

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Some Agri-Horticultural Crops' Germplasm in Bangladesh



December 2024

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Some Agri-Horticultural Crops' Germplasm in Bangladesh

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FOREWORD

I am delighted to note that Crops Division, Bangladesh Agricultural Research Council (BARC) is publishing a guide line titled ‘Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Some Agri-Horticultural Crops’ Germplasm in Bangladesh’. The immense significance of the Plant Genetic Resources (PGR) as the basic raw materials for crop development propelled to come out of this invaluable document.

Bangladesh is one of the major contributors of the South Asian Mega Centre of genetic diversity. By virtue of her favorable agro-climatic conditions, characterized by tropical and sub-tropical environments, has been bestowed with vast PGR base, which are to be duly conserved and properly utilized. It is important to note that PGR collection and conservation is useless if these are not presented with proper information before the end users. In Bangladesh, a good number of indigenous PGR have been collected and conserved in the genebanks of different crop research institutes and universities. It is essential to standardize unified operating procedure for genebank management for all local organizations aligning national and international standards especially guideline provided by Food and Agriculture Organization of the United Nations (FAO) and Bioversity International (BI).

Scientists of Crops Division, Bangladesh Agricultural Research Council (BARC); Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI); and Genetic Resources and Seed Division (GRSD), Bangladesh Agricultural Rice Institute (BRRI) involved in CGIAR-IRRI funded project titled ‘Working Together to Support the Global System for PGRFA’ have taken a great effort to prepare Standard Operating Procedures (SOPs) for 13 agri-horticultural crops. This SOPs provides step-by-step instructions to guide the scientists involved in germplasm regeneration, characterization and preliminary evaluation, and aims to conduct each operation from seed sample preparation to crop harvesting, seed threshing/extraction and data recording following national and international standards. Such endeavor ultimately ensures safe avoiding duplication of conserved materials without hampering genetic integrity and maintaining seed health. I extend my heartfelt felicitation to all the team members engaged in this project for their hard work in successful completion of project activities and preparing such a valuable guideline for genebank management.

Finally, I would like to appreciate the Consultative Group on International Agricultural Research (CGIAR)-International Rice Research Institute (IRRI) authority for financial and technical support through ‘Working Together to Support the Global System for PGRFA’ project to implement such an important activity and develop this SOP.

Nazmun Nahar Karim

(Dr. Nazmun Nahar Karim)
Executive Chairman

PREFACE

Plant Genetic Resources (PGR) is the raw material indispensable for crop genetic improvement, whether by means of farmers' selection, classical plant breeding or modern biotechnologies. These are essential in developing varieties suiting to unpredictable environmental changes and future human needs. The diverse agro-ecological zones (30 AEZs) of Bangladesh have sustained rich genetic resources of crop plants. It is the abode of about 5,000 species of vascular plants and is the secondary center of origin of a good number of crop plants. Considering its rich reserve of PGR, Bangladesh is still in preliminary phases in the use of the PGR which necessitates wise collection and conservation following national and international standards required for present and future human well-being.

Bangladesh is one of the signatories of Convention of Biological Diversity (CBD) and International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). It has obligation to follow Global Plan of Action for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture. Food and Agriculture Organization (FAO) of the United Nations outlined 'Genebank Standards' for Plant Genetic Resources for Food and Agriculture (PGRFA). Bangladesh is to address the major underlying principles of germplasm conservation in maintaining genetic identity, viability, genetic integrity, germplasm health throughout the various processes, beginning with acquisition through to storage and distribution. Outmost care and appropriate step must be taken throughout the various processes to achieve the ultimate goal. Standard Operating Procedures (SOPs) shortly known as SOPs is essential to instill best practices in performing the allocated function. SOPs is a written document with step-by-step instructions to guide the performer of a process. The purpose of SOPs is not to teach someone how to do a work or to impart a skill, but to imbibe best practices that ensures some sort of standardization in performing a work in a most optimal way. Standard Operating Procedures (SOPs) for regeneration, characterization and preliminary evaluation of thirteen important agri-horticultural crops' grown in Bangladesh have been described in different chapters of this SOPs. This SOPs will provide well defined steps to perform regeneration and characterization trials, standardize activities, improve safety and security in operation and minimize wastages in processes.

This publication will contribute to better planning and implementation of systematic regeneration and characterization procedures of thirteen agri-horticultural crops, thereby assisting genebank staff in promoting the overall efficiency and cost-effectiveness of genebank operations, and minimize or avoid loss of identity of seed sample accessions conserved, maintain viability and genetic integrity toward readily availability of healthy materials for crop improvement efforts.

Editors

ACKNOWLEDGEMENT

The Standard Operating Procedures (SOPs) is an outcome of Consultative Group on International Agricultural Research (CGIAR) Genebank Initiatives, Working Together to Support the Global System for PGRFA project. The project has successfully being implemented by Crops Division, Bangladesh Agricultural Research Council (BARC), Plant Genetic Resources Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI) and Genetic Resources and Seed Division (GRSD) with research from CGIAR-IRRI.

I extend my sincere gratitude to CGIAR-IRRI for spearheading this initiative and providing the essential financial and technical support needed to develop the comprehensive Standard Operating Procedures (SOPs) for thirteen agri-horticultural crops cultivated in Bangladesh. I am deeply thankful to Dr. Venuprasad Ramaiah, Senior Scientist I-Plant Genetic Resources, and Ana E. Cope, Manager-Program Coordination, International Rice Genebank (IRG), International Rice Research Institute (IRRI) for their continuous support throughout the development and finalization of this document.

A good number of scientists working at PGRC, BARI and GRSD, BRRI were involved in collecting information and processing of research data and preparing the SOPs. Without their help, successful execution the project and preparation of this document could not be made possible. Their sincere efforts are highly appreciated. Special thanks are due to Dr. Md. Aziz Zilani Chowdhury, Former Member Director (Crops), BARC, for his consistent engagement in project activities and meticulous review of the manuscript.

I hope this publication will serve as a practical guide for PGR scientists across the country, facilitating the systematic execution of germplasm characterization and regeneration while preserving genetic integrity and preventing mechanical admixture.

I also express my sincere apologies and gratitude to any individuals who may have contributed to the preparation of these SOPs but were inadvertently omitted from this acknowledgement.



(Dr. Md. Abdus Salam)

Member Director (Crops), BARC and
Collaborating Scientist
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CHAPTER 01

1. Introduction and Purpose

Plant Genetic Resources (PGR), also known as plant germplasm, are a priceless asset to humanity. They are the foundation of all agriculture and a basic ingredient right at the centre of all agriculture related value chains. They are the most valuable and essential basic raw materials for crop improvement providing biological basis for world food security supporting the livelihoods. Cultivated varieties, obsolete varieties, primitive cultivars (landraces), breeding materials, wild and weedy species, near relatives of cultivated species etc. are considered as component of Plant Genetic Resources (PGR). Bangladesh is characterized by a mixture of tropical and sub-tropical environments offering congenial growing conditions for numerous agri-horticultural crops. It is bestowed with immense agro-biodiversity and rich diversity of landraces, traditional /farmers' varieties in several agri-horticultural crops with a good number of timber and medicinal plants which are indigenous to the country. The diverse 30 agro-ecological regions of the country have sustained rich genetic resources of crop plants. Systematic collection, conservation, characterization and regeneration of the plant genetic diversity is essential for present and future human well-being.

The Genebank of different Research Institutes and Agricultural Universities in Bangladesh conserved a considerable number of accessions of Agri-Horticultural crops belonging to more than 200 species and originating from different countries both in seed storage (LTS and MTS) and field genebank (eg. BRRI: 9006 Germplasm of cultivated Rice including 46 germplasm of 11 wild species; BARI: 12873 germplasm of 152 Agri-Horticultural crops; BJRI: 6093 germplasm of Jute, Kenaf, Mesta and 253 allied fibre crops including wild species; BINA: 2222 germplasm of Rice and 68 other Agri-Horticultural crops; BSRI: 1159 germplasm of Sugarcane; CDB: 562 germplasm of Cotton. Germplasm of same crops are being conserved and managed in different organizations, eg. rice in BRRI, BINA and agricultural universities. Unified operating procedures should be followed for regeneration, characterization and preliminary evaluation of germplasm in the country. Care should be taken to ensure that the identity of seed sample accessions conserved in genebank is maintained throughout the various processes, beginning with acquisition through to storage and distribution. But utmost care is not always ensured during most of the genebank operations. It is essential to provide guidelines for each crop in the country following underlying genebank standards for plant genetic resources for food and agriculture (FAO, 2014). This SOPs is intended as a guideline for genebanks conserving plant germplasm collections of some agri-horticultural crops in the country. SOPs is a written document with step-by-step instructions to guide the performer of a process or a function or an activity. The purpose of SOPs is not to teach someone how to do a work or to impart a skill, but to imbibe best practices that ensures some sort of standardization in performing a work in a most optimal way (Narayanan, 2015 and Eisner, 2022).

An essential element of seed regeneration is the maintenance of genetic integrity of the original sample, and need to keep the conserved seeds free from seed-borne diseases. The two concerns are maintaining the occurrence of different alleles and maintaining the frequency of these alleles. Therefore, knowledge on reproductive system, growth cycle and

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growth habit of an accession are key elements for regeneration process (ICARDA, 2021). There is no standard criterion in Bangladesh for seed quantity and viability in medium-term storage to follow for regeneration. Knowledge gap is also acute in some institutes. International standards are often ignored. Regeneration is also undertaken for the newly introduced, collected or received accessions to allow to conduct characterization and multiply seeds to replenish active and base collection and to send samples for safety duplication haphazardly. It is, therefore, urgent need to formulate standard procedure for regeneration, characterization and preliminary evaluation for conserved accessions, and multiplication of the newly introduced, collected or received accessions to replenish active and base collection and to send samples for safety duplication.

This SOPs is prepared with the following objectives

- i. To give step-by-step instructions to guide the scientists involved in germplasm regeneration, characterization and preliminary evaluation.
- ii. To ensure consistency on the regeneration and characterization activities of cultivated and wild germplasm maintained at different genebank of the country in compliance with national and international treaties and conventions.
- iii. To instill best practices in performing a specific function of PGR management starting from acquisition of germplasm for exploiting in crop improvement program.
- iv. To imbibe best practices that ensures some sort of standardization in performing PGR management activities in a most optimal way.

2. Scope

This SOPs will be applied to the regeneration, characterization and preliminary evaluation of cultivated and wild species germplasm of agri-horticultural crops maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in germplasm regeneration, characterization and preliminary evaluation. The SOPs aims to:

- i. Inspire the genebank curators of all organizations to prepare database of all activities (passport data, characterization, regeneration, conservation, monitoring etc.) performed in respective centres/divisions;
- ii. Provide detail guidelines to procure necessary materials and equipment for regeneration and characterization of newly collected and conserved germplasm;
- iii. Ensure safety duplication of conserved materials in different genebanks of the country without hampering genetic integrity of the accessions and maintaining seed health;
- iv. Ensure utmost care in every operation of germplasm regeneration and characterization trials especially shifting of seed, seedling and harvested materials, and placing of accessions onto the respective field plots to avoid mechanical admixture and miss placing;
- v. Conduct each operation from seed sample preparation to crop harvesting, seed threshing/extraction and data recording following national and international standards;

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- vi. Adopt appropriate management practices for insect pests and diseases to maintain seed health and
- vii. Encourage genebank management authorities to ensure occupational health and safety, and continuous capacity building of staffs.

3. Terms, Definitions, Abbreviations and Acronyms

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

Collector's Number	Original number assigned by the collector(s) of the sample normally composed of the name or initials of the collector(s) followed by a number. This item is essential for identifying duplicates held in different collections and should always accompany sub-samples wherever they are sent.
Passport descriptors	These provide the basic information used for the general management of the accession (including the registration at the genebank and identification information) and describe parameters that should be observed when the accession is originally collected.
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.
Vernacular name (local script, Unicode text)	Name given by farmer to crop, cultivar, landrace, clone or wild form. Use Unicode text to specify the name using the standard script of the local language. This serves as the definitive version of the name.
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.
Accession number	This number serves as a unique identifier for accessions and is assigned when an accession is entered into the collection. Once assigned this number should never be reassigned to another accession in the collection. Even if an accession is lost, its assigned number is still not available for re-use. Letters are used before the number to identify the genebank or national system (eg. BD indicates an accession that comes from the genebank at PGRC, BARI; GRSD, BRRI indicates an accession from the genebank at BRRI; BJRI indicates an accession from the genebank at BJRI; BINA indicates an accession from the genebank at BINA; CDB indicates an accession from the genebank at CDB, etc.
Management descriptors	These provide the basic information used for the general management of accessions in the genebank and assist with their multiplication and regeneration.



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Environment and site descriptors	These describe the environmental and site-specific parameters that are important when characterization and evaluation trials are held. They can be important for the interpretation of the results of those trials. Germplasm collecting site descriptors are also included here.
Characterization	Characterization is 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 (FAO, 2014). This activity provides information on traits that allows discrimination among accessions and facilitates the verification of identity. It also includes the taxonomic identification and verification when needed.
Characterization descriptors	These describe the environmental and site-specific parameters that are important when characterization and evaluation trials are held. It can be important for the interpretation of the results of those trials. Germplasm collecting site descriptors are also included here.
Preliminary evaluation	Consists of recording a limited number of additional traits thought desirable by a consensus of users of the particular crop.
Evaluation descriptors	Many of the descriptors in this category are susceptible to environmental differences but are generally useful in crop improvement and others may involve complex biochemical or molecular characterization. It includes yield, agronomic performance, stress susceptibilities and biochemical and cytological traits.
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 (also called “multiplication”) and/or to increase the viability of the seeds equal to or above an agreed minimum level, which is referred to as the regeneration threshold. The latter case is often termed as “seed rejuvenation”.
Orthodox seed	Orthodox seeds can be dried to low moisture content (2% - 5%) without damage. The seed can be stored in long periods, in line with decreasing of moisture content and temperature of storage room. These seeds can survive extreme freezing or drying conditions during <i>ex-situ</i> conservation. Germplasm of this type of crops are conserved in LTS and MTS.
Recalcitrant seed	Recalcitrant seeds cannot be dried until the optimum moisture content for survival varied from 12% to 31%. They cannot thrive in freezing or drying conditions for a long duration. Thus, their <i>ex-situ</i> conservation is comparatively difficult. Desiccation

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	of such seeds can lead to oxidative damage or deterioration of its cells. Recalcitrant includes large seeds, such as Mango, Avocado, Cocoa, Litchi, Jackfruit, etc. Germplasm of this type of crops are conserved in field genebank.
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 Program.
GRSD	Genetic Resources and Seed Division of BRRI and BJRI.
ICARDA	International Center for Agricultural Research in the Dry Areas.
ID Number	Identification number provided by DONOR.
BD (Bangladesh)	BARI's germplasm number. It is a unique identifying number that applies to each accession conserved in BARI's genebank.
IPGRI	International Plant Genetic Resources Institute.
IPR	Intellectual Property Rights
IRRI	International Rice Research Institute
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
LTS/Base collection	Long term storage for base collection and safety duplicates collections.
MOS (Most Original Seed)	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/Active collection	Medium term storage for active collection
Ne	Effective population size (Ne) 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.
Aus	Rice growing season covering the period from 15 April to 15 July
Aman	Rice growing season covering the period from 15 July to 15 December
Boro	Rice growing season covering the period from 15 December to 15 April
NARS	National Agricultural Research System
RH	Relative Humidity
SMTA	Standard Material Transfer Agreement
SOPs	Standard Operating Procedures
SD	Safety Duplicate Collection
TTT	Trueness to Type
BI	Bioversity International



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BARI	Bangladesh Agricultural Research Institute
BRRI	Bangladesh Rice Research Institute
BINA	Bangladesh Institute of Nuclear Agriculture
BJRI	Bangladesh Jute Research Institute
BSRI	Bangladesh Sugarcrop Research Institute
BTRI	Bangladesh Tea Research Institute
BSRTI	Bangladesh Sericulture Research and Training Institute
BAU-GPC	Bangladesh Agricultural University-Germplasm Centre
CDB	Cotton Development Board
PGRC	Plant Genetic Resources Centre of BARI
GRSD	Genetic Resources and Seed Division of BRRI
NBPGR	National Bureau of Plant Genetic Resources
UPOV	International Union for the Protection of New Varieties of Plants
IPPC	International Plant Protection Convention
WTO	World Trade Organization
WARDA	West Africa Rice Development Association
SPS	Sanitary and Phytosanitary

4. Materials and Equipment

Materials

The following materials, equipment and reagents are needed to carry out this SOPs

- Aluminum foil tube
- 8 x 11 cm, thickness of 0.10 mm paper craft foils
- Ribbons for bar code labeling
- Paper envelop
- Thread ball, Tag label
- Gum tape
- Facial tissue/newsprint paper/sphagnum moss
- Permanent ink marker
- Pencil, eraser
- Cardboard boxes
- Plastic crate
- Gloves
- Facemasks
- Safety glasses
- Chemical protective clothing

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- Gumboots
- Bamboo sticks
- Ropes
- Metal sticks
- Cotton bags
- Royal Horticultural Society Colour Chart
- Isolation net
- Doubled layer paper sacks
- Plastic sacks
- Trays with screen wire or burlap bottoms
- Chemicals (fertilizers, herbicides, pesticides, fungicides, drying chemicals etc.)

Equipment

- Electronic balances (max 2000 g, d=0.01 g)
- Scientific calculator
- Aluminum bag sealer
- First aid box
- Electric tool box
- Scissor
- Knife
- Measuring tape
- Secateurs
- Icebox
- Geographical positioning system (GPS)
- Digital camera
- Stapler with ample number of pin
- Seed drying sheet
- Hand refractometer (0-30% scale)
- Seed counter
- Barcode printer
- Bush cutter
- Cultivator
- Disc harrow
- Rotovators



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- Tractor
- Plot tractor
- Sickle, hand weeder, axe, spade,
- Sprayer (power and knapsack sprayer)
- Panicle thresher
- Hand rotovators
- Laboratory thresher
- Moisture meter
- Hose pipe

All essential genebank equipment is to be monitored and verified by trained staff, calibrated by a certified third party and included in the genebank's maintenance schedule. All field equipment is maintained by the station manager. A hard copy of any equipment manual is stored in a drawer close by where the equipment is operated, while a soft copy is stored on desktops of authorized staff and operators.



Figure 1. Medium term storage (having capacity of conserving about 100,000 germplasm at 4-6⁰C) of PGRC, BARI



Figure 2. Long term storage (having capacity of conserving about 100,000 germplasm at -18 to -21⁰C) of PGRC, BARI

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Figure 3. Germination test of base collection of black gram 21 years after conservation (germination 75-100%)



Figure 4. *In vitro* conservation lab. PGRC, BARI Figure 5. Molecular biology lab. PGRC, BARI



Figure 6. Conservation activities of collected rice germplasm at BRRI

5. Occupational Health and Safety

- All activities performed in this SOPs comply with the requirements and recommendations in National Health and Safety.
- For the activities of seed dressing with chemicals during seed preparation and for chemical application at the field, genebank dress code requires from staff the use of gloves in natural latex, facemasks and safety glasses, as well as dressing with chemical protective clothes.
- Any untoward incident is reported to the supervisor and/or to the farm manager for proper action and investigation.
- Laboratory chemicals and waste are to be disposed carefully to avoid any type of health hazards and environmental pollution.

6. Procedure

The Regeneration and Characterization procedure is initiated when scientists handling germplasm conservation unit of the respective genebank review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of the accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration and characterization activities:

- Review of data and information available in database (eg. seed stocks, seed viability, seed health status, characterization data, etc.)

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- Selection of accessions and generate lists for accessions to be regenerated and/or characterized: Based on last germination test (viability), seed amount at the collection (base or active) and demand of users, the accessions are flagged for regeneration.
- Seed sample preparation
- Distribution of responsibilities
- Preparation of experimental register describing all operations, descriptor list, experimental design, plot dimension, etc.
- Field layout preparation, density and distance
- Field selection (choice of environment and planting season) and preparation
- Seed sowing (eg. seed bed preparation, sowing, nursing, seed rate, method, time, etc.) and labelling
- Preparation of land and pit
- Manure and fertilizer doses and application method
- Transplanting of seedling (age of seedling, spacing, lifting of seedling, establishment of seedling, etc.) and labelling.
- Crop management (fertilization, staking/trellising, irrigation, weed control, insect pest and disease management, isolation, pollination, roguing off-types, etc.)
- Characterization at the field and at the laboratory.
- Harvesting, threshing, seed extraction, drying and cleaning etc.
- Fumigation
- Data validation and uploading on Genebank Documentation System (GDS).

7. Related Flowcharts, Documents and Links

- Related flowcharts of SOPs for regeneration and characterization of cultivated and wild genetic resources rice and some other Agri-Horticultural crops in Bangladesh is constructed showing all operations from beginning to end of this SOPs systematically.
- Flow chart for development and implementation of new procedural SOPs in Bangladesh is also constructed keeping scope of revision of the SOPs for regeneration and characterization as per requirement in future.
- Documents and links for regeneration and characterization of rice at BRR and some Agri-Horticultural crops at BARI are shown in respective chapter.

8. Staff Training and Competency

Training and competency requirements for different level staffs to perform this SOPs efficiently are to be met regularly by the experienced PGR scientists and university teachers.

	SOPs for Regeneration, Characterization and Preliminary Evaluation of Some Agri-Horticultural Crops' Germplasm in Bangladesh	
	SOPs No.: BRRI-BARI-REG & CHAR_A-HC001	Version: 1.0
SOPs Owner: PGRC, BARI and GRSD, BRRI	SOPs Approver: Crops Division, BARC	

9. References

These SOPs have been developed following different standard international documents and standard procedures for crop production developed by BARI, BRRI and other national research institutes.

10. Revision History

Revision history of with effective date, version, description and reviewer has been shown at the end of each SOPs.

Crop wise SOPs for regeneration, characterization and preliminary evaluation of thirteen Agri-Horticultural crops' germplasm in Bangladesh are described below in different chapters.

References

- Eisner C. 2022. The Beginner's Guide to Standard Operating Procedures (SOPs).
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CHAPTER 02

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Rice Germplasm in Bangladesh

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Rice genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Rice genetic resources management.

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6.1 Procedure for Rice

The regeneration and characterization procedure is to be initiated when the scientists handling the conservation unit of BRRI genebank review the data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of the accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.1.1 Database review: Review of data and pertinent information on rice germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.1.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<85% of the initial viability of the stored seeds for cultivated rice germplasm or <75% of the initial viability of the stored seeds for its wild relatives' germplasm) and inadequate amount of seeds (below 1,500 seeds) or have not yet been characterized is generated for regeneration/characterization in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

Each of these lists should include the following information:

- Accession number
- Full taxon name (Genus, Species, Subspecies)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. growing season, wild types and pollination type requiring special seed pre-treatments are to be flagged)

6.1.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



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- When monitoring procedure ascertains for a specific seed stock that viability in LTS is below international threshold value, then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
- Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 100 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
- Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
- Seeds are placed in paper craft foils and identified with the following information:
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Country of origin
 - Number of seeds
- Seeds are treated with appropriate fungicides to control seed borne disease.
- Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the planting date (ICARDA, 2021).



Figure 7. Germination test of rice germplasm for monitoring viability of conserved germplasm in base and active collection

6.1.4 Distribution of responsibilities: As per generated list, Head of the Division, GRSD, BRRRI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific activity.



6.1.5 Preparation of experimental register: Experimental register is prepared by responsible scientist enlisting all operations to be done and all information to be recorded from seedbed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.

6.1.6 Experimental design: Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.

6.1.7 Plot dimension

The prime objective through multiplication and regeneration is to produce about 1 kg of high quality, high viability rice seeds through the least number of cultivation cycles in the field, while maintaining the genetic integrity of the germplasm samples. For most accessions in the collection this can be accomplished in a single growing season. The following plot dimension is sufficient to fulfill the purpose.

- 3.2 m × 3.0 m plots (excluding border rows) providing 100 cm wide channel in between two plots for characterization trial, and 300 cm isolation distance for regeneration.

6.1.8 Field layout

- Prepare field layout hard copy according to experimental design for seedbed and main field following seed sample preparation.
- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly border rows, row to row and plant to plant spacing, and planting spot/hole.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.1.9 Field selection and preparation

- Due attention should be paid to the environment in which cultivated and wild rice germplasm is grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.
- Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and pests.
- Selection of land for nursery: Select the land on which paddy nursery or paddy crop was not grown in the previous season. This is necessary to avoid varietal admixture due to volunteer plants in the nursery itself (Agrawal, 1999).
- Selection of land for crop production
 - Select a fertile field which will produce good quality and healthy seed.
 - Select a field where rice was not grown in the previous season. This is to restrict off type plants arising out of drop out seeds of the last season.
 - Select a field in such a way that you have provision of three meters isolation distance all around the plot. This is required for restricting unwanted cross fertilization due to wind and insects.
 - Land to be used for regeneration and characterization shall be free of volunteer plants and objectionable weeds. The selected plots should be leveled and soil preferably clay loam.



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- Early enough before planting, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the planting date to remove the weeds.

6.1.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for seedbed and main experimental field. Information printed on the tag includes the trial code, bed section number and accession/collector's number.

6.1.11 Seed sowing (eg. sowing rate, method, etc.) and labeling (BRRI, 2022).

- Time of sowing
 - Aus: 15 March to 28 April (B. Aus); 29 March to 18 April (T. Aus)
 - Aman: 29 June to 30 July
 - Boro: 04 November to 15 December
- Seed sprouting
 - Seeds should be sprouted before sowing by loosely packing in cloth bags (labeled with water-proof thermal plastic tags) and soaking them in fresh water for 16 to 20 hours, after which these are removed from water receptacle and the water allowed to drain completely.
 - The seed bags are kept damp by covering them with wet gunnies to ensure optimum condition for germination.
 - Seeds so soaked and damped sprout in about 48 hours in aus and aman seasons, and 72 hours in boro season.
- Preparation of nursery bed
 - The soil should be well pulverized by repeated ploughing.
 - Then flood the nursery plot, puddle and leave it for two days to set with a thin layer of water.
 - Prepare the raised beds measuring 6.0 m × 1.5 m with 0.5 m wide channels all around to facilitate drainage.
 - The loose soil from the channel is taken out to a depth of 15-20 cm and thrown on either side of the bed.
 - Finally the beds are leveled after applying fertilizer. Allow 3 to 5 cm water to stand in the bed.
 - Divide the seedbed into sections for each accession.
- Sowing of sprouted seeds is done by hand keeping in mind that seeds should be distributed evenly and strictly within the section boundaries.
- Immediately after sowing a final check is done for correspondence to the trial layout and any planting error is noted on the hard copy.
- Guard the seedbed from bird damage for about one week.
- Take care of the seedbed. Irrigate as per requirement. Take plant protection measures if needed. Apply fertilizer if the plot is not fertile enough.



- Protect the seedlings from cold injury during winter for boro rice accessions.
- If any line is affected by disease/insect, the line can be discarded
- Labeling: Layout of the seedbed should be prepared during seed sample preparation, and divide the bed into sections according to the number of accessions to be regenerated/characterized; each individual nursery bed section is to be labeled with water-proof thermal plastic tag before seed sowing. Information printed on the tag includes the plot number, accession/collector's number.

6.1.12 Preparation of land for transplanting

- Land is ploughed repeatedly (three to four times) followed by harrowing to obtain a fine tilth.
- Leave the field for 15-20 days.
- The ploughed field should be kept flooded for several days and then puddled to make it soft enough to ease transplanting of seedlings.
- The field should be leveled to facilitate distribution of irrigation water equally all over the field (BRR, 2022).

6.1.13 Fertilizer doses: Fertilizers are to be applied @ 60-20-40-6-1.3 kg N-P-K-S-Zn/ha.

Application method

- All the fertilizers except urea are to be applied at the time of final land preparation. S and Zn can be top-dressed if needed.
- N is to be applied in three equal splits at 10, 25 and 35 days after planting.
- Under direct seeded culture fertilizer should be applied in two equal splits, the first one as basal and the second one at maximum tillering stage; and should be mixed thoroughly with the soil as soon as possible for better utilization (Ahmed *et al.*, 2018).

6.1.14 Transplanting of seedling

- Age of seedling:
 - Aus: 20-25 days
 - Aman: 25-30 days
 - Boro: 35-45 days
- Seedling uprooting
 - Before seedling uprooting, water the seedbed well so that the soil will soften and uprooting will be smooth and easy.
 - Generally, seedling uprooting from sandy seedbed is easy where seedling damage is minimum; but where the seedbed is not sandy, there more time is required for softening of the soil after irrigation and there is more chance of seedling damage.
- Preparation of seedling bundles: Seedling bundles are prepared very carefully causing no injury to any seedling with proper labeling.



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- Placing the seedling to experimental field: Place the seedling bundles with water-proof thermal plastic tags from the nursery bed onto the respective field plot according to field layout. Information printed on the tag includes the trial code, bed section number and accession /collector's number. Transplant the seedlings in rows at recommended spacing.
- No. of seedlings/hill: Plant one healthy seedling per hill by hand
- Spacing: Row to row = 25 cm; plant to plant = 20 cm; plot to plot = 100 cm for characterization and 300 cm for regeneration trials.
- Keep 2-3 cm of water in the field after transplanting (BRRI, 2022).
- Keep the plastic tags in respective field plots hanging with bamboo sticks for final checking.
- Immediately after transplanting a final check is to be done for correspondence to the field trial layout and any planting error is noted on the field layout on the hard copy.
- First and final field plots are marked with sticks as soon as final check is confirmed.



Figure 8. Raising of seedling in the nursery bed and transplanting of rice seedling in field plot

6.1.15 Isolation distance

- Rice is a self-pollinated crop. Both male and female organs are present inside one flower for which fertilization occurs within the flower. So, it is easy to maintain genetic purity. Though strictly self-pollinated, windy conditions and movement of insects from one plant to other result in around 2-5% cross pollination effected by the pollen of other varieties planted nearby (Sahu *et al.*, 2020).
- Provide 300 cm isolation distance among the accessions.
- Avoid seed collection from four boarder rows all around each plot or
- Provide 2 m high plastic sheet barrier around each accession for about two weeks.

6.1.16 Labelling: Seedling bundles of the accessions are placed onto the respective field plots based on the field layout created as per design during seed preparation.

- Once transplanting in the main plot is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes the plot number and the accession number.



- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of sowing, date of transplanting, number of germplasm/accessions, number of checks, experimental design, implementing division etc.).

6.1.17 Weed management: All cultural practices applied during field preparation are intended to prepare a uniform and weed-free seedbed/experimental field immediately prior to sowing/transplanting.

- Weed out the plots twice or thrice by hand weeding as needed before heading.
- Keep water in the field to a particular level as advised, which will reduce weed.
- Weed control with the use of selective post-emergence herbicides is also recommended.
- All weeds between plots and within the alley ways are controlled using hand weeder, Japanese rice weeder or hand hoe.

6.1.18 Water management

In comparison to other crops, rice cultivation requires more water. Therefore, effective irrigation as per requirement of the crop results in higher yield.

- After transplanting maintain 2-3 cm depth of water in the field for a month. It will restrict weed growth. Do not keep more water that will affect the normal tillering of the rice plant.
- Once tillers come up, maintain 3-5 cm depth water in the field till milking stage of the panicles. Shortage of water during panicle initiation and milking stage leads to more chaffy grains in the panicle.
- After milking stage, reduce the water level in the field to 2-3 cm only.
- Once tips of panicle ripen or before 15 days of harvesting drain the total water from the field and allow it to dry.

6.1.19 Roguing off-types plants

- Meticulous monitoring for off-type plants within each plot is to be conducted several times throughout the growing season.
- Utmost care is to be taken to discard only the plants that are well confirmed to be contaminants or volunteers within the plots (eg. by searching in the database for previous collection and/or characterization data).
- Rogue out wild rice plants, plants infested by stem borer, and diseased plants e.g. plants affected by tungro virus and false smut from time to time as required.
- Seedlings that germinate outside a row in the seedbeds and in the field are rogued out.

6.1.20 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is conducted by genebank scientists and the Head of the Division, GRSD, BRRI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from pathology and entomology division of BRRI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.



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- For the diseases that are of major risks and/or are consistently present almost every growing season, a preventive strategy with systematic chemical applications is adopted.
- For diseases occurring occasionally, chemical treatments are decided based on the severity of the disease and the prevalent weather conditions.
- Insects and pests are effectively controlled with appropriate crop management applied during the whole growing season
- However, in case of severe insect infestations appropriate soil or foliar insecticides (depending on insect biology) can be applied to avoid any potential risk for the regeneration of the accessions.
- For the special case of bird damage, bird watchers are used during the grain filling and maturity period to generate noise by using improvised structures or devices.
- Viruses are controlled mainly through vector control and roguing out of infected plants immediately to prevent contamination of healthy plants.
- Seed borne diseases are managed using appropriate seed treatment before seed sowing, and roguing out the infected plants immediately from the plots and covered by soil.



Figure 9. Rejuvenation activities of rice germplasm during T. Aman season.



