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Validation of diagnostic tests to support plant health

Global food security is the most significant challenge mankind is facing in the 21st century due to the 'perfect storm' of a growing population (estimated to exceed 9 billion by 2050), climate change, demand for energy, increased pressure on natural resources, slowing of agricultural productivity growth and decline in the land area for agriculture. Additionally, although it is estimated that a 50% increase in food production will be needed by 2050, currently a quarter of the world's crops are lost to pests, causing major economic losses and social impacts globally. Protecting crops against these losses from farm to fork is critical for achieving sustainable and competitive agriculture as well as for the protection of biodiversity and ecosystems. Establishing smart surveillance mechanisms is essential for the fulfilment of this important goal, as these enable effective monitoring and control of introduction and spread of plant pests.

Early diagnosis and a rapid response are crucial to reduce the risk of entry and spread of plant pests and ultimately their impacts. Furthermore, it is recognized that plant pests can be managed most effectively when control measures are implemented at an early stage of infestation. National Plant Protection Organizations (NPPOs) routinely conduct inspections supported by pest diagnosis for export certification, import, pest surveillance and eradication programs. In 2016, the Commission on Phytosanitary Measures of the International Plant Protection convention adopted a recommendation on diagnostics recognizing that 'pest diagnosis is a cross-cutting issue that underpins most International Plant Protection Convention (IPPC) activities. In order to take action against a pest, it must be accurately identified. To enable safe trade, pest diagnosis must further be completed quickly and to a high level of confidence'.

Validation is essential to provide information on the performance of the tests that are used in diagnostics. However, most detection and identification tests are currently only validated on an intra-laboratory basis or through limited test performance studies (TPS), and there is a need to further harmonize practices and enhance collaboration with kit producers.

What is VALITEST and achievements so far

What is VALITEST?

VALITEST aims at producing validation data for the detection and identification of plant pests and will include two rounds of Test Performance Studies (TPS).

The first round will include combinations of pest/test/matrix, prioritized based on the expertise of the project's consortium for the following pests: *Erwinia amylovora*, *Pantoea stewartii* subsp. *stewartii*, *Citrus tristeza virus*, *Plum pox virus*, *Fusarium circinatum* and *Bursaphelenchus xylophilus*.

The second round will include other combinations based on the needs expressed by various stakeholders. Priorities for validation will then be better aligned to their needs and to the market.

To maximize the impact of the project, calls of interest will be organized to include in the validation programme, kits from suppliers outside the consortium and allow participation in the TPS of interested, proficient laboratories.

Current harmonized procedures in Plant Health for validation and organization of TPS will be improved by including appropriate statistical approaches and by adapting the process for new promising technologies, such as High Throughput Sequencing (HTS) also called Next Generation Sequencing (NGS).

Liaison with regional and international standardization bodies will allow large dissemination of validation data obtained in this project especially by their inclusion in harmonized diagnostic protocols.

The outcomes of the project will stimulate, optimize and strengthen the interactions between stakeholders in Plant Health for better diagnostics and lay the foundations for structuring the quality and the commercial offers for plant health diagnostics tools thanks to a dedicated association and a quality charter.

Activities

Validation of tests for identified needs and specific pests

VALITEST will coordinate (prepare and organize) the tests' validation and running of test performance studies for prioritized pests in a range of matrices and for a range of diagnostic technology related platforms (both laboratory and on site-based). The main objective of this work is to produce validation data for the identified tests where no or limited validation data is currently available.

Improvement of the validation process

VALITEST will improve validation approaches for diagnostic technologies to maximise their usefulness for users (diagnosticians) and decision-makers (at National, European or Regional levels) and their use in routine diagnostics.

In details, the project aims at:

1. Improving the current EPPO Standards for validation of tests for plant pest diagnostics (PM 7/098) and for the performance of interlaboratory comparisons (PM 7/122) by incorporating new statistical tools and predictive models;
2. Developing best practice guidelines;
3. Improving generic approaches for the validation and developing best practice guidelines for the validation and application of non-targeted (generic) diagnostic procedures, using next generation sequencing procedures (NGS) for viruses detection, in plant pest diagnostics as a model.

Quality assurance of reference materials for validation purposes

VALITEST will establish and evaluate guidelines for quality assurance and Standard Operating Procedures (SOPs) for the production of the different types of reference materials used in validation studies for phytosanitary tests including possible quantification of targets in reference material.

Analysis of demand for testing and impacts

This activity relates to a better understanding of the demands for current and future testing options:

1. To support plant health policies by engaging with stakeholders to ascertain views on and demand for existing tests and operating procedures as well the attributes that lead to adoption for future tools.
2. Assess the end markets for tests including their potential market (e.g. reduction in yield losses) and non-market (e.g. reductions in woodland losses) impacts.

