GLOBAL PUBLIC GOODS Phase 2 (GPG2)

Principles and strategy for safety duplication of plant genetic resources for food and agriculture

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Principles and strategy for safety duplication of plant genetic resources for food and agriculture

Rationale for safety duplication

Over 700,000 samples of plant genetic resources are in the in-trust collections of CGIAR Centres. The germplasm and the information that comes with it represent important global public goods to be used for the well being of the present and future generations. As collection custodians, the Centres have expertise in the management of conservation and delivery of services on the use of the germplasm. They are primary agents in creating a global system for the proper preservation, accurate documentation, efficient distribution and facilitated access of genetic resources.

The Global Public Good Project aims to develop a programme to further secure the in-trust collections to perpetuity and promote their use through the upgrading of genebank operations and facilities. The project is guided by the Developmental Goal, which states "crop genetic resources and associated biodiversity are put to use in developing countries to fight poverty, enhance food security and health, and protect the environment". It aims to achieve the following: secure conservation, effective genebank management, facilitated access to the in-trust collection, and CGIAR Center involvement in the development of the global biodiversity conservation and use system.

The collective action for the rehabilitation of global public goods in the CGIAR Genetic Resource System (GRS) includes principles and strategies on safety duplication. These are guided by the following elements: interest and political will, technical issues, economic analysis and legal procedures. Sound management of genetic resources rests on understanding its value and strong political will to act on its protection and conservation. This means provision of adequate facilities and scientific procedures in the light of present economic realities.

Definition of safety duplication

An important aspect of genebank management is to secure duplicates of germplasm for safety backup to mitigate the risk of its partial or total loss caused by natural or man-made catastrophes. The safety duplicates are genetically identical to the base collection and are referred to as the secondary most original sample (Engels and Visser, 2003). This is deposited in a base collection at a different location, usually outside the country.

Safety duplication is generally under a 'black box' approach (Rao and Bramel, 2000; Engels and Visser, 2003; CGN, 2006). This means that the repository genebank provides the best possible storage facilities, but beyond that, has no entitlement for the use and distribution of the germplasm. It is the depositor's responsibility to ensure that the deposited material is of high quality, to monitor seed viability over time and to use their own base collection to regenerate the collections when they begin to lose viability. The germplasm is not touched except for return on request from responsible genebank when the original collection is lost or destroyed. Recall of the deposit is also possible when it is replaced with newly regenerated germplasm.

Safety duplication should not only include security backups, but also proper documentation. However, aside from the export-import permits (Rao and Bramel, 2000), no detailed information is usually supplied from the CGIAR because all passport data of the in trust collections is available through the System-wide Information Network for Genetic Resources (SINGER). Documents simply record what is held (Engels and Visser, 2003).

Standards for long-term storage of seeds are described in two documents namely, International Board for Plant Genetic Resources (IBPGR) Advisory Consultation on Genebank Standards and International Standards for Germplasm Management (ISGM) Framework for Seed Collection. The Svalbard Global Seed Vault (SGSV) management has provided a description of safety deposits and guidelines for the processing and transport of materials to Svalbard, Norway (SGSV, 2007).

Selection of facility to hold safety duplicates

Criteria for the selection of the repository genebank are in place to ensure that the safety duplicates are properly secured. Primary consideration is given to the geographic location and environmental conditions of the location. The geographic location determines the type of climate required for minimum energy input in the maintenance of the cooling facilities. Geologic formations must ensure low radiation (radioactivity) and stability (low probability of earthquakes). The facility must be situated at an elevation that guarantees proper drainage during seasonal rains and eliminates the risk of flooding in the event of rising sea levels due to global warming. Equally important is economic stability and socio-political certainty. A stable economy guarantees the constant flow of capital inputs for the costly maintenance of facilities, genebank operations and personnel. This is unlikely in the presence of social unrest and political uncertainty. Koo et al. (2004) suggested that safety duplicate should be located outside the risk of political embargo, military action or terrorism that could disrupt international access.

The physical requirements for the safety and security of germplasm in collections are described in the Genebank Standards (FAO/IPGRI, 1994). These are as follows:

- Power supply to the seed store must be stable and continuous. An alternative back-up generator with adequate fuel supply is preferred.

- Fire precautions should be undertaken and appropriate fire fighting equipment tested periodically. Personnel must be trained to use this equipment. Installation of lightning conductor rod, alarm system and high temperature cut out for the cooling system is recommended.

- The installation should be designed for high security and adequate security arrangements for the protection of the facility.

- Refrigeration standards and equipment should conform to the Design of Seed Storage Facilities for Genetic Conservation (DSSF) (IBPGR, 1982) specifications. There should be trained personnel and available spare parts for repair and maintenance. Routine preventive maintenance should be carried out.

- The construction and installation standards should follow the DSSF guidelines (IBPGR, 1982), taking into account the local conditions and whenever possible using locally available material. The size of the store should reflect the numbers and sizes of germplasm samples to be stored for efficiency. The use of modular units to increase flexibility and safety is appropriate.

- Protective clothing should be provided and used in the store. Personnel should be aware of and trained in safety procedures. Devices to open doors from inside drying rooms and refrigerated rooms should be installed.

Current practices for safety duplication

A. Seeds

1. Quantity of seeds per accession

The standard quantity of orthodox seeds for long-term storage depends on the genetic composition and breeding system of plants. For homogenous and self-fertilizing plants, 1000 seeds is enough material for a minimum of four times the number of seeds required for one regeneration cycle (Rao and Bramel, 2000). However, Dulloo and Engels (2003) noted that this number is the absolute minimum and the preferred number is 1500 to 2000 seeds, calculated as the minimum sample size for one generation, plus one generation for an active collection and several viability monitoring tests. More seeds are needed for genetically heterogeneous accessions. While FAO/IBPGR set the number to 1500 to 2000 seeds, Rao and Bramel (2000) suggested 4000 seeds for cross fertilizing plants. The number of seeds can be reduced for safety

duplication since this is only a back-up as a last resort and no monitoring is possible from seeds in black box storage.

The number of seeds recommended for storage for safety duplication is less than the given standard for long-term storage because sufficient seeds should be stored for at least 2 independent regenerations and there is usually no monitoring of seed viability in black box storage. Regeneration of germplasm is undertaken when viability drops to 85% of the initial value. It requires 100 plants or more to reduce the probability of large losses of alleles (FAO/IBPGR, 1994). With this in perspective, the accession size for safety duplicates is estimated to be at least 400 seeds, a more conservative number compared to the 500 seed limit set for Svalbard deposits which presupposes an additional amount as a safety factor.

Non-CGIAR genebanks use a definite number of seeds for safety duplicates. The Millennium Seed Bank (MSB) and Nordic Gene Bank (NGB) store 150 and 500 seeds respectively for all of their accessions regardless of weight and breeding type. The Centre for Genetic Resources, Netherlands (CGN) determines the number of seeds for each type of plant. It ranges from 100 to 600 except for flax (4000) and potato (100 to 1000).

The number of seeds per accession deposited as safety duplicates is not uniform in CGIAR genebanks and varies according to the type of crop (Table 1). It ranges from 300 for most cereals and pulses to 4000 for millet with very small seeds. CIMMYT and IITA pack maize in different quantities. A packet of maize at CIMMYT contains 2500 seeds. It is four times greater than the ones at IITA. Phaseolus beans and forages are part of both CIAT and ILRI collections. At CIAT the number of seeds ranges from 100 to 1000, while at ILRI 1000 seeds are stored per accession.

Table 1. Accession size of safety duplicates of CGIAR mandated crops.

Genebank	**Mandated Crop**	**Number of seeds**
1. CIAT, Colombia	Phaseolus beans & Forages	100 – 1000
2. CIMMYT, Mexico	Maize	2500
	Wheat, Triticale, Barley, Rye	300
3. ICARDA, Syria	Chickpea	300
4. ICRISAT, India	Chickpea, Groundnut	300
	Pigeon pea	800
	Sorghum	2000
	Pearl millet	3000
	Other millet	4000
5. IITA, Nigeria	Cowpea, Bambara, Wild vigna, Soybean, African yam bean, Maize	250-600
6. ILRI, Ethiopia	Forages	1000
7. IRRI, Philippines	Rice	1000

Table 2 shows that some crops have met the criterion for the minimum number of seeds for long-term storage. The highlighted items are within the acceptable limits of accession size for long-term storage with reference to breeding systems and regeneration requirements.

Table 2. Types of crop, pollination mechanism, quantity of seeds deposited as safety duplicates and standard quantity of seeds per accession for long-term storage.

Type of Crop	Type of Pollination	Quantity of seeds deposited for storage		
		Number & weight of seeds deposited as safety duplicates	Standard number of seeds for long-term storage	
			Minimum	Maximum
Barley	self	300 (10g)	1000	2000
Chickpea	self	300 (80g)	1000	2000
Groundnut	self	300 (80g)	1000	2000
Flax	self	4000	1000	2000
Forages	cross, self	1000	1000	4000
Maize	cross	2500 (1000g) 250 - 600	1500	4000
Pearl millet	cross	3000 (25g)	1500	4000
Other millet	cross & self	4000 (25g)	1000	4000
Pigeon pea	cross & self	800 (80g)	1000	4000
Potato	cross & self	100 – 1000 (500g)	1000	4000
Rice	cross & self	500 (10g) 1000 (20g)	1000	4000
Rice (wild)	cross & self	50 (1g)	1000	4000
Rye	cross & self	300 (10g)	1000	4000
Sorghum	self	2000 (25g)	1000	2000
Triticale	cross & self	300 (10g)	1000	4000
Wheat	self	300 (10g)	1000	2000
Cowpea Bambara groundnut Wild vigna Soybean African yam bean	self	250 – 600	1000	4000
Phaseolus bean Forages	cross & self	100 – 1000 (5 to 200g)	1000	4000

2. Quality of seeds

Seeds for use as safety duplicates must meet the highest quality standard for long-term, low temperature storage. The quality of seeds is measured by percent viability. High viability and appropriate moisture content and temperature during storage will ensure that the genetic integrity of the germplasm is preserved for a long period of time. Most genebanks store seeds with moisture contents of 3 to 7 percent, except for maize (6 to 8%) and a germination threshold of 85%. The germination limits for wild types of plants is 60%.