6.1.21 Harvesting

- Prior to harvest a second plastic tag same with the ones used for labeling the field plots is prepared for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with the plot number and accession number.
- Accessions are harvested by hand and placed into the respective labeled plastic sacks. The plot tag is placed into the plastic sack and the new one prepared is firmly tightened outer of the sack.
- Crop should be harvested when the grain is ripe. This stage is determined visually from the loss of the green color of the uppermost internode of the peduncle, which turns into very light green or yellow. At this stage transportation of water and nutrients to the spike is cut off and accession is reaching maximum grain fill.
- Harvesting is done by cutting panicles only and placing them in clean plastic sacks with proper labels (Reano *et al.*, 1998).

6.1.22 Threshing and cleaning

- Seeds are threshed and winnowed very carefully to avoid mechanical admixture.
- Initial seed cleaning is done right after threshing to remove any unfilled grains and stubble before transfer to smaller cloth bags for drying.
- Then the seed must be dried to below 12% moisture content for sending to conservation unit.

6.1.23 Fumigation: Threshed and cleaned seeds may be fumigated to prevent insect damage prior to process for conservation.

6.1.24 Data validation and uploading onto BRRI Genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Season
5. Date of sowing
6. Date of transplanting
7. Number of rows per plot and seeding rate per plot
8. Row length, Row width, Plot to plot distance
9. Date of first effective rainfall or first irrigation

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe cold, hail storm, severe drought, inundation due to flood, severe storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. Total grain weight per plot (kg)

C. Comparisons with previous passport or morphological data

- Compare each accession with the following characterization data previously recorded for the accession:
 1. Leaf blade colour
 2. Ligule colour
 3. Culm: Anthocyanin colour
 4. Spikelet: awns in the spikelet
 5. Lemma and palea colour
 6. Seed coat (bran) colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for Bangladesh's rice accessions is considered finalized when threshed grains have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.1.25 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

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Characterization of accessions at the field is done as soon as the accession will be acquired by GRSD, BRRI genebank, and germination test of each accession is done every three years. Accessions having less than 85% viability is selected for regeneration.

Descriptors for cultivated rice (*Oryza sativa* L.) (BRRI, 2018) is used for characterization of cultivated rice (*Oryza sativa* L.). Some additional descriptors are included from IRRI and IBPGR (1980) and BI, IRRI and WARDA (2007) descriptors for rice (*Oryza sativa* L.) and wild and cultivated rice (*Oryza* spp.), respectively. The following information and descriptors are recorded during characterization:

1. Name (long & short) and address of the institute:
2. Geographical position
Latitude- degree and minutes followed by N (North) and S (South)
Longitude- degree and minutes followed by E (East) and W (West)
Altitude (metre):
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:



Figure 10. Morphological characterization of rice germplasm during Aman season



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6.1.26 Descriptor list

Season..... Date of sowing..... Date of transplanting:

Descriptor ¹	Code Guide	Growth stage
1. Accession number	
2. Name	
3. Former designation	
4. Seed source	
5. Country of origin	

Descriptor ¹	Code Guide	Growth stage
6. Variety group	1 <i>indica</i> (Boro/Aus/T.Aman/ DW rice/Jhum) 2 <i>sinica</i> (<i>Japonica</i>) 3 <i>javanica</i> 4 intermediate (hybrids)	
7. Life cycle (Wild species).	1 annual 2 perennial 3 intermediate	Completeness of plant growth in a growing season after ratooning.
8. Seedling height (cm)	² 3 short (<30 cm) 5 intermediate (~45 cm) 7 tall (> 60 cm)	5-leaf stage, approximately 20 days after seeding
LEAF (below the flag leaf)		
9. Leaf blade: length (cm)	² 1 very short (<21 cm) 3 short (~30 cm) 5 intermediate (~50 cm) 7 long (~70 cm) 9 very long (> 80 cm)	early reproductive stage
10. Leaf blade: width (cm)	² 3 narrow (<1 cm) 5 intermediate 7 broad (>2 cm)	early reproductive stage
11. Blade pubescence ³	1 glabrous 2 intermediate 3 pubescent	
12. Blade colour ³	1 pale green 2 green 3 dark green 4 purple tips 5 purple margins 6 purple blotch 7 purple	booting to heading stage

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Descriptor ¹	Code Guide	Growth stage
13. Leaf blade pubescence on blade surface (wild species).	1 glabrous (no hairs) 2 hairy on upper surface 3 hairy on lower surface 4 hairy on both sides	specify location of hairs present on the surface of the blade. Stage: late vegetative
14. Leaf margin pubescence (Wild species).	1 glabrous (no hairs) 2 hairy or ciliated	assess at late vegetative stage
15. Leaf sheath: anthocyanin color	1 absent 9 present	late vegetative stage
16. Basal leaf sheath colour ³	1 green 2 purple lines 3 light purple 4 purple	late vegetative stage
17. Angle ³	1 erect 5 horizontal 9 drooping	prior to heading
18. Flag leaf angle ³	1 erect (<30) 3 intermediate or Semi erect (30-45) 5 horizontal (46-90) 7 descending (>90)	stem elongation to booting
LIGULE		
19. Length (mm)	2	after anthesis
20. Colour ³	1 white 1 purple lines 3 purple	stem elongation to booting
21. Shape ³	1 acute to acuminate 2 2-claft 3 truncate	late vegetative stage
22. Ligule margin shape (wild species)	1 Entire 2 Scalloped or toothed 99 Other	stage: after anthesis
23. Ligule pubescence (Wild species)	1 glabrous 2 partially hirsute: hairs covering less than 50% of the ligule 3 mostly or generally hirsute: hairs covering more than 50% of ligule	visual assessment using hand lens. Stage: after anthesis
24. Collar colour ³	1 pale green 2 green 3 purple	late vegetative stage
25. Auricle colour ³	1 pale green 2 purple	stem elongation to booting
DAYS TO HEADING		
26. Number of days from effective seeding date to 50% heading	2	at flowering
	1 very early (>70 days) 3 early (70-85 days)	



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Descriptor ¹	Code Guide	Growth stage
	5 medium (86-105 days) 7 late (106-120 days) 9 very late (>120 days)	
CULM		
27. Length (cm) (measure from the base of the plants to the neck of the panicle)	² 1 very short (<40 cm) 3 short (41-60 cm) 5 medium (61-80 cm) 7 long (81-110 cm) 9 very long (>110 cm)	after flowering
28. Culm: Anthocyanin colour	1 absent 9 present	after flowering
29. Number (Total tiller)	²	after flowering
30. Culm angle ³	1 erect 3 intermediate 5 open 7 spreading 9 procumbent	
31. Culm diameter (mm) (from 5 mother tillers in the lowest internode)	² 1 small (<5.0 mm) 3 medium (5.1-6.0 mm) 5 large (6.1-7.0 mm) 7 very large (>7.0 mm)	after flowering
32. Internode colour ³	1 green 2 light gold 3 purple lines 4 purple	after flowering
33. Culm: strength (lodging resistance)	1 strong (no lodging) 3 moderately strong (most plants leaning) 5 intermediate (most plants moderately lodging) 7 weak (most plants nearly flat) 9 very weak (all plants flat)	after heading by gently pushing the tillers (30 cm from the ground) back and forth a few times. at harvest (standing position of plants)
34. Lodging incidence (% of plants that lodged)	heading, milk or dough stage
PANICLE		
35. Length (cm) (measured from the neck to the tip of the panicle of main tillers without awns).	² 1 very short (<11 cm) 3 short (~15) 5 medium (~25 cm) 7 long (~35cm) 9 very Long (>30 cm)	dough stage

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Descriptor ¹	Code Guide	Growth stage
36. Number of panicle per plant	² 3 low (<6) 5 intermediate (6-10) 7 high (>10)	early ripening
37. Type ³	1 compact 5 intermediate 9 open	dough stage
38. Panicle: texture of main axis (Wild species).	1 scabrous 2 smooth	assess by rubbing fingers from the base towards the tip of the panicle axis at full panicle exsertion stage
39. Secondary branching ³	0 absent 1 light 2 heavy 3 clustering	dough stage
40. Exsertion ³	² 1 enclosed 3 partly exerted 5 just exerted 7 moderately well exerted 9 well exerted	near maturity
41. Axis ³	1 straight 2 droopy	at maturity
42. Shattering	1 very low (<1%) 3 low (~3%) 5 moderate (~15%) 7 high (~35%) 9 very high (>50%)	at maturity of harvest
43. Threshability	1 difficult (<1%) 3 moderately difficult (1-5%) 5 intermediate (6-25%) 7 loose (26-50%) 9 easy (51-100% grains removed)	at maturity
GRAIN (spikelet)		
44. Spikelet: awns in the spikelet	0 none (awnless) 1 tip only 2 upper quarter only 3 upper half only 4 upper three-quarter only 5 whole length	flowering to maturity



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Descriptor ¹	Code Guide	Growth stage
45. Anther: colour (Wild species).	1 Yellow 2 Brown	stage: at anthesis
46. Length of the longest awn	1 very short (<5 mm) 3 short (~8 mm) 5 intermediate (~15 mm) 7 long (~30 mm) 9 very long (>40 mm)	flowering to maturity
47. Awn colour ³	1 straw 2 gold 3 brown (tawny) 4 red 5 purple 6 black	at maturity
48. Apiculus colour ³ (Colour of the tip of lemma)	1 white 2 straw 3 brown (tawny) 4 green 5 red 6 red apex 7 purple 8 purple black 9 black	at maturity
49. Stigma colour ³	1 white 2 light green 3 yellow 4 light purple 5 purple	at flowering (between 9am and 2 pm)
50. Lemma and palea colour ³	0 straw 1 gold and or gold furrows on straw background 2 brown spots on straw 3 brown furrows on straw 4 brown (tawny) 5 reddish to light purple 6 purple spots on straw 7 purple furrows on straw 8 purple 9 black 10 white	at maturity
51. Lemma and palea pubescence ³	1 glabrous 2 hairs on lemma keel 3 hairs on upper portion 4 short hairs 5 long hairs (velvety)	flowering to maturity

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Descriptor ¹	Code Guide	Growth stage
52. Sterile lemma colour ³	1 straw 2 gold 3 red 4 purple	at maturity
53. Sterile lemma length ³ (measure at post-harvest stage)	1 short (<1.5 mm) 3 medium (1.5-2.5 mm) 5 long (2.5 mm) 7 extra-long (equal to or longer than lemma) 9 asymmetrical	at maturity
54. Spikelet sterility ³	1 completely sterile (0%) 2 highly sterile (1-49% to trace) 3 partly sterile (50-74%) 4 fertile (75-90%) 5 highly fertile (>90%)	at maturity
55. Sterile lemma shape (Wild species)	0 Absent 1 Linear (long and slender) 2 Subulate or setaceous (linear and tapering to a fine point, set with or consisting of bristles) 3 Triangular (and very small)	at maturity
56. 1000 grain weight (g) (fully developed grains adjusted at 12% of moisture)	²	at maturity
57. Length: (mm) (without dehulling)	²	at maturity
58. Width: (mm) (without dehulling)	²	at maturity
59. Brown rice: Length (mm) (after dehulling, before milling)	² 7 extra-long (>7.5 mm) 5 long (6.6-7.5 mm) 3 medium (5.51-6.6 mm) 1 short (<5.5 mm)	at maturity
60. Brown rice: width (mm) (after dehulling, before milling)	² 3 narrow 5 medium 7 broad	at maturity
61. Decorticated grain: shape (Length-width (widest point) ratio of de-hulled grain)	1 slender (L: W >3.0) 3 medium (L: W = 2.1-2.5) 5 bold (L: W = 1.5-2.0) 9 round (L: W <1.5)	at maturity
62. Seed coat (bran) colour ³	1 white 2 light brown	at maturity



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Descriptor ¹	Code Guide	Growth stage
	3 speckled brown 4 brown 5 red 6 variable purple 7 purple	
63. Endosperm type ³	1 non-glutinous (non-waxy) 2 glutinous (waxy) 3 indeterminate	at maturity
64. Decorticated grain: Scent (aroma)	0 non-scented 1 lightly scented 2 scented	at flowering or at maturity-by cooking test
65. Leaf senescence ³ (Penultimate leaves are observed at the time of harvest.)	1 very early (all leaves lost their green colour before grain maturity) 3 all leaves have lost their green colour at harvest 5 intermediate (one leaf still green at harvest) 7 late and slow (two or more leaves still green color at harvest) 9 very late (all leaves still green at harvest)	at maturity
MATURITY		
66. Days from seeding (when 80% of grains on panicle are mature)	²	at maturity
67. Yield (g)	²	after harvesting
68. Phenotypic acceptability (PACP)	1 excellent 3 good 5 fair 7 poor 9 unacceptable	at maturity
GEU (Genetic Evaluation and Utilization) TRAITS		
69. Drought tolerance.....		81. Bakanae
70. Submergence tolerance.....		82. Sheath blight.....
71. Recovery from submergence.....		83. Sheath rot.....
72. Cold tolerance.....		84. Rice tungro virus.....
73. Elongation ability.....		85. Ufra.....
74. Tidal submergence.....		86. Brown plant hopper.....
75. Photoperiod sensitivity.....		87. White-backed plant hopper.....
76. Salinity.....		88. Green leaf hopper.....
77. Bacterial blight.....		89. Protein content (%).....
78. Brown spot.....		90. Amylose content (%).....
79. Leaf scald.....		91. Alkali digestion.....
80. Blast.....		
92. Other distinct special character (if any)		

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Bold words serve as the main heading for the various descriptors, when arranged in a tabular form

² Enter actual measurements (in metric units) or counts.

³ Use X for a mixture of different types.

6.1.27 Identity is monitored by comparing the accession with previous passport or morphological data.

6.1.28 All characterization information is verified by the head of conservation unit of GRSD and the genebank manager.

6.1.29 Characterization data is uploaded on the GRSD, BRR Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to GRSD, BRR Genebank Documentation System.



Figure 11. Data collection for morphological characterization of rice germplasm

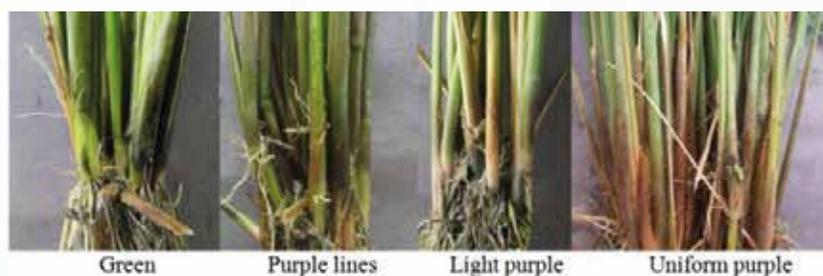


Figure 12. Variation in basal leaf sheath colour of rice germplasm



Figure 13. Variation in awn



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Figure 14. Variation in decorticated grain colour



Figure 15. Variation in decorticated grain length



Figure 16. Monitoring of the field experiment by GRSD scientists



Figure 17. Deep water rice and different crops including rice in shifting cultivation



Figure 18. Diversity of Local rice cultivar in Bangladesh

7.1.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.1.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild rice genetic resources at BRRI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.1.3 Adherence to legal and regulatory frameworks: The regeneration and characterization of cultivated and wild rice genetic resources at BRRI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- IRRI
- UPOV

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8.1.1 Staff Training and Competency

Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of GRSD, BRRI.

Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.1.1 References

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10.1.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Aziz Zilani Chowdhury

CHAPTER 03

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Brinjal Germplasm in Bangladesh

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Brinjal genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Brinjal genetic resources management.

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6.2 Procedure for Brinjal

The regeneration/characterization procedure is initiated when the scientists handling the conservation unit of PGRC, BARI genebank review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of brinjal accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.2.1 Database review: Review of data and pertinent information on brinjal germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.2.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<80% of the initial viability of the stored seeds for cultivated brinjal germplasm or <60% of the initial viability of the stored seeds for its wild relatives' germplasm) and inadequate amount of seeds (below 1500 seeds) or have not yet been characterized is generated for regeneration/characterization in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

Each of these lists should include the following information:

- Accession number/Collector's number
- Full taxon name (Genus, Species, Sub-species)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly winter types, strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged).

6.2.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



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- When monitoring procedure ascertains for a specific seed stock that viability in LTS is below international threshold value (<1500 seeds), then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
- Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 20 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
- Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
- Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Country of origin
 - Number of seeds
- Seeds are treated with appropriate fungicides to control seed borne disease.
- Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the sowing date.



Figure 19. Seed sample preparation for field trial

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6.2.4 Distribution of responsibilities: As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species for PGRC, BARI genebank.

6.2.5 Preparation of experimental register: Experimental register is prepared by responsible scientist enlisting all operations to be done and all information to be recorded from seedbed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.

6.2.6 Experimental design: Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.

6.2.7 Plot dimension: 4.8 m × 3.0 m plot having 100 cm wide and 15-20 cm deep drain in between two beds. A total of 24 seedlings are to be planted per plot at 100 cm × 60 cm spacing (Rashid *et al.*, 2006 and BARI, 2019).

6.2.8 Field layout

- Prepare field layout hard copy according to experimental design for seedbed and main field following seed sample preparation.
- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot/hole/pit.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or random table.

6.2.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated and wild brinjal germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and pests.

- Selection of nursery bed: Select nursery bed (pre-constructed) on which brinjal or other Solanaceous vegetables seedlings were not grown in the previous season. This is necessary to avoid soil borne diseases especially bacterial wilt as well as to avoid varietal admixture due to volunteer plants in the nursery itself.
- Selection of land for crop production: The land should be free of volunteer plants and objectionable weeds. Select the land on which brinjal or other Solanaceous vegetables were not grown in the previous season. This is necessary to avoid soil borne diseases especially bacterial wilt. The soil of the selected field should be fertile, rich in organic matter, sandy loam in texture and well drained (Agrawal, 1999).



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- Early enough before planting, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the planting date to remove the weeds.

6.2.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for seedbed and main experimental field. Information printed on the tag includes accession/collector's number.

6.2.11 Seed sowing (eg. sowing rate, method, etc.) and labeling

- Preparation of seedbed: Seedbed should be well prepared mixing sand, soil and FYM in equal ratio (Rashid *et al.*, 2006). Each bed is divided into several sections; each section is labeled with water-proof thermal plastic tag. Information printed on the tag includes bed section number, accession/collector's number. Provide 20 cm distance between two adjacent bed sections.
- Time of Seed Sowing
 - Brinjal seeds are sown in nursery during July to August.
 - Sowing of seed crop should be so adjusted that maturity does not coincide with rains (Rashid and Singh, 2000).
- Method of sowing: Seed samples are placed in respective sections. Seeds are sown densely in one row of each section at a depth of 1.0-1.5 cm; 10-12 days after germination, the young seedlings are transferred to adjacent rows at 15 cm row to row and 10 cm plant to plant spacing to have uniform, healthy, strong and stout seedlings.
- Immediately after sowing seeds final check is done for correspondence to the trial layout and any planting error is noted on the hard copy.

6.2.12 Preparation of land

- Prepare the land to a fine tilth by deep ploughing, three to four harrowing followed by leveling.
- The plots are raised by 15-20 cm lifting the soil from 100 cm channel kept around each plot.
- Pits of 15 cm diameter and 15 cm depth are to be prepared at 100 cm × 60 cm spacing and recommended manure and fertilizers are applied at 7-10 days before transplanting of seedlings.

6.2.13 Manure and Fertilizer doses: 135-36-105-15-2.0-1.0 kg/ha N-P-K-S-Zn-B and FYM @ 4 t/ha (Ahmed *et al.*, 2018).

Method of application

- a) Half of FYM and all of P, S, Zn and B should be applied as basal during final land preparation. Remaining FYM should be applied in pits 7-10 days before transplanting of seedlings and mixed thoroughly with the soil.

- b) N and K should be applied in three equal splits at 20, 40 and 60 DAT around the plant as side dressing under moist soil condition and mixed thoroughly with the soil as soon as possible for better utilization.

Instead of urea, Urea Super Granules (USG) might be applied 7-8 cm deep in to the soil and 8-10 cm apart from the plant as ring method.

6.2.14 Transplanting of seedling

- Age of seedling: 30-35 days (5-6 leaf stage).
- Spacing: Row to row = 100 cm; plant to plant = 60 cm; plot to plot = 100 cm (Rashid *et al.*, 2006).
- Lifting of seedling: Apply light irrigation with water can before lifting of seedling from seedbed. Lift the seedling carefully with some soil attached to the roots and make bundle carefully with soft rope.
- Bringing the seedling to experimental field: Label the seedling bundle with waterproof thermal plastic tag. Information printed on the tag includes the plot number, accession/collector's number. Bring the labeled seedling bundles to the experimental field and place them to respective field plots.
- No. of seedlings/pit: Plant one healthy seedling per pit. Gap filling may be done immediately after death of any seedling.
- Transplant the seedling at evening time. Irrigate immediately afterwards.
- Keep the label of seedling bundles in respective field plots for final checking.
- Immediately after transplanting, a final check is to be done for correspondence to the field trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with stakes as soon as final check is confirmed.



Figure 20. Raising of brinjal seedling in polybag, pit preparation, manure and fertilizer application, and transplanting of seedlings in field plots



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6.2.15 Labeling

- Once transplanting in the main plot is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of transplant, number of accessions, number of checks, experimental design, etc.).

6.2.16 Staking and shoot pruning: The plants are to be staked with bamboo sticks to keep it upright. All the side shoots below the first flower except immediate below one are to be pruned out (Rashid *et al.*, 2006).

6.2.17 Isolation

- In most varieties the perfect flowers are borne singly and opposite the leaves.
- The stamens dehisce at the same time the stigma is receptive so that self-pollination is the rule although there is some cross-pollination by insects (often cross-pollinated) (Rashid and Singh, 2000).
- The extent of cross-pollination has been recorded from 0 to 48%.
- To avoid cross pollination, isolation nets (preferably 40-60 mesh polyvinyl net) are to be used before flowering stage (one net for each unit plot). Out-cross can also be controlled by covering the inflorescence by butter paper bag before anthesis.

6.2.18 Weeding and mulching: The field should always be kept free of weed.

- Three to four hand weeding may be required during crop period to keep the field clean of weeds.
- Mulching are also to be done following irrigation to keep the soil loose and conserve soil moisture.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.2.19 Irrigation and drainage

- Light irrigation with water can for 3-4 days after transplanting is essential.
- Enter irrigation water into the drain and allow it to stand until the soil around the plant is soaked; avoid flood irrigation.
- Excess irrigation/rain water is to be drained out sharply.

6.2.20 Roguing: Three times field inspection for roguing has been suggested:

- Before flowering by examining plant colour, growth habit and foliage characteristic such as shape, size and posture.
- At early flowering and fruit development-by observing general plant habit, vigour, degree of spinyess.
- At fruiting off-types can be identified on the basis of fruit characteristics like shape, size, colour etc. (Rashid and Singh, 2000).

In addition to off-types, plants affected by diseases such as *Phomopsis* blight and little leaf etc. should be removed from the field as soon as they are noticed.

6.2.21 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices. Sex pheromone trap may be used to control brinjal shoot and fruit borer.

- Regular monitoring of insect pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre (CSO, PGRC). Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology division of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Virus and mycoplasma are controlled mainly through vector control and roguing out of infected plants immediately as they are noticed to prevent contamination of healthy plants.

6.2.22 Harvesting

- Prior to harvest several extra plastic tags same with the ones used for labeling the field plots are printed for each accession, including information on trial code, plot number, accession number/collector's number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with the plot number and accession number/collector's number.
- Fully ripe fruits (this stage is determined visually from the turning of the skin colour into yellow) of each accession are harvested periodically by hand and placed into the respective labeled plastic sacks. Respective extra tag is firmly tightened outer of the sack.
- Seeds should be collected from first or second tier fruits as those have a higher seed weight and germination rate than seeds collected from fruits collected beyond second tier (Rashid and Singh, 2000).
- The sacks are then brought to field laboratory for seed extraction and processing.



Figure 21. Proper stage for harvesting fully ripe fruits of brinjal



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6.2.23 Seed extraction, cleaning and drying

- The harvested fruits are dried in the sun keeping in labeled plastic sack until they shriveled. The fruits are then hand beaten gently to loosen the seeds. The fruits are then cut longitudinally and dipped in water kept in plastic bowl to separate the seed from the pulp (Rashid and Singh, 2000).
- Fresh water should be used for each accession following cleaning of the bowl.
- After separation, the seeds are dipped into water. Those which float should be rejected. The separated seeds are kept in plastic bowl labeled with same plastic tag.
- The seeds are then cleaned and dried in partial shade for few hours for one or two days up to a moisture content of eight percent or below (Agrawal, 1999), before sending to conservation unit.
- Care should be taken to avoid mechanical admixture.

6.2.24 Data validation and uploading on PGRC, BARI Genebank Documentation System:

Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Date of transplanting
6. Number of rows per plot
7. Row length, Row width, Plot to plot distance

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe storm, hail storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. 1000 seed weight (g)
8. Total seed weight per plot (g)



C. Comparisons with previous passport or morphological data

- Compare each accession with the following characterization data previously recorded for the accession:
 1. Plant growth habit
 2. Petiole colour
 3. Corolla colour
 4. Fruit shape
 5. Fruit colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for brinjal accessions in Bangladesh is considered finalized when extracted and dried seeds have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.2.25 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

- Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors of Agri-Horticultural Crops, Part II: Vegetable Crops, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated brinjal (*Solanum melongena* L.)].
1. Name (long & short) and address of the institute:
 2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
 3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
 4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
 5. Name of Person in Charge of Characterization:



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6.2.26 Descriptor List

Date of sowing: Date of Transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Plant growth habit
To be recorded at peak fruiting stage
 - 3 Upright
 - 5 Intermediate
 - 7 Prostrate
 - 99 Others (Specify in the 'Remarks' descriptor)
7. Plant height (cm)
To be recorded at peak fruiting stage
 - 3 Small (≤ 50 cm)
 - 5 Medium (50-100 cm)
 - 7 Tall (>100 cm)
 - 99 Others (Specify in the 'Remarks' descriptor)
8. Plant spread (cm)
To be recorded at peak fruiting stage)
 - 1 Very narrow (≤ 30 cm)
 - 3 Narrow ($>30-40$ cm)
 - 5 Intermediate ($>40-60$ cm)
 - 7 Broad ($>60-90$ cm)
 - 9 Very broad (>90 cm)
 - 99 Others (Specify in the 'Remarks' descriptor)
9. Number of primary branches per plant
To be recorded at peak fruiting stage
Quantitative
10. Petiole length (cm)
To be recorded on 5th leaf from top at full foliage stage
Quantitative



11. Petiole colour

To be recorded on 5th leaf from top at full foliage stage

- 1 Green
- 2 Greenish violet
- 3 Violet
- 4 Dark violet
- 5 Dark brown
- 99 Others (Specify in the 'Remarks' descriptor)

12. Leaf blade length (cm)

To be recorded on 5th leaf from top at full foliage stage

Quantitative

13. Leaf blade width (cm)

To be recorded on 5th leaf from top at full foliage stage

Quantitative

14. Leaf blade lobing

To be recorded on 5th leaf from top at full foliage stage

- 1 Very weak
- 3 Weak
- 5 Intermediate
- 7 Strong
- 9 Very strong
- 99 Others (Specify in the 'Remarks' descriptor)

15. Leaf blade tip angle

To be recorded on 5th leaf from top at full foliage stage

- 1 Very acute (≤ 15 degree)
- 3 Acute ($> 15-45$ degree)
- 5 Intermediate ($> 45-75$ degree)
- 7 Obtuse ($> 75-110$ degree)
- 9 Very obtuse (> 110 degree)
- 99 Others (Specify in the 'Remarks' descriptor)

16. Leaf blade colour

To be recorded at full foliage stage

- 1 Light green
- 2 Green
- 3 Dark green
- 4 Greenish violet
- 5 Violet
- 99 Others (Specify in the 'Remarks' descriptor)



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17. Number of leaf prickles (in upper surface)

To be recorded at full foliage stage

- 0 None
- 3 Few (1-5)
- 5 Many (>5)
- 99 Others (Specify in the 'Remarks' descriptor)

18. Days to 50% flowering

To be recorded as number of days from date of transplanting to date when at least 50% percent plants show first flower open

Quantitative

19. Corolla colour

To be recorded at flowering stage

- 1 White
- 2 Greenish white
- 3 Pale violet
- 4 Light violet
- 5 Bluish violet
- 99 Others (Specify in the 'Remarks' descriptor)

20. Calyx colour

To be recorded at flowering stage

- 1 Green
- 2 Light purple
- 3 Dark purple
- 99 Others (Specify in the 'Remarks' descriptor)

21. Calyx spininess

To be recorded at flowering stage

- 3 Smooth
- 5 Medium thorny
- 7 High thorny
- 99 Others (Specify in the 'Remarks' descriptor)

22. Fruit pedicel length (cm)

To be recorded as average of 5-10 random fruits at marketable stage

Quantitative

23. Fruit pedicel prickles

To be recorded on marketable fruits

- 0 None
- 1 Few (1-5)
- 2 Many (>5)
- 99 Others (Specify in the 'Remarks' descriptor)

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24. Fruit length (cm)
To be recorded as average of same 5-10 fruits at marketable stage
Quantitative
25. Fruit Breadth (cm)
To be recorded as average of same 5-10 fruits at marketable stage
Quantitative
26. Fruit length-breadth ratio
To be recorded as average of same 5-10 fruits at marketable stage
- 1 Broader than long
 - 3 As long as broad
 - 5 Slightly longer than broad
 - 7 Twice as long as broad
 - 8 Three times as long as broad
 - 9 Several times as long as broad
27. Number of fruits per plant
To be recorded as average of cumulative yield of all pickings on same 5-10 plants at marketable stage
Quantitative
28. Fruit curvature
To be recorded on marketable fruits
- 1 None (fruit straight)
 - 3 Slightly curved
 - 5 Curved
 - 7 Snake shaped
 - 8 Sickle shaped
 - 9 U shaped
 - 99 Others (Specify in the 'Remarks' descriptor)
29. Fruit shape
To be recorded on marketable fruits
- 3 Long
 - 5 Round
 - 7 Oblong
 - 9 Oval
 - 99 Others (Specify in the 'Remarks' descriptor)
30. Fruit apex shape
To be recorded on marketable fruits
- 3 Protruded
 - 5 Rounded
 - 7 Depressed
 - 99 Others (Specify in the 'Remarks' descriptor)



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31. Fruit colour

To be recorded on marketable fruits

- 1 Milky white
- 2 Green
- 3 Deep yellow
- 4 Fire red
- 5 Scarlet red
- 6 Lilac red
- 7 Purple
- 8 Purple black
- 9 Black
- 10 Light purple
- 99 Others (Specify in the 'Remarks' descriptor)

32. Fruit colour distribution

To be recorded on marketable fruits

- 1 Uniform
- 3 Mottled
- 5 Irregular striped
- 7 Regular striped
- 99 Others (Specify in the 'Remarks' descriptor)

33. Fruit flesh density

To be recorded on marketable fruits

- 1 Very loose (spongy)
- 3 Loose (crumbly)
- 5 Medium compact
- 7 Compact
- 9 Very compact
- 99 Others (Specify in the 'Remarks' descriptor)

34. Number of fruit harvest

To be recorded as total number of fruit pickings

Quantitative

35. Fruit position

To be recorded at marketable stage

- 3 Pendant
- 5 Semi-pendant
- 7 Erect
- 99 Others (Specify in the 'Remarks' descriptor)

36. Fruit yield per plant (kg)

To be recorded as average of cumulative yield of all pickings on same 5-10 plants at marketable stage

Quantitative

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37. Fruit weight (g)
To be calculated on the basis of fruit yield and number of fruits per plant
Quantitative
38. Seediness
To be recorded on marketable fruits
- 3 Low
 - 5 Medium
 - 7 High
 - 99 Others (Specify in the 'Remarks' descriptor)
39. Seed colour
To be recorded on mature and dried seed
- 1 White
 - 2 Light yellow
 - 3 Grey yellow
 - 4 Brownish yellow
 - 5 Brown
 - 6 Brown black
 - 7 Black
 - 99 Others (Specify in the 'Remarks' descriptor)
40. Seed size
To be recorded on mature and dried seed
- 3 Small (~2 mm)
 - 5 Intermediate (~ 3 mm)
 - 7 Large (~4 mm)
 - 99 Others (Specify in the 'Remarks' descriptor)
41. 100 seed weight (g)
To be recorded at the time of marketable stage on random dried seeds
Quantitative
42. Biotic stress susceptibility
Specify the infestation or infection using any 1-9 scale.
Note: For Additional information as common name(s) of disease(s)/pest(s) and casual organism(s) may be appended in the Biotic notes descriptor.
- 1 Very low or no visible sign of susceptibility
 - 3 Low
 - 5 Intermediate
 - 7 High
 - 9 Very high



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Figure 22. Characterization of brinjal conserved accessions of brinjal

- 6.2.27 Identity is monitored by comparing the accession with previous passport or morphological data.
- 6.2.28 All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.
- 6.2.29 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.



