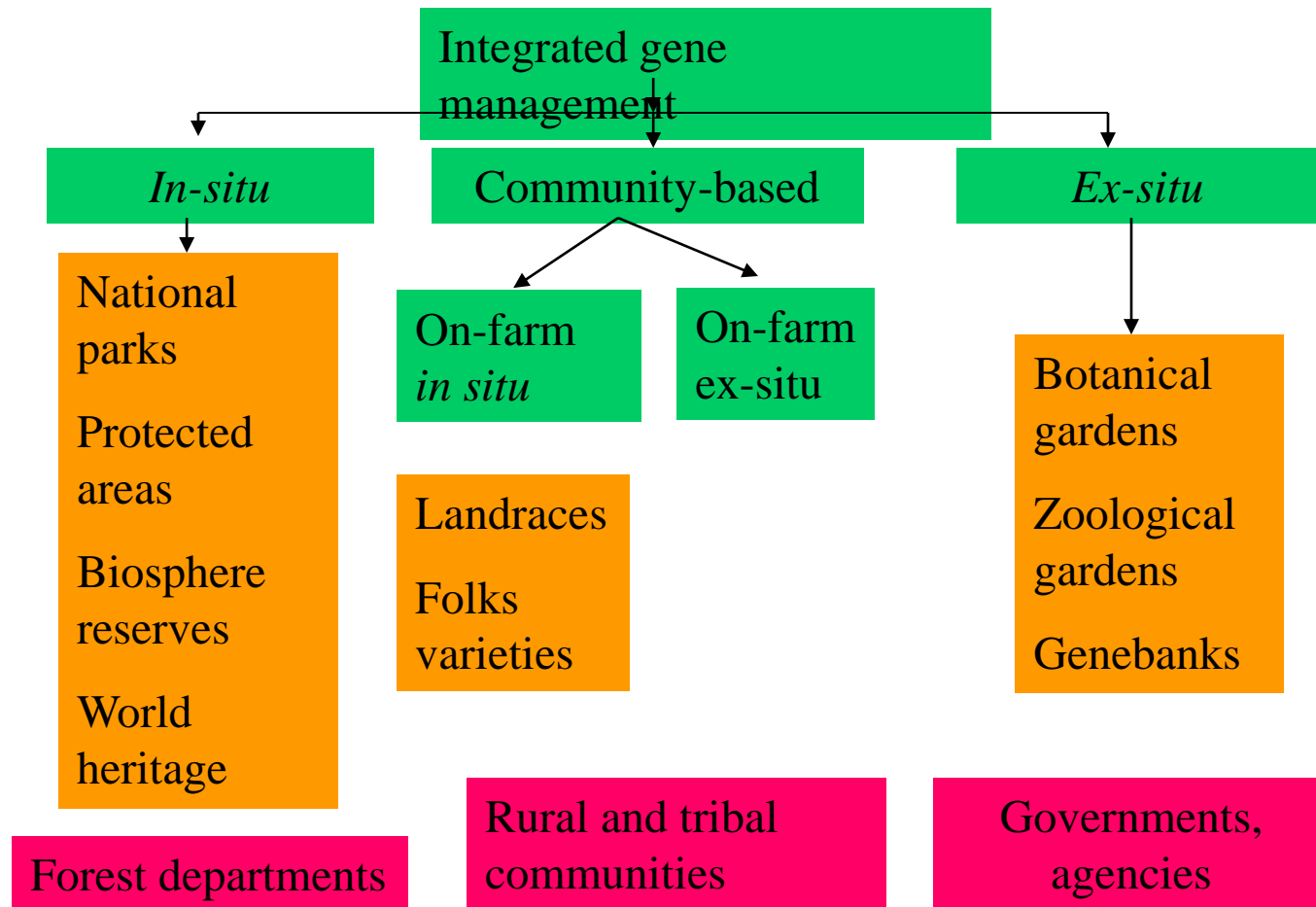



Approaches to conservation



Ex-situ and *in-situ* conservation

- *Ex-situ* conservation means the conservation of components of biodiversity outside their natural habitats;
- *In-situ* conservation means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in case of domesticates and cultivated species, in the surroundings where they have developed their distinctive properties;
- *In situ* conservation on farm refers to the continuous cultivation and management of a diverse set of populations by farmers in the agroecosystems where a crop has evolved (Bellon et al 1997). It includes cultivated species, forages and agroforestry species and their wild relatives
- *Circa situ* used for trees when trees are removed to managed agroforest areas or on farm-based conservation areas identical to their natural habitats.





