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FOOD QUALITY AND SAFETY



ResistVir

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

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Co-ordination Action

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Mechanisms and sources of resistance***

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Mechanisms and Sources of Resistance

Expert group list

Mechanisms and Sources of Resistance
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▪ Aim

To identify how the collaborative programme of ResistVir could result in improved resistance to plant viruses (as specified within Annex 1).

▪ Summary of actions and conclusions

As no budget was available to invite external speakers to this meeting, we organized short presentations from several members of this expert group to promote discussions between consortium members. Thus, C. Caranta, A. Maule, O. LeGall, M. Aranda and M. Boulton each presented a selected area of their research (see Bibliography section). The presenters highlighted potential areas that could benefit through collaborative studies between ResistVir partners. The expert group then met to define the hot topic for this group. It was agreed by all members that this should be: “**NATURAL SOURCES OF DURABLE RESISTANCE TO PLANT VIRUSES**”.

The group felt that genetic resistance provided the most cost-effective and reliable approach to virus resistance in crops. This is as opposed to disease prevention through the use of environmentally-damaging insecticides and nematicides directed against virus vectors. In the current socio-political atmosphere, where the use of GM technology is not generally accepted, this should be derived by the exploitation of known resistant varieties and the exploration of natural genetic diversity in crop germplasm. If this attitude should change, however, the potential for the rapidly advancing knowledge of gene silencing to deliver novel and effective virus resistance is clear. The Expert Group also emphasized that the resistances should ideally provide broad spectrum and durable resistance and, to this end, technical approaches that revealed these additional characters should be encouraged.

The following sections provide the background to these discussions.

▪ State of the art:

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i) Dominant genes conferring resistance to plant viruses

To date, the majority of characterized resistance (R) genes have provided monogenic dominant resistance. Those characterized at the molecular level mostly confer resistance to fungal or bacterial pathogens (Hammond-Kosack & Parker, 2003), but there are currently nine examples of such genes conferring resistance to viruses (Table 1), which have been identified from both crops (e.g. potato, tomato and tobacco) and model species (*Arabidopsis*). In the majority of these cases, the examples fall into the NBS-LRR class of resistance genes, a characteristic that often provides a shortcut to their characterization through homology searches. In common with the responses to fungi and bacteria, these major dominant genes mostly confer complete resistance (qualitative) and are also mostly associated with cell or tissue necrosis. While, conceptually, complete resistance appears to be an attractive option, it suffers from the drawback that it is generally highly specific, being effective against few genetic variants of the virus. Although there has been little formal analysis, these genes tend to be less durable probably because of the strong selection pressure placed upon the pathogen population. However, this may be less of a problem for viruses than for some other pathogens (Garcia-Arenal & McDonald, 2003; Kang et al., 2005) and plant breeders have utilised dominant R genes for virus resistance when they have been available. A major limiting factor, however, is that such dominant R genes controlling a complete resistance are not available for a large number of economically important viruses (for example, for resistance against *Plum pox virus* in *Prunus* sp., for resistance against *Tomato yellow leaf curl* in tomato...)

Table 1. Dominant virus resistance genes

Gene	Virus	Plant sp.	Reference
<i>N</i>	TMV (<i>Tobamovirus</i>)	Tobacco	Whitham <i>et al.</i> , 1994
<i>Rx1</i>	PVX (<i>Potexvirus</i>)	Potato	Bendahmane <i>et al.</i> , 1999
<i>Rx2</i>	PVX (<i>Potexvirus</i>)	Potato	Bendahmane <i>et al.</i> , 2000
<i>Sw5</i>	TSWV (<i>Tospovirus</i>)	Tomato	Brommonschenkel <i>et al.</i> , 2000
<i>Tm2</i>	ToMV, TMV (<i>Tobamovirus</i>)	Tomato	Lanfermeijer <i>et al.</i> , 2003
<i>HRT</i>	TCV (<i>Carmovirus</i>)	<i>A. thaliana</i>	Cooley <i>et al.</i> , 2000
<i>RTM1</i>	TEV (<i>Potyvirus</i>)	<i>A. thaliana</i>	Chisholm <i>et al.</i> , 2000
<i>RTM2</i>	TEV (<i>Potyvirus</i>)	<i>A. thaliana</i>	Whitham <i>et al.</i> , 2000
<i>RCY1</i>	CMV (<i>Cucumovirus</i>)	<i>A. thaliana</i>	Takahashi <i>et al.</i> , 2001

