Flow chart of CIAT genebank operations

Daniel G. Debouck

Many *ex situ* conservation facilities exist around the world (227 in Latin America and the Caribbean alone according to FAO, Anonymous 1998), with different activities because of differences in their mandates. Since its agreement with the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture of 16 October 2006, the genebank operated by the Genetic Resources Program of CIAT has mandate to distribute samples of genetic resources of beans, cassava and tropical forages, according to norms defined by the countries in the Governing Body (Anonymous 2001). Along these norms, the distribution is for purposes of conservation, direct utilization, agricultural research, plant breeding and education. And since CIAT genebank has been formally established in 1978, the distribution has been quantitatively important: as seed samples the bean collection has been distributed more than eight times, and the forage and cassava collections have been distributed about four times each (Gaijy & Debouck 2008). The distribution has implied almost the entire collections, since almost every accession has been distributed at least once. Distribution is thus the *raison d' être* behind the operations carried out for these collections, with different implications such as availability, physiological, phytosanitary, genetic quality, and proper documentation. As detailed elsewhere, the documentation refers to all properties of any accession kept in the genebank but also to all operations that have been performed on any particular accession throughout its handling and conservation in the genebank in order to ensure its availability. The purpose of this note is to briefly describe the reasons behind a series of complex operations and their sequence (Figure 1).

The rationale for immediate availability, a time baseline and five conservation purposes

Keeping in trust the largest and most diverse collections in the world of their kind, one would anticipate from CIAT genebank a quality service in delivery of germplasm. This has three implications: **first**, a timely delivery (or: material that is conserved means material that is available). Since the multiplication of the germplasm may take three or more years to guarantee seed availability, the process does not start upon receiving a request for germplasm of a particular accession. Therefore usually 3-4 samples of any accession are kept available for distribution, and usually more than six samples in the case of core collections. In this case the 'historic' record of past distributions may help: materials for which there has been no requests for germplasm to date would make a poor justification for preparing large seed stocks for future distribution. In contrast, materials under high and steady demand would be multiplied so that there are 10-20 representative samples ready for distribution.

Second, since receptors are expecting plants with known characteristics, the germplasm should be alive, sent (thus approved by the plant quarantine authority) and delivered with such traits. This means different kinds of quality control, namely in seed viability, absence of disease of quarantine importance, and absence of genetic alterations. This means also access to a minimal information, usually passport and characterization data. One should note that along Art. 12.3.c of the International Treaty (Anonymous 2001) it is obligation of germplasm providers to disclose the non-confidential descriptive information associated with the germplasm provided.

