SIXTH FRAMEWORK PROGRAMME

THEMATIC PRIORITY 5

FOOD QUALITY AND SAFETY

ResistVir

Co-ordination of Research on genetic resistance to plant Pathogenic Virus, and their Vectors in European Crops

Project number: FOOD-CT-2005-006961

Co-ordination Action

Deliverable 44: Recommendations report for future research policy

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PU public	
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission services)	RE
CO Confidential, only for members of the consortium (including the Commission services)	

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Executive summary and recommendations

A key deliverable from the RESISTVIR Programme for the Co-ordination of research on genetic resistance to plant pathogenic viruses and their vectors in European crops was the production of a report that summarises the current state of research and makes recommendations about future research priorities. This report provides those recommendations. The analysis highlights the importance of resistance strategies being aimed at both viruses *per se* and their arthropod or fungal vectors. Ideally this would be in an integrated approach that attempts to understand the impact of genetic and practical protection of crops on pathogen and vector populations in a way that will lead to an increased understanding of the principles of durability of resistance. Necessarily this will also include practical factors such as virus detection, diagnosis and epidemiological studies. The Recommendations vary in scope and scale with respect to the current funding instruments available through FP7. Reflecting the diverse nature of the emerging needs in this area across the EU, specific targets of intervention are not identified in the Recommendations although current and emerging priorities are described in the Report. Rather the Recommendations identify principle areas of future activity. The following Recommendations are suited to a programme of support under the definition of 'Collaborative Projects':-

Recommendation 1: *The EU should invest further in the identification of natural resistance genes, including those conferring quantitative resistance and those targeted against virus vectors.*

Recommendation 3: *The EU should monitor progress in the field of virus diagnosis and encourage industry-led support for the refinement of the technology.*

Recommendation 4: *The EU should invest in an integrated approach to understanding and managing population dynamics for viruses and their vectors in EU agricultural systems.*

Recommendation 5: *The EU should continue to support fundamental research on plant viruses but in a way that encourages greater co-operation between parties with common interests and with a credible strategy for delivery to key crop species.*

Recommendations 6 and 7 are less specific and are intended to highlight two important general aspects of funding.

Recommendation 6: *The EU should continue to support research related to improving agriculture in developing countries, especially in the area of disease amelioration.*

Recommendation 7: *The EU should invest in training in the area of plant pathology.*

Recommendation 2 is different in that it seeks to address an outstanding scientific, political and socio-economic issue that relates to the acceptability of plants carrying

GM-mediated virus resistance in the EU. This ambition would be a large multidisciplinary project that would bring together very different scientific, sociological and economics disciplines in a manner which lies outside practices of current EU funding. The proposal is that the EU considers a special Collaborative Project designed specifically to address the hiatus in the low acceptance of GM technology within Europe:-

Recommendation 2: *The EU should invest in the development, from laboratory to market, of a high value GM virus-resistant crop that can act as an exemplar for the utility of the technology, the rigour of the risk assessment and the value to the consumer.*

The recommendations recognise the potential generic practical value of previous research, especially in areas of identification of natural resistance genes and RNA silencing for delivery of virus resistance to EU crops. They also more generally recognise the need for translation of fundamental research to improved crop productivity. The recommendations recognise relative gaps in the EU research portfolio and identify ways in which new technologies may be brought to bear on complex and important agronomic problems in plant pathology. Knowledge gained from the proposed studies will be essential for the future competitiveness of European agriculture.

ResistVir Report

D44 Remit:-

1. "The report should also provide an in-depth analysis of research projects funded, and define those areas that have been extensively covered in the past."

2. "The impact of EC research projects will be covered in a specific section."

3. "This report will aim to highlight the different needs in each European member state, identify synergies or overlaps with other research areas, and encourage development in the most important areas."

1. Background

In line with human need to maximise crop productivity plant viruses have been, and always will be important in agriculture. What is less certain is the precise nature of the threat from these important pathogens in the face of expanding global trade, changing agricultural practices, food security demands of an expanding population, demand for bioenergy, climate change and the genetic plasticity of viruses themselves. Since viruses are transmitted by animal, arthropod or fungal vectors, each of these will also bring a unique set of challenges relating to their respective biologies.

From an EU perspective, assessments of threat should take into account the size of Europe, its geographic, environmental and meteorological diversity, and its diversity in agricultural practices associated with economic benefits and societal preferences. The EU extends from subtropical conditions of the Mediterranean basin to the northern arctic tundra and from the mild, moist conditions of the Atlantic Coast to the summer/winter extremes of the mid-continental climate in the east. The climatic differences dictate the diversity of crops in the EU. These include annuals and perennials (notably vines and trees), and vary from sub-tropical members of the Solanaceae (peppers, aubergines, tomatoes) to mixed grasslands for grazing in the north. Equally, agricultural practices associated with open prairies, intensive arable, and glasshouse systems bring unique environments with unique problems associated with viruses and their vectors. Also, intensive monocultures result in very different selective pressures on viruses and vectors when compared to mixed culture systems. The growing interest in lower input (e.g organic) yet productive farming adds a further dimension.

Legislative constraints on farming, in the form of lower insecticide thresholds, have probably taken little account of indirect impact on the spread of virus diseases. Equally, societal interest in organic farming has not recognised potential for generation of a pool of virus pathogens that can persist in crop or native species and could be transmitted to nearby organic and non-organic sites. Last, and of major significance in the context of virus resistance, is rejection by much of the EU of GM technology. Technology for delivery of pathogen resistance through GM has

progressed furthest with respect to viruses but public nervousness is preventing its effective deployment.

The combination of factors listed above present an enormous challenge to researchers in establishing the underlying biological principles behind durable virus resistance and in its deployment to economic benefit with the EU. The instability of all of these factors means that the optimal research strategy is to ensure preparedness and flexibility to respond to diverse challenges. At present, this could be delivered through GM technology but, without a change in public and political attitudes, more time needs to be spent on more conventional approaches to understand durable virus resistance. As a consequence, there is an increasing demand for effective solutions to virus problems that carry public acceptability, and it is certainly timely to devote more effort in this direction.

1.1 Viruses as pathogens

Plant viruses generally have small genomes with limited coding capacity, which typically encode a capsid protein, a replicase protein, a movement protein to assist in symplastic trafficking through the rigid plant tissues, a vector transmission factor, and a suppressor of gene silencing; some of these functions may overlap within a single protein. Most plant viruses have single stranded (ss) RNA genomes, although viruses with double stranded (ds) RNA, ssDNA or dsDNA also occur. Subclasses of viruses that have more complex genome arrangements and others that have dispensed with one or more functions also exist. An additional class of pathogens, viroids, which lack all protein coding functions, can also be the causes of serious crop diseases. In the general comments below viroids should be considered synonymous with viruses.

Most viruses are transmitted by arthropod vectors (e.g. aphids, hoppers, whiteflies) although fungal- and mechanically-transmitted viruses are also important. These vectors and insects in particular are markedly affected by climate and we can expect that changes in the climate will bring new challenges through the effects on insect pests. Viruses may be circulative and even replicative (multiplying in vector cells), persistent or non-persistent in their vectors. The transmission characteristics differ in each of these situations. A review of key problems and strategies relating to vector transmission of viruses is available of/on? the RESISTVIR website: http://www.RESISTVIR-db.org/key_reports.htm -Expert Group 5 Report. Vertical transmission of viruses through seed occurs for many virus-crop interactions but is variable in its impact on productivity.

The impact of virus infection in crops is through reduced yield and reduced product quality, especially for fruit crops. In annual crops, losses may be restricted to one sowing although creation of contaminated soils and seed transmission can both lead to significant losses in subsequent generations. Also establishment of a population of pathogens in the local environment (e.g. weed hosts) can also have follow-on consequences. For perennial crops, the problem is more significant. This applies particularly to vines and fruit trees. However, the potential interest in perennial grasses (e.g. *Miscanthus* spp.) and trees as biofuel crops raises new topics for research. An overview of key virus problems in Europe is available on the RESISTVIR website: http://www.RESISTVIR-db.org/key_reports.htm -Expert Group 1 Report.

Viruses exist within the environment as metastable populations which are influenced in character by the nature of specific virus-host (incl. crop and weed species), virus-vector and vector-host interactions. Genetic variation in these populations comes about through errors in genome amplification and genome recombination, and provide a 'quasi-species' population of genetic variants which is subject to environmental selection. Genetic plasticity of viruses is one of the main factors influencing the scale of threats from disease. Breakdown of resistance by adapted (*i.e.* virulent) virus variants involves different evolutionary forces. The most obvious one is the selection pressure exerted by the resistant plants in favour of the virulent virus variants. Additionally, the appearance of the virulent virus variants and their future dispersal in the agro-ecosystem depends on mutation frequencies of viruses, bottlenecks exerted on virus populations and migration capacity. These latter three evolutionary forces are largely influenced by the dynamics of populations of virus vectors and can be the target of various control methods.

1.2 Protection against viruses

Currently, protection against damaging effects of virus diseases is achieved either through the deployment of crop lines with heritable resistance to virus infection or through use of expensive and often less sustainable agronomic practices. The latter strategies are often targeted against arthropod vectors, rather than viruses themselves (e.g. insecticide sprays, physical screens), although the rogueing of virus-infected perennials is important. Legislative constraints on insecticide use and increasing occurrence of insects resistant to chemical treatments mean that much greater attention will need to be given to heritable genetic resistance to virus infection.

Heritable virus resistance can be divided into natural virus resistance genes (RESISTVIR website: http://www.RESISTVIR-db.org/key_reports.htm -Expert Group 2 Report and Review) and resistance engineered into plants (RESISTVIR website: http://www.RESISTVIR-db.org/key_reports.htm -Expert Group 3 Report). The latter usually takes advantage of knowledge obtained from fundamental studies of viral processes in plants and uses GM approaches. The former relates either to major dominant or recessive genes conferring resistance to specific viruses or to the products of selection identified through plant breeding. Traits selected through plant breeding need not always confer complete resistance, partial resistance often providing significant value.

Plant breeding remains a largely empirical process although the advent of relatively low-cost genetic marker systems provides a welcome element of predictability to the process. This provides for better characterisation and cataloguing of existing breeding stocks and pedigrees, which is particularly valuable when the genetic identity of the desired resistance trait is known. For elite material, especially perennial and vegetatively propagated lines, the use of tissue culture methods for the production of virus-free material has significant commercial value.

At a more practical level, physical exclusion of virus vectors from crop plants and use of molecular and genetic strategies to interfere with vector transmission of viruses provide great potential for integrating disease limitation with constraints on virus population diversity.

Key elements in understanding virus pathology in crops are diagnosis of the infecting agent(s) and need to understand the underlying epidemiology. Ideally, the latter studies would identify virus-vector-plant relationships over time such that predictions of disease progression and potential epidemics could be made. For the fullest understanding, this knowledge would be supplemented by data that integrated impact of host genetics on virus quasi-species structure that might predict the durability of resistance.

1.3 Research into virus resistance

This will be described in detail in later sections. However, in summary, this can be divided into five main areas: 1. Mechanisms and potential for RNA silencing to be harnessed as an antiviral resistance strategy; 2. Identification, characterisation and exploitation of natural resistance factors; 3. Agronomic and environmental studies to understand the generation of virus genetic diversity, and to identify and predict the occurrence of resistance-breaking virus variants; 4. To identify physical and molecular barriers to vector-mediated transmission, and 5. Fundamental research into plant-virus-vector interactions aimed at identifying novel strategies for virus resistance. This last area, which appears to be tangential to the main thrust of virus resistance research, reflects need to understand basic biological systems in order to identify tangible targets for resistance strategies. Good examples here are identification of RNA silencing targets within viral genomes and identification of host factors required for viral transmission and infection.

1.4 Viruses as research paradigms

An important aspect of virus research directly or indirectly related to resistance is the ‘added value’ for understanding basic biological systems, reaching beyond the immediate relevance to viruses and resistance. The power of viruses as biological probes comes from close integration of these obligate intracellular parasites into normal cellular processes. Hence, studies with plant viruses were arguably primary drivers in unveiling the processes underlying the phenomenon of RNA silencing, which is now recognised as a core epigenetic mechanism underpinning almost all areas of biology. Similarly, dissection of natural dominant resistance genes and their viral avirulence partners has informed research related to resistance to other types of pathogens in plants. Lastly, the process of cell-to-cell communication remains one of the least understood areas of plant biology. Here need for viruses to exploit cell wall channels (plasmodesmata) to create spreading infections has again placed viruses at the centre of research into plasmodesmata.

1.5 Virus Problems in the EU

This topic has been researched and an excellent report produced as part of the RESISTVIR programme (http://www.RESISTVIR-db.org/key_reports.htm -Expert Group 1 Report). It is not the intention of this report to list again the many crops and viruses for which there are significant negative economic consequences for EU agriculture. However, it is of value to comment on some general points. The first is that, although viruses are having a major impact on EU crop productivity and quality,

this is neither monitored nor recorded in a systematic way. Hence, much evidence for particular problems may be accurate but is also anecdotal. Connected to this point, it would appear that agricultural policy in the EU is established without access to accurate data for judging the likely consequences. Hence, changes in the legislation relating to pesticide usage could not have been based upon data about changes in virus transmission. Third, although EU politicians encourage technological solutions to the problems of EU development, many retain a ambiguous view as to the value of GM in the context of agriculture.

2. Previous research activity in the EU

The following sections summarise the funding patterns and research topics and outputs of the RESISTVIR partners. The report considers national funding patterns, national funding priorities, Europe-wide scientific focus and achievements (including the identification of effective transnational synergies), EU-funded activities and an overall analysis of outputs. The analysis comes with several qualifications although generally the author believes the trends to be representative. The qualifications are that: 1. The data collected came from most but not all RESISTVIR partners; 2. Some EU countries and some 'virology' groups are not involved in RESISTVIR, even as associate members; 3. It was not always possible to collect or retrieve complete national and EU data on specific funding; and 4. Inability to access commercially sensitive data. Where possible a differentiation was made between data relating to virus resistance and data relating to more general and fundamental aspects of plant-virus-vector interactions. Sometimes, although the stated long term rationale for the work was to achieve improved resistance to virus diseases, this may not have been explicitly tested. Occasionally it was also necessary to attempt separate funding directed towards 'virus resistance' from a broader programme of improvement for a particular crop.

