##### **PRT-CGN-PG-108B PROTOCOL FOR THE MULITPLICATION OF HORTICULTURAL CRUCIFERS**

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
* All Horticultural Cruciferous species are cross pollinators. Accessions are therefore multiplied in isolation cages, isolation chambers or isolation tunnels. The plants are tied up such that no flowers touch the netting.
* Population size
* Multiplication is done on 80 plants on average (minimum 25 plants).
* In planting out, the required number of plants are chosen non-selectively from the population. However, plants that lag considerably behind in growth may be left out because their poor performance could mean that these plants eventually do not contribute to seed multiplication of the accession.
* The number of plants that have been used in multiplication is recorded for every accession. These data are copied into the Multiplication logbook.
* Sowing
* The number of seeds to be sown is determined by CGN. If material germinates poorly or very slowly, these findings are recorded and copied into the Multiplication logbook.
* Cultivation
* Horticultural Cruciferous crops are mostly biennial and need a vernalisation period. The sowing period is determined in consultation with CGN.
* No selection takes place. However, in case an accession is more heterogenic than expected from the passport data or in case of a mixture of different species or crop types, CGN needs to be consulted and CGN decides if and how selection will take place. This is being recorded and copied into the “Multiplication logbook”.
* Pollination
* Plants are isolated before or as soon as the plants develop flowering stalks The plants are being pollinated by flies or bumblebees, which are placed in the cages/tunnels as soon as 50% of the plants are flowering.
* It is checked regularly if enough flies or bumblebees are active during flowering.
* Harvest
* All inflorescences with ripe seed is being harvested and collected in bags. Some species may be dehiscent and ripe pods should be harvested regularly.
* All produced seed needs to be sent to CGN.

Maintaining identity

* Registration
* During sowing, cultivation and harvesting, accessions should be clearly marked with a label indicating the field number. All field numbers assigned prior to seed sowing should remain unchanged up to and including harvesting and seed cleaning.

Maintaining seed quality:

* Seed decontamination before sowing
* Prior to sowing, the seed may be decontaminated with permitted seed decontaminants.
* Inspection
* The crop should be inspected regularly. Pests and diseases are being treated and recorded and copied into the Multiplication logbook. When a pests or diseases might jeopardize proper multiplication of the seed CGN is notified.
* Seed treatment after harvest
* Bags with harvested plants need to be pre-dried.
* The method of seed cleaning is being decided in consultation with CGN.
* **Concluding actions**
* Recorded deviations from this protocol should be sent to CGN along with the seed. These records are copied into the Multiplication logbook.
* Harvested seed is sent to CGN as soon as possible, in any case no later than six months after harvest. The seed bags are identified by their field number and their CGN number.