1. Content of the 'Topic Description' document

1.1. Topic area

Diagnostics, field detection, surveillance.

1.2. Topic title

Development of molecular test for simultaneous detection of potato viruses Y, M, L, S, X, A and *Potato tuber spindle viroid* (PSTVd) in potato seed tubers.

1.3. Description of the problem the research should solve

Due to the vegetative propagation, the potato crop is vulnerable to many viruses and a quarantine pathogen, the potato spindle tuber viroid (PSTVd). The most important viral diseases are caused by: *Potato virus Y* (PVY), *Potato leafroll virus* (PLRV), followed by *Potato virus A* (PVA) and *Potato virus X* (PVX), and to lesser extent by *Potato virus M* (PVM) and *Potato virus S* (PVS). The pathogens may cause no or mild symptoms, amplify through several generations of seed tubers and produce significant yield loss. Therefore, the effective control requires sensitive and reliable detection methods. Many diagnostic protocols using polymerase chain reaction such as reverse transcription PCR (RT-PCR) and real-time reverse transcription PCR (real-time RT-PCR) are currently available for uniplex or multiplex detection, but none facilitates simultaneous detection of PVA, M, S, X, Y, PLRV and PSTVd. Moreover, contradictory results of practical applications of RT-PCR and real-time RT-PCR for virus detection in dormant tubers over grow-out test have been reported. This results from low virus concentration in dormant tubers and from difficulties in preparing good quality RNA from tuber, which is mainly composed of starch, and also contains polyphenols and redox enzymes. The isothermal methods of amplification of nucleic acids are less sensitive to a number of impurities having an inhibitory effect on the PCR, thus may offer promising alternative for direct tuber testing. This project aims to develop a multiplex isothermal assay based on loop-mediated amplification of nucleic acids (LAMP), helicase-dependent amplification (HDA) or recombinase polymerase amplification (RPA) with multiplex real-time RT-PCR as a reference method. Since there is no amplifying equipment with 7-detection chanels, each RNA sample will be amplified in two separate reactions (one triplex and one quadruplex). The most promising procedure will be optimized for efficient detection of investigated pathogens in extracts from potato tubers.

1.4. Description of the expected results

The project is expected to result in optimized and EU-validated molecular tests for the simultaneous detection of potato viruses A, M, S, X, Y, PLRV and PSTVd. Existing real-time RT-PCR tests will be modified to develop an assay for multiplex detection of investigated pathogens. The multiplex isothermal test (LAMP, RPA, HDA) will be developed for investigated pathogens. The efficacy of viruses and viroids detection of developed tests will be compared.

1.5. Beneficiaries of this research product

Scientists will benefit from optimised protocols for the simultaneous detection of PVA, M, S, X, Y, PLRV and PSTVd by isothermal (RT-LAMP, RT-HDA, RT-RPA) and real-time RT-PCR tests.
NPPOs may adopt developed protocols into their policies of potato certification.
Farmers and companies will have an option to simplify and shorten the procedure of potato seed tubers certification.

1.6. Research funders and research contribution/ distribution

Funding organisation	Research activity and researchers involved
1. Plant Breeding and Acclimatization Institute – National Research Institute, Poland Krzysztof Treder k.treder@ihar.edu.pl Janina Butrymowicz j.butrymowicz@piorin.gov.pl	-Project coordination. -Adapt and optimize existing real-time RT-PCR assays to develop multiplex assay for investigated pathogens. -Design primers for multiplex isothermal assay (RT-LAMP, RT-HDA, RT-RPA). -Select kit for reliable purification of RNA from potato tubers. -Compare efficacy of pathogen detection by developed assays. Contact person: Krzysztof Treder E.mail address: k.treder@ihar.edu.pl
2. Ministry of Rural Affairs, Estonia Sirli Pehme sirli.pehme@agri.ee	-Participation in ring testing and validation of Real-Time PCR multiplex method. We don´t use LAMP technique in our laboratory, but as this is easier than PCR and there is no need of special equipment, LAMP could be also a possible method to test. Contact person: Helena Lasner E.mail address: Helena.Lasner@pmk.agri.ee -Providing plant materials (plant leaves, tubers from storage, in vitro propagated microtubers) necessary to develop reliable nucleic acid (RNA) extraction procedures to determine the optimum method for a particular sample type, partial virus and viroid purification prior to RNA isolation, and assist with data analysis. Contact person: Andres Mäe E.mail address: andres.mae@etki.ee
3. The State Plant Service under the Ministry of Agriculture, Lithuania Arunas Beniusis arunas.beniusis@vatzum.lt Silvija Pupeliene silvija.pupeliene@vatzum.lt	-Adapt and optimize existing real-time RT-PCR tests to develop multiplex test for investigated pathogens. -Select kit for reliable purification of RNA from potato tubers. -Compare efficacy of pathogen detection by developed tests. Contact person: Arunas Beniusis E.mail address: arunas.beniusis@vatzum.lt Contact person: Silvija Pupeliene E.mail address: silvija.pupeliene@vatzum.lt
4. The Norwegian Institute of Bioeconomy Research, Norway	-Contribution to be detailed.

Hanne Skomedal hanne.skomedal@nibio.no	Contact person: Carl Jonas Jorge Spetz E.mail address: carl.spetz@nibio.no
5. All-Russian Plant Quarantine Centre, Russia Natalia Sherokolava natalia_sh@mail.ru	Contribution to be detailed. Contact person: Yuri Shneyder E.mail address: yury.shneyder@mail.ru
6. Fédération Nationale des Producteurs de Plants de Pommes de Terre, France Yves Le Hingrat yves.lehingrat@fnpppt.fr	-Contribution to be detailed. Contact person: Laurent Glais E.mail address: laurent.glais@fnpppt.fr

1.7. Research project partnership outside Euphresco

Euphresco funding ensures a certain level of transnational collaboration among Euphresco member countries. It is possible, if the funding consortium is interested, to contact funding organisations or research groups outside the geographical area covered by Euphresco members. The Euphresco coordinator could advertise the research topic in order to have an enlarged collaboration. If funders are interested in this possibility, please check the case below:

☐The funding consortium of the topic mentioned in section 1.2 requires to advertise the topic outside the Euphresco network

1.8. Any other relevant information on content

2. *Euphresco management aspects of the project*

2.1. Indication of the topic budget

Funding organisation[a]	Mechanism [b]	Total Budget [c]
1. IHAR (PL)	VP	€ 735 981
2. MEM (EE)	NC	€ 120 000
3. VATZUM (LT)	NC	€ 20 000
4. NIBIO (NO)	NC	€ 10 000
5. VNIIKR (RU)	NC	€ 10 000
6. FN3PT (FR)	NC	€ tbc
total		€

2.2. Expected duration of the project

36 months.

2.3. Identification of project coordinator

Has the research project coordinator been identified?
☒Yes
☐No

2.4. Any other relevant information on topic organisation and management

[a] First member is project coordinator. A minimum of two partners are necessary for each proposal. Add lines as needed.
[b] Please indicate the preferred mechanism (e.g. real pot RP; virtual pot VP; non-competitive NC), or several mechanisms if there is flexibility.
[c]Optional, as this amount can still change in the next phase. In-kind contribution should also be indicated in this column.