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## ***1. Introduction & Purpose***

ICARDA Genebank holds large and unique collections of crops of global importance including the mandate crops such as wheat, barley, chickpea, faba bean, lentil and grasspea, along with their crop wild relatives, as well as the temperate forage and range species (see Annex 1 for collection composition). All taxa conserved at ICARDA Genebank are known to have orthodox seed behavior therefore the conservation method has been chosen accordingly.


The collection conserved at ICARDA is part of the Multilateral System (MLS) and is kept and maintained in trust for humanity through an agreement with the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), under the auspices of the Food and Agriculture Organization of the United Nations (FAO).

The aim of ICARDA Genebank is to conserve and maintain the genetic resources of its in trust collection under conditions that meet or exceed agreed international standards based on current technologies and scientific knowledge, and to promote their sustainable use through responding to requests.

ICARDA's collection is currently maintained in two locations:

- 1) Rabat, Morocco (33° 58' 79" N, 6° 51' 64" W, 75 m alt.)
- 2) Terbol, Lebanon (33° 49' 05" N, 35° 58' 59" E, 900 m alt.)

The purpose of this SOP is to give a step-by-step description of the operations pertaining to conservation activities for ICARDA's holdings while demonstrating compliance with international genebank standards.

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## ***2. Scope***


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This SOP applies to the conservation of genetic resources at ICARDA and describes the following activities:

- Seed processing
  - Seed drying and monitoring of seed moisture content
  - Seed characterization and identity verification
  - Preparation of samples for seed health testing and seed viability testing
  - Seed counting and weighing
- Seed packing
  - Preparation of samples for Base, Safe Duplication and Active Collections
  - Labelling
- Process of samples that do not achieve regeneration standards
- Storage
- Monitoring
- Database update

This SOP does not describe the following activities:


- Seed characterization of genetic resources maintained at ICARDA Genebank, described in the characterization section in “Regeneration and Characterization of cultivated and wild cereal genetic resources at ICARDA” (SOP-ICARDA-REG & CHAR\_CE003) and in “Regeneration and Characterization of cultivated and wild food legume germplasm at ICARDA” (SOP-ICARDA-REG & CHAR\_FoodLegum\_1.0).
- Seed viability testing of genetic resources maintained at ICARDA Genebank, described separately in “Seed viability testing of plant genetic resources at ICARDA” (SOP-ICARDA-SVT-001).
- Seed health testing and pathogen elimination, described separately in “Phytosanitary certification of plant genetic resources at ICARDA” (SOP-ICARDA-PHYTO-001).

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### ***3. Terms, definitions, abbreviations, and acronyms***

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

Accession	A distinct uniquely identifiable sample of botanic seeds representing a cultivar, breeding line or a population of a particular plant species, which is maintained in storage for conservation and use.
Active collection	A collection of germplasm maintained for immediate distribution. This collection can serve also for other purposes in terms of genebank activities, such as characterization and evaluation. It is conserved in short- to medium- term storage at 2-4° C and 25% RH.
Base collection	A collection of germplasm conserved for the long term, which is used to replenish the active collection as needed, but from which no distribution to users is done. It is conserved in secure storage at -18 to -20°C.
Breeder's line	Materials developed and fixed by a breeding method.
CGIAR	Consultative Group on International Agricultural Research
DOI	Digital Object Identifier. It is a unique alphanumeric string for each genebank accession created by the GLIS (Global Information System) of the ITPGRFA (International Treaty of Plant Genetic Resources for Food and Agriculture) to improve tracking and traceability of germplasm accessions.
Drying room	A chamber with controlled space environment, where the air temperature is adjusted between 15-18°C and the relative humidity of the air at 15%.
FAO	Food and Agriculture Organization of the United Nations
Genebank material	Materials imported by the genebank and introduced into the collection for conservation and use.
GRS	Genetic Resources Section at ICARDA
H&S	Health and Safety: the right of every employee to carry out his daily work in a safe environment.
IG	ICARDA's germplasm number. It is a unique identification number that applies to each accession conserved in ICARDA's genebank.
IPR	Intellectual Property Rights
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
I&WE	Infrastructure and Work Environment
Long Term Storage (LTS)	A cold room at -18 to -20° C for the conservation of base collection.

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Medium Term Storage (MTS)	A cold room at 0-4° C and less than 25% RH for the conservation of active collection.
MLS	Multilateral System of the International Treaty on Plant Genetic Resources for Food and Agriculture
NARS	National Agricultural Research System
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.
Safety back-up collection	A copy of a collection conserved off site as an insurance against loss.
SMTA	Standard Material Transfer Agreement
SOP	Standard Operating Procedure
SGSV	Svalbard Global Seeds Vault
TTT	Trueness to Type

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#### ***4. Occupational Health and Safety***

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All activities performed in this SOP comply with the requirements and recommendations in ICARDA Health and Safety manual.

