

# An operational genebank manual for the Germplasm Resources Unit at the John Innes Centre

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## 1. **Germplasm Acquisition and Accessioning**

Genebanks can obtain the germplasm they want to conserve through a number of different ways. Conducting collecting missions is possibly the best way of acquiring germplasm material in the most reliable manner. Germplasm exchange with other genebanks is a third route to add genetic diversity to the collection. Obtaining and storing germplasm from researchers and plant breeders is another route to acquire genetic material. Such acquisitions should be guided by a formal mandate that the genebank concludes with its host organization or government and that provides the basis for a genebank acquisition policy. The actual accessioning of acquired germplasm samples, i.e. formally including it into the collection with its unique accession number, is a complex process during which the curator has to check a number of aspects such as the verification of the identity of the material, the health status, the availability of pertinent information, etc. It is further understood that also legal aspects form part of this activity, e.g. was the material collected/obtained in legal manner, are there any restrictions on its use, etc.

### **Box 1.1. Germplasm Acquisition and Accessioning**

**GA1** – Briefly describe any formal mandate that your genebank might have concluded with or received from your “mother organization” (e.g. institute, governmental body).

*This description should include details on:*

- a) which species you conserve and make available;*
- b) who decides on what your mandate is and, if different,*
- c) from whom do you received the mandate;*
- d) the main aspects of the mandate; and*
- e) legal considerations on PGR as foreseen in national legislation.*

#### *a. Which species are conserved and made available:*

The Germplasm Resources Unit (GRU) at the John Innes Centre (JIC) houses biological collections of seeds. The GRU aims to capture the broadest possible gene-pool diversity of the UK's major strategic crops and crop wild relatives, to support plant science and crop improvement through breeding. Within the UK crop conservation community, the genebank national remit covers cereal and legume crop diversity in addition to brassicas induced and derived diversity sets (e.g., Mutagenised TILLING populations, mapping populations).

We focus mainly on wheat, pea, barley and oat germplasm. The collections include crop wild relatives, traditional landrace, adapted and elite cultivars as well as derived lines, mapping populations and induced (mutagenized) diversity panels.

#### *b. and c. Who decides on the genebank mandate and from whom it is received.*

The mandate is historic and is affirmed periodically by three authorities:

1. The John Innes Centre, an independent research institute in which the genebank delivery is one form of its science and social impact.
2. The UK Research and Innovation Biotechnology and Biological Sciences Research Council (UKRI-BBSRC). BBSRC is the main funder of JIC and funds the genebank core activity within the institute through a National Bioscience Research Infrastructure (NBRI) grant.

3. The UK government Department for Environment, Food & Rural Affairs (DEFRA) funds the maintenance of the JIC *Pisum* collection and has statutory responsibility on the delivery of the genebank under the International Treaty for Plant Genetic Resources for Food and Agriculture.

The GRU is custodian of the UK Small Grain Cereal collection (wheat, barley and oats) and the JIC *Pisum* collections. Historically, the cereal collections were assembled (mainly) by the Plant Breeding Institute, Cambridge and the Scottish Crop Research Institute (SCRI). These collections were managed and conserved as part of a government owned breeding industry. The public germplasm custodianship in JIC followed the sector privatisation and started in 1990. The *Pisum* collection were historically assembled in JIC as a model crop and later incorporated important international collections (e.g., of mutation stocks from NordGen and Crop Wild Relatives from various expeditions)

Since 2012, the historic mandate is reviewed and renewed in a five-year cycle as part of JIC Institute Assessment Exercise (IAE) when past activities are summarised and reported, future strategic science programmes are articulated and reviewed, and overall institute funding boundaries are proposed. During the IAE, as a component of JIC strategic delivery, the GRU planned activities are proposed by the GRU manager with inputs from community stakeholders in a discussion with JIC management and are evaluated by JIC strategy committee with inputs from the Science and Impact Advisory Board (SIAB). The GRU five-year funding plan proposal, derived from a 10-year vision, are then communicated, amended as necessary, and once accepted, formalised by UKRI-BBSRC following a peer review process and international assessment panel discussion.

c. Included in b above

**d. The main aspects of the mandate**

**Overarching Aim**

The Germplasm Resources NBRI will conserve the UK cereal and legume crop gene-pool resources and their associated data and enhance utilisation to overcome current and future challenges to global food security.

**Specific Objectives**

1. Continue to develop the GR-NBRI as a centre of best practice, provide advice and support for crop germplasm curation, management, and distribution.
2. Conserve, document, enhance visibility and increase the usability of the UK cereal and legume crop gene-pools.
3. Sustainably increase the deposition of new resources and associated data by the UK biosciences community (and beyond) into GR custodianship.
4. Develop SeedStor database further by adding new functionalities to improve collection management and increase links to external initiatives to generate synergistic impact.
5. Define, develop, and characterise germplasm panels amenable for genebank-genomics to accelerate gene discovery and to enhance the utilisation of germplasm in precise crop breeding.
6. Extend the use of molecular methods to assist quality assurance and enhance germplasm utility.

**Outputs**

Delivering the above objectives (**O**) will generate the following main outputs.

The GR-NBRI will:

1. Provide safe and legal seed distribution (**O1,2**).
2. Develop, publish, and disseminate knowledge relating to seed preservation and germplasm resource management (**O1,5,6**).

3. Report to the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA) governing body on germplasm inventory and use **(O2)**.
4. Ensure that the UK strategic crop collections are readily available and accessible by the user community via a user-friendly optimised database **(O1,3,4)**.
5. Provide effective quality assurance for users and stakeholders **(O2,4,6)**.
6. Maintain and increase its capacity to distribute germplasm collections with clear links to genotype and genome sequence information **(O4,5,6)**.
7. Maintain existing networks and establish new relationships with germplasm providers and user communities to ensure the continuous knowledge exchange with conservationists, researchers, educators, industry, and the wider community **(O1,3,4,5)**.
8. Leverage external income to sustainably increase the collection value **(O3-6)**.
9. Build the genebank community and future leaders and offer training to users **(O1-6)**.

**Outcomes** stemming from the planned outputs. the GR-NBRI will:

1. Ensure the provision of technical policy and regulatory advice.
2. Enable the UK adherence to the ITPGRFA.
3. Operate as a sustainable capability to effectively manage the resources and public funds to provide good value for money and highly qualified staff.
4. Enable scientists and breeders to use the GR resources to develop new knowledge and improved crop varieties to support food security.

e. *Legal considerations on PGR as foreseen in national legislation*

The UK-Plant Genetic Resources group (UK-PGRg) is a collaborative effort of coordination and collaboration between UK genebanks, conservationists and related policy makers in the government Department of Environment, Food and Rural affairs (DEFRA). As part of the UK-PGRg, DEFRA Genetic Resources policy makers are routinely communicating matters related to the UK related legislation with the genebank manager.

**GA2** – Specific agreements. Does your genebank have any specific formal agreements with other genebanks regarding the conservation of specified germplasm?

*This should include:*

*whether or not your genebank has any international agreements to conserve specified germplasm on behalf of other countries,*

- a) *a specific region, and/or*
- b) *the world, and*
- c) *which crops or gene-pools fall under these agreements?*

No such specific international binding agreements exist.

**GA3** – In case your genebank has a germplasm acquisition policy, what does the policy entail? *Please specify which crops or which geographic area, if applicable.*

- The geographic area is not limited, and accessioning is open to global activity.
- Crop Accessioning remit is similar to the genebank remit (Legume cereal and brassica gene-pools as explained above)

Currently, there are four main routes for germplasm acquisition. First, a long-standing collaboration with the British Society of Plant Breeders (BSPB), under which a sample is deposited in the genebank for every cereal line tested as a registered cultivar candidate. The sample is sent in early stages of the legal process that determines whether it is distinct from previously registered named cultivars, sufficiently uniform and stable (DUS test), a prerequisite for national listing. Second, the GRU operates as the depository for wheat germplasm developed as part of the BBSRC wheat strategic programmes (Designing Future Wheat Academic Toolkit and Breeder Toolkit collections or ATK and BTK germplasm, in short). Third, the GRU has a pathway for JIC scientists to deposit recently developed or collected germplasm as part of the Deposited Published Research Material (DPRM) collection. DPRM is primarily designed to support the accessibility for germplasm reported on in published research paper thus facilitating best practice in open reproducible crop science. Fourth, the GRU leads on, collaborates with, and acts in support of national and international consortiums that develop or characterise germplasm (chiefly through application of genomics).

