



**European Cooperation
in the field of Scientific
and Technical Research
- COST -**

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COST 097/14

MEMORANDUM OF UNDERSTANDING

Subject : Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA1407: Application of next generation sequencing for the study and diagnosis of plant viral diseases in agriculture

Delegations will find attached the Memorandum of Understanding for COST Action FA1407 as approved by the COST Committee of Senior Officials (CSO) at its 191th meeting on 12-13 November 2014.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as
COST Action FA1407
APPLICATION OF NEXT GENERATION SEQUENCING FOR THE STUDY AND
DIAGNOSIS OF PLANT VIRAL DISEASES IN AGRICULTURE

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4114/13 “COST Action Management” and document 4112/13 “Rules for Participation in and Implementation of COST Activities”, or in any new document amending or replacing them, the contents of which the Parties are fully aware of.
2. The main objective of the Action is to coordinate and raise the European capacity to apply Next Generation Sequencing (NGS) technologies for the study and diagnosis of viral diseases in vegetatively propagated plants, seeds and seedlings and other matrices (insect, water, soil).
3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 68 million in 2014 prices.
4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.
5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Section 2. *Changes to a COST Action* in the document COST 4114/13.

A. ABSTRACT AND KEYWORDS

The objective of the Action is to coordinate and raise the European capacity to apply Next Generation Sequencing (NGS) technologies for the **study and diagnosis of viral diseases** in vegetatively propagated plants, seeds and seedlings. The Action is highly timely and needed because viral diseases are currently a major economic problem in agriculture throughout the world. The increasing importance of vegetative propagation in plant production and the intensified global plant trade are further increasing the risk of viral diseases in Europe. NGS enables rapid and reliable holistic virus identification (indexing), which is needed for the development of innovative, knowledge-based solutions for plant production. By bringing together a multidisciplinary and multi-actor consortium, the Action will ensure cost-effective research and build up a strong Pan-European knowledge-based network for better control of established, emerging and exotic viral plant diseases. The Action will deliver new scientific knowledge about viral plant diseases that are currently poorly understood, and contribute to the development of more effective surveillance of stock material health and to the improvement of quarantine procedures. The Action thus implements the European strategy of integrated pest management and protection against harmful plant pathogens and contributes to the securement of food production.

Keywords: plant virus, diagnostic, high throughput sequencing

B. BACKGROUND

B.1 General background

Plant viruses and virus-like agents (viroids, satellites...) pose an important threat to many important food crops production and are responsible of production losses which have been estimated at 15-45 billion €per year. For example, Plum pox virus, responsible of the Sharka disease of Prunus crop, causes losses of 3 billion €per year (Cambra et al., 2006). The grapevine fanleaf disease (caused by Grapevine fanleaf virus and by Arabis mosaic virus) causes an estimated 1.2 billion €annual losses to the French grapevine industry alone, despite prophylactic measures and certification schemes ((Fuchs, 2006). The economic impact of Grapevine leafroll-associated virus ranges between 300 \$ and 2,300 \$ per hectare worldwide (Maree et al., 2013). On potato, Potato leafroll virus (PLRV) and Potato virus Y (PVY) can affect yield substantially, with up to 80% losses (Palukaitis, 2012). The availability of virus-free seeds has increased average

sweet potato yield by at least 30 % (Fuglie et al., 1999). Overall, plant viruses and virus-like agents compromise the quality of the product and the growers income in developed countries but also impact food security in many developing countries. Moreover, the intensification of worldwide trade of agricultural goods and climate change are rapidly increasing the probability of introduction and establishment of new viruses in the EU.

The ability to provide fast, inexpensive and reliable **diagnostics** for any viral infection is a key measure for cost-effective control of these ubiquitous pathogens. This is particularly important for vegetatively propagated crops, for which thousands of infected plantlets can be produced from a single infected individual, or for viruses vectored by insects, soil or water which are extremely hard to control after their introduction into a new region or country. ELISA (Enzyme Linked ImmunoSorbent Assay) and real-time PCR are currently the two most widely used technologies in plant virus diagnostics. Nevertheless, they are restricted to already known and well-characterized viruses, because information on the virus traits (e.g. serology or sequence) is required to prepare serological reagents or to design PCR primers and probes. Moreover, these tests require to make pre-test assumptions about the set of pathogens whose presence is to be verified by a particular test. Currently, unknown viruses or genetically distant strains of existing viruses are only partially identified, mainly by biological indexing or electron microscopy.

A solution to these problems is provided by Next Generation Sequencing (NGS) technologies. **NGS technologies have the ability to detect and characterize by a holistic approach the complete virome (virus, viroid and any virus-like agent) infecting any plant sample and potentially even in vectors or various substrates** and could be applied in a range of diagnostic situations: quarantined plant material, border control, certification programs, quality assurance of seed lots etc. These include single or mixed infections of characterized as well as unknown agents with a harmful potential.

