##### **PRT-CGN-PG-125 PROTOCOL mulitiplication beet**

This protocol applies to all parties involved in the multiplication of CGN material.

### **Introduction**

Multiplications have to fulfil minimum quality requirements to ensure maintenance of genetic identity and integrity (avoiding seed mixing among accessions and minimizing loss of diversity through genetic drift) and high seed quality (absence of diseases and sufficient germination ability).

Contamination with Genetically Modified Organisms (GMO) should be prevented.

*Any deviation from this protocol should be reported to CGN, after which it will be recorded by CGN in the Multiplication logbook (FOR-CGN-PG-002).*

**Multiplication**

Maintaining genetic integrity

* Isolation
* Beet (*Beta vulgaris*) is a wind- and cross-pollinating species. It is therefore multiplied by accession isolated in mesh isolation cages, isolation chambers or in isolation tunnels.
* Population size
* About 50 plants are multiplied in each accession with a minimum of 25 plants.
* It is recorded how many plants per accession participated in multiplication. These data are recorded in the Multiplication logbook.
* Sowing
* Any low germination rate of the seed is taken into account.
* The number of seeds to be sown will be determined by the CGN. Germination recommendations sent along by the CGN or the breeder's own germination methods are followed. If material germinates poorly or very slowly, these findings are recorded and copied into the Multiplication logbook.
* Sowing is done in March.
* Vernalisation
* Beets are biennial and need a cold period (vernalisation) to flower in the second year of cultivation. Wintering of beets takes place in pots in a cold greenhouse.
* Cultivation
* No selection is made during plant growth. If an accession is more heterogeneous than what is expected from the passport data, or if it is a mixture of different types, the CGN is notified. The CGN determines whether and how selection may be made. This is recorded and copied into the Multiplication logbook.
* Storage during winter months
* The pots containing the potted beet plants stay in a cold greenhouse during the winter months where the pot is kept slightly moist. The foliage may (partially) die. To prevent rotting or mould growth, (part of) the foliage can be removed without damaging the beet or roots in the pot.
* Pollination
* In the second cultivation year, pollination is carried out by adding flies in the mesh cage where the accession is potted. If necessary, flies are added at several times, for sufficient pollination of the later flowering flowers. Care should be taken to ensure that the mesh cage where the plants per accession are potted is completely closed, so that no flies or other insects can enter.
* As beet is generally a wind-pollinator, this can be anticipated by having the plants in a spot in the greenhouse where there is a lot of air circulation, or circulating the air more at this spot with a fan. Care should be taken here to avoid cross-pollination between accessions that flower simultaneously.
* Seed harvest 2nd year
* Stems with seeds are harvested once they have dried and the cluster of corky material containing the seeds has turned dry and brown. The harvested seed can be bulked per accession. All seed produced will be returned to the CGN.

Maintaining identity

* Characteristics
* During sowing, cultivation, winter storage and harvesting, accessions should be clearly marked with a label indicating the field number. The field number given before sowing remains the same until seed cleaning.
* Harvest
* When harvesting, care is taken to avoid mixing with seed of other accessions.

Maintaining seed quality:

* Seed decontamination before sowing
* Not relevant.
* Pruning
* Remove foliage that dies during winter if necessary.
* Cultivation
* Not relevant.
* Inspection
* The crop is closely monitored. Diseases or pest problems are controlled and recorded and copied into the Multiplication logbook. When diseases that threaten good seed multiplication are detected, the CGN is alerted.
* Seed treatment after harvest
* The harvested plant parts are dried and the seed is kept in dry conditions at all times.
* The method of seed cleaning is being decided in consultation with CGN.

**Concluding actions**

* All deviations during cultivation are noted and sent with the seed. These notes are copied into the Multiplication logbook.
* The harvested seed is sent to the CGN as soon as possible, but no later than 6 months after harvest, with the seed bags bearing the CGN number and field number.