Figure 23. Diversity in fruit shape, size, colour, colour distribution and curvature of brinjal

7.2.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).



7.2.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild brinjal genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.2.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild brinjal genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.2.1 Staff Training and Competency

Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.

Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.2.1 References

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10.2.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Aziz Zilani Chowdhury

CHAPTER 04

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Okra Germplasm in Bangladesh

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Okra genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Okra genetic resources management.

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6.3 Procedure for Okra

The regeneration and characterization procedure of okra is initiated when the scientists handling the conservation unit of PGRC, BARI genebank review the data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of okra accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.3.1 Database review: Review of data and pertinent information on okra germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.3.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<65%) and inadequate amount of seeds (below 1500) or have not yet been characterized is generated for regeneration/characterization of okra accessions in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

- Each of these lists should include the following information:
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Germination (%) of last seed viability test
 - Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
 - Whether characterized or not
 - Pedigree history (only for the breeding and genetic stocks material)
 - Remarks (eg. strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged)

6.3.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is to be reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.
- When monitoring procedure ascertains for a specific seed stock that viability in LTS is below international threshold value, then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.



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- Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 40 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
- Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
- Seeds are placed in paper craft foils and identified with the following formation:
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Country of origin
 - Number of seeds
- Seeds are treated with appropriate fungicides to control seed borne disease.
- Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the sowing date.

6.3.4 Distribution of responsibilities: As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.

6.3.5 Preparation of experimental register: Experimental register is prepared by responsible scientist enlisting all operations to be done and all information to be recorded from land preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.

6.3.6 Experimental design: Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.

6.3.7 Plot dimension: 4.0 m × 3.0 m plots having 100 cm wide and 15-20 cm deep drain in between two plots.

6.3.8 Field layout

- Prepare field layout hard copy according to experimental design for experimental field following seed sample preparation.
- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.



6.3.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated and wild okra germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and pests.

- The land should be free of volunteer plants and objectionable weeds. Select the land on which okra was not grown in the previous season. This is necessary to avoid soil borne diseases especially root knot nematode. The soil of the selected field should be fertile, rich in organic matter, sandy to clay in texture and well drained having pH between 6.0 to 6.8 (Rashid and Singh, 2000).
- Early enough before seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the sowing date to remove the weeds especially perennial weeds.
- Prepare the land to a fine tilth by ploughing, three to four harrowing followed by leveling one or two days before sowing.

6.3.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for experimental field early enough before seed sowing. Information printed on the tag includes the block number, plot number and accession /collector's number.

6.3.11 Manure and Fertilizer doses: 90-30-60-15-2.0-1.4 kg/ha N-P-K-S-Zn-B and FYM @ 3 t/ha (Ahmed *et al.*, 2018).

Method of application

- a) All of FYM, P, K, S, Zn and B; and one-fourth of N should be applied as basal during final land preparation.
- b) Remaining N should be applied in three equal splits at 20, 40 and 60 days after sowing under moist soil condition and mixed thoroughly with the soil as soon as possible for better utilization.

6.3.12 Seed sowing (eg. sowing rate, method, etc.) and labelling

- Time of seed sowing: Seeds should be sown during mid-June to mid-July and mid-February to mid-March for regeneration (Rashid and Singh, 2000) and 15 April to 15 May for characterization.
- Spacing: Row to row: 60 cm; Plant to plant: 40 cm for April to July sowing and 50 × 25 cm for February to March sowing; plot to plot: 100 cm.
- Seed soaking: Seeds should be soaked in clean water for 24 before sowing. Seeds which will not absorb water during imbibition should be discarded. Seeds are placed into cloth bags labelled with water proof thermal plastic tags, firmly tightened outer of the bag and soaked into clean water in plastic bowl (Rashid *et al.*, 2006 and BARI, 2019).
- Placing the soaked seeds to experimental field: Withdraw the cloth bags from water and allow extra water to dropout and partially dry. Bring the cloth bags with soaked seeds from the field laboratory to the experimental field and place to respective field plot according to field layout.



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- No. of plants/hole: Sow 2-3 seeds at 40 cm distances of 60 cm apart rows to a depth of 2.0 to 2.5 centimeter. Keep the cloth bags in respective field plot for final checking.
- Thinning: Allow one healthy seedling to grow per hole and remove extra ones (if there are more than one seedling per hole) at 4-5 leaf stage.
- Immediately after sowing of seed a final check is to be done for correspondence to the layout and any planting error is to be noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed (ICARDA, 2021).

6.3.13 Labelling

- Once seed sowing in the main experimental field is finished, each individual field plot is labelled with a water-proof thermal plastic tag. Information printed on the tag includes block number, plot number and accession /collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of transplant, number of accessions, number of checks, experimental design, etc.).

6.3.14 Isolation requirement: Okra is practically self- and cross-pollinated (often cross-pollinated). Cross pollination is done mainly by insects. The extent of cross pollination varies from 4 to 19%. Isolation of seed field is necessary for production of pure seed. The plot of one genotype should be isolated from the plots of other genotypes by 40 mesh polyvinyl net at flowering stage.

6.3.15 Weed management: The field should always be kept free of weeds.

- Three to four hand weeding may be necessary during crop period to keep the field clean of weeds.
- Mulching are also to be done following irrigation to keep the soil loose and to conserve soil moisture.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.3.16 Irrigation and drainage

- Overhead light irrigation with water can 3-4 days after sowing is essential for facilitating germination.
- Light irrigation is to be applied as and when necessary.
- Excess irrigation/rain water is to be drained out sharply.

6.3.17 Roguing: Roguing of the seed crop should be begin with uprooting and destroying of yellow vein mosaic affected plants soon after they are noticed. This should be continued up to three fruit stage. Sub-sequent roguing of off-type plants should be done prior to flowering. This should continue during the flowering and fruiting stage also (Agrawal, 1999).



6.3.18 Removal of initial fruit: Initial two to three fruits should be removed just after setting to promote vegetative growth and enhancing fruit as well as seed yield.

6.3.19 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology divisions of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Okra yellow vein mosaic virus disease (OYVMV) is controlled mainly through vector (whitefly) control and roguing out of infected plants immediately to prevent contamination of healthy plants.

6.3.20 Harvesting

- Prior to harvest several extra plastic tags same with the ones used for labeling the field plots are printed for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labelled with plot number and accession number.
- The fruits should be harvested periodically when they have dried (about 35 days old). Genotypes with angular fruits, which open along sutures, should be harvested promptly to avoid shattering and damaging of seeds due to entry of rain water (Rashid and Singh, 2000).
- The pedicel of dried fruits are to be cut by scissors, and placed carefully into the respective labelled plastic sacks. Respective extra tag is firmly tightened outer of the sack. The sacks are then tightened and brought to the field laboratory.
- Three to four pickings may be required to complete harvesting.
- Harvesting of fruits should not be done during rainy days, which may cause severe deterioration of germination rate of seeds.
- Immature fruits are harvested at marketable stage (7-8 days after anthesis) for recording fruit descriptors, yield components and yield. For recording seed descriptors mature and dried fruits are harvested.

6.3.21 Seed threshing and drying

- Harvested fruits are dried and accumulated in labelled plastic sacks. Seeds are threshed at the end of ripe fruit harvesting.
- Threshing is done by flailing seed by hand after the fruits are sufficiently dry, and kept in the respective labeled plastic sacks. Light seeds are then removed by cleaning and winnowing. Care should be taken to avoid mechanical admixture. Seeds should be dried to at least 10% moisture content before sending to conservation unit.

6.3.22 Data validation and uploading onto PGRC, BARI genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:



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Version: 1.0

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A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Number of rows per plot and seeding rate per plot
6. Row length, Row width, Plot to plot distance

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe drought, hail storm, severe storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. 100 seed weight (g)
8. Total seed weight per plot (g)

C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Plant growth habit
2. Branching habit
3. Immature fruit colour
4. Number of ridges per fruit

Note:

1. The regeneration procedure for okra accessions in Bangladesh is considered finalized when dried seeds have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).



6.3.23 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

➤ Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors (For Characterization and Evaluation) of Agri-Horticultural Crops (Part I), National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated okra (*Abeimoschus esculentus*)].

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:

6.3.24 Descriptor List

Date of sowing: Date of Transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Early plant vigour
To be recorded after 25 days of sowing
 - 1 Poor
 - 2 Good
 - 3 Very good



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7. Plant growth habit
To be recorded at completion of vegetative stage
 - 1 Erect
 - 2 Medium
 - 3 Procumbent
 - 99 Others (Specify in the "Remarks" descriptor)
8. Branching habit
To be recorded at full foliage stage
 - 1 Low
 - 2 Profuse
 - 99 Others (Specify in the "Remarks" descriptor)
9. Days to 50% flowering
To be recorded as the number of days from date of sowing to the day when 50% plants flowered in a row
Quantitative
10. Number of epicalyx segments
To be recorded at the flowering stage
 - 1 From 5-7
 - 2 From 8-10
 - 3 More than 10
 - 99 Others (Specify in the "Remarks" descriptor)
11. Shape of epicalyx segments
To be recorded at the flowering stage
 - 1 Linear
 - 2 Lanceolate
 - 3 Triangular
 - 99 Others (Specify in the "Remarks" descriptor)
12. First flowering node
To be recorded, at flowering stage
Quantitative
13. First fruit producing node
To be recorded at maturity stage
Quantitative
14. Immature fruit colour
To be recorded at fruiting stage, when fruits are tender and marketable
 - 1 Yellowish green
 - 2 Green
 - 3 Dark green
 - 4 Red
 - 5 Dark red
 - 99 Others (Specify in the "Remarks" descriptor)

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15. Fruit length (cm)
To be recorded at fruiting stage, when fruits attained full length, still tender and marketable
Quantitative
16. Fruit width (cm)
To be recorded at maturity stage
Quantitative
17. Number of fruits per plant
To be recorded during full range of harvesting (add all picking) up to near maturity stage
Quantitative
18. Number of ridges per fruit
To be recorded at near maturity stage
 - 1 None
 - 2 From 5 to 7
 - 3 From 8 to 10
 - 4 More than 10
 - 99 Others (Specify in the "Remarks" descriptor)
19. Fruit pubescence
To be recorded at fully grown green fruit stage
 - 3 Downy
 - 5 Slightly rough
 - 7 Prickly
 - 99 Others (Specify in the "Remarks" descriptor)
20. Plant height (m)
To be measured from ground level to tip of the main shoot (average of 5 plants).
Quantitative
21. Days to 80% maturity
To be recorded from the date of sowing to the date when 80% plants have complete mature fruits in a row
Quantitative
22. Mature fruit colour
To be recorded at near maturity stage
 - 1 Yellowish green
 - 2 Green
 - 3 Green with red patches
 - 4 Dark green
 - 5 Dark red
 - 99 Others (Specify in the "Remarks" descriptor)



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23. Seed shape
To be recorded after harvesting of the crop
 - 1 Round
 - 2 Reniform
 - 99 Others (Specify in the "Remarks" descriptor)
24. Number of seeds per fruit
To be recorded at maturity stage
Quantitative
25. 100 seed weight (g)
To be measured as weight of hundred random seeds in grams (average of 10 random plants)
Quantitative
26. Yield per plant (g)
To be recorded as average of 10 random plants, under full span of picking by adding weight of green and tender fruits
Quantitative
27. Biotic Stress Susceptibility (Specify the infestation or infection using any 1-9 scale)
 - 1 Very low or no visible sign of susceptibility
 - 3 Low
 - 5 Intermediate
 - 7 High
 - 9 Very high



Figure 24. Characterization of okra germplasm

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6.3.25 Identity is monitored by comparing the accession with previous passport or morphological data.

6.3.26 All characterization information is to be verified by the head of the conservation unit of PGRC, BARI and the respective genebank manager.

6.3.27 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to the PGRC's Genebank Documentation System.

7.3.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.3.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild okra genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.3.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild okra genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV



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8.3.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.3.1 References

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10.3.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Abdus Salam

CHAPTER 05

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Ridge Gourd Germplasm in Bangladesh

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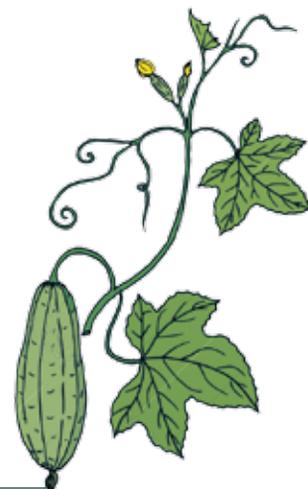
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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Ridge Gourd Genetic Resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Ridge Gourd genetic resources management.

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6.4 Procedure for Ridge Gourd

The regeneration and characterization procedure is initiated when the head of conservation unit of PGRC, BARI genebank review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of ridge gourd accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.4.1 Database review: Review of data and pertinent information on ridge gourd germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.4.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<80% of the initial viability of the stored seeds for cultivated ridge gourd germplasm or <60% of the initial viability of the stored seeds for its wild relatives' germplasm) and inadequate amount of seeds (below 1500 seeds) or have not yet been characterized is generated for regeneration/characterization in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

Each of these lists should include the following information:

- Accession number
- Full taxon name (Genus, Species, Sub-species)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged) (ICARDA, 2021).

6.4.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS (ICARDA, 2021).



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- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.
 - When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value (<80%), then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 10 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
 - Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Country of origin
 - Number of seeds
 - Seeds are treated with appropriate fungicides to control seed borne disease.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the planting date.
- 6.4.4 Distribution of responsibilities:** As per generated list, Head (CSO) of PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.
- 6.4.5 Preparation of experimental register:** Experimental register is prepared enlisting all operations to be done and all information to be recorded from seedbed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.4.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.4.7 Plot dimension:** 8 m × 4 m plots having 100 cm wide and 15-20 cm deep drain in between two plots. Eight pits of 50 cm diameter and 50 cm depth are to be prepared per plot at 2 m × 2 m spacing at 7-10 days before transplanting of seedlings.
- 6.4.8 Field layout**
- Prepare field layout hard copy according to experimental design for seedbed and main field following seed sample preparation.



- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.4.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated and wild ridge gourd germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and pests.

- Selection of site for nursery: Ridge gourd seedlings are raised in 8 cm × 10 cm polythene bags. Polybags are arranged on well prepared land, which is free of objectionable weeds and volunteer plants, receive sunshine all the day, and well drained. Ridge gourd seed are also sown directly in pits of the main field. In such case, land for polybag arrangement is not required (Halim *et al.*, 2006 and BARI. 2019).
- Selection of land for crop production: The land should be free of volunteer plants and objectionable weeds. Select the land on which ridge gourd or other cucurbitaceous vegetables were not grown in the previous season. This is necessary to avoid soil borne diseases especially root knot nematode. The soil of the selected field should be fertile, rich in organic matter, sandy loam in texture and well drained.
- Early enough before planting of seedling/seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the planting date to remove the weeds especially perennial weeds (Agrawal, 1999).

6.4.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for seedbed and main experimental field. Information printed on the tag includes the bed section/plot number and accession /collector's number.

6.4.11 Seed sowing (eg. sowing rate, method, etc.) and labeling

- Preparation of polybag and nursery plot: 8 cm × 10 cm poly-bags are used. The perforated polybag should be filled with a mixture 50% soil and 50% compost/FYM (Halim *et al.*, 2006). The land of nursery plot is prepared to a fine tilth by deep ploughing, three to four harrowing followed by leveling. Nursery plot is divided into several sections as per requirement and labeled with water-proof thermal plastic tags. Information printed on the tag includes bed section number and accession /collector's number. Required number of filled up polybags are arranged in each section burying one-third of the bag under soil.



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- Time of Seed Sowing: Optimum time for seed sowing is 15 March to 15 April. Two seeds are to be sown in each 8 cm × 10 cm poly-bag filled with 50% soil and 50% compost (Halim *et al.*, 2006 and BARI. 2019).
- Seed soaking: Twenty four hours seed soaking in clean water before sowing accelerates germination. Seeds are placed into cloth bags labeled with water proof thermal plastic tags, and soaked into clean water in plastic bowl.
- Method of sowing: Soaked seeds are placed in respective section of nursery bed and two seeds are dibbled per polybag at a depth of 1.5 to 2.0 cm. In case of direct sowing in the main experimental field, soaked seeds are placed in respective field plots, and two to three are seeds dibbled at a depth of 2.0 to 2.5 cm in each of well-prepared pits.
- Immediately after sowing of seed a final check is done for correspondence to the trial layout and any planting error is to be noted on the hard copy.

6.4.12 Preparation of land and pit

- Prepare the land to a fine tilth by deep ploughing, three to four harrowing followed by leveling.
- The plots are raised by 15-20 cm lifting the soil from 100 cm channel kept around each plot.
- Pits of 50 cm diameter and 50 cm depth are to be prepared at 2 m × 2 m spacing, and recommended manure and fertilizers are applied at 7-10 days before transplanting of seedlings (Halim *et al.*, 2006 and BARI. 2019).

6.4.13 Manure and Fertilizer doses: 75-36-60-21-2.0-1.4 kg/ha N-P-K-S-Zn-B and FYM @ 4 t/ha (Ahmed *et al.*, 2018).

Method of application

- a) All of FYM, P, K, S, Zn and B should be applied in pits 7-10 days before transplanting and mixed thoroughly with the soil.
- b) N should be applied around the plant as side dressing at 15, 35, 55 and 75 DAT under moist soil condition and mixed thoroughly with the soil as soon as possible for better utilization.

6.4.14 Transplanting of seedling

- Age of seedling: 16-17 days after germination
- Spacing: Row to row- 200 cm; plant to plant- 200 cm; plot to plot- 100 cm (Halim *et al.*, 2006 and BARI, 2019)
- Placing the seedling to experimental field: Place the seedlings from the nursery bed to labeled plastic crates/trays, bring the plastic crates/trays to the experimental field and place to respective field plot according to field layout.
- No. of seedlings/pit: Plant seedling(s) of one polybag per pit and irrigate by water can immediately afterwards. Allow one healthy seedling to grow per pit and remove extra ones (if there are more than one seedling per bag) one week after transplanting.
- Keep the plastic crates/trays in respective field plots for final checking.



- Immediately after transplanting of seedlings a final check is to be done for correspondence to the field trial layout and any planting error is to be noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.4.15 Labeling:

- Once transplanting in the main plot is finished, each individual field plot is labelled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and the accession /collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e. title of the experiment, date of sowing, date of transplanting, number of accessions, number of checks, experimental design, implementing centre, etc.).

6.4.16 Trellising: Vine crops like ridge gourd plants are allowed to grow on trellis made of bamboo sticks or other materials.

6.4.17 Weeding: The field should always be kept free of weeds.

- Three to four hand weeding at the time of side dressing of fertilizers may be required. Mulching are also to be done following irrigation to keep the soil loose and to conserve soil moisture.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.4.18 Irrigation and drainage:

- Overhead light irrigation with water can might be required in direct seeded pits 4-5 days after sowing to facilitate germination.
- Necessary irrigation water is to be entered in the drain instead of flooding the whole plot as per requirement. Excess irrigation/rain water is to be drained out sharply.

6.4.19 Desuckering: All the branches up to 40-45 cm of the base of the plant are to be pruned out (Halim *et al.*, 2006).

6.4.20 Roguing: Off-type plants are usually detectable fairly early in cucurbits. The early shape of fruits and even the shape of the ovary at flowering sometime reveal off-type plants. Although some damage may already result from cross-pollination, such off-type plants should be immediately rogued out (Agrawal, 1999).

6.4.21 Control pollination

- Hand pollination in the afternoon time promotes fruit set as well as yield.
- For pure seed production, both male and female flowers are covered with butter paper bags before anthesis.
- Distinguish covered female flower from male flower by cross (X) mark with a bold marker pen (preferably red ink) on the bag
- Dusting of pollen is done by shaking the male flower over the stigma or rubbing the anthers with the stigma. The pollinated female flowers are kept covered with the same butter paper bags for two to three days.



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Figure 25. Covering of male and female flowers of ridge gourd with butter paper bags and hand pollination

6.4.22 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology division of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.

6.4.23 Harvesting, seed threshing and drying

- Prior to harvest several extra plastic tags same with the ones used for labeling the field plots are printed for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with the plot number and accession number.
- Fully ripe fruits (this stage is determined visually from the drying of the fruit skin and fruits rattle when shaken) of each accession are harvested periodically by hand and placed into the respective labeled plastic sacks. Respective extra tag is firmly tightened outer of the sack.
- The sacks are then brought to the field laboratory. Harvested fruits in plastic sacks are allowed to dry for two to three days and accumulated in labeled plastic sacks.
- Seeds are threshed in sunny days at the end of harvesting. Fully dried fruits are cut longitudinally and seeds are threshed out by flailing.
- Threshed seeds are cleaned and dried to moisture content of 10-12% before sending to conservation unit.
- Immature fruits are harvested at marketable stage (8-10 days after anthesis) for recording fruit descriptors, yield components and yield. For recording seed descriptors mature and dried fruits are to be harvested.

6.4.24 Data validation and uploading on PGRC, BARI Genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:



A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Date of transplanting
6. Number of rows per plot
7. Row length, Row width, Plot to plot distance

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe storm, hail storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. 100 seed weight (g)
8. Total seed weight per plot (g)

C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Plant growth habit
2. Fruit shape
3. Fruit skin colour
4. Fruit ridge (rib) shape

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for Ridge gourd accessions in Bangladesh is considered finalized when extracted and dried seeds have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).



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6.4.25 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

➤ Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors of Agri-Horticultural Crops, Part II: Vegetable Crops, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated ridge gourd (*Luffa accutangula*)].

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:

6.4.26 Descriptor List

Date of sowing: Date of Transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Early plant vigour
To be recorded after 30 days of sowing
 - 3 Poor
 - 5 Good
 - 7 Very good
 - 99 Others (Specify in the 'Remarks' descriptor)
7. Plant growth habit
To be recorded on fully grown plant
 - 3 Short viny
 - 5 Medium viny
 - 7 Long viny
 - 99 Others (Specify in the 'Remarks' descriptor)

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8. Stem pubescence

To be recorded at peak fruiting stage

- 1 Smooth
- 2 Pubescence
- 99 Others (Specify in the 'Remarks' descriptor)

9. Stem shape

To be recorded at peak fruiting stage (as recorded from the cross section)

- 1 Rounded
- 2 Angular
- 99 Others (Specify in the 'Remarks' descriptor)

10. Tendril

To be recorded at flowering/full blossom stage

- 0 Absent
- 1 Present

11. Tendril type

To be recorded at flowering stage

- 1 Coiled
- 2 Straight
- 99 Others (Specify in the 'Remarks' descriptor)

12. Tendril branching

To be recorded at flowering stage

- 1 Unbranched
- 2 Branched
- 99 Others (Specify in the 'Remarks' descriptor)

13. Leaf margin

To be recorded at full foliage stage

- 1 Entire
- 2 Serrate
- 3 Multifid
- 99 Others (Specify in the 'Remarks' descriptor)

14. Leaf shape

To be recorded at full foliage stage

- 1 Cordate
- 2 Oblong
- 3 Ovate
- 4 Obovate
- 5 Orbicular
- 99 Others (Specify in the 'Remarks' descriptor)



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15. Leaf size

To be recorded at full foliage stage

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the 'Remarks' descriptor)

16. Leaf pubescence nature

To be recorded at full foliage stage

- 3 Soft
- 5 Intermediate
- 7 Hard
- 99 Others (Specify in the 'Remarks' descriptor)

17. Leaf pubescence density

To be recorded at full foliage stage

- 0 No hairs
- 3 Sparse
- 5 Intermediate
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)

18. Petiole length (cm)

To be recorded as average of 5-10 random leaves in the middle region of the vine at full foliage stage

Quantitative

19. Node number at which first female flower appears

To be recorded at first appearance of female flower

Quantitative

20. Days to 50% flowering

To be recorded as number of days from sowing/transplanting date to the date when at least 50% of the plants show first female flower open

Quantitative

21. Sex type

To be recorded at flowering/full blossom stage

- 1 Monoecious (male and female flowers on same plant)
- 2 Gynomonoecious (female and hermaphrodite flowers on the same plant)
- 3 Andromonoecious (male and hermaphrodite flowers on the same plants)
- 4 Androgynomonoecious (male, female and hermaphrodite flowers on the same plants)
- 5 Hermaphrodite (male and female on same flower and on same plant)
- 6 Androecious (only male flower on the plant)
- 99 Others (Specify in the 'Remarks' descriptor)



22. Sex ratio
To be recorded as ratio of female (including hermaphrodite) to male flowers at flowering stage
Quantitative
23. Peduncle length (cm)
To be recorded as average of 5-10 random fruits at marketable stage
Quantitative
24. Peduncle shape
To be recorded at marketable stage
- 3 Round
 - 5 Smoothly angled
 - 7 Sharply angular
 - 99 Others (Specify in the 'Remarks' descriptor)
25. Peduncle attachment
To be recorded at marketable stage
- 1 Hard, not flared
 - 2 Hard and flared
 - 3 Soft, not flared
 - 4 Soft and flared
 - 99 Others (Specify in the 'Remarks' descriptor)
26. Fruit shape
To be recorded at fruiting stage
- 1 Cylindrical
 - 2 Club shaped
 - 3 Spindle shape
 - 4 Elliptical
 - 5 Globular
 - 6 Elongate
 - 7 Heart shaped
 - 99 Others (Specify in the 'Remarks' descriptor)
27. Fruit skin colour
To be recorded at marketable stage
- 3 Light green
 - 5 Green
 - 7 Dark green
 - 99 Others (Specify in the 'Remarks' descriptor)
28. Fruit skin lustre
To be recorded at marketable stage
- 3 Matt
 - 5 Intermediate
 - 7 Glossy
 - 99 Others (Specify in the 'Remarks' descriptor)



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29. Skin hardness of the fruit

To be recorded at marketable stage

- 3 Soft
- 5 Intermediate
- 7 Hard
- 99 Others (Specify in the 'Remarks' descriptor)

30. Fruit ridge (rib) shape

To be recorded in cross section at marketable stage

- 1 Superficial
- 2 Rounded/Grooved
- 3 Intermediate
- 4 Deep grooved
- 5 Narrowly winged
- 6 Deeply winged
- 99 Others (Specify in the 'Remarks' descriptor)

31. Continuity of ridge

To be recorded on fruits at marketable stage

- 1 Continuous
- 2 Discrete
- 99 Others (Specify in the 'Remarks' descriptor)

32. Number of ridges (ribs) per fruit

To be recorded at marketable stage

Quantitative

33. Flesh texture

To be recorded at marketable stage

- 1 Smooth
- 2 Soft/spongy
- 3 Fibrous-gelatinous
- 4 Fibrous dry
- 5 Grainy
- 99 Others (Specify in the 'Remarks' descriptor)

34. Number of primary branches

To be recorded as average of same 10 plants at the end of flowering stage (the branch that arises from the main vine/stem is known as primary branch)

Quantitative

35. Days to first fruit harvest

To be recorded as number of days from date of sowing/transplanting to the date of first marketable fruit harvest

Quantitative

36. Days to last fruit harvest

To be recorded as number of days from date of sowing/transplanting to the date of last marketable fruit harvest

Quantitative



37. Number of fruits per plant
To be recorded as total number of fruits in each picking
Quantitative
38. Yield of marketable fruits per plant (g)
To be recorded as average of cumulative yield of all pickings in same 10 plants
Quantitative
39. Fruit weight (g)
To be calculated on the basis of fruit yield and number of fruits per plant
Quantitative
40. Fruit length (cm)
To be recorded as average of 5-10 random fruits at marketable stage
Quantitative
41. Fruit width (cm)
To be recorded as average of same 5-10 fruits at marketable stage
Quantitative
42. Seediness
To be recorded at marketable stage
- 3 Low
 - 5 Medium
 - 7 High
 - 99 Others (Specify in the 'Remarks' descriptor)
43. Seed lustre
To be recorded on mature and dried seeds
- 3 Matt
 - 5 Intermediate
 - 7 Glossy
 - 99 Others (Specify in the 'Remarks' descriptor)
44. Number of seeds per fruit
To be recorded as average of 5-10 random mature fruits
Quantitative
45. 100 seed weight (g)
To be measured as average weight of 100 random dry seeds
Quantitative
46. Biotic stress susceptibility
Specify the infestation or infection using any 1-9 scale
- 1 Very low or no visible sign of susceptibility
 - 3 Low
 - 5 Intermediate
 - 7 High
 - 9 Very high

- 6.4.27 Identity is monitored by comparing the accession with previous passport or morphological data.
- 6.4.28 All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.
- 6.4.29 Characterization data is to be uploaded on the PGRC, BARI Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.



Figure 26. Characterization of ridge gourd germplasm

7.4.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.4.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild ridge gourd genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.4.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild ridge gourd genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA

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- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.4.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.4.1 References

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Srivastava, Umesh, Mahajan RK, Gangopadhyay KK, Mahendra Singh, Dhillon BS. 2001. Minimal Descriptors of Agri-Horticultural Crops. Part II: Vegetable Crops. National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, ix + 262 p.

10.4.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Abdus Salam

CHAPTER 06

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Chilli Germplasm in Bangladesh

Compiled and Edited by

Dr. Md. Abdus Salam
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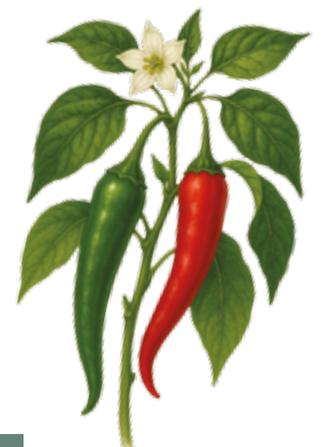
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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Chilli genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Chilli genetic resources management.

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6.5 Procedure for Chilli

The regeneration and characterization procedure is to be initiated when the scientists handling the conservation unit of PGRC, BARI genebank review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of chilli accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.5.1 Database review: Review of data and pertinent information on chilli germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.5.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<70% of the initial viability of the stored seeds for cultivated chilli germplasm) and inadequate amount of seeds (below 1500 seeds) or have not yet been characterized is generated for regeneration/characterization in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

Each of these lists should include the following information:

- Accession number/Collector's number
- Full taxon name (Genus, Species, Sub-species)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly winter types, strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged).

6.5.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



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- When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value (<1,500 seeds), then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 50 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
 - Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Country of origin
 - Number of seeds
 - Seeds are treated with appropriate fungicides to control seed borne disease.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the planting date (ICARDA, 2021).
- 6.5.4 Distribution of responsibilities:** As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species for BARI genebank, respectively.
- 6.5.5 Preparation of experimental register:** Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.5.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.5.7 Plot dimension:** 4.8 m × 3.0 m plot having 100 cm wide and 15-20 cm deep drain in between two beds. A total of 60 seedlings are to be planted per plot at 60 cm × 40 cm spacing.
- 6.5.8 Field layout**
- Prepare field layout hard copy according to experimental design for seedbed and main field following seed sample preparation.



- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or random table.

6.5.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated chilli germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Chilli is very sensitive to biotic and abiotic stresses. Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and pests; water logging/inundation history of the field is also to be considered.

- Selection of nursery bed: Select nursery bed (pre-constructed) on which chilli or other solanaceous vegetables seedlings were not grown in the previous season. This is necessary to avoid soil borne diseases especially bacterial wilt as well as to avoid varietal admixture due to volunteer plants in the nursery itself.
- Selection of land for crop production: The land should be free of volunteer plants and objectionable weeds. Select the land on which chilli or other solanaceous vegetables were not grown in the previous season. This is necessary to avoid soil borne diseases especially bacterial wilt and varietal admixture due to volunteer plants. The soil of the selected field should be fertile, rich in organic matter, sandy loam in texture, free of inundation and well drained (Rashid and Singh, 2000).
- Early enough before planting, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the planting date to remove the weeds (Agrawal, 1999).

6.5.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for seed bed and main experimental field. Information printed on the tag includes the bed section/plot number and accession /collector's number.

6.5.11 Seed sowing (eg. sowing rate, method, etc.) and labelling

- Preparation of seed bed: Seed bed should be well prepared mixing sand, soil and FYM in equal ratio (Rashid *et al.*, 2006 and BARI, 2019). Each bed is divided into several sections; each section is labelled with water-proof thermal plastic tag. Information printed on the tag includes plot number, accession/ collector's number.
- Time of Seed Sowing: In Bangladesh chillis are grown round the year, but for seed crop chillis are to be grown during winter. The best time for sowing of seed is from 1st week of September to 15 October (Rashid and Singh, 2000). For summer crop seeds are sown between 15 March and 15 April.
- Method of seed sowing: Seed samples are placed in respective sections of the seed bed. Seeds are sown densely in one row of each section at a depth of 1.0-1.5 cm; 10-12 days after germination, the young seedlings are transferred to adjacent rows at 10 cm row to row and 5 cm plant to plant spacing to have uniform, healthy, strong and stout seedlings.



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- Immediately after sowing, final check should be done for correspondence to the trial layout and any planting error is noted on the hard copy.