For the activities of seed characterization and seed packing, genebank dressing code requires from the staff the use of laboratory coats.

When staff performs activities inside the drying and cold rooms, [genebank dressing code](#) requires from the staff to comply with the following:


- Hard hats of cap style with shell material of high-density polyethylene for all the activities inside the drying chamber, MTS and LTS chambers.
- Cold coats of high tenacity water repellent nylon (7.5 oz. high-loft insulation), appropriate for up to -10°C for all the activities inside the MTS chamber.
- Freezer coveralls of high-density water-repellent nylon (11.5 oz. high performance insulation) appropriate for up to -50°C for all the activities inside the LTS chamber.
- Insulated work gloves synthetic fiber thermal insulation (80 grams) with waterproof inner bladder, appropriate for up to -30°C for all the activities inside the LTS chamber.
- Freezer hoods of soft polyester fabric and soft fleece interior for all the activities inside the LTS chamber.

Any untoward incident is reported to the genebank manager for proper action and investigation.

Regarding the human activities into the cold and drying chambers, for the MTS chamber staff is not allowed to work inside the chamber more than 30 minutes daily during the pick period (i.e., September – December) and 30 minutes twice per week the rest of the year. For the LTS the allowed time for the staff to remain within the chamber does not exceed 15 minutes twice per week during the pick period and 15 minutes twice per month the rest of the year. As far as the drying chamber is concerned, the same rules are valid as in the MTS and staff is not allowed to work inside the chamber more than 30 minutes daily during the pick period and 30 minutes twice per week the rest of the year.

No tasks in the chambers are allowed to be implemented from independent individuals and a group of a minimum of two personnel staff is entering each time into the chambers to deal with each necessary activity.

Detailed instructions regarding appropriate dressing code for each of the chambers and time limitations for human activities within the chambers have been issued by the Genebank Manager and are shared with all the staff dealing with the conservation process.

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## 5. *Materials, Equipment and Reagents*


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

### Materials:


- 8 x 11 cm, thickness of 0.10 mm paper craft foils (locally purchased)
- Ribbons for bar code labeling (Zebra 5095 High performance Resin; [Zebra ribbons](#))
- Polyester white labels for bar code labeling (Zebra, Z-Ultimate 3000T; [Zebra labels](#))
- Stack plastic containers with hinged lid, dimensions 600 x 400 x 250 mm ([Integra ALC](#))
- Cardboard boxes (locally purchased)
- Laminated aluminum pouches (dimensions: 130 x 260 mm, 130 x 140 mm, 100 x 120 mm) ([Moore & Buckle LTD](#))
- Squared graduated plastic containers of heavy duty (capacity 250, 1000 and 2000 ml) ([Azlon](#) brand in Morocco, locally purchased in Lebanon)
- Gloves (locally purchased)
- Cotton bags (locally purchased)
- Permanent markers size F (locally purchased)
- Permanent markers size M (locally purchased)
- Permanent markers waterproof with round and thick point (locally purchased)
- Transparent resistant scotch (locally purchased)
- Resistant metallic staplers (locally purchased)
- Staples (non-colored) (locally purchased)
- Staple removers (locally purchased)
- Resistant blade cutters (locally purchased)
- Packing rubber bands (locally purchased)
- Stainless steel forceps (locally purchased)
- Stainless steel scissors (locally purchased)

### Equipment:

- Printers for bar code labeling (model [Zebra 110Xi4](#) high performance printer)
- Seed counters ([Wintersteiger S 25 plus](#) and [Pfeuffer contador](#))
- Electronic hygrometer for measuring seed water activity ([Rotronic HYGROLAB-C1-SET-40](#) and [HP23-AW-A-SET-40](#))
- NIR spectroscopy Lab Analyzer ([Metrohm, FOSS NIRS DS 2500](#))
- Dehumidifiers (Munters, [MLT1400](#), [MLT350](#) and [MCS300](#))
- Vacuum and packing machines ([Henkelman Lynx 32](#))
- Precision balances ([RADWAG, WLC-2E0-001](#))
- Insulated cold wear clothing (coveralls, coats, gloves, hoods) ([ASA safety supplies](#))
- Hard caps ([ASA safety supplies](#))

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All essential genebank equipment is monitored and verified by trained staff, calibrated by a certified third party and included in the genebank's maintenance schedule. 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.