Currently, NGS technologies are already applied in various relevant areas: etiology of viral diseases, taxonomy, viral population genetics and plant-virus interactions (Barba et al., 2014; Massart et al., 2014). They are currently raising a growing interest for virus diagnostics. Since their first commercial availability in 2007, they have evolved considerably with significant improvements both for the laboratory and bioinformatics processes. **Thus, we have entered an era where NGS can be translated into practical solutions for virus control in plant production.** Nevertheless, there is still much room for improvement and coordination efforts are now needed to transform this burgeoning field of research into useful and innovative diagnostic tools for the control of existing and emerging plant viruses. This transformation requires the integration of a

number of unique skills in a multidisciplinary and multi-actors international collaboration. Currently, research groups are working on this topic without coordinated networking, leading to poor collaboration and exchange of information between them. The COST Action will thus have excellent possibilities to profoundly raise the quality and efficiency of European research in the topic, to optimize the use of resources, to avoid duplication of efforts and to provide channels for exchange of materials, protocols and knowledge. Elaborated workflows established by multidisciplinary teams will enable application of these new NGS technologies by diagnosticians in laboratories in Europe and worldwide, improving the EU strategies for Integrated Pest Management (2009/128/EC) or protection against harmful plant pathogens (2000/29/EC and 2009/143/EC). In addition to the implications for plant production and phytosanitary practices, the suggested networking of leading scientists in plant virology will set up exchange and discussion platforms accelerating the advancement of science and facilitating the adaptation of phytosanitary regulations in the future. With NGS technologies we will be able to raise new scientific questions which, in the future, will impact the way we see **plant-virus interactions, virus ecology and phytosanitary regulations**. This will create a strong, positive feedback loop from basic science to innovative applications for pest risk assessment (PRA).

B.2 Current state of knowledge

NGS technologies became available at the onset of the 21st century. They provide a highly efficient, rapid, and low cost DNA sequencing platform beyond the reach of the standard and traditional DNA sequencing technologies developed in the late 1970s. They are continually improving and becoming faster, more accurate and cheaper. In 2009, NGS technologies began to be applied to several areas of plant virology and produced key advances in the **etiology** of diseases (identifying the causal agent and allowing its characterization) and viral **ecology** (metagenomics). More than one hundred new viral sequences have been identified from symptomatic and non-symptomatic (latent infection) tissues of agronomic herbaceous plants, grasses and fruit trees, or from non-symptomatic tissues coming from wild plants of different species, over these last 5 years (Barba et al., 2014). We can reasonably anticipate that the pace of discovery of novel viral agents in plants, either mycoviruses or plant viruses, will accelerate in the near future to allow the **complete virome characterization of the plants**. For example, there are still at least 35 diseases of unknown etiology that affect stone fruits alone and a similar number that affect pome fruits (Howell et al., 2011).

An emerging question, which will become more acute as more results accumulate, is the **biological**

significance and the implications of the identification of a novel virus. This question is particularly acute in the case of well characterized or high value plant materials such as mother plants or 'top tier' plants in clean plant programs that serve or have served as the source of plants for international trade, exported from or imported in EU (Massart et al., 2014). The finding of a novel virus in such plant material raises very practical questions that the diagnostician will need to address, in particular the impact of the virus identified on the plant and, ultimately, the potential need to halt the distribution of the plant(s) concerned (MacDiarmid et al., 2013). At the same time, we must acknowledge the fact that very little information will be available, besides the complete or even partial sequence of the novel agent, to assist the decision making process. It is already clear that some of the agents identified are not plant viruses, but are in fact mycoviruses infecting fungal agents associated with the plant, insect viruses, bacteriophages or even mammalian viruses coming from irrigation with contaminated water. Moreover, some plant viruses could be found in water, clouds or soil, implying that such viruses could be only adsorbed to the plant and not in infective interaction with it. Any new viral sequence discovered must therefore be carefully analysed to try to determinate its origin. This strengthens the importance to develop in parallel efforts aimed at the understanding of the biological significance of all these newly characterized virus species, in order to be able to make the most relevant **diagnostic decisions** in the future.

These scientific discoveries are also progressively leading to a paradigm shift in our understanding of **plant-virus relationships**, from pathogenicity-only to other trophic relationships like symbiogenesis or conditional mutualism (Roossinck, 2011). This new paradigm will have an impact on the phytosanitary regulations, for example, by necessitating a revision of the term “virus-free” plant and of the corresponding concept.

The continuous harvest of new viral sequences also have an **impact on viral taxonomy**. Indeed, the Executive Committee of the International Committee for the Taxonomy of Viruses (ICTV) recently agreed to accept, with certain safeguards, species or higher taxa proposals based only on sequence data from NGS studies (Gorbalenya, 2014).

Currently, research groups are each using their own protocol, with differences in sample preparation, sequencing platforms and data analysis pipelines. The comparison of these protocols and of the obtained results is therefore very difficult. International networking will strengthen exchanges to fully understand and evaluate NGS in a diagnostic context, facilitating the comparison between protocols and speeding up the development of standardized protocols.