Key examples of (open ended long standing) projects generating new genebank accessions (fourth germplasm source above) include:

- Defra Wheat Genetic Improvement Network (WGIN: <http://www.wgin.org.uk/>)
- Defra Pulse Crop Genetic Improvement Network (<https://pcgin.org/>),
- The Open Wild Wheat Consortium (<https://openwildwheat.org/>)

The acquired germplasm is then made available internationally through dedicated SeedStor collection page (<https://www.seedstor.ac.uk/>) where collection details are briefly summarised for users and future curator activities and each accession is given a unique StorCode and URL link. The newly acquired accessions are conserved according to the assigned importance decided by its uniqueness, usability and conservation value (i.e., can it be recreated or collected if it was lost from the genebank) which is divided to four levels of custodianship (also called curation level) as explained in the following table and paragraph policy guidance for depositors:

**The Germplasm Resources NBRI offers four levels of germplasm curation.**

Curation level	Collection type	Examples	Custodianship Model
1	ITPGRFA collections  UK legacy collections	<ul style="list-style-type: none"> <li>• BBSRC Small-Grain Collection</li> <li>• A.E Watkins Collection of Landrace wheat</li> <li>• JI-Pisum Collection</li> </ul>	<p>This germplasm is conserved for perpetuity as a UK legacy or as part of the International Treaty for Plant Genetic Resources for Food and Agriculture.</p> <p>Under the treaty rules, germplasm is distributed via the multilateral system agreement (S-MTA). <a href="http://www.fao.org/plant-treaty/areas-of-work/the-multilateral-system/the-smta/en/">http://www.fao.org/plant-treaty/areas-of-work/the-multilateral-system/the-smta/en/</a></p>
2	GRU public collections	<ul style="list-style-type: none"> <li>• Breeder toolkit (DFW collections)</li> <li>• Wheat TILLING</li> <li>• RevGen Collections</li> </ul>	<p>Germplasm is held and managed by GRU according to the cost recovery model based on user demand. The germplasm would be regenerated only if the demand remains steady.</p>

3	GRU managed private lab collection	Deposited Published Research Material (DPRM)	The GRU distributes the originally deposited stocks and monitors seed stock levels but does not regenerate them. The initial cost is covered by the depositor. Minor costs may be incurred from end-users (handling and posting).
4	Private lab collections	Private Lab collections	The GRU monitors the location of the collection in the seed storage room.

### **Which level of curation best fits my project?**

1. To consider the first (highest) level of custodianship, the germplasm would need to be unique and of high international importance for future food security. The germplasm would usually be naturally occurring diversity that would become extinct if a genebank did not conserve it. This level requires a government guarantee for long term funding which currently is less likely for most newly generated/ collected germplasm in the UK.
2. The second level is appropriate for derived germplasm such as mapping populations, and induced diversity such as genotyped/ phenotyped mutagenised germplasm. It is only relevant for germplasm of importance for the bioscience community when high demand is anticipated.

- To maximise the GR-NBRI impact, the genebank team will proactively promote the exploitation and improve usability of and accessibility to the deposited collections of the two higher curation levels.
- Only non-GMO cereals legumes and brassicas can currently be accepted for curation levels 1 and 2

3. **Custodianship level three.** If you publish a paper and want to claim that the generated germplasm is publicly available, you can deposit it with the GRU in a dedicated Lab Collection, the Deposited Published Research Material (DPRM) <https://www.seedstor.ac.uk/search-browseaccessions.php?idCollection=34>.

You will receive a GRU store-code for each of the deposited accessions. Users could order the material directly from GRU by browsing SeedStor for the published store-codes or for known accession names. this would save the progenitor lab the burden of seed distribution. However, the GRU cannot hold enough expertise to advise users regarding all newly generated germplasm, so the corresponding author would be contacted by users when such service is required. This option would usually be appropriate for smaller amounts of accessions (typically less than 50). It suits any type of diversity and any crop species for which seeds can be stored in cool/dry conditions. GMO can also be deposited.

The GRU will only use the original deposited stock for future distribution and will not regenerate it. Therefore, an adequate amount of seeds must be deposited. This changes between collections but should be sufficient for at least 11 orders. Please bear in mind that seed viability does decline over the years even when kept in good conditions.

4. Relevant for Norwich Research Park Scientists and local companies only who manage private germplasm collection in the genebank seed storage room. While the GR-NBRI team has no responsibility or any level of ownership over the germplasm, it monitors its location

and available related data. From conservation point of view, this could help when a science group leader moves to another institute or retire or when a company holding valuable non-restricted germplasm cease to exist or lose interest in its seed collection for any other reason.

### **Guide for depositing new GRU seed collection in SeedStor database**

The following items will need to be completed and discussed if your team and the GR-NBRI team agreed to generate a **new** publicly available germplasm collection (usually under curation level #2).

1. Level of curation: Unlike National Collections (curation level #1) we will usually not be able to offer an immortality to your collection unless requested and discussed. Instead, we will regenerate on a supply / demand basis under the cost recovery model implemented. A suitable Material Transfer Agreement (MTA) for seed distribution will be drafted or adapted from other collections.
2. Fill in required sections of the Minimum Data Spreadsheet <http://v0415.nbi.ac.uk/JIC/JIC-germplasm.php>. We are looking for mainly obvious pieces of information. We are prepared to sit down and discuss any required information.
3. If applicable, please describe each accession in the following format
  - a) Background material (name of cultivar/ GRU store code/ other genebank ID)
  - b) Donor material (name of cultivar/ GRU store code/ other genebank ID)
  - c) Backcrossing detail (amount of backcross before bulking, bulks growing conditions)
  - d) Targeted genotypic area (Chromosome #/ linkage group details)
  - e) Trait of interest (e.g. Grain size/ flowering time/ disease resistance)
  - f) Line identity (any synonym name or identifier used for the line)
4. Will this be standalone collection or will it, potentially, be added to later?
5. Provide viability though a germination rate of the deposited material.
6. Please do not deposit treated seed.
7. Provide a short paragraph describing the collection and its benefit to research and an appropriate logo for the collection. See examples here: <https://www.seedstor.ac.uk/search-browsecollections.php>
8. Provide details of funding bodies involved with the collection and any current/past MTAs in use.
9. Provide any internet links that may be required to be uploaded or be helpful. This may mean resource supports such as project information or genotype/ sequence data links.
10. How you would like to deal with requests from your own group and ex group members. Will you keep your own stock for personal use or request from GRU as others will?
11. Please provide any phenotype data that you would like to tag on, in a separate file. This is strongly encouraged if the collection value arises from a phenotypic screen.
12. Genotype/ genome sequence data. We would like to know if the material was genotyped. To ease user search we would need you to specify the method used and detail if each of the accession could potentially be linked to a unique genomic data. Please provide specific links to acc data in addition to general links to related publications.

13. For wild material: Any expedition data that you can supply (country of origin, exact geographic sampling point, date, participant MTA, Nagoya Protocol Due diligence).
14. Prior to deposition we will need to discuss and agree a cost recovery model to sustainable conserve the germplasm.
15. For cereals cultivars, we would usually send 20 seeds per request. For legumes, we would send six to twelve seeds. For some types we may send less. (E.g. Crop wild relative seed or germplasm in precise stabilised homogeneous state with verified high germination rate). Do you have views on how many seed should be sent per-request?
16. We will usually require at least 200-250 fresh seeds of **high germination rate** to be deposited.
17. The GRU team will test germination in proxy or direct test, depending on the nature of the germplasm. The cost of the test will be recovered by the depositing project unless otherwise agreed.

**GA4 – How do you verify the identity of the germplasm material received (e.g. relying on the donor’s information, comparing material with other accessions, involving (taxonomic) expertise, etc.)?**

The above-mentioned deposition questioner/ discussion process ensures the material identity through knowledge of the depositor. In addition, for wheat, pea and barley, the genebank team is equipped with in-house developed molecular markers that can be applied to identify the germplasm (by comparison to a known source) and often indicate on its uniqueness.

**GA5- Describe if and how you conduct an assessment of the various quality aspects of the seeds, ~~tissue culture or plant material received~~.**

*This description includes:*

- a) *quality aspects related to the correct identification of a given accession, but also*
- b) *health*
- c) *purity aspects of the sample/accession), and*
- d) *use of a quality control system (e.g. ISO).*

a. The correct identification of a given accession:

High proportion of the newly deposited germplasm is genotyped (usually with saturated molecular markers) or genome-sequenced (either fully, i.e, WGS or via a form of reduced complexity such as exome capture sequencing). This material can be verified cost-effectively by application of a sub-set of highly selective markers, previously designed for each crop. These markers can be used for quality control as part of the regeneration (or bulking up) processes or as a quality assurance for seed distribution.

- b. **Health** (seed quality): Deposited seed are tested by a seed specialist in the GR-NBRI team. When possible, 50 seed are tested. If seed are deposited in relatively small quantities, 25 seeds are sampled instead of 50 from each accession. For deposited large populations, generated in high standard horticultural facilities (in one single environment), and maintained in cool dried conditions prior to the seed deposition, a proxy- germination test can be used to save effort. A *proxy germination test* protocol exists. In short, one or two seeds are sampled from each individual acc and the germination % of the collection is indicated. Proxy germination test ensures that the

overall germplasm that is deposited > 90% germination and cannot replace direct test that are used as QA for seed users.

- **Health** (Phytosanitary). We only allow deposition of clean seed, generated in conditions that can be track-recorded for issuing a UK plant passport or phytosanitary licence for seed export. Phytosanitary testing is done by the Animal and Plant Health Agency (APHA) in DEFRA. A regular visit of APHA officer to the GR-NBRI include sub-sampling for lab testing of various diseases as well of seed visual inspection. The genebank technicians accompany the APHA officer and as we hold a >7-year track record of 0% phytosanitary test failure, it is highly expected that the technician assessment will agree with the pathologist government expert approved test.

c. Purity aspects of the sample/accession

The genebank sample can only be as pure as the deposited first sample. For genebank-genomic project we recommend that the deposited seed are generated from the plans sampled for DNA. This is usually, but not always the case. When that is not possible (for technical or biological reason) we request that the deposited seed is the closest possible progeny available. This seed (termed internally as *gold standard*) is not distributed but maintained for future references. A single seed descent of these are often generated as distribution stocks). For different genetic make-up of germplasm, different approach to purity check and maintenance is taken.

