SADC Plant Genetic Resources Centre

GeneBank Standard Operating Procedures

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SADC Plant Genetic Resources Centre (SPGRC)
Farm # 6300, off Great East Road
Private Bag CH6
LUSAKA
Zambia
Tel: +260 211 399 200-10
Fax: +260-211-233746
Email: registry@spgrc.org.zm; spgrc@zamnet.zm
URL: http://www.spgrc.org.zm

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List of Acronyms

BP	Between-paper (method of seed planting for germination test)
NPGRC	National Plant Genetic Resources Centre
PGR	Plant Genetic Resource
SADC	Southern African Development Community
SDIS	SPGRC Documentation and Information System
SOP	Standard Operating Procedure
SPGRC	SADC Plant Genetic Resources Centre
TP	Top of paper (method of seed planting for germination test)

Foreword

The SADC Plant Genetic Resources Centre (SPGRC) is a network of sixteen countries, which have established a regional genebank where plant genetic resources from all the participating countries is stored. These genebank standard operation procedures were developed for the SPGRC Genebank and its network of National Plant Genetic Resources Centres (NPGRCs) in SADC Member States. NPGRCs and ultimately the SPGRC, hold large numbers of seed samples from diverse parts of the Southern African Development Community (SADC) region. Because of the diversity of original habitats, the seed samples often have different quality regimes but they have to be stored in the same storage facilities. The intention of these procedures are to standardize the seed handling process and eventually quality of the seed samples regardless of their diverse origins. The Standard Operating Procedures (SOPs) have been tailor made to genebank set up in the SADC region. The objective of developing these procedures is to ensure that at least seed kept in the SADC genebanks for conservation purposes meets the minimum quality standards that guarantee long-term storage even in an environment of limited resource availability to provide the best handling and storage conditions. It is our hope that the SPGRC regional genebank and the NPGRCs will find these guidelines useful in their work. We wish you the best in your effort to conserve the invaluable PGR of the SADC region forever.

Introduction

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. Before the advent of farming by our prehistoric ancestors, PGR have always been the basis of direct food security for humankind in their natural diversity maintained by the forces of natural selection. Even after the introduction of organized agriculture by man, plant genetic resources continued to be sources of invaluable genes that allowed early crop breeders to improve crops for the benefit of humankind. In their diversity, that include the crop wild relatives, plant genetic resources are a source of genes for all the traits useful to humanity directly when consumed at home or used as raw materials in industrial production processes. PGRs are an embodiment and a package of all the beneficial characteristics of plants to people that only need the provision of a conducive environment for them to be expressed so that man can harvest the fruits. They are a source of potential genes to unlock hunger puzzle that has thrust upon humanity by climate change that has resulted in increased frequencies of droughts and occurrence of new forms of pests and diseases that have proved to be difficult to handle. Despite its value and benefits to humankind, plant genetic resources have always been threatened by extinction either from their natural habitats or from homes, in farmer granaries or other storage facilities that have established the aim of conserving them. Although, in the past, there was threat of plant genetic erosion, the threat has been heightened since the 1950s because of rapid global industrialization and notable advances in scientific methods in agriculture as well as negative shifts in climatic conditions. The introduction of improved crop varieties, especially the high yielding hybrid varieties developed using scientific approaches has resulted in a major shift by world farmers in abandoning farmer varieties that have been for years the store of plant genetic resources diversity. This scenario coupled with devastating climate shifts has warranted serious investment in plant genetic resources conservation across the world. The Southern African Development Community also set up the SADC Plant Genetic Resources Centre to coordinate conservation and sustainable utilization of PGR in the region.

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I. Seed Handling And Genebank Management

1.1 Germplasm Acquisition

Objective

To ensure that germplasm arriving at the genebank are fit for introduction into the genebank and that they are properly documented in incoming seeds register, with all passport and other information provided by the collector or donor.