ii) Recessive genes conferring resistance to plant viruses

Viruses depend upon host factors to complete their infection cycle and therefore the study of host susceptibility factors provides a unique opportunity to identify mutant alleles that could confer recessive resistance to plant viruses. This approach has recently led to the identification of a range of plant species (e.g. tomato, lettuce, pepper, pea, melon, barley, *Arabidopsis*) in which mutations of the translation initiation factor, eIF4E, result in resistance to specific plant viruses (for review see Robaglia and Caranta, 2006). The majority of the resistances function against potyvirus infection (Table 2), although recently eIF4E has also been implicated in recessive resistance to *Barley yellow mosaic virus* (*Bymovirus*, related to potyviruses), *Melon necrotic ringspot virus* (MNSV; *Carmovirus*; Aranda, unpublished data) and *Cucumber mosaic virus* (CMV; *Cucumovirus*). In the last case, separate mutations in eIF4E and eIF4G of *Arabidopsis* conferred recessive resistance to CMV. The eIF4E paralogue, eIF(iso)4E, has also been shown to be necessary for infection of *Arabidopsis* by several potyviruses (*Turnip mosaic virus*, TuMV; *Tobacco etch virus*, TEV; *Lettuce mosaic virus*, LMV; *Plum pox virus*, PPV), and recent studies (Sato *et al.*, 2005) suggest that potyviruses selectively utilize either eIF4E or eIF(iso)4E to infect this plant. Similarly, in pepper (*Capsicum* spp.), whereas

resistance to *Potato virus Y* (PVY, *Potyvirus*) and TEV (*Potyvirus*) is dependent on the mutation of a single *eIF4E* gene, resistance to *Pepper vein mottle virus* (PVMV, *Potyvirus*) requires mutations in both *eIF4E* and *eIF(iso)4E* (Ruffel *et al.*, unpublished). Therefore, some potyviruses can use several *eIF4E* isoforms. For the potyvirus-translation initiation factor interaction, the avirulence determinant was shown to be the virus-genome linked protein, VPg. Intriguingly for MNSV, the determinant was found to be the RNA itself (Diaz *et al.*, 2004, Aranda, unpublished data).

Although these resistances can be qualitative, in many cases they are quantitative and/or are a component of polygenic resistance indicating that allelic variants of the host susceptibility factor can provide a partially supporting function for virus multiplication in host tissues. Also, from the *eIF4E* example, it appears that single mutant alleles can confer resistance to diverse related viruses. Hence, the *sbm1* locus (*eIF4E*) in pea confers resistance to various isolates of *Pea seed borne mosaic virus*, to *Clover yellow vein virus* and to *White lupin mosaic virus* (all potyviruses). Another positive feature of these recessive resistances is their presence in very diverse crop and model species although it is puzzling at this time why the characterised resistances relate solely to *eIF4E* and its paralogues, or other genes (e.g. *eIF4G*) associated with the translational machinery. Nevertheless the recessive resistances offer some important complementary features when compared with the dominant resistance genes.

Table 2. Recessive resistance genes

Gene	Virus	Plant sp.	Reference
<i>pvr1</i> , <i>pvr2</i> ¹ , <i>pvr2</i> ² + <i>pvr6</i>	PVY, TEV PVMV	<i>Pepper (Capsicum spp.)</i>	Ruffel <i>et al.</i> , 2002 ; Kang <i>et al.</i> , 2005
<i>pot-1</i>	PVY, TEV	<i>Tomato (Lycopersicum spp.)</i>	Ruffel <i>et al.</i> , 2005
<i>sbm1</i>	PSbMV	<i>Pea (P. sativum)</i>	Gao <i>et al.</i> , 2004
<i>mo1</i> ¹ <i>mo1</i> ²	LMV	<i>Lettuce (L. sativa)</i>	Nicaise <i>et al.</i> , 2003
<i>AteIF4E</i>	CIYVV	<i>A. thaliana</i>	Sato <i>et al.</i> , 2005
<i>AteIF(iso)4E</i>	TuMV, LMV, TEV, PPV	<i>A. thaliana</i>	Duprat <i>et al.</i> , 2002 ; Lellis <i>et al.</i> , 2002; Decroocq <i>et al.</i> , in press
<i>rym4/5</i>	BaMMV, BaYMV, BaYMV-2	<i>Barley (H. vulgare)</i>	Stein <i>et al.</i> , 2005 Kanyuka <i>et al.</i> , 2005