Moreover, and despite their huge potential as **diagnostic tools**, the sensitivity, specificity, repeatability, reproducibility, robustness and accuracy of existing and future standard protocols will have to be thoroughly evaluated and proper controls will need be defined, such that diagnostics

meet international standards such as EPPO standard PM 7/98. Indeed, such evaluation is mandatory for any new technology to enter the diagnostic field. Evaluation criteria and procedures can be harmonized only through international collaboration between laboratories.

B.3 Reasons for the Action

The setting up of a COST Action on Next Generation Sequencing (NGS) and diagnostics is timely and necessary in plant virology. The rapid expansion of the NGS applied to virus detection has been leading to the emergence of small research groups independently trying to transfer the technological benefits of high-throughput sequencing toward reliable diagnostic protocols of plant viruses. Unfortunately, these groups are currently working each on their own analytical pipeline without central coordination, providing for an overall non optimal use of time, resources and efforts. Considerable benefits, in terms of resource use, economy and scientific quality could be gained by harmonized knowledge-base, concerted actions and increased communication.

Moreover, the establishment of a new technology in routine practice is a challenge and requires dialogue with all the stakeholders (scientists, diagnostic laboratories, quarantine laboratories, National and supra-National Plant Protection Organisation, Policy Makers, Inter-professional Growers Associations, and Nurseries Consortia) on a wide trans-national basis. A COST Action provides therefore the ideal forum to gather expertise and knowledge across Europe and to reach an increased scientific, regulatory and economic expertise for NGS-based plant virus diagnostic within European stakeholders.

By facilitating the transfer of scientific and technological advances into reliable diagnostic protocols carried out within an adapted regulatory framework, the Action will answer specific economic needs, as the availability of healthy plant material for growers and seed producers, of sensitive and reliable diagnostic tools for diagnostic and quarantine laboratories and NPPOs which control existing and incoming plant material.

The COST Action will also stimulate more fundamental and cutting edge research within Europe. It will also strengthen the exchanges and collaboration on an emerging question, which will become more acute as more results accumulate: the biological significance and the implications of the identification of a novel virus or a novel host for a known virus. A coordinated effort is needed to address this more and more critical question and to provide a research framework and clear decision schemes for the diagnostician and policy makers. This would benefit of standards definition in laboratory and bioinformatics pipelines to reach a consensus in research and diagnostic activities

across different European research groups.

It should be underlined that the development of NGS-based approaches for diagnostic, leading to the discovery of novel or poorly characterized viral agents and new trophic relationship, may today complicate the **decision-making** process for the diagnostician and the regulatory authorities.

Nevertheless, in the long run and through the coordinated efforts of the diagnostic stakeholders, these advances will clearly lead to an improved situation of better informed decisions and to the safer movement and trade of plant material.

The benefits of the COST Action for the stakeholders are resumed in the following table:

Stakeholder	Benefits from the COST Action
Researchers (diagnostic tools development)	Speeding up and coordinating current and future efforts for the development of NGS-based diagnostic protocols for plant virus detection and control
Researchers (Fundamental plant virology)	Strengthening exchanges and providing a forum to answer emerging questions linked to plant-virus and vector-virus trophic relationships, virus epidemiology and to the biological significance of newly identified viruses
Diagnostician	Improving the availability of new validated NGS-based protocols for a better virus diagnostic. Scientific framework for better informed decisions
National and European regulatory authorities	Improvement of the regulation based on novel scientific advances Decision schemes for better control of the plant viruses at EU scale
Industry (nurseries and seed companies)	Safer trade of high value plants and seeds for multiplication
Farmers	Improved guarantees of planting healthy plants and seeds on field
Inspection agencies	New technologies available for better control at borders

The COST Action will therefore facilitate the cross-flow of information and the exchanges between currently compartmentalized research collaborations into 4 specific areas:

- Comparison and validation of NGS-based protocol for virus diagnostic in different matrices
- Etiology of graft-transmissible diseases and biological impact of newly discovered agents.
- Taxonomy and viral population genetics

- Socio-economic and regulatory impact - Dissemination

An underlying goal of this coordinated effort is to continue the collaboration and research under different funding schemes after the 4-years COST program.

B.4 Complementarity with other research programmes

The COST Action tangents several ongoing research programs (see the list below). However, to our knowledge, there are no programs focusing on viral diseases on such a broad scale or with similar technological and multi-actor approach as this network.

