Certification Operation Directions for Pathogen Tested Seedlings of Passion Fruit

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1. In order to prevent the spread of diseases through the passion fruit seedlings and improve the quality of passion fruit seedlings and their products, the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ), Council of Agriculture, Executive Yuan establishes this operation directions.

2. Definition of the term:
   2.1 Passion fruit: It denotes *Passiflora edulis*, which is a perennial vine plant, belonging to the genus *Passiflora*, family Passifloraceae.
   2.2 Virus: It denotes *East Asian passiflora virus* (EAPV) and *Cucumber mosaic virus* (CMV) that can infect passion fruit.
   2.3. Parent plants (G1): It denotes the plants provide for the production of scion-supply plants (G2)
   2.4. Scion-supply plants (G2): It denotes the plants that provide the scion for the production of grafted seedlings (G3).
   2.5. Grafted seedlings (G3): It denotes the use of scion and root stock to complete the grafting and domestication, and used for cultivation.

3. In order to conduct certification for pathogen tested seedlings of passion fruit, the following agencies are responsible for relevant operations:
   3.1 Receiving agency: Taiwan Seed Improvement and Propagation Station, Council of Agriculture, Executive Yuan is responsible

for the application and certification.

3.2. Inspection agency: The Taichung District Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan is responsible for the inspection of the operation and management of each nursery.

3.3. Verification agency: Taiwan Agricultural Research Institute, Council of Agriculture, Executive Yuan is responsible for virus verification.

4. Those who apply for the certification schemes of parent plants (G1) and scion-supply plants (G2) shall apply to the receiving agency two months before picking the scions.

Those who apply for the verification of grafted seedlings (G3) shall apply to the receiving agency one month before selling.

5. Those who apply for seedling certification at each stage shall fill in the application form, pay the inspection fee, and submit an application to the receiving agency. The receiving agency will notify the applicant of verification result after the applicant has paid the verification fee. Seedlings that meet the certification requirements, a certificate will be issued.

Application for scion-supply plants (G2) and grafted seedlings (G3) shall attach the certificate of previous stage.

6. The setting and operation managements of nurseries at each stage shall meet the following requirements:

6.1. Nursery conditions:

6.1.1. Parent plant (G1): It is cultivated as a single plant in pots or soil, with plants spaced apart and not contact with the

other plant.

6.1.2. Scion-supply plants: Cultivated by single plant or trellis. For plants cultivated by trellis, the trellis shall be separated, and the plants in the trellis which are cultivated by at least 20 cm apart may be sampled for testing.

6.1.3. Grafted seedling (G3): It is cultivated as a single plant with a pot and placed on a rack 30 cm above the ground.

6.1.4. The nursery facility at each stage shall have an insect-proof screen house covered by screens/meshes with diameter of 4.5mm or less, and the entrance/exit for staff must be equipped with double doors. Nurseries for parent plants (G1), scion-supply plants (G2) and grafted seedlings (G3) must be rain-proof.

6.1.5. The nursery of each stage shall be planted alone in a facility, other crop cannot be planted in the same facility. Seedlings of different stages shall be separated by appropriate equipment in the same facility.

6.2. Operation management:

6.2.1. The source of scion of seedlings at each stage must be certified from plants within the valid period of verification.

6.2.2. If the parent plants (G1) or the scion-supply plants (G2) must be replanted, and the scion of the parent plants (G1) within the valid period of verification shall be used for propagation.

6.2.3. A management record shall be installed at each stage of the nursery to establish information such as species, planting date, valid timeline for supplying seedlings or scions, virus verification results, management personnel, facility

maintenance, and pests and diseases control measures, and so forth. These will be presented to the inspector as requested during the on-site inspection.

6.2.4. After the grafting on the rootstock, the grafted seedlings (G3) shall be moved into a rain-proof, insect-proof and weed-proof nursery for healing, and maintained in the isolated screen house.

6.2.5. To facilitate seedling management and process control, seedlings at each stage must be numbered or marked and recorded in the management logbook. The parent plant (G1) is numbered as a single plant while the scion-supply plant (G2) is numbered as a single plant or trellis, and the plants grown in each trellis must be from the same parent plants (G1).