Germplasm characteristic data is collected from depositor or germplasm is characterise by genebank curators as part of deposition process. This characterisation data set is then compared to the plant performance and seed characteristic every time that the stock is regenerated (as part of deposition of new stock of an existing accession. (This is part of the Regeneration SOP).

D. use of a quality control system (e.g. ISO).

All the major genebank activities (including germplasm and data deposition) are streamlined on SeedStor management system<sup>1</sup>. Related Standard Operation Procedures (SOPs) and data (generated by the team or deposited) are stored on a JIC-GRU group shared folders LIMS (backed up daily). The data storage and documentation is done in accordance with established SOP and data management plan, audited annually by NBI QA officer. Data is separated into raw/analysed and machine-generated/human-generated data.

**GA6 – Describe whether and how the SMTA is being implemented:**

- a) extent of materials covered by SMTA (crops, numbers of accessions)*
- b) ways of SMTA implementation and documentation of transfers of PGR*
- c) other aspects (e.g. monitoring, supervision).*

The UK signed and ratified the ITPGRFA in May 2005. The standard terms of the multilateral system (S-MTA for the MLS) were agreed internationally on June 2006. The S-MTA was consequently adopted for JIC public collections in 2008 (these include all the germplasm whose conservation is funded by the genebank core funding or government long term conservation grant.

- About 27,000 accessions of the GR-NBRI ~50,000 publicly available accessions are registered as MLS and form part of the UK registered national lists (curation level 1,

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<sup>1</sup> RSP Horler, AS Turner, P Fretter, M Ambrose, SeedStor: A Germplasm Information Management System and Public Database, *Plant and Cell Physiology*, Volume 59, Issue 1, January 2018, Page e5, <https://doi.org/10.1093/pcp/pcx195>

conserved for perpetuity and reported annually to EURISCO. These accessions are solely distributed under the SMTA.

- The S-MTA is also the preferred terms for other legacy collections, not registered with the MLS germplasm (for these, users and genebank can opt to use other form of bilateral agreements for various reasons).
- The S-MTA (as well as other MTAs) is generated automatically when the seed are ordered online using the check-out details of the user and the ordered accessions are stored on the database.
- The S-MTA use is reported every year to the FAO governing body by the GR-NBRI manager.
- The genebank management system (SeedStor <sup>1</sup>) generates an automated report based on the seed-order track record data linked to the recorded job orders and users institute data.

### **Box 1.2. Germplasm Collecting**

**GC1** – Describe here the details of the strategy that you follow in implementing germplasm collecting missions.

*This description should include:*

- a) general aspects of planning and implementing a collecting mission,*
- b) the criteria you use for priority setting;*
- c) the actual strategy followed in sampling material from farmers' fields, from nature, etc.; and*
- d) how your germplasm acquisition policy underpins the mission.*

Not applicable in recent years

**GC2** – Provide any additional information on the germplasm collecting activities of your genebank, including the collaboration with others.

Not applicable in recent years

## 2. Ensuring Security

This chapter refers to the security of the genebank structure itself (i.e. its physical security), the safety of its germplasm (i.e. the maintenance of viability) as well as the institutional and personnel security, aspects which together will ensure the long-term conservation of the entire collection.

### 2.1. Physical Security

To ensure the physical security of the collections, the following aspects are regarded as essential elements for achieving the objective

#### **Box 2.1.1. Safety Duplication (of long-term conserved germplasm)**

**SD1** – Please describe how your genebank implements the safety duplication of your germplasm material.

*This description should include the following aspects:*

- a) the type of safety duplication (e.g. black-box; no specific arrangement; other);*
- b) the location(s) where you store your safety-duplicates (country; genebank);*
- c) whether or not you are using a formal agreement with the genebank(s) that store your duplicates?*
- d) whether the safety-duplicates are stored under conditions comparable to your own?  
Please provide details;*
- e) do you maintain safety-duplicates from other genebanks at your genebank? If so, do you know any details of that material?*

*a. Type of safety duplication and its (b) location:*

A systematic duplication plan has started in 2020. It consists of prioritisation of the germplasm for duplication (on site in a different building) and triplication (as a *black-box* in the Svalbard Global Seed Vault). The highest priority is given to unique germplasm that if not conserved, will likely go extinct and that cannot be generated de-novo (such as mapping populations and breeder lines).

- c. **Formal agreements** are in place for the triplicated germplasm in Svalbard Seed vault (managed by the Global Crop Trust on behalf of NordGen)*
- d. **Duplication conditions:** both the duplication and the triplication stocks conservation conditions exceed the quality of the main conservation chamber. The bulk of the diversity is stored in paper envelopes in cool dry conditions (4°C +/-2 and 12% RH +/-2) generally agreed to be for medium term and are therefore regenerated in a 20–25-year cycle. On site safety duplications are stored in hermetic aluminium foils seed envelopes and at -20°C. Triplication in Svalbard are also dried seed in hermetic packets stored at -18°C.*
- e. No black boxes of other genebanks are formally held.*

**SD2** – Do you have a safety duplication policy? If so, please provide essential details.

A plan for all the unique diversity to be duplicated within a decade (2023-2033) was laid out. To exemplify the duplication prioritisation approach we can look into The A.E Watkins germplasm resources. The Watkins collection of landraces (LR) wheat is a unique set of germplasm, for which the GR-NBRI is the primary custodian. The germplasm was collected in the 1920-1930s from an agrobiodiversity environment, largely extinct in present days. In addition to the 1168 landrace accessions (that are used occasional) and can be found here (<https://www.seedstor.ac.uk/search-browseaccessions.php?idCollection=4>), 1418 single seed descent (SSD) lines were derived from the original LR and are also held as a genebank collection <https://www.seedstor.ac.uk/search-browseaccessions.php?idCollection=39>. The derived LR set are highly utilised for wheat genomics and breeding globally. In addition, a resource of some ~8500 accessions of Nested Association Mapping Recombinant Inbred Lines (NAM RILs) that were originated from crosses between Watkins LRs and reoccurring modern elite line were also deposited and conserved <https://www.seedstor.ac.uk/search-browseaccessions.php?idCollection=47>. The NAM RIL populations are routinely used for wheat genetics, genomics, agronomic and nutritional studies.

If we prioritised safety duplication based on contemporary community use and source of cost recovery, the derived SSD lines should be duplicated first, the mapping populations second and the original LRs lastly. However, our approach to conservation looks at the cost and feasibility of resurrecting lost germplasm, following a disaster in the genebank. Therefore, the original source of diversity is first to be duplicated and triplicated, the SSD are secondary in order and the NAM RILs are third. Accordingly, the NAM RILs derived populations will only be prioritised to duplication after all the unique LR in the genebank (of all gene-pools) were duplicated (some 4500 accessions).

In monetary view, generating the NAM RIL resources was a major 10-year effort of a numerous groups in a UK-wide consortium. Its £ replacement value would be a six-digit number. Yet, the original LR diversity cannot be re-collected and therefore is priceless.

As for January 2025, the entire prioritised *Pisum* diversity (of ~3000 accessions) and two sub-set of wheat prioritised diversity of landraces (totalling ~1300 accessions) were triplicated. In addition, the entire Cereal Crop Wild Relatives (*Triticeae*) collection <https://www.seedstor.ac.uk/search-browseaccessions.php?idCollection=3> (935 accessions) was duplicated, and so was the hexaploid wheat TILLING collection (1761 accessions). At the current speed, the entire unique diversity will be duplicated by 2033. Plans to its acceleration are underway and depend on additional funding and on regeneration (bulking up) capacity.

Importantly, newly deposited germplasm is deposited as duplicates (or triplicates if unique and non-recoverable) so the proportion of safely held germplasm can only increase over time.

### **Box 2.1.2. Structure**

**SS1** – Please provide details on how your genebank building has been designed to resist natural disasters (e.g. earthquakes; flood; storm).

- The genebank was not designed to resist natural disasters as such. It is however built as a one floor industrial building (to avoid options of leaks etc). It is made of metal and therefore fire resistant. It is relatively isolated from other buildings to lower chance of floods, chemical or electric fire.
- Chances of earthquakes, flood and storms are not particularly high in the area. Flood could not be however ruled out. The safety duplicates freezer is built in a second floor and will therefore be more flood resistant.

**SS2** – Please describe the security arrangements that you have in place to protect your genebank against burglars, fire and others.

The building is within JIC fenced campus to which access is allowed for access card holders only. Out of hours and permanently security presence is on-site 24 hours a day, 7 days a week. CCTV cameras cover the building exterior access and its front and back (fire exit) doors. A security door is equipped with restricted card access and employees' entrance is therefore automatically recorded and monitored. The genebank access is restricted to trained individuals only, however it is not limited to genebank employees only (JIC and TSL scientist who hold private seed collection in our shared seed storage room have access too). The seed storage room is locked out of hours and inspected by genebank employees during office hours.