Most CGIAR genebanks use the ISTA standard for testing the viability of seeds. They may also conduct germination experiments using a sequential test. The number of seeds for each test is 200, 100, 50 or 20. Monitoring seed viability of safety duplicates is generally done every 10 years. The interval for testing is shortened to 5 years for species known to have a short longevity.

3. Packaging and labelling

The laminated aluminium foil used to pack seeds consists of three layers: outer polyester, middle aluminium and outer polythene. The thickness of each layer varies slightly depending on the brand. Polyester has a thickness of 2 or 12 µm or weighing 12 gm^{-2}. The aluminium foil is 8, 9 or 12 µm thickness or 24 gm^{-2}. The inner sheath of polythene has a thickness of 55, 70, 75 or 80 µm, or 34 gm^{-2}. The size of seed packets depends on the size of the seeds. The packets are either heat sealed or vacuum sealed. Table 3 shows the specifications of different qualities of laminated aluminium foil currently used to pack seeds in genebanks.

The packets of seeds should be labelled both outside and inside to ensure clear identification of accessions. This is important in case outside labels are removed or ink fades so packets of seeds are mixed up. Six out of 11 genebanks surveyed place only outside labels on seed packets. The label is moisture proof and can withstand low temperature. The labelling material is either sticky plastic, computer generated sticky label or self-adhesive paper. The technology used for printing labels can be as sophisticated as thermal transfer printer (CGN) or a simple marker pen with indelible ink. If labels are placed inside the packet, preferred material is computer-printed thick paper or card.

The information in the label of seed packets can either be accession number only (CIMMYT, ICRISAT, NGB), accession number and bar code (CIAT), accession number and year of harvest (IPK), unique serial number (MSB) or includes other crop-related information (CGN, IITA, ILRI, WARDA). ISGM recommends that outside labels of seed packets in long-term storage should contain the following information: accession number, batch reference, scientific name, seed storage location code, date of storage and the packet number if there are several packets or accessions.

4. Transport

The shipment of seeds to the depository genebank is within a larger container. Usually the packets of seeds are placed in sealed cardboard boxes. Some make use of galvanized iron box (ICRISAT), others plastic boxes (IPK) or wooden boxes (NGB). If the destination of the seeds is within few hundred kilometres, transport is by land and takes only a few hours. Generally, genebank curators prefer to air courier the package so avoiding long transit at ports where high temperature is detrimental to the viability of the seeds. Whatever the type of transport, the seeds are in ambient condition. There is no uniform frequency of shipment among genebanks: bi-annual, annual, every 2 to 4 years or occasional.

Table 3. Quality of laminated aluminium bags used in genebanks.

Genebank	**Specification of bags used**				**Supplier and address**
	Inner layer PE* (µ)	Middle layer (ALU)**	Outer layer PET*** (µ)	Extra layers	
CIAT, Colombia	Bags with flat seal				INCODI – Mcdellin Colombia Fax 216 425-9800
CIMMYT, Mexico	Wheat 34 g m^{-2}	24 g m^{-2}	12 g m^{-2}	38 g m^{-2}	DRG Malago, Argus Rd. Bedminster, Bristol B53 3BP – England
	Corn – bags with folded seals				Packaging Aids Corp. 469 Bryant St., San Francisco CA 94107 – USA, Tel. 415 362 9202
CIP, Peru	Screen matrix seal bags				Hokuto Shoji Kaisha Ltd. No. 20 – 21 Meikiminami 1-chome Nakamuru-ku Nagoya, Japan
ICARDA, Syria	70	12	12		Leeuwarder Papier Fabriek (LPF) Verpakkingen, 890 Leeuwarder The Netherlands Fax: +31 58 122 047

Genebank	Specification of bags used				Supplier and address
	Inner layer PE* (µ)	Middle layer (ALU)**	Outer layer PET*** (µ)	Extra layers	
ICRISAT, India	70	12	12		Silces SpA, Via Val Lerone 5 16011 Arenzano, Genova, Italy Fax: +39 10 911 1110
ILRI, Ethiopia	55	9	12		Moore & Buckle, Sutton Rd. St. Helens WA9 3DY, UK
IRRI, Philippines	75	12	23		Barrier Foil Products, CCE Business Park, Windmill Lane, Denton Manchester M343 QS, UK
Nordic Genebank, Sweden	Type 1 75 LDPE****	9	12		Curevac AB, Foreningsgatan 4A 41127 Goteborg, Sweden
	Type II 70 LDPE	9	12		
EMBRAPA/CENARGEN Centro Nacional de Pesquisa de Recursos Geneticos e Biotechnologia, Brazil	75	12	23		Barrier Foil Products, CCE Business Park Windmill Lane, Denton Manchester M343 QS, UK
Center for Genetic Resources, The Netherlands (CGN)	75 or 80 (Polyethylene)	8 or 12	2		*No information on supplier; information on quality of packaging material was obtain from Quality Management System (internet)*

*PE – Polythene, **ALU – Aluminium, ***PET – Polyester, ****LDPE – Low density polythene

Sources: SGRP, 1996; Center for Genetic Resources, (CGN). 2006.

5. Storage

Depository genebanks maintain good facilities for long-term storage of germplasm. Infrastructures are designed to withstand natural or man-made catastrophes. Germplasm is maintained either in a vault or cold room with a temperature of -18 to -20^{o}C (NCGRP, ICARDA, CIMMYT, ICRISAT, MSB, HRI, LINFOA, IPK-Malchow, SASA) or -15^{o}C (IPK-Gatersleben). The seed deposits are shelved as they arrive in the depository. Table 4 summarizes the CGIAR genebank facilities for long-term storage of safety duplicates.

Table 4. CGIAR genebank facilities for long-term storage of safety duplicates.

Genebank	Type of germplasm	Storage container	Temp. (^{o}C)	Moisture (%)	Remarks
CIAT, Colombia	*Phaseolus* beans Tropical forages	Aluminium foil bags	-15 to -20	6 to 8	Area: 260m^{3} Seed longevity: 30 to 50 yr
CIMMYT, Mexico	Maize & Corn	Aluminium foil bags	-18	ambient	Area: 240 m^{3}
CIP, Peru	Seeds	Aluminium packets	-15	Dried 2 weeks @ 17 ^{o}C	Minimum no. of 2000 potato seeds
ICARDA, Syria	Seeds		-20	20%	No RH control in storage, moisture content of dried seeds at 25^{o}C
ICRAF, Kenya	Seeds		-20	10 to 15	Upright freezers; moisture content of dried seeds at 15^{o}C
ICRISAT, India	Seeds	Vacuum sealed aluminium packets	-20	4 to 6	Area: 3 x 125m^{3}
IITA, Nigeria	Seeds	Sealed containers	-20	5 to 7	
	Yam tubers		20	50 to 60	
ILRI, Ethiopia	Forage seeds	Aluminium packets	-20	5 to 8	Upright freezers
IRRI, Philippines	Rice seeds	Sealed aluminium cans	-18 to -20	6	2 cans per accession; air-cooled condensing unit, automatic system to operate cooling units; red light indicator signals open door

Source: SGRP, 1996

6. Data arrangement

CGIAR genebanks comply with all the data requirements of the depository genebanks and legal procedures of the host country. Phytosanitary certificates and import-export documents are necessary for each shipment. In the case of MSB and IPK wherein the black box is deposited in an institution located in the same state, data requirements do not exist. The depositor regularly updates databases and no information is sent to the depository.

7. Conditions for replacement and return of seed deposits

The Letters of Agreements between CGIAR genebanks (i.e. CIAT- CIMMYT, ICRISAT – ICARDA, ILRI – CIAT, CIAT - CIP) include conditions for the replacement and return of safety duplicates. Seed deposits are replaced based on the information provided by the depository institution and the viability test results conducted by the depositor. The seeds can be returned to the depositor upon request.

B. Clonal material

The information on safety duplication of clonal germplasm was provided by the Centro Internacional de Agricultura Tropical (CIAT) for cassava;, International Institute of Tropical Agriculture (IITA) for banana, cassava and yam, International Musa Germplasm Transit Center (ITC) for banana and plantain and International Livestock Research Institute (ILRI) for forage grasses. No data was collected on Andean roots and tubers from the Centro Internacional de la Papa (CIP).

Vegetatively propagated germplasm that cannot be stored as seeds is preserved as living material in field genebanks or by in-vitro slow growth or cryopreservation techniques. In-vitro plantlets of cassava, banana and yam are stored in slow growth chambers, while banana and plantain samples are frozen in liquid nitrogen. Plants of all these and other species can be conserved in field genebanks but the level of risk is higher when keeping material in the field owing to exposure to pests and diseases and environmental conditions.

1. Number of samples per accession

For field genebanks, a minimum of 5 up to 30 plants of each accession are normally maintained (Saad and Rao, 2001). Where space and funds allow more plants can be maintained for additional security. ITC prepares 3 tubes for each accession of banana and plantain for safety storage. Samples are sent to France and Belgium for cryopreservation. For every accession of cassava, 2 to 3 (CIAT) or 5 (IITA) samples are deposited as safety duplicates. Each plantlet is placed in individual tubes and maintained in the cold room for slow growth.