This engagement with stakeholders incorporates elements of the multi-actor approach in considering the demand for and benefits from the validation of existing tests but also with the examination of their requirements for future tests. In addition to the validation of the tests, this approach introduces an element of co-design with end-users that can inform the design of tests and procedures and prioritisation of targets that could assist in bringing such products to the market more rapidly.

Optimization of proficiency evaluation for a horizontal assessment

VALITEST first of all aims at validating diagnostic tests available for a selection of relevant plant pests. The goal of using validated tests is to ensure the quality of the results based on which control decisions will be taken. However, in the case of tests performed in laboratories, the targeted level of performance of a validated test is only ensured if it is performed by a laboratory regularly showing its proficiency through proficiency tests which cannot be organized for all the diagnostic tests on the market. VALITEST will develop guidelines following a horizontal approach allowing proficiency testing to be undertaken without the laboratories having to participate in proficiency tests for all the tests used. The outcome of this activity will represent an option for end users of validated tests, but also possible new services for SME.

Dissemination, communication and training

VALITEST will:

1. Disseminate the validation data generated during the project and gather additional validation data available in plant pest diagnostic laboratories and make these data publicly (and freely) available.
2. Disseminate the results of the project to a wide public including researchers, policy makers and other stakeholders, via different meetings organized at EPPO and EU levels, webinars and through a project website.
3. Ensure future harmonization of validation processes across the EU region by building capacities on validation processes.
4. Build capacities of diagnostics laboratories to perform tests validated in the project by targeted training.

Market exploitation of the project results

One of the project's main aims is to swiftly bring onto the market tests validated according to international standards and produced by the SMEs manufacturing diagnostic kits.

To achieve this goal, the project's work will be made widely known in order to commercially exploit the results from the project, to ensure market sustainability and to enhance competitiveness of the SMEs internationally.

In this activity, the establishment of an EU Association of the Plant Health Diagnostic Industry will ensure the market sustainability of the SMEs by facilitating dialogue with decision makers. The development of an EU Plant Health Diagnostics Charter will permit SMEs to increase their competitiveness and will contribute to the quality and reliability of their product worldwide.

Some achievements on 2020-07-22

WP1: Validation of tests for identified needs and specific pests

Two rounds of tests performance studies (TPS) have been organized. Different partners were involved in the organization of the TPS which includes the selection of the tests to validate and of the laboratories that can participate to the TPS, the preparation of the validation plan, the preliminary assessment of the tests, the preparation of the material, the dispatch of the samples, the completion of the TPS and the exploitation of the results. Harmonization was ensured using common criteria for the selection of tests and laboratories, shared templates and procedures defined in conjunction with other work-packages.

The first round of TPS organized in 2019 targeted six pests identified as important by the consortium before the start of the project.

Pest	TPS organizer	Tests	Number of participants	Number of samples prepared
<i>Erwinia amylovora</i>	NIB	6 tests (real-time PCR, LFDs and LAMP)	32 (from 20 countries)	~900
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	NIB	6 tests (real-time PCR, PCR)	23 (from 16 countries)	~450
<i>Citrus tristeza virus</i>	ANSES	11 tests (ELISA, TPIA, Conventional RT-PCR, Real-time RT-PCR, RT-LAMP and ImmunoStrip)	17 (from 11 countries)	~1650
<i>Bursaphelenchus xylophilus</i>	ANSES	5 tests (conventional PCR, real-time PCR, LAMP)	21 (from 18 countries)	~430 DNA extracts ~280 spiked wood extracts
<i>Plum pox virus</i>	NVWA	8 tests selected (RT-PCR, real-time RT-PCR, DAS-ELISA)	17 (from 12 countries)	~700
<i>Fusarium circinatum</i>	FERA	6 tests (plating, PCR, real-time PCR)	20 (from 15 countries)	~640

For the second round of TPS organized in 2020, the pests were selected based on the need of stakeholders identified in conjunction with WP4 using surveys.

Pest	TPS organizer	Tests	Number of participants	Number of samples prepared
<i>Tomato spotted wilt tospovirus</i>	NIB	8 tests (DAS-ELISA, on-site tests, conventional and real-time RT-PCR)	21 (from 12 countries)	~1540
<i>Xylophilus ampelinus</i>	FERA	9 tests (ELISA, IF, conventional and real-time RT-PCR)	12 (from 11 countries)	~570
<i>Cryphonectria parasitica</i>	UNITO	3 tests (conventional and real-time PCR)	11 (from 8 countries)	~220
<i>Plum pox virus</i>	ANSES	3 tests (LFD RPA, LFD)	15 (from 12 countries)	~640
<i>Tomato brown rugose fruit virus</i>	CREA	5 tests (conventional and real-time RT-PCR)	34 (from 18 countries)	~850
<i>Xanthomonas citri</i> pv. <i>citri</i>	ANSES	13 tests (conventional and real-time PCR, LAMP and direct molecular tests performed from ImmunoStrips or Whatman™ FTA cards)	19 (from 14 countries)	~960

The validation data produced are being used to revise specific EPPO diagnostic protocols and will be made in the renewed version of the EPPO database on diagnostic expertise.

