##### **PRT-CGN-PG-110 PROTOCOL MULTIPLICATION POTATO**

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.

Only material tested for Q-organisms may be used for multiplication. This may be seed from a previous multiplication, or freshly tested plant material or tubers from NVWA's Q-facilities or elsewhere, which have been Q-tested and declared healthy by NVWA or an NVWA-accredited body. Testing methods must be approved by NVWA or the accredited body.

*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

The material tested for quarantine diseases (in accordance with EU Council Directive 2000/29/EC; see pages 25-26 of: <http://eur-lex.europa.eu/legal-content/NL/TXT/PDF/?uri=CELEX:32000L0029&from=EN>) is multiplied in a greenhouse or in isolation fields in grain.

* Population size
* A population size of 20-25 plants per accession is targeted. This number can be adjusted, in consultation with the CGN, if necessary due to poor germination or low seed quantity.
* 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
* Any dormancy or low germination capacity of the seed is taken into account. Germination advices sent along by the CGN or the propagator's own germination methods are followed.
* If material germinates poorly or very slowly, these findings are recorded and copied into the Multiplication logbook.
* Pollination
* In the case of hand pollination in the greenhouse, a pollen mixture is made for each accession. In the isolation fields, pollination is left to insects. If necessary, additional hand pollination can be decided there if fruiting leaves much to be desired in an isolation field and the plants do flower and produce pollen.
* Harvest
* An overall equal amount of seed is harvested per plant to obtain a representative seed mixture. This is achieved by harvesting the same number of berries per plant. If this is not realistic, this will be passed on to the CGN as feedback.
* If more than 10,000 seeds were produced, a mixing sample of about 10,000 seeds should be returned. If very little seed has been produced, this should also be returned.

Maintaining identity

* 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.

Plant care

* Pruning
* Measures are taken to disinfect hands and pruning knives (such as skim milk method) to prevent virus transmission during pruning. One option to avoid the use of knives is to manually break out the tops.
* Monitoring
* Control against diseases and pests takes place. Diseases or pest problems are recorded and copied in the Multiplication logbook. When in doubt, the CGN is consulted.

**Concluding actions**

* All deviations during cultivation are noted and sent to CGN 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.

**CGN**

CGN then dries and packs the seed in accordance with its internal procedures. The vacuum-sealed and individually bar-coded seed bags (containing the newly multiplied seed, status Q-checked) are always stored in a different place in the -20 storage facilities than unchecked source material (e.g. seed from a collection expedition). Accidental mix-up of these seed batches is not possible. Transfer of pathogens between source material and CGN accessions is also not possible, as the source material is in vacuum-sealed bulk bags, but without a barcode.