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# Story of Johnsongrass mosaic virus in Australia

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Plant pathology is concerned primarily with finding practical solutions to disease problems in agriculture, horticulture and forestry. Projected world population growth makes this a priority. In recent years, scientists have focused on molecular aspects of plant-pathogen interactions to provide new insights into pathogenesis and plant defence systems. As part of an overall strategy to combat viral diseases, it is hoped that detailed studies will allow molecular biologists to minimize and prevent the development of symptoms in infected plants.

Relative to world output, Australia is only a minor producer of sorghum and maize cereals but has the potential to make significant contributions in the way of intellectual property. Johnsongrass mosaic virus (JGMV) has caused considerable yield losses in sorghum and maize in Australia in past decades, with up to 100% infection of the crop causing large losses. In Australia JGMV is still considered one of the major diseases in sorghum. Tropical monocotyledonous weeds such as Johnsongrass (*Sorghum halepense*) are good hosts for JGMV inoculum ensuring that the virus can infect nearby crops. In 1959 a Krish sorghum was introduced to Australia from India, which proved to be remarkably resistant to the Johnsongrass strain of JGMV (JGMV-Jg). However, in 1985 an apparently new strain of JGMV evolved, later named the JGMV Krish-infecting strain (abbreviated here as JGMV-Kr), (and appeared in Queensland infecting the Krish sorghums). It is suggested on theoretical grounds that sequence changes in the coat protein (CP) of the RNA virus were responsible for breaking the resistance

of the Krish sorghums. Subsequent comparisons of nucleotide sequences between the two strains of the virus, and between infectious and non-infectious cDNA clones of the virus, have identified a number of amino acids in the N-terminus of the coat protein which are likely to be responsible for the changed biological activity of the Krish-infecting strain.

There are four known JGMV strains; the Johnsongrass strain (Jg), the Krish-infecting strain and the Kansas and Texas strains, originally known as the MDMV-KS1 and MDMVO strains respectively. The Krish-infecting strain is able to infect Krish sorghum cultivars, which are resistant to the other 3 strains. There are no serological relationships between JGMV-Jg and JGMV-Kr. The JGMV-Texas strain is very close serologically and in host response, but distinguishable using monoclonal antibodies. The Kansas and Texas strains share 99% amino acid homology in the coat protein. The oat-infecting Texas strain and the Johnsongrass strain are closely related serologically but can be distinguished using monoclonal antibodies.

A number of species belonging to the family *Gramineae* are hosts for JGMV, including maize, sweet corn, grain fodder and sweet sorghums. *Phaseolus vulgaris* L. appears to be the only dicot host. Its hosts include 24 species in 10 genera within the same family. However, there are only about 14 natural hosts recognized, including; *Sorghum halepense, S. macrospermum, S. stipiodeum, S. sundanense, S. vulgare, Brachiria milliformis, Chencrus ciliaris, Panicum miliaceum, P. urbiculare, Pennisetum typhoideum* and *Zea mays*. *S. vulgare* and *Z. mays* are regarded as original hosts for JGMV in Australia.

JGMV is transmitted to a narrow range of hosts in the *Gramineae* by several aphid species such as *Aphis craccivora, A. gossypii, Myzus persicae* and *Rhopalosiphum maidis* in a non-persistent manner, and can also be transmitted through seed from infected maize but not from sorghum.

Symptom development on infected plants depends on the host genotype, virus strain and environmental conditions. Maize and sweet corn infected by JGMV develop mosaic symptoms, ringspots and chlorosis, while in sorghum there can be mosaic, ringspot or necrotic symptoms sometimes accompanied by stunting. JGMV-Jg induces necrotic red stripes in sorghum OKY8 and SA8735, while three SCMV strains (SCMV-SC, SCMV-BC and SCMV-Sabi) generate only mosaic symptoms.

JGMV belongs to the (RNA) potyvirus group, which is the largest and economically most important group within the 35 plant virus groups, containing at least 181 viruses (83 definite and 98 possible members). They cause significant losses in agricultural and horticultural production and can also affect plants grown for ornamental purposes. Potyviruses are transmitted in a non-persistent manner by many aphid species, and some members have mite, fungus, or whitefly vectors. A "pinwheel" cylindrical inclusion (CI) characteristically formed in the cytoplasm of infected cells has been used as the single most important phenotypic criterion to assign a particular virus into the potyvirus group. Typical potyviruses are flexuous rods with lengths ranging between 600-900 nm and a diameter of 11-15 nm, the dimensions depending somewhat on the host plant. JGMV is a single stranded positive sense RNA virus about 750 nm long and 12 nm in diameter. Potyviruses are included in the picorn-like supergroup of RNA viruses where a VPg protein is attached covalently at the 5′ end of its RNA, while the 3′ end has a poly (A) tail. The potyvirus genome consists of a single-stranded positive sense RNA (~10 kb, Mr $3.0\text{-}3.5\times10^6$) containing a single open reading frame (ORF), which is translated into a large polyprotein, ranging between Mr 30,000-37,000. The polyprotein is subsequently cleaved by 3 *cis*- or *trans*-acting proteinases (P1, HC-Pro and NIa-Pro) to yield at least 8 functional proteins. These are the first protein (P1), the helper component-proteinase (HC-Pro), the third protein (P3), the