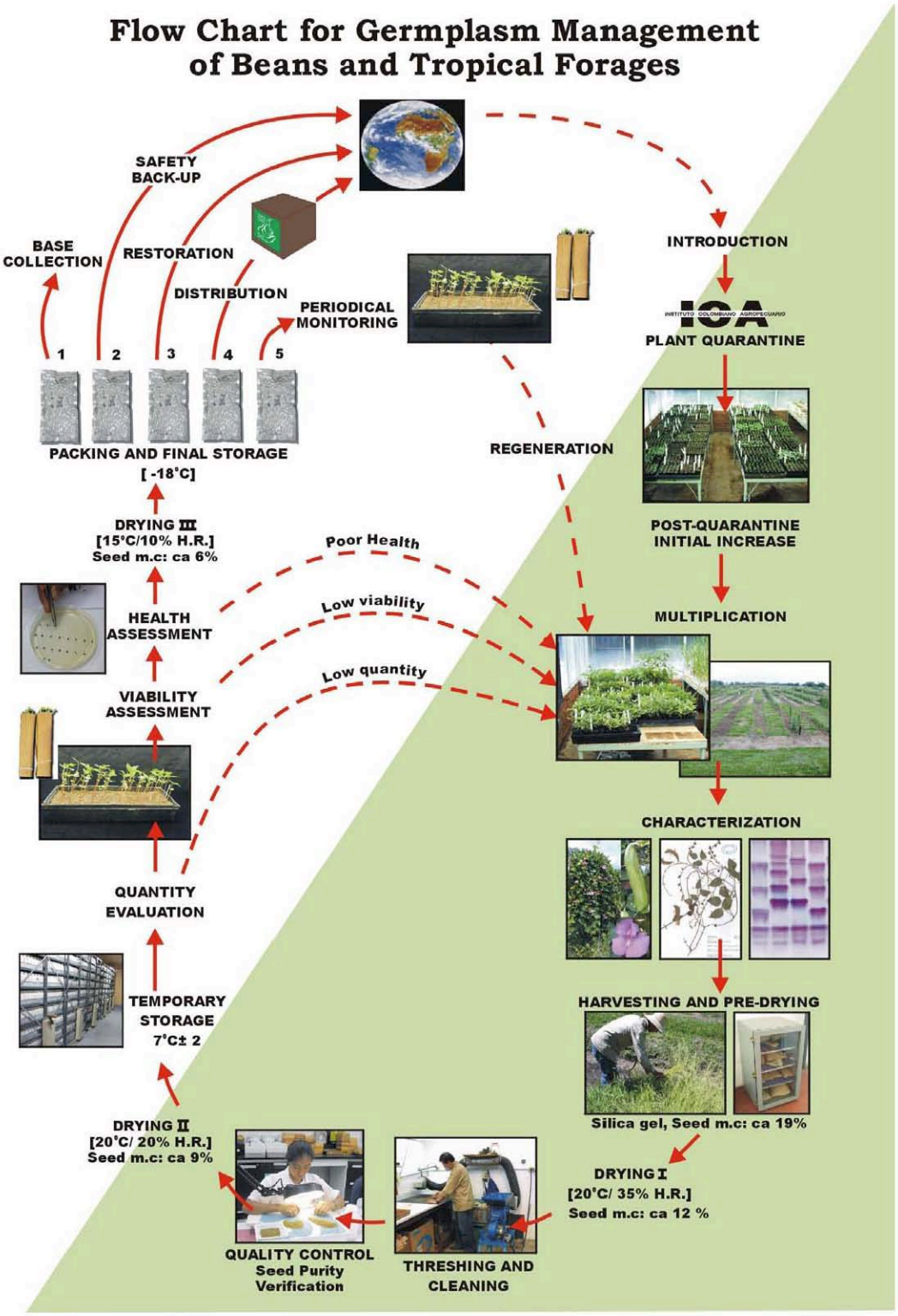

Figure 1 – Flow chart of CIAT genebank operations for the seed collections.

As seed viability erodes with time, and since for many tropical species, seed behaviour under conservation is still largely unknown, it is preferable to check seed viability periodically. Given the large number of wild species kept by CIAT genebank, it was decided to follow an experimental approach and to check viability on year 0, that is just after multiplication or regeneration, and then after five years, in contrast with the norm for genebanks (Anonymous 1994). Each sample is called back for a viability check by the Seed Viability Lab. The testing is done on a single accession basis, not at random across accessions harvested in the same conditions, because of observed variations between accessions (also as noted by Ford-Lloyd & Jackson 1986). After five years of storage, dormancy if it was present at the first check in the weeks following harvest, is gone (Baskin & Baskin 2001). If the two readings at years 0 and 5 coincide with a viability higher than 85%, then the next testing can be done ten years later (at year 15; so, 0; 5; 15; 25; 35; 45), thus saving some seeds for additional future testing. If there are reasons to suspect a relatively fast seed deterioration, then seed viability can be tested at ten years, or every five years (so, 0; 5; 10; 15; 20; 25). Since the seeds for testing are expensive to produce, and likely not to be used, unless the seed stocks for distribution are dangerously low, by this way a compromise is found; as experience is gained on the different species, sampling could even be further delayed – every fifteen years, but it has been defined on an experimental basis. One should note that the seed for viability testing has to be produced, and thus the amounts of seed for at least six testing trials should be separated from the mass of seed separated for distribution and conservation. One should also note that the seed for viability testing is from the same seed lot, also reserved for conservation and distribution. By operating this way, there is no need to implement additional separate seed testing but the mainstream one (see Figure 1), and one knows how seed physiological quality evolves across the different samples if they are kept in the same conditions.