2. Percent viability of propagules

This parameter is applicable only for the cryopreservation. ITC has set a criterion for determining the quality of the propagules. After one hour in liquid nitrogen, a minimum of 3 cryotubes are tested for viability. Each batch of propagules must have at least a 95% certainty that a minimum of one plant can be regenerated.

3. Packaging and labelling

Field genebanks are usually divided into well labelled plots. Labels must be permanent and can be polyester computer printed tags or painted metal or wood posts or boards. A good field map to scale is needed as a backup.

For in vitro slow growth, the plantlets are placed in sterile glass or plastic tubes (2mL-cryotubes at ITC) and packed in polyethylene bags. The size of the package depends on the number of culture tubes.

Labels are directly written on the culture tubes with pencil (ITC) or marker pen (IITA) or on tape placed outside the bags. The label information are as follows: ITC - accession ID, freezing date and experiment number; IITA – accession number, line number and date of last subculture; CIAT – accession number and bar code.

4. Transport

Cuttings, roots or tubers for planting in field genebanks are transported in cool conditions in plastic boxes. Root cuttings can be kept moist but not wet to avoid fungal growth and rotting during shipment. Roots and tubers are packed in paper bags inside ventilated strong paper or plastic boxes.

Materials for cryopreservation and in-vitro slow growth preservation are placed in liquid nitrogen and plastic boxes respectively for transport to the safety duplicate site. Shipment is done by air courier, hand carried or by land transport. The transport must take the shortest time to ensure the viability of the germplasm, most especially those that are deposited for slow growth preservation.

5. Storage

Field genebanks are maintained in a different area or country facing different risks. Sites for field genebanks should have low disease and pest incidence, a good water supply, readily available labour source and good security (Saad and Rao, 2001).

Banana and plantain safety duplicates for cryopreservation are deposited in IRD France and LU Leuven Belgium. Both institutes have facilities for the long-term cryopreservation of clones. CIAT deposits cassava clones at CIP while IITA Ibadan Nigeria sends their duplicates of yam, banana and cassava clones to IITA Cotonou, Benin. The clones are placed in the a growth room at 18 ^{o}C (IITA) and 23 ^{o}C (CIP) with controlled photoperiod. The life expectancy of clones frozen in liquid nitrogen is indefinite. Clones kept in slow growth chambers can survive for 10 to 24 months (yam), 8 to 18 months (cassava) and 4 to 6 months (banana). Table 5 shows the CGIAR facilities for in-vitro long-term storage of clonal germplasm.

Table 5. CGIAR genebank facilities for *in-vitro* long-term storage of clonal germplasm.

Genebank	Type of germplasm	Storage container	Temp. (^{o}C)	Moisture (%)	Remarks
CIP, Peru	Potato	Tubes	6 to 8	60 to 70	16hr light at 1000 lux; 4 tubes of 25 x 150mm
	Sweet Potato	Tubes	16 to 18	60 to 70	16hr at 2000 lux; 3 tubes of 18 x 150 mm
	Andean root and tuber	Tubes	18 to 22	60 to 70	16hr at 3000 lux; 6 tubes of 16 x 150 mm
IITA, Nigeria	Cassava and Yam	Tubes	18 to 25	50	5 to 10 tubes per clone
INIBAP, Belgium	Banana	Tubes	-196		Liquid nitrogen tank

Source: IPGRI,1996

6. Data arrangement

The shipment of banana, cassava and yam germplasm to Benin requires an importation permit and reports from the Plant Quarantine Service (PQS) offices. ITC safety deposits are sent with a list of germplasm for identification and an inventory of box contents.

7. Conditions for replacement

The replacement of germplasm is determined by the information provided for by the depository. ITC replaces its safety duplicates when there is less than 95% certainty that a minimum of 1 plant can be regenerated per experiment.

8. Conditions for return

The safety duplicates of germplasm are returned to the depositor upon request in cases of loss. IITA Benin requires a repatriation permit when clones are returned to Nigeria.

Considerations for principles for safety duplication

A. Technical Analysis to Mitigate Risks

Technical procedures for the preparation, documentation, transportation and storage of safety backups are vital in the successful conservation of genetic resources. This section focuses on the analysis of technical options for safety duplication in order to mitigate risks. It proposes sound alternatives as well as recommendations on best practices. It covers both seeds and vegetative (field and in-vitro) materials.

Some general principles apply to safety duplication:

- Maintain at least one duplicate of each accession as a safety back-up. It is ideal to have two sets of safety duplicates stored in different locations, one maintained locally in the same country and the other in another region subject to low risk.
- Select a location with suitable environment, good security and low risk for the samples.
- Consider mitigating risks to loss of genetic integrity within accessions and loss of accession as well as cost in decision making.

1. Accession size

a. Seeds

Analysis is based on the assumption that the initial population size is large enough to represent the diversity of genetic material to conserve and in the case of in-breeders, genotypes are homogeneous within the population.

Out-breeders

The accession size for out-breeders should be sufficient for regeneration to restore the base collection in case of loss. For each regeneration trial, 100 plants or more are required to avoid the probability of large losses of alleles (FAO/IPGRI, 1994; van Hintum and Hazekamp, 1993). However, Crossa (1995) suggested a larger sample size of 200 or more plants that will give a high probability of retaining rare alleles at low frequencies in most of the loci avoiding greater loss of diversity due to genetic drift. Furthermore, Rao et al. (2006) recommended that there should be sufficient seeds for at least three regeneration attempts to recover the accession. Since 100% viability cannot be assured, the accession size of safety duplicates was recommended to be calculated to achieve the target of three regeneration attempts using a minimum of at least 200 seeds each.

300 seeds - The expected number of plants to be recovered from 300 seeds is 255 if viability is 85%. Conserving 300 seeds per accessions would allow only one attempt of regeneration using a sample size of 200 plants. This number is insufficient and could place the collection at risk if the regeneration attempt was unsuccessful. The use of a smaller number of plants per accession for regeneration will lead to loss of alleles and genetic diversity within accessions. The effects of genetic drift, gene flow, and small population size are more pronounced in out-breeders than in-breeders (Del Rio et al, 1997).

500 seeds - An accession size of 500 seeds with 85% germination will provide 425 plants. This number would allow two attempts of regeneration with a sample size of 200 plants each. Although the number of regeneration attempts is less than three recommended by Rao et al. (2006), this should be enough to recover the accession.

1000 seeds - With 85% viability, the expected number of plants recovered from 1000 seeds is 850. Conserving 1000 seeds per accessions would allow three attempts of regeneration of a sample size greater than 200. The sample size would minimize the occurrence of genetic drift and according to Crossa (1989) is enough to retain two, three, or four alleles even when some of them

occur at a frequency of 1%. However, because this is a large number, the curator must follow random sample preparation of seeds for planting to avoid unintentional selection.

Cost implications of seed size and ease of seed production must be taken into account in determining the accession size for safety duplication. For species having large seeds (e.g. groundnut), storing 1,000 seeds requires large storage containers and space, while species which produce few seeds demand more time and labor for the production of 1,000 or more seeds.

Greater than 1000 seeds - Conserving more than 1000 seeds per accessions provides more than enough seeds for three regeneration attempts and would ensure maintenance of genetic integrity of heterogeneous accessions. However, a large accession size would make the safety duplication expensive to manage. Many accessions of crop wild relatives and forage species have limitations to produce large quantities of seeds, therefore this option may be unrealistic for many genebanks.

In-breeders

FAO/IPGRI (1994) specifies 100 or more plants for each regeneration attempt of in-breeders to avoid large losses of alleles. Rao et al. (2006) recommended to place sufficient seeds in safety duplicate storage for at least three attempts at regeneration to recover the accession.

300 seeds - An accession size of 300 with 85% viability can provide 255 plants for two regeneration attempts. Although the number of regeneration attempts is less than what is recommended by Rao et al. (2006), it is enough to recover the accession. The use of a smaller sample size for regeneration does not pose a problem in maintaining the genetic integrity of homogeneous plants. Self-compatibility in in-breeders is expected to produce homozygous and homogeneous populations. Since intra-accession diversity is relatively insignificant, the probability of the loss of genetic integrity by drift or selection is low (Loveless and Hamrick, 1984). A small sample size for regeneration is easily managed, reducing the probability of unintentional selection and genetic contamination.

500 seeds - The expected number of plants recovered from 500 seeds is 425 based on 85% germination. Conserving 500 seeds per accessions would allow four attempts of regeneration of a sample size of 100 plants, which fulfills the recommendation of Rao et al. (2006). This sample size is sufficient to maintain genetic integrity of the accession.

1000 or more seeds - For a minimum of 1000 seeds with 85% viability, the expected number of plants recovered is 850. Conserving 1000 seeds per accession would allow eight attempts at regeneration using a sample size of 100 plants. Using large numbers of seeds would increase costs and is not necessary for safety duplication. It is cost effective to have a small accession size for homogeneous populations since genetic drift or shift is less likely to occur.

Best practice

The recommended accession size for safety duplicates is at least 500 viable seeds for outbreeders and heterogenous accessions with high diversity., A minimum of 300 seeds may be used for genetically uniform accessions. More seeds should be stored for accessions with seeds of low viability.

b. Vegetative materials

Number of replicates for field genebanks

The number of replicates needed in a field genebank depends on the genetic diversity within the accession and the risk of loss during long-term maintenance based on correct selection of planting material, environmental conditions, cultivation practices, management of pests and diseases and correct labeling of plants (Reed et al., 2004). More replicates are needed in field genebanks with high risk or for maintenance of accessions with high inter-accession diversity. For clones, 5 to 30 plants per accession are usually maintained depending on the size of the plants. For example, 10 clones per accession are maintained for cassava (Fukuda et al., 2005), 30 plants per accession for small forage grasses and 9 plants per accession for larger grasses

(ILRI). Maintaining a large number of replications is not feasible in large perennial species due to space limitations and 3 plants per accession is used in crops such as coconut (Santos et al., 1996).

