

ANNEX 10. Mother Stock Nursery disease indexing procedures

The following procedure describes the mandatory virus indexing activities to be adopted by the Certification Authority to certify planting material as “virus tested”.

This procedure applies for all the planting material eligible for certification. Virus indexing should be performed prior the approval of each new MSN and for the monitoring of the existing ones.

Procedures for sampling and testing of planting material eligible for certification

a) Prerequisites:

- All the planting material should be originated from basic material coming from the Afghan National Collection of Fruit Trees and Nuts.
- All the planting material should be kept and maintained in one of the registered Mother Stock Nurseries.
- In case of new established MSN, The Certification Authority should perform the indexing before the release of the approval certificate.

b) Sampling:

- 1) Trained personnel should perform the sampling in the appropriate period of the year, according to the agro-climatic conditions of the sampling site and taking in consideration the specific characteristics of the different species planted in the MSNs.
- 2) According to the different type of tests to be performed, the samples could be taken from the following sources: leaves, buds, stem, flowers, bark and soil.
- 3) Sampling should be conducted on a random basis, and the number of samples taken from each MSN should be statistically significant in relation to the number of trees planted in the MSNs.
- 4) The operator should spot the sampled trees on the official MSN hard copy map
- 5) Immediately after the removal of the samples, the operator should bundle, label and preserve the material in a portable refrigerator.
- 6) The samples should reach the testing facility in the same day of the sampling, and they should be immediately registered with a unique identification number in the laboratory database and kept in an appropriate preserving environment.
- 7) Testing procedures should start according to the needs of different type of test.

c) Type of tests

The approved indexing methods to screen the MSNs are:

- 1) Viruses
 - a. ELISA (Enzyme-Linked Immunosorbent Assay)
 - b. PCR (Polymerase Chain Reaction)
 - c. RT-PCR (Real-time Polymerase Chain Reaction)
 - d. Biological Indexing
- 2) Bacteria and fungi
 - a. Symptom observation and diagnosis
 - b. In vitro culture

c. Microscopy

3) Nematodes

a. Baermann Techniques

d) Test results

Test result should be filed in the testing facility database. The basic information to be kept are:

- 1) MSNs registration number
- 2) Date of sampling and testing
- 3) Updated MSN map (showing the sampled trees)
- 4) Type of test performed
- 5) Test result
- 6) Recommendations

Test results and recommendations should be officially notify to

- 1) The Certification Authority
- 2) The MAIL
- 3) The NGA chairman
- 4) The MSN owner

e) List of virus to be indexed for certification of plant propagation materials

Test methodology: ELISA, RT-PCR

1) Stone fruits

- ACLSV: *Apple chlorotic leaf spot virus*
- ApMV: *Apple mosaic virus*
- PDV: *Prune dwarf virus*
- PNRSV: *Prunus necrotic ringspot virus*
- PPV: *Plum pox virus*

2) Pome fruits

- ACLSV: *Apple chlorotic leaf spot virus*
- ApMV: *Apple mosaic virus*
- ASGV: *Apple stem grooving virus*
- ASPV: *Apple stem pitting virus*
- ApP: *Apple proliferation phytoplasma*

3) Citrus

- CTV: *Citrus tristeza virus*
- Biological indexing by indicator plants

4) Grape

- ArMV: *Arabidopsis mosaic virus*
- GFkV: *Grapevine fleck virus*
- GFLV: *Grapevine fanleaf virus*
- GLRaV-1: *Grapevine leafroll associated virus 1*
- GLRaV-2: *Grapevine leafroll associated virus 2*
- GLRaV-3: *Grapevine leafroll associated virus 3*
- GVA: *Grapevine virus A*