the width

3.11 Collecting and recording the information

Field data are preferably collected in the morning. This is the best time as the sun is still not so bright to distract the observer especially that the tablet screen may produce glare. In the event that mid-day characterization cannot be avoided, use of dark umbrella to cover the screen of the tablet may be used.

Information is recorded paperless using data sheets in an Android tablet with codes and images of the traits. The following fields are viewable in the tablet:

- Accession identification: information in data sheets that is useful in describing the accession
- Accession number: a unique identification number assigned to a sample once it has satisfied the minimum storage requirements of the genebank
- Variety name: a local or vernacular name of the sample
- Country of origin: country from which the sample came
- Seed File Information: used for heterogenous populations to guide the evaluator in authenticating the sample being characterized.

This information is automatically included when data management staff uploaded the field lab apps containing the entries and the data sheet to be filled in during the characterization process.

3.11.1 During the actual characterization process, observed traits are recorded by touching the images or the codes that best describe the accession being characterized in case the photo of the trait is not available.

3.11.2 The records are uploaded first into a temporary file then to the information system after verification and confirmation, which is after the season when the seeds samples have been compared with the seedfile.

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3.12 Morphological Descriptors

Morphological descriptors are used to describe the accession. Plant, stem, leaf, flower, pod and seed traits are scored or measured and expressed in numeric values. Descriptive traits such as flower colour can be expressed as a numeric value by using codes (Bioversity descriptors for crops). The descriptive traits used will vary with the crop and species.

3.13 Data verification and validation

3.13.1 After every stage of characterization, all the information taken is submitted to the data management team for uploading into a temporary file. This is done until the final verification of the recorded traits had been done.

After samples have been collected from the characterization plots of the cultivated species, they are discarded as a general rule except if the purpose is for regeneration and characterization as well. The seed processing group may request that selected plots be harvested to provide extra seed for distribution.

3.13.2 All characterization information is uploaded to a temporary file. Data are verified by the supervisor in coordination with the data curator before uploading.

3.13.3 The characterization procedure is completed and finalized when all pertinent information has been confirmed and entered into the information system.

3.13.4 Characterization data and other germplasm information are made available to public through the www.nacgrab.gov.ng.

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5. *Staff Training and Competency*

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

- Adetiloye Ibidun Assistant Director Research, Seed Genebank Unit.
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- Aladele Sunday, Director/CEO NACGRAB.
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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*

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7. *Revision History and frequency*

Effective Date of the SOP	Version #	Description	Reviewed By
12/09/2021	1.0	Original SOP	Ibidun, Adetiloye
15/11/2021	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, Copyright, Access agreement

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