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## 6. Procedure

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6.1 The **Conservation** procedure is initiated when an accession arrives at the genebank premises from the regeneration trials following threshing, cleaning, and fumigation. For the newly introduced accessions, (i.e., those coming from collecting missions and/or new acquisitions) the **Conservation** procedure starts upon the assignment of a temporary unique identifier for each accession (IG number for ICARDA Genebank) after validation of its passport data and clearance from seed health laboratory. These steps prompt the initiation of the following conservation activities:


- Seed processing
  - Seed drying and monitoring of seed moisture content
  - Seed characterization and identity verification
  - Preparation of samples for seed health testing and seed viability testing
  - Seed counting and weighing
- Seed packing
  - Preparation of samples for Base, Safe Duplication and Active Collection
  - Labelling
- Process of samples that do not achieve regeneration standards
- Storage
- Monitoring
- Database update

### Seed Processing

6.2 Seed processing is initiated when new accessions with assigned IG numbers or accessions from the regeneration trials arrive at the Genebank facilities. Accessions are received in cotton or paper bags, placed in ascending order into perforated plastic boxes and they are accompanied by the respective list containing the following pertinent information. This information is also included in the barcode label, one placed at the inner side and another one firmly tied outside each accession bag. Accessions are checked and confirmed against the list as quality control measure.

- Accession number (IG)
- Experiment code (for the material received after regeneration)
- Plot number (for the material received after regeneration)
- Full taxon name (Genus, Species, Subspecies)
- Country of origin
- Year of regeneration (for regenerated material) or introduction in the Genebank (for newly acquired material)



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### ***Seed drying and measurement of seed moisture content***

6.3 The perforated plastic boxes containing the seed samples are transferred into the drying room at 15°C and 15% relative humidity (RH). Boxes are put sequentially onto the shelves of the drying room and seeds are allowed to dry for a period of 6-8 weeks. The day of entrance into the drying room is marked with an adhesive label attached at the side of each plastic box.

Drying is one of the critical activities in prolonging the seed longevity and allows the prediction of the potential storage life of each seed sample. For ICARDA seeds (winter cereals, winter food legumes, the crop wild relatives and the temperate forage and range species), seed moisture content (MC) should not exceed 7% and not fall below 4%.


6.4 For quality assurance, the laboratory apparatus used to determine the seed moisture content (Hygrometer) is calibrated once every year, before applying any measurement to the seeds stored into the drying room. Furthermore, as an additional quality assurance measure, 50 samples from each crop are taken randomly to determine the seed moisture content using the standard laboratory method (high constant temperature oven-drying method; ISTA, 2007). For the same seed samples, moisture content is also determined by the Hygrometer and NIR machine and the results are compared with the ones of the standard method to confirm accuracy of the measurements.

6.5 Based on the entrance date into and provided that accessions have completed a minimum of 6-8 weeks deposit in the drying room, one accession per box (or in case of Lebanon 5% of the accessions from each trial) is taken randomly for monitoring the seed moisture content. Seed moisture content is determined using a non-destructive method, using appropriate Hygrometer (Rotronic Hygrometer). The following situations can occur:

6.5.1 The seed moisture content of the sampled accession is at or below the 7% threshold. In this case, the seed sample is sufficiently dry, and the accessions included in the box of the representing accession are considered ready to be processed for packing.

6.5.2 The seed moisture content of the sampled accession is above the 7% threshold. In this case, the seed sample returns to the drying room and along with all the accessions of the respective box is left for further drying. The seed moisture content of the same sample will be checked again after ten days, and the process will be repeated in regular intervals until the seed moisture content reaches or falls below the 7% threshold.

6.6 Boxes with accessions that reached required seed moisture content threshold are withdrawn gradually (two boxes at a time) from the drying room and moved to the seed processing area for further process. For monitoring purposes, the exit date from the drying room is also recorded for each box.

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### ***Seeds characterization and identity verification***

6.7 Accessions are taken gradually from the drying room and entered into the seed processing area (T=20°C, RH=25%) in lots of 40 (i.e., two plastic boxes per time, each including 20 accessions). A representative random sample of 200 seeds from each accession is received and placed separately for seed characterization and identity verification.

6.8 Seed characterization is done for morphological traits based on the appropriate per crop official descriptor lists (IPGRI descriptors). Seed characterization for each crop is described in detail in the pertinent Regeneration and Characterization SOPs.

For the accessions of cereal and food legume crops, seed characterization is also completed in terms of quality traits. For this purpose, the Near Infrared spectroscopy (NIR) is employed as a non-destructive method by the use of appropriate equipment (NIR machine) and accessions are characterized for protein, starch, and crude fiber seed content (cereal crops) or for protein, oil, and crude fiber seed content (food legume crops).

6.9 Further verification of identity of the accessions is carried out during the seed processing phase. In this case the representative sample of 200 seeds kept apart from each accession is compared with the reference set and photos. The reference set comprises the remaining seeds from the accessions retrieved from Svalbard or the remaining original seeds in case of newly acquired germplasm. Identity is also monitored by comparing the accession with previous passport or morphological data. More details on identity verification of the accessions are described in the pertinent Regeneration and Characterization SOPs.

6.10 Following seed characterization and confirmation of trueness to type the sample of 200 seeds is divided in two equal lots of 100 seeds each, for viability testing (details are described in the SOP for Viability Testing) and seed health testing (details in the SOPs of Seed Health Testing).


