

# COST

Domain Committee "Food and Agriculture"

## COST Action FA0806

Start Date (30/03/2009)

End Date (30/09/2013)

Plant virus control employing RNA-based vaccines:  
A novel non-transgenic strategy

## FINAL EVALUATION REPORT

This Report stems from the relevant Domain Committee.  
It contains four parts:

- I. Management Report prepared by the COST Office/Grant Holder*
- II. Scientific Report prepared by the Chair of the Management Committee of the Action.*
- III. Evaluation Report prepared by the "ad hoc" Evaluation Panel, established by the Domain Committee, and edited by the COST Office.*
- IV. DC General Assessment prepared by the Domain Committee*

### **Annex:**

- Part I:** Innovative knowledge resulting from COST networking through the Action
- Part II:** Collection of viral strains on which the relevant research work has been performed
- Part III:** Grants obtained by members of FA0806 Action
- Part IV:** List of COST FA0806 associated labs hosting ESRs working in relevant fields
- Part V:** Publications
- Part VI:** COST FA0806 Pamphlet
- Part VII:** List of inducer molecules tested
- Part VIII:** Map of COST FA0806
- Part IX:** Recommendations for end-users

Confidentiality: the documents will be made available to the public via the COST Action web page except for chapter *II.D. Self-evaluation* and *III. Evaluation Report*.

**Executive summary of the Scientific Report (max.250 words):**

COST FA0806 (PlantiVax) brought together researchers from academia and industry, working in plant virus control. The members of the three Working Groups (WG) reached significant research achievements towards developing the innovative strategy of controlling plant viruses by 'RNA-based vaccines' and enhanced our knowledge on the: molecular plant-virus interaction, production/purification of dsRNA molecules, movement of siRNAs in plants, determination of target genes for silencing and the diversity/origin of prevalent viral strains. The many pathosystems studied reflect the wide range of application the 'RNA vaccination' strategy may have.

FA0806 implemented all available tools focusing its activities on Early Stage Researchers (ESRs). There were 25 STSMs (including one RSTSM and one nonCOST STSM) and 6 Training Schools (TS) performed during the four years. Three TSs were co-organized with other COST Actions. There were 6 well-attended MC meetings, always held in conjunction to either WG or an international conference. FA0806 has supported the participation of its members to international conferences in order to disseminate FA0806 and expand the collaborative network. The Action web site ([//costfa0806.aua.gr](http://costfa0806.aua.gr)) played a key role in communicating the aims, activities and workshop presentations of FA0806 to the wider scientific community.

The high number of publications produced by FA0806 members on the topic shows the scientific impact of their research. The network obtained a significant amount of funding to pursue research on RNA silencing. A coordinated effort of the research community such as the one performed in this Action could contribute towards protecting plant health, thus achieving food security and quality.

## Action FA0806 Fact Sheet

### Title

Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy

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### Details

Draft Mou:

Mou: 257/08

Start of Action: 30/03/2009

Entry into force: 23/01/2009

End of Action: 29/03/2013

CSO approval date: 24/11/2008

### Objectives

The main objective of this Action is to strengthen existing networks in order to develop suitable, efficient and cost-effective methods for reactive and proactive response to viral diseases of plants for a sustainable agriculture. Specific primary objectives of this Action are: 1. Co-ordinate frontier European research on plant virus control through gene silencing approaches. 2. Help develop novel non-transgenic control strategies for managing plant viral diseases in Europe. 3. Optimize protocols for high-throughput production and delivery of suitable resistance inducer molecules. 4. Transfer innovations to end-user groups (i.e. SMEs, plant protection organizations, growers groups). 5. Disseminate results via publication of all meeting proceedings, developing a web site with bibliography and announcements for long-term networking.

### Parties

Country	Date	Country	Date	Country	Date	Country	Date
Austria	10/06/2009	Belgium	15/09/2009	Bulgaria	17/03/2009	Croatia	20/06/2011
Cyprus	10/08/2009	Czech Republic	31/07/2009	Denmark	01/04/2009	Estonia	24/02/2009
Finland	24/02/2009	France	23/01/2009	Germany	23/01/2009	Greece	23/01/2009
Hungary	18/06/2009	Israel	26/01/2009	Italy	23/01/2009	Netherlands	03/02/2009
Norway	27/03/2009	Poland	25/02/2009	Portugal	16/02/2009	Romania	13/05/2009
Serbia	14/03/2011	Slovakia	13/05/2009	Slovenia	31/07/2009	Spain	25/02/2009
Sweden	22/04/2009	Switzerland	24/02/2009	Turkey	20/10/2010	United Kingdom	23/01/2009

Total: 28

### Intentions to accept the MoU

Country	Date	Country	Date	Country	Date	Country	Date

Total: 0

<b>Participating Institutions from non-COST countries</b>	
Argentina	Instituto de Biotecnologia. CICVyA. INTA
Argentina	Biotechnology Institute, Argentine Institute of Agricultural Technology (INTA)
Argentina	Instituto de Biotecnologia y Biologia Molecular (IBBM) CCT- La Plata CONICET
New Zealand	The New Zealand Institute for Plant & Food Research Ltd
South Africa	University of Cape Town
Mexico	Instituto Politecnico Nacional. CIIDIR Unidad Sinaloa
Australia	The University of Queensland
China	Hangzhou Normal University
Peru	International Potato Center (CIP)

<b>Working Groups</b>
WG1: Development of novel non-transgenic strategies for plant virus control WG2: Application of novel non-transgenic strategies for plant virus control WG3: Socio-economic evaluation of impact of the novel application methods

<b>Website</b>
<a href="http://www.aua.gr/COSTFA0806">http://www.aua.gr/COSTFA0806</a>

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## I.C. Overview activities and expenditure

DISSEMINATION	2011-09-09	(n.a.)	(n.a.)	Web page update		1000
DISSEMINATION	2011-09-30	(n.a.)	(n.a.)	Abstract book-Research presented until now in WGs		469,48
DISSEMINATION	2011-09-30	(n.a.)	(n.a.)	Publications in popular magazine and newspaper for national dissemination		938,97
DISSEMINATION	2012-03-29	(n.a.)	(n.a.)	Server development for Bioinformatics software (to be used by FA0806 members)		0
MEETING	2011-05-18	Vienna	Other COST relevant meeting	Small RNA microsposium	1	500
MEETING	2011-09-11	Montpellier	Core Group Meeting	Strategic meeting	12	5014,3
MEETING	2011-09-16	Sapporo	Other COST relevant meeting	International Congress of Virology	1	500
MEETING	2011-09-14	Montpellier	Working Group Meeting	WG1-3: 4th WG1, 3rd WG2 & 3rd WG3 meetings	41	31204,79
MEETING	2011-11-04	Munich	Workshops/Conferences	Workshop on Value Communication of Novel Agrotechnologies	9	5060,11
MEETING	2012-05-01	Antalya	Other COST relevant meeting	International Symposium on Biotechnology and other Omics in Vegetable Science	1	0
STSM	2011-08-31	Cambridge	STSM from Spain to United Kingdom	Role of ARGONAUTE proteins in RNA silencing mediated antiviral defense against Plum pox virus infect	1	2500
STSM	2011-09-30	Wageningen	STSM from Slovenia to Netherlands	Training for different methods for high-throughput studying of plant-virus interaction	1	2200
STSM	2011-09-28	Basel	STSM from Greece to Switzerland	Small RNA detection in the interaction of CMV with tobacco upon application of dsRNA::CMV-CP.	1	2050
STSM	2011-11-17	Warsaw	STSM from Slovenia to Poland	Localization and interactions of proteins involved in host response to PVY infection	1	785
STSM	2012-01-20	Helsinki	STSM from France to Finland	Production of Banana streak virus dsRNA in vitro and in vivo	1	1300
STSM	2012-01-26	Basel	STSM from France to Switzerland	Validation of banana sRNA deep-sequencing	1	1250
STSM	2012-03-31	Strasbourg	STSM from Israel to France	Host expression of dsRNA targeting TYLCV CP and V2 for generating TYLCV- resistant tomato and Arabid	1	0
STSM	2012-05-03	Godollo	STSM from Italy to Hungary	Characterization of AGO-siRNAs complex involved in antiviral silencing	1	0

## **II. Scientific Report prepared by the Chair of the Management Committee of the Action (same layout as in the Monitoring Progress Report)**

describing results achieved during the Action operation in this period, in no more than 3 pages (the report is “cumulative”). All items listed in Sections A, B, and C, below, must be addressed.

Additional documentation such as extended scientific reports, proceedings of workshops, seminars or conferences may be provided separately as an annex to this report, and should be referenced in the report.