- Q-DETECT - Developing quarantine pest detection methods for use by national plant protection organizations (NPPO) and inspection services (GA-245057) Q-bol
- Q-BOL - Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health (GA-226482). Q-Bank is the public website for the dissemination of Q-BOL tools and the identification of regulated (and non-regulated) plant pathogens and pests.
- TESTA: Treatment methods, Evidence for Seed Transmission and Assessment of seed health) - FP7-KBBE-2012-1.2-05: a European project to study the mode of seed transmission of pathogens and to develop pathogen-detection methods and alternative seed treatments.
- DECATHLON: Development of Cost efficient Advanced DNA-based methods for specific Traceability issues and High Level On-site applications. FP7-KBBE.2013.3.5-01
- INFECT-MET: Metrology for monitoring infectious diseases, antimicrobial resistant and harmful micro-organisms: This project aims to develop novel measurement procedures and validation frameworks to support current and emerging molecular approaches for efficient, harmonised and rapid diagnosis, surveillance and monitoring of infectious diseases. EMRP projects HLT08
- SHARCO: Sharka Containment, FP7-KBBE-2007. Grant agreement 204429/ 2008
- COST Action BM 1006: Next Generation Sequencing Data Analysis Network
- COST Action FA 1013: Endophytes in Biotechnology and Agriculture
- COST Action FA 0806: Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy

Furthermore, this COST Action will complement many on-going national research programs.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The main objective of the Action is to coordinate and raise the European capacity to apply Next Generation Sequencing (NGS) technologies for the study and diagnosis of viral diseases in vegetatively propagated plants, seeds and seedlings and other matrices (insect, water, soil).

C.2 Objectives

This COST Action has four main objectives:

1. Coordinate scientific efforts and provide a **research framework** to reach a comprehensive characterization of the plant virome, including new and emerging viruses, and to understand its impact on plants of key economic importance for Europe. The target is in agriculture, but, for example, forestry will benefit of this Action as similar problems are encountered hampering its ecosystemic services..
2. Coordinate and harmonize European knowledge-base for **technological standards and validation** of reliable NGS protocols for use in relevant areas of plant virus diagnostic.
3. Discuss, agree and disseminate **decision schemes on plant virus diagnostic** for policy makers, European and National Plant Protection Agencies and diagnostic laboratories.
4. Discuss, agree and disseminate a **scientific position and expertise** on the impact of NGS on virus taxonomy and on the plant-virus interactions for other researchers, for plant and seed producers and for regulatory agencies.

C.3 How networking within the Action will yield the objectives?

This COST Action will create a new and needed network of European experts and rationalize and streamline their research efforts in plant virus detection and characterization, in plant-virus interactions, in plant virus ecology and in the development of plant virus diagnostic tools. By creating a joint platform and channels for knowledge and competence exchange, it will boost the technological developments of NGS towards diagnostic tools including both wet lab and

bioinformatics data analysis. The Action will thus bring together a critical mass of experts with various backgrounds related to the use of NGS for detection and identification of plant viruses and its scientific and regulatory consequences. Because of the complexity of the NGS laboratory protocol, of the data analysis pipelines and of the biological interpretation of the results, an effective and broad network of knowledge and expertise is felt absolutely critical for the harmonization of the plant protection practices within Europe. Key features to achieve the objectives will be:

1. Developing a collaborative community of multidisciplinary researchers through thematic Workshops and Short Term Scientific Missions between partners.
2. Establishing a joint platform and concrete channels for harmonizing and continuously updating NGS protocols for plant virus diagnostics, including laboratory and bioinformatics workflows, through data, sample and results exchanges and discussions in meetings and e-meetings.
3. Organising meetings, Workshops focused on the distinct but inter-related topics for the Working Groups of the Action, and Short-Term Scientific Missions to meet specific challenges, targeted to specific stakeholder groups.
4. Running Technical and Scientific Training Schools in NGS for plant virus diagnostic and in the consequence of NGS for plant-virus interaction frameworks by the leading institutes of the Action. These Training Schools will disseminate basic and advanced methodologies, especially for Early Stage Researchers.

C.4 Potential impact of the Action

The expected benefits of this Action will be obtained at three levels:

(A) **Improving the technological level:** networking and developing a collaboration platform to produce validated protocols for specific NGS applications in diagnostics. These protocols will also be useful for basic and applied research in etiology, taxonomy, population genetics and plant-virus interactions.

(B) **Streamlining the basic research:** Associating on-going research projects to the Action: i) to increase basic knowledge of viromes in targeted host plants species, fruit trees and small fruit plants, grapevine, tomato, potato but also to a lesser extent to forestry, by building an efficient

network of dedicated researchers, ii) to improve knowledge in etiology, epidemiology and diagnosis of viral diseases, iii) to update virus taxonomy and iv) to increase knowledge of plant diseases present in the EU, which will improve control strategies

(C) Technical and social innovations: Improving plant virus control at European borders and within European territories through scientific and evidence-based recommendations for a safer plant trade inside and outside Europe to Policy Makers, National and European Plant Protection Organisation and diagnostic laboratories.

These various levels will address the development of rapid molecular diagnosis of new viral agents in the frame of the certification schemes or of the European borders and points of entry controls.

A major bonus for the Action will be the sharing of knowledge among institutions and countries, spreading high-level scientific knowledge to a variety of stakeholders. Therefore, this COST Action will also focus its efforts on bridging the gap between cutting-edge scientific advances and practical application of innovative technologies in the complex regulatory and economic frameworks of plant virus diagnostic.

