FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS

INTERNATIONAL BOARD FOR
PLANT GENETIC RESOURCES

FAO/IBPGR TECHNICAL GUIDELINES
FOR THE
SAFE MOVEMENT OF VANILLA GERMPLASM

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INTRODUCTION

Collecting, conservation and utilization of plant genetic resources and their global distribution are essential components of international crop improvement programmes.

Inevitably, the movement of germplasm involves a risk of accidentally introducing plant quarantine pests* along with the host plant material; in particular, pathogens that are often symptomless, such as viruses, pose a special risk. In order to minimize this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of pests that are of quarantine concern.

The ever-increasing volume of germplasm exchanged internationally, coupled with recent rapid advances in biotechnology, has created a pressing need for crop-specific overviews of the existing knowledge in all disciplines relating to the phytosanitary safety of germplasm transfer. This has prompted FAO and IBPGR to launch a collaborative programme for the safe and expeditious movement of germplasm, reflecting the complementarity of their mandates with regard to the safe movement of germplasm. FAO has a long-standing mandate to assist its member governments to strengthen their Plant Quarantine Services, while IBPGR's mandate - *inter alia* - is to further the collecting, conservation and use of the genetic diversity of useful plants for the benefit of people throughout the world.

The aim of the joint FAO/IBPGR programme is to generate a series of crop-specific technical guidelines that provide relevant information on disease indexing and other procedures that will help to ensure phytosanitary safety when germplasm is moved internationally.

In general, the technical guidelines are produced by meetings of panels of experts on the crop concerned, who have been selected in consultation with the relevant specialized institutions and research centres. The experts contribute to the elaboration of the guidelines in their private capacity and do not represent the organizations to which they belong. FAO, IBPGR and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines. By their nature, they reflect the consensus of the crop specialists who attended the meeting, based on the best scientific knowledge available at the time of the meeting.

The present guidelines for vanilla were not developed by a panel meeting; instead,

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* The word 'pest' is used in this document as it is defined in the revised edition of the International Plant Protection Convention. It encompasses all harmful biotic agents ranging from viroids to weeds.

a contract was awarded to a principal coordinating author, Dr M.N. Pearson of the University of Auckland, who collaborated with Dr G.V.H. Jackson and Dr F.W. Zettler. The final editing was carried out by the Joint Programme.

The technical guidelines are written in a short, direct, sometimes 'telegraphic' style, in order to keep the volume of the document to a minimum and to facilitate updating. The guidelines are divided into two parts: The first part makes recommendations on how best to move germplasm of the crop concerned and is divided into general and technical recommendations. Institutions recovering and maintaining healthy vanilla germplasm are listed at the end of this first part. The second part gives descriptions of the most important pests that could be of quarantine concern.

The information given on a particular pest or disease does not pretend to be exhaustive but concentrates on those aspects that are most relevant to quarantine. At the end of each description, a few key references are given, referring mainly to geographical distribution, transmission and indexing methods.

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GENERAL RECOMMENDATIONS

- Material should be collected, processed and shipped with the necessary precautions to avoid accidental movement of pests.

- Under no circumstances should germplasm be moved as rooted plant material, except for *in vitro* plantlets.

- Vanilla germplasm should be moved as seed, *in vitro* pathogen-tested plantlets, or as cuttings re-established from pathogen-tested *in vitro* plantlets grown under containment.

- Only under special circumstances should the movement of untested *in vitro* plantlets or cuttings be considered.

- All germplasm should be collected from healthy-looking plants and, where possible, from areas where pathogens of quarantine concern are not known to occur.

- If it is necessary to import untested germplasm, intermediate and post-entry quarantine should be required, and plants should be tested for the presence of viruses.

- The transfer of germplasm should be carefully planned in conjunction with the relevant plant quarantine authorities. The material should be accompanied by the necessary phytosanitary documents and be in amounts that allow satisfactory examination.

TECHNICAL RECOMMENDATIONS

1. Movement of seed

- Seed of vanilla poses a minimal risk of introducing exotic pests. Consequently, seed can be used to transfer germplasm safely, as none of the pests of quarantine concern are reported to be seed-borne. However, seed is not a usual method of propagation of vanilla and the percentage establishment from seed *in vivo* is extremely low.

- Preferably, seed should be germinated *in vitro* and transferred as sterile plantlets, according to established methods (Arditti, 1982).