6.5.12 Preparation of land and pit

- Prepare the land to a fine tilth by deep ploughing, three to four harrowing followed by leveling.
- The plots are raised by 15-20 cm lifting the soil from 100 cm channel kept around each plot.
- Pits of 15 cm diameter and 15 cm depth are to be prepared at 60 cm × 40 cm spacing and recommended manure and fertilizers are applied at 7-10 days before transplanting of seedlings.

6.5.13 Manure and Fertilizer doses: 96-45-75-15-1.5-1.4 kg/ha N-P-K-S-Zn-B and FYM @ 3 t/ha (Ahmed *et al.*, 2018).

Method of application

- All of P, K, S, Zn and B; and half of FYM and N should be applied as basal during final land preparation.
- Remaining FYM should be applied in pit 7-10 days before transplanting of seedlings and mixed thoroughly with the soil.
- Remaining N should be applied in three equal splits at 25, 50 and 70 DAT under moist soil condition around the plant as side dressing and mixed thoroughly with the soil as soon as possible for better utilization.

6.5.14 Transplanting of seedlings

- Age of seedling: 30-35 days in both seasons
- Spacing: Row to row- 60 cm; plant to plant- 40 cm; plot to plot- 100 cm
- Lifting of seedling: Apply light irrigation with water can before lifting of seedling from seedbed. Lift the seedling carefully with some mud soil attached to the roots and make bundle carefully with soft rope.
- Bringing the seedling to experimental field: Label the seedling bundle with waterproof thermal plastic tag. Information printed on the tag includes plot number, accession/collector's number. Bring the labeled seedling bundle to the experimental field and place them to respective field plots.
- No. of seedlings/pit: Plant one healthy seedling per pit. Gap filling may be done immediately after death of any seedling. Transplant the seedling at evening time. Irrigate immediately afterwards. Keep the label of seedling bundles in respective field plots for final checking.
- Immediately after transplanting a final check is to be done for correspondence to the field trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.5.15 Labeling

- Once transplanting in the main plot is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, number of accessions, number of checks, experimental design, date of sowing, date of transplanting, etc.).

6.5.16 Isolation requirement: Chilli is partially self- and cross-pollinated (often cross-pollinated), but self-pollination is more common. Cross pollination is done mainly by insects. The extent of cross pollination up to 68% in India (Rashid and Singh, 2000). Isolation of seed field is necessary for production of pure seed. The plot of one genotype should be isolated from the plots of other genotypes by 40-60 mesh polyvinyl net before flowering stage (one net for each unit plot).



Figure 27. Application of polyvinyl net for isolation of one genotype from another to protect cross pollination

6.5.17 Weeding and mulching: The field should always be kept free of weeds. Three to four weeding at 20 days interval may be required. Mulching is also to be done following irrigation to keep the soil loose. All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.5.18 Irrigation and drainage: Light irrigation with water can for 3-4 days after transplanting is essential. The uniform soil moisture is essential to blossom and prevent fruit drop. Generally 8-9 irrigations are given, depending upon rainfall, soil type, humidity and prevailing temperature. Enter irrigation water into the drain and allow it to stand until the soil around the plant is soaked; avoid flood irrigation. Excess irrigation/rain water is to be drained out sharply.



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6.5.19 Roguing: Three times field inspection at vegetative (before flowering), flowering (while the first fruit is still only partially developed) and fruit bearing stage (when each plant has several more or less mature fruits) is to be arranged for roguing. In addition to off-types, plants affected by leaf blight, anthracnose and virus diseases should be removed from the field as soon as they are noticed.

6.5.20 Insect pest and disease management: The crop should always be kept free of insect pests and diseases applying recommended management pesticides.

- Regular monitoring of insect pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology division of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Virus and mycoplasma are controlled mainly through vector control and roguing out of infected plants immediately as they are noticed to prevent contamination of healthy plants.

6.5.21 Harvesting

- Prior to harvest several extra plastic tags same with the ones used for labeling the field plots are printed for each accession, including information on plot number, accession number/collector's number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with plot number and accession/collector's number.
- Fully ripe fruits (this stage is determined visually from the turning of fruit colour into red; early harvest of immature fruits will affect germination) of each accession are harvested periodically by hand and placed into the respective labeled plastic sacks. Respective extra tag is firmly tightened outer of the sack.
- The sacks are then brought to field laboratory for drying.
- Green chillis are harvested at marketable stage for recording fruit descriptors, yield components and yield. For recording seed descriptors mature fruits are harvested.

6.5.22 Seed threshing and drying

- Harvested fruits are dried and accumulated in labeled plastic sacks. Seeds are threshed at the end of ripe fruit harvesting.
- Fruits are dried to make them brittle and crushed keeping in labeled plastic sacks. Seeds are separated from pulp and skin. After separation, seeds are dried in partial shade to moisture content of ten percent or below before sending to conservation unit. Care should be taken to avoid mechanical admixture.

6.5.23 Data validation and uploading on PGRC, BARI Genebank Documentation

System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:



A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Season
5. Date of sowing
6. Date of transplanting
7. Number of rows per plot and number of plants per plot
8. Row length, Row width, Plot to plot distance

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe drought, hail storm, severe storm, severe cold, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. 1000 seed weight (g)
8. Total seed weight per plot (g)

C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Plant growth habit
2. Leaf colour
3. Corolla colour
4. Mature (green chilli) fruit colour
5. Fruit shape

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for chilli accessions in Bangladesh is considered finalized when threshed and dried seeds have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.



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2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.5.24 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

- Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors of Agri-Horticultural Crops, Part II: Vegetable Crops, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated chilli (*Capsicum spp.*)]. Some characters are also recorded as per IPGRI, AVRDC and CATIE (1995) descriptor list.

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:

6.5.25 Descriptor List

Season Date of sowing: Date of Transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Life cycle
 - 1 Annual
 - 2 Biennial
 - 3 Perennial
7. Cotyledonous leaf colour
 - 1 Light green
 - 2 Green
 - 3 Dark green

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- 4 Light purple
 - 5 Purple
 - 6 Dark purple
 - 7 Variegated
 - 8 Yellow
 - 99 Others (Specify in the 'Remarks' descriptor)
8. Stem colour
To be recorded at full foliage stage
- 1 Green
 - 2 Green with purple stripes
 - 3 Purple
 - 99 Others (Specify in the 'Remarks' descriptor)
9. Plant height (cm)
To be recorded as average of 5-10 random plants when the first fruit in 50% of the plants began to ripe
Quantitative
10. Plant canopy width (cm)
To be recorded simultaneously with height
Quantitative
11. Plant growth habit
To be recorded at fruit maturity
- 3 Prostrate
 - 5 Intermediate
 - 7 Erect
 - 99 Others (Specify in the 'Remarks' descriptor)
12. Branching habit
To be recorded when plants have ceased its growth
- 3 Sparse
 - 5 Intermediate
 - 7 Dense
 - 99 Others (Specify in the 'Remarks' descriptor)
13. Leaf size
To be recorded at full foliage stage
- 3 Small
 - 5 Medium
 - 7 Large
 - 99 Others (Specify in the 'Remarks' descriptor)
14. Leaf shape
To be recorded at full foliage stage
- 1 Deltoid
 - 2 Ovate
 - 3 Lanceolate
 - 99 Others (Specify in the 'Remarks' descriptor)



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15. Lamina margin

To be recorded at full foliage stage

- 1 Entire
- 2 Undulate
- 3 Ciliate
- 99 Others (Specify in the 'Remarks' descriptor)

16. Leaf colour

To be recorded at full foliage stage

- 1 Yellow
- 2 Light green
- 3 Green
- 4 Dark green
- 5 Light purple
- 6 Purple
- 7 Variegated
- 99 Others (Specify in the 'Remarks' descriptor)



Figure. 28. Cotyledonous leaf colour, plant growth habit and leaf colour of chilli accessions

17. Leaf pubescence

To be observed on the youngest mature leaf

- 0 Absent
- 3 Sparse
- 5 Intermediate
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)

18. Pigmentation at node

To be observed on the youngest mature leaf

- 0 Absent
- 1 Present

19. Days to 50% flowering

To be recorded as number of days from date of transplanting to date when at least 50% plants show first flower open

Quantitative



20. Number of flowers per axil

To be observed as average of 5-10 random axils at flowering stage

- 1 One
- 2 Two
- 3 Three or more
- 99 Others (Specify in the 'Remarks' descriptor)

21. Flower position

To be recorded at anthesis

- 3 Pendant
- 5 Intermediate
- 7 Erect

22. Corolla colour

To be recorded immediately after blooming

- 1 White
- 2 Light yellow
- 3 Yellow
- 4 Yellow green
- 5 Purple with white base
- 6 white with purple base
- 7 White with purple margin
- 8 Purple
- 9 Others (Specify in the 'Remarks' descriptor)

23. Anther colour

To be observed immediately after blooming but before anthesis

- 1 White
- 2 Yellow
- 3 Pale blue
- 4 Blue
- 5 Bluish yellow
- 6 Purple
- 99 Others (Specify in the 'Remarks' descriptor)

24. Stigma exertion

In-relation to anthers at full anthesis

- 3 Inserted
- 5 Same level
- 7 Exerted

25. Male sterility

To be observed immediately after blooming

- 0 Absent
- 1 Present

26. Calyx margin

To be recorded on fully blossom stage

- 1 Entire
- 2 Intermediate
- 3 Dentate
- 99 Others (Specify in the 'Remarks' descriptor)



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27. Days to 50% fruiting

To be recorded as number of days from the date of transplanting to the date when at least 50% plants bear mature fruits at the first and second bifurcation

Quantitative

28. Mature fruit colour

To be recorded at mature fruit stage

- 1 White
- 2 Yellow
- 3 Green
- 4 Orange
- 5 Purple
- 6 Deep purple
- 7 Black
- 99 Others (Specify in the 'Remarks' descriptor)



Figure 29. Pedicel position and mature green fruit colour of chilli accessions

29. Ripe fruit colour

To be recorded on ripe fruits

- 1 White
- 2 Lemon yellow
- 3 Pale orange yellow
- 4 Orange yellow
- 5 Pale orange
- 6 Orange
- 7 Light red
- 8 Red
- 9 Dark red
- 10 Brown
- 11 Purple
- 12 Black
- 99 Others (Specify in the 'Remarks' descriptor)



30. Fruit shape
To be recorded at mature fruit stage
- 1 Long
 - 2 Very long
 - 3 Tapering
 - 4 Conical
 - 5 Oval
 - 99 Others (Specify in the 'Remarks' descriptor)
31. Fruit length (cm)
To be recorded as average of 5-10 random fruits
Quantitative
32. Fruit width (cm)
To be recorded as average of same 5-10 fruits
Quantitative
33. Fruit position
To be recorded at mature fruit stage
- 3 Pendant
 - 5 Semi pendant
 - 7 Erect
 - 99 Others (Specify in the 'Remarks' descriptor)
34. Adherence of calyx to fruit
To be recorded at mature fruit stage
- 3 Loose
 - 5 Semi hard
 - 7 Hard
 - 99 Others (Specify in the 'Remarks' descriptor)
35. Fruit pedicel length (cm)
To be recorded as average of same 5-10 fruits at marketable stage
Quantitative
36. Fruit shape at pedicel attachment
To be recorded at mature fruit stage
- 1 Acute
 - 2 Obtuse
 - 3 Truncate
 - 4 Cordate
 - 5 Lobate
 - 99 Others (Specify in the 'Remarks' descriptor)
37. Blossom-end fruit shape
To be recorded at mature fruit stage
- 1 Pointed
 - 2 Blunt
 - 3 Shrunken
 - 4 Shrunken & pointed
 - 99 Others (Specify in the 'Remarks' descriptor)



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38. Fruit surface

To be recorded at mature fruit stage

- 1 Smooth
- 2 Semi wrinkled
- 3 Wrinkled
- 99 Others (Specify in the 'Remarks' descriptor)

39. Placenta length (cm)

To be recorded as average of same 5-10 fruits at fully matured stage

Quantitative

40. Organoleptic test

To be recorded on fully matured fruits

- 3 Mild pungent
- 5 Intermediate
- 7 Pungent
- 9 Highly pungent
- 99 Others (Specify in the 'Remarks' descriptor)

41. Number of fruits per plant

To be recorded as average of same 5-10 plants

Quantitative

42. Fruit yield per plant (kg)

To be recorded as average of cumulative yield of all pickings at mature green fruit stage of same 5-10 plants

Quantitative

43. Fruit weight (g)

Average weight of 10 ripe fruits of second harvest

Quantitative

44. Seed colour

To be recorded at dry seed stage

- 1 Light yellow
- 2 Deep yellow
- 3 Brown
- 4 Black
- 99 Others (Specify in the 'Remarks' descriptor)

45. Seed surface

To be recorded after threshing and drying of seed

- 1 Smooth
- 2 Rough
- 3 Wrinkled

46. Seed size

To be recorded after threshing and drying of seed

- 3 Small
- 5 Intermediate
- 7 Large

47. Number of seeds per fruit

To be recorded as average number 5-10 random fruits at ripen stage

- 1 < 20
- 2 20-50
- 3 >50

48. 1000 seed weight (g)

To be recorded on dry seeds

Quantitative

49. Biotic stress susceptibility

Specify the infestation or infection using any 1-9 scale

- 1 Very low or no visible sign of susceptibility
- 3 Low
- 5 Intermediate
- 7 High
- 7 Very high



Figure 30. Some yield promising accessions of chilli



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6.5.26 Identity is monitored by comparing the accession with previous passport or morphological data.

6.5.27 All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.

6.5.28 Characterization data is to be uploaded on the PGRC, BARI Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.

7.5.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.5.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated chilli genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.5.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated chilli genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV



8.5.1 Staff Training and Competency

Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.

Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.5.1 References

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Srivastava, Umesh RK Mahajan KK, Gangopadhyay, Mahendra Singh, Dhillon BS. 2001. Minimal Descriptors of Agri-Horticultural Crops. Part II: Vegetable Crops. National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, ix + 262 p.

10.5.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Rezwan Molla

CHAPTER 07

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Tomato Germplasm in Bangladesh

Compiled and Edited by

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Tomato genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Tomato genetic resources management.

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6.6 Procedure for Tomato

The regeneration and characterization procedure is to be initiated when the scientists handling the conservation unit of PGRC, BARI genebank review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of tomato accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.6.1 Database review: Review of data and pertinent information on tomato germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.6.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<80%) and inadequate amount of seeds (below 1,500 seeds) or have not yet been characterized is generated for regeneration/characterization in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

Each of these lists should include the following information:

- Accession number/Collector's number
- Full taxon name (Genus, Species, Subspecies)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly winter types, wild types and pollination type requiring special seed pre-treatments are to be flagged)

6.6.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



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- When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value (<1,500 seeds), then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 30 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity (ICARDA, 2021).
 - Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Country of origin
 - Number of seeds
 - Seeds are treated with appropriate fungicides to control seed borne disease.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the MTS area and to be kept until the planting date.
- 6.6.4 Distribution of responsibilities:** As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.
- 6.6.5 Preparation of experimental register:** Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.6.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.6.7 Plot dimension:** 4.0 m × 2.4 m plots having 100 cm wide and 15-20 cm deep drain in between two plots. A total of 32 seedlings are to be planted per plot at 60 cm × 50 cm spacing.
- 6.6.8 Field layout**
- Prepare field layout hard copy according to experimental design for seed bed and main field following seed sample preparation.
 - Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.



- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.6.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated tomato germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Tomato is very sensitive to biotic and abiotic stresses. Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and insect pests; water logging/inundation history of the field is also to be considered.

- Selection of nursery bed: Select nursery bed (pre-constructed) on which tomato or other solanaceous vegetables seedlings were not grown in the previous season. This is necessary to avoid soil borne diseases especially bacterial wilt as well as to avoid varietal admixture due to volunteer plants in the nursery itself.
- Selection of land for crop production: The land should be free of volunteer plants and objectionable weeds. Select the land on which tomato or other solanaceous vegetables were not grown in the previous season. This is necessary to avoid soil borne diseases especially bacterial wilt and varietal admixture due to volunteer plants. The soil of the selected field should be fertile, rich in organic matter, sandy loam in texture, good water holding capacity, free of inundation and well drained (Rashid and Singh, 2000).
- Early enough before planting, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the planting date to remove the weeds (Agrawal, 1999).

6.6.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for seed bed and main experimental field. Information printed on the tag includes bed section/plot number and accession /collector's number.

6.6.11 Seed sowing (eg. sowing rate, method, etc.) and labeling

- Preparation of seed bed: Seed bed should be well prepared mixing sand, soil and FYM in equal ratio (Rashid *et al.*, 2006 and BARI, 2019). Each bed is divided into several sections; each section is labeled with water-proof thermal plastic tag. Information printed on the tag includes the trial code, plot number, accession/collector's number.
- Time of Seed Sowing: 15 September to 15 October (Rashid and Singh, 2000)
- Method of seed sowing: Seed samples are placed in respective sections of the seed bed. Seeds are sown densely in one row of each section at a depth of 1.0-1.5 cm; 8-10 days after germination, the young seedlings are transferred to adjacent rows at 10 cm row to row and 5 cm plant to plant spacing to have uniform, healthy, strong and stout seedlings.



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- Keep the seed sample packets in respective section of seed bed for final checking.
- Immediately after sowing seed, final check should be done for correspondence to the trial layout and any planting error is noted on the hard copy.
- Virus management: Each seed bed is covered with 40-60 mesh nylon/polyvinyl net to protect the seedlings from white fly (vector of leaf curl virus) infestation (Rashid *et al.*, 2006).
- Covering the seedlings with polythene shade: the seedlings are also covered with transparent polythene shade to protect them from excessive rain and scorching sunshine.

6.6.12 Preparation of land and pit

- Prepare the land to a fine tilth by deep ploughing, three to four harrowing followed by leveling.
- The plots are raised by 15-20 cm lifting the soil from 100 cm channel kept around each plot.
- Pits of 15 cm diameter and 15 cm depth are to be prepared at 100 cm × 60 cm spacing, and recommended manure and fertilizers are applied at 7-10 days before transplanting of seedlings.

6.6.13 Manure and Fertilizer doses: 120-45-60-21-2.0-1.0 kg/ha N-P-P-S-Zn-B and FYM @ 5 t/ha (Ahmed *et al.*, 2018).

Method of application

- a) Half of FYM and all of P, S, Zn and B should be applied as basal during final land preparation. Remaining FYM should be applied in pits 7-10 days before transplanting of seedlings and mixed thoroughly with the soil.
- b) N and K should be applied in two equal splits at 15 and 35 DAT around the plant as side dressing under moist soil condition and mixed thoroughly with the soil as soon as possible for better utilization.

6.6.14 Transplanting of seedlings

- Age of seedling: 30-35 days
- Lifting of seedling: Apply light irrigation with water can before lifting of seedling from seed bed. Lift the seedling carefully with some soil attached to the roots and make bundle carefully with soft rope.
- Bringing the seedling to experimental field: Label the seedling bundle with water-proof thermal plastic tag. Information printed on the tag includes plot number, accession/collector's number. Bring the labeled seedling bundle to the experimental field and place them to respective field plots.
- No. of seedlings/pit: Plant one healthy seedling per pit. Gap filling may be done immediately after death of any seedling.
- Spacing: Row to row: 60 cm; plant to plant: 50 cm; plot to plot: 100 cm
- Transplant the seedling in the late afternoon. Irrigate immediately afterwards.

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- Keep the label of seedling bundles in respective field plots for final checking.
- Immediately after transplanting a final check is to be done for correspondence to the field trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.6.15 Labeling

- Once transplanting in the main plot is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and the accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of transplant, number of accessions, number of checks, experimental design, etc.).

6.6.16 Staking and shoot pruning: The plants are to be staked with bamboo sticks to keep them upright. All the side shoots below the first flower except immediate below one are to be pruned out during weeding. Water suckers emerging from leaf axils are also to be removed (Rashid *et al.*, 2006 and BARI, 2019).

6.6.17 Isolation: Tomato is highly self-pollinated. To prevent varietal mixes and avoid cross pollination that may occur, especially where pollinators such as bees are present. Isolation net (preferably 40-60 mesh polyvinyl net) may be used at flowering stage (one net for each unit plot).

6.6.18 Weeding and mulching: The field should always be kept free of weeds.

- Two to three hand weeding may be required during crop period.
- Mulching is also to be done following irrigation to keep the soil loose.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.



Figure 31. Regeneration of conserved accessions of tomato at PGRC, BARI



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6.6.19 Irrigation and drainage

- Light irrigation with water can for 3-4 days after transplanting is essential.
- Tomato cannot tolerate water stagnation more than 48 hours. Water management in tomato field should be done carefully. Enter irrigation water in to the drain and allow it to stand until the soil around the plant is soaked; avoid flood irrigation.
- Excess irrigation/rain water is to be drained out sharply.

6.6.20 Roguing

Plants showing different characters to the type must be removed. Roguing is done at different stages of crop growth:

- Before flowering- Plants showing different growth habit and foliage characteristics than the particular genotype should be rogued out.
- Early flowering and fruit setting stage- Off-types are rogued out judging the size and shape of immature fruits.
- Fruiting stage- The off-types are identified examining the fruit characteristics like shape, size, colour etc.
- Plants affected by tomato yellow leaf curl virus (TYLCV) and tomato spotted wilt virus (TSWV) must be eradicated soon after they are noticed.

6.6.21 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of insect pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology divisions of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Virus diseases are controlled mainly through vector control and roguing out of infected plants immediately as they are noticed to prevent contamination of healthy plants.

6.6.22 Harvesting and seed extraction

- Prior to harvest several extra plastic tags same with the ones used for labeling the field plots are printed for each accession, including information on plot number, accession/collector's number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with the trial code, plot number and accession /collector's number.
- Seed fruits are allowed to ripen to maturity on the plant. Only completely coloured and mature seed fruits are harvested periodically by hand and placed into the respective labeled plastic sacks. Respective extra tag is placed onto the plastic sack and the new one prepared is firmly tightened outer of the sack. The sacks are then brought to field laboratory for seed extraction and processing.



6.6.23 Seed extraction and drying

- In the field laboratory the harvested fruits are kept in labeled plastic containers for two to three days until the fruits become soft.
- They are then crushed by hand and no fruit juice is allowed to drain out.
- Entire mass is kept for 24 to 48 hours depending upon temperature. Flesh will float at the top and seed will settle down at the bottom. The fermented mass is removed and the seeds are sieved and cleaned with fresh clean water and dried in partial shade to a moisture content of ten percent or less, before sending to conservation unit.
- Care should be taken to avoid mechanical admixture.

6.6.24 Data validation and uploading on PGRC, BARI Genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Date of transplanting
6. Number of rows per plot
7. Row length, Row width, Plot to plot distance

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants).
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe fog, hail storm, severe storm, etc.).
5. Any other event related to the accessions planted (e.g. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.).
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. 1000 seed weight (g)
8. Total seed weight per plot (g)



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C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Plant growth habit.
2. Stem pigmentation
3. Fruit shape
4. Immature fruit skin colour
5. Fruit colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for tomato accessions in Bangladesh is considered finalized when extracted and dried seeds have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.6.25 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

- Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors of Agri-Horticultural Crops, Part II: Vegetable Crops, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated tomato (*Solanum lycopersicum*)].

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:



6.6.26 Descriptor List

Date of sowing:

Date of transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....

6. Plant growth habit

To be recorded at full foliage stage

- 1 Determinate
- 2 Semi-determinate
- 3 Indeterminate
- 99 Others (Specify in the 'Remarks' descriptor)

7. Leaf type

To be recorded at full foliage stage

- 1 Small/narrow
- 2 Potato leaf
- 3 Standard
- 4 Peruvianum type
- 5 Pimpinellifolium type
- 6 Hirsutum type
- 99 Others (Specify in the 'Remarks' descriptor)

8. Leaf colour

To be recorded at full foliage stage

- 1 Light green
- 2 Green
- 3 Dark green
- 99 Others (Specify in the 'Remarks' descriptor)

9. Leaf pubescence

To be recorded at full foliage stage

- 0 Absent
- 3 Sparse
- 5 Medium
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)



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10. Leaf/foliage cover

To be recorded at full foliage stage

- 3 Poor
- 5 Moderate
- 7 Good
- 9 Excellent
- 99 Others (Specify in the 'Remarks' descriptor)

11. Petiole Pubescence

To be recorded at full foliage stage

- 0 Absent
- 3 Sparse
- 5 Medium
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)

12. Stem type

To be recorded at full foliage stage

- 1 Round
- 2 Angular
- 99 Others (Specify in the 'Remarks' descriptor)

13. Stem thickness

To be recorded at full foliage stage

- 3 Thin
- 5 Medium
- 7 Thick
- 99 Others (Specify in the 'Remarks' descriptor)

14. Stem pubescence

To be recorded at full foliage stage

- 0 Absent
- 3 Sparse
- 5 Medium
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)

15. Stem pigmentation

To be recorded at full foliage stage

- 1 Green
- 2 Anthocyanin (red)
- 99 Others (Specify in the 'Remarks' descriptor)



16. Flower size

To be recorded at full blossom stage

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the 'Remarks' descriptor)

17. Flower colour

To be recorded at full flowering stage

- 1 Light yellow/cream
- 2 Deep yellow
- 3 Reddish yellow (Orange gold crimson)
- 99 Others (Specify in the 'Remarks' descriptor)

18. Style position

To be recorded at full blossom stage

- 1 Inserted
- 2 Same level as stigma
- 3 Slight exerted (stigma above the anther cone)
- 4 Highly exerted (stigma much above the anther cone)
- 99 Others (Specify in the 'Remarks' descriptor)

19. Fruit size

To be recorded at near maturity stage

- 1 Very small (≤ 20 g)
- 2 Small ($>20-30$ g)
- 3 Medium ($>30-80$ g)
- 4 Medium large ($>80-100$ g)
- 5 Large ($>100-175$ g)
- 6 Very large (>175 g)
- 99 Others (Specify in the 'Remarks' descriptor)

20. Fruit shape

To be recorded at near maturity stage

- 1 Flat round
- 2 Slightly flattened
- 3 Round
- 4 Oval
- 5 Heart shaped
- 6 Lengthened cylindrical (banana type)
- 7 Pyriform
- 8 Plum shaped
- 99 Others (Specify in the 'Remarks' descriptor)



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21. Immature fruit skin colour

To be recorded on fully developed fruit

- 1 Greenish white
- 2 Light green
- 3 Green
- 4 Dark green
- 5 Very dark green
- 99 Others (Specify in the 'Remarks' descriptor)

22. Presence of green (shoulder) trips on the fruits

To be recorded on fully matured fruits

- 0 Absent (uniform ripening)
- 1 Present (fruit shoulders-upper part fruit around calyx green while pistil area of fruits are red)

23. Fruit colour

To be recorded at near maturity stage

- 1 Yellow
- 2 Green
- 3 Orange
- 4 Red
- 5 Crimson
- 6 Pink
- 7 Tangarine
- 8 Yellow and red
- 9 Tangarine and red
- 10 Yellow, tangarine and red
- 99 Others (Specify in the 'Remarks' descriptor)

24. Fruit surface

To be recorded at near maturity stage

- 1 Smooth
- 2 Corrugated
- 99 Others (Specify in the 'Remarks' descriptor)

25. Shape of stem end/pistil scar

To be recorded at near maturity stage

- 1 Dot
- 2 Stellate
- 3 Linear
- 4 Irregular
- 99 Others (Specify in the 'Remarks' descriptor)



26. Stem-end fruit shape

To be recorded at near maturity stage

- 1 Indented
- 2 Flat
- 3 Round
- 4 Pointed (nipped)
- 99 Others (Specify in the 'Remarks' descriptor)

27. Blossom-end fruit shape

To be recorded at near maturity stage

- 1 Indented
- 2 Flat
- 3 Pointed/nipped
- 99 Others (Specify in the 'Remarks' descriptor)

28. Type of fruit cracking

To be recorded at near maturity stage for radial/concentric cracking

- 0 None
- 3 Slight
- 5 Medium
- 7 Severe
- 99 Others (Specify in the 'Remarks' descriptor)

29. Fruit abnormality

To be recorded at near maturity stage

- 0 Absent
- 1 Present

30. Fruit firmness

To be recorded at near maturity stage

- 3 Soft
- 5 Medium
- 7 Firm
- 99 Others (Specify in the 'Remarks' descriptor)

31. Pulpiness

To be recorded at fruit maturity stage

- 1 Juicy
- 2 Pulpy
- 3 Highly pulpy
- 99 Others (Specify in the 'Remarks' descriptor)

32. Seediness

To be recorded at fruit maturity stage

- 3 Low
- 5 Medium
- 7 High
- 99 Others (Specify in the 'Remarks' descriptor)



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33. Plant height (cm)

To be measured as average of 5-10 random plants from the ground level to the tip of the main stem just before last harvest

Quantitative

34. Number of primary branches

To be recorded as average of same 10 plants at the end of flowering stage. The branch that arises from the main stem is known as primary branch.

Quantitative

35. Days to 50% flowering

To be recorded as number of days from sowing date to the date when at least 50% of the plants show flower open. Stigma emergence on the main branch is considered as flowering

Quantitative

36. Days to first fruit set

To be recorded as number of days from the date of transplanting to date of first fruit set

Quantitative

37. Days to first fruit harvesting

To be recorded as number of days from the date of transplanting to date of first fruit harvest at breaker stage (80% maturity)

Quantitative

38. Days to first fruit maturity

To be recorded as number of days from the date of transplanting to date of plant attaining physical maturity (turning stage)

Quantitative

39. Number of clusters per plant

To be recorded as average of same 5-10 plants at flowering stage

Quantitative

40. Number of flowers per cluster

To be recorded as average of 5 random clusters at flowering stage

Quantitative

41. Number of fruits per cluster

To be recorded as average of 5 random clusters at marketable stage

Quantitative



42. Number of fruits per plant
To be recorded as average of same 5-10 plants at near maturity stage
Quantitative
43. Locule number per fruit
To be recorded as average of 5-10 random fruits at near maturity stage
Quantitative
44. Fruit weight (g)
To be recorded as average weight of 5-10 fruits at near maturity stage)
Quantitative
45. Fruit yield per plant (g)
To be recorded as average of cumulative yield of all pickings in same 5-10
selected plants at near maturity stage
Quantitative
46. Pericarp thickness (mm)
To be recorded as average of same 5-10 fruits from an equatorial section of the
fruit by using Vernier calipers at near maturity stage
Quantitative
47. Organoleptic test
To be recorded at fruit maturity stage
 - 1 Extreme sour
 - 2 Sour
 - 3 Medium sweet
 - 4 Sweet
48. Total soluble solids (%)
To be recorded at maturity stage by using refractometer
Quantitative
49. Seed shape
To be recorded on fully dried seeds
 - 1 Globular
 - 2 Ovate
 - 3 Triangular with pointed base
 - 99 Others (Specify in the 'Remarks' descriptor)
50. Seed colour
To be recorded on fully dried seeds
 - 1 Light yellow
 - 2 Dark yellow
 - 3 Grey
 - 4 Brown
 - 5 Dark brown
 - 99 Others (Specify in the 'Remarks' descriptor)



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51. Biotic stress susceptibility

Specify the infestation or infection using any 1-9 scale

Note: For Additional information as common name(s) of disease(s)/pest(s) and casual organism(s) may be appended in the Biotic notes descriptor

- 1 Very low or no visible sign of susceptibility
- 3 Low
- 5 Intermediate
- 7 High
- 9 Very high



Figure 32. Tomato plants affected by a) Bacterial wilt, b) Tomato Yellow Leaf Curl Virus (TYLCV) and c) Root Knot Nematode

- 6.6.27 Identity is monitored by comparing the accession with previous passport or morphological data.
- 6.6.28 All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.
- 6.6.29 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.

7.6.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.6.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild tomato genetic resources at BARI is in compliance with the following standards and policies:



- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.6.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild tomato genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.6.1 Staff Training and Competency

Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.

Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.6.1 References

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10.6.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Rezwan Molla

CHAPTER 08

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Pumpkin Germplasm in Bangladesh

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Pumpkin genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Pumpkin genetic resources management.

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6.7 Procedure for Pumpkin

The regeneration and characterization procedure is initiated when the head of conservation unit of PGRC, BARI genebank review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of pumpkin accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.7.1 Database review: Review of data and pertinent information on pumpkin germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.7.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<80% of the initial viability of the stored seeds for cultivated pumpkin germplasm or <60% of the initial viability of the stored seeds for its wild relatives' germplasm) and inadequate amount of seeds (below 1,500 seeds) or have not yet been characterized is generated for regeneration/characterization in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/ characterization.

Each of these lists should include the following information:

- Accession number
- Full taxon name (Genus, Species, Subspecies)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly winter types, strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged).