### **II.A. Innovative networking**

- *Innovative knowledge resulting from COST networking through the Action. (Specific examples of Results vs. Objectives)*

The scientific network of COST FA0806 links researchers from several EU laboratories with the aim to boost research on inducing anti-viral silencing in crops by the transient application of dsRNA, siRNAs and/or artificial small RNAs (collectively designated as “RNA-based vaccines”). FA0806 participants are focused in plant sciences, including plant molecular biology, molecular virology, plant pathology, plant physiology, plant breeding, plant biotechnology, organic chemistry, etc and have collaborated with scientists specialized in high-throughput methods for production and external delivery of RNAs onto plants.

Since novel biotechnological strategies are likely to be regarded as risky technologies by consumers, specialists on the value-laden risk and benefit communication are also taking part in the Action to inform the research community about the societal concerns and to help develop an effective communication strategy in an open-minded and transparent way.

The Working Groups of the Action have accomplished significant research achievements that boost progress in developing the innovative strategy of controlling plant viruses by ‘RNA vaccines’, the main goal of this Action. These research achievements enhance our knowledge on: the molecular events during plant-virus interaction, the production/purification of dsRNA molecules, the movement of siRNA in plants, determination of possible target genes for silencing and ‘hot spots’ for siRNA production as well as the diversity and origin of prevalent viral strains. Work has included studies with many different viruses in several pathosystems.

In the life-span of the Action strong research ties were developed by several research groups such as AUA-UniBas, UniBas-CIRAD, NIB-PAS, NIB-WUR, AIPlanta, IVV-CNR (IT)/ABC (HU).

In the list below, the research topics of the FA0806 participants are summarized, describing the innovative knowledge acquired in their labs. In parentheses, the number of the specific example described in **Annex Part I** is provided.

1. The molecular events during plant-virus interaction:
  - a) The movement of siRNAs in plants (43).
  - b) The impact of virus infection on host’s short RNA profile (15, 32, 33, 52, 54).
  - c) The effect of virus infection on plant mRNAs (56) & miRNAs (29, 42).
  - d) The Banana streak virus genetic information in banana genome (45, 47).
  - e) *In vivo* RNA labeling (30).
  - f) The novel host protein (CDC48) interaction with viral movement protein at viral inclusion bodies formed at the ER (16, 51).
2. Studies on RNA dependent RNA polymerases (RdRPs) (1).
3. The RNAi inducer molecules:
  - a) The dsRNAs (2, 13, 14, 25, 31, 41, 53).
  - b) The siRNAs (2, 10, 49).
  - c) The amiRNAs (2, 20).
  - d) The defective DNA (34).
  - e) The hairpin molecules (18, 38).
4. The development of new purification platforms of RNAi inducer molecules (44).
5. The delivery machinery (3, 7, 11, 23, 27).
6. The mechanism of RNA silencing (4, 6, 8, 22, 61).
7. The viral siRNAs & Next generation sequencing (5, 32, 37, 46).

8. The viral silencing suppressors (17, 24, 26, 39, 40).
9. The viral expression systems (21, 60) & Virus Induced Gene Silencing (VIGS) (36, 59).
10. The DNA methylation (35).
11. The genetic variability of several plant viruses (9, 12, 28, 48, 50, 55).
12. The generation of viral infectious clones (57).
13. The diagnosis of plant virus (19).

A list (database) of viral isolates/strains available in the participating FA0806 laboratories has been created (secondary objective no2) (**Annex Part II**). Such a database of well characterized or reference viral isolates will be highly useful to all participants in the future research activities. The material could be available upon request to the owner. Each virus entry in the list is accompanied by the reference, sequence data, and certain main epidemiological data (original host/county/year of isolation and virulence).

COST FA0806 has contributed in networking and scientific collaboration between research Institutes in EU countries and Israel, resulting in the initiation of test vaccination employing PepMV vaccine (named PMV<sup>®</sup>-01) against PeMV in field trials (**Annex Part I**).

- **Significant scientific breakthroughs as part of the COST Action. (Specific examples)**

Through the **collaboration of COST FA0806 associated labs in the frame of STSMs**, the RNA-based vaccination (RbV) strategy was applied in several pathosystems. Work carried out involved the identification of putative effective targets for RNA-based vaccines (RbVs), the efficient production and purification of RbVs, as well as the investigation of the molecular events triggered by the application of these vaccines in the pathosystems under study. Novel knowledge has been acquired regarding various aspects of the RbV strategy, **supporting the development of non-transgenic control strategies for managing plant viral diseases in Europe (primary objective no 2)**.

**Specific results** of this work are listed below:

- New composite RNA-based vaccines were designed and produced against *Cauliflower mosaic paparetrovirus* (CaMV) for testing in Arabidopsis. Production was based on *in vitro* protocols taught in the 1<sup>st</sup> TS of FA0806. These vaccines were further 'enhanced' by incorporating components derived from host target-genes known to support viral infection. This study investigated also possible regulation of these host genes via RNA silencing and the modulation of this regulation by the plant viruses. (STSM: COST-STSM-FA0806-6460)
- RNA-based vaccines derived from selected viral sequences were developed against the EU quarantine virus: *Citrus tristeza virus* (CTV), using an *in vitro* rapid and robust methodology. These products are being applied on CTV-infected Mexican Lime trees and evaluation of their application is in process. (STSM: COST-STSM-FA0806-6873)
- An *in vivo* (in specialized bacterial cells) dsRNA production system was optimized in order to produce an RNA-based vaccine against the *Cocksfoot mottle virus* (CfMV). Innovative materials were used in the process of cleaning of dsRNA derived from the CfMV. (STSM: COST-STSM-FA0806-6806). High quantity of dsRNA targeting P1, the RNA silencing suppressor of CfMV, was achieved using the *in vitro* system developed in Bamford's lab. Inoculation of this dsRNA together with CfMV reduced in some experiments the systemic infection rate of the virus in oat plants.
- Preparation of small RNA libraries from tobacco plants after the application of a *Cucumber mosaic virus* (CMV)-derived RNA-based vaccine, and use of this library for deep sequencing, with the aim to elucidate the underlying mechanism that triggers a resistant plant phenotype. Bioinformatics analysis of the obtained results is ongoing. (STSM: COST-STSM-FA0806-6840)
- Standardization of RNA extraction protocols to obtain good quality RNA and analysis of virus-derived small RNAs from banana plants infected with *Banana streak virus* was achieved and has paved the way for downstream experiments either northern blotting or deep sequencing. (STSM: COST-STSM-FA0806-7200)
- Characterization of the *Sweet potato chlorotic stunt virus* (SPCSV) RNase3 was performed to elucidate its function in the RNA silencing mechanism. The study aimed to identify the activities of RNase3 and its cleavage deficient mutant on several putative substrates *in vitro* employing artificial dsRNA, siRNA, miRNA with different chemical modifications. Advanced

methodology including Electrophoretic Mobility Shift Assay (EMSA) and combination of *in vitro* binding studies with immunoprecipitation of the defective mutant were used to “fish” targeted endogenous small RNA (sRNA) species from total plant RNA. (STSM: COST-STSM-FA0806-7153)