Third, there should be continuity in the service and its quality: a particular accession requested one year should be the same if requested twenty years afterwards. One should note further that along Art. 12.3.g of the International Treaty (Anonymous 2001) it is obligation for germplasm providers to maintain available any germplasm that has been registered into the Multilateral System. Given the size of the collections at CIAT, this means that there is the possibility to maintain viability, health and genetic integrity of samples over long periods of time (also for cost implications; Koo et al. 2004). The size of the collections and complexity of operations would dictate a time baseline of twenty-five years. This practically means that once all operations are completely done for a particular accession there would be no reasons to get back to it – apart from servicing the periodical checking of seed viability and possible distribution, from pre-existing samples to these purposes – within the twenty-five years to come. This practically means also that all needs of seed for a particular accession should be secured once for all and for that duration. There is thus less need to put the collection back to the field to maintain it alive or to be able to offer an extra service (or if so, it will be only for few materials, and that will be possible, because there will be time for it). The quality service in delivery of germplasm implies thus a series of operations that should be carried out under high standard of quality. For efficiency reasons such operations are not carried out at random but organized along a sequential flow (Figure 1); other genebanks follow similar sequenced operations (Kameswara Rao et al. 2006).

As the mandate is a worldwide service of germplasm distribution, requests may come from users of any country, and since CIAT genebank does not know in advance which samples may be

required at any time in a given year, thus one seed stock should be reserved for distribution. As stated above, the size of the seed stock reserved for distribution could theoretically be fixed by the study of the distribution record over the past twenty years. Normally, it should not be lower than 3-4 samples ready for distribution at any time. In fact, from the moment that one seed stock is reserved for distribution, this means automatically three seed stocks: distribution, conservation, and seed viability monitoring. The seed stock reserved for conservation (hereafter named base sample) is the one aimed at the regeneration of the accession when seed viability is dropping below the threshold of 85% (Anonymous 1994), or when the seed stock reserved for distribution is exhausted. The threshold of 85% has been fixed towards the high side in order to maintain the genetic make-up of any particular accession and to buffer the drift to the extent possible.

For safety reasons the size of the base sample should be at least twice the amount of seeds needed for a standard regeneration. So, if the first regeneration fails, there is still another amount of at least the same size and genetic quality to do the regeneration, drawing lessons from the first attempt. The three seed stocks are coming from the same seed lot, so that they have been given exactly the same treatment along all operations. So, the monitoring of seed viability actually measures the physiological status of the seed stocks kept for conservation and for distribution. It is therefore important that the seed stocks kept for conservation and distribution (and for testing seed viability as well) are maintained all under the same conservation conditions.

Because it is good practice in genebank operations to make safety backups, one additional seed stock (or two, if there are two safety backup copies) will be prepared precisely for that purpose. In addition, because it does not cost much (but the preparation of another bag and the cost of the pouch), another seed stock will be prepared for the country of origin of the accession, in case this country requires the accession. So, five conservation purposes are considered. As indicated above, and because there are not enough human, financial, and physical resources to implement two monitoring systems of seed viability, the five seed stocks are made from the same seed lot. As expected, the seed stocks for safety backups and for the country of origin (often named ‘repatriation’) will be leaving the genebank at some date; yet, the genebank will be able to monitor indirectly their physiological quality, and to replace them if viability (tested over time on the same original seed lot) drops below the standard threshold.

The rationale for securing once for all the five conservation purposes

The rationale for five conservation purposes (i.e. base for conservation, seed viability monitoring, distribution, safety backups, and country of origin) is also dictated by the nature of the biological materials. Beans, being landraces or wild *Phaseolus* species, cassava and wild *Manihot* species (as botanic seeds), and tropical forages, are all producers of either low amounts of seed, or large but delayed or erratic amounts of seed. One reason is that all these genetic materials are raw genetic resources that have not yet been much selected by farmers nor improved by breeders for increased or stabilized yield. If the complexities and the costs are in planting and maintenance of the materials in the field in order to obtain their flowering and seed setting after many months or many years, then all amounts of seeds required for the five conservation purposes should be secured if possible in a single production cycle.

