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

Brussels, 21 November 2012

FA1208

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA1208: Pathogen-informed strategies for sustainable broad-spectrum crop resistance

Delegations will find attached the Memorandum of Understanding for COST Action as approved by the COST Committee of Senior Officials (CSO) at its 186th meeting on 20 - 21 November 2012.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action FA1208
PATHOGEN-INFORMED STRATEGIES FOR SUSTAINABLE BROAD-SPECTRUM CROP RESISTANCE

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 4154/11 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to develop innovative pathogen-informed strategies to obtain sustainable and broad spectrum resistant varieties of cereal and solanaceous crops and to become a platform for the development of durably resistant crops for Europe and developing countries.

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 60 million in 2012 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 Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

Restrictions on the use of pesticides mean that there is a need for new, sustainable pest control methods. Exploiting natural plant disease resistance is highly attractive, as it reduces the dependency on pesticides. However, the use of crop resistance is bound by two factors: the limited number of resistance sources against important diseases in major crops and the frequent breakdown of resistance due to rapid evolution of pathogens. Both issues can now be addressed by innovative and powerful approaches developed on the basis of recent and unprecedented progress in research on plants and their pathogens fostered by the revolution in next generation sequencing and the investigation of pathogen effector proteins. The challenge is to implement these novel pathogen-informed strategies for the generation of sustainable broad-spectrum crop resistance. Hence, this COST Action aims to create a European network of scientists and breeders for the translation of breakthroughs in plant-pathogen interaction research into effective breeding strategies for durable disease resistance in cereal and solanaceous crops which are of primary importance for European agriculture.

A.2 Keywords: sustainable agriculture, plant diseases, crop protection, crop improvement, durable plant resistance

B. BACKGROUND

B.1 General background

Plant diseases threaten global food security and cause losses of billions of Euro to the EU economy each year. Disease control by pesticides is often not possible, e.g. in the case of bacteria or soil-borne pathogens, is in general not durable due to the emergence of pesticide resistance in pathogens and raises continued ecological concern. In 2009, the Directive 2009/128/EC was adopted which requires member states to “take all necessary measures to promote low pesticide-input pest management”. The use of disease resistant crop varieties constitutes the economically and ecologically most viable alternative to pesticides in plant pest control. Unfortunately, the current number of resistance genes in major food crops is limited, while their action spectrum is often restricted to individual strains of pathogens. More worrying, from an agronomic point of view, is the ability of pathogens to accelerate their rate of evolution in response to the high selection pressure imposed by resistance genes, which frequently results in rapid breakdown of plant

resistance. Break-down of resistance can occur after only a few growing seasons and is difficult for plant breeders to estimate the agronomic lifespan of a resistance gene without prior knowledge of the matching effector. . Consequently, breeding for durable disease resistance in crop varieties by trial and error is slow and costly.

The on-going sequencing of pathogen and plant genomes is revolutionizing plant pathology and is generating for the first time the insights into the molecular and evolutionary mechanisms of pathogen virulence, necessary to efficiently breed for durable disease resistance. European research has a leading position in this field and national, multinational and EU-funded projects were and are key in the acquisition and exploitation of the genomes of the most important plant pathogens. Linking the generators and potential users of this research will bring multiple benefits to European seeds companies and European research. In addition, cutting edge knowledge on effector biology and innovative pathogen-informed strategies for resistance breeding and genomics developed by European researchers as well as new insights into strategies on how to deploy plant resistance in a sustainable way in agriculture must be brought to the profit of European Economy and consumers. Therefore, the Action will intensify exchange between public researchers and European breeding and biotechnology companies.

A major problem is that research communities in the EU are largely centred on host plants or specific pathogen types (e.g. fungi, oomycetes or nematodes) and fragmented by approaches (functional versus population genetics or evolutionary studies). Given the tremendous progress of the last years and the shift to the unifying concept of pathogen effector proteins that manipulate central cellular host processes, it is timely to break down these barriers and to coordinate European research on the molecular interplay between plants and their pathogens. A COST Action, funding networking between researchers that work with different pathogens and crops, between molecular biologists and population geneticists and between researchers and crop breeders is therefore the ideal mechanism to ensure that advances in fundamental understanding are translated into practical benefits. This Action will focus on major pathogens of cereal and solanaceous crop plants. These two taxonomic groups of plants include food and feed crops of outstanding importance for European economy like wheat, barley, maize and potato. In addition, they include both rice and tomato which are considered as model plant species and allow the accelerated translation of results from fundamental research to applications.

B.2 Current state of knowledge

The genomes of the most important pathogens of cereals and solanaceous plants have been

sequenced and were published during the last five years, generally in extremely high impact journals. These projects have been conducted by international consortia and European researchers and European research funds were generally of key importance. The genome of the powdery mildew fungus, one of the most important pathogens on wheat and barley, was e.g. unravelled and analyzed by an international consortium involving researchers from 5 European countries and was coordinated by European researchers. The genome of the oomycete *Phythophthora infestans*, a major pathogen of potato and tomato, has been sequenced by an international consortium involving many European researchers and was used for comparison with the genomes of related plant pathogenic species.