WP2: Improvement of the validation process

Statistical methods that could be used to improve the reporting of test performance criteria have been identified and evaluated by WP1 and WP2 partners using the results of the first round of TPS. Those methods will be further evaluated by EPPO diagnostic Panels before being proposed for possible inclusion in the EPPO PM 7/98 and PM 7/122.

A draft version of the guidelines for the selection, development, validation and routine use of High Throughput Sequencing (HTS) in plant health diagnostic laboratories has been produced by WP2 with the support of several experts of the consortium. The draft guidelines were sent to 36 reviewers from 18 different countries in June 2020.

WP3: Quality assurance for reference materials for validation purposes

A list of general minimum criteria for the production of reference materials (RMs) to be used in interlaboratory studies (including validations through test performance studies (TPS)) was developed as well as a general standard operating procedure (SOP) for the production of reference material (RM) for use in plant health diagnostics. These documents were used to prepare the reference materials for the second round of TPS and will be used to draft an EPPO diagnostic standard on the production of reference materials.

WP4: Analysis of demand for testing and impacts

A general prioritisation framework was created in collaboration with different work-packages to identify stakeholder priorities in term of pests for which validation data is needed. The results were used to select the pests for the second round of TPS.

WP5: Optimisation of proficiency evaluation for a horizontal assessment

A range of data has been collected using three different approaches, i) the study of accreditation scopes of some laboratories involved in diagnostics, ii) a workshop with diagnostic experts, iii) a survey sent to laboratories listed in the EPPO database on diagnostic expertise. The data collected allow a better understanding of the expectations of laboratories, of what they would consider as acceptable and of the applicability of the horizontal proficiency testing approach.

The results are being used to develop guidelines on a horizontal approach allowing the laboratories to undertake proficiency testing (PT) without having to participate in proficiency tests for all the tests they use.

WP6: Dissemination, communication and training

The training and dissemination plan presenting the objectives of the training and dissemination activities and the tools provided to ensure that the objectives will be met has been established.

The EPPO Database on Diagnostic expertise (<https://dc.eppo.int>) includes a specific section on validation data for diagnostic tests. Laboratories can deposit online validation data that they have generated on specific tests and these can be made visible to all users of the database. One of the objectives of VALITEST was to improve the searching capacity of the database to ensure optimal use. To evaluate the needs of users, a survey was organized. Further needs were identified during meetings with users. The new redesigned and more user-friendly database was launched in May 2020.

Regional training workshops on the concept of validation for plant health diagnostic laboratories will be organized in 2021 (see 1st announcement here: <https://www.surveymonkey.com/r/JVDG9MV>).

WP7: Market exploitation of the project results

The key exploitable results and exploitation routes have been identified for several work packages (WP1, WP5 and WP6). For those work packages, the results of the project could be used for further research activities, for creating and providing services and in standardisation activities.

Besides two general exploitation guidelines (one for industrial partners and one for research institution partners) have been developed to help partners reach the expected exploitation of the results.

Keywords

Biochemistry and molecular biology, Quality management, Plant pests, Diagnostic technology, Certification, Verification, Validation, Technical Compliance, Standards, Plant health, Pest, Diagnostic, Detection, Identification, Test Performance Study, Validated protocols, Next Generation Sequencing, Training, Reference material, Proficiency, High Throughput Sequencing.

The Consortium

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2. University of Turin, Centre of Competence for Innovation in the agro-environmental field (AGROINNOVA), Italy
3. Federal Department of Economic Affairs, Education and Research EAER – Agroscope, Switzerland
4. Bioreba AG, Switzerland
5. Loewe Biochemica GmbH, Germany
6. European And Mediterranean Plant Protection Organization, (International Organization)
7. Fera Science Limited, United Kingdom
8. National Institute of Biology, Slovenia
9. Gembloux Agro-Bio Tech - University of Liège, Belgium
10. Netherlands Food and Consumer Product Safety Authority, Netherlands
11. ClearDetections B.V., Netherlands
12. Stichting Wageningen Research, Netherlands
13. International Plant Analysis and Diagnostics SRL (Ipadlab), Italy
14. Sediag, France
15. Council for Agricultural Research and Economics (CREA), Italy
16. The Main Inspectorate of Plant Health and Seed Inspection (PIORIN), Poland

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<https://www.valitest.eu/>

<https://twitter.com/ValitestProject>

<https://www.flickr.com/people/154720643@N08/groups/>

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