Responsibility

Senior Programme Officer – *Ex situ* Conservation

Procedure

1. All the accessions must come through the germplasm sorting room
2. Arrange the accession samples received in alphabetical order based on the crop species name or in ascending order based on Collector numbers written on the accession packets and verify that the number of seed packets match the accompanying accession packing list
3. If no accession list was provided or arranged accession packets do not correspond with the accompanying list, prepare a new list
4. Verify if samples are accompanied by the necessary passport data such as Common name of crop, scientific name of the crop, NPGRC number, Collectors' name and the sample quantity (grams or number of seeds)
5. Check again to confirm if all the accession packets have been included and if accompanying information and the accession packet information tally
6. If information provided is not tallying with the actual, inform

the Supervisor who will notify the seed donor about it within 48 hours of receiving the consignment

7. Open each packet of seeds and inspect their condition by checking for any insect-damage, fungal growth, damaged seed, empty or shriveled seeds which probably would be unviable

8. Check for moisture content of the seeds that are sound and send for drying if the content is above 7%

9. If the seeds are in poor condition, reject the sample and immediately furnish the donor with the reasons for the rejection and request for a new sample that is in a better condition

10. If the moisture content is below 7% or after drying the seeds to a moisture content below 7%, register the accessions first in the manual registration book allocating the received accessions an SPGRC Number and then enter data in the SPGRC Documentation and Information System (SDIS)

11. Test for the initial viability and accept samples with >85% germination

12. If viability is below 85%, notify the depositor and send the material for regeneration

13. Package, seal, label and send accessions with viability of 85% and above for storage in the genebank

The end

1.2 Obtaining a Working Sample of Germplasm

Objective

To obtain a representative sample of an accession for viability assessment.

Responsibility

Technical Officer – *Ex situ* Conservation

Procedure:

(a) Mechanical Method

(Using the centrifugal divider, soil divider, conical divider, riffle divider)

i) Pour the whole sample into the hopper of the seed divider

ii) Seed will flow through the attached channels into the receiving pans, resulting in approximately equal portions of seed in the pans

iii) Withdraw the pans and pour back the seed into the divider at least twice so as to evenly mix the seed

iv) Withdraw one pan and discard the seed by returning it into the sample bag

v) Replace the pan and pour back the seed in the retained pan into the divider for further subdividing, while discarding seed obtained in the other pan simultaneously

vi) Repeat the process until you obtain a workable seed quantity for various lab tests

(b) Non-Mechanical Method

(Halving Method)

Procedure:

i) Place the sample on a clean surface and thoroughly mix the seeds by hand

ii) Divide the seed into quarters with a sharp-edged spatula and discard opposite quarters at a time by returning it back into the sample bag

iii) The process is repeated until a final sample of the approximate weight for lab tests required is obtained

The end

1.3 Accessing the GeneBank

Objective

To safeguard the SADC region's germplasm and ensure proper maintenance and care of the gene bank equipment as well as promoting accountability in the use SADC Plant Genetic Resources

Responsibility

It is the responsibility of the Senior Programme Officer – *Ex situ* Conservation to ensure that this procedure is adhered to

Procedure:

i) The Genebank is a controlled-access area

ii) Keys to Genebank are always safely kept in the key cabinet by the Senior Programme Officer – *Ex Situ* Conservation

iii) Always seek permission to enter the Genebank from the Senior Programme Officer – *Ex Situ* Conservation

iv) When authorized to enter the Genebank, always fill in the GeneBank access register

v) Germplasm accessions stored in the Genebank are arranged in systematic order; always seek assistance when unsure of what you are looking for. Mixing or contaminating germplasm is a serious offence in Germplasm Conservation.

vi) Log out in the Genebank access register and leave the keys in the care of the responsible officer after every access to the Gene bank

The end

1.4 GeneBank Monitoring

Objective

To maintain the Genebank under optimum operating conditions for long-term storage of germplasm

Responsibility

It is the responsibility of the Technical Officer – *Ex Situ* Conservation to ensure that this procedure is adhered to