Fire detectors (tested weekly) and alarm system alerts for site and to 24h manned reception/security/ engineers (connected to cell phones) re fire or change in temperature, or in relative humidity outside the defined range. Generator power exists as back-up to overcome power cut. Currently, (2025) during site-wide re-construction, The main seed storage room isn't connected to the automatic backup generators system, however should there be a catastrophic failure of mains supply, on-site contractors are equipped with temporary generator that could be delivered and installed at short notice. Electricity to the duplication stock freezers is backed up.

**SS3** – Please provide information on any other structural security aspects that you might have in place.

The seed location within (a very large) room, is kept under highly secured database system with four levels of restriction to access. Therefore, only those who must know where a specific collection is would have the information in hand. This, together with the above physical restrictions make theft less likely. User of the seed storage room that are not part of the genebank team sign a declaration that they will only use their teams allocated shelves. Padlocks for shelves racks exist, to allow extra level of safety if requested.

### **Box 2.1.3. Security Equipment**

**SE1** – Provide details on the kind of emergency (back-up) equipment or arrangements that you have in place to ensure permanent electricity and cooling.

The seeds are conserved in cool dry conditions. Both, the cooling units and dehumidifiers work in 200% capacity. In other words, only half of them are working in any given time to generate the required conditions. A change-over takes place every two weeks so that all the units' depreciation is synchronised.

The cooling units and dehumidifiers are maintained regularly by a third-party expert company. This is the responsibility of JIC Facilities as part of their core grant to maintain JIC infrastructure as a UK National Capability (this grant is renewed every 5 years).

**SE2** – Describe how you monitor temperature and relative humidity in your cold stores and drying room.

The main cold seed storage area (4°C +/-2 and 12% RH +/-2) is monitored by JIC Facilities via the Building Management System (BMS). In the event of temperature or humidity falling outside of this area, a critical alarm will be sent to Facilities. As an additional measure an audible alarm and beacon, which is located externally to the unit, will also activate to alert the building users or any passers-by of seed storage environment issues. The cooling and dehumidification work on individual systems and have been designed with N+1 redundancy, so should one piece of equipment fail there's resilience in the system to maintain the environment within the seed vault.

The site has 24hr on call site engineers who will respond to emergencies out of hours. If further assistance is needed the external contractors Pitkin and Ruddock also provide an on-call service. The Genebank team also run in house monitoring in the cold store with remote Tiny Tags™.

These record temperature and humidity every 30 mins and offer an insight to a particular period.

We run three of these in different locations in seed storage room.

## **Box 2.1.4. Institutional and Personnel Security**

**IPS1** – Provide details on the “institutional security”, in particular with respect to the provision of financial means to operate the genebank

*Aspects to consider are:*

- a) *timely transfer of funds from the “mother” organization to the genebank;*
- b) *do you have direct access to the “mother” organization that provides the budget?;*
- c) *internal “security” of accessing these funds;*
- d) *long-term security and stability of funding (compensation of inflation rates, avoiding variation in years)*
- e) *any other observations that are relevant in this context.*

As explained in AG1, the genebank is a unit within JIC science institute and a team within the Crop Genetics department. Therefore, the mother organisation is JIC. UKRI-BBSRC funds the JIC core activities as a UK National Capability for plant and microbial sciences, this includes a dedicated 5-year renewed budget to the genebank.

- a. Payment Timely transferred.

There are no issues with funds timely transfer.

- b. Do you have direct access to the “mother” organization that provides the budget?

Yes. The genebank has a direct and steady access to the originally allocated budget. In addition, as a research genebank, the team has access to JIC additional research and facilities resources. For example, the genebank team leader can bid for funding of new equipment (Science Infrastructure Committee funds), to ad-hoc annual projects of crop conservation science fund (Institute Development Grants). The genebank can also bid to alternative ‘soft’ sources of funding in a form of science grant (Horizon Europe and BBSRC external science grants). This is supported by the JIC (the mother organisation) as any other JIC faculty member is supported (administratively by a dedicated Grant office, training, etc)

- c. *internal “security” of accessing these funds;*

No issues as such.

- d. *long-term security and stability of funding (compensation of inflation rates, avoiding variation in years)*

The UKRI-BBSRC 5-year funding cycle is the long-term backbone of operation. Defra also offers long term stability by funding (the direct costs) maintenance of the *Pisum* collection. The definition of the genebank as tier-1 GR-NBRI (previously termed National Capability), implies that the facility is essential for UK science delivery and therefore unlikely to be written off in the future. While not being open ended funded, this is probably the closest a UK research entity can get to stability for perpetuity.

- Correction to inflation were carried out for some, but not all funding allocations and indeed, some pots of money indeed saw a decline in real terms. However, the overall funding (in real terms) is on annual increase trajectory since 2012.
- The institute Finance department allows flexibility and carryover between budget years so that a 5-year activity plan is carried out and can prioritise fluctuated needs of regeneration cycle.

**IPS2 – Describe how you secure adequate staffing of your genebank.**

Three genebank employees are employed through the UKRI-BBSRC five-year funding cycles. Continuation of employment is secured between the funding cycles. A fourth employee is funded by DEFRA for the maintenance of the Pisum Collection (a 4-year cycle grant that was renewed continuously since 2007). The statuses of (currently 7) other employees, students, and post- doctoral researchers in the genebank team is less permanent and is linked to cost recovery by germplasm users and science projects' competitive funding.

Within the core funding, cost is also allocated to additional JIC teams (JIC technology platforms). This includes Hort Service team, Experimental Field Trial team, Bioinformatics and computational support teams, Genotyping platform team. So JIC employees time is allocated within those team in support of genebank delivery aspects. This is a very efficient way because it allows access to highly skilled workers for whom full time work cannot be justified for the genebank needs. It is also an advantage when applying to grants for germplasm development and maintenance.

### **Box 2.1.5. Contingency Plans**

CP1 - Describe the kind of emergency or contingency plan that your genebank has in place to cope with disaster situations.

As part of the institute and of the research park contingency plans, the genebank has contingency plan to cover all conceivable risks. The plans were operated and well functioned during the Covid-19 pandemic and lockdown periods.

CP2 - Provide information on the kind of training, security drills and other activities that your genebank gives to its staff to deal with emergency situations, if any.

Staff have regular fire safety training and fire drills to ensure they know what to do in case of an emergency. Some of the staff are also first-aid trained and attend regular refreshers courses to maintain their skills. From a security point of view, JIC Gene bank is restricted access, only suitably trained workers are allowed inside.

The NBI Partnership HSEQA team provide professional health, safety, environment and quality assurance services to support the scientific activities in the JIC Genetic Resources Unit. Authoritative advice, day-to-day work support, project guidance, and regular inspections and monitoring activities to ensure regulatory compliance with key legislation such as the Health and Safety at Work Act 1974, Genetically Modified Organisms (Contained Use) Regulations 2014, Plant Health (England) Order 2015 and Control of Substances Hazardous to Health Regulations 2002. Comprehensive health check programme and training is aimed for handling allergens according to national regulations. Periodic, mandatory individual online training programme is in place and deals primarily with fire, health hazards, data protection and online data handling as well as culture matters such as enhancing and praising inclusivity and rejecting any form of bullying or discrimination in the workplace.

The HSEQA team also provide quality assurance services to the Genetic Resources Unit, through monitoring compliance to the Joint Code of Practice for Research and the JIC Quality Code of Practice. To achieve this regular auditing and quality checking activities are performed to ensure facilities, systems and processes are maintained to the highest possible quality standards whilst embracing a commitment to continuous improvement.

## 3. Germplasm Maintenance

This chapter deals with key aspects of managing germplasm in a genebank, i.e. the maintenance of the viability, the genetic integrity, the availability of the conserved germplasm as well as the management of the corresponding information.

### 3.1. Maintenance of Viability

This section refers to the maintenance of the longevity of the seeds or of tissue cultures or living plants in storage. A high initial viability is the most important pre-condition for achieving the longest lifespan of seed accessions in storage, hence maximum efforts need to be taken to ensure that seeds to be stored have the highest possible viability. Optimum growing conditions when multiplying/regenerating the accessions, efficient management of the preparatory steps before storing the germplasm, adequate storage conditions as well as proper monitoring of the viability are critically important.

#### A. Seed Collections

##### **Box 3.1.1.A. Initial seed viability**

**IV1** – Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your seed, in particular during regeneration and post-harvest (e.g. cultivation practices, pollination aspects, use of specific equipment as shelters, storage of harvested seeds, cleaning, etc.).

While each species requires adjusted conditions, for a general successful regeneration:

- 1) Selection of environment. Growing under glass in stable conditions gives control over maturation and harvest environmental conditions.
- 2) High quality support from Horticultural services. Correct soil type, correct plant density. Good plant care e.g. minimise pathogen damage, watering from below to help this
- 3) Pollination is carried out in cellophane bag. Ensures self-pollination. Little effect on initial viability as long as correct bag types are used (size and material)
- 4) Avoidance of harvesting too early. Allow plants to dry off naturally
- 5) Harvested seeds allowed to stand in drying location post-harvest
- 6) Processing seeds. If carried out mechanically take care to avoid over threshing (drum damage) to minimise negative effects on embryo conditions.
- 7) After processing conduct germination test to assess initial viability

**IV2** – Describe procedures how you deal with a) dormancy and b) hard seeds. For wild legume with hard seed we devised a drill that can efficiently penetrate the outer layer without damaging the embryo. The ‘chipped’ seed have usually enhance viability and they germinate uniformly. We do not treat cereal seed to break dormancy.