- Application of laboratory-scale (*in vitro*) and industrial-scale (*in vivo*) techniques to produce dsRNA and small interfering RNA (siRNA) fragments based on specific *Pepino mosaic virus* (PepMV) sequences, as an attempt to develop a safe method to control PepMV in tomato crops. (STSM: COST-STSM-FA0806-7420)
- Molecular characterization of vsiRNAs to elucidate their function in the *N. benthamiana*-CMV pathosystem. The methodology used was a VIGS-based genetic dissection of *N. benthamiana* RNA silencing factors involved in antiviral RNA silencing and in Sat-RNA-driven silencing of the endogenous gene. The same system was tested on Arabidopsis plants, mutant for the given RNA silencing host factors (RDRs, DCLs and AGOs), to confirm the data acquired in the *Nicotiana* system. (STSM: COST-STSM-FA0806-7618)
- Investigation of the reasons why *N. benthamiana* plants accumulate a higher amount of 22nt vsiRNAs when they are infected with Cym19stop rather than with CymRSV strains. (STSM: COST-STSM-FA0806-7675)
- Application of methods already established for high-throughput evaluation of expression of genes in the interaction of potato-*Phytophthora infestans*, to study genes in a new pathosystem: potato-PVY. Methods such as PVX agroinfection, VIGS (virus-induced gene silencing) and ATTA (*Agrobacterium* transient transformation assay) were tested with the aim to enable fast selection of appropriate targets for improving plants resistance to viruses using non-transgenic approaches. (STSM: COST-STSM-FA0806-8860)
- Investigation of the molecular mechanism underlying the plant resistance phenotype against *Cucumber mosaic virus* (CMV) observed when the exogenously applied dsRNA derived from the gene encoding the CMV capsid protein (dsRNA:CMV-CP) was applied to plants. Detection and quantification of the short interfering RNAs (siRNAs), the hallmarks of RNA silencing produced during the interaction of tobacco with CMV, was achieved by northern blot and probe hybridisation. (STSM: COST-STSM-FA0806-8835)
- Identification of the AGO proteins involved in antiviral defence against infection by *Plum Pox Virus* (PPV). Understanding what, how and when the AGO proteins are involved in the antiviral defence against PPV opens the way to develop new plant resistance strategies. (STSM: COST-STSM-FA0806-8185)
- Various protein kinases involved in potato response to PVY infection were studied. After cloning into several different vectors, the localisation and possible interactions between selected proteins were observed. The study was performed using two methods for transient transformation of plants (biolistic bombardment and agroinfiltration) and confocal microscopy. (STSM: COST-STSM-FA0806-9280)
- High quantity of dsRNA targeting different *Banana Streak Virus* (BSV) intergenic regions using the *in vitro* production system developed in D. Bamford’s lab was achieved. Such molecules are to be tested for particle bombardment of BSV-infected *M. acuminata* banana plants in order to see whether this non-transgenic strategy is efficient to trigger BSV-directed resistance in banana plants. (STSM: COST-STSM-FA0806-9569)
- In collaboration with Dr L. Farinelli (Fasteris SA), analysis of deep sequencing data from banana was performed creating a complete map of *Banana Streak Virus* (BSV) and eBSV siRNAs. Bioinformatics analyses are on the way and indicate that vsRNA produced in virus free PKW (BB) are enriched in 24nt class whereas vsRNAs produced in BSV infected Cavendish banana plants (AAA) are enriched in 21nt class. Validation of the sequencing data was performed via PCR and Rolling circle amplification (RCA). (STSM: COST-STSM-FA0806-9439)
- Production of dsRNA molecules against TYLCV was achieved. Two methods (agroinfiltration and particle bombardment) to establish TYLCV infection in tomato have been tested but proved unsuccessful. The study of the sub-cellular localization of TYLCV proteins has been initiated. To this aim, TYLCV-encoded proteins were fused to fluorescent proteins and ectopically expressed in *N. benthamiana*. Upon expression of the fused TYLCV proteins, the analysis has led to test the involvement of the cytoskeleton and the secretory pathway in V2 interaction with tomato, the natural host of TVLCV. (STSM: COST-STSM-FA0806-9541)

- Experiments were performed on the plants *N. benthamiana* wild type and RDR6i, which were sap inoculated with *Cymbidium ring spot virus* (CymRSV), or with its relative virus unable to express the silencing suppressor p19 (CymRSV 19stop). siRNAs libraries were generated and indexed by following the instruction provided by Illumina. The libraries are being further analysed for quantification before sending them to Illumina sequencing. Protein analyses of the immuno IPs has also been performed and are under further analysis. (STSM: COST-STSM-FA0806-10060)
  - A modified protocol was developed regarding the *in vitro* RISC system, which allows the isolation of evacuated protoplasts from a special cell line (BY-2) of *Nicotiana tabacum*. This is a crucial step of the method, because in contrast with other organisms, plant cell extracts contain nonspecific nucleases and proteases derived mainly from vacuoles, which make difficult to recapitulate the examined molecular processes. The use of tobacco BY-2 evacuated protoplasts for the study of *in vitro* system allows the investigation of molecular mechanisms, such as plant RISC formation. In addition, the effect of different viral silencing suppressors on the RISC machinery was examined. (STSM: COST-STSM-FA0806-11410)
  - Effort was made to: a) silence PDS via external application of dsRNA::PDS on *A. thaliana*, and compare PTGS efficiency between wild type plants and enhanced PTGS mutants, b) silence PDS via the application of TRV::PDS on *A. thaliana* and its PTGS enhanced mutants, c) test resistance of *A. thaliana* and its enhanced PTGS mutants to CMV infection. Based on symptomatology, *A. thaliana* mutants *cbp80* and *se* were found to be CMV resistant and highly resistant, respectively. In contrast, *eri1* and *dhh1* were more susceptible to CMV as compared to Col plants. Mutants *xrn4*, *dcp1*, *mtr4* exhibited similar to Col response against CMV. (STSM: COST-STSM-FA0806-10648)
  - An RSTSM was performed during which two lectures (the first, on the 600 nt long leader of *Cauliflower mosaic virus*; the second, on the observation that almost all Cassava plants in Southern India are infected with *Sri Lanka Cassava Mosaic Virus*) were delivered and were intensively discussed. Interesting discussion was triggered related to the mechanism of translation in plants and to the characterization of plant viruses in the field via modern techniques (mass spectroscopy, dsRNA isolation and deep sequencing. (This is a RSTSM). (STSM: COST-STSM-FA0806-11562)
  - The characterization of AGO-siRNA complex involved in antiviral silencing was studied. (STSM: COST-STSM-FA0806-13361)
  - The operation of Plant Scanalyzer 3D (LemnaTec, DE) and the data obtained from images collected by the machine were analysed. In this way the Phenotyping facility at Agrobios was made familiar to the STSMee. Possible collaborations in phenotyping plant infected with viruses and treated with dsRNA was discussed. Furthermore, the use of tilling platforms developed at Agrobios was discussed for future collaboration. (STSM: COST-STSM-FA0806-14660)
  - The initial steps in the study of DNA methylation in CMV-infected tobacco were performed. The MeDIP protocol developed at FMACH (IASMA, Trento, IT) was followed in order to prepare the libraries for next generation sequencing. (STSM: COST-STSM-FA0806-14661)
  - Bayesian network modeling was applied to integrate experimental time-course omics data of plant-virus interaction to the existing qualitative model of plant defense signaling. (STSM: COST-STSM-FA0806-14881)
  - Taking advantage of deep sequencing of virus-derived siRNA for virus detection. The work included: a) Extraction method and subsequent preparation, and barcoding and bulking of low molecular weight RNA (siRNA) for Illumina GA II system sequencing. b) Analysis of the information obtained using bioinformatics in order to assemble complete viral genomes of viruses previously uncharacterized. (STSM: COST-STSM-FA0806-15348)
- *Tangible medium term socio-economic impacts achieved or expected. (Specific examples)*  
 The project aims at exploiting scientific advancements in the field of plant-pathogen interactions for the development of a disease control strategy that is environmentally friendly and cost-effective. The RNA-based vaccination of plants to induce their resistance against viruses could be envisaged to become an integral part of a crop management strategy to secure plant health. This approach would reduce the need for application of pesticides against viral vectors, thus decrease the chemical residues in agricultural products intended for

consumption, the environmental impact, as well as the cost of agricultural production. Furthermore, due to the fact that the molecular base of the dsRNA-mediated resistance shares similarities between plants and animals, scientific breakthroughs in one field could promote interdisciplinary advancement. For instance, research performed by FA0806 participants on the anti-HIV gene therapy via RNAi could have a significant impact not only in medicine worldwide (providing an additional tool in controlling this devastating human disease) but also in plant protection.

- *Spin off of new EC RTD Framework Programme proposals/projects. (List)*

Promoting collaboration among interested parties has been a high priority of FA0806 Action (secondary objectives no 5, 6 and 7). During the meetings of the Management Committee and the Working Groups, there have been several opportunities to discuss collaboration and joint proposals to funding calls. In addition, all STSMs have created strong links between the participating laboratories, and there are many ongoing experiments that are performed in close collaboration of FA0806 researchers as well as the ESRs in their groups. In parallel, FA0806 members have contribute into the FP8 planning by proposing to national representatives in Brussels, a list of interesting topics as a fact sheet to impact the future calls on plant viruses. Programme proposals/projects developed during the FA0806 Action include the following:

- CNRS-AUA-Syngenta project: “Application of dsRNA vaccination for non-transgenic RNA silencing in crops”, EUR 259.000 (successful, period: 2011-2012).
- Slovenia-Poland bilateral collaboration on “Study of gene expression on potato - PVY interaction” (successful, period: November 2010 to February 2011).
- Erasmus Mundus project (successful, period: 2013-2017).
- Melinda and Bill Gates Foundation grant entitled “Killing a virus with another virus? Tales of a suicidal virus”, proposed by Carl Spetz (Norwegian FA0806 MC member) and Dr Kreuze (Peru, nonCOST member), \$ 100.000 (successful, period: 2012-2013).
- FP7 proposal: “Development of cost-effective biocatalytic production methods for industrial application of dsRNA-based bio-control agents to protect economically important crops against viral infections” (not funded, resubmitted at the beginning of 2011, not funded)
- Grant proposal to The Swedish Research Council Formas on: “Application of dsRNA for control of virus infections in sugarbeet”(not funded)
- Proposal for a new COST Action on a related topic, but beyond that of the present FA0806 Action, is under development.
- ERC Consolidator grant application “Multiscale systems analysis to reveal key components of potato defence signalling” (qualified for the second stage of the evaluation). Slovenian national project “Functional genomics of potato-PVY interaction” (funded 2011-2014).
- Project C.I.S.I.A.Genes and mechanisms for mediterranean plant degfence agaist pathogens - Italian Ministry of Economy (Successfull 2011-2013);

- *Spin off of new National Programme proposals/projects. (List)*

See **Annex Part III** for a list of grants by COSTFA0806 participants (secondary objectives no 5, 6 and 7).