Methods of conservation of plant genetic resources

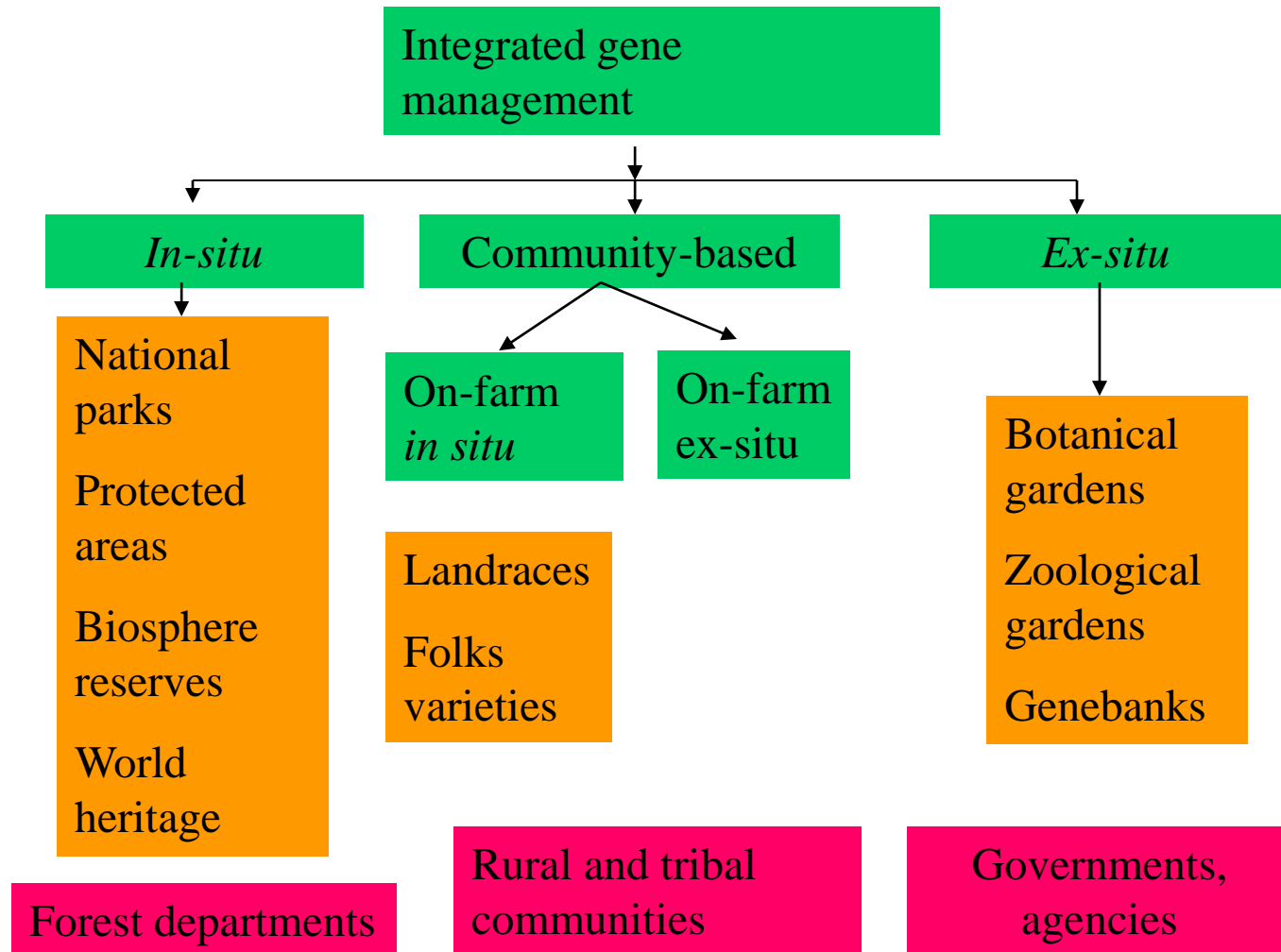
In situ

- Dynamic conservation benefiting from natural and farmers selection;
- Larger genetic base conserved;
- Conservation of related local knowledge;
- Suitable for recalcitrant species; or species that cannot be regenerated or established outside natural habitat
- Success depends on the involvement/commitments of various stakeholders and requires a holistic approach and strengthening of scientific basis;
- Benefits and costs for/by farmers;.

Ex situ

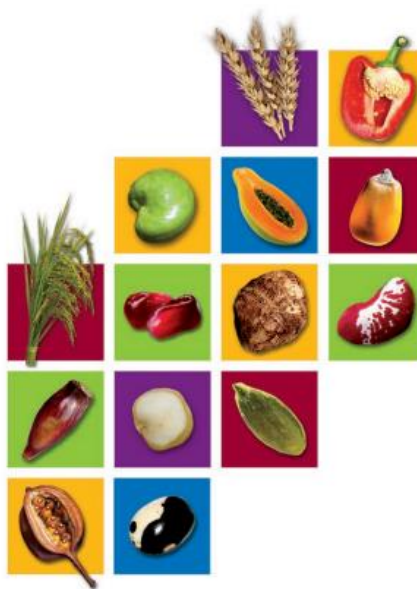
- Static conservation for long periods in genebanks;
- Diversity only sampled;
- Good evaluation and readily available for use in crops improvement programs;
- Source of seeds under disasters for restoration and rehabilitation;
- Benefits to breeders and costs by genebanks;
- Requires skilled staff and can be affected by regeneration and genetic drift and shift.

These should be regarded as complementary methods



Genebank Standards

for Plant Genetic Resources
for Food and Agriculture



COMMISSION ON
GENETIC RESOURCES
FOR FOOD AND
AGRICULTURE





Underlying principles of Genebanking

- Identity of accessions: the identity of seed sample accessions conserved
 - Proper identification
 - careful documentation of data and information
 - recording passport data
 - Herbarium voucher specimen
 - seed reference collections
- Maintenance of viability:
 - Maintaining viability, genetic integrity and quality of seed samples, and making them available for use
 - Collecting as close as possible to maturation but prior to natural dispersal, avoiding collection from the ground