2.1 Scale and trends in national research investment in plant virus research

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These analyses were based upon successful competitive grant awards and exclude core public or private support to tenured scientists (salaries etc). Data sets representing national funding patterns for the UK, France, Italy and Germany are used. These include grants with direct or indirect connection to 'virus resistance'. Lastly, data based upon the sum of all returns are analysed. Attempts to carry out a similar analysis for EU funding patterns were frustrated by lack of complete data recorded on the EU Cordis website. The expectation is that collectively these analyses give a picture of all investment in research across the EU directly or indirectly

connected with virus resistance. Data were collected for the period 1998-2008 or 2000-2008, dependent upon data available. Because of the duration of funding cycles, generally 3-5 years, annual expenditure for the first two years may be lower than actual figures.

Analyses are displayed graphically in Fig. 1.

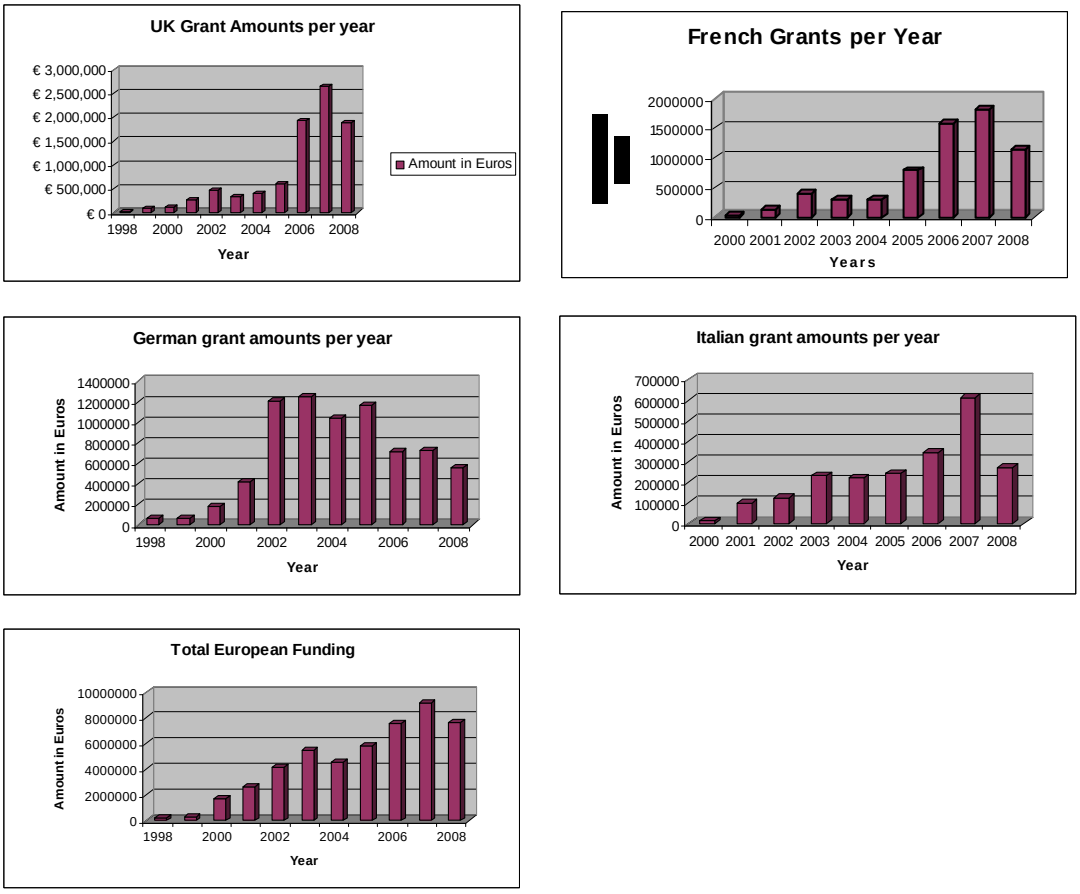


Figure 1 Trends in national (plus EU national) competitive funding for plant virus research

The pattern of funding showed a remarkable consistency across the selected EU States and in the analysis of all nations. The data show that there has been a significant increase in competitive research funding in the area through the 2000's although funding in Germany appears to have peaked in 2004/5 and has subsequently declined. **Overall this reflects recognition of the importance of plant virus research in preparing for agriculture in the 21st Century.** However, anecdotally, this positive trend probably masks a less favourable trend in the numbers of publicly-funded research groups (as opposed to competitively funded research projects) in some countries with 'plant virology' as their primary research focus.

Recent events in global financial markets will have a major impact on public finances. While scientists would argue strongly that spending on research should be

treated as strategic investment for the future and be protected, realistically funding will become tighter. Therefore, it is timely to review priorities for the research area.

2.2 National Priorities in Virus Resistance Research (2000-2008)

This section provides a summary of national research activities taken from returns of the RESISTVIR survey.

(*NB. Virus abbreviations used in this section are listed here. Names are included to illustrate the scope of the research and do not necessarily infer priorities:-*

BaMMV, Barley mild mosaic virus; BaYMV, Barley yellow mosaic virus; BYDV, Barley yellow dwarf virus; CaMV, Cauliflower mosaic virus; CMV, Cucumber mosaic virus; CTV, Citrus tristeza virus; CVYV, Cucumber vein yellowing virus; GFLV, Grapevine fanleaf virus; PLRV, Potato leaf roll virus; MNSV, melon necrotic streak virus; PepMV, Pepino mosaic virus; Potato leaf roll virus; PMTV, Potato mop top virus; PPV, Plum pox virus; PSbMV, Pea seed-borne mosaic virus; PSTVd, Potato spindle tuber viroid; PVX, Potato virus X; PVY, Potato virus Y; RBDV, Raspberry bushy dwarf virus; SBCMV, Soil-borne cereal mosaic virus; SBWMV, Soil-borne wheat mosaic virus; SPCSV, ; SPFMV, Sweet potato feathery mottle virus; TLCV, Tomato leaf curl virus; ToMV, Tomato mosaic virus; TSWV, Tomato spotted wilt virus; TuMV, Turnip mosaic virus; TuYV,Turnip yellows virus; TYLCV, Tomato yellow leaf curl virus; TYMV, Turnip yellow mosaic virus; WDV, Wheat dwarf virus; WSSMV, Wheat spindle streak virus)

Austria

The focus of activity in Austria lies particularly with viruses of stone fruit trees (*Prunus spp.*; plum, peach, apricot) and grapevine, mainly PPV and GFLV. This relates both to epidemiological work (detection, virus characterisation etc) and resistance breeding using molecular approaches. Strategies for the regeneration and selection of transgenic *Prunus* and grapevine have been established. Transgenic grapevines carrying coat protein constructs are being assessed for potential risk of transencapsidation with incoming viruses.

Belgium

Stone fruit trees are also important for Belgium research, notably indexing infection rates in plum and peach trees and generation of virus-free material by meristem tip culture.

Bulgaria

Bulgarian research relates to virus diseases of vegetables and Sharka disease (caused by PPV) of stone fruit. Pepper and tomato lines resistant to the respective virus

pathogens are being established through marker-assisted selection. Tomato lines resistant to CMV, TSWV and/or ToMV are close to market release. The detection and biological properties of PPV (e.g. insect transmission and seed transmission) have been studied. In a collaborative programme funded by the French Government, potential for recombination between PPV strains was also investigated.

Czech Republic

The Czech Republic invests in virus research into vegetables (potato, garlic, tomato, pea), cereal crops (barley), grapevine, stone fruit, top fruit (apple), soft fruit (blackcurrant) and viruses of pasture (clover). Much of this work relates to epidemiological studies of causative agents including virus detection and characterisation, and also includes development of microarray-based detection systems. A further aim is to generate virus-free material, especially for perennial species. An ambitious programme to develop transgenic peas resistant to key virus pathogens is nationally funded.

Estonia

The main practical focus of virus resistance work is on potato viruses and the protection of germplasm collections using biotechnological approaches to protect against a range of diseases. This endeavour is complemented by fundamental research at Tallinn Univ. of Technology into the role of virus suppressors of gene silencing and the impact they might have for development of disease.

Finland

Potato is the fourth most important food crop in the world and an important crop in Finland, and much of the research relates to understanding the molecular process of infection of this crop by the economically important viruses PVA, PVY and PMTV. Fundamental research is carried out on the molecular biology and virus-host interactions of these viruses. Resistance gene-specific markers are developed for use in resistance breeding. The Academy of Finland also funds a programme to combat the problems of the severe virus disease caused by mixed infection with SPCSV and SPFMV in sweet potato, a mandate food crop for developing countries. The work supports larger programmes of activity where genomic tools and resources are being used to develop breeding material resistant to these viruses and to understand how viruses circumvent or suppress antiviral defense responses such as RNA silencing in plants. Studies are also on-going to understand the significance of viruses infecting economically important tree species in the boreal forests and their ecology under climate change.

France

France has supported a large and diverse mix of fundamental and translational research connected with virus resistance. Mandated crops include grapevine, stone fruits, sugar beet, vegetable crops (pepper, tomato, potato, lettuce, melon and squash). Significant effort is devoted to the impact of potyviruses in these crops. Identification

of translation factors as susceptibility proteins led to identification of proteins eIF4E and eIF4G for their role in recessive resistance against potyviruses in pepper, tomato and lettuce. The possibility that such a mechanism could operate in other crops, including the important *Prunus spp.* against PPV, is under investigation by studying allelic variation in these genes or by application of TILLING technologies. These genes, and dominant resistances from other host-virus interactions, also provide valuable resources for studying the impact of resistances as evolutionary selection pressures on pathogen populations and consequences for disease durability. The role of insect transmission could also be important in virus population dynamics. Different mechanisms of virus insect transmission are being studied for CaMV and TYLCV, and dominant resistances in plants to aphids have been identified. France has also been exploring the potential for the delivery of virus resistance through transgenesis. Transgenic *Prunus* trees and grapevines with resistance to PPV and GFLV, respectively, have been tested in the field and the impact of transgenic virus resistant plants on virus population structure and environment tested. This is part of an international programme on risk assessment for these crops, funded by the EU (see below). In addition French groups are exploring novel sources of resistance in model species and novel strategies based upon a fundamental understanding of virus biology in host plants, including mechanisms of gene silencing, virus replication and virus movement. A programme of research addressing importance of rice yellow mottle disease in Africa illustrates the value of connecting European states with the developing countries.

Germany

Relevant research in Germany was associated with potential to generate resistance to viruses in cereals, vegetables, and, to a lesser extent, grapevine, stone fruit and top fruit. Germany also hosts one of the few groups studying virus disease problems of non-fruit trees. Significant attention was given to virus epidemiological studies (detection and population studies) to complement the characterisation of resistant germplasm for barley (against BaYMV, BaMMV, and BYDV), for rye (against SBCMV, SBWMV and WSSMV) and for wheat and durum wheat (against SBCMV, SBWMV, WSSMV, and WDV). Virus detection and characterisation has been used for a range of vegetable crops, including potato, tomato, lettuce, and sugar beet. With a direct connection to resistance, wild potato species have been analysed for resistance to PVY, PLRV and other pathogens. For PVY, there has been a particular focus on the identification of dominant resistance genes. PVY resistance was transferred into hybrid BC_1 clones and a number of BC_2 clones. Within the Brassicaceae, genetic markers and new breeding lines identifying useful resistances to TuMV and TuYV have been provided to breeding companies. Transgenic approaches to developing resistant crops have also been explored. Furthermore, there have been strong fundamental programmes in the areas of RNA silencing and virus movement that seek to underpin biotechnological approaches to virus resistance.

Greece

Greek agriculture is very diverse, often involving small producers. Hence a large amount of the research directly connected with virus resistance involves virus epidemiological studies, particularly detection and characterisation, and

characterisation of natural resistance traits, for example for *Prunus armeniaca* varieties However, in addition to these more applied aspects there are programmes exploring the novel aspects of RNA silencing in relation to the development of transgenic virus resistant plants. The latter includes a) potential for development of plants resistant to viroids and b) identification of host factors supporting virus multiplication as targets for resistance strategies.

Hungary

The most important crops in Hungary are wheat and other cereals. Traditional breeding is very advanced but for viruses where there is no known resistance (e.g. WDV) the potential for biotechnological solutions is being explored. This is supported by a strong programme of work relating to RNA silencing, especially with respect to the balance between defence and counter-defence seen through action of viral suppressors of gene silencing.

Israel

Israel has supported research on practical and basic aspects of virus diseases in vegetable crops, grapevine, and ornamental crops. On the practical side, government and industrial support has given high priority to understanding the biology of TYLCV for development of resistance in tomato. Many different resistant cultivars with natural or transgenic resistances have been produced. Transgenic virus resistant crops for a wider variety of vegetable crops have also been produced, including use of transgenic root stocks for perennial species. At present these are mostly experimental materials. The studies are especially targeted at potyvirus, tobamovirus and newly emerging geminivirus pathogens, and cucurbit crops. Virus-free propagation material is also produced for many crops.

Italy

The majority of research funding for 'virus resistance' in Italy has been directed towards diseases of vegetables, grapevine and stone fruit, with some other work committed to improving the phytosanitary arrangements for soft fruit (blueberry) and ornamentals. In tomato and pepper, natural and biotechnological routes to resistance to TYLCD complex (TYLCV and TLCV) and TSWV have been explored. Similarly, novel natural and transgenic resistance traits to CMV in tomato have been described. Transgenic lines resistant to CMV were transferred to an industrial partner. For tomato, these studies are being supported through a genomics contribution to the international tomato sequencing effort. For artichoke and grapevine, significant value has been demonstrated through generation of virus-free stocks and these materials have been distributed to growers.

Lithuania

National funding in Lithuania has been directed predominantly towards the need for efficient technologies for virus detection and characterisation. Priority has been given

to virus detection in vegetables (especially tomato) and soft fruit, although virus detection in ornamentals and cereals has also been important.

Poland

Poland has funded an extensive programme of activities related to virus detection and characterisation in vegetables, cereals, horticultural species, top fruit and stone fruit. With a direct connection to resistance, potato germplasm has been analysed for resistance to resistance to PVY, PLRV and PVM. Resistance genes for these viruses have been mapped on potato chromosomes and DNA markers developed for molecular marker-assisted selection of virus resistance in breeding programmes. Similarly, novel sources of resistance to SBCMV in winter wheat have been explored. The potential for generating transgenic potato resistant to PVY was also analysed.