## **II.B. Inter-disciplinary networking**

- *Additional knowledge obtained from working with other disciplines within the COST framework. (Specific examples)*

The FA0806 Action supported the participation of its members to international scientific fora, so that they disseminate Action’s objectives, publicize their scientific results obtained in the frame of the Action and interact with the wider scientific community working in the field to get feedback as well as to incorporate scientists from disciplines that could promote Action’s aims. In specific:

- During the FA0806 WGs meeting in Helsinki (FI), researchers working in different fields of **biological** and **socio-economic sciences** had the opportunity to interact. Socio-economists illustrated approaches for cost-benefit analysis of the novel method proposed in the project and strategies for effective dissemination of scientific information to the general public, national officers, end-users and media. Participants working on **human or insect viral**

- pathogens** found common grounds for collaboration with **plant virologists** using tools developed by researchers in biotechnology and biotech companies.
- The FA0806 WG meeting in May 2010 was held in conjunction to the **2<sup>nd</sup> ESF-EMBO Conference** entitled “Antiviral applications for RNA interference” (May 2010, Sant Felieu, Spain). The **ESF-EMBO conference was focused** on antiviral means based on RNA interference in diverse pathosystems (e.g. Human-HIV).
  - The FA0806 participants have linked this COST Action with **relevant projects** in which they participate i.e. **SHARCO** project, **SCIROCCO** etc). As an example, FA0806 website was linked with the SHARCO project (funded by EU's FP7).
  - The International BARD Workshop entitled “Induction and suppression of RNA silencing: insights from plant viral infections”, held in Dan Panorama, Eilat, Israel (March 14-17, 2010), was attended by Dr H. Huet. The workshop was focused on **plant silencing, silencing in Human**, amplification and secondary production of **siRNAs in planta**.
  - The meeting entitled “Plasmodesmata 2010”, held on 21-26 March 2010 at the Q-Station in Manly (Sydney), Australia, was attended by Dr Manfred Heinlein. The meeting was focused on structure, function and role in intercellular communication of **plasmodesmata**, cellular pathways for viruses, **silencing signals** and other macromolecules.
  - The EMBO Workshop “Genomic approaches to interactions between plant viruses, their hosts and their vectors” held on 12-16 June 2010 at Fenestrelle, Italy, was attended by Assistant Professor A. Voloudakis and Dr G. Krczal. The workshop focused on **plant molecular biology**, plant-virus interactions, **genome sequence** of plant viruses and of several hosts, high-throughput technologies for **proteomics, functional genomics** to dissecting molecular interactions among plant viruses, their hosts and vectors.
  - The 11<sup>th</sup> International Plant Virus Epidemiology Symposium held in Ithaca, USA (June 20-24, 2010) was attended Prof. J. Valkonen. The symposium was focused on **virus epidemiology**.
  - The “International Congress of Virology” organized by the International Union of Microbiological Societies (IUMS), held in Sapporo, Japan (September 11-16, 2011), was attended by Dr Manfred Heinlein. The congress was focused on **host responses** and resistance to plant viruses, **virus suppression** of RNA silencing, **virus movement** in plants and **viral reproduction and translation**.
  - The “6<sup>th</sup> Microsymposium on Small RNAs” organized by Javier Martinez (IMBA), held in Vienna, Austria (16-18 May 2011), was attended by Dr Andreas Voloudakis. The symposium was focused on **RNA silencing** and application of small RNAs as inducer of the RNAi molecules in **animals and humans**.
  - The “International Symposium on Biotechnology and other Omics in vegetable science” organized ISHS, held in Antalya, Turkey (29 April - 1 May 2012) was attended by Prof. A. Voloudakis (EL). The symposium was focused on **vegetable crops and biotechnology**.
  - The “Plant RNA workshop” organized as a satellite meeting to ICAR 2012, held in Vienna, Austria (8-9 July 2012), was attended by Prof. Thomas Hohn (CH). The workshop was focused on high-throughput technologies for the analysis of **plant transcriptome and systems biology**, transcriptome sequencing, nonsense mediated decay, **alternative splicing**, plant microRNAs, RNA process and modifications, ribosome and nascent enzyme structures.
  - The “Plant Biology Congress” organized by FESPB and EPSO, held in Freiburg, Germany (29 July-3 August 2012) was attended by Prof. Erkki Truve (EE). The congress was focused on **plant biology**.
  - The “International Plant Molecular Biology Congress” organized by the Korean Society for Plant Biotechnology (KSPBT), held in Jeju, Korea (21-26 October 2012), was attended by Dr Manfred Heinlein (FR). The congress was focused on **plant molecular biology**.
  - The “International Citrus Congress” organized by International Society of Citriculture (ISC), held in Valencia, Spain (18-23 November 2012), was attended by Prof. Andreas Voloudakis (EL). The congress was focused on **citrus biology**.
  - The “7<sup>th</sup> EPSO conference” organized by EPSO, to be held in Porto Heli, Greece (1-4 September 2013), to be attended by Prof. Andreas Voloudakis (EL). The meeting is focused on **plant sciences**.
  - The EMBO Workshop “Green viruses, from gene to landscape” held on 7-11 September 2013 at Hyères-les-Palmiers, France, was attended by many members of FA0806. The



workshop focused on **translation, replication and recombination, intra- and inter-host movement, RNA silencing, resistance mechanisms other than silencing, epidemiology, population genetics and evolution, molecular ecology.**

- *Evaluation of whether the level of inter-disciplinarity is sufficient to potentially provide scientific impacts. (Specific examples)*

The scientific network built through the Action involves specialists working in diverse aspects of plant-virus interactions. A wide range of plant DNA and RNA viruses are under study by scientists of FA0806 consortium and this research has also benefited from the achievements in the respective field of human viruses that are brought to the attention of the Action by participants working in the animal health sector. Moreover, participants focused on the plant biology contributed in filling knowledge gaps regarding the host responses to viral pathogens. Besides, the Action included participants working on innovative material to transfer the 'RNA vaccines' to the host (nanotechnology) as well as development and optimisation of high throughput machinery to deliver these vaccines into plants. More interdisciplinary interaction (through trans-COST activities-meetings and training schools) has been sought through collaboration with scientists from other COST Actions, i.e. Staseq (TD0801), INPAS (FA0605), PlantEngine (FA1006), Molecular Farming (FA0804), BIOFLAVOUR (FA0907) and SeqAhead (BM1006). Training Schools and Workshops organised in the frame of FA0806, covered divers aspects of plant-virus interactions, from 'wet lab' and '*in silico*' methodologies, to field application technology, science commercialisation and science communication. Furthermore, interdisciplinary interaction was achieved by a Reciprocal STSM from Switzerland to New Zealand, during which research results with CaMV and cassava viruses were communicated (two lectures) overseas and induced fruitful discussion resulting in new ideas about future research directions. One of the lectures was video-conferenced to other New Zealand research Institutes. Thus, there has been sufficient level of inter-disciplinarity within FA0806 to accomplish the Action's tasks. Moreover, the involvement of a biotech company in the WG2 of the Action supports a practical exploitation of this research to the benefit of crop plants that are hosts to pathogenic viruses.

- *Evaluation of whether the level of inter-disciplinarity is sufficient to potentially provide socio-economic impacts. (Specific examples)*

The Action seeks to investigate whether host innate resistance mechanisms towards pathogenic viruses can be enhanced with the application of RNA vaccines as an alternative to the well-established plant genetic modification method for protection against viruses (virus resistant transgenic plants). This would have a direct effect on crop produce, alleviating the need for pesticide application, and thus reducing crop production costs and supporting environmental conservation and biodiversity.