Best practice

It is best to maintain a minimum of 3 to 5 replicates per accession in the field genebank safety backup. The safety backup collection should be renewed when 30% of the collection is no longer viable.

Number of replicates of in-vitro slow growth materials

The number of replicates of in-vitro slow growth materials depends on the risk of loss during in-vitro multiplication, sub-culturing and storage and is therefore species specific. More replicates are needed when the storage period is long. Multiplication of three replicates per accession can be handled by genebanks with large collections even with economic limitations. The number of explants in each tube depends on the tube size (IPGRI/CIAT, 1994; Reed et al., 2004). In the case of potato, 5 explants are placed in 25 x 150 mm tubes while for sweet potato and cassava 2 explants are placed in 18 x 150 mm tubes.

To promote genetic stability of the collection, it is a good practice to renew plant material from several cultures in order to minimize the chance of somaclonal variation and selection of a variant plant. There is high risk of losing genetic materials of tropical crops stored in vitro since temperatures do not allow storage longer than 10 to 12 months.

Best practice

It is best to store 3 to 5 replicates per accession of in-vitro cultures. **As soon as 1 replicate deteriorates or shows signs of senescence, sub-culturing of the remaining 2 replicates must be done immediately to get enough material for renewal and avoid losing accessions.**

Number of replicates of cryopreserved materials

The parameters used in determining the number of propagules per accession include the number of control samples and the number of plants needed to recover the accession. The number of control samples is determined by the recovery percentage of the accession from the cryobank. The minimum recovery percentage should be within the range of 60% to 70% regardless of the number of cryopreserved progagules, which may be either in large numbers between 100 to 200 or a minimum of 20 control sample and 20 cryobank samples (Dussert et al., 2003). In the case of accessions showing low plant recovery percentages, it is suggested to increase the number of cryobank samples. The number of propagules per accession stored in the cryobank is obtained by multiplying the minimum number of cryobank samples stored by the number of times it is to be recovered.

Cryopreservation imposes a series of physical, chemical and physiological stresses on plant material, which may induce genetic modifications in cryopreserved cultures and regenerated plants. However, to date, several studies have shown that there is no phenotypical, biochemical, chromosomal, or molecular level modifications that could be attributed to cryopreservation procedures (Engelmann, 2004; Harding, 2004).

Best practice

It is recommended to store at least 100 units or propagules per accession. Two batches of accessions must be cryopreserved separately to minimize risk.

2. Quality of seeds

The longevity of seeds during storage depends on the initial seed viability, moisture content of the seeds during storage and temperature of the store. In general low moisture contents between 3-7% and low temperatures reduce viability loss during storage. Different combinations of moisture

content and temperatures can be used to achieve the same results to preserve longevity of seeds during storage for safety duplication.

Initial germination percentage

Heterogeneous populations require that seeds with a high initial viability of 90 percent are stored in order to avoid selection of traits and changes in the genetic composition of the accession. Since seed deterioration of homogeneous populations does not cause genetic change through selection or drift, a lower germination percentage is acceptable. Another important factor to consider is that the loss of viability is correlated with an accumulation of chromosome damage in surviving seed that can be passed on to succeeding generations (Cromarty et al, 1982). It is therefore necessary to regenerate the accessions before viability has dropped dramatically. Seed viability is monitored on seed lots of the same accessions maintained in long term storage in the genebank and extrapolated to the safety duplicate. In some cases, samples for germination testing may be sent in a separate box with the safety duplicate and monitored for germination by agreement with the depository.

Best practice

It is recommended to use conditions that are at least as stringent as those for long term storage of germplasm in a genebank. An initial percentage viability of 90% in the case of heterogeneous populations that are subject to genetic changes, and a percentage of 85% for others is usual. Special consideration should be given to wild species with low viability (40 to 60%).

Seed moisture content

Seed longevity is increased as storage moisture content is decreased. In many species each reduction of 1% moisture content doubles the longevity of the seeds. Seeds should be dried to a safe moisture content to prevent loss of germination. Hermetic storage at the critical moisture content, below which further reduction in moisture content no longer increases seed longevity in hermetic storage, provides maximal seed longevity at a given storage temperature. The critical moisture content is species specific and varies from about 6% for the protein rich legume seeds of pea (*Pisum sativum*) and mungbean (*Vigna radiata*) to 4.5-5.0 for starch rich cereals like rice, wheat and barley, to about 3.5% for the oily seed species of soya bean and as low as 2% for groundnut. Based on this range in common crops, moisture contents of 3-7% are usually recommended. Moisture contents above this range would result in more rapid loss of seed viability with associated potential for loss of genetic integrity through shorter periods between regeneration cycles. Drying below the critical moisture content will result in ultra-dry seeds. Although research is in progress on longevity of ultra dry seeds during storage, there is still discussion on the full effects of very low moisture content on longevity over long storage periods.

Best practice

It is recommended to use conditions that are at least as stringent as those for long term storage of germplasm in a genebank, using 3-7% seed moisture content to maintain seed viability.

Storage temperature of seeds

Seed longevity is increased as storage temperature is decreased. According to Harrington's Thumb Rules, for each 5^{o}C the seed storage temperature is reduced, seed longevity is doubled (Justice and Baas, 1978). Longevity is increased by a factor of 3 if storage temperature is reduced from 20^{o}C to 10^{o}C; by 2.4 from 10^{o}C to 0^{o}C; by 1.9 from 0^{o}C to –10^{o}C; but by only 1.5 from –10^{o}C to –20^{o}C (FAO/IPGRI, 1994). Optimum longevity is achieved when seeds are stored at about –18^{o}C or lower. However, the reduction in temperature must be coupled with appropriate moisture content levels (see above). Temperature and water content have interlocking effects on the kinetics of seed ageing. The relationship is specific such that every given temperature is linked to optimum water content (Walters, 2003). FAO genebank guidelines recommended storing

seed collections at usually –18°C to –20°C combined with 3-7% seed moisture content to maintain seed viability (FAO/IPGRI, 1994).

Best practice

It is recommended to use conditions that are at least as stringent as those for long term storage of germplasm in a genebank, storing safety duplicates at –18°C to –20°C.

3. Packaging material

a. Different thickness of packaging materials for seeds

The rate of water vapour that enters or leaves a seed storage container will affect seed moisture content and therefore seed longevity (Gomez-Campo, 2006). Properties of seed storage containers that affect overall operating efficiency include the following: permeance (water vapor barrier performance of the material), permeability to substances (i.e. O_2 and organic volatiles), flexibility, strength, availability of suppliers and costs. Permeance is calculated from the water vapor permeability and the material thickness. It decreases as the thickness of the material increases. A container with thicker walls creates a better water vapour barrier (Walters, 2007).

The laminated aluminium foil used for seed packets consists of three layers: outer polyester, middle aluminium and inner polythene. The thickness of each layer varies slightly depending on the brand. Polyester has a thickness of 2 or 12 µm or weighing 12 g m^{-2}. The aluminium foil is 8, 9 or 12 µm thickness or 24 g m^{-2}. The inner sheath of polythene has a thickness of 55, 70, 75 or 80 µm, or 34 g m^{-2}.

High-density films of polyethylene (polythene) show less permeability to water vapor and gases, reaching one-tenth of the amount of moisture transmitted by a low-density film of polyethylene. Thicker polyethylene layer has also a better puncture resistance.

Aluminum foil has a low water vapour transmission rate. Thin aluminum foil has tiny perforations called pinholes. A single hole of $4x10^{-5}$ square inch would transmit about 0.19 g of water vapor per 24 h at 37.8°C and 100 percent relative humidity. The thicker the foil, the less the number and size of pinholes, thus reducing the rate of water vapour transmission (Justice and Bass, 1978). Aluminum foil of 30µm thick is 'commercial pinhole free' while 40 µm-thick foil is guaranteed hole free (Cromarty, et al, 1982). The type of aluminum currently in use by genebanks has only a maximum thickness of 20µm and may contain small pinholes, through which water can permeate. The impermeable layers of polyester and polyethylene offset this. The inner layer of high-density polyethylene also offers resistance to hard sharp seed penetration and provides the heat-sealing properties.

Best practice

It is recommended that the packaging material of seeds for safety duplication be made of 12 µm of polyester, 30 µm of aluminium foil and an inner layer of high-density polythene of 80 µm thickness.

b. Different containers for safety backup of vegetatively propagated materials in vitro

Glass test tubes, which allow illumination and convenience during inspection or evaluation of in-vitro plantlets, have been traditionally used for in vitro storage. They have the advantage of being repeatedly re-used, but are subject to breakage and contamination. Once the black-box collection is replaced with a new one, test tubes may be returned to the base collection institute requiring an additional cost for shipping.

Gas-permeable, heat-sealable Polyethylene bags are potential alternatives for packaging in-vitro plantlets because they are small in size (reduce storage space) and resistant to breakage. It eliminates the risk of contamination caused by air pressure changes and movement of the medium into the caps as in the case of test tubes (Reed, 1991). Reed (1992) reported that in-vitro cultures of *Fragaria* remained sterile after 15 months of storage using polyethylene bags

compared to 20% contamination and death of plants using test tubes. Storage and identification of cryo-collections is facilitated by the use of plastic cryogenic boxes and holders that prevent mixing or loss of samples.

Polypropylene vials are manufactured to withstand temperatures to -196°C. Cryovials have silicone gaskets or o-ring in the screw caps for a secure seal (Daigger and Company Inc. 2007). When cryopreservation procedure involves filling the tubes containing samples with liquid nitrogen, the cryovials should not be closed completely to avoid explosion when the liquid nitrogen changes to the gas phase for any reason.