Spain

The major activity in Spain directly related to resistance concerns disease prevention associated with a range of viruses in cucurbits, citrus and tomato, amongst other lesser crops. Particularly, markers tightly linked to resistance against CMV, MNSV, CVYV, CTV and viruses of the TYLCD complex, supported by tools for the detection of these and other viruses, were transferred to the breeding industry. In parallel, genomic tools for analysis in cucurbit and citrus species were developed. The recessive resistance gene *nsv* in melon (effective against MNSV; carmovirus) was characterised and shown to be a mutant allele of the gene *eIF4E*. Factor eIF4E was shown in other European labs to be at the root of resistance to many potyviruses. The concept of viruses as quasi-species, subject to selection pressures, is important in predicting the durability of resistant crops in the field. The evolution of viruses in the field and in the laboratory has been assessed for key pathogens, notably CMV, TYLCV and the emerging pathogen of tomato, PepMV. The impact of insect transmission as an evolutionary bottleneck has also been assessed. In a practical sense, one route to virus resistance is to exclude insects from the crop. One programme assesses effectiveness of physical barriers between the crop and the external environment. Three areas of fundamental research support these more strategic directions: the development of genomic resources, studies of the molecular mechanisms controlling virus invasion and transmission of aphids, and studies of the molecular processes underlying the RNA silencing-mediated responses to virus invasion.

Turkey

Turkish national funding has mostly been concerned with the detection and characterisation of viruses from vegetables, grapevine, stone fruit and top fruit. Virus-free grapevine stocks have been generated.

United Kingdom

Cereals, oil seed rape, potatoes, sugar beet, and legumes, in order of importance, are the major UK crops. Other vegetables, soft fruit and top fruit are also valuable. Barley and wheat suffer from the soil-borne viruses BaYMV and BaMMV, and SBCMV and

SBWMV, respectively. A new programme explores novel resistances to these viruses in cereals. Sugar beet and potato similarly suffer from the soil-borne viruses BNYVV and PMTV. Recessive resistances *rym4* and *rym5* to BaYMV and BaMMV in barley and *sbm1* resistance to PSbMV in pea have all been characterised as being mutant alleles of *eIF4E.* In *Brassica*, the possibility that similarly based resistance operates against TuMV (potyvirus), the importance of other *Brassica* viruses (especially TuYV) and existence of novel resistances in wild *Brassica* populations are being investigated. Dominant resistance genes in potato, effective against PVX and PVY, have been characterised and resistance to PMTV in potato and RBDV in raspberry is being investigated. Anticipating a more positive public attitude to transgenic resistant plants significant investment has gone into improved design of transgenic crops, understanding the basic principles of the RNA silencing response to virus infection and to risk assessments for release of transgenic crops. All this research is supported by extensive involvement in the establishment of genomics platforms for cereals, potatoes, brassicas and legumes and by ongoing improvement in technologies for virus detection and epidemiological studies. Fundamental programmes on disease signalling and virus movement have potential to identify new strategies to achieve virus resistance. Work also includes epidemiology and modeling studies to predict the impact of changing climate on disease incidence and resistance durability.

2.3 Common activities including transnational EU-supported research

Remit: 2. "The impact of EC research projects will be covered in a specific section."

The descriptions of national research activities identify a number of common themes and interests. Some of these overlap with EU-funded projects that involve several/many EU partners. Hence, in the discussion of these common themes descriptions of the EU projects are included. (***Annex 1 provides a list of EU-funded projects where one or more objectives were to assess the impact of the research for the development of virus resistance.***)

Epidemiology

It is clear that most EU states have technical resources and expertise for virus detection and diagnosis. In many cases, these are run as publically-funded services. Improvement in efficiency of these services is an ongoing activity but phase changes in underlying technology (e.g. hybridisation-based screening) require additional lines of support. The EU has invested in this area both with respect to more conventional antibody based technologies **(*Annex 1 project Nos. 10, 11, 14***) and for alternative chip-based technologies **(*Annex 1 Project Nos. 16, 23***). These basic and advanced technologies will be crucial for screening crop germplasm in the field for resistance and for understanding the structure and dynamic nature of virus populations under selective pressure **(*Related EU Projects – Annex 1 Project Nos 13, 17, 22, 27, 28*)**. It is in this latter area that there is less critical mass and an urgent need for greater understanding. Durability of resistance is usually a characteristic that can only be assigned with hindsight. Understanding how virus populations change under differential selection pressures, such as with extreme or quantitative resistance, will strongly affect the value of any new resistance traits. Virus characterisation, including

genome analysis and deeper studies of molecular processes that support virus multiplication and infection, is also a widespread and necessary activity especially when it relates to newly emerging viruses. There have been a number of EU projects that have supported fundamental research into understanding basic virological structures and processes. Most of these are not included in Annex 1, which is focussed on projects more directly connected with resistance; however, ***Annex 1 Project No 7*** is relevant.

Emerging diseases

This problem lies in the nature of virus biology and changing opportunities brought about by changes in agricultural practices, climate etc. Nevertheless, the survey of research activities over the period 2000-2008 identify several new but now established disease problems, and some genuinely new emerging problems. In the former category, geminivirus infections of vegetables in the Mediterranean basin and Sharka disease of stone fruits are the most prevalent. The Begomo subgroup of the *Geminiviridae* are transmitted by the *Bemisia tabaci* whitefly, an insect that atypically has a broad host range and the ability to transmit several begomovirus pathogens. This insect is also recalcitrant to insecticide treatments. Begomoviruses TYLCV and TLCV in tomato are of concern in many EU or EU-associated states and urgently require some novel strategies to reduce the risk and improve the value of this important crop.

Sharka disease is caused by the potyvirus PPV and affects plums, peaches and apricots. Occurrence of a number of new damaging strains of PPV and difficulties of breeding in these perennial tree crops have similarly created an urgent need for research-based solutions. Understanding the origin of these strains and evolutionary pressures on the virus in a perennial, long lived crop will be central to deciding the optimal resistance strategy. GM resistance may provide the most flexible strategy but potential for recessive resistance based upon eIF4E is also being explored. The damaging potential of Sharka disease in Europe has been recognised for some years and the EU has supported many projects aimed at ameliorating its effects (***Annex 1 Project Nos 17, 19, 20, 22, 28***)

Three examples of new emerging disease agents are receiving attention: PepMV in tomato, WDV in wheat and the PVY NTN-like variants in potato. PepMV is a member of the genus *Potexvirus*. These are mechanically transmitted viruses and can be highly infectious and are particular problems for crops that are grown with extensive mechanical or manual intervention. Currently, this virus is being characterised with respect to its biology and diversity (***Annex 1 Project No. 27***). WDV is a member of the Mastre- subgroup of the *Geminiviridae* and is transmitted by leafhoppers. It has recently been recognised as a cause of significant crop losses in Eastern and Central Europe and sources of natural resistance or transgenic approaches to resistance are urgently being sought. It is likely that the geographic range of the leafhopper vector will expand with changing climate. PVY (*Potyviridae*) is a pathogen of potatoes across the EU and the NTN-like strains overcome all currently used resistance genes, except *Ry*, causing severe foliar symptoms. Unfortunately *Ry* genes are not yet common in European potato varieties. Only a few cultivars bred in Poland and Germany make an exception in this respect.

While it is by definition not possible to predict the appearance of totally novel emerging diseases, it is likely that changing agricultural practices and, in particular, restricted use of selected pesticides will lead to an increase of previously (more or less efficiently) controlled diseases.

Key crops

Priority crops across the EU differ according to geography, geology and climate. Nevertheless, cereals (wheat, barley, rye, and mostly imported rice) provide the major food source to the EU. Potatoes are also a major crop especially for central and Northern Europe. Southern Europe has a more diverse collection of high value crops, especially vegetables and fruits, including grapes. Many nations have cereal diseases as a focus for research activity, although at present it remains the case that fungal infections have a much larger economic impact than viruses. However, changes in insecticide usage and consequent changes in the distribution of virus vectors could alter these relationships significantly. Breeding is efficient in cereals and it is anticipated that the extensive germplasm collections for cereals or compatible wild species will reveal resistances against many of these viruses (***Annex 1 Project Nos. 1, 25*)**. Virus diseases of potatoes are of concern for many states and industry continues to rely very heavily on the restricted access of aphid vectors to cooler northern latitudes for growing disease free 'seed' material. Even so, there is need to identify new resistances to a diverse mixture of potato viruses especially as new strains emerge and insect vector ranges change with warming temperatures. Grapevines are central to European culture and commerce. They are vulnerable to a number of damaging diseases but especially GFLV, which is nematode transmitted. As nematicides are being banned because of their heavy ecotoxicity, soil-borne diseases will become increasingly important. There are few sources of natural resistance to GFLV; hence grapevine is being developed as a GM resistant crop in several EU States (***Related EU Projects Annex 1 Nos. 17, 22***).

Perennial and vegetatively propagated crops

Where natural genetic variation in crop lines or in compatible wild species can be identified, conventional breeding strategies will deliver resistance into elite germplasm. For some crops this approach is inefficient or is not possible. These are perennial crops with a long generation time and vegetatively propagated crops. This relates mostly to trees, grapevine and ornamentals and especially for those which are obligatory out-crossing species for which heterozygosity is the norm. Crops (e.g. potato) that have a long phase of vegetative propagation before commercial sowing are also problematic. Overall, these crops may be better candidates for GM approaches to virus resistance (***Annex 4, 17, 22***).

Screening for natural resistance

Screening for natural resistance to plant pathogens lies at the heart of most conventional breeding programmes and has been applied successfully for many crops (Reviewed in *RESISTVIR: http://www.RESISTVIR-db.org/key_reports.htm* Published review from Expert Group 2). However, identification of novel sources of resistance

needs access to extended gene pools either from underexploited crop germplasm or from wild species. This search will be greatly enhanced by the rapidly expanding genomic resources for many crops and new technologies for the analysis of highly complex genomes. Many EU States are actively engaged in screening for novel resistances, often in close alliance with commercial interests through which products of research can be moved to market (***Annex 1 Project Nos. 3, 12, 13, 28***). Intermediate benefits to companies are genetic markers for marker-assisted selective breeding. The most likely products of such screens are dominant genes for virus resistance, or QTLs for quantitative resistance, that could be bred directly into crops. For difficult-to-breed crops, cisgenesis provides an alternative route assuming that transformation technology exists. Parallel analysis of model host plants, such as Arabidopsis, also has potential to identify novel genes which would require transgenesis for implementation in crop species. A complementary area that has received relatively little attention is the identification of crop genotypes with resistance to insects of other virus vectors.

Resistance strategies based upon mutant susceptibility factors

Research in a range of crops into natural recessive resistance to potyviruses, bymoviruses and carmoviruses has identified mutant alleles of host translation factors (e.g. eIF4E) as the resistance genes (***Annex 1 Project No. 5***). The logical interpretation of this is that the wild type proteins serve to support an aspect of virus multiplication or spread that is removed in homozygous resistant plants. Such recessive resistance has been found in pepper, tomato, lettuce, pea, barley, melon and Arabidopsis. The precise mechanism by which the susceptibility factor supports the virus may differ between virus groups. Since this appears to be a general property across crop species, at least with respect to potyvirus resistance, the potential for other crops, such as stone fruits and brassicas, which have potyviruses as important pathogens, to have equivalent resistances is being explored. As an alternative, mutant populations of crop plants are being screened for artificially generated mutant alleles using high-throughput genotyping techniques such as TILLING. In theory, this approach could work successfully for other susceptibility factors. The identification of such factors forms part of the fundamental research directed at important pathogens in several states (***Annex 1 Project Nos. 5, 7***).

GM virus resistant crops

The logical outcome of much fundamental research, with the stated aim of enhancing virus resistance in crop plants, is identification of aspects of virus biology that are susceptible to genetic intervention using transgene technology (RESISTVIR website: http://www.RESISTVIR-db.org/key_reports.htm -Expert Group 3 Report and Review). The paradigm for this strategy emerged from fundamental observations of the phenomenon of virus cross protection and led to discovery of homology-based resistance and RNA silencing. However, other approaches have been explored. Development of an antiviral state through expression of immunoglobulin chains targeted at virus proteins has been tested (***Annex 1 Project No. 9, 17***). In many cases, however, dominant responses of plants to the presence of homologous 'foreign' sequences, especially when presented as double stranded RNA, is to mount an RNA silencing defence against the incoming pathogen. The importance of this process has

engaged research groups from many EU States with expectation that outputs from the research will ultimately be tested in transgenic crops (***Annex 1 Projects Nos. 4, 24***). Unfortunately, public and political uncertainty of this technology in Europe has delayed progress in this area, and deterred much commercial interest for such crops in Europe. Nevertheless, co-operative research has led to development of virus-resistant transgenic crops which are being tested in laboratory and field trials for durability of resistance and for risk to the environment (***Annex 1 project Nos. 8, 15, 17, 22***). Most of the results of this research have not been formally published although anecdotal evidence for PPV-resistant plum trees and GFLV-resistant grapevine show stable resistance and no adverse environmental effects. Experiences with these transgenic materials are perhaps the most informative with respect to hurdles faced in deploying transgenic resistance in Europe, and the reader is directed to the relevant references in Annex 2.

Virus resistance for developing countries

Developing countries are ideal environments for viruses. Warmer climates, complex ecosystems intermixing agriculture and natural habitats, low inputs and undeveloped niche crops all contribute an environment where viruses and their vectors thrive. In developing countries there is the greatest need for virus resistant crops and there are a few positive examples (e.g. use of cassava mosaic disease-resistant crops in East Africa). However, generally, there has been little exploitation of virus-resistance especially for orphan crops. It is perhaps surprising therefore that only a few EU States have listed programmes of publicly-funded research on crops of relevance to developing countries. This is not a complete picture and there is much work 'behind the scenes', especially related to virus detection and diagnosis (***Related project Annex 1 No. 12***)

Added value from other EU programmes

Clearly, progress in crop improvement will be achieved most effectively in synergy with related programmes of research. Most direct value will come from interactions with genomics programmes for particular crops, for example, EU-SOL programme for genomics in the Solanaceae, European Triticeae Genomic Inititiative (ETGI) and TRITIGEN (COST Action) programme for grain cereals and the GLIP project for grain legumes. These and national programmes aimed at building diverse genomic resources will provide essential support to efforts to combat virus disease threats.