### ***Seed counting and weighing***

6.11 Upon separating and processing the sample of 200 seeds from each accession, the remaining seed lot is weighed using an analytical balance.

The barcode system has been aligned with the electronic balances used and through the development of a user-friendly interface all measurements in terms of seed weight and seed number are recorded for each crop into an automatically generated xls sheets.

6.12 Total number of seeds is calculated based on the crop. The following cases can be distinguished:

6.12.1 For the faba bean and pea accessions, as well as for the accessions of all forage and range species processed in Lebanon, a representative sample of 200 seeds is counted using a laboratory seed counting machine (Pfeuffer Contador) and the weight is recorded. The total number of seeds in the seed lot is calculated using the following formula:

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$$\text{Total number of seeds} = \frac{\text{Total net weight of clean sample (g)} \times 200}{200 \text{ seed weight (g)}}$$

Similar calculations are used to infer the 100 seed weight for the faba bean and pea accessions and the 1,000 seed weight for the accessions of forage and range species, in order these weights to be recorded in the database.

6.12.2 For the cereals and food legumes (lentil and chickpea) accessions processed in Morocco, a representative sample of 300 seeds, is counted using a laboratory seed counting machines (Pfeuffer Contador and Wintersteiger S-25+) and the weight is recorded. The total number of seeds in the seed lot is calculated using the following formula:

$$\text{Total number of seeds} = \frac{\text{Total net weight of clean sample (g)} \times 300}{300 \text{ seed weight (g)}}$$

Similar calculations are used to infer the 1,000 kernel weight for the cereal accessions and the 100 seed weight for the lentil and chickpea accessions, in order these weights to be recorded in the database.

## Seed Packing


### *Preparation of samples for Base, Safe Duplication, and Active Collections*

6.13 Conditions in the seed processing area are kept in T=20°C and RH=25%. Depending on the available seed quantity the following lots are prepared for packing according to the order described below:

A. Base Collection: Two batches of 600-750 seeds each (depending on the crop; Table 1) are counted and weighed for each accession and placed in laminated aluminum pouches to serve as stock in the Base Collection. For faba bean accessions two batches of 450 seeds each are prepared for Base Collection. Due to the large seed size of the crop, the 450 seeds might be divided between two pouches. The technical specifications of the pouches meet the following requirements: i) an outer layer of 17 g/m<sup>2</sup> Melinex, 4 g/m<sup>2</sup> lacquer; ii) a middle layer 33 g/m<sup>2</sup> (12 µm) aluminum foil, 4 g/m<sup>2</sup> lacquer; and iii) an inner layer of 63 g/m<sup>2</sup> polyethylene.

B. Safe Duplication (level 1): One batch of 600-750 seeds each (depending on the crop; Table 1) is counted and weighed for each accession and placed in laminated aluminum pouches to serve as stock for safe duplication in another reliable genebank (“black box”) after a signed agreement for safety duplication. In the case of faba bean accessions, due to the large seed size, one batch of 450 seeds is prepared to serve the purpose of safe duplication.

C. Safe Duplication (level 2): One batch of 600-750 seeds each (depending on the crop; Table 1) is counted and weighed for each accession and placed in laminated aluminum pouches to serve

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as stock for long-term conservation in SGSV (“black box”). For faba bean accessions, one batch of 450 seeds is prepared.

D. Active Collection: The remaining seed quantity is placed into airtight plastic containers to serve as stock for the Active Collection. The plastic containers are manufactured from high density polyethylene, with an inner lid of the same material and an outer lid from polypropylene. Net weight of seeds that will serve for the Active Collection is recorded and the total number of seeds maintained for the Active Collection is inferred based on the formulas given above for the respective crops (sections 6.12.1 and 6.12.2).


From the remaining seed quantity and for all kind of crops, a lot of 300 (150 for faba bean) seeds per accession will be counted and packed into laminated aluminum pouches to be placed inside the plastic container of the Active Collection. This lot is staying intact for the purpose of being used as a reference set.

If the quantity of seeds is not sufficient to fill all the types of Collection (i.e., Base, Safe duplication (level 1 and 2) and Active) or even if the number of seeds for the Active Collection is below the threshold values of 1,200 seeds for legume and forage and range species or below 1,500 seeds for cereals (cultivated and wild), then the accessions are flagged into the database to be planed for another cycle of regeneration the next season (for more details, see the pertinent Regeneration and Characterization SOPs).

- 6.14 Stock in the database is updated with the seed quantity (in terms of seed weight and seed number) prepared for each accession for the different types of Collection (i.e., Base, Safe Duplication and Active).

