Regeneration guidelines: general guiding principles

Introduction

M.E. Dulloo[1], J. Hanson[2], M.A. Jorge[1,2] and I. Thormann[1]

[1]Bioversity International, Rome, Italy

[2]International Livestock Research institute (ILRI), Addis Ababa, Ethiopia

Background

Genebanks must ensure that their germplasm accessions are kept viable and at high quality for as long as possible. Even under the highest standards of management, however, germplasm deteriorates with time and needs to be regenerated. For many genebanks, maintaining collections at acceptable levels of viability and quality is difficult due to the costs involved and their limited capacity and technical expertise — especially when faced with the complex regeneration procedures required by some species (FAO 1998). This has resulted in regeneration backlogs, which is putting important unique material in danger, as highlighted by both FAO's State of the World Report and the crop conservation strategies developed over the past few years with support from the Global Crop Diversity Trust (the Trust), in many cases facilitated by CGIAR centres. The Trust is therefore supporting the regeneration of such priority threatened material around the world.

While a generic decision-support tool for regeneration exists (Sackville Hamilton and Chorlton 1997), some crop-specific knowledge and expertise is required. Unfortunately, although such knowledge exists in genebanks around the world, including the CGIAR Centres, no attempt has been made - until now - to identify, collate and publish best practices to help collection holders undertake regeneration in ways that maintain the genetic integrity and optimize the viability of the planting material they produce. The Trust has therefore requested Bioversity, acting on behalf of the System-wide Genetic Resources Programme (SGRP), to develop crop-specific regeneration guidelines for the 21 major food crops (banana, bean, breadfruit, cassava, chickpea, coconut, cowpea, faba bean, finger millet, grasspea, maize, major aroids, lentil, pearl millet, pigeon pea, potato, rice, sorghum, sweet potato, wheat and yam) that are being targeted in the Trust's regeneration initiative.

Definition

In these guidelines, the term regeneration is taken to mean the re-establishment of samples genetically similar to the original collection when viability or plant numbers are low. Regeneration is also necessary for new accessions which need long-term conservation if quantities are too low, and may be required for sanitary reasons to eliminate diseases. In the case of clonal crops conserved in field genebanks, re-establishment could either be on the same site or in a different site for security or to avoid diseases and pests.

General guiding principles

Assuring genetic integrity

Germplasm regeneration is a critical operation in genebank management, that which involves the greatest risks to the genetic integrity of germplasm, due to selection, out-crossing or mechanical mixing. The risk of losing genetic integrity is especially high when regenerating genetically heterogeneous germplasm accessions of out-crossing crops. The aim of regeneration is to maintain the original genetic diversity and structure of the accession or collection (see recommendations under 'General regeneration guidelines' below).

Assuring efficiency

The reproductive rate of the species and variety at the regeneration site should be considered in order to produce sufficient seeds or planting materials. The number of seeds, cuttings or other planting materials required for regeneration should be carefully calculated, taking into account the desired number of plants for regeneration, germination and field establishment rates of the accession, the amount of seed required after regeneration and whether or not characterization and evaluation are linked to regeneration.

Assuring quality

The aim of regenerating crops is to produce sufficient quantities of healthy, viable seeds, tubers or other vegetative plant propagules. Before distributing germplasm, genebanks must screen it for the presence of seed-borne or vegetative-borne pathogens and pests to prevent spread of diseases and pests. This germplasm should provide an uncontaminated basic stock for breeding programmes, multiplication or other projects.

General regeneration guidelines

Type of collection

The two types of genebank collections recognized for seed crops are active collections and base collections. Active collections should preferably be regenerated from original seeds taken from base collections. However, using seeds from an active collection for up to three regeneration cycles before returning to original seeds (base collection) is also acceptable (FAO/IPGRI 1994). Base collections should only be regenerated using residual seed from the most original sample in the base collection.

In the case of clonally propagated crops, materials are usually maintained in a field collection (Engels and Visser 2003). Some tropical tree species may only need replanting once every 15 years or longer, while other species need to be replaced every few months.

It is good practice to establish a duplicate collection as there will be no remnant seed to fall back on when plants fail, as in the case of seed-propagated crops. Clonally propagated crops can also be conserved in vitro or by cryopreservation, where these technologies have been developed for the specific crop, and such material can also serve as back up to field genebanks. Regeneration of in vitro or cryopreservation collections is not covered in these guidelines (see Reed et al. (2004) for more information).

When to regenerate

Regeneration is a costly process and should only be carried out as often as is necessary to ensure accessions are available in sufficient quantity and adequate quality.

For most seed crops, accessions are regenerated:

- when seed viability falls below 85% of the initial germination percentage in active collections, as determined by viability monitoring (see FAO/IPGRI 1994; Rao et al. 2006; ISTA 2008 for more details). The initial viability before storage should not be less than 85%, although in some genebanks a lower percentage (<75%) is used, especially for wild species.
- when the number of viable seeds per accession is <1500 in active or base collections of populations and <250 seeds for inbred lines.

Poor-quality (low viability) accessions are more important to regenerate than those with inadequate numbers of seeds. Accessions in base collections take priority over those in active collections.

For clonally propagated crops, the decision to regenerate will depend on:

- maturity and deterioration of the plant materials under conservation
- pest and disease status of the field collection
- need to replace collection due to external hazards (drought, floods, cyclones)
- need to increase availability of propagation materials.

Sample size

The sample drawn from a seed accession for regeneration should be randomly chosen to represent the diversity within the accession or collection and to give a high probability of retaining low frequency alleles. As a rule of thumb, Crossa et al. (1993) estimate a range of 90–210 seeds are needed to retain with a probability of 90–95% alleles with a frequency of 0.003 to 0.05 for a number of loci ranging from 10 to 150. Cross-pollinating species usually require more plants to maintain the genetic variation that exists within the population than self-pollinating species [See Crossa (1995) for more details]. However, this is not always the case and may depend on the degree of within-accession variation in sub-populations of selfing species.

