EUPHRESCO Programme VIRUSCOLLECT

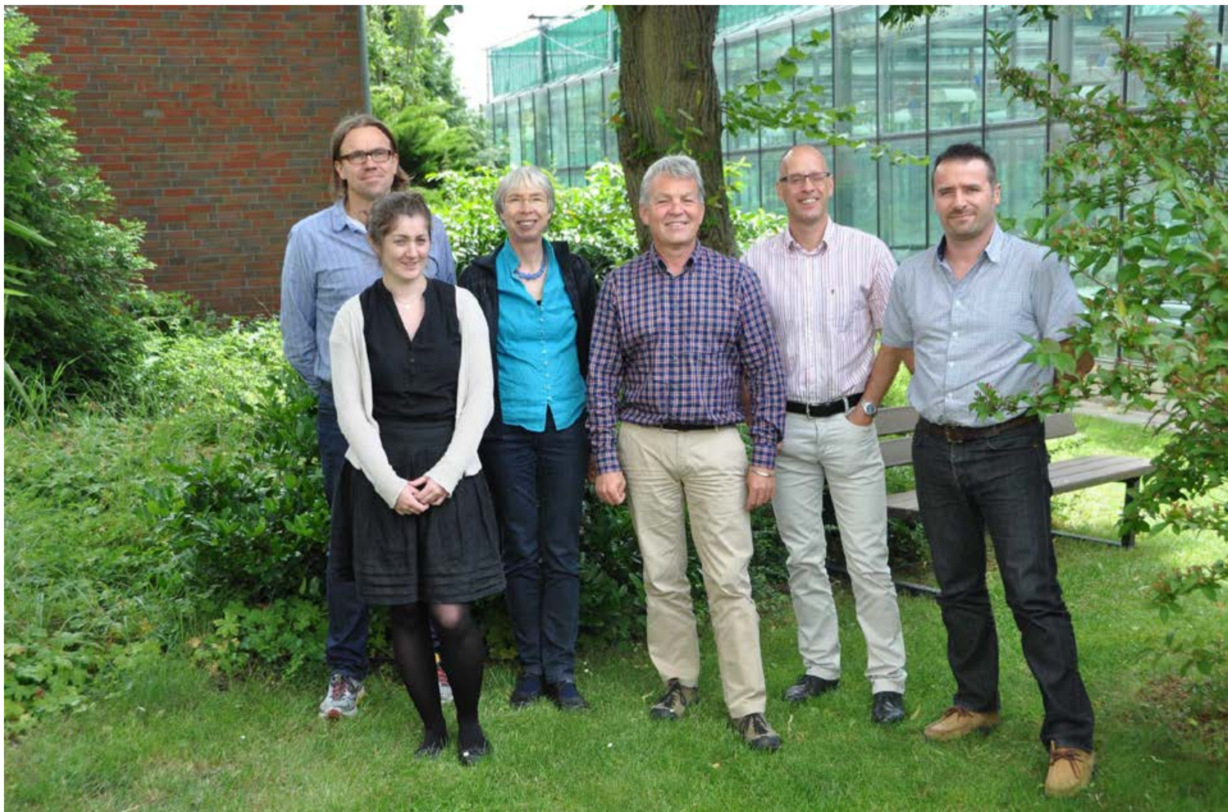
Programme Title Reference collection for regulated viruses and viroids

1st YEAR REPORT

Summary of the 1st year meeting at DSMZ, Braunschweig, 24-25 June 2014

General

The 1st year meeting of the EUPHRESCO project VIRUSCOLLECT was combined with a meeting of the curators of the Q-bank Plant Viruses and Viroids Database. This offered the opportunity to discuss the results and future plans on physical collections in relation to their accessibility. All EUPHRESCO partners* presented the results of their work performed over the last year (WP's 4 to 7). Agreements were made on the choice of isolates to be included in Q-bank. Practical issues on data transfer were addressed, especially for those partners not involved in Q-bank (WP 8). Furthermore, fundamental issues related to the quality and maintenance of a virus collection were discussed (WP's 2 and 3). Finally, DSMZ offered a tour around their facilities and presented on the ISO 17025 and ISO 34 accreditation of the DSMZ Virus collection.



* Particpants: Christophe Lacomme (SASA, UK), Wulf Menzel (DSMZ, DE), Annelien Roenhorst (NPPO, NL), René van der Vlugt (PRI, NL) and Stephan Winter (DSMZ, DE). Unfortunately Thomas Leichtfried (AGES, AT) was not able to join the meeting. Laura Flint (FERA, UK) participated because of her involvement in Q-bank.

The following paragraphs provide the highlights of the discussions and agreements of the meeting.

Collection versus reference material
Before starting the discussion on quality criteria, the term collection should be defined more explicitly. Two terms are relevant, i.e. collection material and reference material. A collection includes virus isolates that fulfil the criteria of authenticity (isolates true to type), purity, viability and preservation. Reference material is a product derived from a collection and related to its use in a diagnostic test. As such reference materials do not have to fulfil the criteria of a collection and may consist of different derivatives of the collection material, e.g. non-infectious virus for ELISA, nucleic acid extract or plasmid for PCR.