Securing the production of all seeds for the five conservation purposes for a good period of time (say, twenty or twenty-five years) in a single step is important from different perspectives. **First,** a single production cycle should help to reduce genetic drift within the accession (Figure 2). In a single accession in a given multiplication site, not all plants will produce equal amounts of seeds; some plants will produce more, others less, and thus they will contribute differently through their progenies to the genetic make-up of the accession into the next generation. This drift can be slowed down by reducing the number of multiplication cycles. One can anticipate that the drift will be low if the multiplication site is very close to the ecology of the original site, so that all plants within an accession have chance to produce something. **Second,** a single production time if well managed should limit risks of disease infections. The more frequent are plantings in the field to increase the amounts of seeds, the higher are the risks of infection by diseases of quarantine importance. This is simply because a multiplication site will anyway select for the pathogens that will preferentially attack the accessions of the crop under multiplication. As we have just seen, choosing the ecology of the multiplication site very close to that prevailing at the original site may favour pathogens that have co-evolved with the crop (e.g. rust or anthracnose in common bean). **Third,** a single production time if well managed should limit risks of genetic contamination by flow of pollens from other conspecific accessions planted nearby. **Fourth,** limiting the number of multiplication cycles contributes to lower the costs involved in all operations related to field work, harvesting, fruit threshing, seed cleaning and drying. If the genebank can obtain net progress, that is to produce all seeds needed for all purposes for the longest period possible, then it will have time to tackle other accessions in the waiting line in need for regeneration or first multiplication.

Given the size of the collections, CIAT genebank cannot operate well if the bottom time line is not a period of twenty or twenty-five years (longer if possible). Processing well throughout all operations (Figure 1) 3,000 to 4,000 accessions yearly means a period of ten to fifteen years for a first round through the entire collection. A way-out is surely not to plant the collection very often to maintain it alive. Therefore all operations should be targeted at securing the germplasm under the shortest duration possible in order to have it fully viable, characterized and available at any time. With a longer time line, there will be possibility to increase the size and diversity of the collections to deliver a better service to users so that they find the variability they need.

