THE PHYTOPATHOLOGICAL AND EPIDEMIOLOGICAL FEATURES OF VIROIDS

R. Flores

Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC),Universidad Politécnica, Avenida los Naranjos s/n, 46022 Valencia, Spain

Despite inducing plant symptoms similar to those accompanying virus infections, viroids have unique structural, functional and evolutionary characteristics: they are composed of a small, non-protein-coding, single-stranded, circular RNA with autonomous replication. Lacking their own proteins, viroids must rely almost entirely on host factors to complete their infectious cycle. This entails differences in the parasitism of viruses and viroids, which can essentially be regarded as parasites of their host translation and transcriptional apparatus, respectively. Viroid species are clustered into the families *Pospiviroidae* and *Avsunviroidae*, whose members replicate in the nucleus and chloroplast, respectively. Viroids replication occurs through an RNA-based rolling-circle mechanism involving synthesis of longer-than-unit strands catalyzed by host RNA polymerases, processing to unit-length (which in the family *Avsunviroidae* is mediated by hammerhead ribozymes), and circularization. Within the initially infected cells, viroid RNA must move to its replication organelle, with the resulting progeny then invading adjacent cells through plasmodesmata and reaching distal parts via the vasculature. To carry out these movements, viroids must interact with host factors, most of which remain unknown. Viroids are the etiologic agents of diseases affecting important crops: potato, tomato, cucumber, hop, coconut, grapevine, subtropical and temperate fruit trees (avocado, peach, apple, pear, citrus, and plum), and ornamentals (chrysanthemum and coleus). Some viroids have wide host ranges but others are mainly restricted to their natural hosts. Certain viroids have destructive consequences, while others affect leaves, stems, bark, flowers, fruits, seeds, and reserve organs, and may have less conspicuous effects including delays in foliation, flowering and ripening. Some viroids only induce symptoms in a particular organ (bark or fruit), whereas others have more general effects. Infections caused by a few viroids result in very mild or no symptoms. Absence of symptoms is common in naturally infected wild plants, which can act as reservoirs. Symptom expression is favored by high light intensity and high temperature, which may explain why viroids mainly affect crops grown in tropical or subtropical areas (and in greenhouses), and also why some viroid infections are recalcitrant to thermotherapy. The mature viroid RNA could be the primary pathogenic effector or, alternatively, viroids could exert their pathogenic effects via RNA silencing. Although most viroids are transmitted mechanically and some through seed or pollen, vegetative propagation of infected material is the most efficient transmission route, thus explaining why certain grapevine and citrus cultivars propagated on infected cultivars or rootstocks contain complex mixtures of different viroids.

E-mail: rflores@ibmcp.upv.es

SPECIFIC CYTOLOGICAL RESPONSES OF HOST CELLS TO PLANT VIRUS INFECTIONS

G.P. Martelli

Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Unità Organizzativa di Bari, Via Amendola 165/A, 70126 Bari, Italy

One of the most conspicuous outcome of virus infections in a host plant is the induction of intracellular modifications which may vary to a great extent in terms of severity and type, in function of the eliciting agent. Most plant viruses induce cytological changes that are readily observed with the electron microscope and may have diagnostic and/or taxonomic value. The products of the direct expression of the viral genome (replicative forms of nucleic acids, structural and non structural proteins, assembled virions) and modifications of normal cell constituents and/or organelles are a consequence of virus multiplication. These products localize intracellularly, and often accumulate in a specific form at preferential cellular sites, giving rise to a variety of cytopathic structures known as "inclusion bodies". These can be defined as intracellular structures produced *de novo* following infection, which may contain virus particles, virus-related material or ordinary cell constituents in a normal or deranged condition, either singly or, more often, in various proportions. Inclusion bodies are veritable "signatures" of the eliciting virus(es), which can be species-specific (rarely) or, more often, genus- or family-specific. Cells newly infected by RNA viruses react quickly, augmenting the amount of their membrane dowry to increase the membranous support for the viral replication complex. This may lead to proliferation of endoplasmic reticulum membranes, which accumulate in the cytosol to give raise to more or less compact structures. An example of this is given by the outstanding cytoplasmic inclusions of members of the former family *Comoviridae,* now subfamily *Comovirinae*, which are virus factories containing viral replication proteins plus genomic ssRNAs and dsRNAs. Alternatively, plenty of vesicles develop at the periphery of chloroplasts or mitochondria of cell infected by members of the family *Tymoviridae*. These vesicles originate from the invagination of the bounding membrane of the organelles and are sites of viral RNA replication. A comparable situation occurs in the genera *Tombusvirus* and *Carmovirus*, where is the peripheral membrane of mitochondria or peroxisomes to be enriched with a profusion of vesicles. The ensuing structures, called "multivesicular bodies", are replication sites for the viral genome. Non structural proteins may aggregate to produce highly family- or genus-specific cytoplasmic or nuclear inclusions (e.g. pinwheels and laminated aggregates of the family *Potyviridae,* made up of either helicase, protease, polymerase or the protein assisting aphid transmission), or cytoplasmic fibrous inclusions composed by one of the viral movement proteins (genus *Necrovirus*), or may enter the composition of complex cytoplamic inclusions containing virus particles embedded in a matrix made up of a protein that activates translation (genera *Caulimovirus, Petuvirus, Soymovirus, Cavemovirus*). Although ordinary electron microscopy has provided a wealth of information on the fine structure and organization of inclusion bodies, cytochemical microscopy and, more recently, gold immunolabelling, whereby the chemical nature of virus-related products can be determined and their intracellular distribution assessed, have provided a better understanding of virus-host relationships correlating structure with function.