- If seeds, other than those germinated *in vitro*, are transferred they should be surface sterilized before shipment by soaking in a saturated calcium hypochlorite solution for 20 min. On arrival in the country of destination they should be inspected under confinement for the presence of insect pests. If found to be infested they should be destroyed.

2 Movement of pathogen-tested *in vitro* cultures

- Stem cuttings should be taken from healthy-looking plants, preferably maintained in a glasshouse or other enclosure, to reduce the microbial contamination present on field-grown plants.

- Sterile cultures should be established from nodal cuttings (Gu *et al.*, 1987). Regeneration of plantlets from aerial root-tip cultures is an alternative method (Philip & Nainar, 1986; 1988).

- Plantlets derived from each explant should be given a separate accession number and grown *in vitro* until they are large enough for indexing according to the procedures recommended in these guidelines.

- All germplasm should be tested for the presence of viruses either in the country of origin, in an intermediate quarantine centre, or under confinement in the recipient country.

- Because some vanilla viruses may occur at low levels in *in vitro* cultures, both the original plantlet and the first generation of subcultures should be tested.

- For the movement of *in vitro* plantlets, neither antibiotics nor charcoal should be added to the culture medium.

- In the recipient country, in vitro plantelets should be examined for microbial contamination, and if this is found to be absent, the plantlets should be grown out and maintained under containment with regular inspection for six months.

3 Movement of cuttings from pathogen-tested *in vitro* cultures

- This method is recommended only in exceptional circumstances where recipient countries are unable to handle *in vitro* material.

- Disease-tested plantlets, produced *in vitro* by the methods described above, should be grown out in an insect-free facility.

- Cuttings from these plants should be washed, surface sterilized in saturated calcium hypochlorite and treated with appropriate insecticides, acaricides and fungicides before despatch.

- In the recipient country, the cuttings should be grown under containment and subjected to regular inspection.

4. Movement of untested vegetative material

- Untested material, either as *in vitro* cultures or as stem cuttings, should only be moved to intermediate or post-entry quarantine facilities where they should be grown as *in vitro* cultures and subjected to the indexing procedures described in these guidelines, before being released. When stem cuttings are moved they must be treated with the appropriate pesticides in the country of origin.

Selected References

Arditti, J. 1982. Orchid seed germination and seedling culture. pp. 358-361. In: *Orchid Biology - Reviews and Perspectives,* Vol. 2. Ed. J. Arditti. Cornell University Press, Ithaca.

Gu, Z., Arditti, J. & Nyman, LT. 1987. *Vanilla planifolia:* callus induction and plantlet production *in vitro: Lindleyana* **2**:48-52.

Philip, V.J. & Nainar, S.A.Z. 1986. Clonal propagation of *Vanilla planifolia* (Salisb.) Ames using tissue culture. *J. Plant Physiol.* **122**:211-215.

Philip, V.J. & Nainar, S.A.Z. 1988. *In vitro* transformation of root meristem to shoot meristem and plantlets in *Vanilla planifolia. Ann. Bot.* **61**:193-199.

INSTITUTIONS RECOVERING AND/OR MAINTAINING PATHOGEN TESTED VANILLA GERMPLASM

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DESCRIPTIONS OF PESTS

Viral diseases

1. Cymbidium mosaic virus

(Potexvirus group)

Symptoms
Plants infected with cymbidium mosaic virus (CyMV) are usually symptomless; occasionally mild mottles or mild chlorotic streaks are observed on leaves of *Vanilla fragrans* and *V. tahitensis.*

Geographical distribution
Worldwide in many orchid species; reported in vanilla only in the South Pacific (Wisler *et al.*, 1987; Pearson & Pone, 1988).

Transmission
The virus is sap-transmissible and is also spread in cuttings used for propagation. No vector is known.

Particle morphology
Flexuous rods, about 480 x 13 nm.

Host range
Systemic infections occur in *Cymbidium* and other genera within the Orchidaceae. Local lesion hosts include *Chenopodium amaranficolor, Datura strramonium, Cassia occidentalis* and *C. obtusifolia.*

Indexing
CyMV is readily detected by ELISA, ISEM and other serological techniques.

References
Francki, R.I.B. 1970. Cymbidium mosaic virus. CMI/AAB Descriptions of plant viruses No. 27. Commonwealth Agricultural Bureaux, Slough.

Pearson, M.N. & Pone, S.P. 1988. Viruses of vanilla in the Kingdom of Tonga. *Australas. Plant Pathol.* **17**:59-60.