**IV3** – Please provide any other information on procedures that you follow to ensure highest possible initial viability. Internal data shows that glasshouse regenerated seed have higher and more stable shelf life in comparison with seed bulks generated throughout the Unit history in the fields. This can be due to the fact that the germplasm is not adapted to UK modern agriculture or due to the relatively wet harvest often experienced in the UK. We therefore regenerate the long-term distribution stocks in pots in glasshouses or semi-controlled conditions greenhouses.

### **Box 3.1.2.A. Seed Viability Monitoring**

**VM1** – Describe the routine seed viability monitoring system that you use.

*The monitoring system should include the following aspects:*

- a) *frequency of testing;*
- b) *Sampling methods;;*
- c) *any thresholds that you use;*
- d) *whether you apply different procedures for crops/species with erratic initial viability or irregular viability lifespan;*
- e) *etc.*

- a. **Frequency of testing:** Initial germination rate is tested within three years of harvest following regeneration and every 10 years thereafter.
- b. **Sampling methods:** Two germination tests methods are used in the genebank; Direct germination tests, and Proxy germination test. Sampling for the Direct tests can be selective or random (depending on the collection use and seed source).
  - **The direct test** samples 50 seed from each re-generated stock (or 25 if stocks are low). It is always the preferred option and used as a default unless otherwise agreed with genebank manger.
  - **Proxy tests** are designed to test quality of large adapted, genetically stable crop populations grown in a single environment in the same time. It is applicable swhen high and stable viability rates are expected and when seed are available in very low numbers or when restrictive time/ costs demand a quicker course of action. Here, one or two seeds represent each accession in each tested batch of (up to) 100 accessions.

Proxy tests are valuable cost-effective tool to ensure seed quality is (on average) high to accept seed from regeneration or a new deposit. In some cases Proxy tests make good biological sense (e.g., when populations are used entirely for QTL mapping or GWAS rather than each individual accession within it).

Proxy test are also used to assess the overall viability decline of stock duplication (on site in -20 freezers), the duplicated population is sampled in advance with four representative bulks to allow future periodic testing. This is expected to allow future users to ensure that the viability remains high without opening all the hermetic deposited samples.

#### **Randon or selective sampling (in direct tests)**

Germination tests are a tool to evaluate seed quality aiming to decide the correct time for regeneration and assessing whether a seed lot can be distributed to users or if it is below the acceptable/ usable standard for distribution. In both end uses (regeneration and seed distribution), seed are manually selected so that no broken or shrivelled seed is used. As the germination tests needs to reflect that, the tested seeds are also chosen in a similar way. However, sometimes germination test in our operation are aimed to assess % of viable seed within a given stock weight (to asses viable seed quantity). In this case, random sampling is applied in accordance with the accepted ISTA protocols. (Importantly, no characteristic such seed size, shape or colour is ever a selective attribute in the uncommon occasion of mixed seed samples).

- c. **Thresholds**

**For regeneration:** Unless there was a biological reason for the low viability (e.g., some mutant or wild populations tend to have lower germination rates), we aspire to regenerate when seed viability falls below 75%.

**For distribution:** There is no hard value as it greatly differs between user communities (scientists, breeders, hobby growers, farmers) and environment of use (lab, glasshouse, or field).

**VM2** – Please describe the information “system” that you might have in place that allows you to make more species- or even accession-specific decisions regarding when the next monitoring should take place.

**For distribution:** At point of seed distribution, *SeedStor* management system (the genebank end only) would show the curator the last germination test results (type of test, date of the test, % viability) of each accession that was ordered. Usually, more than one stock will exist for each accession and *SeedStor* will be able to point the % viability of each of the seed stocks. If a recent test does not exist, seed will be tested prior to distribution. For borderline relatively low viability, seed number can be corrected upwards accordingly and user will be alerted to possible low seed quality and care required for germination and bulking up the seed. If germination/vigour (inflicted from seed age or abnormality in germination) is too low for the seed order stated end use, the curator will communicate possible solutions with the end-user.

**For regeneration**

Every accession below the threshold is marked by the system as needing regeneration. Usually the accession would be regenerated within one or two years according to prioritisation of crop and collections (based on experience demand and known plans for projects).

**VM3** – Please provide information on non-specific thresholds that you might use for viability of seeds (i.e. percentage of germination) and for the amount of seeds left of an accession to initiate regeneration. *In case you differentiate between self- and outbreeding species, please answer for each category separately.*

- A hundred seed is the default low level to trigger regeneration. For stable crop cultivars, or mapping populations with high quality seed, generated under glass, 20 seed would be the lowest seed levels we would agree to deep into before triggering regeneration. The threshold would be higher for field bulks or wild relatives and are adjustable on the seed management level for each sub-collection.
- (Almost) all the germplasm we regenerate is self-breeding.

### **Box 3.1.3.A. Seed Storage Conditions (for the different types of collections, i.e. short/medium- or long-term storage)**

**SC1** – Please provide details on temperature and relative humidity conditions of your storage and drying rooms.

- The main storage room is kept at 4°C +/-2.5, 12% +/-2 RH.
- Safety duplications stocks are dried-up in the above conditions and are then kept in hermetic conditions at -20°C.

**SC2** – Provide details on the type of containers and the packaging procedures (and the corresponding equipment, if any) that you use.

For Short / Medium term packaging: Seeds are cleaned after harvest and amounts recorded. The seed is placed in paper envelopes A6 size, which is suitable for our seed yields. These are labelled with the details of the contents, including barcode, StoreCode, Accession name, and harvest year. The envelopes are then placed in cardboard storage trays (K-bins) which match the dimensions of the shelves in our storage area.

Long term packaging for storage in -20°C Freezers (or for sending to The Global Seed Vault at Svalbard) is by using vacuum packed in Mylar Foil bags. Envelopes are labelled with Storecode and Harvest Year. Airtight packing is done using Turbovac 140-ST Vacuum Packer. When Subsampling for Long term Storage, seeds are taken from the most recent seed stock, which have the best germination percentage. Germination is recorded when re-packing.

**SC3** – What is the range of seed moisture contents (smc) of your stored seeds of different species; what measures do you apply to keep and/or monitor the (low) moisture level? Do you treat different species differently?

SMC is ranging from 5.5 to 9%. In our operation cool dry air is constantly blowing through the paper envelope and ensures the seed mc is maintained constantly low.

**SC4** – Provide data on the total storage capacity (number of containers, number of accessions) and an estimated percentage to which extent this capacity has been filled.

- We are custodian of some 50,000 publicly available accessions of which 27K are MLS covered by ITPGRFA
- We are currently working at full capacity (although, since the genebank only uses 50% of the seed storage room, rationalising the use of science groups on site can slightly increase the national collection storage capacity)

**SC5** – Please include any other aspects regarding storage conditions at your genebank that you regard as important (e.g. anticipated lifespan of freezing and drying equipment and related prudent financial management).

New walk-in freezer was commissioned to the genbank on December 2024, allowing gradual shift to more hermetic packing. Application for completely new genebank infrastructure is underway.

**B. *In vitro* Culture Collections** *Not applicable*

**C. Cryopreserved Collections** *Not applicable*

**D. Field Genebank Collections** *Not applicable*

## 3.2. Maintaining Genetic Integrity

Maintaining the genetic integrity of an accession can be achieved by minimizing genetic drift which may occur predominantly during the process of regeneration, due to too small numbers of individuals being planted, sub-optimal pollination and/or the introgression of alleles from other accessions or commercial crops or crop wild relatives. The following aspects are important for achieving the objectives of maintaining genetic integrity and should be briefly described. Please note that a distinction should be made between seed numbers for an accession and seed numbers for sub-samples per accession. The latter only applies if the seeds of a given accession are being stored and distributed as sub-samples. As genetically modified material gets more widely distributed and as it might have specific (legal, technical, administrative) requirements, a separate box on this type of material is included.

For *in vitro* cultured and cryopreserved material, which are normally maintained as clones, genetic stability is as important as genetic integrity of the seed-stored material.

**SCSS1** – Do you document the initial number of seeds of individual accessions (either as received from collecting missions or through exchange)?

The historic data does not always exist. For more recently added material number of seeds or weight of the original sample can be traced back.

**SCSS2** – Please describe what kind of containers (and equipment) you use, the procedure you follow with respect to sub-sampling, seed numbers per container, etc.

seed storage containers: see above; number of seeds per container/ seed envelope are not fixed but are recorded on the database and data is available for the curators.

**SCSS3** – What is the number of seeds that you use as the minimum threshold per accession? Are these seed numbers of a given accession based on genetic parameters (such as reproduction biology; heterogeneous samples)? Please provide URL of your protocols if these are available online.

The exact threshold is species/ collection/accession specific (In general, amount needed for 10 seed orders + one sowing for regeneration + 20 seed (to account for regeneration failure). The actual number depends on reproduction biology, heterogeneity of accession and its type of use.