The minimum number of seeds for regeneration can be estimated from the standard sample size used for regeneration and the sample viability according to the following equation:

Number of seeds required for regeneration = Desired plant population for regeneration / (percentage of germination[1] x percentage of expected field establishment[2]) (see Rao et al. 2006)

1 *Germination and field establishment percentage should be expressed as decimals: i.e. 95% expressed as 0.95.*

2 *Plant establishment is generally 5% less than the germination percentage in poor conditions and 1% less in good conditions.*

In the case of clonally-propagated crops, since plants should be genetically identical within the accession, the choice of sample size is mostly related to the probability of plant survival in the field and to guarantee that at least a few plants will survive to be harvested so that the accessions can be regenerated again. Often, a minimum 5–10 plants per accession are required, or more if propagules are required for other purposes.

Preparation of seeds / planting material

When preparing seeds stored in a genebank for regeneration, it is necessary to remove the containers from storage and leave them to warm up overnight at room temperature before opening them, to avoid a rapid uptake of moisture.

For clonally propagated crops, different parts of the plant may need to be used for regeneration, from tuberous roots to vines, stems, suckers or other plant parts. For each one, particular methods apply for selection, cutting, disinfestation and short-term storage or pre-conditioning before planting.

Maintaining effective population size

One of the major objectives of regeneration is to ensure that effective population size (N_e) is maintained within the accession. Methods for computing the variance of N_e, for (1) germplasm collection and regeneration of diploid monoecious species, (2) cross-pollinated species and (3) mixed self and random mating species, have been developed by Crossa and Vencovsky (1994); Crossa and Vencovsky (1997) and Vencovsky and Crossa (1999) respectively.

According to Crossa and Vencovsky (1994) and Vencovsky and Crossa (1999), the best strategy for maintaining high N_e is to take an equal number of seeds from the largest possible number of maternal plants. It is more difficult to collect self-pollinating than cross-pollinating species. If there are differences in maturity at flowering and maturity between plants within the accession, it is better to harvest individual plants and mix an equal proportion of seeds from different mother plants to avoid maternal effects.

For clonally propagated crops, the population size is not as important because plants within an accession are usually genetically identical. However, many clonally propagated crops have a significant amount of heterozygosity due to some level of natural out-crossing and perpetuation through vegetative means later, which may be reflected as variation between plants of an accession (Vasil et al. 1994; Lebot and Aradhya 1992). So, 5–10 plants per accession is recommended. When it is proved that they are genetically identical, it is more important to select material from a few healthy and vigorous mother plants than from a large number of inferior plants. The number planted will therefore depend on the level of heterozygosity and intraspecific diversity as well as on cost of conservation and the needs of characterization and evaluation.

Choice of environment

Germplasm accessions should be regenerated when possible in the same ecological region where they originated. Alternatively, select a location that minimizes selection pressures on genotypes or populations. If no suitable sites are found, collaborate with other institutions that can provide suitable sites or facilities for regeneration. Take care during regeneration and when handling seed or plant propagules to avoid contamination from adjacent plots, to minimize gene flow and to prevent inadvertent introduction of transgenes. Plants that can be clearly identified as contaminants and do not belong in the population should be removed. Accessions should not be planted in fields where the same crop has previously

been grown to reduce the risk of volunteer plants, as well as the accumulation of soil pests and diseases.
Regeneration plots should be as uniform as possible and fields should be well drained. Good irrigation should be available even for rain-fed crops to avoid any selection exerted by drought as well as to ensure good yields. A soil nutrient test is also advisable to determine what fertilizers are needed.

Isolation
The breeding system of the particular crop is important. In the case of cross-pollinated species, use proper isolation distances, temporal isolation, bagging, cages and other mechanisms. Since the degree of outcrossing in a number of plant species is related to location, it is a good practice to estimate the outcrossing rate at the place where plants are being regenerated so that the appropriate pollinating technique is used.

Crop management
It is good practice to keep the regeneration plots as clean a possible from alien seeds and plants. Weeding should be carried out regularly to eliminate competition. During the preparation of the regeneration plots, weeds and soil-borne pests and diseases should be eliminated with an appropriate treatment, e.g. herbicides sprays, sterilizing soil, cultivation followed by herbicide and/or deep ploughing to kill emerging weeds.

Monitoring accession identity
Label all accessions individually with long-lasting labels or permanent ink and keep field maps so that the accession identity can be monitored through the crop season. For seed crops, it is recommended to keep an original seed sample for reference in a small plastic bag in a dry environment at 15°C. Whenever the accession is regenerated, the newly harvested seed can be matched to the seed sample to verify that the accession is true to type. The identity of regenerated seed or propagules can also be confirmed by comparing with the original characterization data for specific traits of the accessions, if available.

Harvesting
In general, harvesting is done at optimum maturity (which is after the point of physiological maturity) of the plant, when the maximum number of seeds are ripe, tolerant of desiccation and can be threshed with minimal mechanical injuries and before they deteriorate and disperse naturally [see Rao et al. (2006) for more information].
In the case of clonally propagated crops, the physiological maturity of the mother plant is the most important criterion for the collection of propagules for successful regeneration or short-term conservation. The maturity of the edible part of the plant is usually irrelevant in cases where it does not coincide with the part being propagated.

Common pests and diseases
The guidelines provide a list of the common pest and diseases for each crop but not all the symptoms or control measures. During regeneration (including harvest and post-harvest), crops must be inspected by plant protection specialists, particularly for seed-borne or vegetatively transmitted diseases, to ensure the highest possible health and viability of regenerated material.

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