6.7.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



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- When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value (<85%), then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 8 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity (ICARDA, 2021).
 - Seeds are placed in paper craft foils and identified with the following information:
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Country of origin
 - Number of seeds
 - Seeds need to be treated with Vitavax or Captan at the rate of 2 g/kg or other recommended fungicides at appropriate dose to avoid seed borne diseases.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the planting date.
- 6.7.4 Distribution of responsibilities:** As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.
- 6.7.5 Preparation of experimental register:** Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.7.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.7.7 Plot dimension:** 8 m × 4 m plots having 100 cm wide and 15-20 cm deep drain in between two plots. Eight pits of 50 cm diameter and 50 cm depth are to be prepared per plot at 2 m × 2 m spacing at 7-10 days before transplanting of seedlings.
- 6.7.8 Field layout**
- Prepare field layout hard copy according to experimental design for seed bed and main field following seed sample preparation.
 - Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.



- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.7.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated and wild pumpkin germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and insect pests.

- Selection of site for nursery: Pumpkin seedlings are raised in 8 cm × 10 cm polythene bags (Halim *et al.*, 2006). Polybags are arranged on well prepared land, which is free of objectionable weeds and volunteer plants, receive sunshine all the day, and well drained (Agrawal, 1999). Pumpkin seeds are also sown directly in pits of the main field. In such case, land for polybag arrangement is not required.
- Selection of land for crop production: The land should be free of volunteer plants and objectionable weeds (certain weeds act as harbour of some virus diseases and insect pests by which the crop may be infected). Select the land on which pumpkin or other cucurbitaceous vegetables were not grown in the previous season. This is necessary to avoid soil borne diseases especially root knot nematode. The soil of the selected field should be fertile, rich in organic matter, sandy loam to clay loam in texture and well drained.
- Early enough before planting of seedling/seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the planting date to remove the weeds especially perennial weeds.

6.7.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for seed bed and main experimental field. Information printed on the tag includes seed bed/field plot number and accession/collector's number.

6.7.11 Seed sowing (eg. sowing rate, method, etc.) and labeling

- Preparation of polybag and nursery plot: The polybag should be filled with a mixture of 50% soil and 50% compost/FYM (Rashid and Singh, 2000). The land of nursery plot is prepared to a fine tilth by deep ploughing, three to four harrowing followed by leveling. Nursery plot is divided into several sections as per requirement and labeled with water-proof thermal plastic tags. Information printed on the tag includes bed section number and accession/collector's number. Required number of filled up polybags are arranged in each section burying one-third of the bag under soil.
- Time of Seed Sowing: Seeds may be sown from mid-October to early December. For seed production i.e. regeneration of accessions mid-November is the best time for seed sowing (Rashid and Singh, 2000).
- Seed soaking: Fifteen to twenty hours seed soaking in clean water before sowing accelerates germination. Seeds are placed onto cloth bags labeled with water proof thermal plastic tags, firmly tightened outer of the bag and soaked in clean water in plastic bowl.



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- Seed treatment: Seeds need to be treated with Vitavax or Captan at the rate of 2 g/kg before sowing to avoid seed borne diseases.
- Method of sowing: Labeled cloth bags with soaked seeds are placed in respective section of nursery bed and two seeds are dibbled per polybag at a depth of 1.5 to 2.0 cm. In case of direct sowing in the main experimental field, soaked seeds are placed in respective field plots, and two to three seeds are dibbled at a depth of 2.0 to 2.5 cm in each of well-prepared pits.
- Immediately after sowing of seed a final check is done for correspondence to the trial layout and any planting error is noted on the hard copy.

6.7.12 Preparation of land and pit

- Prepare the land to a fine tilth by deep ploughing, three to four harrowing followed by leveling.
- The plots are raised by 15-20 cm lifting the soil from 100 cm channel kept around each plot.
- Pits of 45 cm × 45 cm × 40 cm size are to be prepared at 2 m × 2 m spacing, and recommended manure and fertilizers are applied at 7-10 days before transplanting of seedlings.

6.7.13 Manure and Fertilizer doses: 75-36-60-21-2.0-1.4 kg/ha N-P-K-S-Zn-B and FYM @ 4 t/ha (Ahmed *et al.*, 2018).

Method of application

- All of FYM, P, K, S, Zn and B should be applied in pits 5-7 days before transplanting and mixed thoroughly with the soil.
- N should be applied around the plant as side dressing at 15, 35, 55 and 75 DAT under moist soil condition and mixed thoroughly with the soil as soon as possible for better utilization.

6.7.14 Transplanting of seedling

- Age of seedling: 16-20 days old seedlings are planted in well prepared pits.
- Spacing: Row to row- 200 cm; plant to plant- 200 cm; plot to plot- 100 cm
- Placing the seedling to experimental field: Place the seedlings from the nursery bed to labeled plastic crates/trays, bring the plastic crates/trays to the experimental field and place to respective field plot according to field layout.
- No. of seedlings/pit: Plant seedling(s) of one polybag per pit and irrigate by water can immediately afterwards. Allow one healthy seedling to grow per pit and remove extra ones (if there are more than one seedling per bag) one week after transplanting.
- Keep the labeled plastic crates/trays in respective field plots for final checking.
- Immediately after transplanting of seedlings a final check is to be done for correspondence to the field trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.7.15 Labeling: Seedling bags of the accessions are kept in labeled plastic crates/trays, and plastic crates/trays are placed onto the respective field plots based on the field trial layout created as per design during seedling preparation.

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- Once transplanting in the main plot is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and the accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of transplant, number of accessions, number of checks, experimental design, etc.).

6.7.16 Straw mulching and placing of earthen plate: Straw mulch are applied on each plot to keep the plants detached from the soil, which also helps conserving soil moisture and controlling weeds. After fruit setting an earthen plate (*sanki*) is placed under each fruit to minimize fruit rotting.

6.7.17 Weeding: The field should always be kept clean of weeds until harvesting.

- Three to four weeding at the time of side dressing of fertilizers may be required.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.7.18 Irrigation and drainage:

- Overhead light irrigation with water can may be required in direct seeded pits 4-5 days after sowing to facilitate germination.
- Necessary irrigation water is to be entered in the drain instead of flooding the whole plot as per requirement. Excess irrigation/rain water is to be drained out sharply.

6.7.19 Desuckering: All the branches up to 40-45 cm of the base of the plant are to be pruned out Halim *et al.*, 2006 and BARI, 2019).

6.7.20 Roguing: Off-type plants are usually detectable fairly early in cucurbits. The early shape of fruits and even the shape of the ovary at flowering sometime reveal off-type plants. Although some damage may already resulted from cross-pollination, such off-type plants should be immediately rogued out (Agrawal, 1999). Roguing is done at 4 stages in pumpkin as follows (Rashid and Singh, 2000):

- Early Vegetative Stage: The plants, whose vegetative characters (e.g. bush or trailing type), foliage and vigour and resistance to specific pathogens are not in accordance with the cultivar/accession description, should be removed.
- Before First Flower Open: Plants having under developed fruit or female flower buds, whose characters are not true to type, should be removed.
- First Fruit Setting: Developing fruits of such a plant which are not typical of the cultivar should be removed along with the whole plant itself.

6.7.21 Control pollination

- Hand pollination in the morning time promotes fruit set as well as yield.
- For pure seed production, both male and female flowers are covered with butter paper bags before anthesis.
- Distinguish covered female flower from male flower by cross (X) mark with a bold marker pen (preferably red ink) on the bag.
- Dusting of pollen is done by shaking the male flower over the stigma or rubbing the anthers with the stigma.



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- The pollinated female flowers are kept covered with the same butter paper bags for two to three weeks until it is ruptured by the pressure of the developing fruit and fruit skin become hard. It is necessary to control fruit fly infestation.



Figure 33. Flower bagging with butter paper bag for control pollination

6.7.22 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology division of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Sex pheromone trap are to be set as per recommendation for controlling fruit fly.

6.7.23 Harvesting

- Prior to harvest several extra plastic tags same with the ones used for labeling the field plots are printed for each accession, including information on, plot number, accession number. Appropriate plastic crates, which provide ample ventilation, are also prepared and labeled with plot number and accession number.
- Fully ripe fruits of each accession are harvested periodically by hand and placed into the respective labeled plastic crates. Respective extra tag is firmly tightened outer of the crate. Several factors are taken into consideration to judge the maturity in pumpkin which are:
 - When the fruit colour changes yellow or yellow-orange or straw colour.
 - When the pedicel of fruit becomes straw coloured and
 - When the vines start drying
- Some fruits are to be harvested at marketable stage of immature fruit (20-25 days after anthesis) for recording immature flesh colour, and at marketable stage of mature fruit for recording mature fruit characters, yield components and yield, and seed characters.
- The crates are then brought to the field laboratory.



6.7.24 Seed extraction, washing and drying

- Before seed extraction fruits should be stored in room temperature for 4 to 7 weeks spreading in one single layer with a space between the fruits (keeping one or two fruits per properly labeled plastic crate) preferably in a cooler dry place.
- Afterward, the fruits are cut into half and scoop out the seed by hand. Some placenta may remain with the seeds which are to be separated by rolling and raking simultaneously. Then the seeds are to be washed with water in troughs.
- The washed seeds should be dried quickly. The seeds are spread on labeled trays and placed in the shade and gradually to sun to dry and continued up to a moisture level of 10 percent. Frequent turning of seeds will ensure uniform drying.

6.7.25 Data validation and uploading on PGRC, BARI Genebank Documentation

System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Date of transplanting
6. Number of rows per plot
7. Row length, Row width, Plot to plot distance

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe storm, hail storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. 100 seed weight (g)
8. Total seed weight per plot (g)



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C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Plant growth habit
2. Leaf margin
3. Leaf shape
4. Sex type
5. Fruit shape
6. Immature fruit skin colour
7. Mature fruit skin colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for pumpkin accessions in Bangladesh is considered finalized when extracted and dried seeds have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.7.26 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

➤ Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors of Agri-Horticultural Crops, Part II: Vegetable Crops, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated pumpkin (*Cucurbita spp.*)].

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)

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4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:

6.7.27 Descriptor List

Date of sowing: Date of transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Early plant vigour
To be recorded after 30 days of sowing
 - 3 Poor
 - 5 Good
 - 7 Very good
 - 99 Others (Specify in the 'Remarks' descriptor)
7. Plant growth habit
To be recorded on fully grown plant
 - 3 Short viny
 - 5 Medium viny
 - 7 Long viny
 - 99 Others (Specify in the 'Remarks' descriptor)
8. Stem pubescence
To be recorded at peak fruiting stage
 - 1 Smooth
 - 2 Pubescence
 - 99 Others (Specify in the 'Remarks' descriptor)
9. Stem shape
To be recorded at peak fruiting stage (as recorded from the cross section)
 - 1 Rounded
 - 2 Angular
 - 99 Others (Specify in the 'Remarks' descriptor)
10. Tendril
To be recorded at flowering stage
 - 0 Absent
 - 1 Present
11. Tendril type
To be recorded at flowering stage
 - 1 Coiled
 - 2 Straight
 - 99 Others (Specify in the 'Remarks' descriptor)



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12. Tendril branching

To be recorded at flowering stage

- 1 Unbranched
- 2 Branched
- 99 Others (Specify in the 'Remarks' descriptor)

13. Leaf margin

To be recorded at full foliage stage

- 1 Entire
- 2 Serrate
- 3 Multifid
- 99 Others (Specify in the 'Remarks' descriptor)

14. Leaf shape

To be recorded at full foliage stage

- 1 Cordate
- 2 Oblong
- 3 Ovate
- 4 Obovate
- 5 Orbicular
- 99 Others (Specify in the 'Remarks' descriptor)

15. Leaf size

To be recorded at full foliage stage

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the 'Remarks' descriptor)

16. Leaf pubescence nature

To be recorded at full foliage stage

- 3 Soft
- 5 Intermediate
- 7 Hard
- 99 Others (Specify in the 'Remarks' descriptor)

17. Leaf pubescence density

To be recorded at full foliage stage

- 0 No hairs
- 3 Sparse
- 5 Intermediate
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)

18. Petiole length (cm)

To be recorded as average of 5-10 random leaves in the middle region of the vine at full foliage stage

Quantitative



19. Node number at which first female flower appears
To be recorded at first appearance of female flower
Quantitative
20. Days to 50% flowering
To be recorded as number of days from sowing/transplanting date to the date when at least 50% of the plants show first female flower open
Quantitative
21. Sex type
To be recorded at flowering/full blossom stage
 - 1 Monoecious (male and female flowers on same plant)
 - 2 Gynomonoecious (female and hermaphrodite flowers on the same plant)
 - 3 Andromonoecious (male and hermaphrodite flowers on the same plants)
 - 4 Androgynomonoecious (male, female and hermaphrodite flowers on the same plants)
 - 5 Hermaphrodite (male and female on same flower and on same plant)
 - 6 Androecious (only male flower on the plant)
 - 99 Others (Specify in the 'Remarks' descriptor)
22. Sex ratio
To be recorded as ratio of female (including hermaphrodite) to male flowers at flowering stage
Quantitative
23. Peduncle surface
To be recorded at marketable stage
 - 1 Soft corky
 - 2 Hard corky
 - 99 Others (Specify in the 'Remarks' descriptor)
24. Peduncle length (cm)
To be recorded as average of 5-10 random fruits at marketable stage
Quantitative
25. Peduncle shape
To be recorded at marketable stage
 - 1 Nearly cylindrical
 - 2 Smoothly grooved
 - 3 Angular grooved
 - 99 Others (Specify in the 'Remarks' descriptor)
26. Peduncle separation from fruit
To be recorded on mature fruit
 - 1 Easy
 - 2 Intermediate
 - 3 Difficult
 - 99 Others (Specify in the 'Remarks' descriptor)



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27. Nature of base of peduncle

To be recorded on mature fruit

- 0 Non-flared
- 1 Flared

28. Fruit shape

To be recorded at marketable stage

- 1 Globular (round)
- 2 Flattened
- 3 Disk
- 4 Cylindrical
- 5 Elliptical (oval)
- 6 Acorn/Heart shaped
- 7 Pyriform
- 8 Dumbbell
- 9 Elongate form
- 10 Terminate superior
- 11 Crowned
- 12 Terminate inferior
- 13 Curved
- 14 Crooked neck
- 99 Others (Specify in the 'Remarks' descriptor)

29. Immature fruit skin colour

To be recorded at marketable stage

- 1 Light green
- 2 Green
- 3 Dark green
- 4 Yellow
- 99 Others (Specify in the 'Remarks' descriptor)



Figure 34. Variation in fruit shape of new and conserved pumpkin accessions

30. Mature fruit skin colour

To be recorded at mature stage

- 1 Creamish
- 2 Yellowish
- 3 Green
- 4 Red
- 99 Others (Specify in the 'Remarks' descriptor)



Figure 35. Variation of matured fruit skin color of Pumpkin

31. Fruit skin colour pattern

To be recorded at immature stage

- 1 Uniform
- 2 Mottled
- 3 Striped
- 99 Others (Specify in the 'Remarks' descriptor)

32. Fruit skin colour intensity

To be recorded at marketable stage

- 3 Light
- 5 Intermediate
- 7 Dark
- 99 Others (Specify in the 'Remarks' descriptor)

33. Fruit skin lustre

To be recorded at marketable stage

- 3 Matt
- 5 Intermediate
- 7 Glossy
- 99 Others (Specify in the 'Remarks' descriptor)



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34. Stem-end fruit shape

To be recorded at marketable stage

- 1 Depressed
- 2 Pointed
- 3 Flattened
- 4 Rounded
- 99 Others (Specify in the 'Remarks' descriptor)

35. Blossom-end fruit shape

To be recorded at marketable stage

- 1 Depressed
- 2 Pointed
- 3 Flattened
- 4 Rounded
- 99 Others (Specify in the 'Remarks' descriptor)

36. Skin hardness of the fruit

To be recorded at marketable stage

- 3 Soft
- 5 Intermediate
- 7 Hard
- 99 Others (Specify in the 'Remarks' descriptor)

37. Fruit ridge (rib) shape

To be recorded in cross section at marketable stage

- 1 Superficial
- 2 Rounded/Grooved
- 3 Intermediate
- 4 Deep grooved
- 5 Narrowly winged
- 99 Others (Specify in the 'Remarks' descriptor)



Figure 36. Variation in rib shape of conserved pumpkin accessions

38. Number of ridges (ribs) per fruit
To be recorded in cross section at full maturity stage

Quantitative

39. Immature flesh colour
To be recorded at marketable stage of immature fruit

- 1 White
- 2 Creamy white
- 3 Yellow
- 4 Deep yellow
- 5 Orange
- 99 Others (Specify in the 'Remarks' descriptor)

40. Mature flesh colour
To be recorded at marketable stage of mature fruit

- 1 Yellow
- 2 Deep yellow
- 3 Orange
- 99 Others (Specify in the 'Remarks' descriptor)



Figure 37. Variation in flesh colour of conserved accessions of pumpkin

41. Flesh thickness (cm)
To be recorded as average of same 5-10 fruits at marketable stage

Quantitative

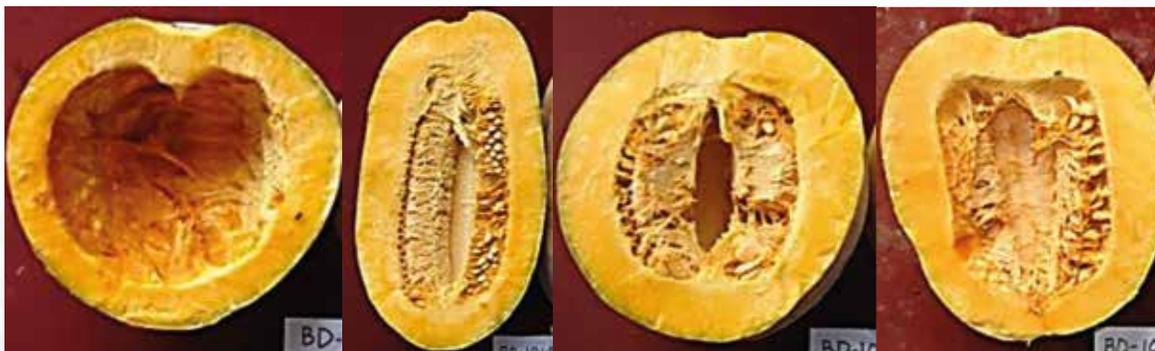


Figure 38. Variation in flesh thickness of conserved pumpkin accessions

42. Fruit length (cm)
To be recorded as average of 5-10 random fruits at marketable stage

Quantitative



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43. Fruit breadth (cm)
To be recorded as average of same 5-10 fruits at marketable stage
Quantitative
44. Flesh texture
To be recorded at marketable stage
- 1 Smooth
 - 2 Soft/Spongy
 - 3 Fibrous-gelatinous
 - 4 Fibrous-dry
 - 5 Grainy
 - 99 Others (Specify in the 'Remarks' descriptor)
45. Number of primary branches
To be recorded as average of same 10 plants at the end of flowering stage. The branch that arises from the main vine/stem is known as primary branch
Quantitative
46. Days to first fruit harvest
To be recorded as number of days from date of sowing/transplanting to the date of first marketable fruit harvest
Quantitative
47. Days to last fruit harvest
To be recorded as number of days from date of sowing/transplanting to the date of last marketable fruit harvest
Quantitative
48. Number of marketable fruits per plant
To be recorded as total number of fruits in each picking
Quantitative
49. Yield of marketable fruits per plant (g)
To be recorded as average of cumulative yield of all pickings in same 10 plants
Quantitative
50. Fruit weight (g)
To be calculated on the basis of fruit yield and no. of fruits per plant
Quantitative
51. Seed lustre
To be recorded on mature and dried seeds
- 3 Matt
 - 5 Intermediate
 - 7 Glossy
 - 99 Others (Specify in the 'Remarks' descriptor)
52. 100 seed weight (g)
To be measured as average weight of 100 random seeds
Quantitative

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53. Biotic stress susceptibility

Specify the infestation or infection using any 1-9 scale

- 1 Very low or no visible sign of susceptibility
- 3 Low
- 5 Intermediate
- 7 High
- 99 Very high

6.7.27 Identity is monitored by comparing the accession with previous passport or morphological data.

6.7.28 All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.

6.7.29 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.

7.7.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.7.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild ridge gourd genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.7.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild ridge gourd genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV



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8.7.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.7.1 References

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- Rashid MA, Singh DP. 2000. A Manual on Vegetable Seed Production in Bangladesh. AVRDC-USAID-Bangladesh Project, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur. 119p
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10.7.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Rezwan Molla

CHAPTER 09

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Bitter Gourd Germplasm in Bangladesh

Compiled and Edited by

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December 2024



First Edition, 2024

This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Bitter Gourd genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Bitter Gourd genetic resources management.

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6.8 Procedure for Bitter Gourd

The regeneration and characterization procedure is initiated when the head of conservation unit of PGRC, BARI genebank review data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of bitter gourd accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

- 6.8.1 Database review:** Review of data and pertinent information on pumpkin germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)
- 6.8.2 Selection of accessions and list generation:** Based on notifications and using pertinent information from the database, list of accessions with lower viability (<80% of the initial viability of the stored seeds for cultivated bitter gourd germplasm) and inadequate amount of seeds (below 1,500 seeds) or have not yet been characterized is generated for regeneration/characterization in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

Each of these lists should include the following information:

- Accession number
- Full taxon name (Genus, Species, Subspecies)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly winter types, strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged).

6.8.3 Seed sample preparation

- Seed foils/containers from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



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- When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value (<80%), then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 12 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
 - Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Country of origin
 - Number of seeds
 - Seeds need to be treated with Vitavax or Captan at the rate of 2 g/kg or other recommended fungicides at appropriate dose to avoid seed borne diseases.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the planting date.
- 6.8.4 Distribution of responsibilities:** As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.
- 6.8.5 Preparation of experimental register:** Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.8.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.8.7 Plot dimension:** 9 m × 3 m plots having 100 cm wide and 15-20 cm deep drain in between two plots. Eight pits of 40-50 cm diameter and 40-50 cm depth are to be prepared per plot at 1.5 × 1.5 m spacing at 7-10 days before transplanting of seedlings.



6.8.8 Field layout

- Prepare field layout hard copy according to experimental design for seed bed and main field following seed sample preparation.
- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.8.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated bitter gourd germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and insect pests.

- Selection of site for nursery: Bitter gourd seedlings are raised in 8 cm × 10 cm polythene bags (Halim *et al.*, 2006). Polybags are arranged on well prepared land, which is free of objectionable weeds and volunteer plants, receive sunshine all the day, and well drained. Bitter gourd seed are also sown directly in pits of the main field. In such case, land for polybag arrangement is not required.
- Selection of land for crop production: The land should be free of volunteer plants and objectionable weeds (certain weeds act as harbour of some virus diseases and insect pests by which the crop may be infected). Select the land on which bitter gourd or other cucurbitaceous vegetables were not grown in the previous season (Agrawal, 1999). This is necessary to avoid soil borne diseases especially root knot nematode. The soil of the selected field should be fertile, rich in organic matter, sandy loam to clay loam in texture and well drained (Rashid and Singh, 2000).
- Early enough before planting of seedling/seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the planting date to remove the weeds especially perennial weeds.

6.8.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for seed bed and main experimental field. Information printed on the tag includes bed section/field plot number and accession /collector's number.

6.8.11 Seed sowing (eg. sowing rate, method, etc.) and labeling

- Preparation of polybag and nursery plot: 8 cm × 10 cm poly-bags are used. The polybag should be filled with a mixture 50% soil and 50% compost/FYM (BARI, 2019). The land of nursery plot is prepared to a fine tilth by deep ploughing, three to four harrowing followed by leveling. Nursery plot is divided into several sections as



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per requirement and labeled with water-proof thermal plastic tags. Information printed on the tag includes bed section number and accession /collector's number. Required number of filled up polybags are arranged in each section burying one-third of the bag under soil.

- Time of Seed Sowing: Two ecotypes of bitter gourd namely *Korola* (summer and rainy season crop) and *Ucche* (late winter crop) are grown in Bangladesh. Ecotypes are to be separated during collection. Optimum time of seed sowing for *Ucche* is 15 to 30 December and for *Korola* is 15 April to 15 May (two seeds are to be sown in each of 8 cm × 10 cm poly-bag filled with 50% soil and 50% compost).
- Seed soaking: Twenty four hours seed soaking in clean water before sowing accelerates germination. Seeds are placed into cloth bags labeled with water proof thermal plastic tags, firmly tightened outer of the bag and soaked into clean water in plastic bowl. Information printed in plastic tag includes bed section number and accession number.
- Method of sowing: Labeled cloth bags with soaked seeds are placed in respective section of nursery bed and two seeds are dibbled per polybag at a depth of 1.5 to 2.0 cm. Each nursery bed section is labelled with plastic tag tightened outer of the cloth bag.
- Immediately after sowing of seed a final check is done for correspondence to the trial layout and any planting error is noted on the hard copy.

6.8.12 Preparation of land and pit

- Prepare the land to a fine tilth by deep ploughing, three to four harrowing followed by leveling. The plots are raised by 15-20 cm lifting the soil from 100 cm channel kept around each plot.
- Pits of 40 cm × 40 cm × 40 cm size are prepared at 1.5 m × 1.5 m spacing, and recommended manure and fertilizers are applied at 7-10 days before transplanting of seedlings.

6.8.13 Manure and Fertilizer doses: 75-30-45-15-1.0-1.0 kg/ha N-P-K-S-Zn-B and FYM @ 3 t/ha (Ahmed *et al.*, 2018).

Method of application

- All of FYM, P, K, S, Zn and B should be applied in pits 5-7 days before transplanting and mixed thoroughly with the soil.
- N should be applied around the plant as side dressing at 20, 40 and 60 DAT under moist soil condition and mixed thoroughly with the soil as soon as possible for better utilization.

6.8.14 Transplanting of seedling

- Age of seedling: 10-15 days after germination
- Spacing: Row to row: 150 cm; plant to plant: 150 cm; plot to plot: 100 cm
- Placing the seedling to experimental field: Place the seedlings from the nursery bed to labeled plastic crates, bring the plastic crates to the experimental field and place to respective field plot according to field layout.



- No. of seedlings/pit: Plant seedling(s) of one polybag per pit and irrigate by water can immediately afterwards. Allow one healthy seedling to grow per pit and remove extra ones one week after transplanting.
- Keep the plastic crates in respective field plots for final checking.
- Immediately after transplanting of seedlings a final check is done for correspondence to the field trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.8.15 Labeling:

- Once transplanting in the main plot is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and the accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of transplant, number of accessions, number of checks, experimental design, etc.).

6.8.16 Trellising: Summer crop (*Korola*) plants are allowed to grow on 1.0-1.5 m high trellis made of bamboo sticks or other materials.

6.8.17 Straw mulching: Straw mulch are applied for winter crop (*Ucche*) on each plot to keep the plants detached from the soil, which also helps conserving soil moisture and controlling weeds.

6.8.18 Weeding: The field should always be kept clean of weeds.

- Three to four weeding at the time of side dressing of fertilizers may be required.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.8.19 Irrigation and drainage:

- Overhead light irrigation with water may be required in direct seeded pits 4-5 days after sowing to facilitate germination.
- Necessary irrigation water is entered in the drain instead of flooding the whole plot as per requirement. Excess irrigation/rain water is to be drained out sharply.

6.8.20 Desuckering: All the branches up to 40-45 cm of the base of the plant are to be pruned out (Halim *et al.*, 2006 and BARI, 2019).

6.8.21 Roguing: Off-type plants are usually detectable fairly early in cucurbits. The early shape of fruits and even the shape of the ovary at flowering sometime reveals off-type plants. Although some damage may already be resulted from cross-pollination, such off-type plants should be immediately rogued out (Agrawal, 1999).

6.8.22 Control pollination

- Hand pollination in the morning time promotes fruit set as well as yield.
- For pure seed production, both male and female flowers are covered with butter paper bags before anthesis.
- Distinguish covered female flower from male flower by cross (X) mark with a bold marker pen (preferably red ink) on the bag.



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- Dusting of pollen is done by shaking the male flower over the stigma or rubbing the anthers with the stigma.
- The pollinated female flowers are kept covered with the same butter paper bags for three four days. Artificial pollination must be completed from 6.00 to 9.00 a.m.

6.8.23 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is to be conducted by genbank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology division of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.

6.8.24 Harvesting

- Prior to harvest several extra plastic tags same with the ones used for labeling the field plots are printed for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with plot number and accession number.
- Fully ripe fruits (this stage is determined visually from the turning of fruit skin colour to yellow) of each accession are harvested periodically by hand and placed into the respective labeled plastic sacks. Respective extra tag is placed into the plastic sacks and the new one prepared is firmly tightened outer of the sack.
- The sacks are then brought to the field laboratory for seed extraction.
- Some fruits are to be harvested at marketable stage of fruit (15-20 days after pollination) for recording immature fruit characters, yield components and yield.
- The sacks are then brought to the field laboratory.



Figure 39. Field view and sex pheromone trap used in the field for controlling insects



6.8.25 Seed extraction, washing and Drying

- The fruits are cut longitudinally and scoop out the seed by hand. Some placenta may remain with the seeds which are to be separated by rolling and raking simultaneously. Then the seeds are to be washed with clean water in troughs carefully to avoid mechanical admixture.
- The washed seeds should be dried quickly. For this, properly labeled trays with screen wire or burlap bottoms may be used. The seeds are spread on trays and placed in the shade and gradually to sun to dry and continued up to a moisture level of 10 percent. Frequent turning of seeds will ensure uniform drying.

6.8.26 Data validation and uploading on PGRC, BARI Genebank Documentation

System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Date of transplanting
6. Number of rows per plot
7. Row length, Row width, Plot to plot distance

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe storm, hail storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. 100 seed weight (g)
8. Total seed weight per plot (g)



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C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Plant growth habit
2. Leaf margin
3. Fruit shape
4. Nature of tubercles
5. Fruit skin colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for bitter gourd accessions in Bangladesh is considered finalized when extracted and dried seeds have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.8.27 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

➤ Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors of Agri-Horticultural Crops, Part II: Vegetable Crops, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated bitter gourd (*Momordica charantia*)].

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)

4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:

6.8.28 Descriptor List

Season: Date of sowing: Date of transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....

6. Early plant vigour

To be recorded after 30 days of sowing

- 3 Poor
- 5 Good
- 7 Very good
- 99 Others (Specify in the 'Remarks' descriptor)

7. Plant growth habit

To be recorded on fully grown plant

- 3 Short viny
- 5 Medium viny
- 7 Long viny
- 99 Others (Specify in the 'Remarks' descriptor)



Fig. 40. Plant growth habit of test bitter melon germplasm

8. Stem pubescence

To be recorded at peak fruiting stage

- 0 Absent
- 2 Present



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9. Stem shape

To be recorded at near maturity stage (as recorded from the cross section)

- 1 Rounded
- 2 Angular
- 99 Others (Specify in the 'Remarks' descriptor)

10. Twining tendency

To be recorded at flowering stage

- 0 None
- 3 Slight
- 5 Intermediate
- 7 Pronounced
- 99 Others (Specify in the 'Remarks' descriptor)

11. Tendril branching

To be recorded at flowering stage

- 1 Unifid
- 2 Bifid
- 3 Multifid
- 99 Others (Specify in the 'Remarks' descriptor)



Figure 41. Tendril branching habit of test bitter gourd germplasm

12. Leaf margin

To be recorded at full foliage stage

- 1 Entire
- 2 Serrate
- 3 Multifid
- 99 Others (Specify in the 'Remarks' descriptor)



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13. Leaf shape

To be recorded at full foliage stage

- 1 Ovate
- 2 Obovate
- 3 Cordate
- 4 Oblong
- 5 Reniform
- 6 Orbicular
- 99 Others (Specify in the 'Remarks' descriptor)

14. Leaf size

To be recorded at full foliage stage

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the 'Remarks' descriptor)

15. Leaf pubescence

To be recorded on fully grown leaf at full foliage stage

- 0 No hair
- 3 Sparse
- 5 Intermediate
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)

16. Internode length (cm)

To be recorded as average of distance between 4th and 5th node on 5-10 random plants at full foliage stage

Quantitative

17. Petiole length (cm)

To be recorded as average of 5-10 random leaves in the middle region of the vine at full foliage stage

Quantitative

18. Node number at which first female flower appears

To be recorded at first appearance of female flower

Quantitative

19. Days to 50% flowering

To be recorded as number of days from sowing /transplanting date to the date when at least 50% of the plants show first female flower open

Quantitative



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20. Sex type

To be recorded at flowering stage

- 1 Monoecious (male and female flowers on same plant)
- 2 Gynomonoecious (female and hermaphrodite flower on same plant)
- 3 Andromonoecious (male and hermaphrodite flower on same plant)
- 4 Hermaphrodite (male and female on same flower and same plant)
- 5 Androecious (male flower only on plant)
- 99 Others (Specify in the 'Remarks' descriptor)

21. Sex ratio

To be recorded as ratio of total number of female (including hermaphrodite) to male flowers on a plant at flowering stage

Quantitative

22. Flower colour

To be recorded at flowering stage

- 1 White
- 2 Light yellow
- 3 Yellow
- 4 Deep yellow
- 99 Others (Specify in the 'Remarks' descriptor)

23. Peduncle length (cm)

To be recorded as average of 10 random fruits at marketable stage

Quantitative

24. Peduncle separation from fruit

To be recorded as nature of peduncle separation from fruit at marketable stage

- 3 Easy
- 5 Intermediate
- 7 Difficult
- 99 Others (Specify in the 'Remarks' descriptor)

25. Fruit shape

To be recorded at marketable stage

- 1 Tapering/spindle shaped
- 2 Elliptical
- 3 Oblong
- 4 Long cylindrical
- 5 Top shaped
- 6 Globular
- 99 Others (Specify in the 'Remarks' descriptor)

26. Fruit surface

To be recorded in cross-section at marketable stage

- 1 Smooth
- 2 Light tubercle
- 3 Deep tubercle
- 99 Others (Specify in the 'Remarks' descriptor)

27. Nature of tubercles

To be recorded at marketable stage

- 3 Sparse
- 5 Medium
- 7 Dense
- 99 Others (Specify in the 'Remarks' descriptor)



Figure 42. Variation in fruit shape and size, surface nature of tubercles of bitter gourd germplasm

28. Blossom-end fruit shape

To be recorded at marketable stage

- 1 Blunt
- 2 Acute
- 99 Others (Specify in the 'Remarks' descriptor)

29. Fruit skin colour

To be recorded at marketable stage

- 1 White
- 2 Milky white
- 4 Light green
- 5 Green
- 6 Dark green
- 99 Others (Specify in the 'Remarks' descriptor)



8. Leaf size

To be recorded at full foliage stage

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the "Remarks" descriptor)

9. Days to 50% flowering

To be recorded from the date of sowing to the appearance of first flowering in 50% plants in a row.