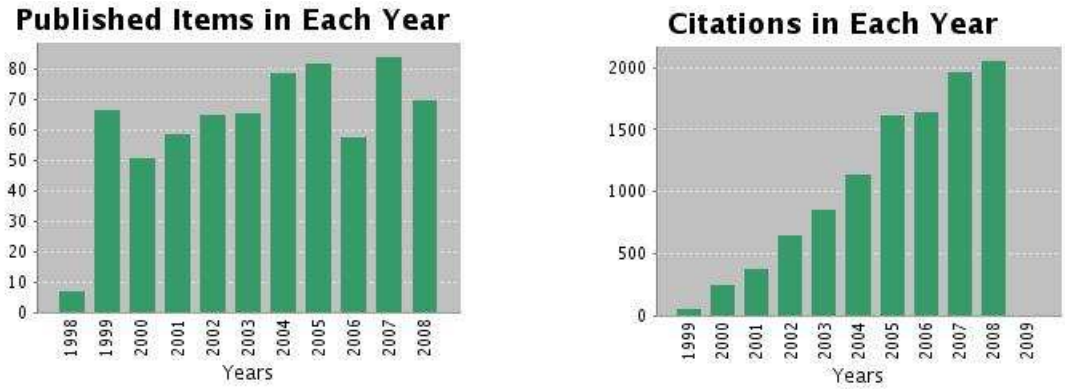
2.4 Outputs

The outputs from research are phased, with earliest measures of success being publications. These overlap with successfully filed patents. In ideal circumstances, reagents, biological materials or even products are passed to industries which eventually lead to products that contribute to increased agricultural efficiency and economic wealth. In the agricultural sector there is, unfortunately, a translational gap in this process where early demonstrations of principle are rarely adopted directly by industry. This is often related to the principles being established in model systems but

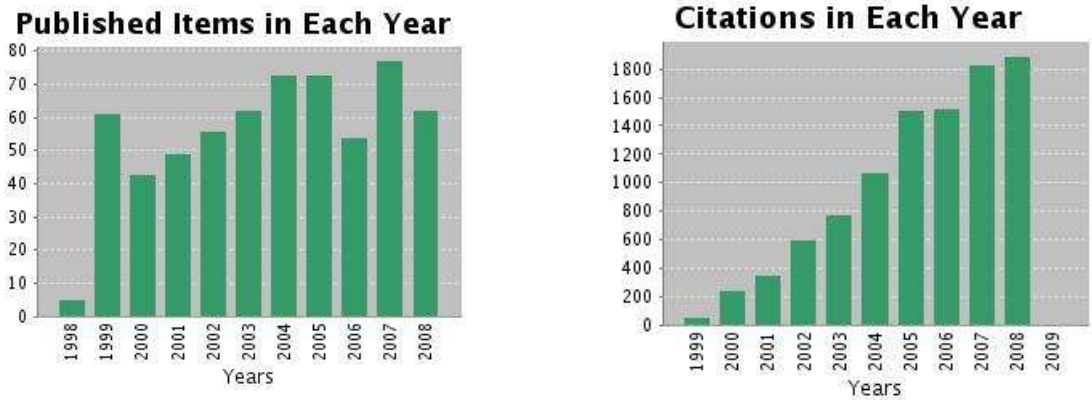
their efficacy in crop species not being demonstrated. This translation from principle to practice is usually too costly in relation to crop value to be done by industry alone. For GM crops, industry is also burdened by the costs of the regulatory process. Hence, while the research community in the area of plant virus resistance has been effective in identification of new principles for indentifying and solving problems of virus disease there are relatively few cases where this has led so far to tangible benefits to society. This situation is changing and the emergence of new sequencing and genomics technologies will reduce both time and costs of moving principles to products, although regulatory costs for GM crops will remain a major hurdle.

Database searches for peer-reviewed publications from EU states within the subject area 'virus+plant+resistance' for the period 1999-2008 identified 688 hits (651 hits for RESISTVIR members that responded to the survey). The distribution of these over time is illustrated in Fig. 2. Relatively few of these relate to technology transfer to the field/market but that probably reflects the nature of the publication system that gives greater weight to the identification of fundamental principles than to application.

Figure 2 Articles from European Labs in the area of 'virus+plant+resistance'



Graphs above relate to all European labs. Graphs below relate to members of ResistVir that responded to the survey. In each case, the *h* factor = ~50



Similar searches of patent databases were carried out within the RESISTVIR activities (http://www.RESISTVIR-db.org/key_reports.htm). The searches included a manual step that excluded cases where the potential for virus resistance was claimed but not demonstrated. Output of the analysis list patents from EU countries for the period

2000 to 2008 (Annex 3). Many of these relate to the demonstration of RNA silencing as an effective tool to deliver virus resistance.

Take-up of these technological advances by industry has been less easy to determine, partly due to the relatively short timescales (eight years) selected and due to confidentiality issues. Nevertheless, the RESISTVIR survey identified a number of examples where industry has benefitted from outputs of the research (Table 1). In addition, value to agriculture generally of service support in the area of virus detection and diagnosis is not quantifiable, but should not be underestimated.

In summary, public investment in the area of 'virus resistance' has been significant (see Figure 1) but benefits to agriculture, while not always quantifiable, are tangible. **The high costs of research and the long lead-times to product delivery, means that it is appropriate that much of this research continues to be publicly-funded.**

Table 1.

Industrial outputs from EU research on plant virus resistance

Country	Approx. Date	Topic	Industrial Output
Bulgaria	2002-2007	Tomato virus pathogens, CMV & ToMV	New line released for variety testing
Czech Rep.	2000-2008	Soft fruit virus diagnosis	Detection methods
France	2007	Certification of potato wrt PVY infection	Certification methods for potato
	2005-2008	Resistance durability to PVY in potato	Knowledge on durability
	2006-2008	eIF4E recessive resistance in Solanaceous crops	eIF4E allele specific markers
Germany	2000-2008	BaYMV, BaMMV and BYDV resistance in barley	Lines with improved resistance
	2002-2007	Soil-borne viruses of wheat	Lines with improved resistance
	2006-2007	Grapevine viruses	Virus-free propagation material
Italy	2002-2005	CMV and TMV as pathogens of tomato.	Transgenic lines with broad range resistance
	2000-2008	Virus pathogens of artichoke	Virus-free material; detection technologies
Poland	2004-2006	Potato viruses	Detection technologies
	2000-2006	PPV in apricot	Hybrids with improved resistance
	2004-2008	Pome and stone fruit viruses	Virus-free propagation material
	2001-2007	PPV resistance in apricot	Molecular markers for improved resistance
Spain	2006-2008	Recessive resistance in cucurbits	Allele specific markers
	2003-2005	Cucurbit viruses	Detection technologies
UK	2000-2008	Recessive resistance in barley	Allele specific markers
	2002-2005	Recessive resistance in pea	Allele specific markers

3. Future challenges and funding priorities

Remit: 3. "This report will aim to highlight the different needs in each European member state, identify synergies or overlaps with other research areas, and encourage development in the most important areas.")

3.1 General Statement

Virus diseases can be counted amongst the range of biotic and abiotic stresses that affect all crops. They present particularly difficult problems due to their absolute integration into processes of their host, ability to show rapid adaptation to selection pressures and due to their complex modes of transmission. Nevertheless, mostly through investment into studies of model virus-plant interactions we now understand a great deal about how viruses work, how they exploit their hosts and the layers of host defence responses mounted against them. These studies will never be complete and there will always be a need for continuing investment in the fundamental research that underpins the potential benefits of this research for agriculture. However, since it is more than 20 years since the first complete viral genomes were cloned and 10 years since the *Arabidopsis* genome was sequenced, it is timely to see the focus of research shifting towards pathogens and crops of significant economic importance to EU agriculture, and to translation of our fundamental advances in virus research to processes and products in EU crops. It is not the intention of this report to identify optimal targets for this translational research, although the reader is referred to RESISTVIR Deliverable 6 'Overview of key virus problems in Europe' (http://www.RESISTVIR-db.org/key_reports.htm Expert Group 1 Report). Amongst the diverse crops produced across the geographic range of the EU, all will have particular virus disease problems. However, potential problems in new crops, such as the perennial biofuel crops should also not be forgotten.

3.2 Technologies

Future research scope and potential across the EU in the area of virus resistance needs to be placed in the context of advances of technology and some unquantifiable variables, including climate change and energy costs in agriculture. The latter are political issues although doubtlessly science will have a role to play. We can, however, anticipate the consequences of technological advances and use those to identify new areas in plant biology that will assist in defending crops against viral attack.

New high-through-put sequencing platforms mean that complete genome sequences for all the major EU crop species could be available within 10 years and that repeat sequencing of breeding lines could become routine for some. In addition, with respect to virus transmission, the genome sequences of some/many virus vectors will also be determined. Increased ease and capacity of DNA of next-generation sequencing means, for example, that the human genome could be re-sequenced within a week. This will make it possible to explore at the sequence level natural communities in agricultural environments, a feature with huge potential for

investigating the relationship of virus pathogens of crops with their wild plant hosts and transmission vectors.

As costs reduce, nucleic acid detection methods (incl. sequencing) could also come to provide the foundation for pathogen detection and characterisation. Already DNA microarrays are being refined for rapid detection of viral pathogens, and have potential to replace specific immunological assays as routine methodologies that give an overall picture of the health status of crops.

We will continue to face the sometimes imponderable challenges of crop tissue culture and genetic transformation but experience has shown that this can usually be dealt with through adequate investment in skills and manpower resources.

3.3 Forward look at the challenges relating to virus resistance

There is no doubt that our understanding of the principles underlying virus resistance has advanced considerably in recent years and that the field has built the confidence and the know-how to take many of the fundamental observations in model virus-plant systems into diverse crop species. The key question remains, however, is how this could best be achieved technologically for particular crops and the strategy for most cost-effective public investment.

Natural virus resistance

Natural virus resistance genes lie at the heart of breeding strategies to develop resistant elite germplasm. Plant pathogens are exposed to several levels of innate immune responses in plants. In many cases these involve the recognition of molecular patterns on the pathogen and triggering of defensive processes. For viruses, as obligate intracellular parasites, many of these processes are not relevant although activation of the RNA silencing machinery by regions of viral dsRNA could be argued to represent an equivalent response. Largely roles of natural resistance genes effective against viruses relates to the specific recognition of viral proteins by the protein products of dominant resistance R genes and the activation of extreme resistance or hypersensitive cell death by processes similar to those observed for other pathogens. Alternatively, recessive resistances occur as mutant alleles of genes encoding proteins necessary to support virus multiplication, or susceptibility factors. Translation factors eIF4E, eIF(iso)4E and eIF4G are the best characterised examples. In contrast, there are almost no studies of the mechanisms controlling quantitative resistance to virus infection even though genes that confer only intermediate resistance (perhaps as low as 10%) may nevertheless have significant value. In addition, there is at least a theoretical argument that partial resistance may exert a weaker selection pressure on the virus and therefore may be more durable.

It should also not be ignored that resistance to crop infection by viruses may be delivered effectively through the control of the transmission vector. There have been only a few examples of characterisation of natural resistances to insect and nematode infestation. It is expected that there will be many other unidentified major genes and quantitative traits that could provide an effective block to virus diseases.

Rapidly expanding knowledge of crop genomes and the development of associated resources mean that more natural virus resistance genes will be identified

and characterised. In addition, allele mining the pool of natural variation using new sequencing technologies will become routine. Similarly, the cost of marker technology needed for marker assisted selective breeding is decreasing rapidly.

Recommendation 1: The EU should invest further in the identification of natural resistance genes, including those conferring quantitative resistance and those targeted against virus vectors.

In the face of continuing resistance to GM technology in crop plants, natural virus resistance genes lie at the heart of breeding strategies to develop resistant elite germplasm. Opportunities in this area are considerable and will reflect local/national agricultural and economic priorities for both governments and SMEs. What is clear is that there is a significant but necessary investment required to undertake QTL mapping and gene cloning for virus resistance traits. However, there is intermediate value in the identification of tightly-linked markers for use directly in breeding programmes. Molecular and genetic characterisation of QTLs brings added value in the potential to directly explore allelic variation within germplasm collections. It will also be possible to identify mutation induced alleles for both the target genes and genes that are functionally related (e.g. in the near signalling pathway). For recessive resistance genes that may be less easily identified through conventional breeding, the expanding technique of TILLING also provides the opportunities for the identification of novel variation.

Separation of resistance strategies into 'engineered' and 'natural' is largely artificial and it is becoming clear that there are opportunities for deploying resistance genes from one species into a heterologous crop species using transgenesis. This is particularly pertinent to dominant R genes. With the use of next generation sequencing technologies comparisons of sequences from resistant and susceptible species have the potential to identify new R-gene loci, which after necessary verification, could be tested in heterologous crop species. Such more speculative strategies may best be directed at crops where conventional breeding is difficult.

Considering the significant financial commitment required for all these studies and the benefit of a collective approach to the generation of necessary resources, this work could effectively done under a crop focussed activity such as "the healthy potato plant", for example. This could bring together pathologists of all flavours to maximise the utility of resources, technologies and experiments (e.g. field trials).

The timescale for this activity depends upon the selection of target crops but programmes running for 10 years should be anticipated.

RNA silencing-based resistance

It is the case, that extensive investments into researching mechanisms underpinning the phenomenon of RNA silencing has often been based around its activity as an antiviral activity and that through this means strategies for combating most of the major groups of viral pathogens have been identified. In fact, notwithstanding the ongoing need to understand the molecular and genetic details of RNA silencing as a central and universal epigenetic process in biological systems, we can probably say that use of RNA silencing to combat viruses is a mature technology.

Hence, through the use of homology-based silencing (sense or antisense transgenes corresponding to viral sequences) or transgenic artificial microRNAs, resistance to any RNA virus could be established. In addition, similar strategies have been shown to be effective against the major groups of DNA viruses, even though here DNA-derived RNA viral transcripts are the targets. If this precept is true, why do viruses continue to be a problem in agriculture?

Crop transgene technology has been widely adopted in the Americas, in parts of Asia and is slowly gaining a foothold in Europe. In the majority of cases, transgenic crops are those carrying genes for herbicide and/or insect resistance, traits for which there is significant market potential for multinational agrochemical companies for whom investment has been significant. However, earlier sections in this report illustrate how virus resistance achieved through transgenic RNA silencing can be effective in both perennial and annual crops. In most cases, these plants have been developed through public funding and public research organisations. Nevertheless, for many countries in the world, and especially in Europe, none of the plants have wide social and political acceptance. This has created a significant barrier to crop development in the area of disease resistance. The recalcitrant European attitude has also had significant repercussions in less developed countries (e.g. Africa) who have taken the socio-political and moral lead from Europe in banning GM crops. Arguably, it is in these countries where virus diseases of major and orphan crops cause most devastation that these issues have most negative impact. This is exacerbated by the high cost of vector eradication through pesticide usage and through the mixed culture of crop and native forest/bush that results in virus and vector emergence from wild plants. Interestingly, the current EU review of pesticide legislation could result in dramatic increases in problems from viruses, insects and other pests in EU crops.