iii) Genes conferring vector resistance

Although most of the Expert Group discussions focussed on genetic resistance targeted at the virus pathogen, it is also relevant to consider the knowledge available for genes that deter infestations with the vectors of virus diseases. In the majority of cases, this is limited to insect resistance. Most of the studies of resistance to insects have been directed towards chemical defences but a few have addressed specific defence responses involving gene-for-gene interactions. To date, two genes conferring resistance to potential virus vectors have been cloned using a positional cloning approach. These fall within the NBS-LRR group of plant R genes. The tomato *Mi-1* confers resistance to potato aphid (*Macrosiphum euphorbiae*), whitefly (*Bemisia tabaci*), and root-knot nematodes (*Meloidogyne* spp.), all of which are virus vectors. The melon *Vat* gene controls resistance to the vector *Aphis gossypii* and also to the transmission of non-persistent viruses (See Patent WO 2004072109, Dogimont *et al.*, unpublished data). However, there is increasing evidence that resistance to many sap sucking and chewing insects may be mediated by single resistance genes of similar structure. For

example, *Nr*, conferring resistance in lettuce against the aphid *Nasonovia ribisnigri*, shows functional similarity to *Mi-1*. It has been suggested (Kaloshian, 2004) that resistance mediated by NBS/LRR genes in insects may be a widespread resource for introducing important insect resistance into plants.

Currently, molecular markers are available for resistance to a diverse range of virus vectors and the efficacy of several are being investigated through breeding programs (e.g. *Dn2* and *Dn4* in wheat against Russian wheat aphid and *H13* in wheat against Hessian fly). Although these do not represent characterised resistance genes, they nevertheless provide valuable technical resources for the incorporation of novel resistances to crop plants. Resistance to viral vectors will be discussed in detail by Expert Group 5.

▪ **Technical Approaches**

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i. Quantitative trait analysis.

It is probable that to date less than 1% of the biodiversity available for disease resistance has been used in commercial varieties. Most agronomic traits in crop plants (and particularly polyploid crops) do not segregate as single defined qualitative characters but as quantitative traits. With regard to virus resistance, this could result from the expression of an incomplete phenotype from a single gene (see eIF4E example above) or, more commonly, from the effect of modifiers of resistance gene functions. Although these traits may confer only moderate resistance, it may nevertheless be extremely valuable within an agronomic context. For many disease resistances, improvement in crop yield of only a few percent can provide the difference between profit and loss.

These quantitative trait loci (QTLs) present particular technical challenges for their identification and their incorporation into crops. However, they provide a relatively untapped source of novel variation for disease resistance.

The study of QTLs does not preclude the identification of the underlying genes. Credible candidate genes co-localizing with QTLs have been identified for several traits, including quantitative disease resistances in rice (Wang *et al.*, 2001, Liu *et al.*, 2002). One of the approaches that facilitates QTL cloning is the increasing availability of expressed sequence tags (ESTs). Many thousands of ESTs have been catalogued for major crop species. It is possible to use a combination of QTL mapping data and mapped EST data to identify genes that underlie quantitative traits.

One limitation of the analysis of QTLs is that it is only readily achieved when using large populations segregating for the phenotypes in question, derived from parents for which a large number of genomic markers are available. This is currently possible for some of the major crops (e.g. the major solanaceous and graminaceous crops) but may be less suitable for mining extensive germplasm collections for novel resistances in a wider range of crop species. It will be essential in the future to take advantage of advances in technology in the development of high through-put quantitative resistance assays to screen larger collections of genetic resources for quantitative / polygenic resistance. The development of these kinds of tool will need strong(er) collaborations between virologists and geneticists. For orphan crops in developing countries the identification of appropriate germplasm may require greater collaboration with private companies.

ii. Marker-assisted selection of virus resistance genes.