Quantitative

10. Flower colour

To be recorded in the early morning when it is fully flowered

- 1 White
- 2 Whitish blue
- 3 Blue
- 4 Pink
- 5 Purple
- 99 Others (Specify in the "Remarks" descriptor)

11. First blossom node

To be recorded from ground level to the node which bears first flower

Quantitative

12. Days to first green pod picking

To be recorded from sowing date to date of marketable green pod

Quantitative

13. Days to last green pod picking

To be recorded from sowing date to date of last picking of marketable green pod

Quantitative

14. Number of pickings

Total number of marketable green pod pickings to be counted

Quantitative

15. Flowering habit

To be recorded at full flowering stage

- 1 Synchronous
- 2 Intermediate
- 3 asynchronous
- 99 Others (Specify in the "Remarks" descriptor)



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16. Number of primary branches per plant

To be recorded as total number of primary branches on a plant (average of 5 random plants)

Quantitative

17. Plant height (cm)

To be measured from the base of the plant (at ground level) to the tip of the main shoot (average of 5 random plants)

Quantitative

18. Number of clusters per plant

To be recorded at completion of pod formation stage (average of 5 random plants)

Quantitative

19. Pod length (cm)

To be recorded at completion of pod formation stage (average of 5 random plants)

Quantitative



Figure 47. Variation in pod length of pea germplasm

20. Number of pods per plant

To be recorded at the time of harvesting (average of 5 random plants)

Quantitative

21. Pod shape

To be recorded when the pod is fully matured

- 1 Blunt
- 2 Pointed
- 99 Others (Specify in the "Remarks" descriptor)

22. Pod thickness

To be recorded at fully grown green pod stage

- 3 Thin
- 5 Medium thick
- 7 Thick

23. Green pod yield per plant

To be recorded as average of 5 random plants

Quantitative

24. Number of seeds per pod

To be recorded on matured pod (average of 5 random pods)

Quantitative

25. Days to 80% maturity

To be recorded from the date of sowing to the date when 80% plants have complete mature pods in a row

Quantitative

26. Seed colour

To be recorded after harvesting

- 1 Cream
- 2 Light yellow
- 3 Whitish green
- 4 Green
- 99 Others (Specify in the "Remarks" descriptor)

27. Seed size

To be recorded at the time of maturity

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the "Remarks" descriptor)



Figure 48. Variation seed colour and seed size of pea germplasm



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28. Cotyledon colour

To be recorded within three months of harvesting

- 1 Light yellow
- 2 Yellow
- 3 Whitish green
- 4 Green
- 99 Others (Specify in the "Remarks" descriptor)

29. Seed surface

To be recorded after harvesting

- 1 Smooth
- 2 Wrinkled
- 99 Others (Specify in the "Remarks" descriptor)

30. Seed yield per plant (g)

To be recorded as average of 5 random plants

Quantitative

31. Protein content (%)

To be recorded on dry weight basis

Quantitative

32. 100 seed weight (g)

To be recorded as weight of hundred random seeds in grams (average of 5 random plants)

Quantitative

33. Biotic Stress Susceptibility

Specify the infestation or infection using any 1-9 scale

- 1 Very low or no visible sign of susceptibility
- 3 Low
- 5 Intermediate
- 7 High
- 9 Very high

Note: For additional information as common name(s) of disease(s)/pest(s) and casual organism(s) may be appended in the BIOTIC NOTE descriptor: 1 Very low or no visible sign of susceptibility, 3 Low, 5 Intermediate, 7 High, 9 Very high

6.10.25 Identity is monitored by comparing the accession with previous passport or morphological data.

6.10.26 All characterization information is to be verified by the head of the conservation unit of PGRC, BARI and the respective genebank manager.

6.10.27 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

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The characterization procedure is considered finalized when all the data has been uploaded to the PGRC's Genebank Documentation System.

7.10.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.10.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild pea genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.10.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild pea genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.10.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.



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10.10.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Aziz Zilani Chowdhury

CHAPTER 12

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Grasspea Germplasm in Bangladesh

Compiled and Edited by

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Grasspea genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Grasspea genetic resources management.

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6.11 Procedure for Grass Pea

The regeneration and characterization procedure of grass pea is initiated when the scientists handling the conservation unit of PGRC, BARI genebank review the data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of grass pea accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.11.1 Database review: Review of data and pertinent information on grass pea germplasm available in database (eg. seed stocks, seed viability, seed demand seed health status, characterization data, etc.)

6.11.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<90%) and inadequate amount of seeds (below 150g or 1,000 seeds) (Hanson and Street, 2008) or have not yet been characterized is generated for regeneration /characterization of grass pea accessions in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration.

➤ Each of these lists should include the following information:

- Accession number
- Full taxon name (Genus, Species, Subspecies)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly winter types, wild types and pollination type requiring special seed pre-treatments are to be flagged)

6.11.3 Seed sample preparation

- Seed foils/containers of grass pea germplasm from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



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- When monitoring procedure ascertains for a specific seed stock that viability in LTS is below international threshold value, then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A population size of at least 80-100 plants for each accession should be used for regeneration of landraces. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Unimproved landraces and accessions showing a large variation in seed colour and size are likely to be more variable. Larger population sizes should be used in order to maintain genetic variation within the accession.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity (ICARDA, 2021).
 - Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Country of origin
 - Number of seeds
 - If the seeds are treated with appropriate fungicide, the pulse crops can be saved from several soil- and seed-borne diseases. Seeds are treated with Provax-200 WP @ 2.5-3.0 g per kg, which minimize incidence of foot rot disease.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the sowing date.
- 6.11.4 Distribution of responsibilities:** As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.
- 6.11.5 Preparation of experimental register:** Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.11.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.11.7 Plot dimension:** Aim for a final plant number of 220 in plots about 10 m² (5.0 m × 2.0 m) at 40 cm × 10 cm spacing having 100 cm wide channel in between two plots.



6.11.8 Field layout: Prepare field layout hard copy according to experimental design for experimental field following seed sample preparation.

- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.11.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated grass pea germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and insect pests.

- The land should be free of volunteer plants and objectionable weeds. Select the land on which grass pea was not grown in the previous three years (Hanson and Street, 2008).
- Grass pea can be grown in all types of soil. Well drained land with loam or clay soils is best for cultivation of grass pea; avoid acid soils (Hanson and Street, 2008).
- Early enough before seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before sowing to remove weeds especially perennial weeds (Agrawal, 1999).
- The soil should be opened and allowed some time for aeration. The land is then ploughed and cross ploughed deeply 2-3 times followed by harrowing and leveling to prepare the land to desired tilth.

6.11.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for experimental field early enough before seed sowing. Information printed on the tag includes plot number and accession /collector's number.

6.11.11 Manure and Fertilizer doses: 15-15-18-10 kg/ha N-P-K-S (Ahmed *et al.*, 2018)

Method of application

- All N, P, K and S should be applied as basal during final land preparation.
- Rhizobium inoculation (@ 40 g/kg seed or 2 kg/ha) must be used if available, and in that case N fertilizer should not be used.

6.11.12 Seed sowing (e.g. sowing rate, method, etc.) and labelling

- Grass pea seeds are to be sown in 5 rows of 5.0 m long, with rows 40 cm apart and 10 cm spacing between plants, giving a density of 250 plants per plot.
- Sow the seeds in field plots directly at a depth of 4-5 cm. 8-10 hours seed soaking in fresh water enhances germination.



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- Time of seed sowing: Last week of October to first fortnight of November is the best time for sowing grass pea seeds (BARI, 2019).
- Spacing: Row to row- 40 cm; Plant to plant- 15 cm; between plots and block- 100cm.
- Placing the seeds to experimental field: Grass pea seeds in paper craft foils are brought from the short term storage area to the experimental field and placed to respective field plot according to field layout.
- No. of plants/hole: Allow two seeds per hole if enough seeds are available because not all of them will germinate. If there are only a few seeds, plant one seed per hole.
- Mark holes for sowing about 5 cm deep, 15 cm along the row. Sow one or two grass pea seeds by hand to a depth of 3.0 to 4.0 cm. Cover with soil and lightly compact the row. Keep the paper craft foils in respective field plot for final checking.
- Thinning: If direct sown, thin to one plant per hole at 2-6 weeks after establishment when plants are about 10 cm tall to give a plant density of about 220 plants per plot and avoid competition that will result in weak plants and low seed yields.
- Immediately after sowing of seed a final check is to be done for correspondence to the trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.11.13 Labeling

- Once seed sowing in the main experimental field is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and the accession /collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the experiment and its contents (i.e., title of the experiment, date of sowing, number of accessions, number of checks, experimental design, etc.).

6.11.14 Isolation requirement

- Grass pea is autogamous and considered predominantly self-pollinating. Rahman *et al.* (1995) reported up to 30% cross pollination.
- In view of this relatively high amount of out-crossing, an isolation distance of at least 20 m (preferably 50 m) between accessions of cultivated grass pea is recommended to maintain genetic integrity during regeneration.
- Cross pollination is done mainly by insects. Isolation of seed field is necessary for production of pure seed. The plot of one accession should be isolated from the plots of other accessions by 40 mesh polyvinyl net at flowering stage.

6.11.15 Weed management: The field should always be kept free of weeds.

- Early growth can be slow so weed by hand 4 weeks after establishment when seedlings are about 10-20 cm tall. Ensure field technicians know what young plants look like so they do not mistake them for weeds (Hanson and Street, 2008).
- Eliminate off-types and plants growing off-row.



- All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.11.16 Irrigation and drainage

- Overhead light irrigation with water can be applied if sufficient moisture is not available in the soil at 3-4 days after sowing for facilitating germination.
- Grass pea is hardy crop. Generally irrigation is not applied to the crop. Light irrigation may be required in severe moisture stress condition.
- Do not allow leaves to wilt at any stage and ensure soil is moist at time of flowering.
- Rapid drainage of excessive rain water is to be ensured.

6.11.17 Roguing

- The off-type plants and diseased plants severely affected by downy mildew should be rogued out from the seed field from time to time as required.
- Careful roguing at flowering and after pod formation need to be done. Off types and plants affected by downy mildew must be removed as soon as observed.

6.11.18 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology divisions of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Grass pea rarely suffers attacks from pests and diseases when grown in dry rainfed conditions.
- Spray with fungicide to control mildew during the rainy season or when using irrigation.
- Tilt 250 EC @ 0.5 ml/L or Sulphur fungicide like Theovit 80 WP or Kumulus 80 DF @ 2 g/L of water is to be applied 2-3 times at 7-10 days interval to control Downy mildew disease.
- Grass pea field may be infested by aphids. Tafgor 40 EC (Dimethoate) @ 1 ml/L of water is to be sprayed to control aphid.

6.11.19 Harvesting

- Prior to harvest second plastic tag same with the ones used for labeling the field plots are printed for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with plot number and accession number.
- When the pods start to turn brown and begin to dry but before fully ripe pods start to dehisce and shatter, the crops are to be harvested.



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- Accessions are harvested by cutting the stems at about 15-20 cm above the ground level (only the vines along with pods), and placed into the respective labeled plastic sacks. The plot tag is placed into the plastic sack and the new one prepared is firmly tightened outer of the sack and brought to the threshing floor. Collect the pods from each plant in labeled plastic sacks and dry under sunshine.

6.11.20 Seed threshing, cleaning and drying

- Thresh the pods on a tarpaulin by gently beating them when sufficiently dry; return the seeds to their labeled sacks.
- Ensure that seed mixing does not occur during threshing.
- The seeds are cleaned of debris by hand picking and hand winnowing.
- Hand pick over the seeds in trays to remove any shriveled, discoloured, infected or damaged seeds from each plant. Incinerate the waste to avoid spread of seed-borne diseases.
- The seeds must be dried to below 10% moisture content for sending to conservation unit for further drying and cleaning.

6.11.21 Fumigation: Threshed and cleaned seeds may be fumigated to prevent insect damage prior to processing to conservation.

6.11.22 Data validation and uploading onto PGRC, BARI Genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Number of rows per plot and seeding rate per plot
6. Row length, Row width, Plot to plot distance
7. Date of first effective rainfall or first irrigation

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe cold, hail storm, severe drought, inundation due to flood, severe storm, etc.)



5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. Total grain weight per plot (kg)

C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Plant growth habit
2. Pod pigmentation
3. Seed size
4. Seed pattern
5. Seed coat colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for grass pea accessions is considered finalized when threshed grains have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.11.23 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

- Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors (For Characterization and Evaluation) of Agri-Horticultural Crops (Part I), National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated grass pea germplasm (*Lathyrus sativus* L.)].



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1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum and minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:



Figure 49. Characterization of conserved accessions of grass pea

6.11.24 Descriptor List

Date of sowing: Date of Harvest:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Early plant vigour

To be recorded after 25 days of sowing.

- 1 Poor
- 2 Good
- 3 Very good

7. Plant growth habit

To be recorded at completion of vegetative stage

- 1 Erect
- 2 Semi-erect
- 3 Spreading
- 4 Bushy
- 99 Others (Specify in the "Remarks" descriptor)

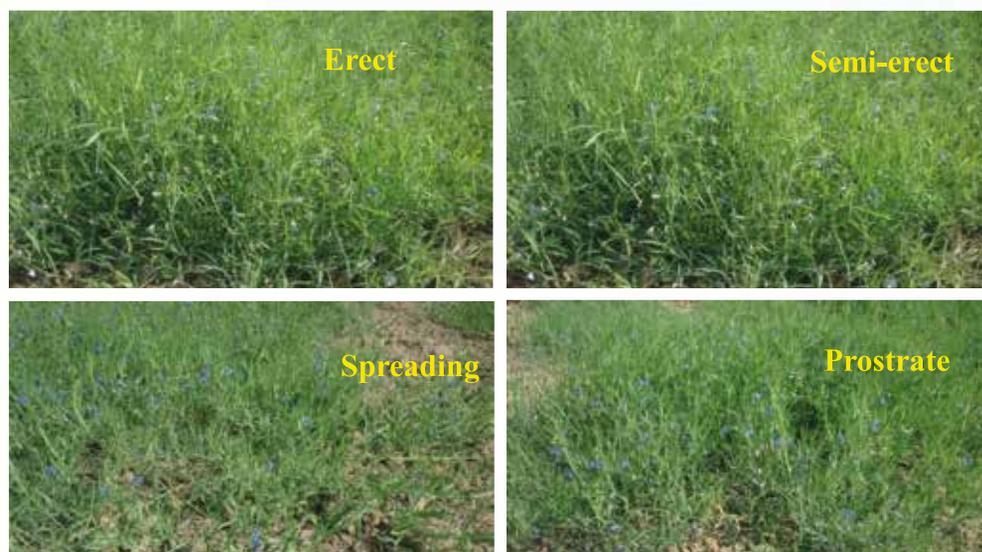


Figure 50. Variation in plant growth habit of conserved accessions of grasspea

8. Number of days to 50% flowering

To be recorded from date of sowing to the stage when 50% plants flowered in a row

Quantitative

9. Flower colour

To be recorded at the fully flowering stage

- 1 White
- 2 Whitish blue
- 3 Blue
- 4 Grey
- 5 Light yellow
- 6 Yellow
- 7 Pink
- 8 Orange
- 9 Red
- 10 Violet blue
- 11 Violet
- 99 Others (Specify in the "Remarks" descriptor)



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Grasspea at BARI

SOPs No.: PGRC-REG & CHAR_Grasspea010

Version: 1.0

SOPs Owner:
PGRC, BARI

SOPs Approver:
Crops Division, BARC

10. Leaf size (cm)

To be recorded on fully extended leaves

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the "Remarks" descriptor)



Figure 51. Variation in flower colour of conserved accessions of grass pea

11. Number of primary branches

To be recorded as total number *at* branches on main stem (average of 5 random plants)

Quantitative

12. Plant colour

To be recorded at full foliage stage

- 1 Light green
- 2 Dark green
- 3 Mottled
- 99 Others (Specify in the "Remarks" descriptor)

13. Pod pigmentation

To be recorded at fully developed pod stage

- 1 Uniformly green
- 2 Uniformly pigmented
- 3 Mottled
- 99 Others (Specify in the "Remarks" descriptor)

14. Pod length (cm)

To be recorded at complete maturity of pods

Quantitative

15. Pod shape

To be recorded when the pod is fully matured

- 1 Oblong elliptical
- 2 Medium oblong
- 3 Curved
- 4 Beaded
- 5 Broad linear
- 6 Broad elliptical
- 99 Others (Specify in the "Remarks" descriptor)

16. Number of pods per plant

To be recorded at completion of pod formation stage (average of 5 random plants)

Quantitative



Figure 52. Variation in pod shape of conserved accessions of grass pea

17. Number of seeds per pod

To be recorded at completion of pod formation stage (average of 5 random pods)

Quantitative

18. Plant height (cm)

To be measured from ground level to tip of the main shoot (average of 5 random plants)

Quantitative

19. Days to 80% maturity

To be recorded from date of sowing to date when 80% of plants in the row have attained maturity

Quantitative



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Grasspea at BARI

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Crops Division, BARC

20. Yield per plant (g)

To be recorded as average yield of 5 random plants in a row

Quantitative

21. 100 seed weight (g)

To be measured as weight of hundred random seeds (average of 5 random plants)

Quantitative

22. Seed coat colour

To be recorded within three months of harvesting

- 1 Grayish white
- 2 Yellow white
- 3 Grey
- 4 Brown
- 5 Yellow green
- 6 Pink
- 7 Red purple
- 8 Black
- 9 Grey mottle
- 10 Green mottle
- 99 Others (Specify in the "Remarks" descriptor)

23. Seed size

To be recorded within three months of harvesting

- 3 Small
- 5 Medium
- 7 Large
- 99 Others (Specify in the "Remarks" descriptor)



Figure 53. Variation in seed coat colour of conserved accessions of grass pea

24. Cotyledon colour

To be recorded within three months of harvesting

- 1 Yellow
- 2 Orange
- 99 Others (Specify in the "Remarks" descriptor)

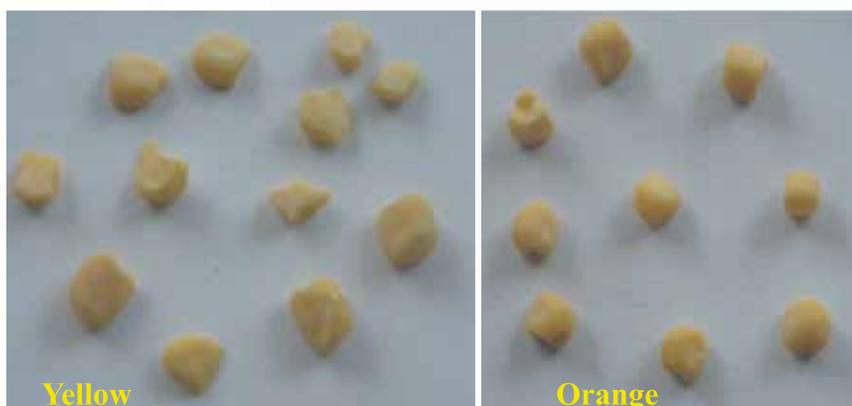


Figure 54. Variation in cotyledon colour of conserved accessions of grass pea

25. Protein content (%)

To be recorded on dry weight basis

Quantitative

26. Lathyrine content (%)

To be recorded as percentage of Lathyrine contents

Quantitative

27. Biotic Stress Susceptibility

Specify the infestation or infection using any 1-9 scale

- 1 Very low or no visible sign of susceptibility
- 3 Low
- 5 Intermediate
- 7 High
- 9 Very high

Note: For Additional information as common name(s) of disease(s)/pest(s) and casual organism(s) may be appended in the BIOTIC NOTE descriptor: 1 Very low or no visible sign of susceptibility, 3 Low, 5 Intermediate, 7 High, 9 Very high



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Grasspea at BARI

SOPs No.: PGRC-REG & CHAR_Grasspea010

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SOPs Approver:
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- 6.11.25 Identity is monitored by comparing the accession with previous passport or morphological data.
- 6.11.26 All characterization information is to be verified by the head of the conservation unit of PGRC, BARI and the respective genebank manager.
- 6.11.27 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to the PGRC's Genebank Documentation System.

7.11.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.11.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild pea genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.11.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild pea genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV



8.11.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.11.1 References

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SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Grasspea at BARI

SOPs No.: PGRC-REG & CHAR_Grasspea010 | Version: 1.0

SOPs Owner:
PGRC, BARI

SOPs Approver:
Crops Division, BARC

Rahman MM, Kumar J, Rahman MA, Afzal MA. 1995. Natural outcrossing in *Lathyrus sativus* L. Indian Journal of Genetics 55:204-207.

10.10.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Abdus Salam

CHAPTER 13

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Proso millet Germplasm in Bangladesh

Compiled and Edited by

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December 2024



First Edition, 2024

This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Proso millet genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Proso millet genetic resources management.

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Correct Citation

Ali MM, S. Rahman, Ahmed I, Hossain MG. 2024. Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Proso millet Germplasm in Bangladesh. In: Salam MA, Molla MR, Hossain MA (Eds.), Standard Operating Procedures for Regeneration, Characterization and Preliminary Evaluation of Some Agri-Horticultural Crops' Germplasm in Bangladesh. Crops Division, Bangladesh Agricultural Research Council (BARC), New Airport Road, Farmgate, Dhaka-1215, Bangladesh. pp. 219-234.



6.12 Procedure for Proso Millet

The regeneration and characterization procedure of proso millet is initiated when scientists handling the conservation unit of PGRC, BARI genebank review the data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of proso millet accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

- 6.12.1 Database review:** Review of data and pertinent information on proso millet germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.) (ICARDA, 2021)
- 6.12.2 Selection of accessions and list generation:** Based on notifications and using pertinent information from the database, list of accessions with lower viability (<80%) and inadequate amount of seeds (below 150g or 1,500 seeds) or have not yet been characterized is generated for regeneration/characterization of proso millet accessions in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.
- Each of these lists should include the following information:
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Germination (%) of last seed viability test
 - Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
 - Whether characterized or not
 - Pedigree history (only for the breeding and genetic stocks material)
 - Remarks (eg. strictly winter types, wild types and pollination type requiring special seed pre-treatments are to be flagged) (ICARDA, 2021)

6.12.3 Seed sample preparation

- Seed foils/containers of proso millet germplasm from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Proso millet at BARI

SOPs No.: PGRC-REG & CHAR_Proso millet011 | Version: 1.0

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SOPs Approver:
Crops Division, BARC

- When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value, then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
- Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A population size of at least 80-100 plant for each accession should be used for regeneration of landraces. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
- Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity (ICARDA, 2021).
- Seeds are placed in paper craft foils and identified with the following information:
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Country of origin
 - Number of seeds
- If the seeds are treated with appropriate fungicide, the pulse crops can be saved from several soil- and seed-borne diseases. Seeds are treated with Provax-200 WP @ 2.5-3.0 g per kg, which minimize incidence of foot rot disease (BARI, 2019).
- Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the sowing date.

6.12.4 Distribution of responsibilities: As per generated list, Head of the Centre, PGR, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.

6.12.5 Preparation of experimental register: Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.

6.12.6 Experimental design: Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.

6.12.7 Plot dimension: Aim for a final plant number of 240-300 in plots about 12 m² (4.0 m × 3.0 m) plots having 300 cm and 100 cm wide isolation/channels in between two plots for regeneration and characterization trials, respectively (Vilas *et al.*, 2015).

6.12.8 Field layout

- Prepare field layout hard copy according to experimental design for experimental field following seed sample preparation.



- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.12.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated proso millet germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes (ICARDA, 2021).

Proso millet can be cultivated in both rich and poor soils. Well drained loam or sandy loam soils rich in organic matter are ideal for cultivation. The selected land should be free from volunteer plants. The land should not be cultivated with same crop in the previous season (Vilas *et al.*, 2015).

- Early enough before seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the sowing date to remove the weeds especially perennial weeds.
- The soil should be opened and allowed some time for aeration. The land is then ploughed and cross ploughed deeply 2-3 times followed by harrowing and leveling to prepare the land to desired tilth (Agrawal, 1999).

6.12.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for experimental field early enough before seed sowing. Information printed on the tag includes plot number and accession/collector's number.

6.12.11 Manure and Fertilizer doses: 60-21-45-10-2.0 kg/ha N-P-K-S-Zn (Ahmed *et al.*, 2018)

Method of application

- a) Half of N and all of P, K, S and Zn should be applied as basal during final land preparation.
- b) Remaining N should be as top-dress in two equal splits after irrigation at 20 and 40 DAS.

6.12.12 Seed sowing (eg. sowing rate, method, etc.) and labeling

- Sow the seeds in field plots directly at a depth of 2.0-3.0 cm
- Time of seed sowing: Third week of November to First week of December is the best time for sowing proso millet seeds (BARI, 2019).
- Spacing: Row to row- 30 cm; Plant to plant- 10 cm; Plot to plot and Block to block- 300 cm for regeneration and 100 cm for characterization (Visal *et al.*, 2015).



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Proso millet at BARI

SOPs No.: PGRC-REG & CHAR_Proso millet011 | Version: 1.0

SOPs Owner:
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SOPs Approver:
Crops Division, BARC

- Placing the seeds to experimental field: Proso millet seeds in paper craft foils are brought from the short term storage area to the experimental field and placed onto respective field plot according to field layout.
- Mark holes for sowing about 3-4 cm deep, 10 cm along the row. Sow two or three proso millet seeds by hand to a depth of 2.0 to 3.0 centimeter. Cover the row with soil and lightly compact. Keep the paper craft foils in respective plot for final checking.
- Immediately after sowing of seed a final check is to be done for correspondence to the trial layout and any planting error is noted on the field layout hard copy.
- Thinning: Thin to one plant per hole at 2-6 weeks after establishment when plants are about 10 cm tall to give a plant density of about 240-300 plants per plot and avoid competition that will result in weak plants and low seed yields.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.12.13 Labeling

- Once seed sowing in the main experimental field is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and the accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of transplant, number of accessions, number of checks, experimental design, etc.).

6.12.14 Isolation requirement

- Proso millet is self-pollinating, but some cross-pollination may occur by the wind (Sheahan, 2014).
- Provide 300 cm isolation distance among the accessions (Vilas *et al.*, 2015).
- Avoid seed collection from boarder rows.

6.12.15 Weed management

- The field should always be kept free of weed. Two weeding and mulching at early plant growth stage may be required.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.
- Eliminate off-types and plants growing outside the row.

6.12.16 Irrigation: One to two light irrigation may be required during prolonged drought due to absence of rains.

6.12.17 Roguing: Roguing should be done often to remove the off-types, volunteer plants and diseased plants from the field to avoid the genetic contamination. Roguing should be done up to the flowering stage. Three times roguing at vegetative, flowering and maturity stage. The off-type plants should be removed from the seed field from time to time as required. This should continue during the flowering and fruiting stage also (Visal *et al.*, 2015).



6.12.18 Insect pest and disease management

- Incidence of insect pest and disease is almost nil in this crop. No fungicide is recommended for this crop.
- Incidence of wire worm and cut worm is noticed some time.
- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology divisions of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Furadan 5G @ 18 kg per hectare may be applied during seed sowing to control wire worm.
- Dursban 20 EC @ ml/L is to be drenched around affected seedling to control cut worm.

6.12.19 Harvesting

- Prior to harvest a second plastic tag same with the ones used for labeling the field plots is printed for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with plot number and accession number.
- Only panicles of accessions are harvested by hand and placed into the respective labeled plastic sacks. The plot tag is placed into the plastic sack and the new one prepared is firmly tightened outer of the sack.
- Crop should be harvested when the seed is ripe. This stage is determined visually from turning of the panicle to straw colour. Seeds mature from the top of the inflorescence to the bottom, and may shatter before harvesting (Baltensperger, 1996).
- Cut the crop when seeds or upper parts of the panicle are ripe and the plants are still green to avoid shattering of seed.

6.12.20 Threshing and cleaning: Seeds are threshed and winnowed on a tarpaulin very carefully to avoid mechanical admixture and return to labeled sacks. Then the seed must be dried to below 12% moisture content for sending to conservation unit.

6.12.21 Data validation and uploading onto BARI Genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Season



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Proso millet at BARI

SOPs No.: PGRC-REG & CHAR_Proso millet011 | Version: 1.0

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5. Date of sowing
6. Date of transplanting
7. Number of rows per plot and seeding rate per plot
8. Row length, Row width, Plot to plot distance
9. Date of first effective rainfall or first irrigation

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe cold, hail storm, severe drought, inundation due to flood, severe storm, etc.)
5. Any other event related to the accessions planted (e.g. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. Total grain weight per plot (kg)

C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Growth habit
2. Plant pigmentation
3. Inflorescence shape
4. Compactness of inflorescence
5. Grain colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for proso millet accessions is considered finalized when threshed grains have been cleaned from main impurities and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Bitter Gourd at BARI

SOPs No.: PGRC-REG & CHAR_Bitter Gourd007 | Version: 1.0

SOPs Owner:
PGRC, BARI

SOPs Approver:
Crops Division, BARC

30. Fruit skin lustre
To be recorded at marketable stage
 - 3 Matt
 - 5 Intermediate
 - 7 Glossy
 - 99 Others (Specify in the 'Remarks' descriptor)
31. Fruit bitterness
To be recorded at marketable stage
 - 3 Mild
 - 5 Moderate
 - 7 Strong
 - 99 Others (Specify in the 'Remarks' descriptor)
32. Vine length (cm)
To be measured at peak fruiting stage from ground level to the tip of main stem on 10 random plants
Quantitative
33. Number of primary branches
To be recorded as average of same 10 plants at the end of flowering stage. The branch that arises from the main vine/stem is known as primary branch
Quantitative
34. Days to first fruit harvest
To be recorded as number of days from date of sowing/transplanting to the date of first marketable fruit harvest
Quantitative
35. Days to last fruit harvest
To be recorded as number of days from date of sowing/transplanting to the date of last marketable fruit harvest
Quantitative
36. Number of marketable fruit harvest
To be recorded as total number of fruit pickings
Quantitative
37. Number of fruits per plant
To be recorded as average of same 10 plants
Quantitative
38. Yield of marketable fruits per plant (g)
To be recorded as average of cumulative yield of all pickings in same 10 plants
Quantitative
39. Fruit weight (g)
To be calculated on the basis of fruit yield and number of fruits per plant
Quantitative

40. Fruit length (cm)
To be recorded as average of 5-10 random fruits at marketable stage
Quantitative
41. Fruit width (cm)
To be recorded as average of same 5-10 fruits at marketable stage
Quantitative
42. Seediness
To be recorded at marketable stage
- 3 Low
 - 5 Medium
 - 7 High
 - 99 Others (Specify in the 'Remarks' descriptor)
43. Number of seeds per fruit
To be recorded as average of 5-10 random mature fruits
Quantitative
44. Seed lustre
To be recorded at marketable stage
- 3 Matt
 - 5 Intermediate
 - 7 Glossy
 - 99 Others (Specify in the 'Remarks' descriptor)

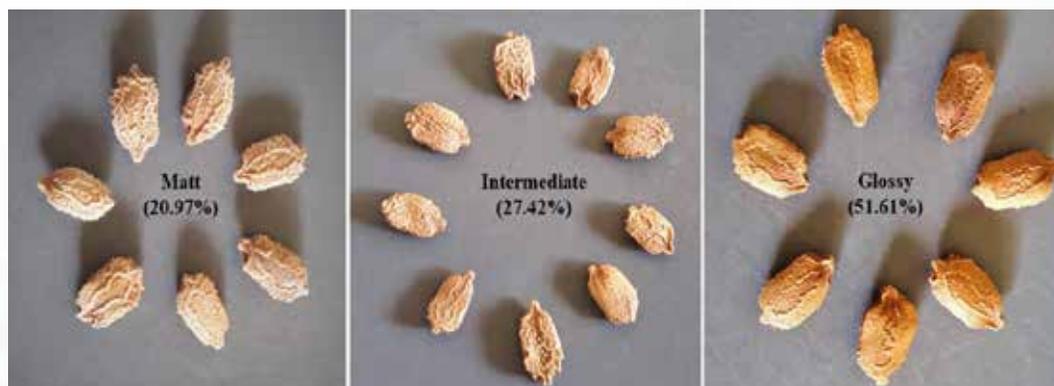


Figure 43. Variation in seed lustre of bitter gourd germplasm.