Purpose of a virus collection
The main purposes of a virus collection are: 1) preservation of particular virus isolates and 2) supply of reference materials for diagnostic purposes. The VIRUSCOLLECT consortium focuses on preservation of isolates fulfilling the criteria, but at the same time produce and offer reference materials for diagnostic purposes. Test laboratories as end users might often be only interested in reference material for diagnostics.

Quality of a virus collection
Ideally a virus collection meets the criteria of authenticity (isolates true to type), purity, viability and preservation. At the same time, the collection has to be kept up to date. This puts high demands on the collection and curation of the isolates. How to select isolates? How to preserve a virus isolate true to type? How to define a particular strain? Moreover, how to keep virus species that cannot be transmitted mechanically or preserved outside their original host. Often only limited amounts of these isolates can be preserved as reference material, but not as collection material.

Conduct codes
Depositors of isolates agree that their isolates will become public available in due time. Purchasers of collection material are fully responsible for its use, either for beneficial or misuse. Curators of public collections should be able to exchange material on the basis of free interchange.

Future collection and accessibility of regulated viruses
In Europe, virus collections are fragmented, often their content is unknown and especially regulated and emerging species are hardly available. The VIRUSCOLLECT consortium aims to characterise relevant isolates and makes them accessible via Q-bank. Since progress in this way is only limited, an alternative strategy has been agreed. Following this alternative approach Q-bank will serve as a central database that provides actual information on especially regulated viruses. On one hand it should list all names of regulated species, on the other hand it should indicate if and where isolates of these species are available. In this way Q-bank offers additional features over other virus databases, i.e. it provides a complete overview of all regulated virus species and at the same time shows if and where they are available. Moreover, it will clearly show which species are missing, and efforts can be initiated to fill these gaps. As a result the Q-bank will no longer show 'complete' data sets for a limited number of species, but limited data for all virus species. However, the key feature remains, i.e. that all data included are checked by the curators.

Q-bank as central database
At the short term, the Q-bank curators will include the names of all plant virus species as well as their quarantine status in the database. Curators will continue on filling the gaps, both on the availability and characterisation of virus isolates. In the mean time, they will include the data generated during the VIRUSCOLLECT project.

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Workpackage 1

Coordination of the VIRUSCOLLECT project

Introduction
Many organisations working in the phytosanitary field are involved in characterisation of new and emerging viruses and the development and validation of diagnostic tests. The fact that the database of Q-bank came available as a tool to share data and information on plant viruses and viroids, offers a chance to profit from data and collections available at other organisations.

Aim
To stimulate the exchange of data and isolates of viruses and viroids of concern in the phytosanitary field via Q-bank database and collections.

Materials & methods
- Coordinate project and topic descriptions, division of work, planning and reporting
- Organisation of a meeting after one year (if budgets available)
- Description and planning of work for the second year (if not decided)

Results
The project description has been finalised in October 2013. On 24-25 June 2014, the first year meeting took place at DSMZ in Braunschweig. This meeting was combined with a meeting of the curators of the Q-bank Plant Viruses and Viroids Database. This offered the opportunity to discuss the results and future plans on physical collections in relation to their accessibility. Details are covered in the different workpackages.

Future plans (2nd year)
Each partner will continue the characterisation of their virus isolates of interest and will provide data for inclusion in Q-bank. In addition, it has been decided to aim a more prominent role of Q-bank Plant Viruses and Viroids Database in the exchange of information and data on (regulated and emerging) virus and viroid species. Q-bank should serve as a platform to provide information on regulated viruses and viroids for researchers, diagnosticians and policy makers. Its unique features including actual information on the quarantine status of virus and viroid species and the availability of isolates. The partners of this EUPHRESCO project will work closely together with the curators of Q-bank Plant Viruses and Viroids Database to achieve this goal in the next year.

Workpackage 2

Identification of gaps in Q-bank with regard to regulated and emerging viruses and viroids

Introduction
To improve Q-bank gaps in its present content should be identified, so that efforts can be made to trace and collect (missing) species relevant to plant health.

Aim
Extend Q-bank by including virus and viroid species relevant to plant health.

Materials & methods
- Make an inventory of (regulated) species available via public accessible collections.
- Select relevant species that are missing and are suitable for inclusion in the collection
- Trace these isolates and try to purchase them for inclusion in the collection (via other workpackages)

Results
During the first year it appeared that the efforts mainly concerned virus species of interest for the participating institutes and countries. Inventories did not yield the desired overviews of the availability of virus and viroid species of interest. Therefore, a new strategy has been proposed for the 2nd year.

Future plans (2nd year)
To improve the availability of (regulated and emerging) quarantine viruses and viroids, the Q-bank Plant Viruses and Viroids Database should serve as the central platform for exchange of information and data. Starting with the inclusion of all relevant species, it should provide comprehensive and actual information on the quarantine status and availability of isolates. This will easily and clearly show which species are not available yet, and therefore offers targets for further development. As a consequence of this strategy, it will be unavoidable that data on individual isolates might be incomplete. This should be clearly stated. However, by showing the lack of data, it also indicates the direction of future work and needs.