The scientific results derived from FA0806 participants related to plant viruses control have been disseminated to stake holders through established contacts of FA0806 participants with NPPOs, biotech companies, nurseries, growers associations, etc. (see Contacts with stakeholders at § "Advancement and promotion of scientific knowledge through publications and other outreach activities" below). In addition, Prof. Anders Kvarnheden (SE) reported projects on viruses infecting cereals and forage grasses that have been carried out in close collaboration with the Swedish Board of Agriculture and farmers. In two workshops dedicated to science communication and commercialisation, WG3 participants have been working in developing a strategy to promote the scientific findings and achievements regarding plant protection. Such commercialization strategies employed by Action members could eventually ensure Action's socio-economic impact to the benefit of agriculture.

## **II.C. New networking**

- *Additional new members joining the Action during its life.*

Colleagues in 14 COST countries supported the proposal for COST FA0806 initially. The active efforts from the Action Chair and the initial FA0806 members resulted in the participation of 28 countries, 63 MC members and substitutes. Furthermore, Institutions from 7 non-COST countries (Argentina, New Zealand, South Africa, Mexico, Australia, China, Peru) have been

collaborating with FA0806. Scientists from Japan, Morocco and Korea have expressed their interest in FA0806 Action's objectives. However, the process of becoming nonCOST members in FA0806 was not completed. Effort has also been put into expanding the network of the Action to incorporate members in the working groups from the already participating countries. Through contacts with ERA (as shown below), FA0806 members had the opportunity to promote the Action and interact with other scientists working in the field.

- *Total number of individual participants involved in the Action work. (Number of participants. Give % of female and of Early Stage Researcher participants)*

During the life of FA0806, 63 MC members and substitutes have been involved in the Action. 41% of them are female.

Regarding participation in the FA0806 activities, there are the following records:

- In the WG meetings, there have been 81 female (30%) in a total of 269 participants.
  - In the Training schools (TS), there have been 42 female (52%) in a total of 81 trainees. ESRs have been 65%.
  - In the Workshops, there have been 5 female (22%) in a total of 23 attendees.
  - In the 25 STSMs implemented there were 11 performed by female (44%) and 15 were performed by ESRs (60%).
- *Involvement of Early Stage Researchers in the Action, in particular with respect to STSMs, networking activities, and Training Schools. In addition, justification should be provided if less than 4 STSMs were carried out during the year.*

After the establishment of the FA0806 STSM assessment committee, the criteria for selection of STSM grantees (candidate early-stage researchers [ESRs]) were decided and announced in the Action's webpage. FA0806 has encouraged the participation of Early Stage Researchers through all available COST tools (secondary objective no4). The **STSM** tool has been promoted in all WG and MC meetings of the Action and MC members put a lot of effort to communicate this information to their work places as well as nationwide. As a result of an efficient coordination between the MC and the STSM committee, 25 STSMs took place as shown in the § "Activities and projects with COST network colleagues". The scientific achievements through these STSMs are significant and are described in § "Significant scientific breakthroughs as part of the COST Action". The **Training Schools (TS)** organised by FA0806 also listed in the § "Activities and projects with COST network colleagues", covered key aspects in the development and application of RNA vaccines, *i.e.* the bioinformatics approach to acquire the knowledge necessary to select putative targets for the RNA vaccines (TS in Athens), the cloning of siRNAs (TS in Cambridge) and the specialised machinery for the delivery of the vaccine molecules (TS in Tel Aviv). The TSs together with the STSMs have contributed to the training of young scientists at selected high-impact topics (secondary objective no 4). The content of the TSs, as described below, has supported the **optimisation of protocols for high-throughput production and delivery of suitable inducer molecules** (primary objective no 3):

- The TS "Double stranded RNA production for plant biotechnology" organised in Helsinki on 7 to 10.9.2009, enabled **25 ESRs** to access hands-on training on key molecular biology techniques for the implementation of the Action's scientific plan, *i.e.* a key approach for producing 'RNA vaccines'. The school also provided a unique opportunity for young researchers to communicate their projects and obtain feedback from FA0806 participants. FA0806 has also supported the Training School participants to attend the FA0806 1<sup>st</sup> WGs meeting, which took place in conjunction with the training school, to allow them to get the most out of their interaction with the scientists attending the meeting.
- The TS "Applied bioinformatics in Plant Sciences" was organized in Athens (GR) with the collaboration of three COST Actions - FA0604 (Tritigen), FA0806 (Plantivax) and TD0801 (Statseq) - together with the FP7 project TriticeaeGenome. The TS program provided **43 ESRs**, coming from various backgrounds of plant science or bioinformatics, with an in-depth overview on the most important issues relevant to current bioinformatics applications for handling mostly high-throughput biological data, enhanced their scientific competence for research and innovation and provided them with a critical advantage in their future careers towards industrial and academic research positions. To ensure a wider dissemination of the

course contents, it was distributed to trainees in electronic format (CD) and will be also uploaded on the COST Actions' website.

- The TS "Small RNA Cloning School" organised in Cambridge (UK), was a hands-on training course for construction of small RNA libraries for high throughput sequencing. The School was held in collaboration with the FP6 Integrated Project SIROCCO LSHG-CT-2006-037900, which conducts research on the role of small RNAs in eukaryotic gene regulation. The aim of bringing the SIROCCO and COST FA0806 consortia together was to strengthen existing networks and increase European cooperation and interaction in the RNA field. The interaction between members of these two groups during the course is expected to lead to lasting collaborations among the next generation of European RNA scientists. **Nine (9) ESRs** were supported by FA0806 to attend this course.
- The TS "Small to large scale vaccination of plants-A hands on training on the vaccine delivery technology" organized in Tel Aviv (IL) by Agrilogics Ltd was held immediately after the 2<sup>nd</sup> WG meeting to allow a better interaction of WG members and trainees. **Fifteen (15) trainees**, composed mainly of young scientists, and four trainers took part in this Training School, that included lectures, practical courses and a demonstration relevant to the delivery devices (Bim apparatuses) designed and developed by Agrilogics, as well as a visit to a commercial nursery.
- The TS "Next generation sequencing analysis methods for life sciences" was attended by **10 ESRs** gaining skills in bioinformatics analysis of deep sequencing results.
- The TS "Virus induced gene silencing in cereal and non-cereal plant species", was attended by **8 ESRs** gaining advanced knowledge in the cutting-edge area of VIGS technology.

FA0806 participants exhibited significant activity related to the training of ESRs on the topics covered by the Action during University teaching or summer schools. For instance, Prof A. Depicker was one of the lecturers in a European epigenetics summer school organised by Mette in Gatersleben (DE). Dr Kvarnheden described the COST project and the training opportunities provided to BSc and MSc students during teaching at Swedish University of Agricultural Sciences and Uppsala University (SE). Similarly, Dr Wassenegger was the lecturer of an advanced training course focused on: 'Application of RNA interference (RNAi) to analyze the function of plant genes' for students of the University of Heidelberg (DE). Besides, ESRs were encouraged to follow networking opportunities such as the Working Group meetings. A list of labs hosting ESRs working in relevant fields is shown in **Annex Part IV**.

- *Involvement of researchers from outside of COST Countries. (Number of participants from non-COST Countries approved by the CSO. Give % of such participants from countries with reciprocal agreements. Specify their contribution)*

Within the life span of FA0806, targeted contacts have been made with research scientists working in the field of RNA silencing, from nonCOST countries. **Argentina (AR), New Zealand (NZ), Mexico (MX), South Africa (SA), Peru (PE), China (CH), Australia (AU)** have established an agreement with COST and scientists from these non-COST countries have been associated with FA0806. All nonCOST FA0806 members have participated in FA0806 activities (mainly WG meetings). Scientists from **India, Cuba, Morocco** and **Korea** have also expressed their vivid interest in Action's objectives.

In specific: Dr Robin MacDiarmid (The New Zealand Institute of Plant and Food Research Ltd) participated in the 1<sup>st</sup> WGs meeting in Helsinki (2009); Dr R. Dietzgen (AU), Dr Assurmendi (AR), Dr K. Cronin (NZ) and Dr J. Mendez (MX) participated in the 3<sup>rd</sup> WG1 meeting in Neustadt (DE) (2010); Dr Kreuze (International Potato Center, Peru) and Dr Hong (Hangzhou Normal University, China) were invited in the annual Working Group meeting in Montpellier (12-14 September 2011); Dr Maria Laura Garcia (AR) and Dr Alejandro Fuentes (Cuba) were invited in the WG meeting in Ljubljana (SL) and presented her work in controlling citrus viruses in Argentina and his work on geminivirus, respectively. The above interaction with the nonCOST participants gave the opportunity to exchange information and discuss the possibility for future collaborations. Both the COSTFA0806 and the above non-COST participants stated that they benefited greatly from such focused meetings organized and the networking. Already a collaborative research has been established between Dr Carl Spetz (Norwegian FA0806 MC member) and Dr Kreuze. Prof. Bencharki Bouchaib from the University of Hassan 1<sup>st</sup> Settat in Morocco and Prof. Kook-Hyung Kim from Seoul National University in Korea, have made

application to join the Action. However, the procedure for these applications was not finalized due to time-restrictions.

