##### **PRT-CGN-PG-129 PROTOCOL mulitiplication fennel**

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
* Fennel (*Foeniculum vulgare*) 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 40 plants per accession; the minimum number of plants is 25.
* 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 can be done early in March. With this sowing time, there is the best chance of flowering in that same cultivation year. If flowering in the second cultivation year is preferred, sowing can be done in July. Optimal germination takes place under a temperature of 20-22⁰C.
* Vernalisation
* Fennel is either annual or biennial (depending on genetic background, sowing time and environmental conditions) and in the latter case needs a cold period (vernalisation) to flower in the second year of cultivation. In this case, wintering of the fennel takes place in a cold greenhouse.
* Cultivation
* No selection is done 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
* If the fennel accession has not flowered in the first year, the plants stay in pots in a cold greenhouse with the pot kept slightly moist during the winter months. The foliage cannot withstand the cold that well and will (partially) die. To prevent rot or mould growth, (part of) the foliage can be removed without damaging the roots in the pot.
* Pollination
* When the flower heads are formed, in the first or second year of cultivation, pollination is carried out 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 flower heads. Care should be taken to ensure that the mesh cage in which the plants per accession are potted 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
* Seed shields are harvested as soon as they are dried (not too early; let them mature well on the plant). Seeds can easily fall from the seed shields, requiring possible harvesting at multiple times. The harvested seed can be bulked by accession. All seed produced will be returned to the CGN.

Maintaining identity

* Characteristics
* During sowing, cultivation, possible winter storage, and seed harvest, the accessions should be clearly marked by means of labels with 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
* Foliage that dies on biennial crops can be removed if necessary.
* 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 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.