This on-going sequencing of pathogen genomes is revolutionizing plant pathology and is generating for the first time the insights into the molecular and evolutionary mechanisms of pathogen virulence, necessary to efficiently breed or engineer for durable disease resistance. The availability of whole genome sequences has enabled progress in the identification of pathogen molecules involved in evading or breaking disease resistance and plant-pathogen interaction research has strongly shifted to the investigation of pathogen virulence effector proteins and their mode of action in host plants.

Effector proteins are secreted by pathogens during infection as key elements in disease and resistance. They are essential for virulence as they allow pathogens to manipulate host immunity and physiology in favour of infection. They are also central to resistance as their recognition in resistant plant varieties triggers strong defence responses preventing disease development. Pathogens can escape host recognition through mutations that modify or delete effectors. Depending on the fitness penalty (i.e. the reduction of virulence) associated with such mutations, resistance breakdown in the field and subsequent successful spread can occur rapidly or more slowly. The availability of increasing numbers of whole genome sequences is starting to generate lists of candidate effectors for major pathogens and will further help to understand the role of genome structure and plasticity in the adaptive evolution of pathogens. They are the basis of innovative, pathogen-informed strategies for durable and broad spectrum crop resistance that are emerging (see section D).

European research in this field is particularly competitive. During the Sixth Framework Program, the Integrated Project BIOEXPLOIT aimed at investigating wild relatives of food crop plants for novel natural resistances to fungal diseases and COST Action872 Nemagenics aimed at **exploiting nematode genomics for the development of new control methods. During FP7,** one ERA-NET program (**Effectoromics) and** two Plant Genomics programs **(PathoNet and PRR-CROP)** have structured European Research communities working on the development of durable resistances.

Research on effector biology, host targets and plant resistance in solanaceae – and to a lesser extent cereals - is centered in Europe and leading groups in these research areas are participating in this Action. Therefore, the Action will contribute to further structure the European Research area in perfect accordance with the Innovative Union Initiative of the EU2020 strategy and maintain the knowledge position of European research groups compared to other regions in the world active in this area (e.g. Asia, USA).

The goal of the Action is to exploit latest knowledge on plant immunity and pathogen virulence for the generation of wide spectrum and long-lasting plant resistance. This will be achieved through two major lines of development and innovation:

- The identification of new resistances (resistance loci/traits) in plant germplasm collections.
- The evaluation of resistance durability by the detailed analysis of pathogen effector proteins in light of their dual role as central players in pathogen virulence and plant resistance protein targets.

Knowledge on durable resistances will be transferred to European plant breeders for the generation of durably resistant crops based on innovative non-GM approaches that will enable sustainable agriculture in Europe and developing countries. In addition to directly contributing to the development of durably resistant crops, the Action will intensify the transfer of concepts and results from academic research to European plant breeders. It will network European research groups in the field to intensify exchange of ideas and people to coordinate future research.

B.3 Reasons for the Action

Due to the progress made in the field of plant pathology and the genome sequencing of major crop plants like rice, wheat, tomato and potato and their pathogens (e.g. late blight and rust), several national and international initiatives have been launched on effector biology and broad spectrum resistance in the past few years. These are focused on specific groups of pathogens or crops, which results in **fragmentation** of the research community and a limitation of the research activities within projects. Taking the biodiversity of plant pathogens into account, it is impossible to combine research activities on their effector biology and broad-spectrum resistance and to address this issue in a single research framework. Therefore, COST is the appropriate framework to build an extensive network that brings together all the different disciplines with the aim to maximize the potential to obtain durably resistant crop plants based on central host targets (productive outcome). By coordinating ongoing research activities of the participating groups new collaborations will arise that will create the opportunity to produce higher impact science and novel insights into durable resistance based on common host factors by sharing complementary knowledge and

expertise. In this way, ongoing research will be more efficient, by joining efforts and preventing redundant research activities.
The network will permit rapid comparative analysis of results (before publication) and thus favour rapid identification of key defense genes in different crop plants or key features of plant defense that will not be identified by analysis in a single pathosystem. The COST framework is also an appropriate instrument to build a network between academic groups and the plant breeding industry as knowledge and expertise can be shared between participating breeding companies in a pre-competitive phase. The knowledge and knowhow generated by the network will be directly available for the participating breeders, which can implement novel insights and targets in their ongoing breeding programs to obtain more durable resistant plants. This will strengthen the market position of the European breeding companies, which have often a leading position worldwide. Participation in this COST Action network will enable them to develop alternative strategies to breed for broad-spectrum resistance without the use of GM technologies, which is not yet accepted by the European public.
The research field explored in the project is **highly competitive** internationally. Creating a European network will reduce competition between European labs, by facilitating and stimulating collaborations between these labs. This will strengthen the European Research Area and the knowledge position of European research groups compared to other competing regions in the world, especially Asia and the US. In addition, COST provides a framework in which young researchers can visit different labs across Europe which is particularly important in an extremely fast moving research field such as effector research and allows them to build their own network, increasing their job opportunities and accelerating research and innovation for sustainable crop protection. In this way, COST contributes to the economic development of Europe and stimulates the mobility of scientists in Europe. COST also provides an excellent framework to share knowledge and expertise on state-of-the art sequence technologies and effector biology with small breeders, which often lack in-house facilities and expertise to implement these technologies in their breeding programs. In this way, this COST Action can contribute to the capacity building of the European breeding industry, as well as academic groups who just enter this research area or lack their own facilities and expertise to do this type of research in their home countries.