- *Advancement and promotion of scientific knowledge through publications and other outreach activities. (Number of publications and other outreach activities that resulted from COST networking through the Action. Complete list should be given in an annex)*

#### **Publications**

A list of publications of the FA0806 participants is given in **Annex Part V**. They constitute **very high impact publications** in scientific journals and books or **communications in international congresses**, indicating the intense effort of the participants' groups in this research field. References regarding this new biocontrol method were included into the monograph of plant viruses published (in Romanian) by Dr. I.V. Pop. Collaboration between members is also indicated by the joint publications.

#### **Webpage of the Action**

The webpage of the Action (<http://costfa0806.aua.gr/>) was launched on May 1, 2009 and has been made freely accessible since its construction aiming at communicating Action's objectives and activities and thus attracting the interest of the scientific community, industry and policy makers. In the web page, information on the objectives of the project, the organization for the implementation of FA0806, the participants, details on all meetings and Training Schools (program, reports), and the Action's poster can be found. The webpage has been successfully used as the main tool for advertising all Action's meetings and training activities, and as an efficient interface for collecting applications and registration forms of participants in Action's activities. It is also used as a source for the poster and pamphlet of the Actions. A copy of the pamphlet can be found in Part VI of the Annex. A password-protected part of the webpage is being developed to facilitate voting between members and to host any exchanged material during the development of joint projects before submission. The site also provided information on participant research profiles in order to boost collaboration with scientists outside the FA0806 Action. Besides, the list (database) of the viral isolates/strains available in the participating FA0806 laboratories that has been created within the Action, including well characterized or reference virus isolates, is uploaded to the website (secondary objective no2). This database will serve as a base for research collaboration through exchange of viral strains and effort will be put to update this list even after the end the FA0806 Action.

Arrangements were made to maintain the Action web page in the server of AUA after the end date of the Action in order to allow long lasting interaction with colleagues worldwide that are interested in the novel method of plant virus control (RbV). The website will keep assembling scientific information on the plant gene silencing (secondary objective no1).

#### **Poster for the Action:**

The poster of the Action has been prepared and uploaded in the FA0806 webpage.

#### **Contacts in the ERA**

The Action has been linked with relevant projects to further support the scientific network opportunities:

- **ESF-EMBO:** A COSTFA0806 WG held jointly with the 2<sup>nd</sup> ESF-EMBO Conference in May 2010, in Spain. Dr V. Pantaleo (a member of FA0806) was awarded **the 1<sup>st</sup> prize** for the best poster presentation during the ESF-EMBO Symposium ('Antiviral Applications of RNA Interference').
- **ResistVir project:** Participants of COSTFA0806 are also members of the ResistVir project.
- **SharCo project:** The COSTFA0806 website has been linked with the SharCo project (funded by the seventh EU framework programme).
- **EFSA:** In 2010 Dr Miroslav Glasa was participating in the EFSA working group on *Plum pox virus*.
- **EPSO:** Prof. Andreas Voloudakis to be participating in the 7<sup>th</sup>EPSO conference (1-4 September 2013).
- **European Society of Virology:** Dr V. Panaleo is a member of ESV.

- **Ibero-American Network on Integrated Virus Disease Management for Vegetable Crops:** Dr Navas-Castillo has contacts with this network.

The **poster** of the Action has been presented in the following international scientific meetings:

- International BARD workshop: 'Induction and suppression of RNA silencing: insights from plant viral infections', Dan Panorama, Eilat, Israel (March 14-17, 2010).
- Plasmodesmata 2010, Manly (Sydney), Australia (21-26 March, 2010).
- EMBO Workshop "Genomic approaches to interactions between plant viruses, their hosts and their vectors" Fenestrelle, Italy (June 12-16, 2010).
- 11<sup>th</sup> International Plant Virus Epidemiology Symposium and 3<sup>rd</sup> Workshop of the Plant Virus Ecology Network, Ithaca, USA (June 20-24, 2010).
- The SharCo Research Workshop, organized together with the International symposium on *Plum pox virus* Sofia, Bulgaria (September 5-9, 2010).
- International Congress of Virology, held in Sapporo, Japan (11-16 September 2011).
- The "6<sup>th</sup> Microsymposium on Small RNAs", Vienna, Austria (16-18 May 2011).
- International Symposium on Biotechnology and other Omics in vegetable science, Antalya, Turkey (29 April – 1 May 2012).
- Plant RNA workshop, Vienna, Austria (8-9 July 2012).
- Plant Biology Congress, Freiburg, Germany (29 July-3 August 2012).
- International Plant Molecular Biology Congress, Jeju, Korea (21-26 October 2012).
- International Citrus Congress, Valencia, Spain (18-23 November 2012).
- 7<sup>th</sup>EPSO conference, to be held in Porto Heli, Hellas (1-4 September 2013)

The following list refers to **other selected scientific events** that members of the Action attended, reflecting their capacity for networking.

- SIROCCO 4<sup>th</sup> Annual Meeting (October 2010, Heidelberg, Germany)
- ESF-EMBO Symposium 'Antiviral Applications of RNA Interference' (May 2010, Sant Feliu de Guixols, Spain)
- EMBO/EMBL Symposium: 'Non-Coding Genome' (October 2010, Heidelberg, Germany)
- BioRN Biotech-Cluster, NTC Partnering Workshop and Annual Meeting (January 2010, Heidelberg, Germany)
- Expert discussion on virus induced gene silencing safety issues, Zentrale Kommission für die Biologische Sicherheit (Central Commission for Biological Safety) (February 2010, Berlin, Germany)
- 'Plant Biotechnology in Germany' Meeting (September 2010, Hannover, Germany)
- High Council for Biotechnologies Symposium on Emerging Biotechnologies (December 2010, Paris, France)
- SFB 648 Meeting on 'Communication in Plants and their Responses to the Environment' (May 2011, Halle, Germany)
- BioRN Biotech-Cluster, Annual Meeting (May 2011, Heidelberg, Germany)
- Plant Biotechnology in Germany (September 2011, Hannover, Germany)
- SFB 796 Meeting on 'Mechanisms of Viral Host Cell Manipulation - from Plants to Humans' (October 2011, Bamberg, Germany)

It should be emphasized that members' research teams have strong collaboration with national research entities of diverse disciplines, e.g. the Polish group led by Prof. Marek Figlerowicz, which include scientists from: Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan (discipline of molecular virology, plant molecular biology, RNA structure), Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw (discipline of plant molecular biology, plant physiology), Institute of Plant Protection, Poznan (discipline of plant virology), Plant Breeding and Acclimatization Institute, Mluchow (discipline of plant breeding, plant biotechnology), Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan (discipline of organic chemistry). In addition, there have been exchanges of biological material (viral strains) between members' lab and other scientists' lab outside FA0806 (e.g. between the Institute of Virology, Slovak Academy of Sciences, Slovakia and the Palacky University of Olomouc in Czech Republic).

### **Contacts with stakeholders:**

On a national level, effort has been put by participants to contact different types of stakeholders (governmental agencies; inter-professional associations; greenhouse farmers; small and medium enterprises) to inform them about the COST FA0806 objectives (primary objective no 4). Active attendance of FA0806 participants in an International agricultural exposition (Agrokomplex, Nitra, August 2009) also helped dissemination of the novel methodology proposed by the Action. Major companies in the field of agriculture and biotechnology are collaborating or in contact with COSTFA0806 participants. For example, Finnzymes Ltd. has partially financed the Training School in Helsinki, while others are working in partnership with participants' labs or developing common projects. Through all these contacts, FA0806 participants sought to stimulate authorities to consider the socio-economic impact of this new approach (RbVs) (secondary objective no 3).