The above-mentioned benefits will provide a competitive advantage for Europe, strengthening the ability to deliver virus-free plant material and to detect quickly any emerging virus for effective and immediate control actions. As a result, farmers and national economies will directly benefit from the Action.

C.5 Target groups/end users

Through all steps, the Action seeks active dialogue with different target groups, in order to reach a true multiactor approach. The main target groups are:

1. **Researchers:** The Action will generate new standardized procedures and guidelines for a smart utilization of high throughput sequencing in virus detection and characterization. This will facilitate the diffusion of these technologies within the plant virology community. The development of NGS-based diagnostic will be facilitated through the coordination of scientists.
2. **Diagnostic laboratories and inspection bodies** will be able to make the most relevant diagnostic decisions based on latest scientific advances and will be able to up-to-date validated NGS technologies.

3. **National and European Plant Protection Agencies** could adapt policies based on scientifically validated assumptions from national and international bodies, and bodies from industry who are seeking information for policy development.
4. **Industry:** plant nurseries, seed and seedlings producers and/or traders, will directly benefit from the availabilities of better controlled plants and of an adapted regulatory framework allowing them to produce and/or commercialize better quality products. The Inter-professional associations and farmers will also benefit from planting healthier material, leading to better plant pathogen control.

All groups will be directly involved in this Action. Several participants are heading official diagnostic laboratories, are involved in National or European Plant Protection Organisations or are working for industry.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

With the constantly growing world trading and the changing climate, the crop production in Europe is exposed to new and emerging viral/viroid pathogens, which represents a major threat to their viability. The efficient and early detection of these pathogens, based on sensitive and unbiased techniques, is a key factor in the establishment of the necessary control measures to protect the European crops. The Action will make use of the latest technological advances in NGS to directly address these issues.

Beside promoting the cooperation and quick information and data exchange within Europe through a network of laboratories and scientists, the Action will address (i) the definition of standard procedures and protocols for the standardization of NGS in plant virus diagnosis and detection within Europe, necessary to the eradication programs which rely on early, sensitive and easy-to-use methods of detection based on the knowledge acquired by study of viromes in different systems (ii) the etiology and biological impact of newly discovered viruses or viroids, their interactions with vectors and hosts, but also synergistic and/or antagonistic interactions between viruses/viroids, necessary to understand expression of symptoms and epidemiology of the diseases (iii) viral/viroid population genetics and taxonomy, necessary to understand the global virus/viroid diversity and evolution, which can impact the efficiency of detection methods and resistance traits (iv) the

dissemination of the results to the scientific community and the European regulatory agencies to ensure that the quarantine organisms' regulations are well in place within the EU members and neighbouring countries, and to find means of effective control of these pathogens.

D.2 Scientific work plan methods and means

WG1: Comparison of NGS protocols for virus diagnostic and standard recommendations

The development and validation of NGS-based protocols for the detection of viral pathogens requires in-depth knowledge of different fields, in particular virology and bioinformatics. Thus, this WG will be divided into two subgroups. Each subgroup will focus on one field with its peculiarities. WG1.1 will address topics linked to the preparation of the material to be sequenced, for example the sampling, nucleic acids extraction, type of molecules to be sequenced, and will also involve itself in the comparison of techniques. A particular focus will be dedicated to the library preparation and bias avoidance to have a reliable picture of the presence of even rare sequences in the samples, in particular using enrichments for viral nucleic acids (dsRNA, viral particles purification, vsRNAs). WG1.2 will focus on the analysis of the sequence data (bioinformatics). Current programs may still generate errors in the analysis, and many efforts are still required to develop more user-friendly programs, which are faster, require less computing power and allow the identification of any viral sequence, even when confronted with highly divergent viruses. The reliability of these programs needs to be improved in order to fit the requirements of the diagnosis field. Protocols including *in vitro* and *in silico* procedures will be established for selected platforms (Illumina, IonTorrent, PacBio and comparison of platforms will be also addressed. The minimum requirements and rules for discovery of new viruses and for confirmation of the presence of known viruses in a sample will be established. In addition, for validation purposes, other methods such as PCR, ELISA or biological indexing will be used to confirm results on the viruses found in selected samples. The WG1 will keep in close contact to the other WGs, in order to provide them with the latest protocols and advances.

WG2: Etiology of viral diseases and biological impact of newly discovered viruses or virus-like agents.

