◆ EPPO Standards ◆

PHYTOSANITARY PROCEDURES

BARLEY STRIPE MOSAIC HORDEIVIRUS

INSPECTION AND TEST METHODS
FOR BARLEY SEEDS

PM 3/34(1) English

Organisation Européenne et Méditerranéenne pour la Protection des Plantes
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APPROVAL

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard.

REVIEW

EPPO Standards are subject to periodic review and amendment. The next review date for this set of EPPO Standards is decided by the EPPO Working Party on Phytosanitary Regulations.

AMENDMENT RECORD

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

DISTRIBUTION

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

SCOPE

EPPO Phytosanitary Procedures are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the inspection, testing and treatment of plants and plant products moving in trade, or for the implementation of surveys against quarantine pests.

REFERENCES

OEPP/EPPO (1996) Glossary of Phytosanitary Terms. *EPPO Technical Documents* no. 1026.

CABI/EPPO (1997) Quarantine Pests for Europe, 2nd edition (Ed. by Smith, I.M.; McNamara, D.G.; Scott, P.R.; Holderness, M.), CAB International, Wallingford, UK.

OEPP/EPPO (in preparation) Specific Quarantine Requirements. Available as electronic documents from the EPPO Web Site.

DEFINITIONS

Phytosanitary procedure: Any officially prescribed method for performing inspections, tests, surveys or treatments in connection with plant quarantine.

Inspection: Official visual examination of plants, plant products or other regulated articles to determine if pests are present and/or to determine compliance with phytosanitary regulations.

Survey: An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area.

Test: Official examination, other than visual, to determine if pests are present or to identify pests.

Treatment: An officially authorized procedure for the killing, removal or rendering infertile of pests.

OUTLINE OF REQUIREMENTS

EPPO Phytosanitary Procedures describe the methods to be followed for performing inspections, tests, or treatments of commodities moving in trade, or surveys against quarantine pests. For many quarantine pests, a reference to the relevant EPPO Phytosanitary Procedure is made in the corresponding EPPO Specific Quarantine Requirements. The development of EPPO phytosanitary procedures started many years ago, and these methods have been published in the Bulletin OEPP/EPPO Bulletin under several titles: ‘Fumigation standards’, ‘Quarantine Inspection Procedures’ and ‘Quarantine Procedures’. All of them are now appearing under the title ‘EPPO Phytosanitary Procedures’ and are being edited into EPPO Standard format. The numbering of these procedures will continue to follow the sequence described in the Bulletin OEPP/EPPO Bulletin 20(2), 229-233, which corresponds approximately to the chronological order of appearance of the Phytosanitary Procedures.

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

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Phytosanitary procedure

BARLEY STRIPE MOSAIC HORDEIVIRUS
INSPECTION AND TEST METHODS FOR BARLEY SEEDS

Specific scope

This standard describes the inspection and test methods for barley stripe mosaic hordeivirus on barley seeds, to satisfy the requirements of EPPO Standard PM 2/88(1).

Specific approval and amendment

First approved in September 1990.
Edited as EPPO Standard in 1998.

Introduction

Barley stripe mosaic hordeivirus (BSMV) is an A2 quarantine organism and details about its biology, distribution and economic importance can be found in Data sheet no. 88 (OEPP/EPPO, 1983).

According to the EPPO Specific quarantine requirements (OEPP/EPPO, 1990) for this virus, importing countries are recommended to require countries exporting barley seeds from any country where barley stripe mosaic hordeivirus occurs to certify that the seed crop has been inspected during the growing season or that representative samples of seed have been tested by an EPPO-recommended method and found free from the virus.

Methods

Detection of BSMV is not reliably performed by field inspection as the presence and severity of symptoms depend very much on temperature, because even when present the symptoms may not be distinct and finally because latent infection is common.

Seed testing by ELISA (Lister *et al.*, 1981; Huth, 1988) is a much more reliable method and can be completed within 24 h. Details are given in Appendix I.

APPENDIX I

Field inspection

Inspection should take place while the plants are still green and can be done as early as the 2-3 leaf stage (GS 12-13), because plants grown from infected seed may show leaf symptoms. Look for chlorotic or necrotic spots and stripes on leaves (Data sheet no. 88: OEPP/EPPO 1983).

Seed test

Sampling

Testing may be carried out prior to export in connection with the issue of a phytosanitary certificate for a seed-production field. In this case, 10-20 seed lots of 50 g each (one lot comprising about 1000 seeds) are randomly taken from each field for testing by the procedure below. Alternatively, testing may be carried out on a seed consignment at any stage in export and import. In this case, 1 kg seed is taken from every 20 t of each consignment and subdivided into 50 g lots to be tested.

ELISA test

Homogenize the seeds for 1 min in an electric mill (e.g. IKA type A-10-S or M 20) connected with a cooling device. Suspend the seed powder in 10 ml phosphate-buffered NaCl solution (16.0 g NaCl, 0.4 g KH_2PO_4, 2.88 g $Na_2HPO_4 \cdot 2H_2O$, 0.4 g NaN_3, 1 ml Tween 20 in 2 litre H_2O, pH 7.4) and hold for 1 h, allowing the liquid phase to separate from the sediment.

Fill 2 x 200 µl per lot of the supernatant into two wells of micro-ELISA-plates coated with anti-BSMV gamma-globulin. Two further wells per plate are reserved for control supernatant, prepared in the same way from seeds free from BSMV, and two more for buffer only. If a positive control is used (one kernel or small parts of symptom-bearing leaves would be sufficient), the washing between the different steps during the ELISA test should be carried out very carefully to avoid other wells becoming contaminated by BSMV from the positive control.

Measure optical density (OD) at 405 nm using a normal micro-ELISA-reader, after storing the plates for 18-20h at room temperature. For highly infected lots, shorter incubation may be sufficient (e.g. 1 h). Samples are considered positive if the OD value is at least 3 times higher than the value measured for samples of healthy seed lots.

References

Huth, W. (1988) [Use of ELISA to detect barley stripe mosaic virus in barley seed]. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **40**, 128-132 (in German).

Lister, R.M.. Carroll, T.W. & Zaske, S.R. (1981) Sensitive serologic detection of barley stripe mosaic virus in barley seed. *Plant Disease* **65**, 809-814.

OEPP/EPPO (1983) Data sheet on quarantine organisms no. 88: barley stripe mosaic hordeivirus. *Bulletin OEPP/EPPO Bulletin* **13** (1).

OEPP/EPPO (1990) Specific quarantine requirements. *EPPO Technical Documents* no. 1008.

Enquiries

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