B.4 Complementarity with other research programmes

The Action is complementary to, and will add value to, numerous ongoing national and multinational programs. In addition, plant health and crop protection is recognized as a central

element for sustainable crop production in FP7 Cooperation Work Programme (Food, Agriculture and Fisheries, and Biotechnologies) but, there is no current EU-wide project that has the breadth or scope of the Action or that links researchers working with such diverse pathogens and hosts or that links in that way researchers with the EU plant breeding industry.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The main objective of the Action is to develop innovative pathogen-informed strategies to obtain sustainable and broad spectrum resistant varieties of cereal and solanaceous crops. The main deliverables are: - Compiled knowledge on effectors in a wide range of economically important cereal and solanaceous plant pathogens (fungi, oomycetes, nematodes and bacteria) and their cellular host targets in these major crop plants (novel insights into effector biology and plant resistance) - Translation of this knowledge into pathogen-informed strategies based on central effectors and common host targets - Implementation of such pathogen-informed strategies in ongoing breeding programs - Dissemination and improvement of novel, cutting edge approaches for resistance engineering. The main outputs for European Research coordination and performance are: - Training of young scientists dedicated to research and innovation for sustainable crop protection and capacity building for small and medium-sized breeding companies and academic groups who just enter this research area or lack expertise in their home countries (, Training Schools, Workshops, Short Term Scientific Missions) - Networking of scientists investigating plant resistance and pathogen virulence (meetings) and better integration of the research community with the plant breeding industry. - Sharing resources, technical know-how, complementary expertise and approaches (Workshops). - Strengthening the European breeding industry committed to crop improvement (innovation).

C.2 Objectives

The main objective of the Action is to become a platform for the development of durably resistant crops for Europe and developing countries by mobilising the expertise of scientists from leading European institutions. Exchange between scientists investigating plant resistance, effector function and effector evolution will be intensified, valuable knowledge on pathogen virulence and plant resistance will be compiled and transfer of this knowledge from academic research to plant breeders in private companies or public institutions will be accelerated.

Four specific Objectives have been defined, which will be addressed in four different Working Groups (**WG1 – WG4**).

Objective 1:

To identify central effectors of important pathogens on cereals and solanaceous crop plants by coordinating research on the functional analysis of pathogen effectors in virulence and avirulence (**WG1**)

Objective 2:

To identify central host proteins and processes in cereals and solanaceous crop plants that are targets of pathogen effectors by coordinating research on the functional analysis of virulence targets in plants (**WG2**).

Objective 3:

To predict the agronomical lifespan of resistance genes and host targets by coordinating research on deciphering the mechanism underlying effector evolution and diversity in major cereal and solanaceous pathogens (**WG3**).

Objective 4:

To identify broad-spectrum resistance genes and other central host targets in crop germplasm collections and breeding material by coordinating research on plant immune receptors that recognise central effectors and allelic variants of central host targets (**WG4**).

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

Detailed knowledge on pathogen effectors, i.e. their contribution to virulence and their natural diversity, is central to identify new and unconventional resistance sources in crops and to predict the agronomic lifespan of disease resistance genes. However, the unexpectedly large numbers of effectors in single pathogen species suggests that the translation of effector action into durable, large spectrum crop resistance is a complex multivariate problem. Solving this problem is now feasible by the ongoing technological revolution in next generation sequencing and high throughput functional screens. However, it demands the instalment of a critical mass in the European research area. A COST Action will be instrumental in coordinating and increasing the impact of fragmented approaches undertaken as part of various national initiatives in the field of effector biology. This Action aims at building a platform that brings together experts from different disciplines in molecular plant pathology and plant breeding that will join their efforts to obtain durable resistant varieties for cereals and solanaceae. This will be achieved by mobilising and training scientists from

major European institutions and breeding industry working on effectors from various plant pathogen types and their targets in crops. At present, the network includes 35 partners from 15 European countries representing 11 universities, 12 academic research institutions and 8 companies (see List of Experts). This network will bring together and stimulate the collaboration between scientists and breeders with the aim to translate knowledge and expertise into applications in order to achieve the main objective of this Action.