Workpackage 3

Description of quality standards for Q-bank accessions and their maintenance and handling in contributing collections

Introduction
A growing demand of diagnostic laboratories, scientists or breeders for well characterized reference isolates can be observed. The characterization and production of reference materials is a key activity of collections. Common quality standards are required to ensure the quality and allow comparability across borders. The availability of standardized reference isolates will allow the end-user to obtain reliable and reproducible results at any time.

Aim
The aim of this work package is to generate a workflow scheme for the development of virus accessions which will later be made available via Q-bank. This workflow will help to identify and define the required quality standards for the characterization, maintenance and distribution of virus isolates. The developed standards will support best practice in the production process in collections and will also include the documentation and traceability. Such defined Q-bank standards will improve the transparency and will facilitate a better worldwide acceptance of the virus isolates provided by the contributing collections.

Material and methods
If applicable and reasonable, the description of quality standards for Q-bank virus isolates is based on internationally recognized and accepted ISO norms/guides (e.g. ISO/IEC 17025:2005, ISO guide 34:2009) or EPPO standards (e.g. PM 7/98), which will be specifically adapted to plant viruses. Although it is clear that not all requirements can be fully implemented in non-accredited institutions, they provide a valuable guideline to derive common quality standards for all reference collections contributing to Q-bank.

Results
A table was generated, listing the most important quality related requirements and factors. The requirements derived from the two internationally recognised ISO standards ISO/IEC 17025:2005 (General requirements for the competence of testing and calibration laboratories) and ISO guide 34:2009 (General requirements for the competence of reference material producers), were adapted to plant viruses and were grouped in the following sections:

- General quality management
- Characterisation and Identification
- Reference material production
- Quality control
- Order processing and shipping

This Quality standards for Q-bank collections of viruses and viroids (Annex 2) was distributed to the different participants of the project in February 2014. The participants commented on this first draft and it was further discussed at the 1st year meeting in Braunschweig in June 2014. The participants agreed to use this 'global standard' as working basis for the 2nd year of the project.

Future plans (2nd year)
The quality standards as described in Annex 1 will be used and evaluated by each laboratory. The experiences will be discussed during the 2nd year meeting, and included in the final version. This standard should define the scientific and technical competence of the reference material producers, and to guarantee the quality and assigned property values of the materials offered at Q-bank. If successfully implemented, end-users of the materials and information provided at Q-bank will use Q-bank with confidence.

Workpackage 4

Characterization of various viruses of stone and pome fruits, cereals and maize

Introduction
Viruses are very important pathogens. For the diagnostic of viruses with molecular tools it is necessary to have a variety of isolates. With different isolates it is possible to improve the quality of the molecular detection. So a Reference Collection of various isolates is very important for virologists working in research and diagnostic laboratories.

Aim
Collect virus species from stone and prome fruits, cereals and maize
Provide isolates of viruses

Material and methods
Isolation and characterisation of various viruses from maize (SCMV, MDMV etc.)
Isolation and characterisation of various viruses from cereals (WSMV etc.)
Isolation and characterisation of various viruses from stone and pome fruits (CRLV, PNRSV, PDV etc.)

Results
In the 1st year of the project different plant viruses from various crops have been collected (Table 1). Viruses were detected by using specific RT-PCR's in cereals and corn, which are important crops in agriculture. These isolates can be used as reference material for diagnostic work.

Table 1. Overview of viruses detected in cereals, corn and fruit crops

Host plant	Virus	RNA
Hordeum vulgare	*Barley yellow dwarf virus*	Yes
Hordeum vulgare	*Barley yellow mosaic virus*	Yes
Prunus avium	*Cherry virus A*	Yes
Prunus domestica subsp. *syriaca*	*Plum pox virus*	Yes
Triticum aestivum	*Wheat dwarf virus*	Yes
Triticum aestivum	*Wheat streak mosaic virus*	Yes
Zea mays	*Maize dwarf mosaic virus*	Yes

Future plans (2nd year)
In the 2nd year more isolates from the same viruses will be collected to improve diagnostic tests.

Workpackage 5

Characterization of various whitefly-transmitted viruses of significance for tomato and cucurbits in Europe

Introduction
To collect and further characterise whitefly transmitted virus species of significance for tomato and cucurbits belonging to the genera Begomovirus, Crinivirus and Carlavirus (especially Cowpea mild mottle virus and Tomato pale chlorosis virus isolates infecting tomato and other solanaceous plants).

Aim
To collect and further characterise whitefly transmitted virus species belonging to the genera Begomovirus, Crinivirus and Carlavirus.

Material and methods
As an accredited laboratory, virus isolates will be characterized according to our documented management system and standard operating procedures which were developed considering ISO/IEC 17025 and ISO Guide 34 standards.