E-mail: martelli@agr.uniba.it

THE CONSEQUENCES OF CLIMATE CHANGE FOR PLANT DISEASES

M. Shaw

School of Biological Sciences, University of Reading, Lyle Tower, Whiteknights, Reading RG6 6AS, UK

The effects of climate change on disease are likely to be complex and hard to predict because they involve changes in host distribution and phenology and changes in plant-associated microflora, as well as direct biological effects on the pathogen in the context of rapid co-evolution. Verification of predictions is a major difficulty, the most convincing method would be to "back-forecast" observed historical changes. However, the long-term dynamics of plant disease have been relatively little studied, due to a lack of empirical data over long time-spans. Most of what is known concerns invasions, in which the involvement of climate is unclear or clearly absent. The long-term prevalence will be a balance between the decline in the unfavourable parts of the season, immigration and the increase during favourable parts; the latter has been most studied because of its economic importance and ease of study. In cases where long-term prevalence can be deduced, climate may seem to have little to do with change. Genetic changes are probably inherently unpredictable. Based on experience with invasives, the worst surprises in plant disease seem likely to arise from host shifts, mediated by altered geographic ranges or chance genetic change unconnected with climate change. For wild plants whose ideal ranges have moved or disappeared, stress may produce entirely novel disease problems, but competition is more likely to be critical. Short-term forecast criteria coupled with weather generated from climate simulations form a basis for projection. However, most of these forecasts account for only a moderate proportion of the annual variance in severity and necessarily ignore parts of the life-cycle which were not limiting in the observation period. Changes in vector-borne virus diseases may be the easiest to predict, because vector activity and range is often related to simple medium-term climatic variables such as temperature sums; however, recent experiences are not promising. Advice to growers, and the politicians regulating markets in land food and commodities, must emphasise the need for systems to be resilient and adaptive to the unexpected. Planning must obviously include "best guesses" and clear predictions, but we must convey the sources of uncertainty effectively.

E-mail: m.w.shaw@reading.ac.uk

FUNCTIONAL GENOMICS AND BIOTECHNOLOGICAL APPLICATIONS OF THE MOLECULAR INTERACTION BETWEEN PLANT, FUNGAL PATHOGENS AND BENEFICIAL MICROORGANISMS

R. Marra

Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy

Biocontrol fungi of the genus *Trichoderma* are widely used in agriculture because of their ability to contrast plant pathogens and simultaneously improve plant fitness. Studies published thus far on plant-pathogen interactions have been focused mainly on the molecular changes in the plant, related to pathogen attack and/or defence response. However, to date, the key factors involved in multiple-component systems that produce beneficial effects to the plant are not known and their effects have not yet been determined. The use of novel tools to investigate these complex processes, such as proteomic analysis, has shown that a molecular cross-talk is established between the antagonist, the plant, and the pathogen, and has permitted the identification of a variety of signalling molecules involved in *Trichoderma*-plant, *Trichoderma*-pathogen or *Trichoderma*-plant-pathogen interactions. New applications have been proposed from such findings, both in agriculture and biotechnology, to exploit the ability of these fungi to change plant metabolism and resistance to biotic and abiotic stresses. In fact, they can be used to improve either the fitness or capacity of the plant to degrade toxic compounds. By using a proteomic approach, the concurrent interactions of the biocontrol agents *T. atroviride* strain P1 and *T. harzianum* strain T22 with different host plants and/or fungal pathogens were studied, in order to identify and analyze proteins differentially produced by the three players. Two-dimensional maps of protein extracts were obtained from the plant and fungi, either singly or in all possible combinations. Differential proteins in the gels were extracted by tryptic digestion, then characterized and identified by mass spectrometry (MS) and *in silico* analysis. The accumulation patterns of putatively important proteins in the single/combined antagonist-plant-pathogen interactions were determined as an indication of the functional role in the associations. In the plant proteome, the production of specific pathogenesis-related proteins and other disease-related factors (i.e., potential resistance genes) was stimulated by the contact with the fungal pathogens and/or *T. atroviride*, which is in agreement with the alleged ability of the biocontrol agent to induce systemic resistance against various microbial pathogens. Many differential proteins obtained from the *T. atroviride* interaction proteome showed interesting homologies with fungal hydrophobins, ABC transporters, virulence factors etc. For instance, cyclophilins accumulated in the pathogen proteome during the interaction with the plant alone and/or with the antagonist, but were also found up-regulated in the proteome of the beneficial microbe interacting with fungal pathogens. Because of the complexity of the system, which is based on the concurrent activity of several players, an integrated approach with other functional genomics techniques was implemented in our research. The results from the proteomic study were effectively matched and compared with data obtained from both metabolomic experiments and the analysis of *Trichoderma* spp. EST libraries prepared by growing the fungus in diverse interaction conditions. The information collected during this investigation was instrumental in the research and to the development of a novel biofertilizer product with plant protection properties, which has been recently placed on the international market.