C.4 Potential impact of the Action

The creation of such a highly interconnected European platform dedicated to the investigation of the molecular interplay between plants and their pathogens will strengthen the European research community and **increase the competiveness** of the European breeding industry. In addition, this interdisciplinary approach will result in **novel scientific insights** into pathogen virulence and plant immunity, which will be described in scientific papers in high impact journals. The presence of both research and development partners will ensure that research is innovative and oriented to the **development of new applications**. In addition, breeding companies will benefit from the training of young scientists. These highly qualified young professionals will be attractive for breeding companies, as they currently face difficulties in recruiting **skilled personnel** in this specific research area. The participation of commercial breeders allows the translation of results into applications in ongoing breeding programmes and boosting the development of pathogen-informed strategies for durable and broad-spectrum crop resistance. Pathogen-informed strategies will allow faster and more targeted selection of new sources of resistance in crop germplasm collections and more efficient breeding for durable broad-spectrum resistant varieties. The outcome of a coordinated approach holds the promise to accelerate the **release of novel and durably disease resistant varieties**, to drastically reduce the use of pesticides. This will contribute to sustainable and save food production, from which the European consumers and environment will benefit. In addition, it will result in a reduction in costs for growers and secures the production of food for an increasing world population.

C.5 Target groups/end users

European breeding companies are stakeholders in this Action and the expected results from this

Action will strengthen the European breeding industry by privileged access to cutting edge knowledge on effector biology and innovative pathogen-informed approaches for durable resistance. Scientists investigating plant resistance and pathogen virulence will profit from networking in the Action allowing sharing of resources, technical know-how, expertise and approaches and setting up of collaborative research projects. Both European scientists and Breeders are actively involved in the Action. Intensified exchange between public research and companies may lead to the adjustment of research goals with respect to economic relevance and market priorities strengthening the European Research area. Young scientists and scientists from developing countries will particularly profit from onsite visits, Training Schools and Workshops. European farmers and farmers in developing countries will benefit from high quality and durable disease resistant crop varieties allowing sustainable and low pesticide input cultivation, which will result in cost reduction and increased crop yield. European consumers will profit from healthy food produced in a sustainable, economically and ecologically reasonable manner.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

Research activities will be coordinated in four Working Groups (WG1-4) to address the main objective of the Action (section C). The scientific focus of each Working Group is described below.

WG1 Understanding the contribution of pathogen effectors to virulence

Phytopathogens secrete effectors during infection to manipulate host functions and to promote pathogenesis. The availability of genome sequences for the pathogens concerned in this project allows the identification of their effector complements and makes it now possible to develop platform-enabled high-throughput strategies for cloning and characterisation of effector genes. This is of particular interest taking in account the high number of effector genes to be dealt with, ranging from 20-70 in bacteria (Génin, 2010) up to several hundred in eukaryotic pathogens (Stassen *et al.*, 2011; Koeck *et al.*, 2011). WG1 will gather scientists from effector biology teams working on distinct cereal and solanaceous pathogen species to conceptualize high-throughput cloning strategies of high numbers of effectors. For innovative crop-improvement, it is of outstanding interest to identify the central effectors with a major contribution to virulence in important pathogens. WG1 focuses on functional approaches (loss-of-function and gain-of-function studies) allowing the identification of such effectors in important pathogens of cereal and solanaceous crops.

In addition it aims at exchanging and compiling results from such studies.

WG2 Host processes and proteins targeted by central pathogen effectors

Plant resistance can rely on the inability of the pathogen to manipulate plant functions. Therefore, protecting or modifying central host proteins or cellular pathways that serve as "susceptibility factors" from manipulation by pathogen effectors presents an innovative and promising strategy for crop resistance. WG2 aims at identifying the host proteins and processes that are targets of pathogen effectors in order to exploit this knowledge for durable and broad spectrum resistance in cereals and solanaceous crops (WG4). Phytopathogenic bacteria such as *Pseudomonas syringae* and *Xanthomonas campestris* have served as excellent model pathogens from which knowledge, especially with respect to effector proteins, has been gained that can be applied to more complex pathogens (Block and Alfano, 2011). Presently, host targets for over a dozen of type III effector families and biochemical functions for a similar number of effector families have been identified, and most type III effectors play a role in suppression of host immunity. For example, the *P. syringae* effectors AvrPto and AvrPtoB target cell surface immune receptor kinases such as FLS2, EFR, and CERK1, or co-receptors such as BAK1 (Shan *et al*., 2008; Xiang *et* al., 2008; Gimenez-Ibanez *et al*., 2009). Other effectors target downstream signalling components, such as protein kinase cascade components, RNA-binding proteins and transcription factors (Zhang *et al*., 2010; Wang *et a*l., 2010; Feng *et al*., 2012). Research on nematode, oomycete and fungal effectors has lagged behind on bacterial effectors, but gradually more knowledge has been gained about the functions of effectors from these important eukaryotic pathogens (de Jonge et al., 2011).
WG2 aims at exchanging on host proteins and processes in cereals and solanaceous crops that are targeted by effectors from diverse pathogens. An important aspect will be exchange on methodological questions. Generic approaches in model organisms or simplified systems facilitate the identification of host proteins and processes targeted by effectors from phylogenetically unrelated pathogens but are frequently prone of artifacts. Establishing and diffusing common standards and protocols and critical evaluation of experimental tools will therefore be a priority. In addition, compilation of validated host targets will be performed as a prerequisite for the identification of future breeding or engineering targets (WG4).