### ***Labelling***


- 6.15 Early in the season and prior to the seed packing, the genebank manager is allocating the positions that the accessions will be placed into the cold rooms (Active and Base Collection). Positions are allocated for all the accessions regenerated at the field and/or introduced into the Genebank as newly acquired material based on the respective trial lists (see Regeneration and Characterization SOPs). In case an accession did not yield enough seed quantity to fill all types of Collections, the allocated position for the particular accession is valid and the accession will be placed in the position reserved, after the next regeneration season. To the extent possible, positions allocated in such a manner, in order to accommodate accessions within the cold rooms into ascending order.

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**Table 1.** Packing material and seed quantity for the different types of Collections used at ICARDA Genebanks.

<b>A. Base Collection</b>					
Crop	Packing material	Number of packs	Pack size	Seeds per pack	Storing conditions
Wheat and Barley (cultivated)	Laminated aluminum pouches	2	130 x 140 mm	750	T: -18 to -20°C RH: no control
<i>Aegilops</i> and wild <i>Triticum</i>	Laminated aluminum pouches	2	130 x 260 mm	750	T: -18 to -20°C RH: no control
Lentil (including wild)	Laminated aluminum pouches	2	100 x 120 mm	600	T: -18 to -20°C RH: no control
Chickpea, Grasspea and Pea	Laminated aluminum pouches	2	130 x 260 mm	600	T: -18 to -20°C RH: no control
Faba bean	Laminated aluminum pouches	4	130 x 260 mm	225	T: -18 to -20°C RH: no control
Forage and Range species	Laminated aluminum pouches	2	100 x 120 mm	750	T: -18 to -20°C RH: no control
<b>B. Safe Duplication</b>					
Crop	Packing type	Number of packs	Pack size	Seeds per pack	Storing conditions*
Wheat and Barley (cultivated)	Laminated aluminum pouches	2	130 x 140 mm	750	T: -18 to -20°C RH: no control
<i>Aegilops</i> and wild <i>Triticum</i>	Laminated aluminum pouches	2	130 x 260 mm	750	T: -18 to -20°C RH: no control
Lentil (including wild)	Laminated aluminum pouches	2	100 x 120 mm	600	T: -18 to -20°C RH: no control
Chickpea, Grasspea and Pea	Laminated aluminum pouches	2	130 x 260 mm	600	T: -18 to -20°C RH: no control
Faba bean	Laminated aluminum pouches	2	130 x 260 mm	225	T: -18 to -20°C RH: no control
Forage and Range species	Laminated aluminum pouches	2	100 x 120 mm	750	T: -18 to -20°C RH: no control
<b>C. Active Collection</b>					
Crop	Packing type	Number of containers	Container capacity	Seed threshold	Storing conditions
Wheat and Barley (cultivated)	Plastic container	1	1,000 ml	1,500	T: 0 to 4°C RH: 20 to 25%
<i>Aegilops</i> and wild <i>Triticum</i>	Plastic container	1	1,000 ml	1,500	T: 0 to 4°C RH: 20 to 25%
Lentil (including wild)	Plastic container	1	1,000 ml	1,200	T: 0 to 4°C RH: 20 to 25%
Chickpea, Grasspea and Pea	Plastic container	1	2,000 ml	1,200	T: 0 to 4°C RH: 20 to 25%
Faba bean	Plastic container	1	2,000 ml	1,200	T: 0 to 4°C RH: 20 to 25%
Forage and Range species	Plastic container	1	250 ml	1,500	T: 0 to 4°C RH: 20 to 25%

\*For the Safe Duplication Collection, seeds are stored temporary at ICARDA's premises, by the time to be sent to another Genebank (level 1) or to the Svalbard Global Seed Vault (level 2).

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6.16 The allocation for positions in the cold rooms is indicated per accession with a code composed of four discrete numbers separated by a dash and a letter A or B (Active or Base Collection, respectively) as a prefix, i.e. “A: xx - xx - xx - xx”.

The first number in the above code stands for the number of rack into the cold chamber, the second number specifies the vertical cabinet out of all that comprise the rack, the third number stands for the number of shelf (starting from the bottom) within the vertical cabinet and the fourth number stands for the number of tray where the accession is positioned within a specific shelf.

Thus, the accession with the position code for example: A: 11 - 03 - 05 - 02, will be placed into the Active Collection at the rack no. 11 into the cold chamber, at the third vertical cabinet, onto the fifth shelf and inside the second tray of this particular shelf.

6.17 Electronic files (xls) with the allocated positions are sent to the documentation specialist and upon validation the new positions are uploaded in the database.

6.18 In parallel with the preparation of seeds for the Base, Safe Duplication and Active Collection, barcode labels are printed including the following information.


- Accession number (IG)
- Full taxon name (Genus, Species, Subspecies)
- Country of origin
- Cropping season
- Position into the collection (for Active and Base collection only)

One barcode label is affixed outside the container/pouch, while a second one is placed inside. For all the containers and pouches a waterproof thermal resistant white polyester type of label, dimensions 7.62 x 5.08 cm is used printed by the use of a high-performance ribbon (for more details see Section 5: Materials and Equipment of this SOP).