Procedure:

i) Genebank conditions are monitored twice a day at 08:00 hours and at 16:30 hours

ii) The Technical Officer collects keys to the GeneBank from the Senior Programme Officer – *Ex Situ* Conservation

iii) Log in the manual and electronic GeneBank access register

iv) Enter the Genebank and check and record in the record book the following parameters:

- Gene bank temperatures from the wall thermometers in the genebank
- Relative humidity from the hygrometers on the genebank walls
- Check the state of each of the freezers to confirm if they are functioning properly by reading off the temperatures from each of the freezers

v) If all is well, log out and return the keys to the Senior Programme Officer – *Ex Situ* Conservation

vi) Monitoring has to be carried out at least three times a week (Monday, Wednesday and Friday)

NB: Report any anomalies in the functioning of the freezers and any other equipment as soon as they are discovered to your immediate supervisor who should immediately request for technical assistance to remedy the problem.

The end

1.5 Testing for Germination

Objective

To determine the germination percentage of accessions before storage and at 3 or 5 year intervals (for oil/legume and cereal crops respectively)

Responsibility

It is the responsibility of the Senior Programme Officer – *Ex situ* Conservation to ensure that this procedure is adhered to

Procedure:

(a) Seed planting

(1) Between-Paper Method (BP)

i) Obtain a representative sample of your seed by using a seed divider (cross reference to how to get a representative sample of an accession SOP)

ii) Sterilize the working table with 70% Sodium Hypochlorite solution (*Jik*) or alcohol

iii) Spread a paper towel on a flat surface, print on one of its corners accession number (Batch ref), date of test and replicate number

iv) Moisten the filter/germination paper towel with water until it is thoroughly damp. Do not dampen to point of runoff or dripping

v) Place 50 seeds (or other sample size) in rows on the paper towel. Make sure you randomly select seeds for your sample; do not cull any damaged, discolored or light seeds, since this will *bias* your germination test

vi) If a vacuum counter is used, the head must be held flat and completely covered with seed before the vacuum is turned on to avoid biased selection of seed

vii) Moisten a second towel and carefully place onto the first paper towel, leaving the seeds sandwiched between the two towels

viii) Roll up the towels with the seeds in-between and place in a container that will retain the moisture

ix) Repeat the process by doing the second replicate

x) Use a rubber band to hold the rolled papers and prevent them from falling apart

xi) Keep the rolls upright bound together as replicate 1 and 2 in a deep-bottom plastic container or tray

xii) Place the container in the germination cabinet with optimum settings for the species. Keep the towels moist by spraying with water (use spray bottles) if necessary, especially when temperatures are high (25°–30°C), but do not over-water

xiii) Run the test according to recommended period for each species

(2) Top of Paper Method (TP)

This method is most suitable for species with seeds smaller than 2 mm in diameter such as small-seeded vegetables and forage grasses. The seeds are germinated on top of moist absorbent paper in containers with fitting lids to prevent moisture loss

i) Sterilize container surfaces by wiping with 70–95% alcohol or soaking in 20% bleach

ii) Label containers with accession number, number of replicate and testing date; use a pencil or permanent marker for labeling

iii) Place the filter paper substrate at the bottom of the container or Petri dish

iv) Add the required volume of distilled water. For Whatman Grade 181 filter paper in 9 cm Petri dishes, 4 ml of water is required. The filter paper should not be so wet that a film of water forms around the finger when it is pressed

v) Firm down the paper substrate in the container using a forceps or tweezers

vi) Place 50 seeds on two wetted filter papers, each labeled in the corner

vii) Using forceps, spread the seeds uniformly on the surface of the paper so that they are not touching

viii) Cover the containers with lids and ensure that there is no air lock resulting from excess moisture on the covers

ix) Place the containers in a germinator maintained at the recommended temperature for germination of the species in question (see guidelines for testing germination of the most common crop species)

x) Run the test according to recommended period for the species being worked on e.g. 4 to 10 days for *Sorghum bicolor*

