EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
Summary sheet of validation data for a diagnostic test

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

Laboratory contact details	Institute for Sustainable Plant Protection via Amendola, 122/D, 70126 Bari, Italy
Short description of the test	Detection of Meloidogyne graminicola by SCAR conventional PCR in rice roots.
Date, reference of the validation report	2017-03-09 - Fanelli et al. 2017 Eur J Plant Pathol 149, 467–476
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	Meloidogyne graminicola (MELGGC)
Detection / identification	detection
Method(s)	Extraction Molecular Extraction DNA RNA Molecular Conventional PCR
Method: Extraction	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Fanelli et al. 2017
Is the test modified compared to the reference test	no
Other information	
Method: Molecular Extraction DNA RNA	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes

As or adapted from an IPPC diagnostic protocol	no
Reference of the test	De Luca et al., 2004 and Fanelli et al., 2017.
Other information	
Other details on the test	Total DNA was extracted from individual IJs or pool of 5-10 IJs as described in De Luca et al., 2004 and Fanelli et al., 2017.
Method: Molecular Conventional PCR	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	no
New test being considered for inclusion in the next version of the EPPO diagnostic protocol?	yes
As or adapted from an IPPC diagnostic protocol	no
Reference of the test	Bellafiore, S., Jougla, C., Chapuis, E., Besnard, G., Suong, M., Vu, P. N., De Waele, D., Gantet, P., & Thi, X. N. (2015). Intraspecific variability of the facultative meiotic parthenogenetic root-knot nematode (Meloidogyne graminicola) from rice fields in Vietnam. Comptes Rendus Biologies, 338, 471–483.
Is the test modified compared to the reference test	yes Lysis buffer and DNA extraction technique as reported in De Luca et al., 2004, PCR kit (GoTaq Flexi Promega) PCR cycling (an initial denaturation at 94◦C for 3 min, followed by 35 cycles: 94◦C for 30 s, annealing at 60◦C for 30 s and extension at 72◦C for 1 min and a final step at 72◦C for 7 min).
Kit	
Is a kit used	no
Other information	
Reaction type	Simplex
Are the performance characteristics included in the EPPO diagnostic protocol?	**no**
Performance Criteria :	
Organism 1.:	**Meloidogyne graminicola(MELGGC)**
Analytical sensitivity	
What is smallest amount of target that can be detected reliably?	One individual infective juvenile (1 J2).
Diagnostic sensitivity	
Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98	100% Italian M. graminicola population 100% for Philippines population
Analytical specificity - inclusivity	
Number of strains/populations of target	Two populations: one from Italy (18 specimens) and

organisms tested	one from Phylippine (13 specimens).
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Non-target organisms tested 4 J2 of Meloidogyne. oryzae, 3J2 of Meloidogyne naasi, 3 J2 of Meloidogyne javanica and 25 J2 of Meloidogyne incognita.
Specificity value	97% Only one cross-reaction occurred in Meloidogyne oryzae (1J2).
Cross reacts with	Meloidogyne oryzae
Reproducibility	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (evaluated with 5 replicates by 1 operator on 5 different days and with 2 different PCR equipment).
Repeatability	
Provide the calculated % of agreement for a given level of the pest (see PM 7/98)	100% (evaluated on 10 replicates).
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	De Luca, F., Fanelli, E., Di Vito, M., Reyes, A., & De Giorgi, C. (2004). Comparison of the sequences of the D3 expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in different populations and species of Pratylenchus from the Mediterranean region. European Journal of Plant Pathology, 110, 949-957. Fanelli, Elena & Cotroneo, Alba & Carisio, Loredana & Troccoli, Alberto & Grosso, Silvio & Boero, Cristina & Capriglia, Francesco & De Luca, Francesca. (2017). Detection and molecular characterization of the rice root-knot nematode Meloidogyne graminicola in Italy. European Journal of Plant Pathology. 10.1007/s10658-017-1196-7. Fanelli, E., Gaffuri, F., Troccoli, A., Sacchi, S., & De Luca, F. (2022). New occurrence of Meloidogyne graminicola (Nematoda: Meloidogyninae) from rice fields in Italy: Variability and phylogenetic relationships. Ecology and Evolution, 12, e9326. https://doi.org/10.1002/ece3.9326

Creation date: 2024-06-18 11:17:01 - Last update: 2024-06-24 18:09:27