 <p><b>Plant Genetic Resources Bank "Mihai Cristea"</b></p>	<p><b>FOR TESTING/MONITORING SEED VIABILITY</b></p>	<p><b>Edition: 2</b></p>
		<p>Page 1 of 3</p>

## SOP.14. TESTING/MONITORING SEED VIABILITY

### 1. Introduction

The genetic material introduced and preserved in the two types of BRGV collections must meet national standards (SR 1634/1999) and international standards (FAO and ISTA, 2023) regarding seed viability in Gene Banks.

Accessions obtained through collection, acquisition, or regeneration/multiplication, after phytosanitary control and drying, are tested for viability. All seed accessions are evaluated both upon entry into the Bank's collections and at regular intervals throughout the storage period by performing the standard germination test.

### 2. Initial viability testing of plant material introduced into the bank

In accordance with current standards, minimum germination requirements are met, which are 85% for cultivated species, while for spontaneous flora species, which normally do not achieve high germination levels, a lower percentage (60%) is accepted.

Depending on the size of the sample, seed dimensions, or low multiplication rates (e.g., wild species), the initial test is performed on samples of 100/200 seeds, randomly taken and divided into two replicates. Each sample is labeled with an entry number to avoid confusion with other samples.

The standard germination test is performed using the following methods: on a layer of paper (TP), between two layers of paper (BP), or on a sand substrate (S). At the end of the test, the average germination percentage is calculated from the two replicates. If the difference between replicates is greater than 10%, the germination test is repeated.


The germination test results are recorded in a special register (ViaT), which includes the entry number, the number of seeds tested, the date of test initiation, the germination method used, and germination percentages, which are later forwarded to the curators in the predefined format.

**Note:** For accessions received from agricultural institutes or stations within the country to be stored in the base/active collection, intended for future use in breeding/research, and which have a stock of fewer than 500 seeds, the germination test will be performed on 10% of the sample.

### 3. Monitoring the viability of plant material preserved in the active collection (+4°C)

This activity is conducted approximately every five years, based on internal and practical considerations for organization and prioritization, and targets seed accessions stored in the active collection with stocks exceeding 150 seeds.

Using the germination interval mentioned above, Excel lists (F-14.5.1) are generated with the help of the Biogen database and forwarded to the conservation department for supplying the samples whose viability is to be tested.

 <p><b>Plant Genetic Resources Bank "Mihai Cristea"</b></p>	<p><b>FOR TESTING/MONITORING SEED VIABILITY</b></p>	<p><b>Edition: 2</b></p>
		<p>Page 2 of 3</p>

The final results obtained for each analyzed accession are recorded in Excel lists (ViaM), which include: scientific name, accession number, year of last germination test, afferent germination percentages, stock, number of seeds used, test start date, number of normal germs, number of hard seeds, number of abnormal germs, number of dead seeds and germination percentages. All germination test information is also transferred to the database, in the dedicated file.

When a decline in viability below the minimum admissible levels is observed, the curators include the respective accessions in a regeneration cycle.

**Note:** For accessions scheduled for multiplication/regeneration in the following year, germination tests will not be performed.

#### **4. Recommendations for methods used to determine germination**

In general, germination capacity determination methods are specific to different plant species, with recommendations regarding the germination substrate, temperature conditions, evaluation duration, treatments to break seed dormancy, all adhering to standard protocols presented by the International Seed Testing Association (ISTA). However, establishing a strict standard for the number of seeds for germination tests is difficult.

The treatments used to break seed dormancy include: manual scarification or removal of the seed coat, soaking seeds in a large volume of water if the seed coat contains inhibitors that prevent or delay germination, pre-cooling (cold stratification), pre-heating (warm stratification), applying a solution of gibberellic acid (GA3) or potassium nitrate (0.2% KNO<sub>3</sub>) on the germination substrate, and adjusting light intensity/presence.

For certain wild species such as clover, alfalfa, melilot, or cultivated species such as beans, peas, lupin, and soybean, breaking seed dormancy can be particularly challenging, as seeds often remain hard or swell but fail to germinate. Therefore, when calculating the germination percentage, the number of water-imbibed or hard seeds is added to the number of normal seedlings in specific proportions shown in the table below.

Species	Common name	Seed Proportion (%)
<i>Glycine max</i>	Soybean	50%
<i>Lathyrus</i> sp.	Sweet pea	
<i>Lupinus</i> sp.	Lupin	
<i>Pisum arvense</i>	Field pea	
<i>Trifolium repens</i>	White clover	
<i>Vicia</i> sp.	Faba bean, Vetch	
<i>Lotus corniculatus</i>	Bird's-foot trefoil	75%
<i>Medicago sativa</i>	Blue alfafa	
<i>Medicago falcata</i>	Yellow alfafa	
<i>Melilotus</i> sp.	Sweet clover	
<i>Onobrychis</i> sp.	Sainfoin	
<i>Trifolium hybridum</i>	Hibrid clover	
<i>Trifolium incarnatum</i>	Crimson clover	
<i>Phaseolus</i> sp.	Bean	100%

**Note:** Abnormal seedlings or dead seeds, which are removed during the initial and final counts, are recorded in ViaT/ViaM, as the information obtained serves as an indicator of seed deterioration for subsequent germination tests.

## 5. Forms

### F-14.5.1. Sample release for viability monitoring