The potential of NGS to detect sequences of new viruses and virus-like agents in plant tissues, even at very low concentration, has raised new questions about the biological significance and the implications of the identification of a novel virus/virus-like agent. The interpretation of the detection of such sequences in a plant, for which no biological data is available, is delicate, as the

sequence can come from the host itself or an organism associated (like endophytes). Furthermore, there is also no information concerning the trophic relation between the virus and its host, i.e. whether it is latent, pathogenic, or beneficial. This WG will address the complex problem of the etiology and biological impact of newly discovered viruses or viroids, and will thus generate invaluable information for fundamental research purposes (gaining insights into the development of viral/viroid diseases) as well as for applied purposes (establishing thresholds for the detection of viruses/viroids by NGS). The WG will focus on the characterisation of newly discovered viruses or viroids, their molecular biology, their interactions with vectors (insects or nematodes) and hosts, but also evaluate synergistic and/or antagonistic interactions between viruses/viroids in plants, which will be necessary to understand the impact and/or epidemiology of the diseases. The pathogen-vector-host triangle will be studied by analysing viromes under natural field conditions for economically significant agricultural crops for the EU, such as fruit trees, grapevine, potato, tomato, but also forest and urban greens which face the same problems, associating this research to currently funded projects. In addition, NGS will also be used to detect and study plant viruses or virus-like agents and their impact in different environments like waters or soils. The holistic character of the NGS detection should indeed enable the identification of new and known plant viruses in such matrices, in which they have been largely overlooked so far due to the limitations of the previous analysis methods (Mehle and Ravnkar, 2012).

Such characterisations might lead to a paradigm change in the way we consider the virus-host relationship, going beyond the pathogenicity and addressing other types of trophic relationships, with a shift from pathogens to pathobiome (Vayssier-Taussat et al., 2014).

The WG2 will provide their findings, especially in terms of new viral sequences, to WG3 in order to allow them to proceed with taxonomical propositions, and to WG4 for the rapid dissemination of important new findings.

WG3: Taxonomy and viral population genetics

Viruses have a high mutation rate within short generation time, consequently genetic variants are produced very often in infected hosts, resulting in a population with a high degree of diversity. This Action will provide an analysis of the intra-isolate, intra-host, intra-vector diversity, which will offer a global view of the viral diversity. This information will play a crucial role in the reliable recognition of virus- and virus-like sequences in NGS data-sets. The Action will also promote work on population genetics. On-going projects will be associated to the Action. Advances in virus taxonomy are expected from these results, especially with questions raised about the rapid pace of discovery of novel viral agents for which very little if any biological data is available and their

impact on viral taxonomy. Hence, the International Committee on Taxonomy of Viruses (ICTV) has recently accepted to include new viral species on the sole base of a complete genomic sequence, without any additional biological data. New collaborations between teams working on different host plants to study the host range of the virus species will be initiated, as well as cooperation with non-European countries for studies on diversity of the pathogens.

WG4: Regulatory and socio-economic impact

To make sure that the benefits of the Action will be as far-reaching as possible, WG4 will coordinate a “dissemination task force”. Risk-benefit analysis of the application of NGS technologies and subsequent data-analysis protocols will be performed. Risk communication activities will be shared with national and EU regulatory authorities such as Health and Consumers Directorate General of the EU and Panel on diagnostics and quality control of the European Plant Protection Organisation. Dissemination of the results obtained by the Action to stakeholders (growers, advisors, private companies, local and national governmental personnel, non-governmental parties) will be achieved through meetings, workshops and a dedicated website. The International Working group on Legume and Vegetable Viruses and similar working groups will be contacted to discuss the scientific position of NGS in the plant virus field and exchange opinions. The results of this Action will also be published in highly ranked journals, to obtain the best dissemination possible among the scientific community. Trainings Schools will be organised for NGS in diagnostics and plant-virus interaction to disseminate basic and advanced methodologies, targeting especially Early Stage Researchers and diagnosticians.

Altogether, the Action will bring together scientists from different horizons, with different and complementary expertise. The specific points addressed by each of the different WGs, the close and permanent interactions between them, will allow to address most efficiently an extremely serious, challenging and common threat.

E. ORGANISATION

E.1 Coordination and organisation

The initial participants involved in the development of this network are coming from 17 countries and include researchers from universities and national R&D institutions, SME, diagnostic laboratories, quarantine laboratories and expert scientists for National and European Plant

Protection Organizations. Participants all have an internationally recognized expertise in plant virology and/or in next generation sequencing.

The Action will provide a flexible framework open to any country and participant. It will allow the implementation of new ideas and activities during the period of the Action.

The organisation and management of the Action include the Management Committee (MC), the Steering Group (SG) and four Working Groups (WGs), responsible for the main tasks and also for the transverse networking activities. The MC will consist of up to 2 representatives from each participating COST country. The MC will meet twice a year (see timetable). The SG will be formed by the Action Chair and Vice-Chair, the 4 WG Leaders and the STSM Manager. The SG will meet twice a year and will also hold 2 to 4 skype conferences to implement at operational level the MC decisions, e.g. preparing and coordinating meetings, Workshops, STSMs and Training Schools, evaluating Action progress more regularly and verifying the adequate dissemination of the Action's results.

