##### 

#### PRT-CGN-PG-101B PROTOCOL FOR MULTIPLICATION OF TOMATO

This protocol applies to all parties involved in multiplication of CGN material.

#### Introduction

Multiplications have to fulfill minimum quality requirements to ensure maintenance of genetic identity and integrity (no unwanted mixing among accessions, no genetic narrowing through drift) and a high quality of seed (no diseases, good germination capacity). Contamination through Genetic Modified Organisms (GMO) must be prevented to the best ability.

Any deviation from this protocol must be reported to CGN and will be recorded by CGN in the Multiplication logbook (FOR-CGN-PG-002).

#### Multiplication

##### Maintaining genetic integrity

* Isolation
* All accessions are multiplied in pollinating-insects free greenhouses.
* population size
* Seven plants are multiplied per accession. When taken into account issues of heterogeneity, self-incompatibility, wild material that is hard to multiply, insufficient germplasm or other considerations, ten plants per accession are used. In consultation with CGN, population sizes can be adjusted when modification is considered necessary.
* In planting out, the required number of plants is chosen non-selectively from the population, although 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 included in a multiplication is recorded for every accession. These data are copied into the Multiplication logbook.
* sowing
* The possibility of dormancy or poor germination capacity of the seed is taken into account. Germination advice sent with the seed from CGN or the methods of breeder are followed.
* If material germinates poorly or very slowly, these findings are recorded and copied into the Multiplication logbook.
* pollination
* By trembling inflorescences, self-pollination is increased which results in a better seed-set.
* Cross-pollinating species like *L. peruvianum* require pollination with a pollen mixture.
* harvest
* From each plant approximately the same amount of seeds is harvested, to get a representative seed mixture. This is realized by harvesting the same amount of fruits per plant. If this is not realistic, CGN is consulted.
* If very little seed is produced, it should also be returned.

##### Maintaining identity

* characteristics
* During sowing, cultivation and harvest, accessions must be clearly marked with a label giving the field number. All field numbers assigned before sowing remain unchanged up to and including harvest.

##### Maintaining seed quality

* seed disinfection before sowing
* Seeds need to be disinfected before sowing with trisodium phosphate, according to the protocol send with the seeds. The crop is kept free of aphids with appropriate means to prevent transmission of viruses.
* pruning
* To prevent transfer of viruses, hands and pruning knives are disinfected (for example with the skimmed milk method).
* inspection
* The crop is monitored closely. Close attention should be paid to seed born viruses and viroids. Where applicable the material will be tested according the instructions, mentioned in the actual EU guidelines/regulations. For tomato this means tests will be performed to check the presence of ToBRFV and a visual check will be done on the presence of *Clavibacter michiganensis ssp michiganensis, Xanthomonas euvesicatoria, X. gardeneri, X. perforans, X. vesicatoria*, Pepino mosaic virus and PSTV. Diseases or plagues are combated; this is recorded and copied into the Multiplication logbook. When a disease is identified that might jeopardize proper multiplication of the seed, CGN is notified.
* seed handling after harvest
* In consultation with CGN, a decision is made as to how the seed will be cleaned after harvest.
* Cleaned seed samples must be dried well and stored under circumstances with low temperature and Relative Humidity.

#### Concluding actions

* Any deviations during cultivation or cleaning are noted in the appropriate records, which are sent with the seed. These notes 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.