**SCSS4** – Please provide details on other aspects that are important in this context.

### **Box 3.2.2.A. Pollination Control**

**PC1** – Please describe the regeneration procedures that you follow for self- and outbreeding species.

*Please include in your description the following aspects:*

- a. any control measures to minimize or avoid cross-pollination between accessions;*
- b. the use of pollination cages for insect-pollinated species;*
- c. the use of specific pollinators for insect-pollinated species;*
- d. strategies to ensure that males and females participate equally in the reproduction;*

*e. strategies to avoid any genetic drift (minimum number of plants, minimum number of plants at flowering stage before pollinators introduction, similar quantity of seeds harvested from each plant, etc.*

a. Minimize cross-pollination

We use cellophane bags or bread-bag covers to separate each inflorescence or plant from their neighbouring plants. (We work mostly with self-breeding species). Every seed that was generated without cellophane bag is marked in the database and on the envelope as Unbagged seed (these can be of value to some end-users but will not serve for regeneration of future stocks nor to support contemporary seed science.

b-e) Not applicable

### **Box 3.2.3.A. Regeneration Environment and Procedures**

**RE1** – Describe the regeneration environment and conditions that you apply. If applicable, you might want to distinguish between different types of germplasm (e.g. wild relatives, landraces, modern varieties, breeding material, genetic stocks, etc.).

*Consider the following aspects:*

*a) in how far are the environmental conditions of the current regeneration of individual germplasm accessions comparable to the environmental conditions that existed at the original collecting or breeding site?*

It is very different. The UK field conditions and standard green-house conditions for regeneration are certainly different than the historic global collection sites.

No special environmental conditions

*b) do you use controlled environments?*

Yes, most of the regeneration work is done in semi-control environment (heated greenhouses)

*c) do you collaborate with other genebanks in Europe?*

Not for regeneration, but yes for knowledge exchange and training.

*d) others.*

### **Box 3.2.4.A. Seed Processing Procedures**

**SPP1** – Describe the protocol(s) that you use for threshing and seed cleaning.

Glasshouse regenerated seed is threshed in two ways a) Mechanical threshing (Flexiseeder single ear / small sample benchtop thresher) or b) by hand using rubber tubing (cricket bat handle cover grips). For some fragile seed hand threshing is preferable.

Cereal Machine Thresher Protocol:

- 1) We use the field/Lab Single Ear Thresher by Flexiseeder  
[https://flexiseeder.com/index.php?id\\_product=9&controller=product](https://flexiseeder.com/index.php?id_product=9&controller=product)
- 2) Training and H&S instructions are given to all users before operating for the first time.
- 3) Ensure the outlet pipe is in place linking the thresher to the extraction system and that the main room extraction is switched on near room entrance.
- 4) Ensure front Perspex cover and main green side door are closed to neutralise safety cut out switches. Check kill switch on top of unit is up to allow operation
- 5) Put a suitable collection tray or container into place under the lower outlet pipe for seed collection.
- 6) Turn on the thresher (green switch) and listen for any running abnormalities as a pre check. Also listen for residue seed unintentionally left behind from previous running. Either blow out or open front to retrieve unwanted seed (see point 9) also)
- 7) A hinged lid is fitted to the entry funnel prevent grain exiting from the inlet when threshing. It must be kept closed apart from when loading ears
- 8) Feed some 'discard' ears into the hinged funnel at top of the threshing machine and watch the passage through the machine via the Perspex front and assess the threshing process. Depending how these discard ears are processed consider the following
- 9) Was the seed satisfactorily separated from the trash? Observing through the Perspex, did the seed thresh out and drop to the collection tray or travel upwards with the trash to the extraction. Adjust the winnowing / air supply to achieve this. Start at 10% open (there is a nut to adjust and lock this). Allow time for the seed to drop to collection tray. Depending on crop consider adjusting the winnowing / air supply.
- 10) Fully opening the winnowing / air supply between samples will allow an air boost to aid a final clean out. Return to initial setting for next sample
- 11) If seed is lodged within the system, first try blowing through by opening air intake fully and closing repeatedly. If still lodged, open front Perspex cover to clear out
- 12) If unsatisfactory samples are still being generated the drum speed may need adjusting. This is completed inside the equipment and will require a JIC Facilities Planon job request.
- 13) Note to Facilities: Check the operating manual in black A4 folder for belt adjustment instructions. The door to access inside works on a kill switch mechanism. Disconnect electric cord from external power supply before opening
- 14) Check your resultant seed sample carefully. It should be threshed relatively clean of trash. Check the condition of seed to ensure the sample was not over threshed leading to damage of embryos. If this is encountered, you could render your seed un viable
- 15) Leave the equipment clean and ready for next user
- 16) A video for correct operating procedure is available for training.

## **Hand Threshing Protocol – Cereals**

1. Take harvest bag from sack and take out cellophane bagged ears.
2. Find and take out all labels – check the code on the harvest bag matches all the labels especially if you are using protocol that means unbagged ears are being left unthreshed.
3. Keep one good example of an unthreshed ear and packet to small white packet. Place this inside the larger packet. Put other unbagged ears back in the brown bag and keep in white sack. These may be used later for phenotyping tests
4. Remove the cellophane bags from the bagged ears
5. Thresh seed out using threshing tube. Depending on ear size possibly 3 or 4 ears can probably be done at once. Do as many as tube will hold in one go. It's worth spending enough time on the threshing stage as unthreshed seed coming out of tube waste time sorting out before packeting
6. Tip seed from the tube to collection plate and use blowing / winnowing and shaking to clear debris leaving only cleaned seed.
7. Keep extraction chute shut until ready to use and close immediately afterwards. It can disastrously suck plates and seed away by mistake
8. Tip the cleaned seed to a labelled white packet. Check code written in black ink in top quarter of packet. Also state bgd (bagged) or ubgd (unbagged) in top right corner). Label the year in bottom right corner S24 = Summer 2024. All this information will eventually be displayed on a barcoded packet label
9. Store in correct sized Kbins. Do not leave Kbins or unthreshed material in threshing room overnight
10. Never have more than one brown bag / seed sample on bench at one time
11. Clean air extracted area of bench, pushing all debris down extractor chute after each separate sample process
12. Check correct bin is being used for all elements of rubbish generated (non-recyclable, green waste, recyclables)

## **Mechanical Legume threshing**

**Peas** or other legumes are harvested from the JIC field as they reach maturity. This may be as whole plants or as bags of pods depending on the size of plot. In any case they are placed into net bags with the field label from the plot for identification. These are stored in the Field Station off the ground with sufficient air circulation and left to dry further. In the circumstances that peas are harvested in damp conditions they will either be placed on the grain drier to have air blown through or if smaller samples, placed in a drying oven at ~30C for 24 hours.

Legume are threshed using a Wintersteiger LD350 thresher adjusted for each crop and each sub diversity set to the appropriate speed and fitted with the correct sieve according to the manual.

If further cleaning is required to remove excess pod the sample is poured through the nearby Sortex winnower. The label coming from the field is placed in the seed bag with the sample.

The peas are put through the R25 seed counter using the QR code to record the data into the correct file.

A duplicate label is produced and stuck on to the seed bag carrying the same information as the field label (which remains in the bag for back-up and verification) plus the seed number, weight and thousand grain weight (TGW) of the sample.

Data is provided to the research lead for the project and the seed samples are stored in the Field Station seed store if not collected by the project team for further analysis.

**Legume grown in greenhouse are hand threshed.**

Pods are harvested when they reach maturity and collected in a labelled paper bag with additional plant label in the bag.

Pod are hand threshed

**SPP2 – Describe the protocol(s) that you use for seed drying, including whether you use different drying procedures for different types of species.**

Seed drying in all cases of GRUs different crops; Wheat, Barley, Oats, Peas, Beans starts on the plant during maturation and ripening. Following that in the Seed Storage area of controlled low humidity the seed keeps on drying until equilibrium with the above stated conditions.

**SPP4 – Please describe how and where you store (in a temporary manner) newly harvested seeds.**

*Please provide details on the temperature and relative humidity of the storage room/space; what type of containers do you use, if any.*

The conditions for post-harvest and post processing are the same. Breathable paper bags are used to hold cut seed heads (wheat barley oats peas beans). These bags are then kept in breathable white sacks. This allows flexibility to allow skilled technicians to steadily work through processing material, rather than relying on the need for temporary staff to speed the task through soon following harvest.

**SPP5 – Describe the criteria you use to decide on seed quantity per accession for the long-term storage.**

The return of seed should ideally allow for: 10 seed distributions amounts + one future regeneration sowing + safety buffer (20 seed) + duplication packet (>two sowing) + Sample for Svalbard Seed Vault (if accession was not yet duplicated).

Ideally regeneration is conducted with minimal waste. Glasshouse pot grown ears of cereals are covered with cellophane bags post ear emergence but pre pollination. This precise stock becomes the primary regeneration material and will be initially long termed stored, threshed in C5 white paper envelopes. These 12x16cm packets can hold up 100g.

Non bagged ears are also kept and GRU attempts to keep all seed produced If possible due to the costs involved in production. Non-bagged seed maybe be used as phenotyping stock in future use or for users such as hobby growers that do not demand high purity.

The strategy for regeneration attempts to avoid multiple regenerations of the same material regularly (with 10s 000s of lines in stock, regenerations must be conducted efficiently meaning as little seed wastage as possible)

#### **Box 3.2.5.A. Genetically Modified Material**

**GMM1** – In case you treat GMO material differently from “normal germplasm”, please provide here the details for each of the deviating procedures (and equipment).