6.19 Laminated aluminum pouches are hermetically sealed under vacuum with the use of appropriate sealing machines (Henkelman, Lynx32 vacuum packing machine). For the wild cereal accessions the sealing is done under reduced vacuum capacity (65-70%), while for some of the forage species with hard prickles (e.g., *Onobrychis* spp.) sealing of pouches is done with no vacuum use. Plastic containers are also hermetically closed with the use of inner and outer lid.

Seed sample preparation for the different types of Collections and sealing process will take place within a short time during the same day that the accessions will enter the seed processing area.

6.20 Once accessions are packed, labelled and sealed they are placed into the respective trays to be deposited directly into the cold rooms at their permanent positions in the LTS (for the Base Collection) and/or MTS (for the Active Collection). In the case of Safe Duplication, the sealed laminated aluminum pouched prepared for each accession, are placed into appropriate plastic boxes, dimensions 600 x 400 x 250 mm and entered for a temporary storage into the LTS, up to the time that they will be sent to the repository genebank or to the SGSV.

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## Process of samples not achieving regeneration standards

6.21 Before accessions deposit into the cold rooms, available seed quantities as well as results of seed viability and seed health tests are known. The following cases can be distinguished:

6.21.1 Accession did not yield enough seeds to fill all the types of Collections (i.e., Base, Safe Duplication (level 1), Safe Duplication (SGSV), Active) or seed quantity deposited into the Active Collection is less than 1,500 seeds for cereal and forage and range species and less than 1,200 seeds for food legume species.

In this case accession is flagged in the database to be included in the regeneration plan of the next cropping season.

6.21.2 Seed viability for the accession is below 85% in case of cultivated germplasm or below 75% in case of crop wild relatives' germplasm.

In this case, accession is flagged in the database to be included in the regeneration plan of the next cropping season.

6.21.3 Accession is detected with quarantine disease (category B1: quarantine recorded with specific tolerance level) or non-quarantine disease (category B2: non quarantine, recorded).

In this case, accession is included to the disinfection process with the supervision of Seed Health Lab before process for storage.


## Storage

### *A. Active Collection (MTS)*

6.22 Plastic containers are arranged in trays, according to the list generated by the database that indicates the final positions into the MTS. A self-adhesive label is placed outside each tray indicating the position of the tray, the crop, as well as the accessions (list of IG numbers) included in the tray.

6.23 Trays are transferred into the MTS and placed onto the shelves at their permanent positions.

Room conditions at the MTS are, T: 0 to 4 °C and RH: <25%. Sensors are placed within the chamber to monitor the conditions in terms of temperature and relative humidity and indications are continuously displayed on a monitor fixed outside the entrance of the SAS area. An alarm set is installed, and an alert indication is received through email and sms at the mobile phones of the genebank manager and crop curators (at both location; Lebanon and Morocco).

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### ***B. Base Collection (LTS)***

6.24 Laminated aluminum pouches are arranged by two (by four in the case of faba bean, see Table 1) for each accession and placed in appropriate plastic boxes, dimensions 600 x 400 x 250 mm, according to the list generated by the database that indicates the final positions into the LTS. A self-adhesive label is placed outside each box indicating the position of the box and the crop.

6.25 Boxes are transferred into the LTS and placed onto the shelves at their permanent positions.

Room conditions at the LTS are, T: -18 to -20 °C and RH: no control. Sensors are placed within the chamber to monitor the conditions in terms of air temperature and indications are continuously displayed on a monitor outside the SAS entrance. An alarm set is installed, and an alert indication is received through email and sms at the mobile phones of the genebank manager and crop curators (at both location; Lebanon and Morocco).

### ***C. Safe Duplication (level 1 and SGSV)***

6.26 Laminated aluminum pouches that have been prepared for safe duplication to another genebank are arranged into appropriate plastic boxes (dimensions: 600 x 400 x 250 mm).

6.27 An additional box is prepared that includes randomly selected accessions, representative of all boxes that will be shipped for safe duplication. Seeds of these accessions are packed in laminated aluminum pouches and placed into the box as extra seed lots. This particular box will be marked with the indication “samples to be used for viability monitoring”, in order to permit the repository genebank to check periodically for viability, or alternatively for these samples to be retrieved by ICARDA and checked for viability at ICARDA Genebank premises.


6.28 Boxes are numbered based on a sequential number given by the documentation specialist, according to the previous numbering of boxes already deposited under “black box” status to other genebanks.

