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Stability in tissue culture and renewal of varieties

Variety renewal in tissue culture—Overview by South Africa *

Submitted by the secretariat

The following document was prepared by the delegation from South Africa. The Specialized Section is invited to discuss the relevance of the protocol and its potential application for other countries.

This document is submitted according to ECE/CTCS/2017/10 section II c, ECE/CTCS/2018/2 section VII a, and A/74/6 (Sect.20), para 20.37; and Supplementary.

* Submitted on the above date for technical reasons.

Potato Certification Service South Africa - Standard operating procedure for the renewal of varieties

1. Background

As potato is a vegetatively propagated crop, it must be maintained vegetatively to have a supply of disease-free material available to the industry. Due to soma clonal variation that may occur, the material cannot be multiplied indefinitely, which makes it necessary to renew the material from time to time. The renewal of clones for mass propagation and seed multiplication is a very important task, which should not be taken lightly as all further propagation of a specific variety is initiated from five tubers. The utmost care is therefore taken to ensure that the selected clones conform to the typical characteristics of the variety to be renewed and that it is disease free.

There are two instances which necessitate the renewal or replacement of a potato variety; firstly, when a variety has been multiplied up to generation 12 in the *in vitro* multiplication process and secondly, when a potato variety shows any defect during the field multiplication.

The preferred method of renewal or replacement of an imported variety is always to import a new clone from the breeder company. However, where renewal of a variety is required, all possible actions must be taken to minimize the risk associated with renewal of clones.

If a defect is detected in a clone, the clone is immediately withdrawn from production and the production continues with the remaining clones. Where a variety has reached generation 12 *in vitro*, Potato Certification Service (PCS) is notified by the Maintenance facility or private company or in collaboration and PCS will then identify a suitable planting to select the new clones from by using the national PCS database. While the selection and initiation takes place, the existing clones can still be used for further multiplication up to generation 18. The clones will only be replaced upon successful completion of the renewal process upon instruction from PCS.

2. Scope

PCS will coordinate a process for the selection of a minimum of five clones for renewal of material in the Genebank. The clones that are selected must conform to the typical characteristics of the variety to be renewed and must be disease free.

3. References

The following documents are used as reference for the SOP for the renewal of varieties:

- Protocol 2013
- The South African Seed Potato Certification Scheme 2013
- SOP for establishment and testing of material as per the approved ICCSP establishment facility.

4. Process for the Renewal of Material

The project for the renewal of material in the Genebank consists of 13 phases which will be overseen by PCS. PCS will be responsible for delegating certain tasks to other stakeholders and provide feedback on the project. The project will only be deemed as completed once 5 clones for the newly selected variety, are released to the Genebank or variety owner for the use by commercial clients for seed potato production.

Stakeholders (person having the interest in the variety or his representative knowledgeable to identify the variety) will accompany PCS in each of the phases. All other relevant parties can be included in the selection and renewal of material and will be assigned certain responsibilities. These parties may include, PCS Technical Manager, PCS Certification

Officials, Variety Owner, Plant Breeder, Genebank Manager, Potatoes South Africa Research Manager, Plantovita Technical Manager and the Grower.

The 13 phases for renewal of material include:

1. Suitable location and eligible planting material
2. First field evaluation and selection
3. Second field evaluation and selection
4. Virus testing of leaves
5. Harvesting of tubers
6. Evaluation of tubers
7. Clone selection
8. In vitro establishment
9. Bacterial testing
10. Virus testing
11. Diffused Light Sprout evaluation
12. DNA-testing
13. Approval and Release of selected clones

4.1 Suitable location and eligible planting material

Making use of the PCS Database, suitable locations with eligible planting material will be identified. It is important to select a location according to its isolation from possible sources of infection with low disease pressure.

Clones will be selected from registered units planted with certified disease-free Generation 1 or Generation 2 seed potatoes, which allows easier phenotypical identification, but which is still early enough to reduce the possibility of infection. Mini tubers / Generation 0-material planted might differ from the typical phenotypic description of the variety, therefore Generation 1 or Generation 2-plantings showing mature characteristics are the material of choice.

4.2 First Field Evaluation and Selection

PCS will accompany Stakeholders to select 20 plants from the variety to be renewed when the potato plants have grown approximately 20 – 30cm high. Plants that represent typical examples, according to the UPOV or variety owner description of the said variety, are selected. These plants are clearly marked, and plants on either side of the selected plants are removed to prevent the tubers of these plants from being mixed with tubers from the selected plants at harvest.

4.3 Second Field Evaluation and Selection

Approximately one month after the first selection, during the flowering stage of the potato plants, a second evaluation will take place. This stage is best for variety identification as the difference in the colour of the flowers makes it easier to identify, however some varieties do not flower.

The morphological characteristics of each plant are evaluated. Characteristics such as the colour of the plants, shape of the leaves, growth of the plants, colour of the stems, etc. are evaluated to ensure that the selected clones represent a typical form of the specific variety.