Preserving samples in liquid nitrogen should be done carefully If cryovials are immersed in the liquid phase, liquid nitrogen can still enter the closed screw-top cryovials during storage. The cryovial may then explode when it is removed from storage due to the vaporization and expansion (700x expansion ratio) of the liquid nitrogen inside the cryovial. Where feasible, the risk of explosion of cryovials stored in the liquid nitrogen liquid phase can be further reduced by moving cryovials to the gaseous phase in the liquid nitrogen container for at least 24 hours before removing (Office of Environmental Health and Safety Cornell University, 2007).

Use of cryopreservation for safety backup requires careful monitoring of liquid nitrogen levels. It is safer to use storage containers with narrow necks in order to avoid rapid vaporization of the liquid nitrogen and an alarm system to indicate low liquid nitrogen levels is highly recommended (Reed et al, 2004).

Best practice

It is recommended to use glass test tubes or gas-permeable, heat-sealable polyethylene bags for in-vitro slow growth cultures and polypropylene vials with a secure seal for cryosamples stored in containers with narrow necks and an alarm system to indicate low liquid nitrogen level.

4. Labelling

a. Labelling seed packets

Number of labels

The risk is high when seed packets have only one outside label, most especially when labels do not have high adhesive properties, are not resistant to chemicals and moisture, and printing fades. Use of an internal label mitigates the risks and is recommended for materials that are being stored in safety back-up for long periods.

Best practice

It is recommended to place an outside and inside label to each packet of seeds to make sure that the germplasm is properly identified.

Type of label

Labels for long-term conservation should be moisture proof and resistant to low temperature and with enhanced smudge and scratch resistance. Polyester strong paper self-adhesive labels are recommended because they are more resistant to below zero temperatures and the adhesive has shown to be permanent for many years, ensuring identity of the collections.

Best practice

Self-adhesive labels that are resistant to below zero temperature with permanent adhesive are recommended.

Type of ink used

A good ink and print quality are important to ensure that the labels can be easily read after many years of storage. Permanent markers may be used or printers, which may be dot matrix, laser, direct thermal and transfer thermal.

Direct thermal printers and transfer thermal printers produce high quality print with clear characters. Transfer thermal printer is a better option for safety duplication labelling because of their long life image stability (Rojas, 2008).

Best practice

Use printers with ink with long life and image stability for printing labels for safety backups.

b. Labelling containers of vegetatively propagated materials

In-vitro slow growth

Medium term conservation lasting two to four years requires permanent labels and printing. Self-adhesive labels that are resistant to below zero temperatures with permanent adhesive should be used. Polypropylene labels are good and reliable option for medium term in-vitro conservation (CIP, 2008). Barcode labelling improves management through efficient monitoring and tracking of different genebank activities and should be used whenever possible.

Best practice

Self-adhesive labels that are resistant to below zero temperature with permanent adhesive are recommended.

Cryopreservation

Cryogenic labels must withstand extreme deep-freezing temperatures of liquid nitrogen (-196^{o}C) with resistance to chemicals. They should also resist moisture during repeated freeze and thaw cycles. Polyester thermal transfer-printed labels are the best option for labelling cryovials. 2D (two-dimensional) barcoding (e.g. Data Matrix), which is a high-density, non-linear is recommended (Symbol Technologies, 1999).

Best practice

Polyester thermal transfer-printed label is recommended for labelling cryovials.

5. Transport

a. Method and duration of transport

Seeds

Black box storage requires that boxes remain unopened and seeds are stored in the same box used for shipping. The box should be durable and strong enough to support the weight of the seed packets, provide protection during shipping and withstand low temperature storage. Sealed cardboard boxes, plastic covered and plastic boxes are currently used. Transport should be by the fastest and most practical method to reduce the shipping period when the seeds are exposed to a range of temperatures.

Best practice

Strong cold-resistant boxes (thick carton or polypropylene box) are the best options for transporting and storing seeds. Boxes should be sealed properly. Shipment should

consider the fastest means of transport available either by air freight, courier or by land to avoid deterioration of seed quality during transit.

In-vitro slow growth

Optimum protection of in-vitro cultures must be guaranteed during transport. In order to protect the cultures from rough motion it is best to use culture medium containing a higher concentration of agar, e.g. 0.95% (Tay and Liu, 1992). Test tubes must be sealed with permeable tape and packed in polystyrene racks with respective fit-caps so they remain stable during shipping and can easily be transferred when they reach their destination. Racks are packed in thick cardboard or plastic boxes for shipping to avoid the risks of drastic environmental changes (over-heat, freeze, heavy rain) or accidents (fire) that could damage the materials. In-vitro cultures can remain viable for up to a month when properly packed and transported under optimum temperatures. Plantlets remain healthy even after lengthy customs delay (Reed et al, 2002).

Best practice

Pack sealed test tubes in polystyrene racks with fit-caps in sterile conditions in strong thick cardboard or plastic boxes.

Cryopreservation

Traditional shipping method involves packing the material with dry ice and using rapid transport. This approach entails high risk with an average product loss of 20% (Cryogenic Consultants LLC, 2005). The best alternative to transport cryopreserved materials is using dry shippers at cryogenic temperatures. These use absorbent material that prevents liquid spill and the temperature remains at approximately -150° C until the liquid nitrogen evaporates from the absorbent material (Roberts Oxygen Company Inc., 2008). The shipping dewar retains liquid nitrogen for 15 days, so it could also be used for international shipments (Reed, 2008).

Best practice

Cryopreserved materials should be transported using liquid nitrogen.

b. Effects of temperature during transport on germplasm viability

Seeds

Rapid deterioration of seeds during storage may occur due to high temperatures during shipping. Shipping by the most direct and fast route in the cooler months of the year can help to alleviate risks during shipping. A data logger can be used to monitor temperature changes during transport.

In-vitro slow growth

Freezing or overheating decreases viability of in-vitro slow growth plants. In-vitro plantlets can survive two or three weeks without light. At high temperature growth of plants accelerates. The increase in growth rate could lead to death of explants since respiration exceeds photosynthesis. Critical differences between culture medium and external temperature could lead to high relative humidity within containers resulting in hyperhydricity in plantlets, affecting their survival. A data logger can be used to monitor temperature changes during transport.

Cryopreservation

The temperature threshold in cryopreservation is specific to the cryopreserving method applied and samples being cryopreserved (e.g. pollen, seeds, meristematic tissue). Conditions during transport of cryopreserved samples should ensure that the liquid phase of nitrogen is maintained ($-196^{o}C$). The change from liquid to gas phase (at $-135^{o}C$) poses high risk because nitrogen gas evaporates easily and results in sample damage by thawing. Cryopreserved cultures should not

be thawed and refrozen during transit. Cryostorage equipments must have alarm devices in case of a change in temperature.

Best practice

It is recommended to ship by the most direct and fast route in the cooler seasons and label boxes clearly to indicate the need for temperature control and proper handling during shipment. It is desirable to include a data logger outside the box to monitor temperature changes during transport.

6. Data to accompany shipment

Each box must be labelled accordingly to ensure proper handling and identification. The label includes the Depositor's name, Consignee's name and box identification number. The use of stickers or stamps stating 'Fragile material', 'Perishable plant material', or 'Do Not freeze' (in case of in-vitro), 'Do not put directly under sun' (in case of in-vitro and seeds), and 'This way up' are also recommended. Original shipping documents such as, Phytosanitary Certificate, Import Permit and Complete List of Materials, must be attached outside each box for easy access during inspection by custom and plant quarantine officials without opening the boxes that may damage the samples. It is suggested to add on each box an "arrival state card" which will be filled by the Consignee immediately after reception. Card information will report the state of material and data, and temperature fluctuation in the box as recorded by the data logger. The card should be returned to the Depositor promptly since the information will help determine the scheme for viability monitoring.

Best practice

Germplasm identification must include the following:

6.1 Packets (seeds), test tubes (in-vitro slow growth), cryovials (crypreservation)

Depositor's accession number and/or identifier

6.2 Storage box

Box identification number
Depositor name & address

6.3 Documentation

Depositor code: WIEWS or other Institute code for the institute holding the genebank accession
Depositor's accession number/identifier
Collecting number
Country of collection or source: Where the accession is originally from.
Crop and full scientific name: *Genus species subspecies*, including authority
Weight or number of seeds
Number of replicates (test tubes, cryovials, number of plants per container)
Date (month/ year) of viability testing & regeneration (seeds) or subculture (in-vitro)
Date of freezing (cryopreservation)