It is not essential to identify and characterise the host genes, rather marker assisted selection (MAS) can be used to identify DNA sequences closely linked to these genes. The sequences can be derived, for example by PCR-based MAS and then tracked in breeding programmes. This has already proven to be a useful technology for introducing disease resistance to pathogens in commercially important crops, with an early example allowing the pyramiding of bacterial blight resistance genes in rice (Singh *et al.*, 2001). Using a technology designated "single large-scale MAS", the targeted genes are fixed by MAS, and the remainder of the genome is selected by conventional breeding techniques (Ribault and Betran, 1999). This proved useful to develop virus resistance in maize, rice, and beans (Singh *et al.*, 2000). Markers for MAS come in different forms and incur various levels of cost, applicability, ease of use and application to high through-put procedures. Although MAS is seen as relatively costly, Morris *et al.* (2003) and Dreher *et al.* (2003) suggest that the costs of MAS are not necessarily appreciably more than when using the conventional approach, especially when the conventional screens for virus infection may be difficult. With improvements in MAS technology costs are likely to decrease. MAS is particularly useful as the resistance locus can be identified in the absence of the pathogen, or in the case of mature plant resistance, when a gene must be selected at the seedling stage.

It is likely that increasingly more markers will be identified in the near future as additional insights are gained into resistance gene structures and mechanisms, and the technology is improved. In a study to produce markers for CAPS (cleaved amplified polymorphic sequence) markers for *Fusarium* and *Papaya ringspot virus*-resistance alleles in melon, based on the mapping of PCR-identified NBS-LRR sequences, it was found that the genes were present in a R gene cluster (Brotman *et al.*, 2005). The method also identified loci responsible for aphid resistance. Allele-specific PCR was used to distinguish the single nucleotide polymorphic (SNP) alleles of the *Rsv1* and *Rsv3* genes that confer resistance to *Soybean mosaic virus* in soybean (Jeong and Maroof, 2004). These allowed the genotyping of eight parental lines and F2 individuals of three mapping populations. MAS has also provided the means to introduce cassava mosaic virus disease resistance into cassava cultivars (Akano *et al.*, 2002), thereby providing a mechanism for improving the yields of a subsistence crop for resource poor farmers in Africa. Such tools together with a better knowledge of the distinct mechanism available in the natural diversity of crops for virus resistance should permit the construction of efficient and durable resistance systems.

iii The value of fundamental approaches in model systems for the identification and deployment of natural virus resistance.

The two sections above address the introgression of natural resistance genes and the identification of novel quantitative resistance. It should be noted, however, that few of the success stories have come about independent of parallel or preceding studies in model systems. Many crop species have the drawbacks of long generation times, large (often polyploid) genomes and limited genetic and bioinformatic resources. There is a strong strategic and financial argument for building on the advances in model species biology while also identifying pipelines whereby new findings can be effectively tested and transferred into crop species. The sequenced genomes of *Arabidopsis* and rice make them the pre-eminent models for dicot and monocot crop plants, although the sequencing of tomato and the legume, *Medicago truncatula*, will expand the repertoire of useful species.

The importance of eIF4E, eIF(iso)4E and eIF4G have emerged largely from fundamental studies including model systems. We anticipate that there will be many more potential resistance targets that function as host susceptibility factors. One question is how these will best be identified? They may emerge from the mining of diverse germplasm collections but this empirical approach is unlikely to be the most efficient strategy. The alternative is to build on existing fundamental work with

model host-pathogen interactions. New technologies (e.g. protein-protein interaction technologies and proteomics, particularly for sequenced plant genomes) make the characterisation of virus-related complexes from infected cells increasingly tractable.

We have established that host factors required for virus replication offer the potential for novel routes to resistance through the identification of mutant alleles (Thivierge *et al.*, 2005) and that, technically, these factors will be identified most readily by using model systems followed by an extrapolation of this information to important crop-pathogen interactions. In this case, it becomes necessary to identify the mutant alleles either as natural genetic variants using specific marker technology or as artificially-induced mutations. The latter is becoming possible for increasing numbers of species through the technology termed TILLING (Colbert *et al.*, 2001). TILLING technologies are currently available for barley, wheat, pea and tomato and further crops are in development.