(b) Seedling Evaluation

i) After the required germination period which is 4 days to 10 days (*Sorghum bicolor*), remove the towels from the container and unroll the paper carefully so that the fragile shoots are not destroyed

ii) Carefully assess the seedlings and count the seeds that have reached a stage of germination with all the essential structures present and record as normal seedlings during the first and final counting

iii) Seedlings without all the essential structures present are counted and recorded as abnormal seeding

iv) Count and record decayed seed as dead seeds. In the first count, remove badly decayed seedlings in order to reduce the risk of secondary infection

v) Sometimes, some seeds may have imbibed some water but look very fresh and sound, record these as fresh seeds.

vi) Some seeds may remain intact as though they were not subjected to moisture, record these as hard seeds

vii) Computation of germination percentage is based on normal seedlings using the formula = Sum of normal seedlings in both replicates divided by 100 (total number of seeds) x 100

viii) Note that seedling evaluation is not always a once off exercise, you may have to count out the germinated (normal) seedlings and return the replicates until no further germination has occurred mainly after two consecutive counts

ix) Recommended and accepted percentage is >85%. Samples with less than 85% to be regenerated/rejuvenated

NB: Report any anomalies in the functioning of all germination equipment as soon as they are discovered to your immediate supervisor who should immediately request for repair works or replacement of equipment to remedy the problem.

The End

1.6 Determination of Seed Moisture Content

Objective

To determine the moisture content of seed lots to determine the level of moisture reduction needed to reach the threshold moisture content ideal for long-term storage

Responsibility

It is the responsibility of the Technical Officer – *Ex situ* Conservation to ensure that this procedure is adhered to

Procedure:

i) SPGRC uses Mettler Toledo HC-103 Moisture Analyzer which is destructive

ii) Set the Analyzer ready for the analyses and clean it thoroughly together with the seed grinder so as to obtain accurate results

iii) Grind a few seeds of the sample to be analyzed using a seed grinder

iv) When the Analyzer is ready for the sample, place 2-3 grammes of the ground material onto the sample pan

v) Replace the cover of the Analyzer, which will automatically commence the analyses

vi) When the analysis is completed, a sound signal will be heard with the percentage of moisture reading showing on the screen of the Analyzer

vii) Record the readings and enter into the database

NB: Report any anomalies in the functioning of the Moisture Analysis equipment as soon as they are discovered to your immediate supervisor who should immediately request for repair works or replacement of equipment to remedy the problem.

2. MULTIPLICATION AND REGENERATION

2.1 Preparation for Planting

Objective

To make sure that accessions for multiplication are identified on time and land for multiplications is made ready on time

Responsibility

It is the responsibility of the Senior Programme Officer – *Ex Situ* Conservation to undertake this process

Procedure:

i) Decide on the number of accessions for multiplication in a specific year based on the resources available and draw out a multiplication list. The list must include important information like the accession number and the species

ii) It is recommended that at this point, a file for the work to be done is created and all documents associated with the work put in the file

iii) Using the multiplication list; prepare well labeled field/plot tags and have them ready

iv) Decide on time on the field where the multiplication of accessions will be conducted for the year

v) Map out the field to identify unsuitable portions of the land like ant heaps, and swampy areas to avoid them when marking out the plots

vi) Mark out plots in the field avoiding unsuitable areas such as swampy portions and ant heaps. The standard plots used at SPGRC are 1.5 m by 3m for cereal crops. Five rows per plot. Rows for creeping cowpea and cucurbits to be 3 – 5m apart

vii) Plots are marked in straight neat ranges leaving working ranges in between ranges. Leave 3 m between ranges for the tractor to pass and 1m between plots in a range

viii) Number the ranges in ascending order using clearly labeled signs from left to right.

ix) Clearly label the plots using the already prepared tags applying the principle of moving from left to right in your counting. The field is ready for planting

x) Maintain the integrity for each accession and control cross pollination by using appropriate pollination bags

xi) Use recommended fertilizer rates

xii) Monitor and control pest and diseases on time

The end

2.2 Seed Preparation for Planting

Objective

To ensure that correct germplasm accessions are prepared for multiplication in the field

Responsibility

It is the responsibility of the Technical Officer – *Ex Situ* Conservation to supervise this process