 - collected germplasm is genetically representative of the original population
- Maintenance of genetic integrity:
 - Maintain viability and diversity of the original collected sample.
 - Ensuring that viability is maintained according to the standards contributes to the maintenance of genetic integrity.
- Maintenance of germplasm health:

icarda.org conserving and distributing are free from seed-borne diseases and regulated pests (bacteria, virus, fungi and insects).



Underlying principles of Genebanking

- Physical security of collections:
 - secure the materials from any external factors, natural disasters and human-caused damage.
 - ensure that genebank cooling equipment, as well as backup generators and equipment to control power outages,
 - ensure materials are safely duplicated in other locations
- Availability and use of germplasm: Conserved material must be available for current and future use.
 - maintain sufficient quantities of seed and
 - related information on the accessions.
- Adherence to the legal and regulatory frameworks at national and international levels,

Types of genebanks

- Seed bank
- Tissue bank (in vitro collections)
- Cryobank
- Field Genebank



I. Seed bank

- A seed bank stores seeds to preserve genetic diversity (for Orthodox Seeds)
 - Cost-effective means of off-site (*ex situ*) conservation
 - Large amount of genetic diversity in small volume
 - Most species produce seeds that are desiccation tolerant when mature; such dried seeds can be frozen
 - Within limits & very approximately, seed life-span doubles for every 1% reduction in moisture content and for every 5°C reduction in temperature → great longevity