6.29 An electronic list of accessions with the accessions included within each plastic box is generated. The list includes the following information:

- Crop ID
- Accession number (IG)
- Full taxon name (Genus, Species, Subspecies)
- Country of origin
- Year of seed production
- Year of safe duplication
- Net seed weight (g)
- Seed number
- Box number

6.30 Printed lists are placed within each of the corresponding plastic boxes and boxes are entered for a temporary storage into the LTS awaiting shipment process.



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6.31 The genebank manager contacts the repository genebank and an agreement is signed between the two genebanks specifying that the material will be deposited and maintained under the “black box” status.

In the case of second level of safe duplication material (i.e., to the Svalbard Global Seed Vault), there is an overall signed agreement between the ICARDA and the Crop Trust.

6.32 The genebank manager sends the inventory list and the pertinent information of the material to the genebank manager of the recipient genebank and requests for the import permit.

In case of safe depositing to the Svalbard Global Seed Vault), the import permit constitutes a statement document for importing seeds issued by the Norwegian Food Safety Authority.

6.33 The genebank manager fills and sends to the seed health laboratory the “Seed dispatch request” forma, with the following information:

- Crop ID
- Full taxon name
- Seed source
- Year of seed production
- Number of boxes
- Total number of accessions
- Net weight of all shipment
- Gross weight of all shipment
- Contact details of the recipient
- Details of last seed health test control for the accessions included in the shipment.
- Fumigation requirement
- Seed treatment requirement


One “Seed dispatch request” forma is filled for each of the different crops that are intended to be deposited for safe duplication.

6.34 The responsible of seed health laboratory submits a phytosanitary certificate request form to the National Plant Protection Service (in Lebanon or Morocco), including all the above information.

6.35 At the same time all shipment related documents are prepared by the genebank manager. These documents are the following:

- Non-commercial value certificate
- Non-GMO declaration

6.36 Upon obtaining the phytosanitary certificate from the national authorities and the import permit from the repository genebank a copy of these, along with a copy of the other two documents (i.e., non-commercial value certificate and non-GMO declaration) is placed inside each of the boxes. An envelope with a copy of all documents is also placed in a “window” outside of each plastic box. The original documents are placed inside one of the boxes and in all other boxes an indication marks the box number in which the original documents can be found.

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6.37 Plastic boxes are closed and secured with appropriate plastic lockers and tares and shipped via international courier.

During the period between documents preparation and/or certificates acquisition and the time of shipping the lots are kept in appropriate conditions (i.e., in the LTS chamber). ICARDA Genebank is striving to reduce the shipment duration to the minimum.

6.38 The repository genebank is informed through an e-mail, attaching a scanned copy of all the documents accompanying the shipment as well as the tracking number of the shipment.

6.39 The genebank manager tracks the delivery of the consignment online using the shipment's tracking number.

6.40 When the consignment is delivered, traced online, an acknowledgement of receipt is requested from the repository genebank to confirm arrival of the shipment.

6.41 Upon receiving acknowledgement receipt from the repository genebank, all information about the new safe duplication deposits is updated into the database.

Same process as above is followed when ICARDA safe duplicates its germplasm through mutual deposits between the two Genbank location sites (i.e., Lebanon and Morocco).

Safe duplication to the Svalbard Global Seed Vault is done when large number of accessions is ready to be shipped.

## Monitoring of seed viability and seed quantity


Monitoring consists of periodically:

1. Testing the viability of accessions maintained under medium term and long-term storage to ensure they did not lose their capacity to produce viable seedlings when needed and remain always above certain thresholds in terms of seed viability rates.
2. Monitoring seed quantity to ensure sufficient seed for conservation and distribution purposes.

Viability monitoring is done periodically, after 5 years' interval in the Active Collection and after 7 to 10 years in the Base Collection. Seed quantity monitoring is done prior to every distribution.

6.42 For viability monitoring: the genebank manager proceeds to a random sampling that captures approximately 5% of the accessions from each one of the shelves of each vertical cabinet of the chambers, based on the year of seed production.

6.43 A list with the candidate accessions is generated, indicating the precise positions of the accessions within the cold chambers and the accessions are withdrawn to be tested for viability.

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6.44 Viability testing is carried out according to the steps described in detail in the related SOP for Viability Testing.

6.45 Based on the results of the viability test the following cases can be distinguished:


6.45.1 Viability is  $\geq 85\%$  for cultivated accessions and  $\geq 75\%$  for wild species germplasm. In this case accessions meet the acceptable standards for viability and the samples remain in the MTS and LTS for an additional period before being tested again.

6.45.2 Viability is  $< 85\%$  for cultivated accessions and  $< 75\%$  for wild species germplasm. In this case, the accessions are added to the list of accessions to be regenerated for the next cropping season. In addition, all the accessions positioned at the same shelves with the accessions monitored and accounted for lower viability than the acceptable thresholds, are withdrawn and subjected to viability testing. According to the results, accessions with reduced viability will be flagged to be included in the regeneration plan the following cropping season.