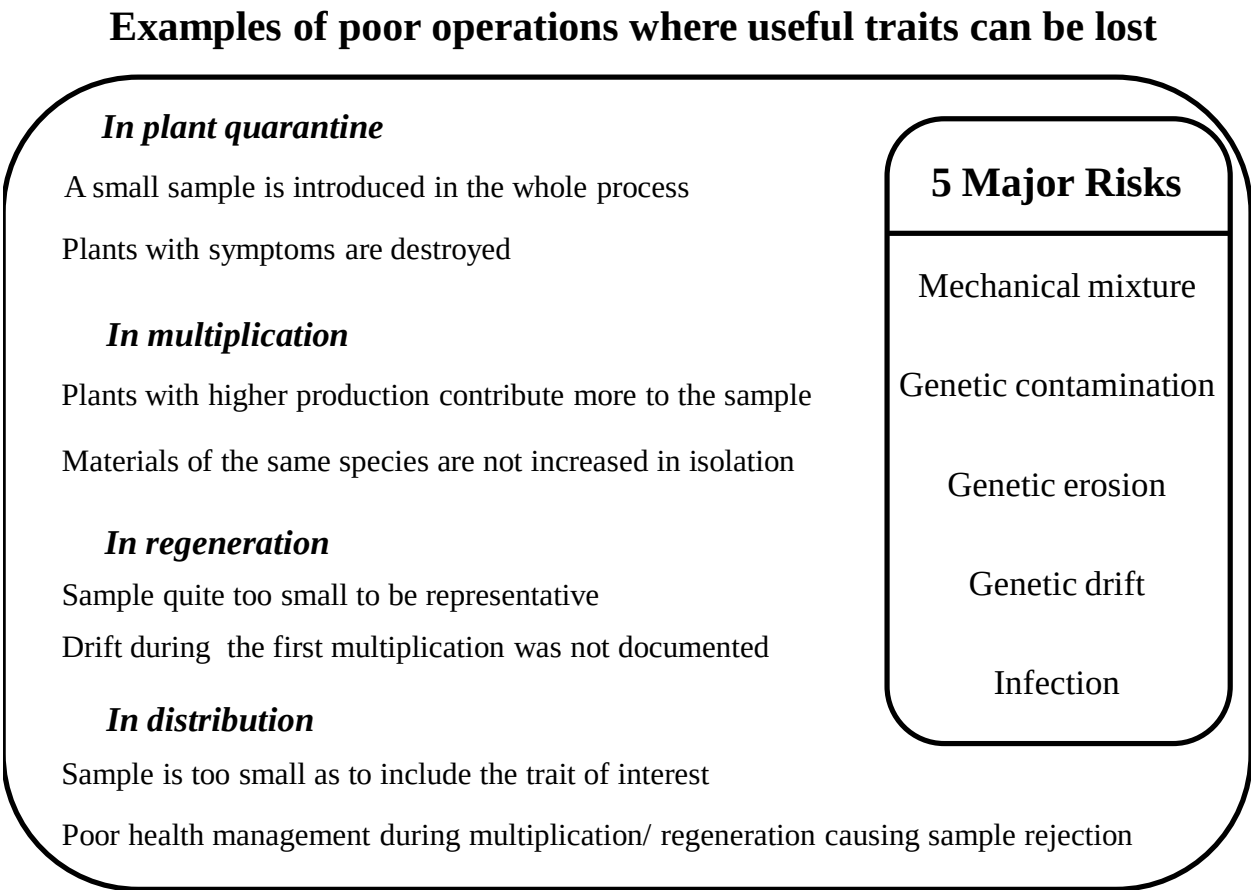

Figure 2 – The 'five capital sins' or major risks that can affect germplasm during operations.

The operations of multiplication, characterization and harvest processing

The germplasm obtained through exchange, donation or specific explorations, is often in limited supply, and/ or of unknown physiological or phytosanitary status for immediate long-term conservation and possible distribution. After the quarantine introduction process, it does require seed increase through cycles of multiplication. The first cycle usually takes place in protected environments (glass-house, mesh-house) in order to secure enough seed for massive multiplication in the field that will secure the amount of seeds for the five conservation purposes. In view of these future multiplication cycles, growth habit, dates of flowering and of physiological maturity are data worth noting. Notes about colors of flowers, stems, and fruits are also taken, since they often help to clear doubts about any accidental mixture. Care is being taken to make sure that each individual contributes equally in amounts of seeds to the sample that is the starting point of the next generation, in order to limit drift. As the first multiplications usually take place in closed environments, the risks of genetic contamination because of accidental crossing with other accessions are low (but so might be the seed setting in the case of many legumes, given the absence of pollinating agents !). For the next multiplications in open environments, care should be taken for cross pollinated species through distances, non

synchronous flowering because of sequenced planting, or special devices (such as cages, mesh, etc; Plucknett et al. 1987; Ashworth 2002).

The seed multiplication cycles after harvesting end with the pre-drying that will allow a smooth threshing of the fruits. Pre-drying (or first drying cycle) that brings the moisture of fruits and inflorescences from 20-22% (or more if harvested in the rainy season) down to 14%, also contributes to stop the spoiling by molds, fungi and bacteria. The time lag between harvest and threshing should not exceed one week. After threshing and cleaning, the seeds are dried in contact with dry air at 20-22^{o}C for one week in Drying Room No. 2, so that seed moisture goes down to 10 to 8%. After one week of drying the seeds are gathered in plastic containers. Because harvesting may extend over a couple of weeks or even months, seeds are in the meantime secured in the Temporary Storage room at + 6 to 8^{o}C. This temporary storage allows keeping insects such as bruchids away, while the different harvests threshed and dried in the same way are progressively put together. The last harvest triggers the determination of seed moisture content; if moisture content is not at 8%, additional drying in contact with dry air at 20^{o}C will be done in Drying Room No. 2.