Results
During the 1st year of the project, main focus was the acquisition and characterization of Begomoviruses and Carlaviruses. Carlaviruses are usually naturally transmitted by aphids, but two species infecting cucurbits and tomato were known to be transmitted by whiteflies. The recently described species Cucumber vein clearing virus and *Cowpea mild mottle virus* (CPMMV) and their serologically related isolates infect tomato and eggplant. Four of these serologically related isolates of the latter species were characterised. Two could be identified to be almost identical, an isolate causing pale chlorosis in tomato from Israel and an isolate causing mosaic in eggplant from Jordan. These isolates, however, represented a putative new species within the Carlaviruses, rather than being isolates of CPMMV. Two other isolates originating from Sudanese tomato samples also differed substantially from CPMMV.
Furthermore, isolates of the important tomato infecting Criniviruses *Tomato chlorosis virus* (ToCV PV-1023) and *Tomato infectious chlorosis virus* (TICV PV-1108) were characterized (one each). For ToCV an ELISA a suitable antiserum was already available, for TICV the coat protein (CP) was cloned in an expression vector in order to raise an antiserum against the recombinant CP.
In addition, two viroid isolates, *Chrysanthemum stunt viroid* (CSVd, PV-1116) and *Chrysanthemum chlorotic mottle viroid* (CChMVd, PV-1009), and two non-European potato virus isolates from pepino samples from New Zealand (*Potato virus X*, PV-1101; *Potato virus M*, PV-1102), were collected and extensively characterised in order to make them available as reference materials.

Future plans (2nd year)
In the 2nd year further efforts are being made to collect more whitefly-transmitted viruses of the genera Begomovirus and Crinivirus. In addition, other viroid species in the genera Pelamoviroid and Apscaviroid will be addressed, which are not yet available in Q-bank collections. The activities will include molecular and biological characterisation as well as investigation of possible means to conserve/maintain the isolates.

Euphresco Phytosanitary ERA-NET

Workpackage 6

Characterization of known and unknown viruses and development of diagnostic standards

Introduction

In an earlier project a limited number of isolates of different regulated viruses currently present in the plant virus collection of DSMZ/NVWA/PRI have been subjected to Next Generation Sequencing (NGS). For a number of isolates substantial sequence information has been generated. In addition, sequences of new virus isolates or even new viruses were identified. For further characterization and to allow a better comparison with already known sequences, the sequences of the isolates under study need to be completed and analysed in more detail.
In addition, reliable standards are needed for validation of diagnostic tests. A number of (regulated) viruses pose problems in this respect because of lower levels of stability. More research on the development of stable standards is required.

Aim

1) To further complete, characterise and analyse the genomic information of regulated viruses, in particular *Strawberry latent ringspot virus* (SLRSV, unassigned species in the family Secoviridae), *Andean potato mottle virus* (APMoV, genus Comovirus) and *Andean potato latent virus* and related viruses (APLV, genus Tymovirus).
2) To develop more stable standards for validation of diagnostics in particular for *Plum pox virus*, *Leek yellow stripe virus* and *Potato virus Y* (genus Potyvirus)

Material and methods

For a selection of viruses partial sequences obtained by NGS will be completed to complete sequences by conventional techniques. If more isolates of a virus are available sequences will be compared and further analysed to gain more insight in the variability of the species and consequences for diagnostics.

Results

For a number of regulated viruses (Table 2) complete sequences of different isolates have been determined using a combination of Next Generation Sequencing (NGS) technology and conventional Sanger sequencing. Sequence data as well as additional biological and serological data on these isolates have been compiled and included in Q-bank.

Table 2. Overview of characterised viruses

Virus	Action	Q-bank
Andean potato mottle virus (APMoV) Strains B, C, H	Isolates compared. Strain C appears a mixture, further analysis needed.	APMoV-B APMoV-H
Tobacco ringspot virus (TRSV)	Isolate from *Mentha* sp. (Bob Martin)	TRSV
Strawberry latent ringspot virus (SLRSV)	Isolate from *Lilium* sp. cv 'Tiber'	To be included after publication of nt sequence.
Chrysanthemum stem necrosis virus (CSNV)	Isolate HiCh06A L1 from Japan	CNSV
Potato black ringspot virus (PBRSV)	Two isolates previously sequenced in Q-Bol project now included in Q-bank.	PBRSV (2)
Arachaca virus B (AVB)	Isolate previously sequenced in Q-Bol project now included in Q-bank.	AVB
Andean potato latent virus (APLV) Strains Col, Col2, Hu, isolate Q	APLV-Hu was identified as the recently renamed Andean potato mild mosaic virus (APMMoV). APLV-Q isolate was found to be co-	To be included after publication of nt sequence.

	infected with *Potato virus T* (PVT). Now subject of further analyses.	
Other Tymoviruses, a.o. *Eggplant mosaic virus, Physalis mottle virus, Scrophularia mottle virus, Turnip yellow mosaic virus*	Collected and (partially) characterised,	To be included after publication of nt sequence.

Future plans (2nd year)
Further work is summarised in Table 3.

Table 3. Overview of viruses to be characterised

Virus	**Action**
Strawberry latent ringspot virus (SLRSV)	Initial sequence analyses have indicated significant differences between isolates. Further sequence studies on additional isolates from diverse origins will be performed to develop a reliable diagnostic test.
Potato virus T (PVT)	The isolate found together with the APLV-Q isolate will be further characterised.
Arabis mosaic virus (ArMV)	Different isolates will be characterised for their nt sequences and included in Q-bank.
Tospoviruses	Different Tospovirus species will be analysed and partial nt sequences determined and included in Q-bank.