cylindrical inclusion protein (CI), the small nuclear inclusion protein (NIa) including VPg at its N-terminus, the large nuclear inclusion protein (NIb), and the coat protein (CP). In addition, two smaller proteins are located on the polyprotein between P3 and CI (6K1) and between CI and NIa (6K2), each of approximately 6 kDa. Only two of the proteins, VPg and CP, are detected in virus particles. The four proteins HC-Pro, CI, NIa and NIb have been isolated and studied, but P1 and P3 have apparently not yet been detected *in vivo* (Dougherty 1988). HC-Pro was recently discovered to interfere with the accumulation of the small RNAs associated with post-transcriptional gene silencing of endogenous genes in plants (PTGS), which is consequently suppressed. The CI of TuMV determines the virulence of the virus in *Brassica napus TuRB01* that possesses a dominant resistance allele. The JGMV genome contains two untranslated regions (UTR or non-coding region) at the 5′ and 3′ end. The 5′ and 3′ UTR consist of 135 and 475 nucleotides respectively. The 3′ UTR of JGMV is considered to be the longest non-coding region amongst the potyviruses.

Coat proteins have long been used for the classification of potyviruses because of their unique sequences and, to a lesser extent, their serology. It is the only gene product found in the virion, apart from VPg. The N- (30 to 95 amino acids) and C-terminal regions (18 to 20 amino acids) of the CP are exposed on the particle surface, yet removal of these exposed regions by trypsin does not affect infectivity of the virus when mechanically inoculated, indicating that the N- and C- termini are not essential for this purpose. The surface-exposed regions of the CP have other important biological functions such as determining cross-protection and vector or host specificity. The N-termini of the coat proteins vary considerably among the distinct potyviruses, both in length and sequence, while at the C-terminal two-thirds of the proteins are highly homologous. The N-terminus of the CP is immuno-dominant and contains virus-specific epitopes. The JGMV-Jg

is serologically close to WMV, but not to SCMV, PVY and CYVV. An amino acid triplet "DAG" in the N-terminus of the CP may be involved in HC-Pro/CP interaction and aphid transmissibility. The DAGX motif is responsible for aphid transmission. They observed that the TVMV-AT (aphid transmissible strain) could not be transmitted following a single nucleotide change of G to A at position 8445, changing the amino acid triplet from DAG to DAE. They also noted that for the first amino acid in the motif, only aspartic acid (D) or asparagine (N) is essential for aphid transmissibility. Mutations in the DAG motif, located in the N-terminus of TVMV CP, were strongly correlated with aphid transmissibility in binding to HC-Pro. The DAG motif is common to potyviruses but occurs at different positions, generally between 5 to 13 residues from the N-terminus. There is no doubt that the CP has a role in host specificity and virulence. The grouping of SCMV strains is well correlated with the reactivities of these strains to different sorghum cultivars. The diversities of the CP N-terminus of SCMV could also be correlated with the host range of the viruses. The CP may also play a role in cross-protection. The similarities in the N-terminal region of the coat protein might determine cross-protection. However, transgenic plants expressing the SMV CP conferred a high resistance to PVY and TEV, even though the sequence identities between the coat proteins of PVY and TEV were only 58% and 61%, respectively. The mechanisms by which protection is achieved in CP-mediated virus resistant transgenic plants are most probably both host and virus specific. Although there is no direct evidence, the surface-exposed N- or C-terminus of the CP could be involved in the infection process, and especially in cell-to-cell or long-distance movement. The CP of TEV is important for virion assembly, cell-to-cell movement and long-distance transport. The core region is essential for cell-to-cell movement, while the exposed N- and C-terminal regions appear to be essential for long distance transport.

One of the most effective ways in which plants resist pathogen infection is through induction of the hypersensitive response (HR). The HR is an active defence mechanism that plants employ to prevent the spread of viral, bacterial, fungal and nematode pathogens. An important feature of the HR is that it is a generalized response. Despite the different characteristics of the various types of pathogens, the same set of biochemical responses ensue: production of pathogenesis-related (PR) proteins, hydrolytic enzymes, callose and lignin precursors, oxidative bursts of $H_2O_2$, activation of systemic acquired resistance, etc. These responses act in concert to restrict pathogen infection and prevent systemic disease. The end result of HR induction is localized cell death and necrosis at the site of pathogen infection. In contrast to the generalized defence responses, induction of HR occurs in a highly specific manner. The ability of a plant to respond to the presence of a particular pathogen and initiate defence mechanisms implies that there is a specific recognition event between the plant and the invading pathogen. The resistance segregated with single dominant loci in plants and single dominant loci in pathogens provided the framework for the current model termed the "gene-for-gene" hypothesis. This hypothesis suggests that plants carry specific resistance (*R*) genes, the products of which directly or indirectly interact with the products of pathogen encoded avirulence (*avr*) genes leading to induction of the HR.