WG3 Evolutionary constraints on pathogen effectors and emergence of new pathotypes

Effectors can evolve rapidly and escape recognition by the plant immune system either by deletion or by changing individual amino acids. In addition, genome rearrangements may participate to the evolution of new effectors to subvert plant resistance. In certain cases, the genomic environment of

effector-coding genes seems important for higher evolution rates (Rouxel *et al*, 2011; Raffael et al., 2010). Effector-coding genes are e.g. found in some species in very specific genomic areas known to favour evolution. Surveying the polymorphism of effectors in natural populations reveals the evolutionary forces operating on them and indicates whether resistance relying on their detection is likely to be durable. Resistance relying on the detection of effectors showing a high presence-absence polymorphism or high natural variability can e.g. be expected to have only a narrow detection range or to break down rapidly. On the contrary, resistance relying on the detection of highly conserved effectors promises to be broad-spectrum and to be more durable.
A primary goal of WG3 is to set up common tools to analyze large datasets in comparative genomics analysis. The network will promote exchanges on methods to characterize the genomic environment of effector-coding genes and to detect selection in these genes. In addition, the WG3 aims at integrating knowledge on effector evolution in the generation of durably resistant crops. Results on the evolution of individual effectors will be connected to functional analysis (WG1 and WG2) in order to identify promising targets for sustainable and broad-spectrum resistance breeding (WG4).

WG4 Plant immune receptors and allelic variants of host targets for sustainable and broad-spectrum resistance breeding

Educated plant breeding benefits from exploiting effectors of pathogens (Ellis et al., 2009). This so-called effectoromics strategy was pioneered for the potato - *P. infestans* interaction (Vleeshouvers *et al*., 2011). Late blight resistance genes and their matching effectors are being identified at unprecedented rate. The effectoromics approach is currently also being embraced for breeding of resistance to various other agronomically important plant diseases of solanaceaous crops and cereals. To target broad-spectrum resistance genes, preferably central effectors and conserved apoplastic proteins of pathogens are exploited (WG1). Identifying the matching resistance genes and pattern recognition receptors will maximize the potential of durability of resistance.
As an alternative approach to use effectors to directly detect resistance genes, the effector targets (WG2) are studied. Natural variants of effector targets that are insensitive to effector manipulation but yet retain their intrinsic function can be a first step to generate disease insensitive plants. Such variants can be obtained by screening genetic resource of a certain plant species, e.g. breeding collections or wild germplasm. Identified allelic variants can directly be used in breeding, or be applied by targeted mutagenesis in cultivated plants. This was recently very elegantly shown for transcription activator-like effectors (TALEs) for bacterial pathogens (Li *et al*., 2012). TALE-nucleases can be generated for modifying specific target sites in crop plants, in a non-GMO

strategy, and the resistance of the crop can be increased.
High-throughput screens allow the identification of resistance genes that recognise central effectors (WG1) in crop germplasms or allelic variants of central effector targets (WG2). WG4 aims to transfer know how and knowledge on effectors and resistance to breeders for the development of durably resistant crops and to foster collaboration between academic research and breeding companies.

D.2 Scientific work plan methods and means

WG1: Understanding the contribution of pathogen effectors to virulence
The objective of WG 1 is to identify central effectors of important pathogens on cereals and solanaceous crop plants by coordinating research on the functional analysis of pathogen effectors in virulence and avirulence.
The specific objectives of WG1 are:
Identification of essential effector genes by gain-of-function assays or loss-of-function analysis.
This will be achieved by the use of the following methods and means:

- High throughput cloning of effector genes into expression vectors
- Transfer of cloned effector genes into pathogen strains with reduced virulence
- Gain of virulence assays based on the inoculation of susceptible host lines
- High throughput cloning of mutagenesis constructs for effector genes (RNAi, REMI, homologous recombination)
- Generation of pathogen mutant lines in strains of interest
- Phenotyping of individual effector gene mutant lines on susceptible host

Workshops will be organized to discuss methodological aspects and results of innovative approaches, including high throughput screening schemes established by participating groups. Comparing results obtained for different pathogens will allow to select for essential effectors important in pathogen virulence. Short Term Scientific Missions of students and scientists will allow the training of people in these methods, so they can apply and implement them in their own pathosystem.

Outcome:

- Repertoire of candidate effector genes cloned in expression vectors
- Repertoire of individual effector genes mutant lines
- List of major virulence effectors for each important pathogen on solanaceous and cereal crops

WG2: Identification of host processes and proteins targeted by pathogen effectors

The objective of WG 2 is to identify central host proteins and processes in cereals and solanaceous crop plants that are targets of pathogen effectors by coordinating research on the functional analysis of virulence targets in plants.

