#### PRT-CGN-PG-301 Protocol viability testing

##### **Introduction**

The ISTA guidelines are generally followed for performing germination determinations, but curators may have made changes to these over time based on practical considerations. These methods are recorded for each germination determination in GENIS and are also listed in the Overview of Methods of Germination Determinations (OVZ-CGN-PG-301).

**Frequency, types and scheduling of germination determinations**

##### Initial germination for acquired or propagated material (A)

Before a sample is included in the genebank, an initial germination is done.

Repeat germination (H)

This means monitoring the germination of collection material over time. It is expected that the germination of seed stored at -20°C with an absolute moisture content between 3 and 7% will deteriorate only slowly.   
Within CGN, the guideline is to carry out the first repeat germination 25 years after inclusion of the relevant accession, followed by intervals of 5 or 10 years, depending on germination results. Better storability is expected in wheat and barley than in other crops, therefore intervals of 5 and 20 years respectively apply to these.  
No more tests are carried out on accessions that are already known to be propagated in future years.  
An annual overview of all germination tests to be carried out is made by the Head of Documentation. This overview is generated from GENIS using the above germination intervals. The curators can use this advice to plan. When determining the execution year, limited flexibility can be applied, for example, to accommodate differences in test numbers between years or combining small numbers of material of the same type.

Duplicate germination

In duplicate germination determination, a portion of the samples is tested for a second time under code number (anonymously). Numbers eligible for a second determination are randomly determined for each crop.

The reason for this check is to test the reproducibility of the results and strive to improve the germination protocols.

Based on the previous year's germination results, a number of crops are selected to be checked in the current year. The CGN aims to have checked all crops once in five years. The crop selection is discussed with the curator of the crop; if no germination tests are scheduled for that crop that year, a decision may be made to select another crop if necessary. Also, if something else needs to be taken into account when selecting accessions (e.g. no wild accessions this year), this is discussed and noted.

After crop selection, a selection of 5% with a minimum of 10 accessions is made from the accessions tested last year (if possible). The procedure of selection is noted.   
The duplicate determinations (from the previous year) are carried out simultaneously with the (repeat) germination of the current year.

As soon as the curator has the bags for the current year's germination determination taken, he/she indicates that duplicate determinations should also be carried out, and the duplicate material is also taken by the Seed Manager. This is immediately repacked by the Documentation Project Officer, and given to the curator in new bags with a code on them. As a result, this process happens 'blind': the curator does not know which accessions are involved, so the assessment is independent.

After performing the duplicate determinations, the results are 'decoded' and compared with the original results. The analysis is reported on.

Group sampling

When scheduling repeat germplasm, there is the option for curators to use group sampling. Here, accessions are grouped by species and regeneration year, and from each group a minimum of 25% of the accessions are tested. The motivation for this is that material of the same species regenerated under the same conditions and having undergone the same procedures after harvesting is expected to show similar germination rates over time.

Within a group, the accessions are tested in order of prior germination results, the lowest first.

In the event that one or more accessions from a group sampling score too low, follow-up germination testing will be done starting with the next 25% of the group based on lowest prior germination. Thus, in the worst case, the whole group will be tested in four steps.

**Conducting germination tests**

To perform germination determinations, the methods drawn up by the various curators are followed. These methods in are the overview Methods germination determinations (OVZ-CGN-PG-301).

This table lists for each crop the most commonly used germination method, including the number of seeds to which the germination is done as well as which characteristics of the seeds and/or seedlings are recorded.   
The ISTA (International Seed Testing Association) guidelines are followed for assessing individual seedlings.

Practical considerations in preparing germination determinations:

* A seed sample must meet the minimum quality requirements as stated in INS-CGN-PG-005. In case of visibly poor seed quality (brown discolouration, broken- or empty seeds), the number is re-cleaned.
* Each germination sample is labelled with an accession or receipt number and a field number to avoid mix-ups with other samples.
* At least one day before the start of germination tests, the vacuum seed bags should be opened and further stored under room conditions so that the seeds can already absorb some moisture.

**Registration and assessment of germination**

The results of the germination determinations are entered into Excel. This file contains the accession numbers, the germination method used and possibly the germination percentages. Based on both the germination rate and a visual assessment of the seedlings, a final score is given. This final score will determine the further treatment of the sample.

The following scores can be given:

1. The seeds are below the minimum germination requirement (80% or 60%) and/or show strong signs of ageing and need to be multiplied as soon as possible.

3. The seeds are around the minimum germination requirement or are likely to drop below it soon and/or show moderate to strong signs of ageing; the seeds should be multiplied within a few years.

2. The seeds are still sufficiently germinating, but show slight signs of ageing and/or are moving towards the minimum germination requirement; it is advised to bring forward the next germination determination.

1. The germination rate is well above the minimum germination requirement. The seeds are very germinative and give nice strong seedlings.
2. Score given in a group sampling if the other accessions within the group scored 1.

**Follow-up germination determinations**

* Score 1 (good) for initial germination (A): advice to test germination again after 25 years.
* Score 1 (good) for repeat germination (H): advice to test germination again after 10 or 20 years, depending on the crop, see previous information in this document.
* Score 2 (sufficient): recommendation to test germination again after 5 years. This applies to both repeat germination and initial germination.
* Score 3 or 4 (insufficient) on repeat germination (H): re-determine germination. If the score is again insufficient, and the seed has been properly cleaned, then multiplication is planned.
* Score 3 or 4 (insufficient) for initial germination (A): redetermine germination. If the result remains below the agreed standard, the steps below are followed.

Germination results below standard

If the result of an initial germination remains below the agreed standard, the following steps are followed sequentially.

* Seed cleaning  
  It is checked whether the seed material is sufficiently cleaned. If this is not the case, the seed material is further cleaned. Please refer to the cleaning protocols PRT-CGN-PG-201 and PRT-CGN-PG-202.
* Sprouting  
  If the curator expects the material to exhibit germ dormancy (e.g. in wild material), consideration is given to whether the material can be brought to germination by other means. In wild species, it is often difficult to break the germ dormancy present. Seeds in germination dormancy swell but do not germinate. When calculating germination percentage, the number of swollen seeds is added to the number of normal seedlings. This is also done in the case of hardiness, a form of germination dormancy that occurs in clover, field bean and pea.
* Additional multiplication   
  If the material cannot be further cleaned and any germ rest cannot be broken, it will be determined in consultation with the curator whether the material will be reproduced again.
* Inclusion as deviant material or destruction  
  In exceptional cases, by written consensus of two curators and the collection management project leader/Cluster leader, the minimum quality requirement may be deviated from. For this, see FOR-CGN-PG-030 Recording with permission. This applies especially to material that is difficult to propagate (wild species), whose new multiplication is very costly, or to landraces that are actually better considered wild, such as some poorer germinating potato landraces. In this case, the result is marked A. In the remark field, the remark recording with permission is added.  
  If the initial germination results remain below the agreed standard, and no exception is made to record the material as deviant, the accession is given the status not-accessed.   
  The curator can then later decide to review the material again or destroy it.