Users can deposit GMO that the genebank will distribute (under the above explained DPRM, custodian level #3), however the genebank team do not regenerate GMO. Their distribution would be under national law and recipient regulation (e.g., triple packing etc)

**GMM2-** Describe the policy and procedures (if any) in your genebank, related to ensuring that distributed samples are not containing GMOs.

As all our growing areas for regeneration as well as the genebank seed processing area contain no GMO seed, we are certain that contamination is extremely unlikely. We therefor issue a ‘No GMO declaration’ upon request.

**B. *In vitro* Culture Collections** *Not applicable*

**C. Cryopreserved Collections** *Not applicable*

**D. Field Genebank Collections** *Not applicable*

### 3.3. Ensuring Availability

An important objective of conservation efforts is to facilitate the effective utilization of germplasm accessions by researchers, breeders and farmers. Thus, ensuring the ready availability of stored germplasm is an important principle. It refers to the ability of genebanks to supply and distribute the stored germplasm, together with any associated information, in an adequate way to users. Aspects that can affect the availability include: (a) policies, (b) seed stock, (c) health status of accessions, and (d) distribution quantity. Although most of the questions are not relevant in the ECPGR/AEGIS context, it was decided to keep the questions and to allow for a comprehensive genebank manual that can be used “globally”.

#### A. Seed Collections

##### **Box 3.3.1.A. Ensuring Availability of Germplasm – Policy Aspects**

**AGP1** – Describe the germplasm distribution policy that you follow at your genebank. *You might want to consider in your response the following aspects:*

- a) *crop/species specificity;*
- b) *whether or not sufficient seed stock is available; who the requestor is;*
- c) *what the purpose of the germplasm request is;*
- d) *any restrictive conditions and/or*
- e) *the total amount of accessions sent per request for distribution of germplasm;*

*use of a formal agreement to distribute the germplasm*

- All the accessions are publicly available to order here <https://www.seedstor.ac.uk/>
- For MLS material, we send 20 cereal grains or 6-12 legume grains per ordered accession. If requested, and available more seed can sometimes be sent.
- For large mapping populations, with high germination rate, three seeds are sent (as the user would normally grow one plant from each to bulk up and use in field the following season).
- For MLS material, we use the S-MTA. Minor cost recovery (currently 2£ per line and £15 per order) is applied, mainly to reduce waist resulting from free online access. Cost recovery statement can be read here <https://www.seedstor.ac.uk/GRU-CostRecovery.php>
- Non-MLS collections have other MTAs that are either not restricted to academic use only or allow full freedom to operate for commercially for one (relatively low) flat fee per accession allowing their full cost recovery (including the investment to generate them and maintain their genomic data in highly usable state).
- MTA for each of the collections can be found under the information button linked to each collection description in the database.
- No restriction on number of accessions sent per seed request (within reason).
- Reason for requesting seed:

In the last 5-year report we reported germplasm use by diverse user groups including archaeologists, botanists, brewers, bakers, bioinformaticians, commercial breeders and pre-breeders, computer scientists, conservationists, crop scientists (e.g., plant pathologists, seed scientists, geneticists, physiologists), educators (in schools, colleges, universities), farmers, and thatches. **During 2017-2023 a total of 33,415 seed samples were sent in response to 1903 online requests received from 51 countries.** Of this effort:

- 65% in support of academic research (in the UK and globally)
- 15 % in support of crop breeding industry
- 5% in support of education

- 15% other, including direct use

**AGP2** – Do you have as part of your service-rendering policy aspects such as a “maximum time” between receiving a germplasm request and distribution of the germplasm?

No.

The operational time to process a ‘standard’ job within the genebank team is usually up to two weeks if germination record is up to date and three weeks if it isn’t. Large seed requests (of hundreds of accessions) require more time to process as they demand allocation of extra worker and re-prioritisation of daily tasks.

A UK ‘standard’ seed request under the MLS (S-MTA non-negotiable terms) would only require the operational time to be delivered.

The timeline required to generate the paperwork for export is highly varied and a case-by-case operation. Agreed and signed MTA, Phytosanitary lab tests, issue a signed phytosanitary license, issue a Certificate of Origin, sign a non-GMO declaration.

Internal SOPs are regularly updated for seed export, but they would still demand time (usually a few weeks but sometimes many months). Changing regulations in the receiving country is often a cause for delay or shipments being returned by courier. Overall, less than 3% of the jobs are not delivered with 6 months for any reason.

**AGP3** – Describe how you treat “related information” about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.

All the “related information” that is relevant for users is accessible online in *SeedStor* database. Each accession has an internet page with a URL that summarise passport data, characteristic, expedition data, related imagery data and links to external database if relevant.

### **Box 3.3.2.A. Ensuring Availability of Germplasm – Seed/Germplasm Stock Aspects**

**AGSS1** – Please provide details on the minimum/maximum amount of seed, plant, *in vitro* samples that you distribute (where relevant, differentiated by species groups, i.e. self-pollinating, cross-pollinating and/or whether an accession is homo- or heterogeneous).

Collection and crop specific as explained in AGP1, as a rule of thumb, 20 grain of cereal and 10 of legume crop. 6-10 propagules of cereal wild relative.

**AGSS2** – Describe how you store the seeds/other germplasm of a given accession with respect to the use of single or multiple bags or containers per accession.

We keep each harvest of a particular accession separate, so there are sometimes multiple stocks for each accession. All are recorded as separate stocks on *SeedStor*, so we know exactly what we have. The Store Code will indicate the accession with an identifier for location (greenhouse, field or other) and a number at the end which makes it unique. This combined with a barcode ensures we can track record of seed use and documentation to a unique seed packet.

**AGSS3** – Describe how you manage the availability of adequate seed/other germplasm stock per accession, including the use of an absolute lower minimum of seeds per accession as the threshold to decide to regenerate.

SeedStor public database system allows users to view details of the lines we hold and order any lines of interest to them. This is the public side of SeedStor.

We also have the production side of SeedStor (or management system) on which the team processes are streamlined. This is available only to members of the genebank. It is the engine for all operations in the maintenance of our germplasm.

This helps us to process seed requests and update seed stocks as we work through a job. This means we can see what size our stocks are at any time and allows us to see the date and value of the % germination for each line.

It also guides us with our regeneration process. Set up the parameters we want for regeneration, and it will screen the stocks to reach that recommended lines to regenerate.

The decision to regenerate depends upon parameters:

<b>Stock amount</b>	We have insufficient stocks to supply 10 seed requests
<b>Seed Age</b>	Stocks are older than xx years from harvest. (this varies with species)
<b>Germination %</b>	Stocks have a % germination of below 75% (or lower as decided in a case-by case manner)
<b>Stock quality</b>	Quality of seed from previous harvest is visually poor due to the season

We have a basic sample size for our core stocks which include varieties, landrace, old lines.

For cereals we usually distribute 20 seeds per line for legumes it is 6-12 seeds per line.

Some collections, such as our precise genetic stocks, mutated or single seed derived stocks have specific requirements agreed following discussion with the germplasm depositors or developers. Therefore, sample number will vary and depending on seed availability and the reliability of the replication of the stocks.

We keep each harvest of a particular accession separate, so there are sometimes multiple stocks for each accession. All are recorded as separate stocks on SeedStor, so we know exactly what we have. The Store Code will indicate the accession with an identifier for location (greenhouse, field or other) and a number at the end which makes it unique. This combined with a barcode ensures we always know our stock levels, track them to past phytosanitary tests and to specific distributions.

**B. *In vitro* Culture Collections** *Not applicable*

**C. Cryopreserved Collections** *Not applicable*

**D. Field Genebank Collections** *Not applicable*

### **Box 3.3.3.A. Ensuring Availability of Germplasm – Health Aspects**

**AGHA1** – Describe how you store seed/other germplasm with respect to germplasm health considerations, including whether you have a “policy” of storing only “disease-free” (as far as you can see or determine) accessions, at least for the quarantine pests and diseases.

One can never ensure ‘no disease’, but we ensure that no restricted diseases are spread with our germplasm. In short, here are the measures we take:

1. For event of infestation in field, we store seed for two weeks in -20 C prior to processing the seed.
2. Seed are tested by Animal and Plant Health (APHA) inspector when they are exported.
3. Seed are often pre-tested by APHA for an entire harvest as a bulk.
4. APHA test include visual screen to ensure that the seed is clean and lab test to rule out an array of virus born diseases.

**AGHA2** – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).

**Plant phytosanitary** rules are very important to prevent spread of disease/pests across the globe. The UK government website gives details of the rules for exporting seed. Any seed leaving the UK will require a phytosanitary certificate unless there is an Import Permit from the recipient country stating it is not required as the seed will go into quarantine on arrival.

APHA has an online phytosanitary certification application process, which we are registered to use.

Only seed should be in the package, no leaf litter, pests or diseases.

We arrange a sampling appointment with the local APHA inspector, who will either inspect the seed himself or send the seed to the official testing laboratory in York. Once the seed has been passed as clean, we are then able to apply for a phytosanitary certificate.