Recommendation 2: The EU should invest in the development, from laboratory to market, of a high value virus-resistant crop that can act as an exemplar for the utility of the technology, the rigour of the risk assessment and the value to the consumer.

Notwithstanding the regulatory and hence financial and time hurdles, GM technology can provide a rapid response to the changing spectrum of virus challenges that we can expect in the face of a changing climate and ever increasing globalisation of food production and supply. Public funding of the development of such a crop will side-step perception in the eyes of the public, the ‘green’ pressure groups and the media that GM crops only benefit large multinational companies, although the ‘golden rice’ story shows that this is evidently need not always be the case ((Potrykus, I. (2001) Golden Rice and Beyond. Plant Physiology 125, 1157-1161). A good candidate for such a crop could be tomato. Tomato is susceptible throughout the Mediterranean basin to a wide range of virus pathogens, including well known pathogens such as *Cucumber mosaic virus*. Two more recently emerged and notable problems are *Tomato yellow leaf curl virus* (TYLCV; Begomovirus) and *Pepino mosaic virus* (PepMV; Potexvirus) for which no known natural resistance exists. TYLCV is a single-stranded DNA geminivirus transmitted by white flies, which are already resistant to most acceptable insecticides. PepMV is a relatively recently emerged pathogen that is devastating tomato crops throughout Southern Europe. The crop largely cannot be grown in the open due to disease pressure, adding significant

additional costs. Such a project would incorporate plant virologists from the RESISTVIR programme, experts in GM regulation and risk assessment, and sociologists to advise on marketing and public acceptance. SMEs should be engaged at an early stage with the expectation that the public investment in research, development and regulatory issues would be reflected in a correspondingly lower cost to consumers. Anecdotal evidence suggests consumers have little fundamental objections to GM technology when the advantages are tangible (RESISTVIR web site http://www.RESISTVIR-db.org/docs/seminars/WP7_WorkShop_GM_crops_Do_they_work_in_Europe_Krczal.pdf), "GM crops - Do they work in Europe? Mechanisms of public acceptance." Krczal, G.). One scientific question to which we have paid relatively little attention to date is the consequence of selection pressure on the virus pathogen population following deployment of GM RNA silencing-based resistance, although preliminary evidence suggests that this technology is at least as good as natural virus resistance genes. The timescale for delivery of the research could be as little as five years, excluding delays for the regulatory processes.

Virus populations and epidemiology

An important component in the fight against virus epidemics is the rapid detection and identification of viruses and, in some cases, the coincident presence of their transmission vectors. Virus identification continues to be heavily dependent on somewhat dated technologies of immunoassay, microscopic visualisation of virus particle morphology and bioassay on specific indicator host plants. As indicated above it is likely that low-cost high through-put hybridisation-based assays will revolutionize screening procedures such that multiple disease problems (and potentially physiological problems) will be analysed in parallel providing a rapid and complete picture of crop plant health. This technology is fairly well advanced. Further development and deployment of this technology might be viewed as a close-to-market activity. Nevertheless……..

Recommendation 3: The EU should monitor progress in the field of virus diagnosis and encourage industry-led support for the refinement of the technology.

In contrast to practical questions of virus diagnosis, our understanding of the factors that regulate the population dynamics of virus pathogens, particular in the context of multi-trophic interactions between virus, plant, vector, environment and agronomic practice, is in its infancy. These are complex issues but ones that lie at the heart of virus disease epidemics and effectiveness of virus resistance in field crops. They relate to questions of size and distribution of virus and vector populations in crop and wild plants, of genetic selection and of the influence of climate and farming practice, and will be relevant to virus resistance whether delivered through GM or using natural resistance. Answers to these questions are crucial if we are to understanding the nature of resistance durability.

Genetic resistance is amongst the most efficient and environmentally-safe methods to control plant viruses and their vectors. However, relying on one or few resistance genes as the only strategy to control plant viruses may end up with

resistance breakdown in a short period of time, especially if the resistance has initially a high efficiency and exerts a strong selective pressure on the pathogen population. Alternatively, combining resistance to virus diseases with other control measures in an integrated management approach will benefit on the long term the durability of resistance.

Recommendation 4: The EU should invest in an integrated approach to understanding and managing population dynamics for viruses and their vectors in EU agricultural systems.

Investment in this area should attempt to draw together virologists, entomologists, population biologists, ecologists and mathematical modellers to integrate multiple approaches that target both viruses and vectors for the delivery of durable field resistance. These issues will strongly depend on the development of mathematical models to integrate the various epidemiological, socio-economical and meteorological parameters involved in these complex control strategies. The aim would be to establish models of disease principles that extend beyond particular crop-virus-vector relationships to stimulate the development of more predictive modes for development of durable virus resistance. An investment programme of 10 years should be anticipated.

New strategies for virus resistance

It is the case that many of the most valuable scientific discoveries arise serendipitously from high quality fundamental research and the creative talents of researchers. A case in point is the discovery of the phenomenon of post-transcriptional gene silencing that has become the foundation of many areas of new biology, including virus resistance. Similarly, the identification of mutant *eIF4E* genes as recessive resistance genes in various crop species stemmed from the fundamental observation that eIF4E interacted with the viral avirulence protein in laboratory studies of protein-protein interactions. Other potential resistance strategies have been identified from fundamental studies of other virus proteins and their functions in supporting virus replication in the host cell.

Recommendation 5: The EU should continue to support fundamental research on plant viruses but in a way that encourages greater co-operation between parties with common interests and with a credible strategy for delivery to key crop species.

One criticism of European fundamental research on plant viruses is one of inadequate co-operation and synergy and a focus on model systems without appropriate thought being given to translational strategies for delivery to key crops. A further consideration is that consequences of virus evolution have resulted in distinct virus groups becoming uniquely specialised pathogens. Hence it is the case that biological properties of one virus group (genus) may not be directly relevant to another virus genus. In this situation, research effort may be better focussed around virus genera (e.g. *Potyviruses* or *Begomoviruses*) than around areas of biological integration (e.g. virus replication or virus movement).

Less Developing Countries

Recommendation 6: The EU should continue to support research related to improving agriculture in developing countries, especially in areas of disease amelioration.

The preceding text considers the major threats to EU agriculture and makes recommendations for the improvement of key EU crops. Less developed countries, suffer to a greater extent from crop diseases and have the greatest need for agricultural development. EU researchers have a huge contribution to make in helping to overcome some of these problems either directly through research or through training.

Investment in people

Recommendation 7: The EU should invest in training in the area of plant pathology.

Recent years has seen a decline in interest in sciences amongst EU graduates, with an increasing proportion of strong graduates and postgraduates coming from Asia and India. One consequence is that there is a danger that local (meaning EU) skill base will be depleted. This is particularly the case in the area of plant pathology where the spectrum of diseases changes with relative frequency and intimate knowledge of crop characteristics are important to recognise, identify and correct disease problems. Crops are also often 'tailor-made' for local conditions meaning that principles in crop design must also be adapted to local needs.

4. EU FP7 Funding instruments

This Report makes a number of Recommendations that should be considered by the EU for further support to maximise the outputs of previously funded research and to harness advances in technology to tackle the challenges of virus diseases in EU crops in a changing world. Within the FP7 funding portfolio most of these Recommendations could be covered by Collaborative Projects of varying sizes. Recommendations 6 and 7, however, are less specific and are intended to highlight two important general aspects of funding. The major exception to the above is the Recommendation for an innovative and multidisciplinary approach to the delivery of transgene-based resistance to viruses in a valuable crop. The combination of experts in plant virology, socio-economics, publicity, agriculture etc is outside of normal EU approaches to scientific funding and warrants specific discussion over the creation of a special project to meet the demands of this activity.

ANNEX 1

EU-funded projects relating to 'virus resistance'

(Owing to incomplete recording on the EU Cordis Website, there may be gaps in this data set.)

1	**Date**:	1996-2000
	Project Code:	**PJ_REF:IC18960049**
	Title:	**Genetic engineering to improve Chinese wheat - introduction of stable resistance to soil-borne mosaic viruses as a prototype system**
	Partners:	UK, Germany, China
		Objective: The main objectives are as follows: * To identify Chinese wheat cultivars and/or synthesise new breeding lines which will be highly amenable to tissue culture and regeneration from protoplasts. * To evaluate the most promising cereal transformation to determine the best for use with Chinese wheat cultivars. * To assemble information on the molecular variation amongst isolates of both wheat spindle streak mosaic bymovirus (WSSMV) and soil-borne wheat mosaic furovirus (SBWMV) from different sites in Europe and China, and to design a pathogen-encoded antiviral gene strategy. * To develop a suitable expression vector for wheat transformation and use constructs with these virus products to produce genetically-stable transformed wheat.
2	**Date**:	1997-2000
	Project Code:	**PJ_REF**:IC15960907
	Title:	**Molecular exploitation of sobemoviruses**
	Partners:	UK, Estonia, Finland, Russia & Latvia
		Achievements: Foreseen Results The goals 1-3 will generate information that will be of general importance for the efficient expression of foreign proteins in transgenic plants. The goal 4 will generate virus expression vectors that will be generally useful in plant molecular plants. These vectors will allow high level expression of foreign proteins in plants without the need to resort to time consuming transformation technology. The sobemovirus vectors will also have practical importance that will be tested in this project by the expression of vaccine proteins in infected plants. This work reflects a novel approach to the production of oral

		vaccines for use ultimately in both animals and humans. The final goals 5 and 6 will lead to the production of virus resistant plants and are a potential solution to the disease problems posed by the two viruses used in this project. The recent development of transformation technology in monocotyledonous plants means that the transgenic resistance is now a feasible target of plant biotechnology. The analysis of natural resistance will have a longer term benefit in the production of plants resistant to sobemoviruses and other pathogens.
3	**Date**:	1997-2001
	Project Code:	**PJ_REF:IC18970172**
	Title:	**Application of biotechnology for genetic improvement of oilseed rape**
	Partners:	Germany, China, UK, Sweden, Canada
		The main objectives are as follows : * To identify the loci controlling oil content in Chinese and European cultivars of oilseed rape. * To develop molecular tags for a self-incompatibility system to provide breeders in China and Europe with a new production system and a broader genetic base for hybrid varieties. * To identify sets of genes for resistance to all known strains of Turnip Mosaic virus (TuMV). * To transfer the TuMV resistance genes from Brassica rapa (turnip rapeseed) into B. napus (oilseed rape) and B. oleracea (broccoli, cauliflowers and European cabbages).
4	**Date**:	1997-2000
	Project Code:	**PJ_REF**:IC15970900
	Title:	**Molecular Study of the Role of Potyvirus encoded Proteins P1,P3,NIB (Replicase) and Coat Protein in Virus Replication and Host Response to Infection:Studies towards Biotechnological Methods of Disease Control**
	Partners:	UK, Estonia, Finland, Russia
		No report given
5	**Date**:	**Start date:**1997-09-01 - **End date:**2000-08-31
	Project Code:	**PJ_REF**:BIO4972356
	Title:	**Characterization of recessive genes that control natural resistance to potyviruses**
	Partners:	Denmark, UK, Spain, Finland
		General information: Characterization of plant resistance genes have

		significantly advanced our understanding of plant-pathogen relationships. This raises the possibility to engineer new resistance specificities or broad range resistance through the manipulation of the resistance gene product or its functional pathway. Very little is known about the nature and function of recessive genes that provide highly effective and durable resistance through the suppression of virus replication or movement, in the absence of tissue necrosis. Peas contain clustered recessive resistance genes effective against a range of potyviruses. The most studied of these are the sbm genes that confer specific resistance against different pea seed-born mosaic virus (PSbMV) pathotypes. Resistance gene sbm-l is of commercial interest for its effectiveness against the most common PSbMV pathotype (P-1). The genetic organization of the sbm-associated clusters suggests that gene-duplication, genetic translocation and functional diversification has occurred leading to the range of potyvirus resistances. Hence, knowledge of the basis of sbm phenotype could provide a route to a common resistance strategy for this large and important group of viruses. Unfortunately map-based cloning in pea has yet to be established and, at this time, would be unlikely to be successful within a three year EU Biotechnology Project. In this project we propose to use biochemical, and molecular biological techniques to:isolate and characterise the sbm-l resistance product, or its dominant counterpart (Sbm-l), and use that information to isolate the resistance gene. characterise the sbm resistance mechanism and test whether the same principle operates for the neighbouring potyvirus resistances in the pea genome. We have already identified the protein, VPg, determining pathotype in PSbMV, i.e. the virus protein that probably interacts with the Sbm-l gene product. VPg is integral to viral RNA replication, and preliminary data indicate that PSbMV does not replicate in cells of the resistant plants. VPg will be used to probe for interacting plant factors by means of the yeast two-hybrid system and protein-protein affinity techniques. The biological activity of the potential Sbm-l genes will be verified by transformation of pea. The use of the resistance genes lies beyond the scope of the proposed three year project but results are expected to contribute to the platform of understanding leading to the next generation of potyvirus resistant plants.
6	**Date**:	1997-2002
	Project Code:	**96/T/18**
	Title:	**Management of insect pests and viruses using ecologically compatible technologies**
	Partners:	Greece, Italy, UK
		Management of insect pests and viruses. (TOBACCO)