B. Economic Analysis

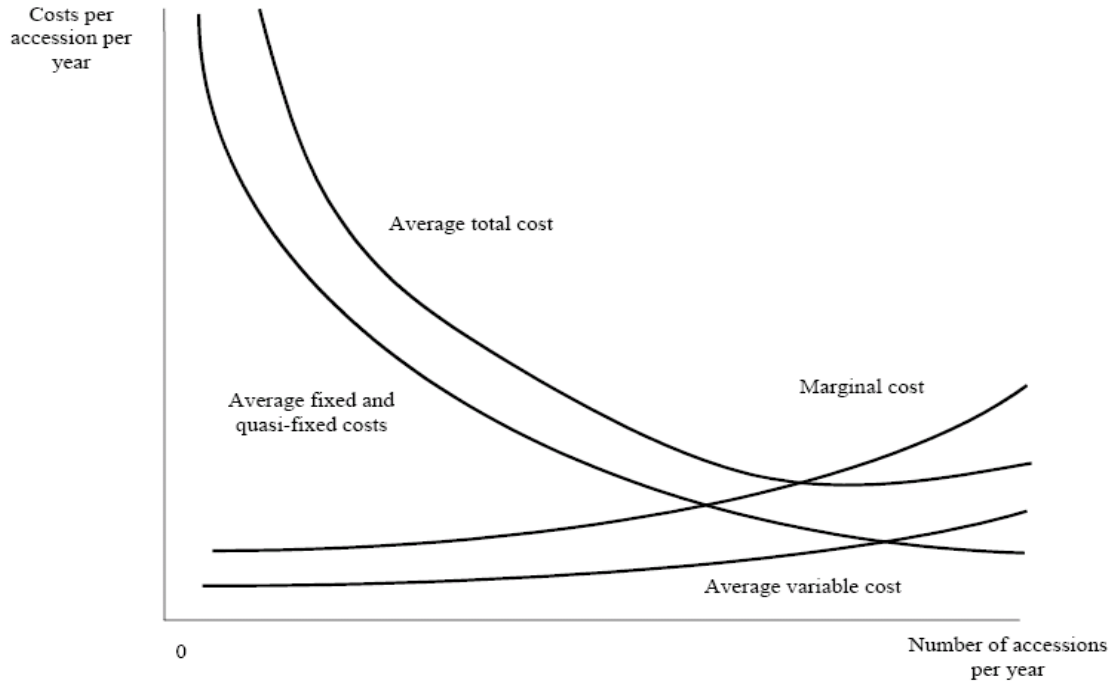
Storing germplasm with the highest quality guarantees longevity and balances the cost incurred in processing and storage. Economic studies (Koo et al. 2004) on the benefits of genebanks have demonstrated the benefits of ex-situ conservation relative to costs and use of “good practices” (Smale and Drucker, 2007; Smale and Koo 2003). These good practices include the maintenance of safety duplicates of the collection. The marginal costs of keeping safety duplicates mitigates the risk of loss of germplasm in ex situ collections.

The economic analysis of safety duplication considers the costs of the technical options of mitigating risks. The main objective of this study was to evaluate the costs effectiveness of safety duplication using different practices of packaging, labelling, transport, documentation and storage. Decisions on how to conserve a material are based on both technical and economic considerations to determine how the economic cost can be reduced without compromising the genetic integrity and longevity of the seeds.

Economic model

In this model the average costs refer to the costs of managing one accession. Marginal costs are the increase in total costs from the addition of one more accession to the genebank. Total costs include costs that vary with accessions and species and costs that are fixed independent from the number of accessions managed. Average fixed or quasi-fixed (genebank management) costs normally decline as output increases. A standard assumption of micro-economic theory is that marginal costs initially decline as more is produced and eventually increase due to diminishing marginal returns to fixed factors (e.g., land, infrastructure). Marginal cost is equal to average total costs when average total cost is at a minimum.

When genebanks operate below capacity; average costs represent only upper bound estimates of the marginal costs. Figure 1 illustrates how average and marginal costs are thought to change with amounts produced (for example, the number of seeds stored, regenerated, disseminated, etc).



Source: Pardey et al. 2001

Figure 1: Genebank average and marginal cost

This evaluation focuses on analyzing the effects of changing the variable non-labor costs on risk reduction given a technological environment. An underlying assumption is that genebank managers and staff in the different centers are qualified and working at their best capacity. Variable labor is needed for packing and labelling of accessions. Quasi-fixed labor is needed for supervision of the different steps and documentation of accessions. It is true that labor skills can be improved and that for instance, the number of accessions packed in a period of time can be increased in order to reduce costs. However, the evaluation of labor costs on safety duplication practices would lack complementary context information on staff performance, technical infrastructure, and location.

Changes of capital costs are not included in this evaluation. Over the years there have been improvements in labelling and packing techniques. The model includes a comparative evaluation across technologies in current use and performs some simulation adding some degree of variation to use and costs of inputs (labels, boxes) and services (shipping).

The economic model is constructed using the EXCEL software. CGIAR centres can use the model by changing the values that are centre-specific. Projections in terms of cost can be made using risk analysis tools (i.e @RISK software) to simulate scenarios or manually by generating a table of values using EXCEL software. Table 6 shows the basic structure of the economic model.

Table 6. Parameters for the estimation of costs for safety duplication (indicative costs used)

Components	**Units**	**Cost USD**	**Remarks**
Basic Information			
Sample size	No. seeds/acc	300	
No. of seeds per gram	No. of seeds	100	
Sample weight	g	3	
DHL box weight	g	1,500	
Aluminum bag weight	g	8.08	Computed based on size of bag purchased
Weight of aluminium bag/ seed type	g	2.02	Depends on the type of seed
Discount rate	%	0.03	
Longevity (replacement period)	**years**	25-50-100	**Equation for computation**
Packing			
Laminated aluminium bag costs	US$/piece		Equation based on current price
Cost of aluminium bag/accession	US$/piece		Equation based on the type of seeds
Labeling			
Outside label – sticky label cost	US$/label		Equation based on cost per box & no. per sheet
Cartridge cost for printing - Epson	US$/cartridge	15	
Cartridge use for printing - Epson	labels/cartridge	80,000	
Cost of outside label (label+printing)	US$/ label		Equation based on cost per label and printing
Labour cost			
Processing of accession (contractual;)	US$/hour	2.5	
Documentation of accession (scientific expertise	US$/hour	50	
Box capacity			
Capacity of DHL box - weight	kg	23.5	
Total weight of seeds + bag/accession	kg		Equation
Maximum no. of accession per box - volume	Number	1000	
Maximum no. of accession per box - weight	Number	1000	
No. of accession ready to be shipped	Number		Equation based on capacity by weight. & volume

Components	Units	Cost USD	Remarks
No. of DHL box to be shipped	Number		Equation based on no. of acc. & box capacity
Total weight of shipment	kg		Equation based on all accessions to be shipped
Shipping			
Weight of accessions + DHL box	kg	25	
Cost of DHL box – 25 kg	US$	508	Fix rate for shipment to CIAT, Colombia
Gross shipping cost	US$	3.15	Equation based on fix DHL rate
Discount rate	%	0.2	Special offer to ILRI
Summary of costs	**Average**	**Total**	**Equation based on the parameters presented above**
Labour cost	**Sub-total**	**Sub-total**	
Labeling	**Sub-total**	**Sub-total**	
Shipping	**Sub-total**	**Sub-total**	
Total average costs			
In perpetuity costs given the longevity of seed			

1.2. Simulation analysis based on the economic model

Costing of each parameter is based on actual costs at ILRI for safety backup of the forage collection as an example. The safety duplicates are deposited in CIAT, Columbia.

1.2.1 The effect of seed size on total cost of safety duplication

Increasing the accession size from 300 to 500 of maize and large seeded legumes (3 seeds per gram) can drastically affect costing. Average cost is increased by 48% while total cost by 53%. For seeds that are smaller the average and total cost remain the same even if accession size is increased to 500 (Table 7).

Table 7. Average and total cost of safety duplication as affected by seed weight and accession size

Seed category	No. of seeds per gram	Cost in USD		Difference (%) in costs			
		Accession size: 300		Accession size: 500			
		Average cost A	Total cost B	Average cost C	Total cost D	Average cost (A-C)/A	Total cost (B-D)/B
1	3	2.52	2250.2	3.74	3441.18	48.4	52.9
2	10	1.53	1431.28	1.53	1431.28	0	0
3	20	1.13	1126.88	1.13	1126.88	0	0
4	100	1.04	1041.88	1.04	1041.88	0	0
5	1000	1.04	1084.38	1.04	1084.38	0	0
6	10000	1.04	1084.38	1.04	1084.38	0	0

1.2.2 Factors affecting average and total cost of safety duplication

The average cost of safety duplication is mainly affected by the cost of the laminated aluminium foil packaging. For every unit change in the cost of aluminium bag, the average cost change is at

0.97. Changes in labeling costs are relatively insignificant despite the assumption that there is 50% variation in label cost. Refer to figures 2 and 3.

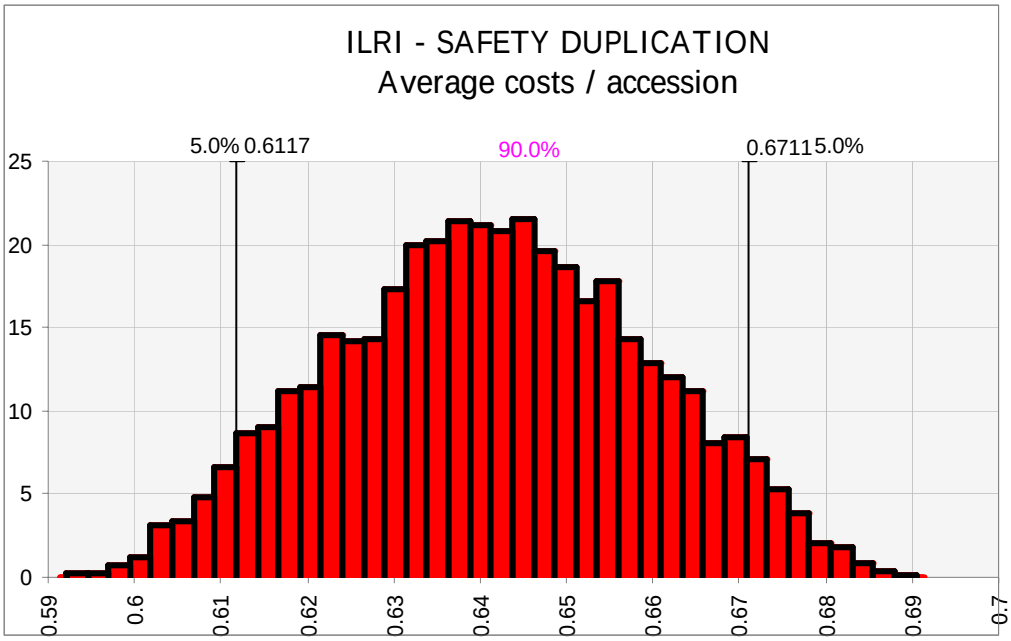


Figure 2. Distribution of average cost per accession in USD of ILRI safety duplication