45. 100 seed weight (g)
To be measured as average weight of 100 random dry seeds
Quantitative



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Bitter Gourd at BARI

SOPs No.: PGRC-REG & CHAR_Bitter Gourd007 | Version: 1.0

SOPs Owner:
PGRC, BARI

SOPs Approver:
Crops Division, BARC

46. Biotic stress susceptibility

Specify the infestation or infection using any 1-9 scale

- | | |
|----|---|
| 1 | Very low or no visible sign of susceptibility |
| 3 | Low |
| 5 | Intermediate |
| 7 | High |
| 99 | Very high |

6.8.29 Identity is monitored by comparing the accession with previous passport or morphological data.

6.8.30 All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.

6.8.31 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.

7.8.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.8.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild ridge gourd genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.8.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild ridge gourd genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)

SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Bitter Gourd at BARI

SOPs No.: PGRC-REG & CHAR_Bitter Gourd007 | Version: 1.0

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- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.8.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.8.1 References

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SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Bitter Gourd at BARI

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Srivastava, Umesh RK, Mahajan KK, Gangopadhyay, Mahendra Singh, Dhillon BS. 2001. Minimal Descriptors of Agri-Horticultural Crops. Part II: Vegetable Crops. National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, ix + 262 p.

10.8.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Aziz Zilani Chowdhury

CHAPTER 10

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Chickpea Germplasm in Bangladesh

Compiled and Edited by

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Chickpea genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Chickpea genetic resources management.

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6.9 Procedure for Chickpea

The regeneration and characterization procedure of chickpea is initiated when the scientists handling the conservation unit of PGRC, BARI genebank review the data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of chickpea accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.9.1 Database review: Review of data and pertinent information on chickpea germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.9.2 Selection of accessions and list generation

- Based on notifications and using pertinent information from the database, list of accessions with viability lower than 75% (at PGRC, 85% is the standard) and inadequate amount of seeds (below 1,000) or have not yet been characterized is generated for regeneration /characterization of chickpea accessions in the current season.
- If more than 25% of seeds are infected by one or more of the following fungi: *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Macrophomina*, *Penicillium*, *Phoma*, *Rhizopus* spp. (Street *et al.*, 2008).
- When seed demand is high.
- These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.
- Each of these lists should include the following information:
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Germination (%) of last seed viability test
 - Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
 - Whether characterized or not
 - Pedigree history (only for the breeding and genetic stocks material)
 - Remarks (eg. cultivated types, wild types and pollination type requiring special seed pre-treatments are to be flagged).



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6.9.3 Seed sample preparation

- Seed foils/containers of chickpea germplasm from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.
- When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value, then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
- Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 100 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
- Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
- Seeds are placed in paper craft foils and identified with the following information:
 - Accession number
 - Full taxon name (Genus, Species, Subspecies)
 - Country of origin
 - Number of seeds
- If the seeds are treated with appropriate fungicide, the pulse crops can be saved from several soil- and seed-borne diseases. Seeds are treated with Provax-200 WP @ 2.5-3.0 g per kg, which minimize incidence of foot rot disease.
- Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials, placed in plastic trays and transferred to the short-term storage area to be kept until the sowing date (Street *et al.*, 2008).

6.9.4 Distribution of responsibilities: As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.

6.9.5 Preparation of experimental register: Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.



6.9.6 Experimental design: Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.

6.9.7 Plot dimension: 4.0 m × 2.5 m plots having 100 cm wide channel in between two plots.

6.9.8 Field layout

- Prepare field layout hard copy according to experimental design for experimental field following seed sample preparation.
- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.9.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated chickpea germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and insect pests.

- The land should be well-drained, free of volunteer plants and objectionable weeds having a good reserve of soil moisture. For best results, plant in normal soil types, with pH 7.5.
- Select the land on which chickpea was not grown in the last three years. This is necessary to avoid soil borne diseases especially foot rot. The soil of the selected field should be clay to clay loam in texture and well drained (Agrawal, 1999).
- Early enough before seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by two or three harrowing should also be done one or two weeks before the sowing date to produce a fine tilth and an even, flat seedbed, and to remove the weeds especially perennial weeds.

6.9.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for experimental field early enough before seed sowing. Information printed on the tag includes the plot number, block number and accession /collector's number.

6.9.11 Fertilizer doses: 27-18-24-12-2.0-1.2-0.8 kg/ha N-P-K-S-Zn-B-Mo (Ahmed *et al.*, 2018).

Method of application

- All fertilizers should be applied as basal during final land preparation.
- Rhizobium inoculation (@ 50 g/kg seed or 1.5 kg/ha) must be used if available and in that case N fertilizer should not be used.



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6.9.12 Seed sowing (eg. sowing rate, method, etc.) and labelling

- Chickpea seeds are to be sown in 5 rows of 4 m long, with rows 50 cm apart and 10 cm spacing between plants, giving a density of 200 plants per plot.
- Time of seed sowing: Third week of November to First week of December is the best time for sowing chickpea seeds (BARI, 2019).
- Placing the seeds to experimental field: Chickpea seeds in paper craft foils are brought from the mid-term storage area to the experimental field and placed to respective field plot according to field layout.
- Keep the paper craft foils in respective field plot for final checking.
- No. of plants/hole: Count the number of seeds to be planted per row and place in separate envelopes/bags. Allow two seeds per hole if enough seeds are available because not all of them will germinate. If there are only a few seeds, sow one seed per hole to a depth of 3.0 to 4.0 centimeter, cover with soil and lightly compact the row. Finally keep one seedling per hole.
- Immediately after sowing of seed a final check is to be done for correspondence to the trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with stakes as soon as final check is confirmed (ICARDA, 2021).

6.9.13 Labeling

- Once seed sowing in the main experimental field is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number, block number and the accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e. title of the experiment, number of accessions, number of checks, experimental design, date of transplanting and implementing centre, etc.).

6.9.14 Isolation requirement

- Chickpea flowers are normally self-pollinated before they open. Cross pollination is done mainly by insects (Purseglove 1968).
- Isolation of seed field is necessary for production of pure seed. An isolation distance of 10 m is required for foundation seed, which cannot be given because of land scarcity. This is not feasible in regeneration trial, because of land constraints (Agrawal, 1999).
- The plot of one accession should be isolated from the plots of other accessions by 40 mesh polyvinyl net at flowering stage.

6.9.15 Weed management: The field should always be kept free of weeds.

- One hand weeding should be done within 30-35 days after sowing. Second weeding may be required at 50-55 days after sowing depending on weed infestation.



- All weeds between plots and within the alley ways are controlled using spade and bush cutter.
- Eliminate off-types and plants growing outside the row.

6.9.16 Irrigation and drainage

- Overhead light irrigation with water can is to be applied if sufficient moisture is not available in the soil immediately after sowing for facilitating germination.
- Apply supplementary irrigation 10 days after sowing if no rain occurs, to ensure adequate seed yield. Plants should not become so water-stressed that flower or pod abortion occurs or pod-filling is impeded.
- Avoid excessive soil moisture during the cropping season. Rapid drainage of excessive rain water is to be ensured.

6.9.17 Roguing: The off-type plants and diseased plants affected by blight and wilt should be removed from the seed field from time to time as required. This should continue during the flowering and fruiting stage also (Agrawal, 1999).

6.9.18 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology divisions of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Botrytis grey mold (BGM) disease of chickpea is controlled mainly through thinning out of overcrowded plants, roguing out of infected plants and burning of plant debris.
- Virtako 40 WG @ 0.15g per litre of water is to be sprayed at 7-10 days interval in case of severe infestation of pod borer.

6.9.19 Harvesting

- Prior to harvest second plastic tag same with the ones used for labelling the field plots are printed for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with plot number and accession number.
- Crop should be harvested when the pods are dry, i.e., pods rattle when shaken. Older leaves turn yellow and drop, indicating maturity.
- Accessions are harvested by cutting the plants at about 15-20 cm above the ground level (only the shoots along with pods), and placed into the respective labeled plastic sacks. The plot tag is placed into the plastic sack and the new one prepared is firmly tightened outer of the sack and brought to the threshing floor and dried under sunshine.



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Figure 44. Regeneration of conserved accessions of chickpea

6.9.20 Seed threshing and drying

- Threshing of seeds is done by beating with sticks when sufficiently dry.
- Care should be taken during threshing so that the seed coats are not injured.
- Threshed seeds are then taken out of the plastic sacks carefully with plot tag and kept in plastic bowl.
- The seeds are cleaned by hand winnowing very carefully to avoid mechanical admixture.
- Remove remaining debris by hand.
- Then the plastic bowls with seeds are placed in threshing floor for drying under sunshine. Silica gel or another appropriate desiccant may be used for seed drying to desirable moisture content.
- Determine total weight of cleaned seeds.
- Determine 100-seed weight.
- The seeds must be dried to below 8% moisture content for sending to conservation unit for further drying and cleaning.

6.9.21 Fumigation: If signs of insect attack are detected, threshed and cleaned seeds may be fumigated to prevent insect damage prior to processing to conservation. However, this is not generally recommended, especially for long-term storage.

6.9.22 Data validation and uploading onto PGRC, BARI Genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:



A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Season
5. Date of sowing
6. Date of transplanting
7. Number of rows per plot and seeding rate per plot
8. Row length, Row width, Plot to plot distance
9. Date of first effective rainfall or first irrigation

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (e.g. heavy rain, severe cold, hail storm, severe drought, inundation due to flood, severe storm, etc.)
5. Any other event related to the accessions planted (e.g. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. Total grain weight per plot (kg).

C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Growth habit.
2. Plant pigmentation
3. Flower colour.
4. Seed colour.
5. Seed shape.

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.



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Note:

1. The regeneration procedure for chickpea accessions is considered finalized when threshed grains have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.9.23 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

➤ Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors (For Characterization and Evaluation) of Agri-Horticultural Crops (Part I), National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated chickpea germplasm (*Cicer arietinum* L.)] (Mahajan, *et al.*, 2000).

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:

6.9.24 Descriptor List

Date of sowing: Date of Transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....

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6. Early plant vigour
To be measured at seedling stage (25 days after sowing)
 - 1 Poor
 - 2 Good
 - 3 Very good
7. Plant growth habit
To be recorded at completion of vegetative stage
 - 1 Erect (0-15 degrees from vertical)
 - 2 Semi-erect (16-25 degrees from vertical)
 - 3 Semi-spreading (26-60 degrees from vertical)
 - 4 Spreading (61-80 degree from vertical)
 - 5 Prostrate
 - 99 Others (Specify in the "Remarks" descriptor)
8. Plant pigmentation
To be measured at seedling stage (30 days after sowing)
 - 1 No anthocynine, stem and leaves pale green
 - 3 No anthocynine, stem and leaves green
 - 5 Low anthocynine, stem and leaves light purple
 - 7 High anthocynine, stem & leaves predominantly purple
 - 9 Highly purple
 - 99 Others (Specify in the "Remarks" descriptor)
9. Number of leaflets per leaf
To be measured at seedling stage (45 days after sowing)
 - 1 5-7
 - 2 7-9
 - 3 9-10
 - 4 11-13
 - 5 13
 - 99 Others (Specify in the "Remarks" descriptor)
10. Leaflet size
Size of basal pair of leaflets at foliage stage
 - 1 Small (mm long, mm width)
 - 2 Medium (10-15 mm long, 4-12 mm width)
 - 3 Large (15 mm long, 12 mm width)
 - 99 Others (Specify in the "Remarks" descriptor)
11. Plant pubescence
To be observed on stem, leaves and pods at seedling stage (45 days after sowing),
at foliage stage
 - 1 Glabrous
 - 3 Light pubescent
 - 4 Pubescent
 - 7 Dense
 - 99 Others (Specify in the "Remarks" descriptor)



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12. Days to 50% flowering

Number of days from sowing to the day when 50% plants in a row flowered

Quantitative

13. Flower colour

To be recorded at peak flowering stage

- 1 Light blue
- 2 Blue
- 3 Dark blue
- 4 Light pink
- 5 Pink
- 6 White
- 7 White pink striped
- 99 Others (Specify in the "Remarks" descriptor)

14. Number of primary branches per plant

To be recorded as total number of primary branches on main stem (average of 5 random plants)

Quantitative

15. Biomass

To be recorded at mid-pod filling stage

- 1 Low
- 2 Heavy
- 99 Others (Specify in the "Remarks" descriptor)

16. Plant height (cm)

To be recorded at maturity stage (average of 5 random plants)

Quantitative

17. Number of pods per plant

To be recorded at the time of harvest (average of 5 random plants)

Quantitative

18. Pod shape

To be recorded at complete maturity of pods

- 1 Oblong
- 2 Round
- 3 Cylindrical
- 99 Others (Specify in the "Remarks" descriptor)

19. Number of seeds per pod

To be recorded as average of 5 random plants

Quantitative

20. Days of 80% maturity

To be recorded from sowing date to 80% of the pod turned brown and attained maturity

Quantitative

21. Seed colour

To be observed from mature seeds stored not longer than three months

- 1 Black
- 2 Brown
- 3 Light brown
- 4 Dark brown
- 5 Reddish' brown
- 6 Grayish brown
- 7 Salmon brown
- 8 Grey
- 9 Brown beige
- 10 Beige
- 11 Yellow
- 12 Light yellow
- 13 Yellow brown
- 14 Orange yellow
- 15 Orange
- 16 Yellow beige
- 17 Ivory white
- 18 Green
- 19 Light green
- 20 Variegated
- 99 Others (Specify in the "Remarks" descriptor)



Black



Brown



Light brown



Yellow brown



Dark brown



Ivory white



Reddish brown

Figure 45. Variation in seed colour of conserved accessions of chickpea



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22. Seed shape

To be recorded at maturity

- 1 Angular ram's head (desi)
- 2 Irregular rounded owl's head (kabuli)
- 3 Pea shaped, smooth round
- 99 Others (Specify in the "Remarks" descriptor)

23. Seed surface (Testa texture)

To be recorded at maturity

- 3 Rough
- 5 Smooth
- 7 Tuberculated
- 99 Others (Specify in the "Remarks" descriptor)

24. Grain yield per plant (g)

To be measured as average of 5 random plants at maturity

Quantitative

25. 100 seed weight (g)

To be measured as weight of 100 matured and random seeds (average of 5 random plants)

Quantitative

26. Protein content (%)

To be measured from matured and dry seeds

Quantitative

27. Biotic Stress Susceptibility

Specify the infestation or infection using any 1-9 scale.

- 1 Very low or no visible sign of susceptibility
- 3 Low
- 5 Intermediate
- 7 High
- 9 Very high

Note: For additional information as common name(s) of disease(s)/pest(s) and casual organism(s) may be appended in the BIOTIC NOTE descriptor: 1 Very low or no visible sign of susceptibility, 3 Low, 5 Intermediate, 7 High, 9 Very high

6.9.25 Identity is monitored by comparing the accession with previous passport or morphological data.

6.9.26 All characterization information is to be verified by the head of the conservation unit of PGRC, BARI and the respective genebank manager.

6.9.27 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.



The characterization procedure is considered finalized when all the data has been uploaded to the PGRC's Genebank Documentation System.

7.9.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.9.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated and wild chickpea genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.9.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated and wild chickpea genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.9.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Chickpea at BARI

SOPs No.: PGRC-REG & CHAR_Chickpea008

Version: 1.0

SOPs Owner:
PGRC, BARI

SOPs Approver:
Crops Division, BARC

9.9.1 References

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10.9.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Aziz Zilani Chowdhury

CHAPTER 11

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Pea Germplasm in Bangladesh

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Pea genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Pea genetic resources management.

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6.10 Procedure for Pea

The regeneration and characterization procedure of pea is initiated when the scientists handling the conservation unit of PGRC, BARI genebank review the data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of pea accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.10.1 Database review: Review of data and pertinent information on pea germplasm available in database (eg. seed stocks, seed viability, seed demand, seed health status, characterization data, etc.)

6.10.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<85%) and inadequate amount of seeds (below 1,000) or have not yet been characterized is generated for regeneration/characterization of pea accessions in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

➤ Each of these lists should include the following information:

- Accession number
- Full taxon name (Genus, Species, Sub-species)
- Germination (%) of last seed viability test
- Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
- Whether characterized or not
- Pedigree history (only for the breeding and genetic stocks material)
- Remarks (eg. strictly winter types, strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged)

6.10.3 Seed sample preparation

- Seed foils/containers of pea germplasm from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.
- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Pea at BARI

SOPs No.: PGRC-REG & CHAR_Pea009

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- When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value, then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A minimum of 160 individuals for each accession is required to be established at the field for landraces and wild relative species. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity (ICARDA, 2021).
 - Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Country of origin
 - Number of seeds
 - If the seeds are treated with appropriate fungicide, the pulse crops can be saved from several soil- and seed-borne diseases. Seeds are treated with Provax-200 WP @ 2.5-3.0 g per kg, which minimize incidence of foot rot disease.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the sowing date.
- 6.10.4 Distribution of responsibilities:** As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.
- 6.10.5 Preparation of experimental register:** Experimental register is prepared enlisting all operations to be done and all information to be recorded from seed bed preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.10.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.10.7 Plot dimension:** 3.6 m × 2.8 m plots having 100 cm wide and 10-15 cm deep drain in between two plots. One hundred sixty-eight seedlings will be allowed to grow per plot at 40 cm × 15 cm spacing.
- 6.10.8 Field layout**
- Prepare field layout hard copy according to experimental design for experimental field following seed sample preparation.

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- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.10.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated pea germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

Field is to be selected based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different diseases and insect pests. Peas should not be grown consecutively even for two years in the same land as it will favour seedling diseases, blight and also pea nematodes. It is best rotated with cereals (Rashid and Singh, 2000).

- The land should be free of volunteer plants and objectionable weeds. Select the land on which pea was not grown in the previous season. This is necessary to avoid soil borne diseases especially seedling diseases and pea nematodes.
- Pea can be grown in all types of soil. The crop is best adapted to well drained clay loam soils well supplied with calcium. Soil pH should be in the range of 6.0-7.7 (Rashid and Singh, 2000).
- Early enough before seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the sowing date to remove the weeds especially perennial weeds Agrawal, 1999).
- Thorough preparation of soil is essential for pea because it is an exhaustive and short duration crop. It helps the rapid and free spread of roots. The soil should be opened and allowed some time for aeration. The land is then ploughed and cross ploughed deeply 2-3 times and leveled to facilitate sowing. Large clods are to be broken by harrowing, finally the land should be ploughed to fine tilth.

6.10.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for experimental field early enough before seed sowing. Information printed on the tag includes field plot number and accession /collector's number.

6.10.11 Manure and Fertilizer doses: 45-24-30-12-1.4 kg/ha N-P-K-S-Zn and FYM @ 3 t/ha (Ahmed *et al.*, 2018)

Method of application

- a) All FYM, P, K, S, and Zn, and one-third of N should be applied as basal during final land preparation.
- b) Remaining N should be side dressed in two equal splits at 20 and 35 DAS under moist soil condition and mixed thoroughly with soil as soon as possible for better utilization.



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6.10.12 Seed sowing (eg. sowing rate, method, etc.) and labelling

- Pea seeds are to be sown in 40 cm apart rows of the field plots directly at a depth of 2.0-2.5 cm (BARI, 2006).
- Seed soaking: 8-10 hours seed soaking in fresh water enhances germination.
- Time of seed sowing: First to third week of November is the best time for sowing pea seeds (BARI, 2019).
- Spacing: Row to row- 40 cm; Plant to plant- 15 cm; plot to plot- 100 cm
- Placing the seeds to experimental field: Pea seeds in paper craft foils are brought from the short term storage area to the experimental field and placed to respective field plot according to field layout.
- No. of plants/hole: Sow one or two pea seeds per hole at a depth of 2.0 to 2.5 cm. Allow one healthy seedling per hole to grow and remove extra ones at 4-5 leaf stage (BARI, 2019). Keep the paper craft foils in respective field plot for final checking.
- Immediately after sowing of seed a final check is to be done for correspondence to the trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.10.13 Labeling

- Once seed sowing in the main experimental field is finished, each individual field plot is labeled with a water-proof thermal plastic tag. Information printed on the tag includes plot number and the accession/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the experiment and its contents (i.e., title of the experiment, date of sowing, number of accessions, number of checks, experimental design, etc.).

6.10.14 Isolation requirement

- Pea is largely self-pollinated crop with a little natural crossing. Cross pollination is done mainly by insects.
- Isolation of seed field is necessary for production of pure seed.
- The isolation distance for peas is relatively short and aims mainly to avoid mechanical mixtures. However, the isolation distance may be at least 20 meter from one accession to another (Rashid and Singh, 2000). This is not feasible in regeneration trial, because of land constraints.
- Hence, the plot of one accession should be isolated from the plots of other accessions by 40 mesh polyvinyl net at flowering stage.

6.10.15 Weed management:

- The field should always be kept free of weeds. One hand weeding should be done within 30-35 days after sowing.



- All weeds between plots and within the alley ways are controlled using spade and bush cutter.

6.10.16 Irrigation and drainage

- Overhead light irrigation with water can is to be applied if sufficient moisture is not available in the soil at 3-4 days after sowing for facilitating germination.
- Irrigation should be provided as and when needed. Abundant water supply during flowering should be ensured.
- Late irrigation during warm weather should be avoided which may cause sun-scalding of plants as also plants may tend to lodge and some rotting of vines may occur if the soil is kept too wet (Rashid and Singh, 2000).
- Rapid drainage of excessive rain water is to be ensured.

6.10.17 Roguing:

- Careful roguing at flowering and after pod formation need to be done. Off types and plants affected by blight and pea mosaic must be removed as soon as observed.

6.10.18 Insect pest and disease management: The crop should always be kept free of insect pests and diseases adopting recommended management practices.

- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology divisions of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Sulphur fungicide like Theovit 80 WP or Kumulus 80 DF @ 2 g/L of water is to be applied 2-3 times at 7-10 days interval to control powdery mildew disease.
- In rust disease affected field Tilt 250 EC or Folicur 250 EC @ 1 ml/L of water is to be applied 2-3 times at 7-10 days interval to control the disease.
- During preliminary stage of hairy caterpillar infestation, affected leaves with larva are to be destroyed or buried under soil. Ripcord 10 EC @ 1 ml/L of water is to be sprayed if required.

6.10.19 Harvesting

- Prior to harvest second plastic tag same with the ones used for labeling the field plots are printed for each accession, including information on plot number, accession number. Appropriate plastic sacks, which provide ample ventilation, are also prepared and labeled with plot number and accession number.
- Harvesting of mature pods should be done when 80% of the plants dried and 90% of the pods turn brown. This stage is determined visually from the loss of green colour of the plants, which look like dried straw (Agrawal, 1999).
- To test the maturity a common practice is to squeeze the seed between fingers. If the cotyledons break away from each other and free moisture is not visible, the crop may be considered mature enough for harvest (Rashid and Singh, 2000).



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Pea at BARI

SOPs No.: PGRC-REG & CHAR_Pea009

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- Accessions are harvested by cutting the plants at about 15-20 cm above the ground level (only the vines along with pods), and placed into the respective labeled plastic sacks. The plot tag is placed into the plastic sack and the new one prepared is firmly tightened outer of the sack and brought to the threshing floor and dried under sunshine keeping inside of the plastic sacks.



Figure 46. Regeneration of pea germplasm

6.10.20 Seed threshing and drying

- Threshing of seeds is done by beating with sticks when sufficiently dry.
- Care should be taken during threshing so that the seed coats are not injured.
- Threshed seeds are then taken out of the plastic sacks carefully with plot tag and kept in plastic bowl.
- The seeds are cleaned by winnowing very carefully to avoid mechanical admixture.
- Then the plastic bowls with clean seeds are placed in threshing floor for drying under sunshine.
- The seeds must be dried to below 10% moisture content for sending to conservation unit for further drying and cleaning.

6.10.21 Fumigation: Threshed and cleaned seeds may be fumigated to prevent insect damage prior to processing to conservation.

6.10.22 Data validation and uploading onto PGRC, BARI Genebank Documentation System: Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Date of sowing
5. Number of rows per plot and seeding rate per plot
6. Row length, Row width, Plot to plot distance
7. Date of first effective rainfall or first irrigation



B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe cold, hail storm, severe drought, inundation due to flood, severe storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. Total grain weight per plot (kg)

C. Comparisons with previous passport or morphological data

- Compare each accession with the following characterization data previously recorded for the accession:
 1. Growth habit.
 2. Flower colour.
 3. Pod thickness.
 4. Pod shape
 5. Seed colour.
 6. Seed size.
 7. Cotyledon colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for pea accessions is considered finalized when threshed grains have been cleaned from main impurities, fumigated and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.10.23 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Pea at BARI

SOPs No.: PGRC-REG & CHAR_Pea009

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- Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [NBPGR descriptors: Minimal Descriptors (For Characterization and Evaluation) of Agri-Horticultural Crops (Part I), National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi, India is used for characterization of cultivated pea germplasm (*Pisum sativum*)].

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:

6.10.24 Descriptor List

Date of sowing: Date of Transplanting:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....

6. Early plant vigour

To be recorded at 25 days after sowing

- 1 Poor
- 2 Good
- 3 Very good

7. Plant growth habit

To be recorded at completion of vegetative stage

- 1 Erect
- 2 Semi-erect
- 3 Spreading
- 4 Bushy
- 99 Others (Specify in the "Remarks" descriptor)

6.12.22 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

- Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards (IBPGR. 1985. Descriptors for *Panicum miliceum* and *P. sumatrense*. International Board for Plant Genetic Resources, Rome, Italy. 14p.) and Guidelines for the conduct of tests for Distinctiveness, Uniformity and Stability: Proso millet (*Panicum miliaceum* L.) (Nagehara *et al.*, 2001).

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:
3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:



Figure 55. Morphological characterization of conserved accessions of proso millet



6.12.23 Descriptor List

Date of sowing: Date of Harvest:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Growth habit

To be recorded at 2-4 leaf stage

- 1 Erect
- 2 Erect geniculate
- 3 Decumbent
- 4 Prostrate
- 99 Others (Specify in the "Remarks" descriptor)



Figure 56. Variation in plant growth habit of conserved proso millet accessions

7. Plant height (cm)
To be recorded at the completion of flowering stage (Measure from ground level to tip of inflorescence; in case of decumbent or prostrate plants, length of flowering culm from rooted base)
Quantitative
8. Plant pigmentation at leaf sheath
To be recorded at the completion of flowering stage
0 Not pigmented (green)
1 Pigmented
9. Number of basal tillers
To be recorded at vegetative stage (No. of tillers at ground level or from the basal nodes)
3 Low (<5)
5 Medium (5-15)
7 High (>15)



10. Culm branching
To be recorded at dough stage (Number of culm branches on the main stem)
 - 0 Absent
 - 1 Present
11. Blade length of flag leaf (cm)
To be recorded at the completion of flowering stage (Measured from ligule to tip)
 - 3 Short (<20 cm)
 - 5 Medium (20-35 cm)
 - 7 Long (>35 cm)
12. Blade width of flag leaf (cm)
To be recorded at the completion of flowering stage (Measured at widest point)
 - 3 Narrow (<1.5 cm)
 - 5 Medium (1.5-2.5 cm)
 - 7 Wide (>2.5 cm)
13. Blade pubescence
To be recorded at the completion of flowering stage
 - 1 Essentially glabrous
 - 5 Medium pubescent
 - 7 Strongly pubescent
14. Sheath length of flag leaf (mm)
To be recorded at the completion of flowering stage (measured from internode to ligule)
Quantitative
15. Sheath pubescence
To be recorded at the completion of flowering stage
 - 1 Essentially glabrous
 - 5 Medium pubescent
 - 5 Strongly pubescent
16. Ligule pubescence
To be recorded at the completion of flowering stage
 - 1 Essentially glabrous
 - 5 Medium pubescent
 - 7 Strongly pubescent
17. Degree of lodging at maturity
To be recorded at maturity stage
 - 3 Slight
 - 5 Medium
 - 7 Extensive
18. Senescence
Degree to which plant is still green at time the primary inflorescence on each culm (tiller) reaches maturity
 - 1 Actively growing
 - 6 Dead



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Proso millet at BARI

SOPs No.: PGRC-REG & CHAR_Proso millet011 | Version: 1.0

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Crops Division, BARC



Figure 57. Variation in senescence of proso millet accessions

19. Length of peduncle (cm)
To be recorded at completion of flowering stage (Measured from node to base of panicle)
 - 1 Very short (<10.0 cm)
 - 3 Short (10.0-20.0 cm)
 - 4 Medium (20.1-30.0 cm)
 - 5 Long (30.1-40.0 cm)
 - 7 Very long (>40.0 cm)
20. Peduncle exertion (mm)
Measured from the exposed point of the peduncle from the leaf sheath up to base of the inflorescence
Quantitative
21. Length of panicle (cm)
To be recorded at dough stage (Measured from lowest branch to tip of last branch of inflorescence)
 - 1 Very short (<10.0 cm)
 - 3 Short (10.0-20.0 cm)
 - 4 Medium (20.1-30.0 cm)
 - 5 Long (30.1-40.0 cm)
 - 7 Very long (>40.0 cm)
22. Number of primary inflorescence branches
To be recorded at dough stage (Counted as number of branches originating from the primary axis of the inflorescence)
Quantitative
23. Number of nodes per primary axis of inflorescence
To be recorded at dough stage (Average of 5 plants)
Quantitative
24. Number of secondary inflorescence branches
To be recorded at dough stage (Counted as number of major branches on primary axis of the inflorescence)
Quantitative
25. Inflorescence shape
To be recorded at dough stage
 - 3 Diffused
 - 5 Arched
 - 7 Globose-elliptic



Globose

Diffused

Arched

Figure 58. Variation in inflorescence shape of proso millet accessions.

26. Compactness of inflorescence

To be recorded at dough stage [Each of the inflorescence types (descriptor 20) ranges from open to very compact]

- 3 Open
- 5 Intermediate
- 7 Compact



Open

Intermediate

Compact

Figure 59. Variation in inflorescence compactness of proso millet accessions

27. Seed shattering

To be recorded at mature stage

- 1 Absent
- 9 Present

28. Grain colour

To be recorded at mature stage

- 2 Straw white/cream
- 3 Golden yellow
- 5 Grey
- 7 Dark grey



Cream

Golden yellow

Grey

Dark grey

Figure 60. Variation in grain colour of proso millet accessions



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Proso millet at BARI

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29. Grain shape
To be recorded at post-harvest stage
- 2 Elliptical
 - 4 Oval
30. Length of grain (mm)
To be recorded at post-harvest stage
Quantitative
31. Width of grain (mm)
To be recorded at post-harvest stage
Quantitative
32. Days to flowering
Counted as days from sowing to 50% of plants in flower
Quantitative

FURTHER CHARACTERIZATION AND EVALUATION

33. Uniformity of population maturity (%)
Percentage of plants mature at harvest
Quantitative
34. Uniformity of individual plant maturity (%)
Percentage of inflorescences mature on raceme at full maturity
Quantitative
35. Shattering of inflorescence (%)
Percentage of spikelets remaining on raceme at full maturity
Quantitative
36. Yield of grain (kg/ha)
Quantitative
37. Yield of straw for fodder (kg/ha)
Quantitative
38. Plant aspect
Overall agronomic eliteness of the accession
- 1 Very poor
 - 3 Poor
 - 5 Average
 - 7 Good
 - 9 Very good
39. Biotic Stress Susceptibility
Specify the infestation or infection using any 1-9 scale
- 1 Very low or no visible sign of susceptibility
 - 3 Low
 - 5 Intermediate
 - 7 High
 - 9 Very high



- 6.12.24 Identity is monitored by comparing the accession with previous passport or morphological data.
- 6.12.25 All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.
- 6.12.26 Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.

7.12.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild PGRs of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).

7.12.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated proso millet genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.12.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated proso millet genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.12.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.



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- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of work.

9.12.1 References

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10.12.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Aziz Zilani Chowdhury

CHAPTER 14

Standard Operating Procedures (SOPs) for Regeneration, Characterization and Preliminary Evaluation of Foxtail millet Germplasm in Bangladesh

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This SOPs shall be applied to the regeneration, characterization and preliminary evaluation of Foxtail millet genetic resources maintained in genebank of different research institutes and agricultural universities. The scope of this SOPs is to outline the step-by-step instructions to guide the scientists and technical assistants of different research institutes and universities involved in Foxtail millet genetic resources management.