Inoculation of susceptible host plants with cloned material of many plant virus genomes, including numerous RNA viruses, can result in the establishment of viral infections. Availability of these clones makes it possible to manipulate viral genomes for study and biotechnological applications. For this reason, considerable effort is being devoted to the development of efficient systems of plant infection using cloned versions of viral genomes. In the case of plant RNA viruses, cloned cDNA material can be used to obtain either *in vitro* or *in vivo* infectious

transcripts. Both strategies have been applied successfully to members of the *Potyvirus* genus. *In vitro* derived capped-transcripts of full-length clones of several potyviruses initiate infections on susceptible hosts. The cDNA clones of potyviruses supplied with appropriate plant-recognizable promoters, have been used to produce *in vivo* transcripts able to infect plants. Most plant RNA viruses do not have a DNA intermediate in their life cycle, making molecular studies more difficult because of RNA instabilities. The availability of full-length cDNA clones of the viral genome capable of providing infectious transcripts, prepared either *in vitro* or *in vivo*, has significantly contributed to the study of RNA viruses as cDNA clones can be readily mutated to investigate genomic functions. One of the biggest challenges in preparing a full-length cDNA clone of a potyvirus is to maintain the 10 kb ORF of the polyprotein. Despite successful preparations of full-length cDNAs by RT-PCR in some cases, in practice it is more usual that several overlapping cDNA fragments need to be cloned. Success is dependent on factors such as the type of host cell used, bacterium directed spontaneous mutations, and the general stability of the plasmid in *E. coli*. The instability of some viral sequences in bacteria (or their potential toxicity) can be the most important single factor in some *E. coli* strains. One of the major difficulties when cloning and manipulating potyviral genomes is the apparently high toxicity of some viral products to bacteria. Many of these difficulties could be circumvented by changing the bacterial strain used for cloning. Alternatively, to avoid undesired expression of cloned material in bacteria, the insertion of introns can also be used. The introns interrupting the viral ORF, served to reduce toxicity problems of potyviral full-length clones, since splicing of introns introduced in PSbMV clones, after their inoculation into plants, permitted the production of correct genome-size RNA which resulted in infection. Once a full-length infectious cDNA clone of its RNA genome is available, the nucleotide sequences of the virus can be

manipulated by site-directed mutations, and the mutated transcript inoculated into leaves and tested for infectivity, virus replication and systemic virus spread. The functions of various nucleotide sequences can thus be systematically identified.

A classical method for virus inoculation has been to mechanically inoculate plants by hand. The inoculum is prepared by grinding virus infected leaves in a buffer and using an abrasive such as carborundum powder. The method may not be suitable for all viruses because some plant viruses can quickly lose their biological activity under such conditions. Moreover, chemical compounds (eg. phenolics) produced by damaged plant cells may also damage intact viruses. However, this is the most convenient inoculation method. *In vitro* prepared transcripts are known to be easily degraded, generally leading to low infection rates, probably due to differences in the level of damage caused to the plant in each experiment. From that point of view, at first sight an *in vivo* transcription system appears to be superior to *in vitro* transcription, because of the suspected high stability of *in vivo* transcripts. There are two efficiently applicable methods *in vivo* transcription system: biolistic bombardment and agro-infiltration with a binary vector system.

Infectious *in vivo* and *in vitro* RNA transcripts derived from full-length viral cDNA clones have proved to be powerful and efficient tools for the study of RNA viruses. In addition, reporter genes such as green fluorescent protein (GFP) and -glucuronidase (GUS) have been used to study various aspects of viral gene functions by inserting them as fusion products into infectious cDNA clones, as has been done for TEV and PVX. Infectious transcripts have been used for the clarification of gene(s) or region(s) responsible for breaking the resistance of plants against the potyvirus TVMV-W. Using infectious cDNA clones, the potyviral genomic functions relating to various aspects such as infectivity, virus movement, aphid transmission and polyprotein processing have been studied

extensively by many researchers.

Historically, the study of plant viruses including potyviruses has contributed greatly to the elucidation of eukaryotic biology. Recently, the biotechnology industry has developed an increasing number of disease therapies utilizing recombinant proteins by employing pathogens in the process of creating added value to agriculture is, in effect, making an ally from an enemy. A lot of further studies need to be performed by agriculturists and molecular virologists to fully realize the potential and the value of the potyvirus in plant science.