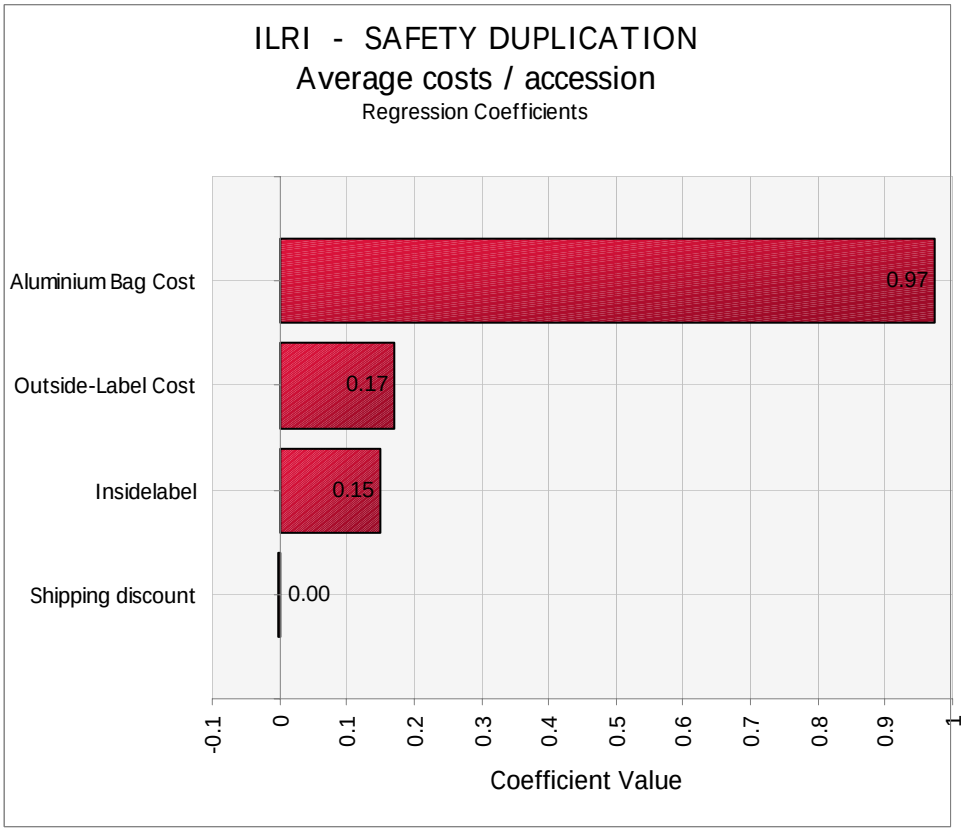


Figure 3. Regression coefficients of average costs per accession in USD of ILRI safety duplication

In the case of total costs, the main cost component is the number of accessions shipped each time. It determines the annual total cost and in turn affects total perpetuity costs. For every unit increase in the number of accessions shipped, there is a 0.99 change in the total cost. It is advisable to optimise number of accessions sent per storage container. Some savings are possible by shipping in bulk. Refer to Figures 4 and 5.

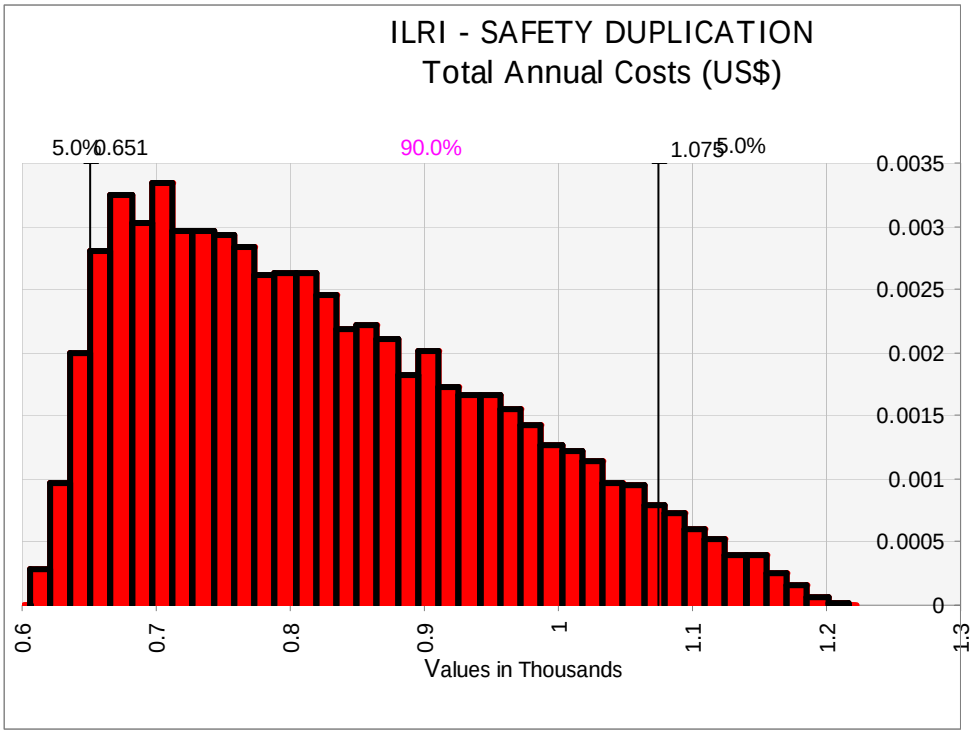


Figure 4. Distribution of total cost in USD of ILRI safety duplication

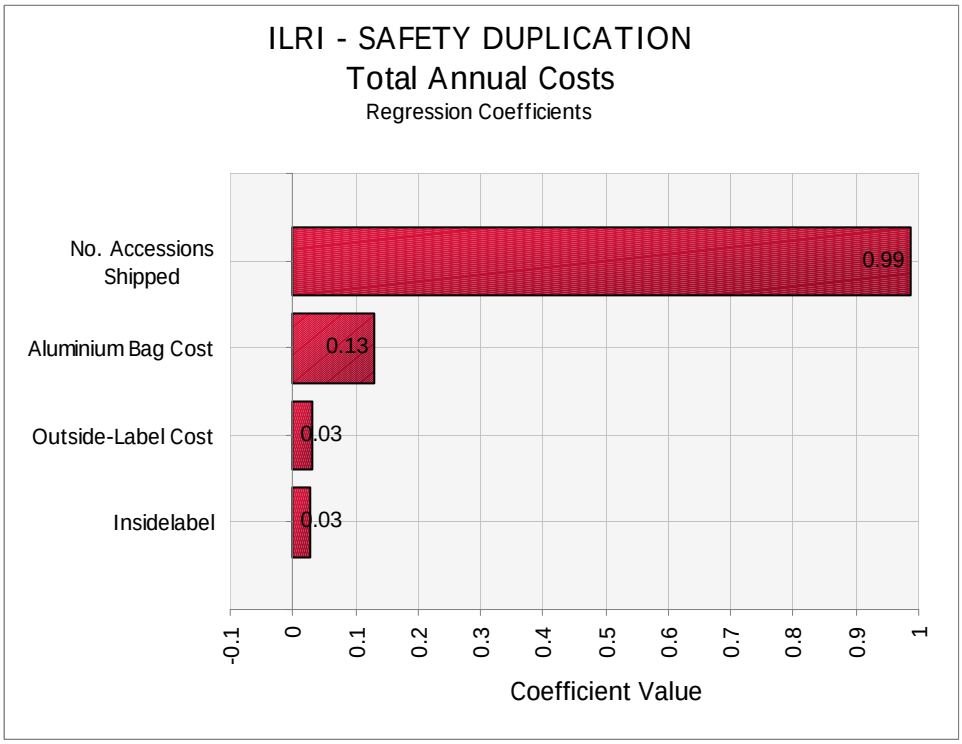


Figure 5. Regression coefficient of total annual cost of ILRI safety duplication

1.2.3 Factors that affect the perpetuity cost of safety duplication

There is an inverse relationship between longevity and average cost per accession. The shorter the longevity of seeds deposited, the higher is the average cost per accession. It is recommended to store seeds of high quality (higher viability) and provide the optimum conditions for storage so that the genetic integrity of the germplasm is maintained for a long period of time, thus reducing the frequency of replacement and generating savings. Packaging and labelling affect perpetuity cost marginally either average or total cost. Refer to figures 6, 7, 8 and 9.