Procedure

Seed preparation for planting is done by the Technical Officer – *In Situ* Conservation.

i) Prepare and label the seed planting envelopes appropriately with plot number, accession number and species name. Envelopes are sorted in ascending order using field plot numbers

ii) Seek authority to enter the genebank from the Senior Programme Officer – *Ex Situ* Conservation

iii) Seed samples to be retrieved from the freezers and kept under ambient room temperature at least two weeks before planting time

iv) Using the multiplication list prepared for the season, extract the seed for multiplication from the gene bank or any storage facility.

v) It is recommended that seed extraction from the storage facilities be done in batches to minimize chances of making mistakes

vi) From the genebank packets/bottles extract only enough seed for planting; 40 - 250 seeds. Always tick on your seed list the accessions whose seed has been prepared.

vii) When all the seed preparation is done, seed is kept in the laboratory waiting for planting

2.3 The Planting Process

Objective

To ensure planting of germplasm is done effectively in a manner that guarantees germination under standard conditions

Responsibility

It is the responsibility of the Technical Officer – *Ex Situ* Conservation to supervise this process

Procedure

1. Planting is only done when the moisture content of the soil is sufficient to support effective seed germination.
2. Prepare a marked planting string by knotting it or painting it to show inter-row and intra-row spacing. For cereals an intra-row spacing of 25 cm and inter-row spacing of 75 cm is appropriate.
3. Open holes using a small planting hoe deep enough to allow seed emergence without challenges. Do not use a stick for drilling holes. The depth of the hole depends on the seed size being planted.
4. Apply basal fertilizer at a rate of 10 g per planting station. Put basal fertilizer at the side of the planting hole to avoid direct contact of seed with fertilizer.
5. Lay the seed packets to corresponding labeled plots first. Always verify numbering on the seed packets if the correspond to numbers on the plot labels.
6. Drop the seed in the centre of the planting hole avoiding the basal fertilizer applied.
7. Cover the hole with enough soil to allow seed emergence.

Immediately apply a pre-emergence herbicide if applicable.

2.4 Pollination and Its Control in Maize (*Zea mays*) and other Out-Crossing Crops

Objective

To do away with germplasm contamination during the multiplication process while at the same time minimising inbreeding

Responsibility

It is the responsibility of the Technical Officer – *Ex Situ* Conservation to supervise this process

Procedure:

i) Always monitor the development of the multiplication plots. Anticipate the period when you are likely to get flowers on your plants

ii) Inspect daily in each plot for plants with shoot buds ready for covering with plastic shoot bag covers and cover them early enough before they produce silk. Always rouge out plants whose female flowers have produced silk before being bagged

iii) Covering shoot buds is the key to the success of the diversity maintenance process and therefore must never be compromised

iv) When sufficient numbers of plants in the plot have produced silk, collect pollen from all the plants in tassel in the plot

v) Pollen collection is done by bagging all the plants in tassel a day before the day of pollination

vi) On the following day before 10:00 hours, remove the pollen bags quickly avoiding possible contamination by foreign pollen and pour all the pollen from the plants in the plot in one pollen bag

vii) Hold tight the open end of the pollen bag, mix the pollen by shaking the bag

viii) Use the pollen mixture to pollinate all the plants in silk in the plot by quickly removing the shoot bud plastic bag cover and sprinkling pollen on the silk and immediately covering with a new pollen bag. The process must be done quickly within 5 seconds!!

ix) For effective execution of the process in 8 above, two people are required

x) The other pollination approach is to randomly pollinate plants in silk with pollen from different plants within the same plot

xi) Care must always be taken to avoid pollination by foreign pollen so the process of pollination has to be done expeditiously

xii) In the case of cucurbits, cover the female flower with a paper cup just before the flower opens up. After pollinating, tie the flower with a rubber band and cover the flower with a big leaf to shade it from direct sunlight

The end

2.5 Harvesting Regenerated Accessions

Objective

To enable the accessions to be harvested at the right maturity stage and handled in a manner that guarantees high quality by avoiding contamination by foreign seeds and loss from pests and diseases attack