The basic and applied research necessary to achieve the tasks will be funded by the participating countries. The Management Committee will be responsible for the coordination of national research and COST budget will support networking and coordination efforts for implementing the Action. The Management Committee will evaluate the topics of the Workshops, Training Schools and conferences during the meetings along with the Scientific Management Committee which will be nominated for each event. The Scientific Management Committee will have the responsibility for the organization of the events by designing the program and selecting invited speakers and selection of students and Early Stage Researchers.

Milestones

M1. Kick-off meeting. Selection of the Management Committee (including the nomination of Chair and vice-Chair) and nomination of the Working Groups composition (month 3).

M2. Validation of methodologies to be used in WG1, WG2, WG3, and WG4 (month 3).

M3. Web site page (month 6).

M4. Draft of validated protocols for NGS analyses of viruses for fruit trees, grapevine, tomato, potato and forest trees in WG4.1 and WG4.2 (month 24)

M5. Identification of new viruses (month 36).

M6. Update of viromes in fruit trees, grapevine, tomato, potato and forest trees and identification of a first list of putative new emergent and invasive viruses (month 42).

M7. Genetic diversity and adaptive variability data for the selected species and populations (month 36).

M8. Database of intra-isolate and/or intra-vector diversity of viruses, for selected species (month

36)

M9. Joint workshop with scientists, stakeholders and policy makers for discussion and approval of recommendations and guidelines (month 44).

M10. Final conference (month 48)

M11: Decision schemes for the use of NGS in plant virus diagnostic (month 48)

M12: Publication on the scientific, regulatory and socio-economic impact of NGS in plant virology and recommendations based on scientific expertise gained during the Action (month 48)

Workshops and Training Schools will be organized according to the needs identified by COST Action participants.

E.2 Working Groups

The organization of WGs is described in detail above in Section D. Within and between the defined WG, the Action will be sufficiently flexible in terms of scope, memberships and activities.

There will be a WG vice-leader for each WG and the WG committees will include 4-8 participants (MC members).

The WG meetings are scheduled on a yearly basis in order to have an optimal exchange of ideas and knowledge, to discuss progresses and to plan future activities. Joint WG meetings will enhance integration of activities from different fields, and promote interface between WGs.

E.3 Liaison and interaction with other research programmes

The Action will actively seek collaboration with other projects and this will be facilitated by the fact that participants of this Action are also involved in these other projects. These connections will be used for discussion and exchange of knowledge on all related topics in particular on comparison of laboratory and bioinformatics workflows and in discussion of scientific position of NGS on different scientific events and by WebPages connections. As the consequence, these activities will also set the proper environment for giving birth to new collaborative Projects, for example within the H2020 framework. The list of these projects is detailed in section B5.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will

also be committed to considerably involve Early-Stage Researchers. This item will also be placed as a standard item on all MC agendas.

The Action does not settle for standard solutions in gender issues but puts a serious and concrete emphasis on promoting gender equality. For example, 50% of WG Leaders and committee members will be females, all meetings will be chaired by both a male and female participants, and the Chairs have special responsibility to promote the voice of less represented gender (e.g. by selecting equally many female and male speakers). The Action will also tender the Early-Stage Researchers in different ways, e.g., each senior participant is encouraged to bring to meetings early stage post docs, PhD or graduate students that work in relevant fields. Training Schools will be specifically announced to PhD students in different countries, and in scientific meetings, at least 1-2 speeches will be reserved particularly for Early Stage Researchers. The gender and age equality will be monitored by the Management Committee that commits to keeping the equality issues on agenda and encourages the members to actively work for promotion of equality.

F. TIMETABLE

		Year 1		Year 2		Year 3		Year 4	
WG	Action	1	2	1	2	1	2	1	2
1	WG1-meeting		X		X		X		X
1	Workshop	X		X		X		X	
1	Final meeting								X
2	WG2-meeting		X		X		X		X
2	Workshop	X		X		X		X	
2	Final meeting								X
3	WG3-meeting		X		X		X		X
3	Workshop	X		X		X		X	
3	Final meeting								X

4	WG4-meeting		X		X		X		X
4	Workshop	X		X		X		X	
4	Final meeting								X
1-4	Short Term Scientific Missions		X	X	X	X	X	X	X
1-4	Training School		X		X		X		X

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, CZ, DE, EL, ES, FI, FR, IT, NL, PL, PT, RO, SE, SI, SK, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 68 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The target audiences for the dissemination of the results of the Action include:

- Partners in the COST Action.
- International researchers working in plant virology (virus characterization, population genetics, plant-virus interaction, virus epidemiology, virus vectors, plant breeding and engineering) and in development of virus diagnostic tools (for plant, animal or human viruses).

- Early Stage Researchers working in plant virology and in development of virus diagnostic tools.
- National and European Plant Protection Organisation having the core mission of publishing recommendations and protocols for plant viruses control, including new and emerging ones, at the borders and within Europe and their respective countries.
- International working groups on plant virus research, i.e. International Council for the Virus and Virus-like diseases of the Grapevine ICVG, and other similar like PPV or citrus virus diseases.
- Policy makers at International, European, national government, and regional levels interested in adapting the policies to the scientific and technologic evolution for improving plant virus control.
- Diagnostic laboratories and quarantine laboratories as end-users of the technological diagnostic tools developed.
- Inspection agencies as end-user of the technological diagnostic tools developed.
- Industry (Seed producers, plant nurseries, farmers, forest owner and manager, providers of diagnostic kits)

H.2 What?