Research work recipient *ex aequo* of the "Professor Giovanni Scaramuzzi" award 2009 for the best Ph.D. (Dottorato di Ricerca) thesis, sponsored by the Italian Society for Plant Pathology.

Corresponding author: R. Marra
Fax: +39.081.7775114
E-mail: robmarra@unina.it

CHARACTERIZATION OF SYNERGY BETWEEN *CUCUMBER MOSAIC VIRUS* AND *POTATO VIRUS Y* IN TOMATO

T. Mascia

Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy

Mixed infections of *Cucumber mosaic virus* (CMV) and a number of host-specific potyviruses are frequent in commercial fields of vegetable crops. As a case study, viral interplay resulting from mixed infections of CMV and *Potato virus Y* (PVY) has been investigated in tomato. The outcome of mixed infection was examined using two strains of CMV representing subgroups IA (CMV-Fny) and II (CMV-LS), a number of CMV isolates carrying mutations in the 3'-terminal region of RNA-2 and the SON41 strain of PVY. PVY-SON41 that, when alone, exhibited a rather limited pathogenicity to tomato, was shown to act synergistically with either the mild CMV-LS or the severe CMV-Fny strains, inducing an aggravation of symptoms, enhancement of CMV systemic movement and increased accumulation of CMV RNAs from 7 up to 60 days post-inoculation (dpi). At early infection time points, the overall RNA accumulation level of PVY-SON41 was lower in plants co-infected with CMV-Fny than in plants infected by the potyvirus alone, while at very late infection stages also PVY received a beneficial effect from double infection, since it appeared more uniformly distributed throughout the whole tomato tissues. The interaction was monitored at the single cell level using tomato protoplasts and at tissue level using immunogold electron microscopy (IGL) and quantitative real-time PCR. In doubly infected protoplasts, the replication of CMV was already in progress at 6 h post inoculation while that of PVY was strongly antagonized. To dissect the possible mechanism(s) underlying the observed behaviour the CMV-FnyΔ2b mutant, which does not translate the 2b protein, was used. This mutant was unable to move from inoculated cotyledons and to invade systemically the plant because, as shown by IGL, it was blocked at the level of the bundle sheath of tomato phloem. Thus, the resistance to systemic infection could be attributed to the inability of the mutant to enter the sieve elements rather than to exit from them. The function needed for invading the phloem was complemented by PVY because in the cotyledons of doubly infected plants the CMV-FnyΔ2b mutant crossed the bundle sheath and penetrated companion cells and sieve elements. The ability of CMV-FnyΔ2b to spread and accumulate systemically in doubly infected plants was probably complemented by PVY, as well. PVY complementation was not necessary in plants grown at 15°C because they were systemically infected also by the CMV-FnyΔ2b mutant. Since RNA silencing is inactive at low temperature, one possible explanation is that RNA silencing was the mechanism responsible for blocking the CMV-FnyΔ2b mutant at the bundle sheath boundary level. In doubly infected plants this function was likely complemented by the PVY HC-Pro silencing suppressor, whereas complementation was unnecessary in plants grown at low temperature because of the absence of the RNA silencing signal. Complementation by PVY in systemic movement did not have any effect on symptomatology, as tomato plants systemically infected by CMV-FnyΔ2b mutant were symptomless. This is the first time that the molecular mechanisms underlying the mixed infection of CMV and PVY in tomato were studied to some details. Since both viruses take some advantage from mixed infection, the results of this study provide a preliminary explanation on why such infections are so frequent in nature in tomato.

Research work recipient *ex aequo* of the "Professor Giovanni Scaramuzzi" award 2009 for the best Ph.D. (Dottorato di Ricerca) thesis, sponsored by the Italian Society for Plant Pathology

Corresponding author: T. Mascia
Fax: +39.080.5442906
E-mail: tizianamascia@email.it