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6.13 Procedure for Foxtail Millet

The regeneration and characterization procedure of foxtail millet is initiated when the scientists handling the conservation unit of PGRC, BARI genebank review the data and pertinent information in the database, based primarily on seed viability and seed quantity thresholds of foxtail millet accessions in the active and base collections, as well as on the status (in terms of viability and quantity) of the newly acquired accessions. This step marks the beginning of the cropping season and triggers the initiation of the following regeneration activities:

6.13.1 Database review: Review of data and pertinent information on foxtail millet germplasm available in database (eg. seed stocks, seed viability, seed demand seed health status, characterization data, etc.)

6.13.2 Selection of accessions and list generation: Based on notifications and using pertinent information from the database, list of accessions with lower viability (<80%) and inadequate amount of seeds (below 150g or 1,500 seeds) or have not yet been characterized is generated for regeneration/characterization of foxtail millet accessions in the current season. These threshold values are in full compliance with the international standards that have been set on seed viability (FAO/IPGRI, 2014). The accessions are flagged for regeneration/characterization.

- Each of these lists should include the following information (ICARDA, 2021):
- Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Germination (%) of last seed viability test
 - Seed amount at the collection (base or active, depending from where the seeds have been retrieved)
 - Whether characterized or not
 - Pedigree history (only for the breeding and genetic stocks material)
 - Remarks (eg. strictly winter types, strictly summer types, wild types and pollination type requiring special seed pre-treatments are to be flagged)

6.13.3 Seed sample preparation

- Seed foils/containers of foxtail millet germplasm from MTS (Active collection) or LTS (Base collection) are withdrawn and allowed to adjust to ambient temperature. As a general rule, seeds from the MTS can be used for up to three cycles of regeneration without returning to the most-original sample, i.e., LTS. After three cycles of regeneration, the MTS is reconstituted from the seeds available in LTS.



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- In the unlikely event that the stock in the MTS is insufficient to cover adequately the minimum sample size required for regeneration, then seeds from LTS are to be used for the regeneration of the accession and replenishment of MTS stock.
 - When monitoring procedure ascertains for a specific seed stock, that viability in LTS is below international threshold value, then half of the seeds stock in LTS is to be withdrawn and prepared for planting, in order to compensate for the lower than optimum viability rates and minimize any risk of genetic drift.
 - Seed samples are to be withdrawn from the containers bearing in mind the minimum sample size required for regeneration and the current percentage of germination. A population size of at least 80-100 plant for each accession should be used for regeneration of landraces. This number will yield enough seeds to replenish the active collection, but also the base and safety duplication collections if needed.
 - Database is to be updated with the remaining seed stocks based on the amount of seeds withdrawn from each of the accessions to be used for the regeneration and/or characterization activity.
 - Seeds are placed in paper craft foils and identified with the following information (ICARDA, 2021):
 - Accession number
 - Full taxon name (Genus, Species, Sub-species)
 - Country of origin
 - Number of seeds
 - If the seeds are treated with appropriate fungicide, the pulse crops can be saved from several soil- and seed-borne diseases. Seeds are treated with Provax-200 WP @ 2.5-3.0 g per kg, which minimize incidence of foot rot disease.
 - Paper craft foils containing treated seeds are organized in consecutive order based on the respective lists of trials and the plot number, placed in plastic trays and transferred to the short-term storage area to be kept until the sowing date.
- 6.13.4 Distribution of responsibilities:** As per generated list, Head of the Centre, PGRC, BARI distributes responsibilities of regeneration and/or characterization of germplasm (new collection and/or accession) among the scientists for specific crop species.
- 6.13.5 Preparation of experimental register:** Experimental register is prepared by responsible scientist enlisting all operations to be done and all information to be recorded from land preparation to seed threshing and cleaning with dates, descriptor list, dates of periodic visit of experts, pest management etc.
- 6.13.6 Experimental design:** Augmented Randomized Complete Block Design with 4-6 check varieties and several blocks.
- 6.13.7 Plot dimension:** Aim for a final plant number of 240-300 in plots about 9.6 m² (4.0 m × 2.4 m) plots having 300 cm and 100 cm wide channels in between two plots for regeneration and characterization trials, respectively.



6.13.8 Field layout

- Prepare field layout hard copy according to experimental design for experimental field following seed sample preparation.
- Show experimental unit (unit plot) marking length and width of plot, plot to plot and block to block distances, and boarder areas.
- Sketch of a single plot is also to be shown marking clearly row to row and plant to plant spacing, and planting spot.
- Distribute accessions and check varieties among the plots using random number from a scientific calculator or from the table of random numbers.

6.13.9 Field selection and preparation

Due attention should be paid to the environment in which cultivated foxtail millet germplasm is to be grown to avoid selection pressure and minimize genetic drift, as well as to produce good seed quality and also avoid any hazardous escapes.

- Foxtail millet needs moderately fertile well drained soil for good yields. Foxtail millet can be grown in all types of soil. Well drained land with sandy loam to loam soils is best for cultivation of foxtail millet; avoid acid soils.
- The selected land should be free of volunteer plants. Select the land on which foxtail millet was not grown in the previous season (Visal *et al.*, 2015).
- Early enough before seed sowing, selected fields are deep ploughed, in order to remove the weeds, the previous year crop residues and also to invert the soil. A second less deep ploughing followed by harrowing should also be done one or two weeks before the sowing date to remove the weeds especially perennial weeds (Agrawal, 1999).
- The soil should be opened and allowed some time for aeration. The land is then ploughed and cross ploughed deeply 2-3 times followed by harrowing and leveling to prepare the land to desired tilth.

6.13.10 Preparation of plot label: Prepare plot label (water-proof thermal plastic tag) for experimental field early enough before seed sowing. Information printed on the tag includes plot number and accession/collector's number.

6.13.11 Manure and Fertilizer doses: 60-24-42-10-2.0 kg/ha N-P-K-S-Zn (Ahmed *et al.*, 2018)

Method of application

- a) Half of N and all of P, K, S and Zn should be applied as basal during final land preparation.
- b) Remaining N should be as top-dress in two equal splits after irrigation at 25 and 55 DAS.

6.13.12 Seed sowing (eg. sowing rate, method, etc.) and labelling

- Sow the seeds in field plots directly at a depth of 2.0-3.0 cm.



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- Time of seed sowing: Third week of November to second week of December
- Spacing: Row to row- 30 cm; Plant to plant- 10 cm; Plot to plot and Block to block- 300 cm for regeneration and 100 cm for characterization
- Placing the seeds to experimental field: Foxtail millet seeds in paper craft foils are brought from the short term storage area to the experimental field and placed onto respective field plot according to field layout.
- Mark holes for sowing about 3-4 cm deep, 10 cm distance along the row. Sow two or three foxtail millet seeds by hand to a depth of 2.0 to 3.0 centimeter. Cover with soil and lightly compact the row. Keep the paper craft foils in respective field plot for final checking.
- Immediately after sowing of seed a final check is to be done for correspondence to the trial layout and any planting error is noted on the field layout hard copy.
- First and final plots are to be marked with sticks as soon as final check is confirmed.

6.13.13 Labeling

- Once seed sowing in the main experimental field is finished, each individual field plot is labelled with a water-proof thermal plastic tag. Information printed on the tag includes the trial code, plot number and the accession number/collector's number.
- A signboard is set at the beginning of each experimental field that has a brief description of the field and its contents (i.e., title of the experiment, date of transplant, number of accessions, number of checks, experimental design, etc.).

6.13.14 Isolation requirement

- Foxtail millet is a self-pollinated crop and should be raised in isolation.
- The isolation distance maintained between the varieties is 3 metres for both foundation and certified seed production to maintain the varietal purity (Vilas *et al.*, 2015).
- Avoid seed collection from boarder rows.

6.13.15 Weed management and thinning

- The field should always be kept free of weed. Two weeding and mulching at plant growth stage may be required.
- All weeds between plots and within the alley ways are controlled using spade and bush cutter.
- Eliminate off-types and plants growing outside the row.
- Thinning: Thin to one plant per hole at 2-6 weeks after establishment when plants are about 10 cm tall to give a plant density of about 240-300 plants per plot and avoid competition that will result in weak plants and low seed yields. Thinning can be done at the same time as the first weeding.

6.13.16 Irrigation and drainage

- Foxtail millet can survive in drought conditions and requires minimal water.

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- However, avoid water stress at the time of grain/seed forming.
- Frequency of irrigation depends on soil moisture level and climate.
- Ensure the soil has well moisture and avoid over watering.
- In case of heavy rains, of floods make sure the field drains out quickly.

6.13.17 Roguing: Roguing should be done often to remove the off-types, volunteer plants and diseased plants from the field to avoid the genetic contamination.

- A minimum of two field inspections should be done between flowering and maturity stages.
- The first inspection is done at the time of flowering to check the isolation and off-types and the second inspection is done during the maturity stage prior to harvest to check the off-types Vilas *et al.*, 2015).

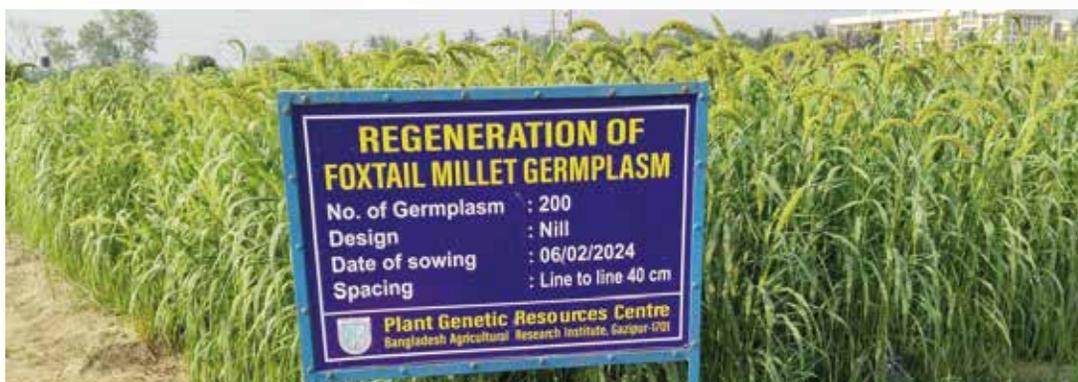


Figure 61. Regeneration of foxtail millet germplasm.

6.13.18 Insect pest and disease management

- No fungicide is recommended for this crop.
- Incidence of wire worm and cut worm is noticed some time.
- Regular monitoring of pests and diseases occurrence is to be conducted by genebank scientists and the Head of the Centre, PGRC, BARI. Periodic visits at the regeneration and characterization field are coordinated also with the experts from plant pathology and entomology divisions of BARI, to assess severity and presence of diseases, especially for those transmitted by seeds, and insects.
- Furadan 5G @ 18 kg per hectare may be applied during seed sowing to control wire worm.
- Dursban 20 EC @ ml/L is to be applied to control cut worm.

6.13.19 Harvesting

- Prior to harvest a second plastic tag same with the ones used for labelling the field plots is printed for each accession, including information on plot number, accession number. Appropriate cloth bags, which provide ample ventilation, are also prepared and labelled with the plot number and accession number.
- Harvesting is done when the earheads are physiologically mature and start to dry. The plants are still green to avoid shattering of seed.



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- Only the earheads are harvested by hand and placed into the respective labelled cloth bags. The plot tag is placed into the bag and the new one prepared is firmly tightened outer of the bag.

6.13.20 Threshing Cleaning and Drying

- The earheads are dried before threshing. The seeds are threshed by hand.
- The threshed grains are further cleaned by winnowing on tarpaulin and return to cloth bag very carefully to avoid mechanical admixture.
- The cleaned seeds are dried under the sun keeping inside cloth bag to attain a safe moisture level of 12%.

6.13.21 Data validation and uploading onto BARI genebank Documentation System:

Scientists handling the conservation unit validates all data from regeneration fields prior to uploading on the database. This process is an essential step prior to conservation procedure and verifies and documents the following types of regeneration data:

A. Seed preparation and planting data

1. Seed source
2. Full taxon name
3. Number of accessions planted and number of checks (if applicable)
4. Season
5. Date of sowing
6. Date of transplanting
7. Number of rows per plot and seeding rate per plot
8. Row length, Row width, Plot to plot distance
9. Date of first effective rainfall or first irrigation

B. Crop management and harvesting data

1. Number of plants established (registered as percentage of total expected number of plants)
2. Type and dates of fertilizers application
3. Type and dates of disease and pest management applications
4. Unexpected events during the cropping season (eg. heavy rain, severe cold, hail storm, severe drought, inundation due to flood, severe storm, etc.)
5. Any other event related to the accessions planted (eg. very low rates of germination for a particular accession, damage of an accession due to an external mechanical factor, etc.)
6. Number of plants harvested (registered as percentage of total expected number of plants)
7. Total grain weight per plot (kg)



C. Comparisons with previous passport or morphological data

Compare each accession with the following characterization data previously recorded for the accession:

1. Growth habit
2. Plant pigmentation
3. Panicle growth habit
4. Inflorescence compactness
5. Seed colour

If the identity of the accession is in doubt, check it against its herbarium voucher specimen. Discard the accession if its identity is not the same as the original accession.

Note:

1. The regeneration procedure for foxtail millet accessions is considered finalized when threshed grains have been cleaned from main impurities and are ready to be transferred to the seed processing unit for further cleaning, authentication and drying and all regeneration data have been validated by conservation unit so as to start the conservation procedure.
2. Further data information for specific traits of the accessions is generated, validated and documented during the characterization process (see below).

6.13.22 Characterization and Preliminary Evaluation

Germplasm characterization is an important operation for a genebank. The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits as well as reaction to biotic and abiotic stresses that are known to affect the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

- Traits are recorded during the different growth stages of the plants: vegetative, reproductive, and post-harvest stages. A set of morphological and agronomical descriptors are observed following internationally agreed standards [ICAR-IIMR, ICAR-NBPGR, ICRISAT and FAO. 2023. Key Descriptors for Foxtail millet. ICAR-Indian Institute of Millets Research, ICAR-National Bureau of Plant Genetic Resources, International Crops Research Institute for the Semi-Arid Tropics, India, and Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. 15p].

1. Name (long & short) and address of the institute:
2. Geographical position
 - Latitude- degree and minutes followed by N (North) and S (South)
 - Longitude- degree and minutes followed by E (East) and W (West)
 - Altitude (metre)]:



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3. Soil texture (according to FAO, 2006) and chemical analysis (organic matter content, pH, electrical conductivity, etc.)
4. Weather data of regeneration/characterization site (maximum & minimum temperature, relative humidity, rainfall, sunshine hours, etc.) during crop season
5. Name of Person in Charge of Characterization:



Figure 62. Morphological characterization of foxtail millet accessions.

6.13.23 Descriptor List

Date of sowing: Date of Harvest:

1. Accession number.....
2. Name.....
3. Former designation.....
4. Seed source.....
5. Country of origin.....
6. Plant Growth habit

To be recorded on plot basis at flowering/heading stage

- 1 Erect
- 2 Erect geniculate
- 3 Decumbent
- 4 Prostrate



Erect



Erect geniculate

Figure 63. Growth habit of foxtail millet accessions

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7. Days to 50% flowering
Record number of days from sowing until 50% plants (main tillers) have begun to flower/anthesis on plot basis
Quantitative
8. Plant height (cm)
Record the height of main tillers of five randomly selected plants from ground level to tip of inflorescence at physiological maturity. In case of decumbent or prostrate, length of flowering culm from rooted base at physiological maturity
Quantitative
9. Number of basal tillers
Record number of main tillers at ground level or from the basal nodes on five randomly selected plants at physiological maturity
Quantitative
10. Plant pigmentation
Record the pigmentation on five randomly selected plants at flowering
 - 0 Not-pigmented or green
 - 1 Pigmented
 - 2 Deep purple
11. Culm branches
Record the number of culm branches on the main stem of five randomly selected plants at physiological maturity
 - 0 Absent
 - 3 Few
 - 7 Many
12. Leaf colour
Record the predominant colour assessed as a single observation on group of plants on plot basis from main tillers at vegetative stage
 - 1 Light green
 - 2 Green
 - 3 Yellow
 - 4 Purple
 - 5 Dark purple
 - 99 Other (specify in the descriptor Notes)
13. Blade length of flag leaf (cm)
Record leaf blade length from ligule to tip of flag leaf on main tillers of five randomly selected plants at flowering
Quantitative
14. Blade width of flag leaf (cm)
Record leaf blade width at the widest point of flag leaf on main tillers of five randomly selected plants at flowering
Quantitative



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15. Sheath length of flag leaf (cm)
Record leaf sheath length from node to ligule on main tillers of five randomly selected plants at flowering
Quantitative
16. Leaf senescence
Record the level of leaf senescence assessed as a visual observation on plot basis at panicle maturity
 - 1 Leaves almost green
 - 2 Leaves moderately green
 - 3 Leaves almost dry
 - 4 Leaves completely dry
17. Peduncle length (cm)
Record length of peduncle from top most node of main tiller to base of inflorescence on five randomly selected plants at maturity
Quantitative
18. Peduncle exertion (cm)
Record length of the exposed portion of the peduncle from the flag leaf sheath up to the base of the inflorescence on main tillers of five randomly selected plants at maturity
Quantitative
19. Inflorescence length (cm)
Record length of inflorescence from lowest branch to tip of last branch on main tillers of five randomly selected plants at physiological maturity
Quantitative
20. Inflorescence width (cm)
Record the width of widest part of inflorescence on main tillers of five randomly selected plants at physiological maturity
Quantitative
21. Inflorescence lobes size
Record the size of lobes on the inflorescence of five randomly selected plants at physiological maturity
 - 0 Absent
 - 3 Small
 - 5 Medium
 - 7 Large



Absent



Small



Medium



Large

Figure 64. Variations in Inflorescence lobes size of foxtail millet accessions

22. Panicle growth habit (in relation to stem)

Record the growth habit on plot basis at flowering

- 1 Erect
- 2 Semi-erect
- 3 Horizontal
- 4 Drooping

23. Panicle branching

Record the branching of panicle on five randomly selected plants at distal end of panicle at physiological maturity

- 0 Absent
- 1 Present

24. Inflorescence bristles length

Record the length of bristles assessed as visual observation at the middle of the inflorescence on five randomly selected plants at flowering stage

- 3 Short (<4mm)
- 5 Medium (4-8 mm)
- 7 Long (>8mm)



Short



Medium



Long

Figure 65. Variation in inflorescence bristles length of foxtail millet

25. Inflorescence compactness

Record the arrangement of lobes and compactness of inflorescence on five randomly selected plants at physiological maturity

- 3 Loose
- 5 Medium
- 7 Compact

26. Inflorescence shape

Record the predominant shape observed on five randomly selected plants at physiological maturity

- 1 Oblong
- 2 Ovate
- 3 Elliptic
- 4 Obovate



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Open

Intermediate

Compact

Figure 66. Variation in Inflorescence compactness of foxtail millet accessions

27. Seed colour

Record the predominant colour visually assessed as a single observation on a small seed lot

- 1 White
- 2 Yellow
- 3 Orange
- 4 Brown
- 5 Red
- 6 Black
- 99 Other (specify in the descriptor Notes)



White



Yellow



Orange



Red



Brown



Black

Figure 67. Variation in seed colour of foxtail millet accessions



28. Seed shape
Record the shape visually assessed as a single observation on a small seed lot
- 1 Round
 - 2 Elliptical
 - 3 Oval
 - 4 Ovate
29. 1000-Seed weight (g)
Record weight of 1000 seeds after drying the seeds at about 11-12% moisture content
Quantitative
30. Shattering of inflorescence (%)
Record percentage of spikelets remaining on racemes at full maturity from five plants selected at random
- 3 Low
 - 5 Medium
 - 7 High
31. Grain yield per plant (g)
Record the average grain yield of five plants randomly selected
Quantitative
32. Straw yield per plant (g)
Record the average straw yield of five plants randomly selected
Quantitative
33. Grain carbohydrate content (%)
Record percentage of carbohydrate content from seed samples randomly collected from the plot.
34. Grain crude protein content (%)
Record percentage of crude protein content from seed samples randomly collected from the plot.
35. Grain dietary fibre content (%)
Record percentage of dietary fibre content from seed samples randomly collected from the plot.
36. Grain calcium content (mg/100g)
Record calcium content from seed samples randomly collected from the plot.
32. Seed starch type
Record starch type on seed samples randomly collected from the plot.
- 0 Non waxy
 - 1 Waxy



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Foxtail millet at BARI

SOPs No.: PGRC-REG & CHAR_Foxtail millet012 | Version: 1.0

SOPs Owner:
PGRC, BARI

SOPs Approver:
Crops Division, BARC

34. Biotic Stress Susceptibility

Scored as percentage infection from a specific trial to induce disease or insect infestation, under natural/artificial inoculation conditions to be specified. In each case, it is important to state the origin of the infestation or infection, i.e., natural, field inoculation, laboratory. Record such information in descriptor 46. NOTES. These are coded on a susceptibility scale from 1 to 9:

- 3 Low
- 5 Intermediate
- 7 High
- 9 Very high

34. Abiotic Stress Susceptibility

Scored as percentage survival from a specific trial to induce stress, under conditions which are clearly specified. Drought trials are often performed under greenhouse conditions or rain-out shelters.

- Susceptibility to high temperature
- Susceptibility to drought
- Susceptibility to high soil moisture
- Susceptibility to low temperature

Notes

Specify here any other additional information. Add any additional traits that are important to describe the diversity among accessions within this species.

- 6.13.24** Identity is monitored by comparing the accession with previous passport or morphological data.
- 6.13.25** All characterization information is to be verified by the head of conservation unit of PGRC, BARI and the genebank manager.
- 6.13.26** Characterization data is to be uploaded on the PGRC's Genebank Documentation System by the Documentation specialist.

The characterization procedure is considered finalized when all the data has been uploaded to PGRC's Genebank Documentation System.

7.13.1 Related Flowcharts, Documents and Links

The following flowcharts are pertinent to this SOPs:

- Flowchart of standard operating procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh (Annexure 1)
- Flowchart for Development and Implementation of New Procedural Standard Operating Procedures for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh (Annexure 2).



7.13.2 Compliance with standards and policies: The Regeneration and Characterization of cultivated foxtail millet genetic resources at BARI is in compliance with the following standards and policies:

- FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture (2014)
- International Rules for Seed Testing. International Seed Testing Association, ISTA (2016) SPS

7.13.3 Adherence to legal and regulatory frameworks: The Regeneration and Characterization of cultivated foxtail millet genetic resources at BARI adheres to the following national and international frameworks:

- ITPGRFA
- SMTA
- International Plant Protection Convention (IPPC)
- WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)
- AVRDC
- NBPGR
- UPOV

8.13.1 Staff Training and Competency

- Training and competency requirements to perform this SOPs have been met by the working and retired experienced scientists of PGRC, BARI and university teachers.
- Genebank staff are to undergo periodic training. Competency testing is to be done regularly in an informal way as part of the supervision and ensuring the quality of the work.

9.13.1 References

- Agrawal, RL. 1999. Seed Technology (Second Edition). Oxford & IBH Publishing Co. Pvt. Ltd., Kolkata.
- Ahmed *et al.*, 2018. Fertilizer Recommendation Guide-2018. Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka-1215. 223p
- BARI. 2019. *Krishi Projukti Hatboi* (Handbook on Agro-technology), 8th edition. Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh.
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- FAO/IPGRI. 2014. Genebank standards. Food and Agriculture Organization of the United Nations, Rome and International Plant Genetic Resources Institute, Rome. Available from: FAO Genebank Standards 2014.



SOPs for Regeneration, Characterization and Preliminary Evaluation of Cultivated and Wild Genetic Resources of Foxtail millet at BARI

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PGRC, BARI

SOPs Approver:
Crops Division, BARC

ICARDA, Genetic Resource Section. 2021. Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA (SOPs ICARDA-REG & CHAR_CE001_ver3.0). Contributors: Tsivelikas A, Yazbek M, Kehel Z, Moulakat A, Jawad R, Al-Awar B, Zaher O, El-Miziani I.

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Vilas A. Tonapi, Venkatesh Bhat B, Kannababu N, Elangovan M, Umakanth Raghunath AV, Kulakarni, Kritika V Tonapi, KV Raghavendra Rao, Nageshwar Rao TG. 2015. Millet Seed Technology: Seed Production, Quality control & Legal compliance. 101- 104 PP. ISBN 81-89335-54-5

10.13.1 Revision History

Effective Date of the SOPs	Version #	Description	Reviewed By
December 31, 2024	001	Original SOPs	Dr. Md. Rezwan Molla

SOPs for Regeneration, Characterization and Preliminary Evaluation of Some Agri-Horticultural Crops' Germplasm in Bangladesh

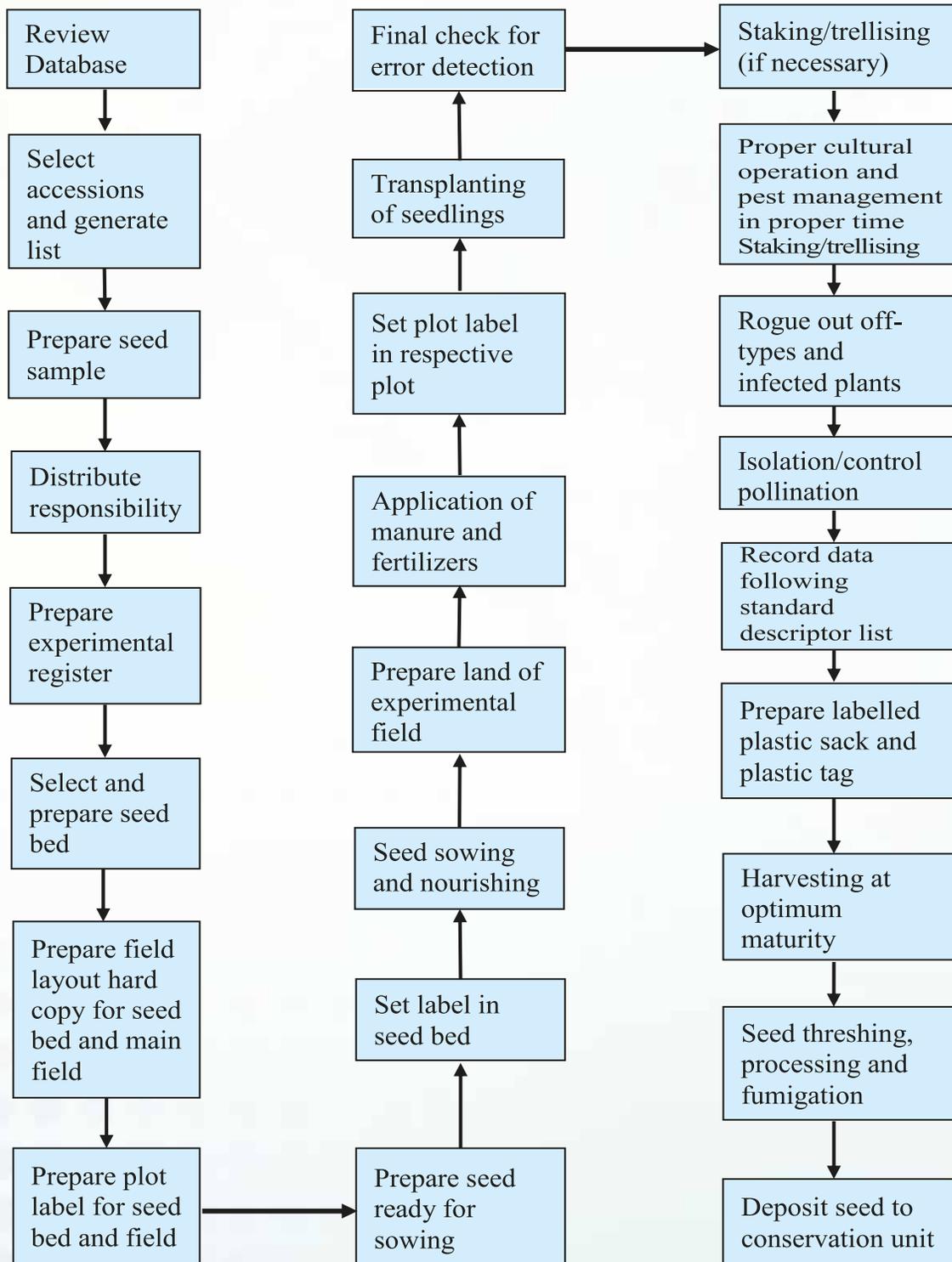
SOPs No.: BRRI-BARI-REG & CHAR_A-HC001 | Version: 1.0

SOPs Owner:
PGRC, BARI and GRSD, BRRI

SOPs Approver:
Crops Division, BARC



Annexure 1. Flowchart of Standard Operating Procedure for Regeneration and Characterization of cultivated and wild plant genetic resources of Agri-Horticultural Crops in Bangladesh





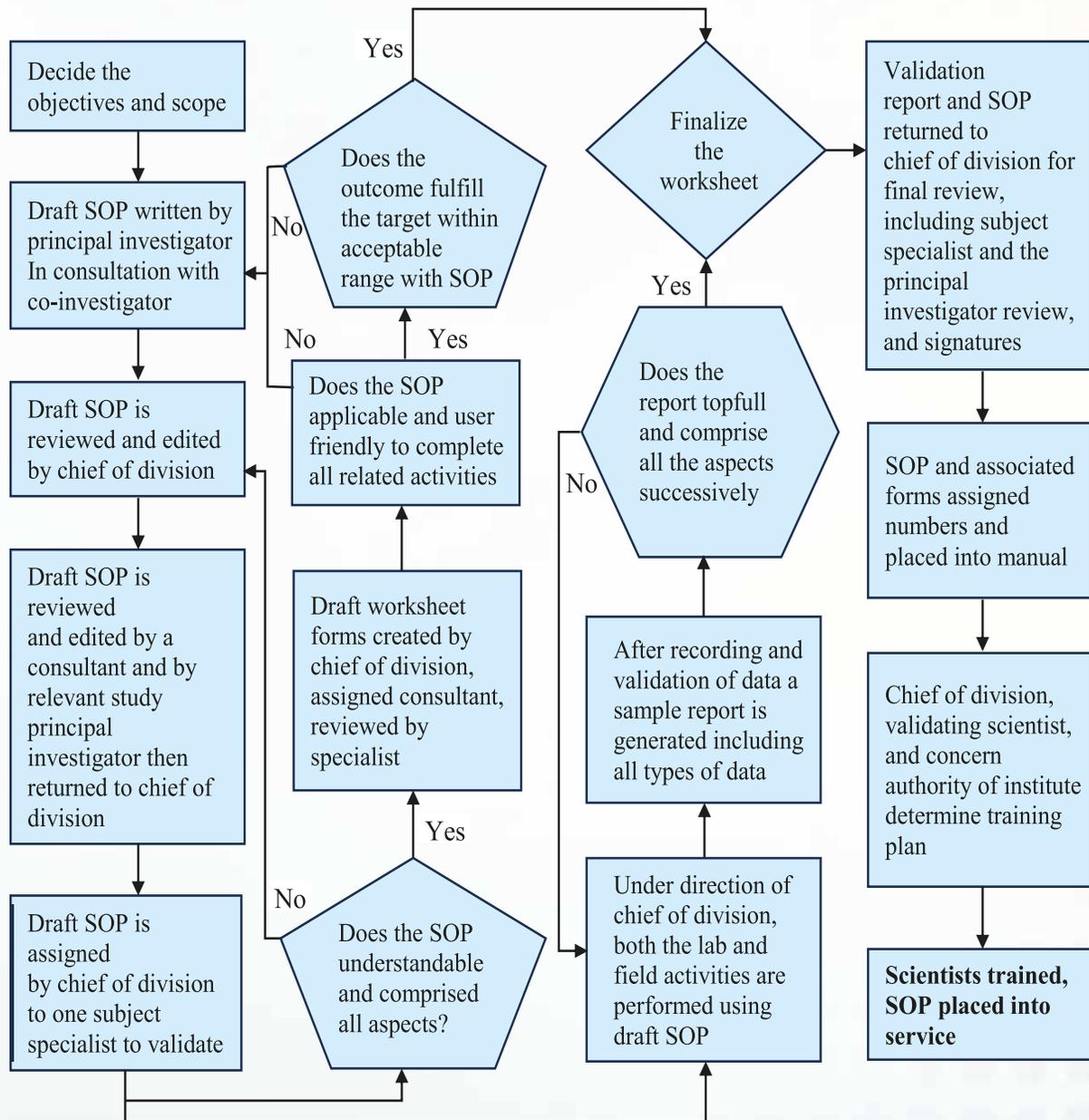
SOPs for Regeneration, Characterization and Preliminary Evaluation of Some Agri-Horticultural Crops' Germplasm in Bangladesh

SOPs No.: BRRI-BARI-REG & CHAR_A-HC001 | Version: 1.0

SOPs Owner:
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Annexure 2. Flow Chart for Development and Implementation of New SOP for Regeneration and Characterization of Agri-Horticultural Crops' Germplasm in Bangladesh.



For More Information

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