6.46. Data from viability monitoring is recorded and uploaded in the database.

6.47. For quantity monitoring and as described in 6.21.1, accessions that fall below quantity thresholds (less than 1,500 seeds for cereals and less than 1,200 seeds for food legume and forage and range species) are flagged in the database to be included in the regeneration plan of the next cropping season.

6.48. One cycle of the conservation procedure is considered finalized when: i) samples are placed in long term and medium-term storage as well as samples have been deposited for safe duplication; and ii) all pertinent information has been entered into the genebank database.

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## ***7. Related Workflows, Checklists, Documents and Links***

The following **workflows** are pertinent to this SOP:

- The procedural workflow for the Conservation of Genetic Resources at ICARDA is in the appendix.

The following **checklists** are used to control the quality of the consignment:


- Checklist for phytosanitary and legal requirements (confirms that all phytosanitary and legal requirements are met prior to an international consignment by checking that accessions are under the MLS status of the ITPGRFA, no IPR, confirmed TTT, clean phytosanitary status, etc.).
- Checklist for all outbound consignments (confirms consistency and completeness of all outgoing consignments by checking the inclusion of the courier waybill, customs invoice, SMTA, phytosanitary certificate, import permit, certificate of donation, non-GMO declaration, non-commercial value declaration, delivery proof, etc.).

**Compliance with standards and policies:** The Conservation of Genetic Resources at ICARDA is in compliance with the following standards (include institutional, national, and international), and policies (include framework and operational):

- [FAO's Genebank Standards for Plant Genetic Resources for Food and Agriculture](#) (2014)
- International Rules for Seed Testing. [International Seed Testing Association](#), ISTA (2016)
- [FAO's Pest Risk Analysis for Quarantine Pests](#)
- [FAO's Requirements for Phytosanitary Certificates](#)

**Adherence to legal and regulatory frameworks:** The Conservation of Genetic Resources at ICARDA 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 \(SPS Agreement\)](#)
- [European and Mediterranean Plant Protection Organization \(EPPO\)](#)
- [UPOV](#)

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## ***8. Staff Training and Competency***

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

- Zakaria Kehel, Research Team Leader, Head of Genetic Resources Section. Contact: [Z.Kehel@cgiar.org](mailto:Z.Kehel@cgiar.org)
- Mariana Yazbek, Senior scientist, Genebank Manager Lebanon, oversight of the conservation procedure in Lebanon, Contact: [M.Yazbek@cgiar.org](mailto:M.Yazbek@cgiar.org)
- Athanasios Tsivelikas, Scientist, Genebank Manager Morocco, oversight of the conservation procedure in Morocco, Contact: [A.Tsivelikas@cgiar.org](mailto:A.Tsivelikas@cgiar.org)
- Adil Moulakat, Research Assistant, Contact: [A.Moulakat@cgiar.org](mailto:A.Moulakat@cgiar.org)
- Rama Jawad, Research Assistant, Contact: [R.Jawad@cgiar.org](mailto:R.Jawad@cgiar.org)
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- Oumaima Zaher, Research Assistant, Contact: [O.Zaher@cgiar.org](mailto:O.Zaher@cgiar.org)
- Khadija Aouzal, Research Assistant – Documentation specialist, Contact: [K.Aouzal@cgiar.org](mailto:K.Aouzal@cgiar.org)

Genebank staff undergoes periodic training and periodic competency testing. Competency testing is done regularly in an informal way as part of the supervision and ensuring the quality of the work.

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## 10. *Revision History*

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Effective Date of the SOP	Version #	Description	Reviewed By
July 1 <sup>st</sup> , 2018	1.0	Original SOP	<ul style="list-style-type: none"> <li>• Dr Ahmed Amri</li> <li>• Dr Janny van Beem</li> </ul>
October 30 <sup>th</sup> , 2023	1.1	Updated version	<ul style="list-style-type: none"> <li>• Dr Zakaria Kehel</li> </ul>

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## ***11. Citation, Copyright and Access Agreement***

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**Annex 1.** ICARDA Genebank holding composition (last update October 2023).

Crop	Number of accessions
Bread wheat	15,090
Durum wheat	20,357
Primitive wheat	1,393
Wild <i>Triticum</i>	2,169
Wheat hybrids	48
<i>Aegilops</i> spp.	5,185
Barley	30,247
Wild <i>Hordeum</i>	2,240
Chickpea	14,833
Wild <i>Cicer</i>	552
Lentil	13,732
Wild <i>Lens</i>	645
Faba bean	9,654
<i>Lathyrus</i> spp.	4,468
<i>Pisum</i> spp.	4,596
<i>Medicago</i> annual	8,930
<i>Trifolium</i> spp.	5,519
<i>Vicia</i> spp.	6,452
Other forage and range species	5,828
Not mandate cereals	285
<b>Total</b>	<b>152,223</b>