7	**Date**:	1997 -2000
	Project Code:	**BIO4972300**
	Title:	**Composition of plant RNA replicases**
	Partners:	Greece, Spain, Netherlands, UK
		The genome of the vast majority of plant viruses consists of RNA. This genome encodes one or more proteins that act as subunits of an enzyme complex, the "RNA-dependent RNA-polymerase" (RdRp) or "replicase, that is responsible for the replication of the viral RNA. Available evidence indicates that, in addition to viral proteins, these replicases also contain subunits encoded by the plant genome. Despite 25 years of research, the composition of a viral replicase or the function of host proteins in the enzyme complex is not known for any plant (or animal) RNA virus. Sequence similarities between viral replicase proteins indicate an evolutionary relationship between the enzymes of different viruses and suggest that different viruses make use of similar host proteins to replicate their RNA genome. The first objective of this proposal is to identify the host subunits in the replicases of three plant viruses, representing the two major superfamilies of plant viruses. The interaction between virus and host proteins will be studied by a number of novel techniques including the yeast two-hybrid system, novel reporter genes and protein tags that permit the purification of replicases by affinity column chromatography. In addition, hoSt proteins interacting specifically with viral RNAs will be identified by a newly developed technique for screening cDNA libraries. The proposal will focus the research of leading experts in the respective fields from four European countries (NL, GB, ES, GR) on common virus/plant interactions. Identification of host proteins involved in the synthesis of viral RNA will represent a major break-through in understanding the replication mechanism of RNA viruses and will offer targets for the engineering of plants with a broad resistance to virus infection. Transformation of plants with these (mutant) host genes will complement current strategies to obtain plants with a highly specific virus-resistance by transformation of plants with viral sequences. In addition to replicases induced by virus infection, plants contain an RdRp activity that is detectable in non-infected plants. The host RdRp appears to be associated with a RNase activity and is believed to play a role in the phenomenon of RNA-mediated resistance of plants to viruses. This resistance is based on the activation of a host activity that specifically degrades transgenic viral RNA and homologous genomic RNA of an infecting virus, and may resemble the phenomenon of co-suppression of host gene expression. The second objective of this proposal is to identify plant genes encoding components of the host RdRp/RNase complex or other factors involved in RNA-mediated resistance to virus infection. Identification of these genes by transposon tagging will shed light on the function of the host RdRp in noninfected plants and will increase the efficacy of strategies to engineer RNA-mediated resistance to virus

		infection in plants. The results accruing from both objectives will be promoted where appropriate by the commercial partner in this proposal. The benefits of this proposal include increased crop yield and quality, and a reduction of the use of pesticides that are currently being applied to control the vectors that transmit viruses from plant to plant..
8	**Date**:	1998-2000
	Project Code:	**PJ_REF:BIO4980374**
	Title:	**Assessment of Risks induced by virus-derived transgene products in plants, using luteoviruses carrying the green flourescent protein as a visible reporter**
	Partners:	Netherlands, Sweden, France
		General information: The large number of genetically-engineered virus resistant plants which is expected to be put forward for approval for marketing in the EU prompts the need for risk assessment studies on the potential interactions between viral transgene products and infecting viruses or viroids. Major concern centres on three main classes of hazards: heterologous-encapsidation, RNA recombination and synergism which may result in viruses and viroids with altered genetic traits, novel combinations of properties, or novel' diseases. Little information is available concerning the probability of the occurrence of the undesired interactions in transgenic plants, making it impossible to reach generally acceptable conclusions about the environmental safety related to the introduction of these plants. The major bottleneck in obtaining sufficiently large data sets on recombination heterologous encapsidation and synergism is to detect these events. The current proposal aims to tackle this problem by using a visible reporter, the jellyfish green fluorescent protein (GFP), which will markedly simplify and speed up the detection of the aforesaid events, thus allowing large numbers of virus-infected transgenic plants to be screened. The GFP-encoding gene will be incorporated into the genome of beet western yellows luteovirus (BWYV) and potato leafroll luteovirus (PLRV). The GFP-carrying viruses will then be used to challenge transgenic plants expressing luteoviral and potyviral sequences which are commonly used to achieve pathogen-derived resistance in a large number of important arable and vegetable crops. Luteoviruses are most appropriate for this type of risk assessment studies because (i) they are known to readily undergo heterologous-encapsidation during co-infections in nature, and encapsidate viroid RNA, (ii) their genetic maps suggest that they arose by RNA recombination between different ancestor viruses and several areas on their RNA are potential 'hot spots' for recombination, and (iii) synergism is a common phenomenon in mixed infection of a luteovirus and a potyvirus, leading to enhanced symptom expression and higher virus litres. A consortium of research partners will combine

		their complementary expertise in order to address the following objectives: 1. To identify heterologous-encapsidation of PLRV and BWYV in transgenic plants expressing the viral capsid-associated proteins of these viruses, and to assess whether potato spindle tuber viroids (PSTVd) will be encapsidated by these transgenic proteins. 2. To identify RNA recombination between luteovirus-derived transgenes and infecting BvVYV or PLRV, and between PVY-derived transgenes and infecting luteoviruses. 3. To identify the occurrence of synergism between potyviral transgene products involved in virus movement and infecting luteoviruses. 4. To evaluate the probability of the aforementioned events, particularly in relation to naturally occurring mixed infections of the viruses and viroids involved. The project is organized in work packages in which each of these points will be addressed. The project starts off with generating the required transgenic plants and the full-length infectious luteoviral clones carrying the GFP gene. Measurable deliverables are (i) well described methods to detect possible interactions between viral transgenic products and co-infecting viruses and viroids, and (ii) procedures to assess the probability of these interactions. These methods and procedures are pivotal for regulatory authorities carrying out risk assessment under Community legislation.
9	**Date**:	2000 -2002
	Project Code:	**SMT4-CT98-2246**
	Title:	
	Partners:	Greece, Italy, UK, Netherlands, Spain
		http://www.springerlink.com/content/h8t8m6e7eqxt9y2t/ http://www.springerlink.com/content/u4211t7q02051q31/
10	**Date**:	2000-2002
	Project Code:	**EU 5th Framework**
	Title:	**Virus Detector:** Improved diagnostic tools for the certification of strawberry propagation material
	Partners:	**Czech Republic, Netherlands, Germany, Poland, Italy**
		Viruses constitute a significant threat to the strawberry industry, causing severe economic losses. These viruses include the causal agents of quarantine diseases of major economic importance, such as crinkle, mottle, vein banding, and mild yellow edge disease. Rapid and simple methods for the detection of the major aphid-borne strawberry viruses are unavailable, due to problems with the purification of these

		viruses from plant material. Control of the quarantine viruses is difficult and relies completely on excluding viruses by meristem culture and rigorous certification programmes to maintain clean planting stock. Most aphid-borne strawberry viruses can only be detected by using time-consuming biological indexing procedures introduced over 40 years ago, such as grafting to sensitive indicator plants. Methods based on the detection of viral nucleic acid would offer an alternative to the expensive and time-consuming procedures currently used.
11	**Date**:	2000-2003
	Project Code:	**QLRT-1999-01116**
	Title:	**AFPTEST** **Standard test kits incorporating novel antibody fusion proteins to detect harmful viruses**
	Partners:	UK, Austria, France, Germany
		Objectives The aims of the project are to produce and validate test kits based on antibody fusion proteins (AFP) to detect and identify three harmful viruses: tomato spotted wilt virus (TSWV), potato leafroll virus (PLRV) and beet necrotic yellow vein virus (BNYVV), and to prove the benefits on a realistic scale. The target viruses are of statutory or quarantine significance and pose a threat to the free movement of plants and plant propagation material in Europe. Currently, laboratories obtain reagents for these viruses from a variety of different sources. With the introduction of plant passports, it is important to have confidence in the results of tests done across the European Union, so the standardisation of tests and harmonisation of test methods is desirable. Yields of functional AFP produced in *Escherichia coli*, *Drosophila* and *Pichia* expression systems will be compared in pilot expression studies. Large quantities of AFP will be produced in the best systems and purified. Prototype kits will be designed and components optimised for stability and performance and these will be extensively evaluated by end users and modified as necessary. Once the effectiveness of the new technology and of the reagents have been demonstrated, the relevant plant health authorities and technology users will be notified and we expect the results to influence policy and assist harmonisation of tests for these viruses.
12	**Date**:	2000-2003
	Project Code:	**ICA4-CT-2000-30007**

	Title:	**Sweet potato viruses** :The identification, incidence and control of sweet potato viruses in East and South Africa and assessment of host plant resistance for sustainable development programmes.
	Partners:	Germany, Uganda, Sweden, South Africa, UK
		Sweet potato is a major staple food in Africa. The proposed work surveys sweet potato crops in Uganda, Kenya and South Africa using methods which seek both known and unknown viruses. Novel viruses and variants of known viruses will be identified and characterised and diagnostic methods will be developed. The yield effects and symptoms of each virus on local Ugandan, Kenyan and South African sweet potato varieties will be recorded; resistance will be sought. The impact of virus disease on the livelihoods of farmers and the potential role of resistance will be assessed. Sweet potato chlorotic stunt, common in Africa, is unusual in that it breaks resistance of sweet potato to at least two other important viruses; the molecular basis and range of this synergism will be elucidated. Training toped level will be provided to 3 African students and links UK, German, Swedish, Kenyan, Ugandan and S African institutes.
13	**Date**:	2000-2004
	Project Code:	**QLRT-1999-01471**
	Title:	**DISCOVAR: Development of diagnostic tools and host plant resistance to control the rapid spread of lettuce big-vein and ring necrosis disease in leafy vegetables**
	Partners:	Germany, Netherlands, Spain, UK
		To combat the soil-borne, fungus transmitted varicosa viruses of lettuce and other leafy vegetables in Europe, the project objectives are: 1) the full characterisation and classification of the viruses causing big-vein (BVD) and ring necrosis disease (RND), and providing insight in the variability of these viruses 2) the development of diagnostics for the sensitive and specific detection, and quantification of the viruses and their fungal vector, *Olpidium brassicae*, in soil, irrigation water and breeding lines 3) the identification, characterisation and exploitation of natural host plant resistance of *lactuca* and *cichorium* to BVD and RND 4) the development of a sustainable system for the production of lettuce and endive through the integration of objectives 1, 2 and 3.
14	**Date**:	2000-2004
	Project Code:	
	Title:	**Demonstration project on novel diagnsotics**
	Partners:	UK
		Development & validation of novel recombinant antibody-based

		assays for virus detection
15	**Date**:	2000-2004
	Project Code:	**QLK3-2000-00361**
	Title:	**VRTP IMPACT :Virus-resistant transgenic plants: ecological**
	Partners:	UK, France, Germany, Hungary
		Virus resistance was among the very first agronomically useful traits to be introduced into transgenic plants, and several virus-resistant transgenic cultivars have already been commercially released in the US and China. In order to provide the necessary science-based risk assessment of such plants before contemplating commercial release in Europe, it is essential to clarify several points concerning potential ecological impact. The most important potential impacts could result from two forms of gene flow, either from plant to virus by recombination, or from plant to plant by sexual outcrossing. VRTP IMPACT will study recombinational plant to virus gene flow in two extremely important groups of plant viruses, the cucumoviruses and potyviruses, and will study plant to plant gene flow in two major crops that have sexually compatible wild relatives in Europe, beet and oilseed rape. At the end of this three year project, it is expected that the results will clarify whether the currently developed types of virus-resistant cultivars can be released with acceptable risk from either of these two forms of gene flow in the absence of transgenic plants.
16	**Date**:	2001-2004
	Project Code:	**QLRT-2000-02270**
	Title:	**DIAG CHIP** **Feasibility of a European Union plant health directive (77/93/EEC): Diagnostic chip**
	Partners:	UK, Spain, Germany, France
		Objectives The objective of the project is to establish methodologies for the direct detection of quarantine pathogens of potato in suspect plant samples using gene chip technology. The gene chip consists of a glass microscope slide with the gene sequences from each of the organisms that need to be detected in a single assay arrayed on its surface. Methods will be established for sample extraction, sample labelling, hybridisation and data capture. The two most important characteristics of a diagnostic protocol will be compared with traditional methods, i.e. sensitivity and specificity. The end point of the project is a 'ring test' where, following a training course to facilitate technology transfer, the

		technology developed will be evaluated by quarantine diagnosticians.
17	**Date**:	2002-2006
	Project Code:	**QLRT-2000-01183**
	Title:	**RESISTANCE IN GRAPEVINE** **Engineering durable pathogen resistance in grapevine: A novel strategy for integrated disease management to overcome environmental impacts of pesticides**
	Partners:	Germany, Portugal, Italy,
		Objectives and Outcomes The aim of the 'Resistance in Grapevine' project was to engineer antibody fragments specifically binding and inactivating viral proteins from selected Nepo- and Closteroviruses. Proof-of-concept for the antibody-based pathogen resistance strategy should be demonstrated by the production of these antibody fragments in transgenic N. benthamiana plants and challenging with viral pathogens followed by the generation of transgenic rootstock and grapevine varieties showing durable resistance.Numerous antibody scFv fragments have been generated by phage display or hybridoma technology binding to the coat protein of the Closteroviruses GLRaV-2 and -3 and the Nepoviruses ArMV and GFLV. Selected scFvs have been expressed in bacteria and purified recombinant proteins were analyzed in detail for their integrity, yield and functionality.Stable transformation of Nicotiana benthamiana confirmed the high accumulation of functional scFvs in the plant cell cytosol. The T1 progeny of selected plant lines producing the GFLV-specific scFvs conferred complete resistance against GFLV and partial resistance against ArMV . Partial pathogen resistance was observed in transgenic N. benthamiana expressing ArMV specific scFvs. Evaluation of the T2 progeny for improved scFv accumulation and durable virus resistance is in progress. Stable transformation and expression of GLRaV-2 and GLRaV-3 scFvs was also obtained in N. benthamiana. Stable transformation of rootstocks and grapevine plants with the virus specific scFvs has been performed using optimized protocols.