In specific, contacts have been established with:

*Agricultural companies and farmers having an interest in FA0806 technologies. All listed companies are keen in testing 'RNA vaccines':*

- Plantex Ltd., Veselé pri Piešťanoch – fruit tree nursery, fruit production
- Agro- Mačaj, Ltd., Senec (Slovakia) – production of vegetables
- L.S.I., Ltd., Horné Saliby (Slovakia) – production of vegetables
- Szalay, Ltd., Veľký Biel, (Slovakia) – production of vegetables
- Asparagus, Ltd., Nové Zámky, (Slovakia) – production of vegetables
- TVO, Ltd., Bratislava (Slovakia) – diagnostic and biochemical trade
- Kientzler GmbH & Co. KG ([www.kientzler.de/english/index.html](http://www.kientzler.de/english/index.html))
- Enza Zaden (The Netherlands) - vegetable seed breeding company
- KWS SAAT AG (Germany) - plant breeding company

*Professional organisation having expressed their interest in FA0806 technologies:*

- Slovak Crop Protection Association
- Slovak Fruit Farmer Union
- Slovak Plant Health Society
- European seed association (Brussels)

*Biotech/Biopharmaceuticals companies as well as companies providing innovative technology:*

- Aboca (Italy, Spain, Poland) - phytotherapy related products
- Aelred SAS (France) - dedicated to targeted plant improvement
- Agritest S.r.l (Italy) - diagnostic kits trade
- AGROAXIS (Spain) - products and services of vertical greenhouses parks, pharmaceuticals and nutraceuticals
- BASF (Germany) - chemical company
- Bayer CropScience
- BIA Separations (Slovenia) - provider of the chromatographic media for RNA purification
- Fasteris SA (Switzerland) - deep sequencing and bioinformatics analysis services
- Febit Biomed GmbH
- Finnzymes Ltd
- InstrAction GmbH (Germany) -production of modern chromatographic phases
- Morflora Ltd. (Israel) - plant protection and enhancement solutions
- Biosphere- plant protection and enhancement solutions
- Syngenta (US) -chemical company, marketing pesticides and seeds
- Affimed Therapeutics AG ([www.affimed.com/](http://www.affimed.com/))-development of new therapeutics
- Agroaxis™ Park ([www.agroaxis.com/](http://www.agroaxis.com/))- commercialization of products and services of vertical greenhouses parks
- Curaxys ([www.curaxys.com/default.htm](http://www.curaxys.com/default.htm))-development of new therapeutics
- Institute for Clinical Research and Development ([www.ikfe.de/](http://www.ikfe.de/))-development of new therapeutics
- Agrilogics Ltd- optimization of the BIM machine
- Tivshtil-production of plant propagation material
- Qiagen

### Governmental organisations

- APHIS and APS (USA) - the IL-60 system is at its final stages to be considered as non-transgenic
- Turkish Ministry of Agriculture (Turkey)
- Participants from the National Institute of Biology in Slovenia are involved in decision and policy making in the field of GMO regulation on national, EU and international level.
- RLP AgroScience GmbH was visited by the Working Committee 'Agriculture and Environment', Social Democratic Party of Germany, in October 2011.

- **Activities and projects with COST network colleagues.**

As planned in the Workplan of each year, the consortium has successfully organized 7 Working Group meetings, 6 Management Committee meetings, 6 Training Schools, 4 Workshops, 25 STSMs, 1 Core group meeting and 1 Strategic meeting. Through these meetings dissemination of results to research community and end-user groups have been achieved (primary objectives no 4 and 5).

### Meetings:

- 1<sup>st</sup> MC meeting, Brussels, Belgium, 30.3.2009 (24 participants)
- 2<sup>nd</sup> MC meeting, Helsinki, Finland, 12.9.2009 (21 participants)
- 1<sup>st</sup> WGs meeting, Helsinki, Finland, 11-12.9.2009 (57 participants)
- 2<sup>nd</sup> WG2 meeting, Tel Aviv, Israel, 18/03/2010 (19 participants)
- 2<sup>nd</sup> WG1 meeting, Sant-Feliu de Guixols, Spain, 1/06/2010 (19 participants)
- 3<sup>rd</sup> WG1 & 2<sup>nd</sup> WG3 meetings, Neustadt, Germany, 20-21/09/2010 (30 participants)
- 3<sup>rd</sup> MC meeting, Neustadt, Germany, 20-21/09/2010 (17 participants)
- Strategic meeting I, Montpellier, France, 11/9/2011 (16 participants)
- 4<sup>th</sup> MC meeting, CIRAD, Montpellier, France, 14/9/2011 (21 participants, 14 countries represented)
- 4<sup>th</sup>WG1 and 3<sup>rd</sup> WG 2 & 3 joint meeting, CIRAD, Montpellier, France, 12-14/9/2011 (38 participants, 9 invited speakers, 21 countries represented)
- 5<sup>th</sup> WG 1 meeting held in Pultusk, Poland (13 June 2012).
- Core group meeting, held in Warsaw, Poland (15 June 2012).
- WG 1,2,3 meeting, held in Ljubljana, Slovenia (3-5 September 2012).
- 5<sup>th</sup>MC meeting, held in Ljubljana, Slovenia (5 September 2012).
- 6<sup>th</sup> MC meeting, to be held in Hyeres, France (14 September 2013).
- WG 1,2,3 meeting, to be held in Hyeres, France (11-14 September 2013).

### STSMs

1. COST-STSM-FA0806-6460 of Dr. Mikhail Shchepetilnikov  
From: Institut de Biologie Moléculaire des Plantes, UPR2357, Strasbourg (FR)  
To: Institute of Botany, University of Basel, Basel (CH), host laboratory co-headed by Prof. Thomas Hohn and PD Dr. Mikhail Pooggin.  
Period: 01/06/2010 to 06/09/2010.  
Topic: RNA-based vaccination against *Cauliflower mosaic virus*
2. COST-STSM-FA0806-6806 of Ms Birger Ilau  
From: Tallinn University of Technology, Tallinn, Estonia (EE)  
To: University of Helsinki, Helsinki (FI), host laboratory headed by Prof. Dennis Bamford  
Period: 02/08/2010 to 30/09/2010  
Topic: Production of dsRNA
3. COST-STSM-FA0806-6873 of Mr Tefkros Iacovides  
From: Ministry of Agriculture, Natural Resources and Environment, Department of Agriculture, Nicosia (CY)  
To: Agricultural University of Athens, Athens (GR), laboratory of Plant Breeding and Biometry, led by Andreas Voloudakis  
Period: 30/08/2010 to 09/12/2010

- Topic: Production of dsRNA for the silencing of *Citrus tristeza virus* Coat Protein, p20 and p23 genes.
4. COST-STSM-FA0806-6840 of Assistant Professor Andreas Voloudakis  
 From: Agricultural University of Athens, Athens (GR)  
 To: FASTERIS SA, Plan-les-Ouates (CH), host laboratory headed by Laurent Farinelli.  
 Period: 09/09/2010 to 30/09/2010  
 Topic: Deep sequencing of small RNAs of *Cucumber mosaic virus* (CMV) in the interaction tobacco/CMV/ dsRNA\_CMV.
  5. COST-STSM-FA0806-7200 of Dr Rajeswaran Rajendran  
 From: Institute of Botany, University of Basel (CH)  
 To: CIRAD, Montpellier (FR), host laboratory headed by Marie-Line Iskra-Caruana  
 Period: 16/11/2010 to 28/11/2010  
 Topic: Analysis of *Banana streak virus*-derived small RNAs
  6. COST-STSM-FA0806-7153 of Ms Isabel Weinheimer  
 From: Department of Agricultural Science, University of Helsinki (FI)  
 To: RLP AgroScience, GmbH AIPlanta-Institute for plant research, Neustadt un der Weinstrasse (DE), host laboratory headed by Gabriele Krczal  
 Period: 1/12/10 to 28/02/2011  
 Topic: Characterization of the viral class I RNase III substrate specificity.
  7. COST-STSM-FA0806-7420 of Ms Anneleen Paeleman  
 From: Scientia Terrae Research Institute (BE)  
 To: University of Helsinki, Helsinki, host laboratory headed by Prof. Dennis Bamford  
 Period: 17/1/2011 to 21/1/2011  
 Topic: Training dsRNA production systems
  8. COST-STSM-FA0806-7618 of Dimitris Kaloudas  
 From: Athens (GR)  
 To: Istituto di Virologia Vegetale (IT), host Vitantonio Pantaleo  
 Period: 01/03/2011 to 15/04/2011  
 Topic: Effect of *Nicotiana benthamiana* DICERS in symptom development in CMV-CMVsatRNA infections.
  9. COST-STSM-FA0806-7675 of Vitantonio Pantaleo  
 From: Istituto di Virologia Vegetale (IT)  
 To: Agricultural Biotechnology Center, Godollo (HU), host laboratory headed by Zoltan Havelda  
 Period: 07/04/2011 to 21/04/2011  
 Topic: Small non-coding RNAs in plant defense: strategies for helping plants against pathogens.
  10. COST-STSM-FA0806-8185 of Dr Carmen Simon Mateo  
 From: Centro Nacional de Biotecnologia (Madrid, Spain)  
 To: University of Cambridge (Cambridge, UK), hosted by Prof. Sir David Baulcombe  
 Period: 1/7/2011 to 31/8/2011  
 Topic: Role of ARGONAUTE proteins in RNA silencing mediated antiviral defense against *Plum pox virus* infection.
  11. COST-STSM-FA0806-8860 of David Dobnik (PhD candidate)  
 From: National Institute of Biology (Ljubljana, Slovenia)  
 To: Wageningen University & Research Centre (Wageningen, The Netherlands), hosted by Richard Visser  
 Period: 30/8/2011 to 30/9/2011  
 Topic: Training for different methods for high-throughput studying of plant-virus interaction.
  12. COST-STSM-FA0806-8835 of Dr Maria Holeva  
 From: Benaki Phytopathological Institute (Athens, Greece)  
 To: Institute of Botany, University of Basel (Basel, Switzerland), hosted by Prof. Thomas Hohn