The preparation for long-term conservation

Three filtering steps next ensure quality of the stored germplasm, in this order: i) seed purity check, ii) viability check, and iii) health testing. Some genebanks add the checking for the presence of transgenes (Kameswara Rao et al. 2006); in the case of CIAT this is still irrelevant. The checking of seed purity is important because storing chaff, broken seeds or soil particles is purposeless; furthermore, the plant quarantine authority will not sign off phytosanitary certificates if seeds are not physically pure. The seed purity checking goes together with the evaluation of amounts of seeds for storage. We have seen about the value of securing once for all the germplasm for the five conservation purposes, and thus numbers of seeds once cleaned must be high enough; to that end, amounts of seeds were pre-fixed, taking into account the frequencies of requests (for instance when the accessions belong to the core collection), and the genetics of the species to ensure the conservation of the intra variability. After counting, once the accession has reached the pre-fixed amount of seeds, two subsamples are separated from the total seed lot, one for the checking of viability and one for the health testing. In the meantime, before results from these two checks are known, the seeds are kept in the temporary storage room at +6 to +8^{o}C maximum (in order to avoid damages by weevils). Because it involves specialized testing by skilled Staff, and is thus expensive, the check for lack of diseases of quarantine importance comes last. It does not make a lot of sense indeed to do this testing if the material has not passed successfully the two prior tests (i.e. seed purity and compliance with pre-fixed amounts of seeds, and seed viability).

If the amount of seeds does not reach the required quantities, then additional multiplication will be performed. It may mean additional harvesting if the crops are perennial such as some forages, because it might be too expensive or risky to plant again the original material. If the material does not comply with the viability check, another multiplication cycle must be done. If the material is not approved by the Germplasm Health Lab (GHL), another multiplication cycle must be done. In both cases the feedback towards those in charge of the seed production is essential in order to make progress. The approval by the Germplasm Health Lab triggers the last step: the packing for long-term storage. The accessions approved for packing will be picked up in the

Temporary Storage Room, and be exposed to dry air at +20°C for one week in the Drying Room No. 3, in order to reach a seed moisture content of 5% maximum, preferably lower. Another control about seed moisture content will take place before the packing. If moisture content still exceeds 5%, additional drying in contact with dry air at +20°C in Drying Room No. 3, will take place. A common rule of thumb indicates 15-15-15 (fifteen days at 15°C in contact with air at 15% relative humidity), letting seeds to adjust naturally to finally reach 5% (Cromarty et al. 1982; Wieland 1995). The packing of the seeds into the five conservation purposes is done in the Packing Room, with one aluminum plastic pouch for each purpose. The size of the pouch has been calculated for the pre-fixed amount of seeds to be stored. A partial vacuum ensures that the sealing has been well done; if not the operator will notice that air comes back immediately in the pouch. Once packed, the seeds are stored in the -18 to -20°C cold room.

No money for active and base collections, but a single one !

Given the critical importance of drying for the long-term storage of seeds (Hong et al. 1996; Roberts 1975), one should note the presence of two controls of seed moisture, before the preparation of the two subsamples for the labs of viability and health testing, and before the final packing. Being done independently by different people, operating different drying rooms, there is some guaranty that at least one check for seed moisture has been done properly.

One can note that the samples for conservation, distribution, viability testing and repatriation (the safety backups being sent periodically to the -18 to -20°C vaults where safety backups are done) are all kept in the same conditions at -18 to -20°C. The conservation is thus long-term for all the samples of the same accession, and there is no need to run an active collection and a base collection, that would suppose two managements (and higher costs). As stated, because the seed viability will be checked periodically on the same seed kept in the -18 to -20°C cold room and in the safety backup cold rooms also at -18 to -20°C, it is anticipated that the behaviour will be about the same in the different places. If viability goes significantly down below the threshold of 85%, after regeneration, the pouches can be replaced in the different places.

One can note that the seed for distribution will have been approved by the Germplasm Health Lab closely after the production in the field, be the initial multiplication or a recent regeneration, and with no possibility of getting infected while stored at -18 to -20°C. The Plant Quarantine Authority of Colombia (ICA) can thus access the GHL files about any tested accession prior to its distribution in order to approve the phytosanitary certificates, as appropriate. Normally a new health test would not be necessary prior to shipping, but some countries may require additional testing, for instance against a special virus or bacteria. In this case, seeds for the testing will be taken out of the stock for distribution, and because it is the same seed lot, the test will be informative, and the information stored for any future need.