▪ **Future directions:**

In the area of resistance to plant viruses, the European research community has divided its efforts between building a fundamental knowledge base and responding reactively to real agronomic problems, the latter being delivered where possible to the agricultural industry, usually in the form of markers for MAS. In the last 5 years, plant biology has moved into a new phase driven by advances in technology. In the next ten years, we will see the genomes of all the major crop species sequenced and the accompanying technologies for faster and larger scale analyses being developed in parallel. These developments provide new opportunities that should change the way we tackle the problem of virus resistance. It will be possible to mine much larger collections of crop germplasm genetically for known and novel resistances, and to deliver these to industry in a more timely fashion. However, as we understand more about the principles that underlie virus replication and genome expression in plants, it should also be possible to move from a reactive to a predictive mode of investigation into virus resistance. In an unstable climate, which will have profound effects upon insect vectors, this will be increasingly important. In an ideal world we should be able to predict the nature of effective resistance to potential pathogens as they appear, and to provide a more rapid response time in delivery of resistance-related tools to industry. Broad spectrum resistance offers obvious advantages here. Equally, as we understand more about virus pathogens as populations and the processes of genetic mutation and selection, we would hopefully be in a position to predict the durability of specific resistances.

In the current socio-political climate in Europe these resistances will not be based upon GM plants but rather will exploit diverse existing and novel stocks of crop germplasm. However, for viruses, GM approaches based upon gene silencing provide extremely powerful opportunities and it would be unwise to ignore their potential for the longer term. It is also important not to overlook the possibility of the convergence of non-GM and transgenic approaches. Where R genes are not available in the crops, or only in wild, or exotic germplasm, the time required for introduction of the gene by conventional breeding may be prohibitive. Dominant R genes from wild relatives could be rapidly transferred into breeding lines and it could be argued that a genomics-based approach to genetic modification of crops may be more acceptable to the public as only “natural” genes are transferred. In many cases these will represent genes that could have been transferred by conventional methods over several generations. A combined molecular breeding approach using MAS and transformation was successful in stacking of two major genes (*Piz-5* and *Xa21*) to provide resistance to blast and bacterial blight in rice (Narayanan *et al.*, 2002). The general utility of the combined genetic and GM approaches is evidenced by the increasing numbers of plants converted to disease resistance by this technique. For example, transformation of barley with the barley gene *Rpg*

(Horvath *et al.*, 2002) confers stem rust resistance. Furthermore, the potential of the method for trans-species transfer was evidenced by the resistance obtained following transformation of tomato with the tobacco N gene (that confers resistance to *Tobacco mosaic virus*) (Whitham *et al.*, 1996). Transgenic resistance will be covered in depth in the report from Expert Group 3.

▪ **Potential for links with other Expert Groups:**

- EG1 - Resistance to economically important European viruses
- EG7 - Novel durable new resistance
- EG3 - Transgenic resistance and associated risks
- EG4 - Functional genomic and proteomic approaches to resistance
- EG5 - Interference with vector transmission

▪ **Potential for links with other (Framework 6) funded projects:**

Although there are no directly relevant projects, the expert group will contact the co-ordinators of the following projects which have tangential relevance to the ResistVir aims.

- TP PLANTS AND HEALTH
- CROPBIOTERROR (assessing risks of pathogens)
- GRAIN LEGUMES (improvement of – various mechanisms)
- CLEANFRUIT (using sterile fly technology, established for med. Sci., for improving quality of fruit and citrus trees)
- EUSOL (genetic and genomic tools for tomato improvement)

▪ **Notes/References**

- ¹ Caranta, C. “Genetic and molecular basis of qualitative and quantitative resistance against viruses in vegetables (Solanaceous crops and Cucurbitaceae) and model species (*A. thaliana*)”
- Maule, AJ. “Recessive resistance to potyvirus infection”
- LeGall, O. “Genetic and molecular bases of host-virus compatibility in model and crop species (arabidopsis, lettuce and stone fruit trees)”
- Aranda, M. “Resistance of cucurbits to agronomically important viruses”
- Spak, J. “Host-virus relationships and sequence variability among fruit ilarviruses”
- Boulton MI. “Biological and genetic characterisation of resistance to geminiviruses in cereals”

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