During this evaluation, the 20 selected plants / clones are closely inspected by PCS and the responsible stakeholders for any infection with viruses, other diseases and deviations. Any off-types or infected plants are removed from the selection.

4.4 Virus testing of leaves

At the end of the normal growing season, leaf samples will be taken individually by a PCS Official from the remainder of the selected clones and tested at Plantovita for the occurrence of Potato Virus Y (PVY) and Potato Leaf roll Virus (PLRV), making use of ELISA as management tool to eliminate any virus infected material prior to initiation.

Composite leaves of each haulm per plant are cut off individually, pooled and packed in a Bioreba extraction bag, clearly marked and placed in the cool bag. Leaves from each plant will be tested individually. Selected clones which tested positive for the occurrence of PVY and/or PLRV will be eliminated. The remainder of the clones will be killed off directly after the leaf samples were taken on the selected plants.

4.5 Harvesting of tubers

After two weeks (\pm 14 days) or as soon as the skins of the tubers have set, all the tubers from each clone are harvested manually by a PCS Official. Each clone's tubers will be placed in a separate polypropylene bag, labelled, sealed and transported to Plantovita.

Plantovita will place the material in a secured Cold Room facility for 2 weeks, maintaining the health and identity of the selected clones.

4.6 Evaluation of harvested tubers

Once the material has arrived at Plantovita, PCS will arrange for a date with the Stakeholders where the tubers of the selected clones are then evaluated based on the UPOV description of the said variety. The clones are then ranked based on the most uniform characteristics of the specific variety which may include number of tubers per plant, tuber size distribution, yield, tuber borne diseases, quality of the tubers, shape, eye depth, colour, internal defects etc.

4.7 Clone selection

All the selected clones are kept at Plantovita, but the five superior clones, based on the most uniform characteristics of each variety, are initiated in tissue culture to replace the previous clones of the variety in the Genebank. Two selected tubers of each clone will be sent to the establishment facility and the sister tubers will be placed in the Light sprout facility.

The establishment facility will keep the selected tubers per clone in a fridge for approximately two weeks, before taking it out for sprouting. One of the selected tubers will be kept as back-up for *in vitro* establishment.

4.8 *In vitro* establishment

The stolon and rose end of the preferred tuber of each selected clone is tested for the presence of the bacterial wilt causing organism, *Ralstonia* spp.

One of two processes could then be followed which includes direct initiation of sprouts, or the initiation of nodal cuttings.

4.8.1 Direct initiation of sprouts

The sprouts of the selected tuber of each selected clone are broken off and initiated *in vitro*, by superficially sterilising the material and placing it on sterile growing medium.

4.8.2 Initiation of nodal cuttings

The eyes of the selected tuber of each selected clone are scooped out and planted in an insect-proof ICCSP approved Greenhouse. Nodal cuttings from these plants are superficially sterilized and initiated into tissue culture for further propagation.

4.9 Bacterial testing

All the *in vitro* established material must be tested for the presence of the bacterial wilt causing organism, *Ralstonia* spp., Soft Rot *Enterobacteriaceae* (SRE) and general microbial contamination. Any clones with infection are discarded and the process is started over with the next suitable clone from the remainder of the selected clones at Plantovita.

4.10 Virus Testing

All the *in vitro* established material must be tested for the presence of virus infection. Potato Leaf Roll Virus (PLRV) and Potato Virus-Y (PVY) must be tested for PCR. All the other viruses, PVX, PVM, PVA, PVS and TSWV will be tested with ELISA. Any clones with infection are discarded and the process is started over with the next suitable clone from the remainder of the selected clones at Plantovita.

4.11 Diffused Light Sprout evaluation

The sister tubers from each selected clone of which the selected tuber was used for initiating *in vitro*, will be used for diffused light sprout descriptions. PCS will place the clearly labelled sister tubers in the Light Sprout Room at Plantovita under diffused lights to allow the sprouts to develop. PCS will be responsible to conduct the Diffused Light Sprout evaluation of the sister tubers in cooperation with the Stakeholders. All clones not conforming to the typical description of the diffused light sprouts of the variety to be renewed will be discarded.

4.12 DNA-testing

DNA tests are performed as a further safety measure to ensure that the material is true to type. DNA-profiles of a number of commercially produced varieties are available. Material from the *in vitro* established clones, with a sister tuber from the each of the clones from the diffused light sprout evaluation, are then compared to the existing profiles or maintenance material (Genebank material) as standard of the said variety. Any clones deviating from the standard are removed from the selected material.

4.13 Approval and Release of selected clones

Only once trueness-to-type is confirmed by the field descriptions, diffused light sprout evaluations, DNA-fingerprinting and tuber evaluations and the clones have passed all the disease tests, can the material be released to the Genebank and be made available to the commercial clients for seed production.

PCS will submit a written report on the selection, renewal and release of the variety for commercial use, upon which the existing clones in the Genebank can be replaced.

5. Acceptance requirements for accessions in the Genebank

Five Selected Clones

Sister tubers for diffused light sprout identification