Workpackage 7

Establishment and maintenance of a potato virus species reference collection for a regulated potato viruses and generation of stable positive controls (non-live reference material) for molecular tests for a range of virus species and genera

Introduction
Currently SASA holds various potato virus collections:

1) Virology and Zoology Section holds indigenous viruses as live material (glasshouse grown potatoes / indicator plants or freeze dried material).
2) Plant Biosecurity and Inspections Section holds non indigenous and some indigenous viruses as live material (*in vitro* microplants / indicator plants or freeze dried material). Nucleic acid and plasmid clones are also available as positive controls for some viruses

Within this workpackage we will
1) Assess the infectivity status of the current collections (freeze dried / microplants) and for other material check that it is still fit for its intended use. Gaps in characterisation data will be identified and where appropriate isolates will be sequenced. A comprehensive data base will be developed of material held (this can be used to populate the project database);
2) Add new potato infecting viruses (particularly to the microplant collection);
3) Include a range of virus isolates (pathotypes/strains/phylogenic groups from specific virus species) mainly potyviruses;
3) Identify the most valuable material and deposit it where relevant with other organisations as a backup;
4) Evaluate the production of stable reference material (DNA/cDNA PCR fragments) / cloned fragments) for use as template controls for molecular tests for selected viruses in a number of virus genera.

Aims
Milestone 1: Establishment of new and maintenance of current virus reference collections: infection of plants with known virus isolates, propagation of plants infected with known live (*i.e.* capable of infection) virus isolates (*e.g.* growing plants (in vivo, in vitro potato microplants), freeze-dried, tubers, *etc…*). Target virus species are listed in Annex 1.
Milestone 2: Validation of virus reference material: Molecular and serological characterization of infected plants (ELISA, PCR-based, partial and/or full-length sequencing).
Milestone 3: Generation and validation of “stable positive control” (*i.e.* DNA/cDNA PCR fragments, cloned fragments for a range of virus species/genera).
- PCR amplification and purification of DNA/cDNA template from reference virus material
- Validation of DNA/cDNA template for a range of molecular tests (species and/or genera specific)
- Define and agree on a common standard to fulfil the requirements for generating and using DNA template as a positive control in molecular test.

Material and methods
- Bioassays on a range of indicator plants.
- Serological methods: DAS-ELISA.
- Molecular methods: conventional RT-PCR, Real-Time RT-PCR, sequencing.

Results

Milestone 1
Establishment of new and maintenance of current virus reference collections: infection of plants with known virus isolates, propagation of plants infected with known live (i.e. capable of infection) virus isolates (e.g. growing plants (in vivo, in vitro potato microplants), freeze-dried, tubers, etc…).

Indigenous viruses
List of the current live collection material propagated is given in Table 4.1 and 4.2.

Table 4.1. Indigenous viruses maintained in potato plants (and frozen leaf material from indicator plants)

Virus	Isolate specification
Potato virus A	PVA (SASA-QC58/59)
Potato leafroll virus	PLRV (SASA-QC55/56/57)
Potato virus M	PVM (SASA-QC68/69)
Potato virus S	PVS (SASA-QC72/73)
Potato virus V	PVV (SASA-QC60/61)
Potato virus X	PVX (SASA-QC74/75)
Potato virus Y	PVY^{O} (SASA-DV71, SASA-QC62/63) PVY^{C} (SASA-QC66/67) $PVY^{N\text{-}Wilga}$ (SASA4388) $PVY^{EU\text{-}NTN}$ (SASA-DV76) $PVY^{NA\text{-}NTN}$ (SASA-DV69) PVY^{E} (SASA10766) PVY^{N} (SASA-QC64/65)
Tomato black ring virus	TBRV (SASA-QC70/71)

Table 4.2. Indigenous viruses maintained in tubers (and frozen leaf material from indicator plants)

Virus	Isolate specification
Potato mop top virus (PMTV)	'Mild' isolates (SASA285 SASA331)
PVA	'Severe' isolates (SASA28, SASA364)

Quarantine viruses
Microplants are established for the viruses listed in Table 4.3.

Table 4.3. Quarantine viruses established in microplants

Virus	Isolate specification
Andean potato latent virus (APLV)	
Andean potato mottle virus (APMoV)	
Potato latent virus (PotLV)	
Potato virus P (PVP)	Also the rough dwarf strain
Potato virus S (Andean) PVS [a]	
Potato virus T (PVT)	
Potato yellowing virus (PYV)	
Potato yellow vein virus (PYVV)	
Tomato chlorosis virus (ToCV)	
Tomato yellow vein streak virus (ToYVSV)	

Milestone 2
Validation of virus reference material: Molecular and serological characterization of infected plants (ELISA, PCR-based, partial and/or full-length sequencing).