Responsibility

It is the responsibility of the Technical Officer – *Ex situ* Conservation to ensure that this procedure is adhered to

Procedure

i) Accessions are harvested from the field once the seed has reached physiological maturity or later

ii) The Technical Officer determines accessions that are ready for harvesting since maturity stage differs by accession

iii) Harvesting bags are prepared and well labeled with plot numbers, accession number and species for only those plots to be harvested

iv) Corresponding labeled empty harvesting bags are laid beside the plots with mature, ready to harvest accessions

v) Individual plants in each plot are harvested into the harvesting bags and full bags placed beside each plot for collection

vi) Only bagged inflorescences in case of cereal crops like maize, sorghum and pearl millet are harvested. Avoid harvesting plants whose pollen bags are missing as it might not be certain whether there was pollen contamination or not

vii) Insert another labeled tag inside each harvesting bag with seeds as a backup label in case the labeling on the outside of the bag is defaced

viii) Tightly secure the open end of the bag with a string and carry the harvesting bags with germplasm to the edge of the field from where they will be collected to the processing shed

The end

2.6 Seed Extraction and Cleaning

Objective

To improve seed purity by removing damaged, immature seeds and extraneous materials to optimize long term storage

Responsibility

Technical Officer– *Ex situ* Conservation

Procedure:

i) It is recommended that the germplasm seed be extracted, processed and cleaned by hand to minimize breakages, losses and accession admixtures

ii) Always clean the working environment before starting seed extraction and after working on any one plot

iii) Handle harvested plots in the bags one at a time and clean the working area after working on a given accession

iv) The Technical Officer will extract and record in the inventory book the accessions to be worked on and give them to the workers

v) The worker will empty each bag in a winnowing tray, shell and separate the seeds from their pods by hand

vi) Separate any debris (broken seeds, inert materials, infected seeds) by hand or using a fine blower

vii) There may be need to extend seed cleaning process in the lab by spreading the seeds on a flat well-lit surface of contrasting colour such as an illuminated table (Purity table) to remove damaged seeds, seeds of different species, *etc*.

viii) When thoroughly clean, seed is then transferred into porous cotton bags with corresponding labels, both inside and outside the bag

ix) Note that small seeds should not be discarded but eliminate off-type seeds if clearly noticeable

x) Close the processing bags loosely to facilitate uniform dehydration

xi) Split a seed lot into several bags when necessary i.e. when the seed bag is too big and tie them together for easy of location. Each bag is labeled separately inside and outside

xii) Record the extracted plot and the quantities of seed extracted in the seed extraction record book and store the seed in readiness for drying

The end

2.7 Seed Drying

Objective

To dry seeds down to a range of 3 - 7% Moisture Content

Responsibility

It is the responsibility of the Technical Officer – *Ex situ* Conservation to ensure that this procedure is adhered to

Procedure

i) Always keep an accession drying record book in the seed drying room

ii) Number the drying shelves/racks to enable easy location of materials in the drying room

iii) Arrange the seed bags in ascending order by field plot number on the drying shelves/racks

iv) Make sure accessions of the same species, harvested in the same season are placed on the same rack. Seed samples should be laid out in single layers so as to allow free air flow

v) Determine the moisture content of seeds before putting them in the drying unit

vi) Record the date of entry in the drying room, accession location on the shelves and number of packets entered per accession

vii) The seed drier has to be set at a temperature of approximately 15°C and 15% Relative Humidity to enable a slow drying process

viii) Turn the seed bags upside down every 2–3 days to allow homogeneous dehydration

ix) Leave the seed samples to dry on the drying racks and at two week intervals extract seed samples for moisture content testing to determine the level of drying

x) Seeds for base collection are said to be dry when their moisture content reaches 3 -7% (oil crops – ground nuts = 5 – 7%)

NB: Report any anomalies in the functioning of the seed dryer as soon as they are discovered to your immediate supervisor who should immediately request for repair works to remedy the problem.

The end