18	**Date**:	2002 -2005
	Project Code:	**CRAFT QLK5-CT-2002-70996**
	Title:	**POTYPROTECT: Biological suppression of severe plant viruses**
	Partners:	Greece, France, Bulgaria, Cyprus, Italy, Israel
		http://www3.interscience.wiley.com/journal/118562537/abstract
		http://www.biomatnet.org/publications/2018broch.pdf
		The aim of this project was to develop cross-protection as an immune-like safeguard from viral diseases in plants
19	**Date**:	2002-2003
	Project Code:	**(B04927)**
	Title:	**InterRegIIIB Aquitaine / Euskadi**
	Partners:	
		The purpose of this project is to make profitable the progress generated for the analyses of genomic and of functional genetics in order to study the interactions plants/pathogenic for the pathosystem Prunus/Sharka. The approaches will identify genes expressed in response to the infection using cDNA-AFLP and the cloning of Gene Analogues of Resistance (AGR) known from other species, herbaceous. The characterization of genes of interest (genes candidates, genes selected for their pattern of expression) will include/understand a structural base (information deduced from the sequence, genic complexity), a transcriptional base (abundance of mRNAs under various conditions of infection and of stress), a functional and genetic base (Co-localization with genomic areas implied in resistance). The collection of results obtained should enable us to dissect at the molecular and cellular level the profile of infection by the virus at a sensitive or resistant plant and to potentially generate markers usable in Selection Assisted by Markers within the framework of the fight against Sharka. Finally, this inter-area project will join teams in Aquitaine and in the Basque Country concerned with the genomic functional calculus and interested consequently set of themes “Study of the interactions plants/virus”. It aims at bringing together research projects relating to a socio-economic problem of first importance to France, Spain and more generally to Europe: Sharka**.**
20	**Date**:	2002-2004
	Project Code:	**QLK5-CT-2001-51880**
	Title:	**Plant recovery and tolerance to plum pox virus: the genetic and cellular basis of the phenomenon in stone fruit trees**

	Partners:	France
		Objectives: The purpose of this project is to analyse the genetic and cellular basis of resistance in plum breeding lines deride from a quantitatively resistant (C.najbolja) cultivars, displaying the plant recovery phenomenon
21	**Date**:	2002-2006
	Project Code:	**QLK3-CT-2001-60032**
	Title:	**Studies of plant virus biology and disease processes**
	Partners:	UK
		http://www.plantphysiol.org/cgi/content/abstract/138/4/2155
22	**Date**:	2003-2006
	Project Code:	**QLK3-CT-2002-02140**
	Title:	**TRANSVIR: Environmental impact assessment of transgenic grapevines and plums on the diversity and dynamics of virus populations**
	Partners:	Italy, France, Spain, Germany, Slovenia, Romania
		The overall goal of proposal is to assess the environmental impact of transgenic grapevines and plums expressing CP genes on the diversity and dynamics of virus populations in the field. Our main objectives are to: 1) Analyze and compare the dynamics and variability of virus populations in transgenic versus non-transgenic plants. 2) Monitor the development, viability, properties, and environmental impact of recombinant virus species. 3) Examine whether transgenic grapevines and plums expressing viral CP genes increase the likelihood of emergence of recombinant viruses beyond that of natural background events. 4) Evaluate if virus infection favors the translocation of virus-derived transgene transcripts from transgenic rootstocks into non-transgenic scions. 5) Determine the reversion of PTGS, and hence of the engineered protection, in plums in the presence of heterologous viruses. Our results will contribute to the development of transgenic grapevines and stone fruit trees that are environmentally safe and exhibit resistance to important viruses, including Grapevine fanleaf virus (GFLV), Grapevine virus A (GVA), and Grapevine virus B (GVB) in the case of grapevines, and Plum pox virus (PPV) in the case of plums. In addition, our studies will be helpful to design CP gene constructs that confer virus resistance while minimizing the emergence of recombinant viruses with undesirable properties.

23	**Date**:	2002-2007
	Project Code:	
	Title:	
	Partners:	Czech Republic
		Development of biomarkers for the detection of fruit viruses by array technology
24	**Date**:	2003-2006
	Project Code:	**QLG2-CT-2002-01673**
	Title:	**Virus-induced gene silencing : unravelling the basis of a mechanism and its exploitation for the analysis of a multitude of individual gene functions in plants (VIS)**
	Partners:	Estonia, Netherlands, Ireland, Greece, Switzerland, Hungary, UK, Spain
		Objectives: The objective of the proposal is to produce a detailed description of the molecular mechanisms underlying different stages of virus-induced gene silencing (VIGS) in plant cells. VIGS is a recently discovered sequence - specific RNA degradation mechanism in plants, that has a huge potential for the functional analysis of individual genes on the genome-wide scale, provided that its general principles are better understood. We plan to investigate the following aspects of VIGS: i) viral features detected by the plant^TMs surveillance mechanism; ii) suppressors of VIGS; iii) host factors required for VIGS; v) role of DNA methylation in VIGS . The fulfilment of the project should pave the path for the construction of generally applicable efficient VIGS vectors for the functional analysis of multitude of plant genes.
25	**Date**:	2005-2007
	Project Code:	
	Title:	**Soil-borne cereal mosaic virus, Wheat spindle streak mosaic virus**
	Partners:	Germany, France, UK
		Soil-borne cereal mosaic virus (SBCMV) belonging to the Furoviruses is a seriousconstraint to winter wheat cultivation in Europe. Due to transmission by the plasmodiophorid Polymyxa graminis, chemical measures are neither effective nor acceptable for economical and ecological reasons. Therefore, the only possibility of controlling this virus is breeding and growing of resistant

		cultivars. Genetic analyses of a set of DH-lines indicated that a single locus from chromosome 5DL controls resistance to SBCMV in related European wheat cultivars 'Tremie' and 'Claire'. Interestingly, the Sbm1 gene for resistance to SBCMV has been recently mapped to the same chromosomal interval in wheat cv. ‘Cadenza’ that shares no common ancestry with cultivars ‘Tremie’ or ‘Claire’. In both populations a previously unmapped SSR fragment is the closest linked marker suggesting that cultivars ‘Tremie’ and ‘Claire’ may also possess Sbm1 or another SBCMV resistance gene very closely linked to Sbm1. The diagnostic value of this SSR was assessed using a collection of SBCMV resistant and susceptible wheat cultivars. Importantly, all of the susceptible genotypes carry a null allele of this SSR, whereas resistant genotypes presumably related to either ‘Claire’ and ‘Tremie’ or ‘Cadenza’ revealed a 152bp or 154bp allele, respectively. Therefore, this SSR fragment is well suited for marker assisted selection for SBCMV resistance in European wheat breeding programmes. In order to identify genes involved in resistance to SBCMV, expression profiling using RNA of inoculated and healthy plants of resistant and susceptible lines and the barley cDNA array comprising 10.000 unigenes was carried out. 14 genes differentiating between resistant and susceptible cultivars after artificial SBCMV infection were identified which are recently analysed in detail.
26	**Date**:	2006-2010
	Project Code:	**FOOD-CT-2006 –016214**
	Title:	**EUSOL**
	Partners:	Bulgaria, Netherlands (virus aspect)
		The aim of the EU-SOL project is to develop high quality tomato and potato varieties with improved traits important for consumers, processors and producers. The project particularly focuses on mapping, isolating and characterizing genes underlying important traits such as healthiness, nutritional value, taste, flavor, fragrance, shelf-life, starch composition, yield and plant architecture.New alleles of key-genes for these traits will be extracted from the rich biodiversity present in the Solanaceae. This natural biodiversity is an under-exploited sustainable resource that can enrich the genetic basis of cultivated plants. Assembly of these genes within new genotypes will boost our knowledge of the factors that control quality. Also, it will provide a blueprint for novel high quality varieties to be developed by EU breeding companies using breeding strategies based on marker-assisted breeding and genetic engineering using exclusively natural plant genes.
27	**Date**:	2007-2010

	Project Code:	**FP6-STREP-044189**
	Title:	**Pepino mosaic virus**
	Partners:	Germany, Poland, Spain
		PEPEIRA is an RTD activity aimed at developing an EU-wide Pest Risk Assessment (PRA) for Pepino mosaic virus (PepMV). The proposal will investigate the epidemiology and economic impact of PepMV in order to allow a robust and scientifically-justified assessment of the risk posed by this pathogen to the European tomato industry.
28	**Date**:	2008- 2012
	Project Code:	204429
	Title:	**Sharka containment**
	Partners:	France, Germany, Italy, Spain, Turkey, Bulgaria, Romania, Czech Republic, Poland, Slovenia, Serbia
		Objective: SharCo is aimed at helping the EU face the accession of Member States known as endemic of sharka disease by providing the EU with tools such as marker-assisted selection, PPV resistant plant materials, guidelines, warning systems, decision-support system. On that purpose, the project will, in the field of epidemiology, identify driving factors of PPV spread and diversification and develop novel and highthrough-put detection systems warning sharka outbreaks. In the field of genetics, it will provide molecular markers for the implementation of marker assisted selection of PPV resistant fruit varieties. In the field of biology, we will assess innovative biotechnological approaches to broaden resistance to PPV in different fruit tree species. Finally, in order to develop a PPV outbreak management, we will elaborate i) guidelines for endusers and policy makers concerning cultivation and risk management, ii) an early warning system coupled with a decision support system. All knowledge and tools developed by the project will be widely disseminated all over Europe with special attention made to PPV endemic countries.

ANNEX 2

References relating transgenic virus resistant grapevine and plums in Europe

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ANNEX 3

Construction of a database of patents on plant virus resistance for the RESISTVIR Consortium

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Patent Search Strategy An Israeli company (Arad-Ophir Ltd. http://www.arad-ophir.co.il/) was selected and tested for the patent search**.** Final results were delivered and maintained in Excel format. The final search was performed in the Claims of the patents of the following Patent Authorities through the Delphion company service (on 22/5/2006):

- US – Granted patents from 1971 to search date
- US – Applications from 2001 to search date
- EP – Granted patents from 1991 to search date
- EP – Applications from 1986 to search date
- PCT – Granted patents and Applications from 1978 to search date
- Japan Abstracts from 1973 to search date

Plant and Virus and Resist** was searched in the Claims of the patents in the above list, yielded 1257 patents, and refined manually by reading.

Searching patent claims presented some problems. Many patents demonstrate resistance to some plant pathogen, and by extension claim resistance to a list of other pathogens, including viruses. Some claim plant cultivars (not virus resistant) that will still be patented if transformed with e.g. virus resistance. Some claim some resistance to a human or animal virus and by extension to similarly created transgenic plants. Also *results* are claimed, not mechanisms, which is scientifically disappointing.

Results From the search of 22/5/2006 for patents worldwide we resolved:
159 different patent groups demonstrating plant virus resistance,
48 Plant Variety Patents claiming virus resistance,
97 groups of patents where plant virus resistance is claimed but not demonstrated.

The accompanying "Database for Plant Virus Resistance Patents by European Inventors (to May 22nd, 2006)" carries 54 groups of patents *demonstrating plant virus resistance* with European authors or co-authors, and a single European-authored Plant Variety Patent which demonstrates resistance to a plant virus vector.

Column headings of the database.

Column heading	Meaning
Title	Full title of the patent
Publication Number and Date	Publication and date number of the patent
Assignee/ Applicant Name	The owner of the patent
Inventor Name	The inventor of the patent
Priority Number and Date	Number assigned pre-publication; may be different from publication number. Original application date;

	different from publication date
Link to full text	Hyperlink giving free direct access to full patent text

To follow the hyperlinks press "Control" and "left mouse click" simultaneously.

Notes A Patent is a granted award, and has a legal status. A Patent Application has not yet been granted. The status of an Application is not defined in our search i.e. we were not supplied with information on the position of an Application in the long patent process, or if the Application has been abandoned. Additionally, it is impossible to know what will remain of an Application's Claims after the Patent is granted. Many items in the database are Applications in a variety of jurisdictions (countries) with different legal requirements and processes.

- US patents currently have numbers in the 5 to 7 million range.
- From 1997 US patent applications were published in the form of USyearxxxxxxxx i.e. US2004000542902P.
- Generally, US patents are granted for 17 years from issue (prior to 1997), and after 1997 for 20 years from filing application, as is the case in most jurisdictions and the EPO.
- EPO (European Patent Organization): Patents ending in A are applications, while those ending in B are patents.
- WIPO (World Intellectual Property Organization) Patent Applications are prefaced WO, and are only applications.

Database for Plant Virus Resistance Patents by European Inventors (to May 22nd, 2006)**.**

Victor Gaba and Amit Gal-On, Dept. of Plant Pathology and Weed Science, ARO Volcani Center, POB 6 Bet Dagan 50250, Israel

Note: Only patents with European inventors are included. Patents by European companies by non-European inventors have been excluded. Some non-European inventors are included working with Europeans.

Country codes used: AT Austria; BE Belgium; CH Switzerland; DE Germany; DK Denmark; ES Spain; FI Finland; FR France; GB Great Britain; IL Israel; IT Italy; NL Holland; PT Portugal; SE Sweden; RU Russia; NZ New Zealand; US U.S.A.

Patents are arranged by country of European author using country codes above (with non-European co-authors' countries listed after), and then alphabetically by senior author's family name.

Title	Publication No. and Date	Assignee/ Applicant Name	Inventor Name	Inventor country	Priority No. and Date	Link to full text
Method for screening for mutant virus movement proteins (MP)	WO04023137 A2; 18/03/2004	ARC Seibersdorf Research GMBH	Waigmann, Elisabeth	AT	AT2002000001 330; 05/09/2002	full text link
Method for inducing viral resistance into a plant	US6297428; 02/10/2001	SES Europe N.V./S.A.	Guilley; Hubert|Jonard; Gerard|Richards; Ken|Bouzoubaa; Salah|Bleykasten-Grosshans; Claudine|Weyens; Guy|Lefebvre; Marc	BE; FR	EP19960008701 06; 19/08/1996	full text link
Process for obtaining and multiplying defective, non infectious virus genomes	EP134536B1; 25/03/1992	Ciba-Geigy AG	Paszkowski, Jerzy, Dr.|Lazar, Gabor, Dr.|Shinshi, Hideaki, Dr.|Rauseo, Isabelle, Dr.|Hohn, Thomas, Dr.|Potrykus, Ingo, Dr.	CH	CH1983000004 235; 04/08/1983	full text link
Disease resistant transgenic plants	WO9943833A 1; 02/09/1999	Universit¸ Bern	Kuhlemeier, Cornelius, Jan|Tadege, Million|Dupuis, Isabelle|Bucher, Marcel	CH	WO1998IB0000 232; 26/02/1998	full text link
Molecular pathogenicide mediated plant disease resistance	EP1123398B1; 10/08/2005	Fraunhofer-Gesellschaft zur F¤rderung der angewandten Forschung e.V.	Fischer, Rainer|Schillberg, Stefan|N¸Hring, J¤Rg|Sack, Markus|Monecke, Michael|Liao, Yu-Cai|Spiegel, Holger|Zimmerman, Sabine|Emans, Neil|Holzem, Achim	DE	EP19980001196 30|IN19983398 0066698; 1998-10-16|1998-10-16	full text link
Inducible virus resistance in plants	EP298918B1; 05/09/2001	Syngenta Participations AG	Hohn, Thomas, Dr.|Bonneville, Jean-Marc, Dr.|F□tterer, Johannes, Dr.|Gordon, Karl, Dr.|Sanfa¬on, H·l¤ne, Dr.	DE	CH1987000002 645; 10/07/1987	full text link
Method of inducing the virus resistance in	US200313943 2A1;	Kohle Harald|Conrath Uwe|Seehaus Kai	Kohle, Harald|Conrath, Uwe|Seehaus, Kai	DE	DE2000010021 190; 03/05/2000	full text link