Wisler, G.C., Zettler, F.W. & Mu, L. 1987. Virus infections of vanilla and other orchids in French Polynesia. *Plant Dis.* **71**:1125-1129.

2. Odontoglossum ringspot virus

(Tobamovirus group)

Symptoms

Plants infected with odontoglossum ringspot virus (ORSV) are usually symptomless; occassionally mild mottles are observed on the leaves of *Vanilla fragrans* and *V. tahitensis.*

Geographical distribution

Worldwide in many orchid species; reported in vanilla only in the South Pacific (Wisler *et al.*, 1987; Pearson & Pone, 1988).

Transmission

The virus is sap-transmissible and is also spread in cuttings used for propagation. No vector is known.

Particle morphology

Straight rods, about 300 x 18 nm.

Host range

ORSV systemically infects numerous orchid genera and produces local lesions in *Chenopodium quinoa, Gomphrena globosa* and *Nicotiana tabacum* cv. Xanthi-nc.

Indexing

ORSV is readily detected by ELISA and ISEM.

References

Edwardson, J.R. & Zettler, F.W. 1986. Odontoglossum ringspot virus. pp. 233-247 In: *The Plant Viruses. Volume 2.: The Rod-Shaped Viruses.* Eds. M.H.V. Van Regenmortel & H. Fraenkel-Conrat. Plenum Press, New York.

Pearson, M.N. & Pone, S.P. 1988. Viruses of vanilla in the Kingdom of Tonga. *Australas. Plant Pathol.* **17**:59-60.

Wisler, G.C., Zettler, F.W. & Mu, L. 1987. Virus infections of vanilla and other orchids in French Polynesia. *Plant Dis.* **71**:1125-1129.

3. Vanilla Mosaic Virus

(Potyvirus group)

Symptoms

Vanilla mosaic virus (VMV) causes leaf distortion and mosaic in *Vanilla fragrans*, *V. pompona* and *V. tahitensis.*

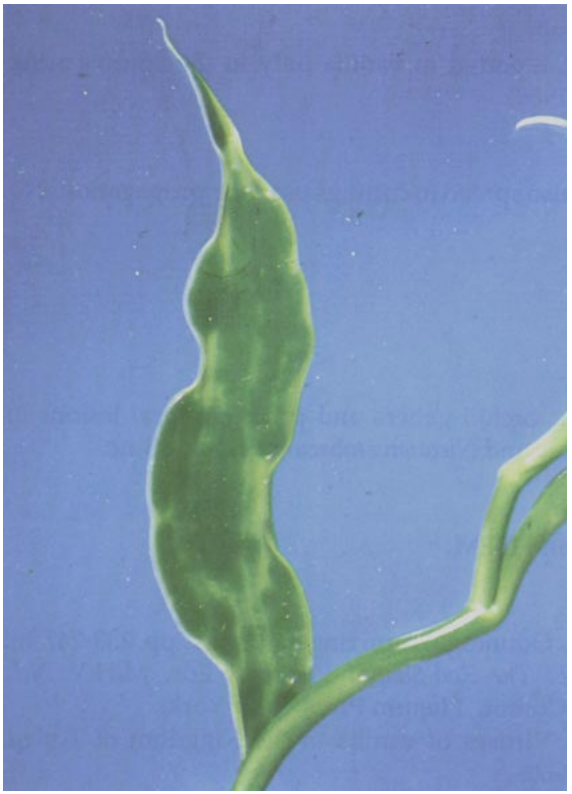

Fig. 1. Symptoms of VMV in *Vanilla tahitensis.* (Prof. F.W. Zettler, University of Florida)

Geographical distribution

French Polynesia (Wisler *et al.*, 1987), Cooks Islands, Fiji and Vanuatu (Pearson, unpubl.).

Transmission

The virus is sap-transmissible and is also spread in cuttings used for propagation. Tests with vanilla have shown that the aphid, *Myzus persicae*, can transmit VMV non-persistently.

Fig. 2. Symptoms of VMV in *Vanilla fragrans.* (Dr M.N. Pearson, University of Auckland)

Particle morphology
Flexuous filaments, about 767 x 11 nm.

Indexing
The virus can be detected by ELISA using a virus-specific polyclonal antiserum or a potyvirus group monoclonal antibody.

References
Wisler, G.C., Zettler, F.W. & Mu, L. 1987. Virus infections of vanilla and other orchids in French Polynesia. *Plant Dis.* **71**:1125-1129.