6.2.6. During the verification process, if plants are found to be infected by the *East Asian Passiflora virus* (EAPV) or *Cucumber mosaic virus* (CMV), they shall be removed and destroyed, and the cleaning and control measures of the nursery shall be strengthened. If mutant plants are found during cultivation, they shall be removed immediately.

6.2.7. Tools used in each cultivation management stage shall be sterilized. When pruning, special knives shall be used for each parent plant (G1), scion-supply plant (G2), separate scion-supply plants in the trellis to ensure clean operation.

6.2.8. The entry and exit of staff shall be strictly controlled in the nursery to avoid virus infection caused by contact of human, and vector insect control and nursery cleaning shall be implemented regularly.

6.2.9. Weed management shall be carried out in all stages of the

nursery to avoid the breeding of wild host plants and vector insects as a source of virus transmission.

6.2.10. If the nursery facility is damaged, it shall be repaired immediately.

7. The nursery inspection, sampling methods and virus verification regulations are as follows:

7.1. Testing virus

East Asian passiflora virus (EAPV) and *Cucumber mosaic virus* (CMV) are tested for the passiflora seedlings.

7.2. Verification method

7.2.1. The parent plants (G1) are to be tested by enzyme-linked immunosorbent method (denoted as ELISA) and reverse transcription polymerase chain reaction method (denoted as RT-PCR).

7.2.2. The scion-supply plants (G2) are to be tested by ELISA.

7.2.3. The grafted seedlings (G3) are to be tested by visual inspection or sampling by ELISA.

7.3. Nursery inspection and sampling method

7.3.1. Nursery inspection: Inspectors shall check on-site to confirm that establishment and management conditions of nursery in each stage meet the aforementioned regulations.

7.3.2. Sampling method

7.3.2.1. The parent plant (G1) is sampled in each, and an appropriate of leaf tissue of the parent plant (G1) is cut; it is placed in a sealed plastic bag with the plant number being labelled.

7.3.2.2. Sampling for the scion-supply plants (G2) is conducted in a single plant pot or via trellis. Single plant is sampled in the same way as the parent plants (G1). Scion-supply plants (G2) without spacing on the trellis are sampled and tested for viruses as if they were the same sample. For every 2.5 meters on the trellis, appropriate amount of leaf tissues are collected at each sampling point and all samples are mixed into one sample; then, they are placed into a sealed plastic bag with the number of the trellis being labelled. If there are partitions within the trellis, the samples will be taken on a partition-by-partition basis.

7.3.2.3. If the grafted seedlings (G3) testing is required, an appropriate amount of leaf or stem tissue is taken from the scion randomly and placed into a sealed plastic bag for testing.

8. The standards for seedlings certification at each stage are as follows:

8.1. For the first application for certification of the parent plants (G1), samples will be collected and tested once every three months. In case *East Asian passiflora virus* (EAPV) and *Cucumber mosaic virus* (CMV) were not detected in two consecutive tests are considered as qualified.

8.2. The scion-supply plants (G2) are sampled and tested within two months before picking scions. If no *East Asian passiflora virus* (EAPV) and *Cucumber mosaic virus*, (CMV) is detected, it is considered as qualified.

8.3. Before the grafted seedlings (G3) are sold, they are visually observed by inspectors, and the incidence of symptoms are less

than 0.1%; or one-thousandth of the seedlings are sampled and tested, and no *East Asian passiflora virus* (EAPV) or *Cucumber mosaic virus* (CMV) is detected, it is considered as qualified.

9. The fees shall be charged as follows:

9.1. Inspection fee: Charge for each application is NT$1,000.

9.2. Testing fee: Each sample contains the *East Asian passiflora virus* (EAPV) and *Cucumber mosaic virus* (CMV). For the detection of two viruses, those who adopt ELISA will be charged NT$30 per sample, and those who adopt RT-PCR will be charged NT$300 per sample.

The income and expenditure of various expenses in this Operation Direction shall be handled in accordance with budget procedures.

10. If the seedlings produced in each stage are certified to meet the requirements, a certificate will be issued. The validity period of the parent plants (G1) and the scion-supply plants (G2) are both half a year. The validity period of grafted seedlings (G3) is 30 days.