plants	24/07/2003					
RNA and DNA molecules for producing virus resistance	EP558944A3; 08/06/1994	Max-Planck-Gesellschaft zur Fэrderung der Wissenschaften e.V.|BAYER AG|HOECHST AKTIENGESELLSCHAFT	Loss, Peter, Dr.|Schreier, Peter, Dr.|Maiss, Edgar, Dr.|Schneider, Rudolf, Dr. c/o Hoechst AG	DE	DE1992004203 441; 06/02/1992	full text link
Transgenic plants displaying virus and phosphinothricin resistance	US5633434; 27/05/1997	Hoechst Aktiengesellschaft	Schneider; Rudolf|Donn; Gunter| Mullner; Hubert	DE	DE1990004003 045; 02/02/1990	full text link
DNA comprising plum pox virus and tomato spotted wilt virus cDNAS for disease resistance	US5569823; 26/10/1999	Bayer Aktiengesellschaft	Schreier; Peter Helmut|Stenzel; Klaus|Adam; Gunter|Maiss; Edgar	DE	DE1993004317 845; 28/05/1993	full text link
Method for the production of transgenic plants with increased virus resistance by silencing vegetable dnaj-like proteins	US200525187 9A1; 01/03/2006	IPK Institut für Pflanzengenetik und Kulturpflanzenforschung	Hofius, Daniel|B"Rnke, Frederik|Sonnewald, Uwe	DE, DK	DE2002010232 978; 19/07/2002	full text link
Plant proteins	US200204641 6A1; 18/04/2002	Consejo Superior de Investigaciones Cientificas	Gutierrez-Armenta, Crisanto|Sanz-Burgos, Andres Pelayo	ES	WO1996ES000 0130; 13/06/1996	full text link
Transgenic plants displaying multiple virus resistance and a process for their production	US5589625; 31/12/1996	Kemira Oy, Biotech	Saarma; Mart|Kelve; Merikke|Truve; Erkki|Teeri; Teemu	FI	EP19920001046 76; 18/03/1992	full text link
Protein with plant protecting properties	EP868431B1; 29.12.04	Djavakhia, Vitali G.|Batchikova, Natalia|Korpela, Timo|Khomutov, Radii M.|Nikolaev, Oleg	Djavakhia, Vitali G.|Batchikova, Natalia|Korpela, Timo|Khomutov, Radii M.|Nikolaev, Oleg	FI; RU	FI19950000036 88; 2.8.95	Link
Virus-resistant transgenic plants	US5968828; 19/10/1999	Helsinki University Licensing Ltd. Oy	Pehu; Eija|Pehu; Tuula|Maki-Valkama; Tuula|Valkonen; Jari|Koivu;	FI;US	US1996000751 233|US1994000	full text link

comprising cells transformed with a polynucleotide encoding a potyviridae P1 protein or P1 protein fragment			Kimmo|Lehto; Kirsi		246123; 1996-11-18|1994-05-19	
EIF4e gene mutations and potyvirus resistance	US20052554555; 17/11/2005	Genoplante Valor	Caranta C., Ruffel S., Bendahmane A., Palloix A., Robaglia C.	FR	-	full text link
Combination of mutation in both eIF4E et eIF(iso)4E for resistance against Potyvirus	WO 05/118850; 15/12/2005	Genoplante Valor	Caranta C., Ruffel S., Palloix A., Fabre N.	FR	-	not available
Transgenic plants belonging to the species Cucumis melo	US5789656; 04/08/1998	Biosem	De Both; Michoel|Ben Tahar; Sophia|Noel; Marianne|Perret; Joel	FR	FR1989000010848; 11/08/1989	full text link
Regeneration and genetic transformation of sugar beet	EP517833B1; 02/11/1995	Biocem	Gerentes, Denise|Perez, Pascual|Kallerhoff, Jean|Ben Tahar, Sophie|Perret, Jo[L	FR	FR1990000002686|FR1990000002687; 1990-03-02|1990-03-02	full text link
Means for identifying the locus of a major resistance gene to the rice yellow mottle virus, and their applications	US20030938300A1; 15/05/2003	Ghesquiere Alain|Albar Laurence	Ghesquiere, Alain|Albar, Laurence	FR	FR1999000007834; 21/06/1999	full text link
Method of genetic modification of a wild type viral sequence	US6835538; 28/12/2004	SES Europe N.V./S.A	Lauber; Emmanuelle|Guilley; Hubert|Richards; Ken|Jonard; Gerard	FR	FR1998000870159; 10/07/1998	full text link
DNA virus ribozymes	WO9503404A1; 02/02/1995	Biocem; Commonwealth Scientific And Industrial Research Organisation; Lenee, Philippe; Gruber, V'Ronique;	Lenee, Philippe|Gruber, V'Ronique|Baudino, Sylvie|Mason, John|Comeau, David|Rezaian, Mohamad, Ali|Dry, Ian, Barry|Rigden, Justin, Ellis	FR	WO1993EP0001946; 22/07/1993	full text link

		Baudino, Sylvie|Mason, John; Comeau, David; Rezaian, Mohamad, Ali; Dry, Ian, Barry; Rigden, Justin, Ellis				
Polyribozyme capable of conferring on plants resistance to cucumber mosaic virus and resistant plants producing this polyribozyme	US6265634; 24/07/2001	Gene Shears Shears Pty. Ltd.	Lenee; Philippe|Perez; Pascual|Gruber; Veronique|Baudot; Gaelle|Ollivo; Catherine	FR	FR1993000002269; 26/02/1993	full text link
Method for inducing viral resistance into a plant	EP1038961A1; 27/09/2000	Centre National De La Recherche Scientifique	Jonard, G'Rard|Lauber, Emmanuelle|Guilley, Hubert|Richards, Kenneth	FR	EP1999000200773; 16/03/1999	full text link
Method of conveying BNYVV resistance to sugar beet plants	US6956149; 18/10/2005	SES Europe N.V./S.A.	Richards; Kenneth|Jonard; G'rard|Guilley; Hubert|Van Dun; Cornelis Maria Petrus	FR; NL	EP1999000200236; 27/01/1999	full text link
Methods and DNA constructs for gene silencing in transgenic plants	US6635805; 21/10/2003	Plant Bioscience Limited	Baulcombe; David Charles|Angell; Susan Mary	GB	GB1997000003146|GB1998000000442; 1997-02-14|1998-02-12	full text link
Virus resistant plants	WO9321329A1; 28/10/1993	The Gatsby Charitable Foundation|Baulcombe, David|Longstaff, Marian	Baulcombe, David|Longstaff, Marian	GB	GB1992000008545; 21/04/1992	full text link
Plant-derived resistance gene	WO9954490A2; 28/10/1999	Plant Bioscience Limited	Bendahmane, Abdelhafid|Baulcombe, David, Charles|Kanyuka, Konstantin Valerievich	GB	GB1998000008083; 16/04/1998	full text link
Novel plant virus sequences	WO9322345A1; 11/11/1993	Unilever Plc|Unilever N.V.|Boulton, Robert, Edwin|Brears, Timothy|Foulds, Ian, Jeffrey|Jack, Peter, Liam|James, Christopher, Michael|Lea, Vincent, John|Sidebottom, Christopher,	Boulton, Robert, Edwin|Brears, Timothy|Foulds, Ian, Jeffrey|Jack, Peter, Liam|James, Christopher, Michael|Lea, Vincent, John|Sidebottom, Christopher, Michael|Slabas, Antoni, Ryszard|Stratford, Rebecca	GB	GB1992000009669; 02/05/1992	full text link

		Michael|Slabas, Antoni, Ryszard|Stratford, Rebecca				
Improvements in or relating to disease-resistance of plants	WO9504825A1; 16/02/1995	Unilever Plc|Unilever N.V.|Stratford, Rebecca|Boulton, Robert, Edwin|Higgins, Elaine, Susan	Stratford, Rebecca|Boulton, Robert, Edwin|Higgins, Elaine, Susan	GB	GB1993000016384; 06/08/1993	full text link
Virus resistance in plants	WO9203539A1; 05/03/1992	Imperial Chemical Industries Plc|Buck, Kenneth, William|Hayes, Robert, James	Buck, Kenneth, William|Hayes, Robert, James	GB	GB1990000018646; 24/08/1990	full text link
Raspberry plant named `Glen Ample`	USPP011418; 13/06/2000	Scottish Crop Research Institute	McNicol; Ronnie J.|Jennings; Derek L.	GB	US1998000069762; 30/04/1998	full text link
Induction of resistance to virus diseases by transformation of plants with a portion of a plant virus genome involving a read-through replicase gene	US5596132	Cornell Research Foundation, Inc.	Zaitlin; Milton|Golemboski; Daniel|Lomonossoff; George	GB;US	US1995000488672|US1994000198182|US1992000894064|US1990000491473; 1995-06-07|1994-02-15|1992-06-08|1990-03-12	full text link
Vectors capable of imparting herbicide resistance and viral cross protection and methods	WO02059334A1; 01/08/2002	Virogene Ltd.|Friedman, Mark, M.	Gal-On, Amit|Shiboleth, Yoel, Moshe|Arazi, Tsachi	IL	US2001000263521P; 24/01/2001	full text link
Engrafted plants resistant to viral diseases and methods of producing same	WO05079162A2; 01/09/2005	State Of Israel, Ministry Of Agriculture	Gal-On, Amit|Zelcer, Aaron|Wolf, Dalia|Gaba, Victor, Paul|Antignus, Yehezkel	IL	US2004000546173P; 23/02/2004	full text link
Resistance to virus infection using modified viral movement protein	US5898097; 27.4.99	Calgene LLC	Beachy; Roger N.|Lapidot; Moshe|Gafny; Ron	IL, US	US1994000231209; 19.4.94	full text link
Method of preparation of transgenic plants	US5959181; 28/09/1999	Metapontum Agrobios S.c.r.l.	Cellini; Francesco|Grieco; Pasquale Domenico	IT	IT1996MI000092 7; 09/05/1996	full text link

resistant to viral infections and so obtained plants						
PMMoV resistant Capsicum plants	EP1647182A1; 19/04/2006	De Ruiter Seeds R&D B.V.	Allersma, Anton Pieter|Hofstede, Ren„© Johannes Maria|Vreugdenhill, Dirk	NL	EP20040000777 44; 01/10/2004	full text link
Methods for generating resistance against CGMMV in plants	US200423713 6A1; 25/11/2004	De Both Michiel Theodoor Jan|Fierens Onstenk E.V.	De Both, Michiel Theodoor Jan|Fierens, Onstenk E.V.	NL	EP20010002004 48; 08/02/2001	full text link
Plant virus DNA constructs and virus resistant plants comprising said constructs	US5919705; 06/07/1999	Novartis Finance Corporation	De Haan; Petrus Theodorus	NL	GB1993000011 593; 04/06/1993	full text link
Virus resistant or tolerant cells	WO9509920A 1; 13/04/1995	Sandoz Ltd.|Sandoz-Patent-Gmbh|Sandoz Erfindungen Verwaltungsgesellschaft Mbh|De Haan, Petrus, Theodorus	De Haan, Petrus, Theodorus	NL	GB1993000020 548; 06/10/1993	Full text link
Plants resistant to tospoviruses	US6057492; 02/05/2000	Novartis AB	De Haan; Petrus Theodorus	NL	GB1995000005 907; 23/03/1995	full text link
A method for producing plants which are resistant to closteroviruses	EP1188833A1; 20/03/2002	De Ruiter Seeds C.V.	De Ruiter, Wouter Pieter Johannes|van der Knaap, Bernardus Josef|Klapwijk, Abraham Alexander|Hofstede, Ren„ Johannes Maria	NL	EP20000002031 91; 14/09/2000	full text link
Methods for generating resistance against CGMMV in plants	WO02063019 A2; 15/08/2002	Keygene N.V.	Onstenk E.V. Firens, Bernarda, Gerharda, Johanna|De Both, Michiel, Theodoor, Jan	NL	EP20010002004 48; 08/02/2001	full text link
Constructs containing impatiens necrotic spot tospovirus RNA and methods of use thereof	US5773700; 30/06/1998	Andoz Ltd	Van Grinsven; Martinus Quirinius Joseph Marie|De Haan; Petrus Theodorus|Gielen; Johannes Jacobus Ludgerus|Peters; Dirk|Goldbach; Robert Willem	NL	GB1992000006 016; 19/03/1992	full text link
Lettuce variety nascent	US200602107 7A1; 26.1.06	-	Van Schijndel; Johannes Theodorus	NL	US2005000178 842; 11.7.05	full text link
Lettuce variety	US200602107	-	Van Schijndel; Johannes Theodorus	NL	US2005000178	full text

nasimento	8A1; 26.1.06				913; 11.7.05	link
Methods for coupling resistance alleles in tomato	EP1563727A1; 17.8.05	Seminis Vegetable Seeds, Inc.	Braun III, Carl Joseph|Hoogstraten, Jacobus Gerardus Joannes	NL; US	US2004000777984; 12.2.04	full text link
Methods for coupling resistance alleles in tomato	WO05079342 A2; 1.9.05	Seminis Vegetable Seeds, Inc.	Hoogstraten, Jacobus, Gerardus, Joannes|Braun, Carl, III	NL; US	US2004000777984; 12.2.04	full text link
Method for conferring resistance or tolerance against furovirus, potyvirus, tospovirus, and cucomovirus to plant cells	US7019195; 28/03/2006	Syngenta Participations AG	Heifetz; Peter Bernard|Patton; David Andrew|Levin; Joshua Zvi|Que; Qiudeng|De Haan; Petrus Theodorus|Gielen; Johannes Jacobus Ludgerus	NL;FR; US	US1999000309038|US1998000150705P; 1999-05-10|1998-05-26	full text link
A method to control the ripening of papaya fruit and confer disease resistance to papaya plants	WO02082889 A1; 24.10.02	Cornell Research Foundation, Inc.|PAIS, Maria, Salom', Soares	Gonsalves, Dennis|Balde, Aladje|Chiang, Chu-Hui	PT;US	US2001000283022P; 11.4.01	full text link
Genetic method for producing virus resistant organisms	WO9844136A; 18.10.98	ZENCO (NO. 4) LIMITED	Atabekov, Jossif Grigorievich|Dorokhov, Youri Leonidovich|Morozov, Sergey Yurievich	RU	GB1997000006469; 27.3.97	full text link
Transgenic plants with resistance against tobacco rattle virus and corresponding nucleotide sequence	WO02077209 A1; 03/10/2002	Plant Science Sweden Ab	Melander, Margareta	SE	SE2001000001048; 26/03/2001	full text link
Virus resistance in plants	WO0250281A1; 27/06/2002	Plant Science Sweden Ab	Melander, Margareta|Lee, Michael	SE; NZ	SE2000000004755; 21/12/2000	full text link