The specific objectives of WG2 are:

1. To identify host proteins and host processes targeted by pathogen effectors in cereals and solanaceae
2. To study the subcellular localisation and dynamics of effectors, host targets and processes
3. To analyse the function of host targets in (a)virulence and effector-target interaction and dynamics

This will be achieved by the use of the following methods and means:

Pathogen effectors have become excellent tools to dissect the plant immune system and can be used as probes to identify novel host targets and pathogenicity processes. For host-translocated effectors, yeast two-hybrid analysis can be used to identify potential effector targets. For extracellular effectors, other interaction screens such as the yeast split-ubiquitin system are more appropriate.

A complementary approach to identify host interactors of pathogen effector proteins are immuno-precipitation assays and purification of affinity-tagged effectors, allowing the detection of *in planta* interacting partners. Mass spectrometry is generally employed to identify the interactors, which is greatly facilitated by the recent release of crop plant genome sequences (i.e. rice, tomato, potato, wheat).

Functional analysis of the host interactors (e.g. by gain or loss of function approaches using transgenic plants) is needed to assess whether the identified interactors indeed support pathogen proliferation. Further functional analysis can reveal the exact role of the interactor in the establishment of disease by the respective pathogens.

The Action will coordinate, stimulate and facilitate the use and availability of tools (Short Term Scientific Missions, Training Schools, Workshops) and platforms to identify (common) host targets and processes and to examine their subcellular distribution and function in crop plants.

Outcome:

- Host proteins (from solanaceous plants and cereals) necessary for infection by one or multiple pathogens
- Particular physiological processes that are targeted by multiple pathogen effectors

WG3 Evolutionary constraints on pathogen effectors and emergence of new pathotypes

The objective of WG3 is to predict the agronomical lifespan of resistance genes and host targets by coordinating research on effector evolution and diversity in major cereal and solanaceous pathogens.

The specific objectives of WG3 are:

1. To decipher the mechanisms underlying effector evolution and diversity
2. To identify central effectors (to be studied in WG1) based on selection signatures and conservation.

This will be achieved by the use of the following methods and means:

Genome sequencing and whole genome gene expression analysis is beginning to identify the full complement of effectors of the most important solanaceous and cereal pathogens. From the whole genome sequences, the genomic environment of effectors can be characterized. When several whole genome sequences of the same pathogen are available, the presence/absence pattern and the amino acid polymorphism of effectors can be studied directly. When only one genome is available, the polymorphism of effectors can be studied in chosen populations by PCR amplification and sequencing or other appropriate methods. Based on the detected polymorphism, signatures of selection can be detected. Comparative genomic studies allow the identification of effectors with peculiar selection profiles (highly conserved for example; Bart et al. 2012; Stergiopoulos et al. 2012) which may be particularly interesting for functional analysis and durable resistance breeding. The main goal of WG3 will be to set up common tools for the analysis of large comparative genomics datasets. The network will promote exchange on methods to characterize the genomic environment of effector genes and to detect selection in these genes. These exchanges will be realized by meetings, Workshops, Training Schools and Short Term Scientific Missions.

Outcome:

- central effectors for which a long lifespan of their corresponding resistance proteins are predicted based on their evolutionary profiles

WG4: Selection of immune receptors and allelic variants of plant targets for resistance breeding

The objective of WG4 is to identify broad-spectrum resistance genes and other central host targets in crop germplasm collections and breeding material by coordinating research on plant immune receptors that recognise central effectors and allelic variants of central host targets.

The specific objectives of WG4 are:

1. Identification of resistance receptors that recognise central effectors (WG1) in crop germplasm collections.

2. Identification or generation of variants of central effector targets (WG2) in crop germplasm

This will be achieved by the use of the following methods and means:

To address specific objective 1, conserved effector proteins or central effectors (identified in WG1 and WG3 by effector biology groups) will be functionally tested in high-throughput screens in crop germplasm collections by plant breeding groups to identify corresponding resistance receptors. Functional assays, such as agroinfiltration or agroinfection are well established for solanaceous plants. For cereal crop species, ballistic bombardments of effector constructs can be used as a to screen germplasm collections. Plant material for genetic research, such as mapping populations generated by participating groups, will be used to isolate resistance genes or develop molecular markers for applied breeding purposes.

To address specific objective 2,plant genetic resources that are available in plant breeding groups will be screened for their allelic variants of central effector targets (that are identified by research groups in WG2). Identified allelic variants are introduced in breeding material, by generation of breeding populations or by targeted mutagenesis in cultivated plants.

Effector-driven plant breeding acts at the interface of effector genomics and plant genetics. To guarantee success of such co-operative approach, the interaction between research groups that specialize in effector biology and in plant breeding should be optimized. In this Action such interaction is greatly supported by organizing visits and meetings between the scientists of both sides. The Action will thus coordinate and stimulate the exchange of information between research

groups on effector genomics, virulence targets, resistance receptors and plant breeding to address the objective of WG4.