The seed for distribution will have had at least one viability check, if distributed between year 0 and year 5, and two checks if distributed after year 5 and before year 10 or year 15, and more afterwards. The genebank can thus be confident about the physiological quality of the distributed accessions, without the need to perform an additional test prior to the shipping. The genebank may however decide to perform a viability test, for instance for accessions at year 9, that would substitute for a test at year 10; because it is the same seed lot, again the test is informative of the overall behaviour under conservation.

The flow chart as a management tool

One can note that the decision to send a material for regeneration is triggered either by the shipping of the penultimate sample reserved in Distribution (which practically includes Repatriation), or by a drop of the viability below the treshold of 85%. In the latter, the genebank will use the Base sample for the regeneration, or in order to have additional safety the samples left over from the Distribution, if still available. The products of the regeneration will cause a new cycle, with eventually the replacement of the safety backups copies by pouches of novel seed. The former situation is a bit more tricky. If we are towards the end of a cycle, with one sample left in Distribution and likely few in Viability Checking, then the decision is easy and swift: regeneration, using whatever sample left before using the Base sample. If we are at the beginning of a cycle, the situation happens because there have been many requests for that accession. The genebank manager may decide to take one (or two) sample out of Viability Checking and to "move" it to Distribution, keeping at least one sample in Viability Checking. In the best situation, the genebank gains a leeway of 2-3 years (that could be of interest if field operations are overworked). However the accession has been under high demand, and this situation is likely to repeat itself. So, with seeds from the last sample of Viability and from the Base sample, regeneration is of order.

A frequently asked question is: what to do with the remaining samples of an accession, once regeneration has been decided? These are part of the Base samples and the safety backup copies, as the regeneration will use first whatever may be left in Distribution and in Viability Checking, before using the Base sample. Regeneration may have however to use part of (or the whole) Base sample, in order to have enough internal diversity to minimize changes in the genetic makeup of the accession. A cautious approach however would invite to approach regeneration in two steps, that is, to keep a second sample of equal genetic quality in case the first attempt of regeneration fails. It is thus most likely that an experienced genebank will have seed samples from the previous cycle. The writer would recommend as far as possible to keep the old samples in the long-term vault; if the viability is gone, the DNA and the seed proteins might be still useful for different studies in the future. The safety backup copies will have to be done again with the fresh seed from the regeneration, and a full new cycle will be re-initiated.

The flow chart (Figure 1) is a sequence of operations on the germplasm towards a goal – capacity to distribute – that hiddens a series of cycles of operations under each step along the main sequence. Each step has also its separate and interlinked module of documentation in the database. In other words, germplasm production or viability checking implies each a sequential series of operations before the next step along the main sequence can start. But there is first an important division and organization of tasks. Because the designation process towards the Secretariat of the Treaty implies a conservation responsility allowing a distribution capacity, it was found convenient to identify a pool of activities under a Conservation Group (in white to the left in Figure 1). Because it is the Conservation Group that has responsibilities for the distribution (and periodically, the safety backups), it must know the status of availability and viability of seed stocks for distribution. The Conservation Group will thus 'contract' services to the Production Group for the seed increase of the materials. And it will afterwards contract services to the Seed Viability Lab, the Germplasm Health Lab, and the Genetic Quality Lab. Such a division and organization of tasks allows the different members of Staff to specialize and to perform better what they know best (an important criterion for Staff allocation). A pool of

conservation activities is a bare minimum for a genebank, since there are possibilities, on a permanent or temporary basis, to contract services outside (production to farmers, seed viability checking or germplasm health to specialized laboratories).