Acquisition of germplasm

- Samples have been acquired legally with relevant technical documentation.
- Collecting should be made close to the time of maturation and prior to natural seed dispersal
- Period between seed collecting and transfer to a controlled drying environment should be
- Samples should be accompanied by at least a minimum of associated data/passport descriptors.
- The minimum number of plants from which seeds should be collected is between 30-60 (up to 150) plants, depending on the breeding system of the target species

Drying and storage

- Samples should be dried to equilibrium in a controlled environment of 5–20 °C and 10-25 (**15%**) percent of relative humidity.
- After drying, Samples need to be sealed in airtight container for long-term storage (LTS); for frequent access to seeds, store in non–airtight containers (MTS: Medium Term Storage)
- Most-original-samples (MOS) and safety duplicate samples should be stored under long-term conditions at a temperature of -18 ± 3 °C and relative humidity of 15 ± 3 %.
- For medium-term conditions (active collection), samples should be stored under refrigeration at 5–10 °C and relative humidity of 15 ± 3 %



Seed viability monitoring

- Initial seed viability test should be conducted after cleaning and drying the accession or at the latest within 12 months after receipt of the sample at the genebank.
- Initial germination value: $\geq 85\%$ for cultivated crop species. 75% for Wild and forest species
- Viability monitoring test intervals: Varies: 5, 10, 15 years?
- Viability threshold for regeneration: 85%

Regeneration

- when the viability drops below 85 % **OR**
- when the remaining seed quantity is less than what is required for three sowings of a representative population of the accession. The most-original-sample should be used to regenerate those accessions.
- Regeneration should ensure that the genetic integrity of a given accession is maintained.
 - Species-specific regeneration measures should be taken to prevent admixtures or genetic contamination arising from pollen gene flow that originated from other accessions of the same species or from other species around the regeneration fields.
- At least 50 seeds of the original and the subsequent most-original samples should be archived in long-term storage for reference purposes



Characterization

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



Documentation

- Passport data of 100 percent of the accessions should be documented using FAO/Bioversity multi-crop passport descriptors.
- Data and information generated should be recorded in a suitably designed database.

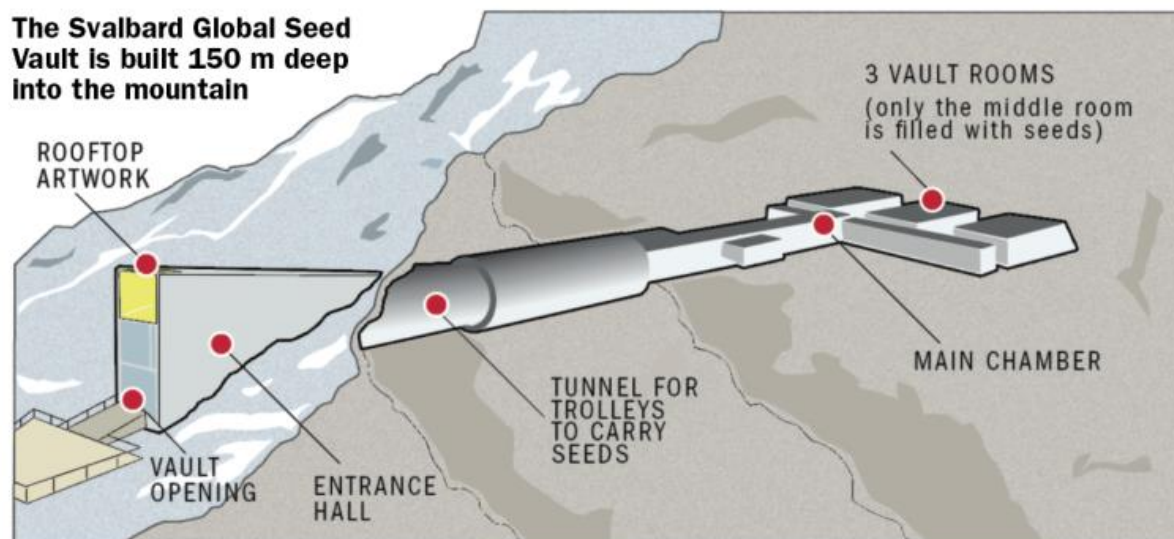
Distribution and exchange

- Seeds should be distributed in compliance with national laws and relevant international treaties and conventions.
- Seed samples should be provided with all relevant documents required by recipient country.
- a sample of a minimum of 30–50 viable seeds should be supplied if available; if not → multiplication



Safety duplication

- Safety duplicate sample for every original accession should be stored
 - in a geographically distant area
 - under the same or better conditions than those in the original genebank
- Each safety duplicate sample should be accompanied by relevant associated information.