Indigenous viruses
All abovementioned viruses have been characterised either serologically (DAS-ELISA SASA monoclonal antibodies) and for some by Real-Time RT-PCR (PVY, PVA, PVV, PVX, PLRV, PMTV, TRV). Partial sequencing of target viral gene of relevance for molecular diagnostics has been achieved for the viruses listed in Table 5.1. Full-length sequencing has been achieved for the virus isolates listed in Table 5.2

Table 5.1. Partially sequenced indigenous viruses

Virus	Sequence data
Potato mop top virus	Partial sequence Coat protein read-through sequence RNA2
Potato virus A	Coat protein (SASA-QC58/59)
Potato virus X	Coat protein (SASA-QC74/75)
Potato virus V	Coat protein (SASA-QC60/61)
Potato virus Y^N	Coat protein (SASA-QC64/65)
Tobacco rattle virus	Partial sequence 16K RNA1

Table 5.2. Fully sequenced indigenous viruses

Virus	Sequence data
Potato virus A	Mild isolates (SASA285 SASA331)
	Severe isolates (SASA28, SASA364)
Potato virus Y	PVY^O (SASA-DV71) PVY^{EU-NTN} (SASA-DV76) PVY^{NA-NTN} (SASA-DV69) PVY^E (SASA10088)

Quarantine viruses

Partial sequencing of target viral genes of relevance for molecular diagnostics has been achieved for the quarantine viruses listed in Table 5.3

Table 5.3. Partially sequenced quarantine viruses

Virus	Sequence data
Beet curly top virus (BCTV)	Not specified
Potato latent virus (PotLV)	
Potato virus P (PVP) also the rough dwarf strain	
Potato virus S (Andean) PVS [a]	
Potato yellowing virus (PYV)	
Tomato chlorosis virus (ToCV)	
Tomato yellow vein streak virus (ToYVSV)	

Milestone 3

Generation and validation of 'stable positive controls' (i.e. DNA/cDNA PCR fragments, cloned fragments for a range of virus species/genera). Define and agree on a common standard to fulfil the requirements for generating and using DNA template as a positive control in molecular tests.

Indigenous viruses

Cloning, sequencing, collection of plasmid DNA with target cDNA and corresponding *E. coli* glycerol stocks has been achieved for the virus genes listed in Table 6.1.

Table 6.1. Indigenous viruses for which sequences are available as cloned material

Virus	Cloned sequence
Potato mop top virus	Partial sequence Coat protein Read-Through RNA2
Potato virus A	Coat protein (SASA-QC58/59)
Potato virus X	Coat protein (SASA-QC74/75)
Potato Virus V	Coat protein (SASA-QC60/61)
Potato virus Y^N	Coat protein (SASA-QC64/65)

Tobacco rattle virus	Partial sequence 16K RNA1 (SASA-Q Spey)

Quarantine viruses
Plasmids are prepared for the viruses listed in Table 6.2.

Table 6.2. Quarantine viruses for which sequences are available as cloned material

Virus	Cloned sequence
Beet curly top virus (BCTV)	Not specified
Potato yellow mosaic virus (PYMV)	
Tomato chlorosis virus (ToCV)	

Validation is on-going for all DNA control material.

Future plans (2nd year)

Indigenous viruses
Complete the molecular characterisation (partial sequencing) of listed viruses (PVM, PVS, PAMV, TBRV). Complete the cloning of virus (and endogenous) target genes (PVY^C, PVY^O, PVM, PVS, PAMV, TBRV, COX, NAD5). Complete validation of DNA control material. Complete live-material collections and validation according to set standards.

Quarantine viruses
Complete molecular characterisation (sequencing) of listed viruses. Complete the cloning of virus target genes. Complete live-material collections.

Workpackage 8

Data and isolates in Q-bank

Introduction
Q-bank is a European initiative to create a public database on plant pests and pathogens which is linked to physical collections. This will make them both accessible and available as reference material for diagnostic and research purposes. Both NVWA and PRI were closely involved in setting-up Q-bank and are currently, together with DSMZ (DE) and FERA (UK), responsible as curators for the plant virus section.

Aim
Make isolates studied in this project as well as the biological, molecular and additional data (i.e. on diagnostics) available to (European) NPPO's, research and other interested parties via Q-bank.

Material and methods
Virus isolates and data generated by the different work packages of this project will be collected, curated and included in Q-bank.

Results
By the end of 2013, the Q-bank Plant Viruses and Viroids database has been redesigned. Data from the former tables have been checked and transfered to the new tables for about 100 virus and viroid isolates. Data generated by the NPPO and PRI in the Netherlands and DSMZ in Germany have been included. Work is ongoing to include data from the other EUPHRESCO VIRUSCOLLECT partners.

Future plans (2nd year)
To continue the inclusion of species and data in Q-bank.

Annex 1. Summary of deliverables (Q-bank data and products)

green	completed
yellow	partially completed
orange	just started
blue	still to be started