Zettler, F.W. & Wisler, G.C. 1990. Vanilla mosaic virus. p. 593. In: *Viruses of Tropical Plants*. Eds. A. Brunt, K. Crabtree & A. Gibbs. CAB International, Wallingford.

4. Vanilla necrosis potyvirus

(Potyvirus group)

Symptoms

Plants infected with vanilla necrosis potyvirus (VNPV) show distorted younger leaves with diffuse chlorotic patches, and necrotic lesions on older leaves and on stems, causing defoliation and death.

Fig. 3. Leaf symptoms of VNPV in *Vanilla fragrans.* (Dr. M.N. Pearson, University of Auckland)

Geographical distribution

In *Vanilla fragrans* in Fiji, Tonga and Vanuatu.

Transmission

The virus is sap-transmissible and is also spread in cuttings used for propagation. Tests using the host plant *Nicotiana clevelandii* have shown that the aphids *Myzus persicae* and *Aphis gossypii* can transmit VNPV non-persistently. Transmission to vanilla has not been successful.

Fig. 4. Vine defoliation caused by VNPV in *Vanilla fragrans.* (Dr. M.N. Pearson, University of Auckland)

Particle morphology
Flexuous filaments, about 765 nm long.

Host range
VNPV causes systemic infections in *Vanilla fragrans, Nicotiana benthamiana* and *N. clevelandii* and produces local lesions in *Chenopodium amaranticolor* and *C. quinoa.*

Indexing
The virus can be detected by ELISA using a virus-specific polyclonal antiserum or a potyvirus group monoclonal antibody.

References
Pearson, M.N. & Pane, S.P. 1988. Viruses of vanilla in the Kingdom of Tonga. *Australas. Plant Pathol.* **17**:59-60.

Pearson, M.N., Brunt, A.A. & Pone, S.P. 1990. Some hosts and properties of a potyvirus infecting *Vanilla fragrans* (Orchidaceae) in the Kingdom of Tonga. *J. Phytopathol.* **128**:46-54.

Pearson, M.N. & Pone, S.P. 1990. Vanilla necrosis potyvirus. p. 594. In: *Viruses of Trapical Plants.* Eds. A. Brunt, K. Crabtree & A. Gibbs. CAB International, Wallingford.

5. Uncharacterized potyvirus (es)

Symptoms
Distorted leaf margins, sunken chlorotic patches and mosaic.

Geographical distribution
In *Vanilla fragrans* in Fiji and Vanuatu.

Particle morphology
Flexuous filaments, about 760-800 nm long.

Transmission
Unknown.

Indexing
ELISA using a potyvirus group monoclonal antibody.

6. Uncharacterized rhabodvirus-like particles

Symptoms
Distorted leaf margins, sunken chlorotic patches and spreading necrotic lesions.

Fig. 5. Symptoms associated with rhabdovirus-like particles in *Vanilla fragrans.* (Dr. M.N. Pearson, University of Auckland)

Fig. 6. Symptoms associated with rhabdovirus-like particles in *Vanilla tahitensis.* (Dr. M.N. Pearson, University of Auckland)

Geographical distribution
In *Vanilla fragrans* in Fiji and Vanuatu, and in *V. tahitiensis* in Vanuatu.

Particle morphology
Rhabdovirus-like particles of about 420 x 75 nm.

Transmission
Unknown.

Indexing
Electron microscopy.

Reference
Pearson, M.N. 1990. Virus diseases of vanilla in the South Pacific. Working paper 16. Sixth Regional Technical Meeting on Plant Protection, Auckland, New Zealand, Feb. 1990. South Pacific Commission, Nouméa.

Other pests

Descriptions for other pests are not included here since it is recommended to move vanilla germplasm as *in vitro* cultures or to subject it to tissue culture in intermediate or post-entry quarantine and that the risk of accidentally introducing known, non-viral pests of quarantine importance, together with vanilla germplasm, is avoided by good tissue culture practices.

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IBPGR is an autonomous international scientific organization under the aegis of the Consultative Group on International Agricultural Research (CGIAR). IBPGR was established by CGIAR in 1974. The basic function of IBPGR is to promote and coordinate the collecting, conservation, documentation, evaluation and use of plant genetic resources and thereby contribute to raising the standard of living and welfare of people throughout the world. Financial support for the core programme is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, France, FRG, India, Italy, Japan, the Netherlands, Norway, Spain, Sweden, Switzerland, the UK and the USA, as well as the United Nations Environment Programme and the World Bank.

Citation:

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