The certificate is valid for 14 days from the date of signing. It must be visible from the outside of the package and easy for any customs officer to open and read if required.

The various steps required for this process are also recorded on SeedStor.

Some species will require a growing season inspection, as you are unable to check if a disease is present at any other time.

To speed up the process and reduce cost, we do use bulk testing. We may distribute a single population from a single year for many years and so by doing a bulk test, for an area that would suffer the most, (e.g., Antarctica). The seed is checked for all the common diseases and pests for that species and for which a lab tests exist in the UK. We can then use the bulk for as long as we have stocks from that harvest year.

We keep a record of all these bulk samples, which we can use to apply for a phytosanitary certificate alone without testing every time. A matching record is also kept with the local Inspector for reference.

**AGHA3** – Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a “plant passport”.

**Plant Passports** are required for all plants and seed of certain species for distribution within the UK. Our genebank has been registered to issue Plant Passports. This requires strict record keeping ensuring that any plant or seed can be traced back to its origin packet of seed. They need to be visible on the outer container, so visible, whilst in transit.

### **Box 3.3.4.A. Germplasm Supply**

**GS1** – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes, including whether you differentiate between germplasm from self- or outbreeding species, heterogeneous accessions, and possibly other aspects.

As a genebank holding different forms of material, we carefully consider what the amount to send out should be on a case-by-case approach across the collections. This is considering the different type of users and different forms of utilisation of the different collections. For a collection of heterogeneous type material (e.g. mutagenised population), the number of seed would take in account germination % to ensure that a mutation of interest (if found in the lowest possible 25%) would still be found, this usually amounts to at least 20 seeds. Similar approach is relevant for diverse landrace accessions in historic landraces collections. For derived material that has been through a process of Single Seed Decent (SSD) or highly stable modern cultivar we send 7 seeds (or less if the user would only attempt to grow one plant). On the other side of the spectrum, when seed bulks available (e.g., from field demonstrations of heritage varieties), high amounts can be offered and supplied to hobby growers or artisan farmers.

In summary, the genebank aim is to supply the correct amount to deliver the property that the user has requested while preventing unnecessary waste of effort, resources and energy.

**GS2** – As GS1 above, but in case your germplasm samples do not possess the minimum viability, would you increase the number of seeds?

Yes, but only to a minimum limit of 50% viability. The user would be pre warned that this approach was being followed. They would have the choice to decline the offer. A typical situation for this would be in dealing with mutagenised F5 seed. At a minimum rate germination rate of 85%, potentially double the seed could be sent for a known germination rate of 50%.

**GS3** – Please provide information on any other aspects related to seed supply.

Seed supply is always carried out as swiftly and efficiently as possible. Staff in the GRU are embedded in research and pre-breeding project and therefore realise the importance to deliver seed orders promptly. Orders are logged on the in-house operating system after being received via the Genebank email account. At this point the job is logged to the system and the correct member of staff is assigned the job.

This ensures a dated record is made of the seed request avoiding leaving the job undone. The efficiency of the inhouse operating system requires that the job processing tasks are clearly shown at the stage of requiring attention.

Only when all tasks are completed; seed sampling, stocks updated, MTAs created and returned signed, cost recovery arranged, phytosanitary certification applied for, does the system allow to proceed to dispatch.

Within the UK the postal service is used for small order < 10 packets for seed that's easily replaced. For larger orders, all international (except odd cases e.g. Japan) and those where seed is more irreplaceable an international courier (DHL or FEDEX) is used. Resealable poly

bags are used to hold the seed. These are clear and can easily be seen to carrying seed. Inspectors can easily open check and reseal

**B. *In vitro* Culture Collections** *Not applicable*

**C. Cryopreserved Collections** *Not applicable*

**D. Field Genebank Collections** *Not applicable*

## 4. Providing Information

The lack of adequate information on a given accession may well decrease the value of that accession to the user. The information on individual accessions should be as complete as possible in order to facilitate the identification of duplicates and/or to select accessions with desirable characteristics. A genebank should have a documentation system in place that allows to optimize management of the collections as well as to provide access to information about the collection to users.

### **Box 4.1. Genebank Documentation System**

**GD1** – Please provide details on the technical aspects of the genebank information management system(s) that you use.

- a) On which software is the system based (i.e. Oracle, Fox Pro, MS Access, MS excel, MS Word, other?).
- b) In case you use a manual information management system, please provide details.
- c) In case your “internal” database(s) is/are different from the publicly available database(s), please provide details on both,
- d) Describe which activities of the genebank are covered by the system.

We use a bespoke in-house developed public database mirrored by internal genebank management system. The public side of the system can be freely browsed here <https://www.seedstor.ac.uk/>. For more information see Horler et al 2018 <https://www.seedstor.ac.uk/>. All the activities of the genebanks and all the historic and updated accessions and stock information is managed through the database and management system.

**GD2** – Provide details on which types of data you handle in your documentation system, e.g. passport data, characterization & evaluation data, cultivar data, material distribution, etc.

All the information is available here <https://www.seedstor.ac.uk/>

For each accession, tabs of data exist for [Passport Data](#) [Taxonomy Data](#) [Expedition Data](#) [Phenotype Data](#) [Accession Images](#) [Supplemental data & Links](#)

**GD3** – In case your internal database(s) is/are different from the publicly available database(s), please provide details on both.

The internal database includes information that is not relevant for end users but necessary for the curators. The internal Germplasm Information Management System allows team members to track and process germplasm requests, determine regeneration priorities, handle cost recovery and Material Transfer Agreement paperwork, manage the Seed holdings and easily report on a wide range of the genbank tasks.

All the information usable for user (on the accession level) is publicly available and browsable <https://www.seedstor.ac.uk/>.

**GD4** – Describe in which form you send accession specific data (e.g. as hard copy, electronically – if the latter, please specify (in plain text) which file format, i.e. Excel, Access, others is used).

All data is browsable (and downloadable) online by users. No data needs to be sent.

**GD5** – Provide information on how technical support for development and maintenance of the documentation system is arranged.

The current system was launched in 2014 following a two-year development process. The system was developed by requesting a computer engineer to follow an experienced genebank curator through all her daily routine and streamline them to support the work, ensure full track record and efficient usability of data. The development process also included amalgamating all the previously existing data (in various forms and files) into the new database. Since its creation it is updated regularly to the latest background operation technology (e.g., to the supported version of MYSQL and PHP) by the NBI group research Computing for Science, that provides support to several technological and science groups in JIC. The developer of the system (Dr Richard Horler) keeps on developing minor improvement of the system (officially on a 10% FTE) so that the system is updated with the genebank operational changes.

**GD6** – Describe your genebank policy with respect to backing-up of the database contents, including with which frequency.

All onsite LIMS systems including SeedStor is backed-up on a daily basis and exist on local hard disks as well as on a cloud based data duplication.

**GD7** – Provide any other information on your information management system that is not covered in one of the above questions.

The SeedStor genebank management system supports the genebank manger in report preparation, e.g., reporting S-MTA use to FAO/ annuar national list report to EURISCO, Genebank Indicators reports to institute management and funders.

The phenotyping data held in SeedStor (>500,000 phenotypic data point) is a unique strength of the collection and enable cogent choice of germplasm for science and breeding.

#### **Box 4.2. Information Exchange**

**IE1** – Please describe how you make your passport data available to users (i.e. as hard copy; via the internet; other?).

All the data is available online. Each accession has a URL with the passport data.

**IE2** – Please indicate if your data is available as machine-to-machine web-services. In case it is, describe:

- a. what types of data (passport data, characterization & evaluation data etc.) and
- b. which web-service interfaces are available (i.e. GBIF IPT, BioCase, TapirLink).

SeedStor passport and characterisation data can be machine-to machine harvested using BrAPI (<https://brapi.org/>). Currently, the Designing Future Wheat project database grassroots-genomics

communicated with SeedStor behind the scene to describe studied accessions.  
<https://www.earlham.ac.uk/research-project/grassroots-genomics>

SeedStor is also linked to the plant genomic browser of EMBL- European Bioinformatics Institute (EnsamblePlant) where user can browse mutation lines and order them on SeedStor. Plans are underway (funded UKRI-BBSRC grant) to link natural genomic diversity of wheat and pea landrace and modern cultivars too.

**IE3** – Please indicate if your data is published to EURISCO. Describe which data is published to EURISCO and at which intervals.

The ~27,000 accessions that form part of the UK MLS contribution are reported annually to EURISCO.

**IE5** – Describe the kind of information you distribute together with the germplasm to persons that request germplasm.

*Please consider the following data types: Passport, Characterization; Evaluation, and/or Germplasm management data (e.g. viability percentage; protocols followed for routine operations; etc.*

Accession data (passport and characterization) is available online.

Information that is sent to user includes:

- 1) labelling with the unique GRU Code and the Accession name.
- 2) Advice for any lines being sent in excess rates due to lower germination rates
- 3) MTAs
- 4) Invoices
- 5) Phytosanitary Certification or Plant Passport (if required).
- 6) Import Permit (if required)
- 7) Inventory of seed lines enclosed
- 8) Guidance to good seed care and use (to avoid misuse of low vigoured old seed)
- 9) Request for user to provide information on safe arrival, germination rates, photographs and reports of the material.
- 10) Acknowledgment and Notification for publications arising from the use of the seed.