1st year

Partner	virus or viroid species/ genus	# isolates	biological data (test plants)	serological data (antiserum used)	molecular tests (type & design)	sequence data (gene/region)	production of standard (control)
AT–AGES	Potyvirus	c. 5			+		
	CRLV	1			+	+	
	Viruses of stone and pome fruits	c.5			+	+	
	Viruses of cereals and maize				+		
DE–JKI/DSMZ	*Begomovirus*	c. 20	+	+	+	full sequence	+
	Crinivirus	c. 3	+	+	+	partial sequence (minimum CP)	+
	Carlavirus	c. 3	+	+	+	almost full sequence	+
	Viroids		+		+	full sequence	+
NL–PRI	SLRSV	c. 6	+	+	+	full sequence (1) partial sequence (5)	+
	APLV/Tymovirus	c. 8	+	+	+	full sequence	
	APMoV	c. 3	+	+	+	full sequence	
	Potyviruses, to start with PPV, LYSV, PVY, etc						+
UK–SASA Indigenous (non quarantine)	PVY / Potyvirus PVY strains E, N, O, C, NTN, N-Wilga	6	+	+ (SASA MAb PVYN & MAb PVYO/C)	+	full sequence (2 isolates) partial (4 isolates - CP)	+ (stable positive control)
	PVA / Potyvirus Mild & Severe isolates	6	+	+ (SASA MAb PVA)	+	full sequence (2 isolates) partial (4 isolates - CP)	+ (stable positive control)
	PVM / Carlavirus	1	0	+ (SASA MAb	+	partial sequence	+ (stable positive

				PVM)		(minimum CP)	control)
	PVS / Carlavirus	1	0	+ (SASA MAb PVS)	+	partial sequence (minimum CP)	+ (stable positive control)
	PMTV / Pomovirus	1	0	+ (SASA MAb PMTV)	+	partial	+ (stable positive control)
UK–SASA Non indigenous (quarantine) Years 1 and 2	ToYVSV (Begomovirus	1	Not mechanically transmitted	+		+	micropropagation (mp) & plasmid clone to be produced
	PYMV / Begomovirus	0 (plasmid only)			Begomovirus genus conventional RT-PCR with sequencing		plasmid clone available
	PotLV / Carlavirus	2	Published (Brattey et al.)	Mab (SASA)	Carlavirus	partial	mp available plasmid clone to be produced
	PVP / Carlavirus	3 (including PRDV)	Published (Nisbet et al)	Pab (Julio Daniels	Carlavirus genus conventional RT-PCR with sequencing	partial	mp available plasmid clone to be produced
	PVS (Andean) / Carlavirus	1	+	Mab (SASA)	Carlavirus genus conventional RT-PCR with sequencing	partial	mp available plasmid clone to be produced
	APMoV / Comovirus	1	+	Pab (PRI)	Comovirus genus conventional RT-PCR with sequencing	partial	mp available plasmid clone to be produced
	ToCV / Crinivirus	1	Not mechanically transmitted		ToCV specific real time RT-PCR	partial	mp & plasmid clone to be produced
	PYVV / Crinivirus	1	Not mechanically transmitted		PYVV specific real time RT-PCR	partial	mp & plasmid clone to be produced
	BCTV / Curtovirus	3	Not mechanically transmitted		Curtovirus genus conventional PCR with sequencing	partial (all isolates)	Plasmid clone available
	PYV / Ilarvirus	1	Not easily/ reliably transmitted	Pab (CIP, SASA)	PYV specific conventional RT-PCR	partial	mp & plasmid clone to be produced
	AVB-O / Nepovirus	1	+	Mab (SASA)		+	mp & plasmid

							clone to be produced
	PBRSV / Nepovirus	1		Mab (SASA)	using sub group A primers with sequencing	+	mp & plasmid clone to be produced
	PVT / Trichovirus	1		Mab (SASA)			mp available
	APLV / Tymovirus)	3		Pab (PRI)	+	+	mp available

2nd year

Partner	virus or viroid species/ genus	# isolates	biological data (test plants)	serological data (antiserum used)	molecular tests (type & design)	sequence data (gene/region)	production of standard (control)
AT–AGES	Potyvirus	c. 5			+		
	CRLV	1			+	+	
	Viruses of stone and pome fruits	c.5			+	+	
	Viruses of cereals and maize				+		
DE–JKI/DSMZ	*Begomovirus*	some	+	+	+	full sequence	+
	Crinivirus	1-2	+	+	+	partial sequence (minimum CP)	+
	Viroids	c. 3 (additional)	+		+	full sequence	+
NL–NVWA/PRI	SLRSV	c. 5			+	partial sequence	
	PVT	c. 2				full sequence (1)	
	ArMV	c. 3			+	full/partial sequences	
	Tospoviruses	c. 15				partial sequences	
UK–SASA Indigenous (non quarantine)	PVX / Potexvirus	1	0	+ (SASA MAb PVX)	+	partial sequence (minimum CP)	+ (stable positive control)
	PVV / Potyvirus	1	0	+ (SASA MAb PVV)	+	partial sequence (minimum CP)	+ (stable positive control)
	TRV / Tobravirus	1	0	+ (SASA MAb TRV)	+	partial sequence (minimum CP)	+ (stable positive control)
	PAMV / Potexvirus	1	0	+ (SASA MAb PAMV)	+	partial sequence (minimum CP)	+ (stable positive control)
	TBRV / Nepovirus	1	0	+ (SASA MAb TBRV)	+	partial sequence (minimum CP)	+ (stable positive control)