Genebank standards for field genebank in vitro culture and cryopreservation

- Phenomenon of non-orthodoxy
 - Understanding the desiccation tolerance and sensitivity in orthodox compared to non-orthodox (intermediate and recalcitrant) seeds is of fundamental importance for conservation.
 - At maturity, orthodox seed water content would generally be in the range[0.05 – 0.16 g/g] (5 % - 14 %), although some species are shed at much higher water content, undergoing substantial dehydration after this.
 - Unlike recalcitrant seeds, all orthodox seeds acquire desiccation tolerance, which is genetically-programmed and entrained before, or at the start of maturation drying.
 - Recalcitrant seeds do not dry during the later stages of development and are shed at higher water contents in the range of 0.3–0.4 – >4.0 g/g. Because they are desiccation-sensitive, the loss of water rapidly results in decreased vigour and viability, and seed death at relatively high water contents.

II. Field genebank

- Field genebank is **essential for those crop species having recalcitrant seeds for conservation, characterization, evaluation and utilization**





Choice of location of the field genebank

- The agro-ecological conditions (climate, elevation, soil, drainage) of the field genebank site should be as similar as possible to the environment where the collected plant materials were normally grown or collected.
- located so as to minimize risks from natural and manmade disasters and hazards such as pests, diseases, animal damage, floods, droughts, fires, snow and freeze damage, volcanoes, hails, thefts or vandals.
- located so as, to minimize risks of geneflow and contamination from crops or wild populations of the same species to maintain genetic integrity.
- should have a secured land tenure and should be large enough to allow for future expansion of the collection.
- should be easily accessible to staff and supplies deliveries and have easy access to water, and adequate facilities for propagation and quarantine.



Acquisition of germplasm

- Propagating material should be collected from healthy growing plants whenever possible, and at an adequate maturity stage to be suitable for propagation.
- The period between collecting, shipping and processing and then transferring to the field genebank should be as short as possible to prevent loss and deterioration of the material.
- Samples acquired from other countries or regions within the country should pass through the relevant quarantine process and meet the associated requirements before being incorporated into the field collection



Establishment of field collections

- A field genebank should have a clear map showing the exact location of each accession in the plot.
- The appropriate cultivation practices should be followed taking into account micro-environment, planting time, rootstock, watering regime, pest, disease and weed control



Field management

- Plants and soil should be regularly monitored for pests and diseases.
- Appropriate cultivation practices such as fertilization, irrigation, pruning, trellising, rootstock and weeding should be performed to ensure satisfactory plant growth.
- The genetic identity of each accession should be monitored by ensuring proper isolation of accessions wherever appropriate, avoiding inter-growth of accessions, proper labelling and field maps and periodic assessment of identity using morphological or molecular techniques.



Regeneration and propagation

- Each accession in the field collection should be regenerated when the vigour and/or plant numbers have declined to critical levels in order to bring them to original levels and ensure the diversity and genetic integrity is maintained.
- True-to-type healthy plant material should be used for propagation.
- Information regarding plant regeneration cycles and procedures including the date, authenticity of accessions, labels and location maps should be properly documented and included in the genebank information system.

In vitro collection & Cryopreservation

- Cryopreservation or Cryostorage: The storage of plant organs in liquid nitrogen (-196°C) or above (maximum -140°C).
- In the genebank context, it is used for buds, shoot tips, other meristematic and embryogenic tissue, explants from recalcitrant and (in special cases) entire orthodox seeds, pollen and somatic embryos.
- In most cases in vitro phases before and/or after the storage phase proper are involved.