The main sequence allows itself for a series of quality controls. Being alerted by the arrival of new materials (by germplasm exploration or acquisition from other collections, under the SMTA of the Treaty or a Germplasm Acquisition Agreement), the Conservation Group proceeds with the introduction with the plant quarantine authority (Instituto Colombiano Agropecuario, ICA, in the case of Colombia) and starts hiring services from the Production Group for the early increases of the material, and from the GHL for the phytosanitary controls during introduction. Upon a successful introduction, the Conservation Group can make a pre-registration towards the formal designation to the Secretariat of the International Treaty (with the recording of passport data and digital images); meanwhile it hires services to the Production Group for a full seed increase. A practical way of hiring such services is through seed multiplication contracts identified by year and if applicable semester (e.g. 2010A for a contract of seed multiplication celebrated during the first semester of 2010, 2010B in the second semester, for 999 number of accessions and detail thereof). The Production Group will consider its multiplication contract terminated once seed are given back to the Conservation Group at the harvest receipt step. This step is critically important because it will start compiling the different harvests for the different accessions, and provide feedback on the first criterion for progress: that there was seed harvested. Through Harvest Receipt, the Conservation Group will bother the Production Group in order to know whether seeds can still be harvested from accessions involved in a particular contract. The Harvest Receipt staff conducts meanwhile the pre-drying, so that the staff involved in the fruit threshing gets materials to clean in an orderly way. The staff in charge of the seed purity check will ask for seed from the staff doing the threshing, in order to advance the seed purity control, the verification of the quantity threshold and the seed moisture content test. The coordinator of the Conservation Group must control the smooth advance of these steps for passing contracts timely with the Viability Testing Lab and the Germplasm Health Lab. Please note that these two labs have access through the internal computer network to the history of multiplication of each accession, namely place and dates. This is important for their own work of quality control, but also in order to provide appropriate feedback to the Production Group, about the level of compliance on each multiplication contract. This type of follow-up is critical to the net progress mentioned above: for example, the Production Group must know if fruits are harvested at the wrong moment, because harvesting too early or too late significantly affects seed viability. Lastly, the staff in charge of the final packing will get the clearances from the Viability Testing Lab and the Germplasm Health Lab, and will re-check the moisture content of the seeds to be packed; verification of seed purity and number target will be immediate at the moment of the final packing for the five conservation purposes.

The flow chart (Figure 1) is also a risk management line, where the next operation in the line can control quality and performance of the former, while the coordinators of major groups manage risks. At the beginning when seeds are few, the multiplication is performed in closed and protected environments (glass-/ mesh-houses). Digital images of seeds have been already taken in order to prevent mixtures, and if the material is particularly scarce a backup has been made using *in vitro* culturing. The first purpose of conservation to be filled in is obviously the Base sample, and once constituted and well dried is at no risk in the Temporary Storage room at + 6^{o}C. It can sometimes stay there for a couple of years, while the waiting line clears up for

additional seed increase. Being a new operation, i.e. additional seed increase, it will be the object of another contract, in order to allow both the Production Group and the Conservation Group to make a tracking of each material. Some of the major risks affecting seed germplasm are summed up in Figure 2. While mechanical mixture or genetic contamination are relatively easy to control, drift can be mastered if enough (100 plants per generation, as most of the legumes in the collection are somewhat outbreeders) seeds are planted for increase or regeneration, and if harvests are levelled to the lower seed producer in the planting line. It is rarely done that way, and thus some buffering is obtained through the planting of a large number of individuals for multiplication/ regeneration. Drift of course is a problem if the original population entering the genebank is itself a large population; it is much less a problem if the original population is just of a few seeds. Sometimes it is possible to make the genebank accountable because it is possible to separate variants (with letters A,B,C, after the accession number) themselves easy to identify and to describe. Genetic erosion is another problem, when the genebank decides to not multiply all original propagules (a sort of contradiction in view of its role). The bottleneck might be at the quarantine level, but increasingly fast and reliable PCR methods of disease indexing could help to fasten the process while giving the host country the warranty of absence of diseases of quarantine importance. Please note that in order to control infection, namely in the multiplication plots/ facilities, it is the interest of all three – the Production Group, the Germplasm Health Lab and the Conservation Group – to have GHL Staff visiting such plots/ facilities for early diagnostic. It will help the Production Group to have higher productivity of the accessions under multiplication, and thus help to reduce the number of contracts, which in turn will also reduce the workload of the Conservation Group. GHL will have already an insight on potential problems for their fast identification. The proper documentation of the health status within each contract also helps towards increased perfomance and the reduction of risks: if a problem comes back in another year, the previous documentation can help to overcome it fast and possibly at low costs.

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