

## SOP 02. ANNEX 4. STRATEGY FOR COLLECTING PLANT GENETIC RESOURCES

For seed samples to be conserved in the bank, the collection team must determine the taxonomy of the target species using herbarium samples, local or regional plant identification guides, or by consulting colour illustrations of virtual herbaria.

Thus, before the actual collection, a visit to the collection site is conducted, during which several phytosociological surveys are carried out (depending on the size of the area). This allows for the analysis of the floristic composition of the existing phytocoenoses, qualitative phytopopulations indices (individual vitality, phenological state), and quantitative indices (abundance, coverage, frequency) (SOP 02. ANNEX 6.).

After recording this data, 2–3 individuals belonging to the target species are collected for herbarium purposes, and the area from which the seeds will be collected is marked with flags. Herbarium samples must bear the same collection number as the seed samples of the respective species, which will be collected later.

Since germplasm is intended for long-term conservation, seeds must be collected at an optimal stage of development (optimal maturity), free from insect damage, and not hollow. It is crucial to know the fruiting period of the species to be collected, as well as the period when the seeds are physiologically mature. These periods are influenced by the variability (annual, regional) of climatic conditions.

Full maturity can be identified using herbarium data, which provides information about the flowering period. It is known that most plants in temperate zones bear fruit 4–8 weeks after the end of the flowering stage (flowers drop). The development stages of herbaceous plant seeds are as follows:

- **Milk stage:** If the seeds are crushed between fingers, they release a white, milk-like substance. These seeds are not viable.
- **Soft dough stage:** If the seeds are crushed between fingers, they release a soft dough-like substance resembling endosperm. These seeds have very low viability.
- **Hard dough stage:** If the seeds are crushed between fingers, they release nothing, having a hard endosperm that maintains its shape.
- **Mature stage:** Seeds are usually very hard (high consistency).

Seeds collected at the mature stage exhibit high viability and vigor, with the greatest longevity for ex situ conservation. In contrast to cultivated species, the harvesting calendar for wild flora species presents more challenges for seed collectors. Among many other characteristics, wild species may exhibit:

- Due to genetic variability and environmental factors, wild species may form heterogeneous populations, with phenotypic and physiological variability among individuals, leading to desynchronization of flowering and fruiting periods within the same species.
- The presence of inflorescences with indeterminate growth, where the floral peduncle continues to grow after flowering begins, resulting in multiple flower development stages on the same individual.
- Some fruits release seeds abruptly, while others release seeds gradually over an extended period, requiring careful assessment of the optimal harvesting period.

Regarding seed viability, it must be considered that not all flowers are effectively pollinated, and those that are may form seeds. Additionally, developed seeds are not always full (containing an embryo) or healthy; some may be damaged by insects (*Coleoptera*, *Lepidoptera*, *Hemiptera*) or pathogens (bacteria, viruses, fungi).

The frequency of empty and/or damaged seeds varies depending on the population, species, botanical family (*Cyperaceae*, *Poaceae*), and season. Therefore, the collection team must observe and evaluate the proportion of empty or damaged seeds before proceeding with the actual collection.

Data obtained from seed sectioning tests (using pruning shears, scalpels, or other sharp blades) on a representative sample from the collection area provides information on the collection technique to be used to obtain high-quality seeds by avoiding certain parts of the population or selecting another, more productive and healthier population.

For long-term conservation and to safeguard against the natural loss of the population, the sample must contain at least 500 seeds collected from at least 50 individuals of the same species. This quantity is necessary and sufficient to ensure the species' survival, minimizing the founder effect and random genetic drift.

If the sample is reduced due to operations preceding ex situ conservation (germination tests, ecological, genetic, or botanical studies) or seed distribution to internal or external users, or if seed viability falls below a critical level (75–85%), curators must consider the possibility of recollection or regeneration of the sample.

Regenerating genetic resources from wild flora is a complex activity requiring expertise in propagating wild plant genetic resources and additional financial resources for isolating regeneration plots with fabric isolators and using cages with pollinating insects to eliminate the risk of hybridization between samples or species.

