##### PRT-CGN-PG-106B PROTOCOL FOR THE MULTIPLICATION OF *SPINACIA*

This protocol applies to all parties that regenerate CGN materials. Any deviations should be reported to CGN.

**Introduction**

Regenerations should comply to minimum quality requirements in order to maintain the genetic identity and integrity of accessions (avoidance of unwanted admixture between accessions and genetic erosion by drift), and to guarantee seed quality (absence of diseases and sufficient germination ability). Contamination with genetically modified organisms (GMO) should be avoided as much as possible.

**Regeneration**

maintenance of genetic integrity

* isolation
* Spinach is wind-pollinated and generally dioecious. Therefore, each accession should be regenerated in isolation (sufficient distance between accessions or pollen-proof environments).
* population size
* The number of plants per accession is aimed at 80 plants for cultivated materials and at 120 plants for wild materials. The minimum number per regeneration is 40 plants. In consultation with CGN, population sizes can be adjusted when modification is considered necessary.
* Plants are chosen randomly from an accession when planting material for regeneration. However, plants that fall behind in growth may be discarded if such plants are not expected to contribute to the seed yield of the regenerated accession.
* The number of plants that have contributed to the regeneration of an accession is documented and reported to CGN.
* sowing
* Potential dormancy or low germination ability of the seeds are taken into account. Germination advises provided by CGN or in-house germination methods are followed. Prior to sowing, wild species are pre-treated with low temperature.
* Low or slow germination of material is documented and reported to CGN.
* pollination
* Spinach is wind-pollinated and generally dioecious. Proper distribution of pollen over the female plants should be ensured during the flowering stage of the regeneration.
* harvesting
* Seeds are harvested of at least half the number of plants that were used to start the regeneration (see ‘population size’).
* In case of insufficient seed yield, also these regenerated samples should be send to CGN by third parties.

maintenance of identity

* procedures
* Labels with clear accession identifiers are used during sowing, cultivation and harvesting of the materials. Field numbers should not be changed during the regeneration.
* Off-types are discarded in case one or a few plants are phenotypically strongly different from the other plants of a cultivated accession.
* control
* Materials are regularly checked. Appropriate measures are taken in case of problems with pests and diseases, which are documented and reported to CGN. Serious problems that may compromise the successful regeneration of an accession are communicated with CGN during the regeneration.
* seed treatment after harvesting
* Seed cleaning procedures after harvesting are performed in consultation with CGN.
* When only part of the seed yield is returned to CGN (e.g. in case of very large harvests) a representative sample of the regeneration should be provided.

**Finalization**

* All deviations during cultivation and seed cleaning are documented and send to CGN together with the regenerated seeds.
* Harvested seeds are send to CGN as soon as possible, at the latest 6 months after seed harvesting. Seed bags should be labelled with unique and appropriate accession identifiers, including CGN codes.