Annex 2. Quality standards for Q-bank collections of viruses and viroids

General quality management

the whole quality management system and procedures should be periodically reviewed to keep all up to date (internal audits)
onsite audits/inspections of the production facility should be possible/allowed (transparency)
business continuity should be guaranteed
equipment (e.g. pipets, balance, pH meter) should be periodically calibrated to traceable measurement standards
maintanance and calibration reports should be kept
only trained personnel should be allowed to handle reference material (whole process from characterization to order processing)
facility access control is a prerequisite
appropriate cleaning and decontamination procedures should be installed to avoid contaminations
data (chracterization, production and ordering process) should be stored (including a back up copy), tracability is important
ensure the protection of confidential information
backup storage of the collection should be available (e.g. DSMZ has N_2 storage [seed stock] in one building, freeze dried material [distribution stock] in another)
in order to guarantee sustainability, adequate equipment and reliable funding is needed for long-term stability of the physical Q-bank collections
ensure that personnel is free from undue internal or external pressure that adversely affect the quality
in order to guarantee that the required expertiese is maintained, the head of laboratories/curators of the collections should have a long-term employment

Characterization and identification

isolate origin should be recorded as good as possible (isolation host, country of origin, submitted by, original isolate number/name given by the collector)
procedures for receipt and handling of biological material should be documented
unique numbers have to be assigned which are never reassigned if the material is later discarded
material has to be characterized to the level of its intended use [identity for isolates and specific reaction for controls]
identification with appropriate methods (species level according to demarcation criteria in King et al. 2012-Virus Taxonomy)
at least a partial genome sequence, ideally covering a gene used for species demarcation, should be available (e.g. coat protein gene)
property values have to be assignes
characterization has to include assessment of viability and purity
characterization may include a broad range of techniques, especially to assess purity:
- electron microscopy
- dsRNA extraction
- RCA
- typical symptoms on assay hosts

- groupe specific tests
- transmission trials (mechanical, insect …)

(of course not all have to be applied for each isolate during characterization, but the selection/combination should give confidence)

suitability of selected/specific conservation method has to be assessed

identity and purity also has to be verified for material received from other sources (collections/scientists), irrespective if it is already characterized or not

raw data should be stored (e.g. sequencing, ELISA)

traceable documentation of the whole characterization process is required

Reference material production

procedures should be documented in SOPs to guarantee uniform production and consistent batch quality

SOPs should also contain all required data for relevant buffers, media, PCR-reaction mixture, etc.

changes of the production process should be veryfied by quality

appropriate labeling (collection name, scientific/species name or accepted acronym, no synonyms/colloquial names)

unique collection (accession) number if available (especially important if more than one isolate of a species exists in the collection)

long-term stability: suitability of selected conservation method should be assessed exemplarily for old batches (e.g. 5 years old)

short-term stability: suitability of conservation method should be assessed exemplarily under extreme conditions to simulate transport (e.g. 10 days 37°C)

between vial homogeneity should be assessed exemplarily (in particular for positive controls)

if virus is only available in mixed infection (PC, NA extract and inoculum), this information should be provided to the customer

(not required for only seed transmitted, non-pathogenic viruses of the families Partitiviridae, Endornaviridae and only genome integrated viruses)

traceable documentation of the whole production process is required

Quality control

the processes used in QC should be defined

exemplarily validation (e.g. for DAS-ELISA) and/or participation in interlaboratory comparisons for proof of competence is recommended

each new batch of positive controls should be tested to fulfill QC criteria (should be defined, e.g. PC has at least 5-times higher OD compare to NC/buffer)

each new batch of inoculum or the plant used to produce the batch should be tested to ensure identity of species (e.g. EM, ELISA, PCR)

storage conditions of produced batches should be clearly defined

storage conditions sould be controlled to ensure quality (e.g. temperatur)

batches should be traceable to the production process and date of production

quality of nucleic acid extracts have to be checked (e.g. gel electrophoresis, nanodrop….)

non-conforming work should be controlled and corrective actions taken

traceable documentation of the quality control process is required

Order processing and shipping

it has to be guaranteed that handling always complies with relevant national law and regulations
international safety regulations and Q regulations (letter of authority [EU Directive 2008/61], dual use [EU Council regulation 1232/2011]) have to be considered
appropriate packing and shipping (based on experience "fit for purpose", e.g. dry ice or room temperature, courier service or regular mail)
provide instructions for end-users including storage and handling conditions (eg. Certificate, information sheet or provide information via website)
imformation what and how much customer gets has to be specified (e.g. dried, freeze dried, fresh leaves; 1 cutting, 2 ml PC etc.)
an expiry date has to be defined for the material provided
a free replacement should be provided within that timeframe if material is not appropriate
free access to related data/information should be granted
customer support after purchase of material should be guaranteed
a permanent contact addresse should be provided/published (mail, email and telephone)
traceability of orders should be possible for at least XY years
customer complaints should be tracked
confidentiality for all orders of past and present customers has to be guaranteed
customers have to be informed if nonconformities are realized (e.g. contaminations, misidentification)
information on order processing time (lead-time) should be provided (regular process, not for exceptional cases that might happen)
distributers have to be authorized by the reference material producer (appropriate storage and handling has to be guaranteed)
if resoled by unauthorized third parties/distributers, the material will loose its reference status (no control/influence on handling, storage etc.)
material will loose Q-bank reference status if reproduced by third parties (no influence on production process, QC, storage and handling)
traceable documentation of the order processing is required