When regenerating samples in an area different from the collection site, natural selection may occur due to the inability of some wild species to adapt to the climatic conditions of the regeneration or multiplication site.

The ideal seed quantity for a sample intended for long-term conservation at the Suceava Genebank (BRGV) is between 10,000 and 20,000 seeds. These seeds are used as follows:

- 600 seeds for germination tests;
- 5,000 seeds for the base collection, conserved long-term;
- 5,000 seeds for the active collection, conserved medium-term;
- 1,150 seeds for the duplicate collection;
- For external users (breeders, researchers, individuals), the number of seeds varies depending on seed size and intended use.

Since the reproductive biology of most target species is less studied, capturing rare alleles (with a frequency of 1–5%) from a population requires collecting a larger seed sample representing at least 50 phytosociological individuals from the respective population (when possible).

This collection strategy suggests that a single sample collected in this manner from a population ensures the potential for vegetative restoration of the area from which the sample was collected, as well as the entire natural distribution area of the respective species. To ensure that a seed sample encompasses the greatest possible genetic variability of a target species, the following must be observed:

- Seeds are collected when plants are in their maximum productivity phase (when over 60% of seeds are mature); otherwise, the seeds are of poor quality and physiologically immature.

- Collection is done randomly, maintaining a distance of 5–10 meters between individuals of different species and between individuals of the same species. A smaller distance represents genetic similarity, reducing genetic variability.

- When flowering and fruiting periods differ among individuals of the same species, multiple visits to the area are made to capture the entire genetic variability of that population.

- Equal quantities of fruits/seeds are collected from each individual in the population to ensure that genetic information is equally well represented in the sample. When most seeds in the sample come from the same individual, many genes are similar.

- After collecting a sample, two labels are written (one placed inside the bag and the other attached to the bag), noting: species name, collection date, seed source, number of plants collected, collector's name, weight or estimated number of seeds.

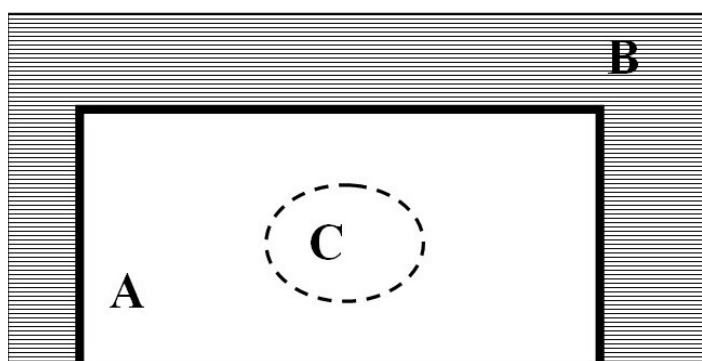
The collection site is identified using local knowledge of the site, publications, and the expertise of specialists and/or administrators from important units and organizations (National/Natural Parks, botanical reserves, local government departments, etc.).

Upon identifying the collection site, several "areas" are established for sampling (*Figure 1.*):

- **Optimal zone (A):** The sample is collected from the area where the individual, species, or population develops optimally in terms of abundance, and the genotype-environment interaction results in a phenotype resistant to various environmental conditions.

- **Intermediate zone (B):** The sample is collected from an area with the same climatic and soil conditions as the location where the seed will be sown (cultivated) or regenerated and multiplied.

- **Marginal zone (C):** The sample is collected from plant populations surrounding the edges of the established site. These plants grow under different environmental conditions than those within the site. In these conditions, due to natural selection and genetic mutations, these populations undergo morphological and physiological changes that provide better adaptation to the environment than populations within the site.



**Figure 1.** Delimitation of areas of the plant genetic resources sample collection site

By collecting seed samples from species in these sites (A+B+C), genetic diversity can be maintained for each identified species. To maintain genetic diversity at the population level, seeds are collected randomly as follows:

- At least 30 phytosociological individuals for self-pollinating species;
- At least 59 phytosociological individuals for cross-pollinating species.