Outcome:

- Breeding material that shows resistance responses to central or conserved effectors.
- Plant material for isolation of resistance genes or molecular markers
- Allelic variants of effector targets in cereal and solanaceous crop species

E. ORGANISATION

E.1 Coordination and organisation

This COST Action will provide the necessary coordination to establish a European platform for scientists and breeders to exchange ideas, people, materials and technologies developed in their own research programs. The aim is to foster exploitation of the unifying concept of pathogen effector proteins manipulating central cellular host processes for the development of sustainable and durable disease resistance in the major European cereal and solanaceous crop plants.
To ensure achievement of these objectives, the Action is organised in accordance to the "Rules and Procedures for implementing COST Actions" and four Working Groups are set up with defined deliverables and milestones (sections D2 and E2).
The **Management Committee** (MC) will coordinate and supervise the action. It is composed of one representative of each country and will meet once a year in conjunction with the annual conference. The MC will have the following responsibilities:

- Appointment of Chairperson and Vice Chair of MC and of Working Group (WG) co-ordinators.
- Organisation of MC meetings.
- Critical appraisal of progress within each WG towards defined objectives.
- Annual reports monitoring activities of the COST Action and integrating reports from the four WGs.
- Decision on Scientific Meetings and Training Schools proposed by WG co-ordinators

- Assessment of applications for Short-Term Scientific Missions after evaluation by the WG co-ordinators.
- Ensuring tight links between WGs.
- Appointment of a person responsible for generating and maintaining a dedicated web site for the COST Action; monitoring of content to ensure that it serves the needs of the COST participants and promotes dissemination of research outputs.
- Appointment of persons acting as contacts with other programs and COST Actions.
- Ensuring gender balance and participation by ESRs (see Section E4 below).

Short-Term Scientific Missions, particularly for young scientists, will be an important part of the Action. Specialized meetings and Workshops will be organized for the different WGs. In addition, joint meetings for all WGs will be organised at least twice during the Action in order to ensure integration of activities.
A dedicated website will be created and kept up to date, both to serve the needs of the participants and with the specific aim of ensuring the dissemination and exploitation of the results of the Action (see Section H2).

E.2 Working Groups

Four working groups with clearly defined objectives (section B, C and D) will established
WG1: Understanding the contribution of pathogen effectors to virulence
WG2: Identification of host processes and proteins targeted by pathogen effectors
WG3: Evolutionary constraints on pathogen effectors and emergence of new pathotypes
WG4: Selection of immune receptors and allelic variants of plant targets for resistance breeding
The working groups will be managed by WG coordinators selected among the MC members and appointed by the MC. The **WG co-ordinators** will have a central role in the achievement of the Action's goals and have the following responsibilities:

- Coordination and supervision of WG activities to ensure progress towards defined objectives
- Annual reporting on WG activities to the MC

- Planning of WG Scientific Meetings and Training Schools
- Evaluation of Short-Term-Scientific Missions
- Facilitating joint research projects
- Establishment of contacts to related European and international research programs and initiatives

E.3 Liaison and interaction with other research programmes

The research groups participating to the Action and having indicated their interest have an excellent track record within this field and are often involved in multiple other national, European and International research programs. They will be encouraged to create links between the Action and these programs.
MC members will be appointed that will act as contacts with other programs and COST Actions and make propositions for joint meetings, Workshops and Training Schools. WG coordinators will also establish contacts to related European and international research initiatives and programs.

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. A gender balanced mixed group of scientists and Early Stage Researchers was involved in the preparation of the Action. It is expected that Early Stage Researchers and female scientists will play leading roles in the Management of the Action and the coordination of WGs. Gender balance will also be observed in the Workshops, Teaching Activities and Short-Term Scientific Missions. The active participation of young scientists in meetings and Workshops will be encouraged and they will have priority for Short Term Scientific Missions. In addition, where appropriate we will run Training Schools focused on the skills required by ESRs to develop a successful career in this field. MC members will be appointed that will monitor gender balance and participation of ESRs in the activities of the Action.

F. TIMETABLE

The duration of the Action is four years. The timetables shown in Table 1 and Figure 1 are punctuated with annual meetings of the MC and the individual WGs. A start-up and final meeting for all WGs will be held. In addition, an inter-WG meeting will be held after two years and individual WG meetings and specific Workshops will be organized after approval by the MC.

Table 1: Timetable of the Action:

	Year 1		Year 2		Year 3		Year 4	
Start-up meeting	Scientific Activities	Individual WG meetings or Specific Workshops MC Meeting	Scientific Activities	Inter WG meeting Specific Workshops MC Meeting	Scientific Activities	Individual WG meetings or Specific Workshops MC Meeting	Scientific Activities	Inter WG meeting MC Meeting

	Year 1				**Year 2**				**Year 3**				**Year 4**			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WORK AREAS	WG1: Identification of effectors															
	WG1: Functional role of effectors in virulence															
	WG2: Identification host targets and processes															
	WG2: Functional role host targets and processes in disease															
	WG2: Cell biology studies effector-host targets combinations															
	WG3: Decipher evolutionary mechanisms of effector diversity															
					WG3: Selection essential effectors based on evolutionary profiles											
					WG4: identification immune receptors in effector screens											
					WG4: identification allelic variants of effector host targets											
	Translation of knowledge into applications to improve ongoing breeding programs															

Comment [m1]: The table is correct

Comment [IS2]: Could you please confirm that the table is correct?

