##### **PRT-CGN-PG-119 PROTOCOL mulitiplication maize**

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**

Maintiaining genetic integrity

* Isolation
* Maize is a monoecious cross-pollinator. For pollination, the female cobs must be bagged.
* Population size
* Multiplication is on 100 plants per accession. The minimum number of plants is 50 plants.
* It is recorded whether fewer than 100 plants participated in multiplication. These data are recorded in the Multiplication logbook.
* Sowing
* Time of sowing depends on the location of propagation; soil temperature should be >10°C.
* The seed spacing between rows is 75cm, in the row there are 16 plants per 5m.
* Low seed germination 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.
* Vernalization
* Not relevant.
* Cultivation
* If possible, multiplication is done at a location that approximates the original environment of the accessions in question, in terms of temperature and day length.
* No selection takes place. If an accession is more heterogeneous than what is expected based on the passport data, or if it is a mixture of different species or types, the CGN will be notified. The CGN decides whether and how selection may take place. This is recorded and copied into the Multiplication logbook.
* To avoid lodging, less fertiliser is used than in commercial cultivation.
* Pollination
* Cross-pollination using the chain-pollination method is used, using both female and male inflorescences from each plant.
* After pollination, the cobs become sheathed.
* Harvest
* One cob per accession is harvested per plant when the cobs are mature; if the accession is heterogeneous for earliness, harvesting can be done at different times and the harvested seed 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. 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
* The seed can be disinfected against soil fungi.
* Pruning
* Not relevant.
* Inspection
* If infestation by infection is so severe that multiplication is endangered, biological control can be applied or spraying with authorised products can be used.
* Seed treatment after harvest
* The bags containing the cobs are pre-dried, 3-7 days at 25-30˚C.
* 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 to CGN. 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.