##### PRT-CGN-PG-121 PROTOCOL mulitiplication corn lettuce

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
* Multiplication is carried out in a greenhouse. As corn salad and related *Valerianella* species are self-fertilising, no specific protective measures are applied to prevent pollination between accessions. To minimize the risk of contamination, a distance of at least two meters between accessions is maintained from the start of flowering.
* Population size
* A harvest of about 50 grams of seed per regeneration is aimed for. Since a greenhouse regeneration yields about 5 grams of seed per plant, ten plants per accession are multiplied when it concerns material for which homogeneity may be assumed, such as for modern cultivated material. For regenerating material with presumed heterogeneity, as may be the case for landraces and wild species, twenty plants per accession are used. In consultation with the CGN, population sizes may be adjusted if it is considered appropriate.
* When transplanting, the required number of plants is chosen without applying selection in the population. However, plants that lag much behind in growth may be omitted if the lag would result in these plants not eventually contributing to seed multiplication of the accession.
* It is recorded how many plants per accession participated in multiplication. These data are recorded in the Multiplication logbook.
* Sowing
* Any dormancy or low germination capacity of the seed is taken into account. Germination recommendations 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.
* Consideration is given to the possibility that material may be difficult to flower. In such cases, an additional vernalisation treatment is implemented.
* Pollination
* No special facilities.
* Cultivation
* No special facilities.
* Harvest
* Seed is harvested from at least half the number of plants with which seed multiplication was started (see 'population size').
* If multiplication has been outsourced to third parties and very little seed has been produced, this seed should also be returned to the CGN.

Maintaining identity

* Characteristics
* During sowing, cultivation and harvesting, accessions should be clearly marked by labels with the field number. The field number given before sowing remains the same until harvest.
* If one or a few plants within an accession of modern cultivated material differ greatly from the rest, they are removed.

Maintaining seed quality:

* No special facilities regarding seed-borne diseases.
* Inspection
* The crop is closely monitored. Diseases or pest problems are controlled, documented and recorded in the Multiplication logbook. The CGN is contacted when diseases are detected that threaten proper seed multiplication.
* Seed treatment after harvest
* 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. 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.