##### **PRT-CGN-PG-127 PROTOCOL mulitiplication cultivated carrot**

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
* Cultivated carrot (*Daucus carota var. sativus*) is a cross-pollinating species. It is therefore multiplied by accession isolated in mesh isolation cages, isolation chambers or in isolation tunnels.
* Population size
* Multiplication is done at about 80-100 plants per accession; the minimum number of plants is 40.
* 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.
* If material germinates poorly or very slowly, these findings are recorded and copied into the Multiplication logbook.
* Sowing is done in April-May.
* Vernalisation
* Cultivated carrots are biennial and a cold period (vernalisation) is needed to flower in the second year of cultivation. Overwintering of the carrots takes place in pots in a mouse-free 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 plants' carrots 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 roots in the pot.
* Pollination
* In the second cultivation year, pollination is done by adding flies (or other pollinating insects) 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 are potted by accession is completely closed, so that no flies or other insects can enter. Plants may grow tall during flowering, which may require support material.
* Seed harvest 2nd year
* Harvest seed pods ('bird's nest') as soon as they have dried (not too early; let them ripen well on the plant). Harvesting can be done at several times. The harvested seed can be bulked. All seed produced is returned to the CGN.

Maintaining identity

* Characteristics
* During sowing, cultivation 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
* If necessary, remove foliage that dies during the winter. Keep main stem if possible.
* Cultivation
* Not relevant.
* Inspection
* Control against diseases and pests takes place. 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 pre-dried and the seed is kept in dry conditions at all times.
* In consultation with the CGN, it will be decided how the seed will be cleaned after harvest.

**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.