- Period: 18/9/2011 to 28/9/2011  
Topic: Small RNA detection in the interaction of CMV with tobacco upon application of dsRNA::CMV-CP.
13. COST-STSM-FA0806-9280 of Ana Lazar (PhD candidate)  
From: National Institute of Biology, (Ljubljana, Slovenia)  
To: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Laboratory of Plant Pathogenesis (Warsaw, PL), hosted by Jacek Hennig  
Period: 10/11/2011 to 17/11/2011  
Topic: Localization and interactions of proteins involved in host response to PVY infection.
14. COST-STSM-FA0806-9569 of Matthiew Chabannes (Researcher)  
From: CIRAD (Montpellier, France)  
To: University of Helsinki (Helsinki, FI), hosted by Dennis Bamford  
Period: 16/1/2012 to 20/1/2012  
Topic: Production of *Banana streak virus* dsRNA *in vitro* and *in vivo*.
15. COST-STSM-FA0806-9439 of Pierre-Olivier Duroy (PhD candidate)  
From: CIRAD (Montpellier, France)  
To: Botanisches Institute der Universitat Basel (Basel, CH), hosted by Mikhail Pooggin  
Period: 16/1/2012 to 26/1/2012  
Topic: Validation of banana sRNA deep-sequencing.
16. COST-STSM-FA0806-9541 of Adi Moshe (PhD candidate)  
From: National Institute of Biology, (Ljubljana, Slovenia)  
To: Institut de biologie moléculaire des plantes (Strasbourg, FR), hosted by Manfred Heinlein  
Period: 01/02/2012 to 31/03/2012  
Topic: Host expression of dsRNA targeting TYLCV CP and V2 for generating TYLCV-resistant tomato and Arabidopsis.
17. COST-STSM-FA0806-10060 of Dr Vitantonio Pantaleo (Researcher).  
From: Istituto di Virologia Vegetale del CNR (Bari, IT)  
To: Agricultural Biotechnology Center (Gödöllő, HU), hosted by Dr Jozsef Burgyan  
Period: 15/04/2012 to 03/05/2012  
Topic: Characterization of AGO-siRNAs complex involved in antiviral silencing.
18. COST-STSM-FA0806-11410 of Ms Viktoria Tisza (PhD candidate).  
From: Agricultural Biotechnology Center (Gödöllő, HU)  
To: Martin Luther University (Halle, DE), hosted by Prof. Behrens Sven-Erik  
Period: 17/09/2012 to 28/09/2012  
Topic: STSM training.
19. COST-STSM-FA0806-10648 of Prof. Andreas Voloudakis  
From: Agricultural University of Athens (Athens, EL)  
To: INRA (Versailles, FR), hosted by Dr Herve Vaucheret  
Period: 15/05/2012 to 15/07/2012  
Topic: dsRNA-mediated RNAi induction in *Arabidopsis thaliana* PTGS enhanced mutants.
20. COST-STSM-FA0806-11562 of Prof. Thomas Hohn (an **RSTSM**).  
From: University of Basel (Basel, CH)  
To: Food and Plant Research Institute (Auckland, NZ), hosted by Dr Robin MacDiarmid  
Period: 02/12/2012 to 06/12/2012  
Topic: Virus infection and green islands and green populations.
21. COST-STSM-FA0806-13361 of Dr Vitantonio Pantaleo (Researcher).  
From: Istituto di Virologia Vegetale del CNR (Bari, IT)  
To: Agricultural Biotechnology Center (Gödöllő, HU), hosted by Dr Jozsef Burgyan  
Period: 19/05/2013 to 07/06/2013  
Topic: Characterization of AGO-siRNA complex involved in antiviral silencing.

22. COST-STSM-FA0806-14660 of Prof. Andreas Voloudakis.  
 From: Agricultural University of Athens (Athens, EL)  
 To: Metapontum Agrobios (, IT), hosted by Dr Francesco Cellini  
 Period: 08/07/2013 to 12/07/2013  
 Topic: Phenotyping-Operation of Plant Scanalyzer 3D and data analysis.
23. COST-STSM-FA0806-14661 of Prof. Andreas Voloudakis.  
 From: Agricultural University of Athens (Athens, EL)  
 To: FMACH, IASMA (Trento, IT), hosted by Dr Azeddine SiAmmour  
 Period: 15/07/2013 to 26/07/2013  
 Topic: DNA methylation analysis in CMV-infected tobacco.
24. COST-STSM-FA0806-14881 of Ms Ziva Ramsak (PhD student).  
 From: National Institute of Biology, (Ljubljana, SI)  
 To: Durham University (Durham, UK), hosted by Prof. Michael Goldstein  
 Period: 19/08/2013 to 08/09/2013  
 Topic: Bayesian network modeling approaches to elucidate key players of the potato defense response.
25. COST-STSM-FA0806- 15348 of Ms Piret Peterson (PhD student) (a **nonCOST STSM**).  
 From: Tallin University of Technology, (Tallin, EE)  
 To: International Potato Center (Lima, PE), hosted by Dr Jan Kreuze  
 Period: 23/09/2013 to 29/09/2013  
 Topic: Tacking advantage of deep sequencing of virus-derived siRNA for virus determination.

### Training Schools

- 1<sup>st</sup> Training School: “Double stranded RNA production for plant biotechnology”, Viikki Graduate School in Molecular Biosciences, University of Helsinki, Helsinki, Finland, 7-10/9/2009. (25 students, 5 teachers). (see Action’s webpage)
- 2<sup>nd</sup> Training School: ‘Small to large scale vaccination of plants - A hands on training on the vaccine delivery technology’, Tel Aviv, Israel, 19-22/03/2010. (15 students, 4 trainers). (see Action’s webpage)
- 3<sup>rd</sup> Training School: ‘Small RNA Cloning School’, Cambridge, England, 6-9/12/2010 (9 students, 2 trainers). (see Action’s webpage)
- 4<sup>th</sup> Training School: ‘Applied Bioinformatics in Plant Sciences’, Athens, Greece, 13-17/12/2010 (43 students, 14 trainers). (see Action’s webpage)
- 5<sup>th</sup> Training School: "Next generation sequencing analysis methods for life sciences" (trans-COST activity of FA0806, TD0801, BM1006), Uppsala, Sweden, 28 May -1 June 2012 (10 students, 3 trainers). (see Action’s webpage)
- 6<sup>th</sup> Training School: “Virus induced gene silencing in cereal and non-cereal plant species”. (A trans-COST activity), Rothamsted, United Kingdom, 25-29/6/2012 (8 students, 5 trainers). (see Action’s webpage)

### Workshops

- The “Science commercialization” Workshop organized by Dr Gabi Krczal in Neustadt, DE (20-21 August 2012) (Trans-COST activity of FA0806, FA0907, FA0804, FA1006). (14 FA0806 participants in a total of 25 participants, 9 invited speakers).
- The “Science Communication Workshop” (Dialogue workshop) ‘Value Communication of Novel Agro-technologies’ organized by Dr Gabi Krczal in Neustadt in the frame of WG3, Munich, Germany (3-4/11/2011) (trans COST Activity of FA0806, FA0804, FA1006 and FP0905) (11 FA0806 participants (18% females), 5 countries represented)
- The “Metagenomics in Virology” Workshop, organized by Prof. Ben Berkhout, Prof. Lia Van der Hoek (University of Amsterdam, NL) and Dr Walter Pirovano (Baseclear, NL) in Amsterdam, NL (22-23 April 2013) (trans-COST activity of FA0806 and TD0801). (12 FA0806 participants in a total of 30 participants, 9 invited speakers).
- The “Plant Epigenetics” Workshop, organized by Dr Etienne Bucher (University of Basel, CH) in Kandersteg, CH (13-14 May 2013), (10 FA0806 participants, 8 invited speakers).