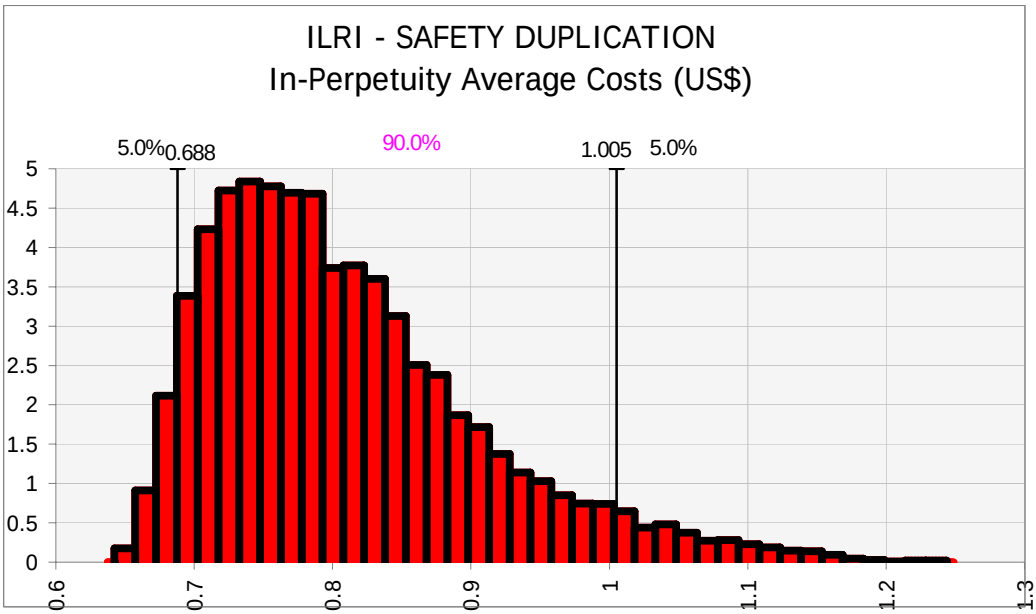


Figure 6. Distribution of in-perpetuity average cost in USD of ILRI safety duplication

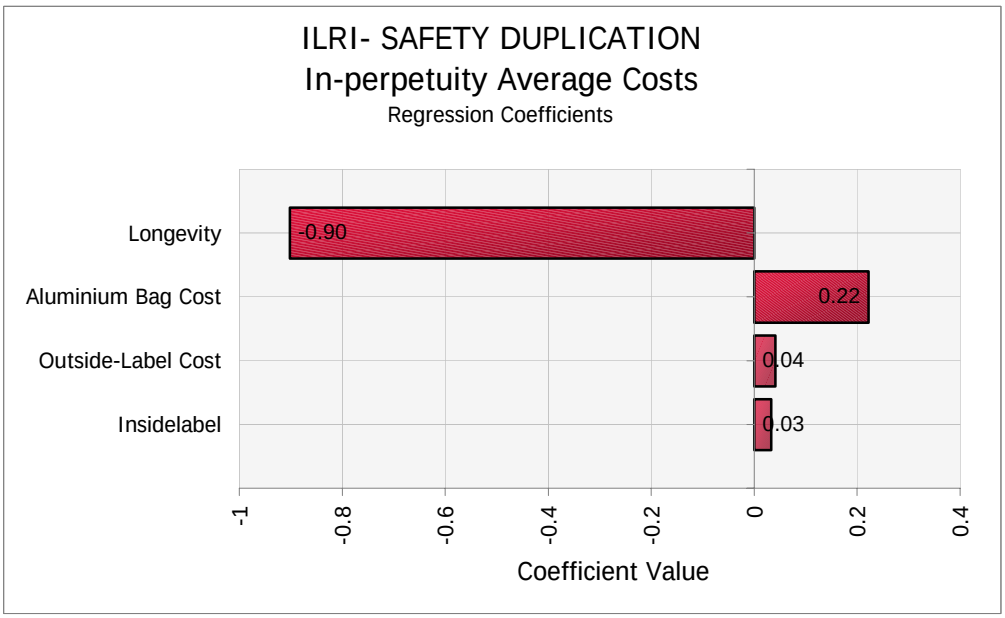


Figure 7. Regression coefficients of in-perpetuity average cost of ILRI safety duplication

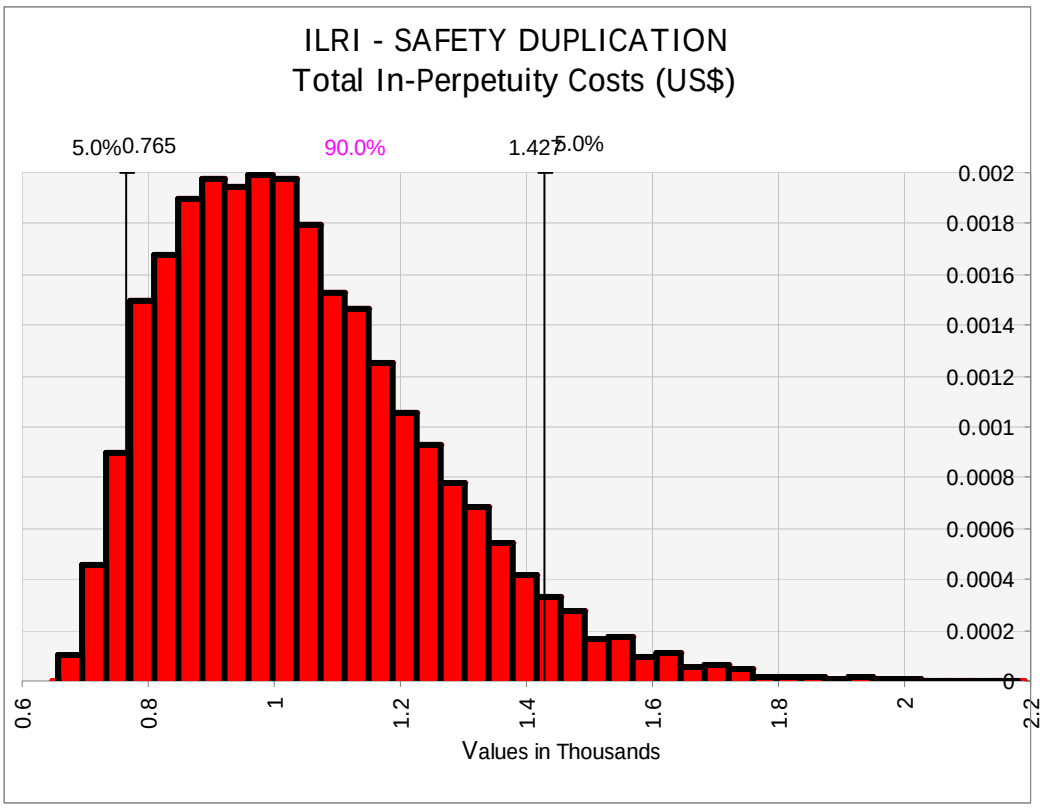


Figure 8. Distribution of total in-perpetuity cost in USD of ILRI safety duplication

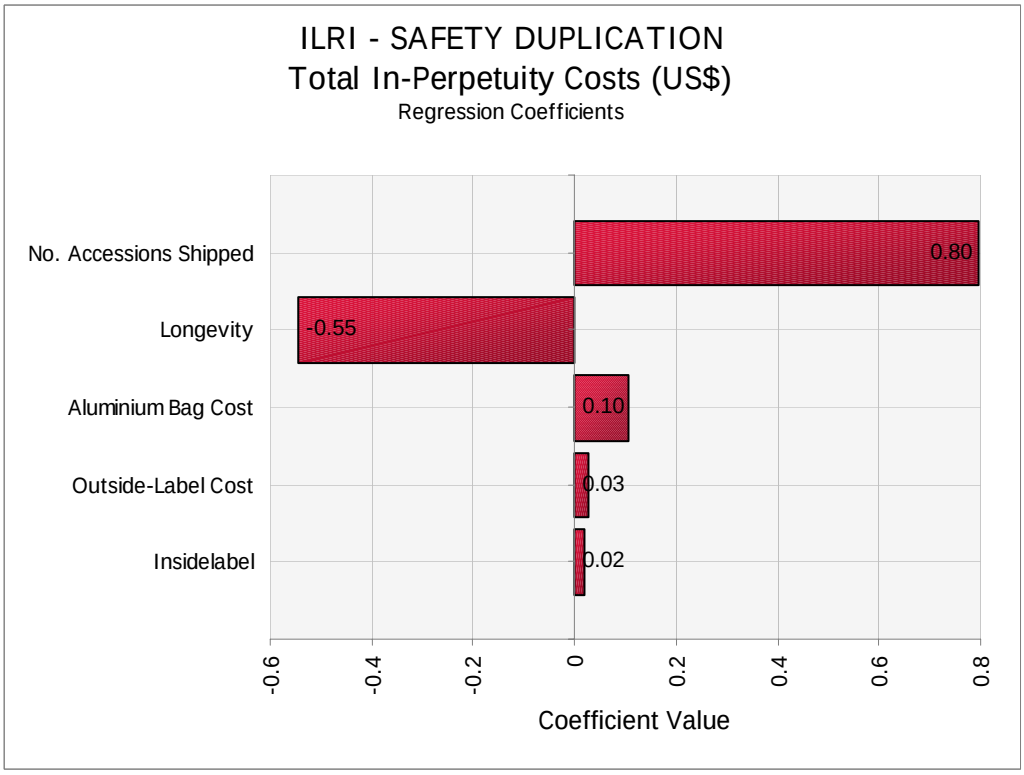


Figure 9. Regression coefficients of total in-perpetuity cost in USD of ILRI safety duplication.

V. LEGAL ASPECTS OF SAFETY DUPLICATION OF GERMPLASM

It is recommended that safety duplication of seeds should be under a black box agreement while duplication of in vitro and cryopreserved samples may be under different agreements that require the recipient to assist in monitoring and reporting. A clear agreement should be signed between the depositor and the recipient of the safety duplicate that sets out the responsibilities of the parties and terms and conditions under which the material is maintained.

Acronyms

CGIAR	Consultative Group for International Agricultural Research
CGN	Center for Genetic Resources, The Netherlands
CIAT	Centro Internacional de Agricultura Tropical
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo
CIP	Centro Internacional de la Papa
FAO	Food and Agriculture Organization
HRI	Horticultural Research Institute
IBPGR	International Board for Plant Genetic Resources
ICARDA	International Center for Agricultural Research in the Dry Areas
ICRISAT	International Crops Research Institute for Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
IPGRI	International Plant Genetic Resources Institute
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung
IRRI	International Rice Research Institute
ISGM	International Standards for Germplasm Management
ISTA	International Seed Testing Association
LINFOA	Bundesamt fur Agrarbiologie, Linz, Austria
MSB	Millennium Seed Bank
NCGRP	National Center for Genetic Resources Preservation
PQS	Plant Quarantine Service
SINGER	System-wide Information Network for Genetic Resources
SASA	Scottish Agricultural Science Agency
SGRP	System-wide Genetic Resources Programme

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