Figure1: GANNT chart giving an indication of the timing of each of the WG activities:

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, CH, DE, DK, ES, FR, HU, IL, IT, NL, PL, RO, SI, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 60 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?

Knowledge and technical advances related to these key components will be disseminated to the **Scientific Community**, including other researchers in this field working in research Institutes, Academia and Industry who are not participating in the network. The Action will disseminate results to the **EU Breeding Industry inside and outside the network**. Novel insights on pathogen (a)virulence obtained in this Action and potential applications and impact on crop protection and pathogen control will be disseminated to national and European **policy makers and bodies (EPPO, EPSO).** The **general public**, including **European consumers**, will be informed about novel non-GM based approaches to obtain durable resistant crops and the benefits of these approaches for a save and environmentally friendly food production The Action will disseminate results to **High school students** and **teachers** to increase to increase their interest in plant pathology and plant breeding.

H.2 What?

Set up of an electronic **Communication Platform** (internet discussion forum, email network, etc.) as an integral part of the Action website. A Communication Platform between participants of the network and other interested parties outside the network will be established to facilitate an effective exchange of information to the public and other interested parties from outside the network, including high school students and teachers, policy makers and representatives of standard bodies. **Publications** (both electronic on the website and hardcopy) on the activities employed by the

Action, including state of the art reports, interim reports, case study reports, proceedings, final reports, etc. Results will be published in (joined) articles in peer reviewed scientific and technical Journals.

Workshops, seminars and conferences open for non-participating groups.

A **Technology Transfer Platform** will be launched which will further support the transfer of knowledge, knowhow and materials between academic groups and breeders, both inside and outside the network. This TTP will be an integrated part of the website of the Action.

The Action will post general information on the Action on an up-to-date and informative **public website**. The websites of the Action will be maintained frequently to provide up to date information about the ongoing activities of the Action and the results obtained (e.g. reports, publications, proceeding). In addition, background information will be made available on the objectives of the Action for both specialists and non-specialists. The participants also share their work through **links** to their institutions' publically accessible web pages and a variety of press releases/news stories, usually coordinated by their relevant Communications teams and widely distributed to policy makers, politicians and other end-users

An attractive and informative **flyer** and a **poster** will be made presenting the goals and achievements of the Action.

Short communications about recent developments and novel insights will be published in journals for specialists and non-specialists, including **Newsletters** of standard bodies like for example EUCARPIA, which is the European society of plant breeders.

Short **training and demonstration projects, practical courses and on-site visits** for high school students and teachers on plant pathology and plant breeding.

H.3 How?

The network aims at publishing its results in **peer reviewed scientific journals** like TAG, MPMI, Plant Journal, Plant Physiology, etc., but also **open access journals** like BMC Plant Biology, PlosPathogens, etc., which will increase the audience substantially. Part of the research is conducted by researchers active at the forefront of this research area (see list of publications in section G for examples), which allows the network to make a significant contribution to this field and disseminate results in **high impact** journals like The Plant Cell, Plos Biology, etc. The network will coordinate and facilitate collaborations between different groups (i.e. by. Short Term Scientific Missions), so it is expected that in the course of this Action outstanding results will be obtained that can be

disseminated in a number of **joined high impact publications**.

News items about ongoing activities and obtained results will be published on the Action's website, but also in **short communications** and **newsletters** of (inter)national journals for specialists and non-specialists.

The Action will contribute to other national and international conferences and symposia by presenting an up-to-date poster on the goals and achievements of the Action and by the distribution of a flyer explaining the objectives of the Action.

Dissemination of results and applications to the breeding industry will be achieved by **stimulating (bilateral) collaborations and contacts** between scientific participants and industrial partners inside and outside the network by making use of existing contacts in ongoing (inter)national research programs.

The Action will **facilitate and promote the translation of results** obtained by the scientific groups in the network into tools, strategies and technologies that can be applied in ongoing breeding programs of the **breeders** that participate in the network or outside the network. Therefore, the activities at the TTP will be monitored and the contents of the Technology Transfer Platform will be maintained frequently to keep it up-to-date.

The Action will **contribute and stimulate local activities and initiatives** organized by participating groups and standard bodies outside the network for specialists in the field of effector biology and host resistance and susceptibility. Likewise, the Action will also contribute and stimulate the development of activities for non-specialists like short training and demonstration projects, practical courses and on-site visits for high school students and teachers by providing information, network infrastructure and expertise.

The Action will encourage routinely use of social media, for instance the webpage "Plants and Microbes" http://www.scoop.it/t/mpmi which has ~6,000 visitors per months.