1. As any other Action, this Action will be accessible in outline in the main menu of the COST website.
2. A specific and dedicated website will be created in line with the best website organization of current and past COST Actions. The web site will provide general information on the Action (objectives, scientific program, Working Groups, Management Committee, participants, scheduled and past meetings, reports, publications, STSMs). More specifically, the web site will strengthen links with other relevant websites like those of EPPO, Q-bank, NPPOs, involved institutions and other

related EU-funded projects. Reciprocity will be asked to maximize audience, knowledge transfer and impact of the COST Action website.

3. The Action will organize themed Workshops and meetings. Some Workshops may be linked to European and International scientific conferences like the International Congress of Plant Pathology (ICPP), the International Plant Protection Congress (IPPC), the Conference of the European Foundation for Plant Pathology or the American Society of Phytopathology annual meeting. The integration of Action's workshop within larger conference and working groups like IWGLVV and PVY WIDE and Virology section in EAPR will broaden and accelerate the diffusion of the results of the Action to the targeted audience. At least one of the Workshops will be focused on the regulatory and socio-economic impact of the NGS in plant virus diagnostics.
4. Action results and Workshop or meeting proceedings will be translated into articles in relevant scientific journals or chapters in collaborative books. Open Access publishing will be preferred whenever possible to ensure accessibility.
5. Dissemination of results toward EPPO and NPPOs expert panels or International working groups on plant virus research will be done through participation and specific talks during the regular meetings of these panels. These talks will inform the experts on the scientific results of the Action and on their regulatory and socio-economic impacts for plant protection.
6. The Short Term Scientific Missions and the Training Schools will allow joint efforts to solve specific questions through strengthened exchanges. They will also promote rapid and direct diffusion of the acquired knowledge of the leading experts toward Early Stage Scientists or senior scientists with limited experience in NGS.
7. Scientists and, in particular Early Stage Scientists, will contribute to diffuse the results of the Action through social networking (Twitter, ResearchGate, etc.) towards targeted and general audience. Social networks used will be mentioned in the webpage.
8. A secured area within the website will be available for the participants of the Action to share document and not publicly available information, e.g. drafts of working documents or preliminary data.

9. Working documents, intermediary reports and final results will be published on the website.
10. A technical Workshop to disseminate the results of the Action will be addressed to the Heads of the National Reference Diagnostic for plant virus diagnostics of the EU and their officers, whatever their status (participant to the Action or not).
11. Specific recommendations based on the results of the Action will be addressed to the regulatory authorities (Food agencies) and inspection agencies, including National Reference Laboratories.
12. Targeted e-mails will be sent to interested partner in various research organizations (universities, research centres, private companies), in National or European Plant Protection Organizations, in Food security agencies to build awareness of the Action and to inform them on the schedules of upcoming events.
13. The Action will invite selected international experts from inside or outside Europe, especially from the USA, Russia, Mediterranean countries and China. This will also enhance the dissemination of the Action's results and the transfer of knowledge worldwide.

H.3 How?

The dissemination and transfer of knowledge is of crucial importance in COST Actions, as well as the utilisation of the results by the stakeholders, including scientists, diagnosticians, policy officers, panel experts and plant health inspectors. The methods of dissemination to these stakeholders summarized in H2 will be use within and outside Europe. Importantly, the participants to the Action are scientists and the majority of them are either experts in National or European Plant Protection Organisations, head of National Reference Laboratory for Plant Virus Diagnostic, advisors for food security agencies and inspection bodies or head of the National Quarantine Station. These affiliations, and the corresponding networks, will significantly enhance the effectiveness of the dissemination throughout the stakeholders (see table).

Methods from H2	How would it be done
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1, 2, 8 & 9	Creation and maintenance of the website
3	Organisation by the MC (Participant belongs to organizing committee of international conferences)
4	Coordinated work of MC and involved scientists
5	Several participants are experts in NPPOs and EPPO panels and in International working groups on plant virus research
6	Call to participant for STSMs and Training school by the MC. Selection of the granted proposals
7	Website and already identified “social-network friendly” participants and ESR
10 & 11	Announcement through mailing list from participants who are members of Panels and through EPPO secretariat, who head quarantine and/or National Reference Diagnostic Laboratory. Request for a themed workshop with EPPO.
12 & 13	Based on the large network of each Participant, coordinated by the MC

The Management Committee will be responsible to ensure the smooth functioning of the COST Action and the dissemination of the results. The Action progresses will be checked at each meeting with specific focus on the dissemination plan. The dissemination strategy will be reviewed and updated regularly by the MC depending on the progresses of the Action.