##### PRT-CGN-PG-105B PROTOCOL FOR THE MULTIPLICATION OF *LACTUCA*

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
* All autogamous species/accessions are regenerated in a greenhouse without isolation measures. The wild, allogamous species such as *Lactuca perennis*, *L. tatarica*, *L. sibirica* and some accessions of *L. viminea* are regenerated in isolation cages in a greenhouse or in outdoor tunnels. This also applies to species for which the reproductive behaviour is not fully understood, such as *L. orientalis*, *L. undulata* and *Chondrilla juncea*.
* population size
* Eight plants per accession are used to regenerate material that can be expected to be largely homogeneous, such as modern cultivars. To regenerate heterogeneous material, as is often the case for landraces and wild species, 16 plants per accession are used. 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.
* Low or slow germination of material is documented and reported to CGN.
* Wild species are vernalized by cold treatment.
* pollination
* Flies or bumblebees are used to regenerate autogamous species, such as *L. perennis*, *L. tatarica*, *L. sibirica* and some *L. viminea* accessions, and the species that are treated as such, including *L. orientalis*, *L. undulata* and *C. juncea*.
* cultivation
* Plants of heading accessions are treated with gibberellic acid (GA3) or other chemicals to trigger plants to shoot.
* 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.
* Off-types are discarded in case one or a few plants are phenotypically strongly different from the other plants of the accession.
* Perforated plastic bags are used to cover the inflorescences of wild species that easily release seeds. Just before ripening of the seeds, the bags are closed at the top in order to prevent wind dispersal to other accessions.

maintenance of seed quality

* virusfree seeds
* To prevent contamination with the seed-born *Lactuca* Mosaic Virus (LMV), it is recommended to sample all materials 1-3 weeks after planting and to test for LMV by means of ELISA.
* Materials are treated with appropriate means to keep the plants free of aphids, and hence to avoid virus transfer.
* 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.

**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.