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Story of Johnsongrass Mosaic (Poty)virus in Australia

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Abstract One of the major aims in studying plant viruses is to minimise the development of symptoms in infected plants. With the advent of *in vitro* transcript mediated research on plant viruses, substantial progress has been made. This article describes the biology of a plant specific RNA virus, Johnsongrass mosaic virus (JGMV), important to Australian sorghum and corn agriculture and, in particular, at a molecular level which of the RNA sequences in its genome that make it possible for the virus to move from cell to cell, and eventually spread systemically throughout the entire plant. The JGMV has caused considerable yield losses in maize and sorghum over a number of years in Australia. Incidents where 100% of the crop has been infected are on record. The use of this virus is convenient under laboratory conditions because it can be readily transmitted by mechanical inoculation with infected leaf sap, which obviates the need for maintaining aphid colonies. The JGMV is a single stranded positive sense RNA virus.

Key words: Potyvirus, JGMV, JGMV-Jg, JGMV-Kr, RNA, RNA transcript, cDNA, Coat protein (CP)

Introduction

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 defense 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 (Shukla 1989c; Gough 1993). In Australia JGMV is still considered one of the major diseases in sorghum. Tropical monocotyledonous weeds such as Johnsongrass 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) (Teakle 1971; Teakle 1973; Conde 1976). 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) (Persley 1986; Persley 1987; Persley 1990). Shukla *et al.* (1994b) 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 (Suranto 1998; Kim 2002).

JGMV taxonomy

The RNA potyvirus group 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 (Shukla 1994a). 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 (Edwardson 1974; Edwardson 1992) has been used as the single most

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important phenotypic criterion to assign a particular virus into the potyvirus group (Hollings 1981a; Hollings 1981b). 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 (Taylor 1968). JGMV is a single stranded positive sense RNA virus about 750 nm long and 12 nm in diameter (Fig 1) (1968; Teakle 1973; Suranto 1998; Kim 2002).

Four strains of sugar cane mosaic virus (SCMV) in Australia were first described and designated as sugar cane strain (SCMV-SC), Queensland blue couch grass strain (SCMV-BC), Sabi grass strain (SCMV-Sabi) and the Johnson strain (SCMV-Jg) (Simmonds 1966; Taylor 1968; Teakle 1973). SCMV-Jg was later reclassified and has been treated separately as Johnsongrass mosaic virus (JGMV-Jg) (Shukla 1987; Shukla 1989d; Gough 1993; Shukla 1994a). The new classification of JGMV-Jg was based on a number of criteria including symptoms on different cultivars, and on the morphological and serological properties of the virion, such as the typical reactions of different host plants and the distinct serological relationship with SCMV and MDMV(maize dwarf mosaic virus) (Shukla 1984; Shukla 1989c; Shukla 1989e; Shukla 1989b; Shukla 1992a; Shukla 1992b). Comparative studies, including comparisons of the amino acid and nucleotide sequences of the CP between JGMV, SCMV and MDMV, clearly showed that JGMV was a distinct potyvirus (Table 1, 2 and 3) (Gough 1981; Gough 1987; Shukla 1987; Handley 1998). Many further studies have since confirmed and supported this classification using the reactions on selected oat and sorghum cultivars (Table 4 and 5), morphology and serology of cytoplasmic inclusions, amino terminal serology of CP, CP sequences, CP high-performance liquid chromatography peptide profiles and 3' non-coding sequence identities and hybridization (Shukla 1983; Shukla 1988e; Shukla 1988d; Shukla 1988c; Shukla 1988d; Shukla 1988a; Shukla 1989c; Tosic 1990;

Table 1. Amino acid sequence homologies between the coat proteins in the SCMV subgroup

Virus Strain	JGMV-Jg	JGMV-MDKS1	JGMV-MDO	SCMV-SC	SCMV-MDB	MDMV-A
JGMV-Jg						
JGMV-MDKS1	99					
JGMV-MDO	99	99				
SCMV-SC	67	68	68			
SCMV-MDB	67	68	68	94		
MDMV-A	68	68	68	88	86	

Adapted from Shukla *et al.* (1992a). JGMV strains (Jg, MDKS1 and MDO) are distinct from other potyviruses (Handley *et al.* 1998).

Table 2. Nucleotide sequence homologies between the 3' UTR in the SCMV subgroup

Virus Strain	JGMV-Jg	JGMV-MDKS1	JGMV-MDO	SCMV-SC	SCMV-MDB	MDMV-A
JGMV-Jg						
JGMV-MDKS1	97					
JGMV-MDO	96	98				
SCMV-SC	44	46	46			
SCMV-MDB	48	47	45	88		
MDMV-A	44	44	44	54	54	

Adapted from Shukla *et al.* (1992a).

Table 3. Grouping of maize dwarf mosaic viruses and sugarcane mosaic viruses from Australia and the United States on the basis of reactivities of affinity purified virus-specific antibodies

JGMV-Jg[a)]	MDMV[b)]	SCMV[c)]	SrMV[d)]
SCMV-Jg (Aust.)[e)]	MDMV-A (Aust.)	MDMV-B (U.S)	SCMV-H (U.S)
MDMV-O (U.S)[f)]	MDMV-D (U.S)	SCMV-A (U.S)	SCMV-I (U.S)
MDMV-KS1 (U.S)	MDMV-E (U.S)	SCMV-B (U.S)	SCMV-M (U.S)
	MDMV-F (U.S)	SCMV-D (U.S)	
		SCMV-E (U.S)	
		SCMV-SC (Aust.)	
		SCMV-BC (Aust.)	
		SCMV-Sabi (Aust.)	
		SCMV-ISIS (Aust.)	
		SCMV-Brisbane	
		SCMV-Bundaberg (Aust.)	

Adapted from Shukla *et al.* (1992a). a) JGMV-Jg; Johnsongrass mosaic virus, b) MDMV; Maize dwarf virus, c) SCMV; Sugarcane mosaic virus, d) SrMV; Sorghum mosaic virus, e) Aust.; Australia, f) U.S.; United States

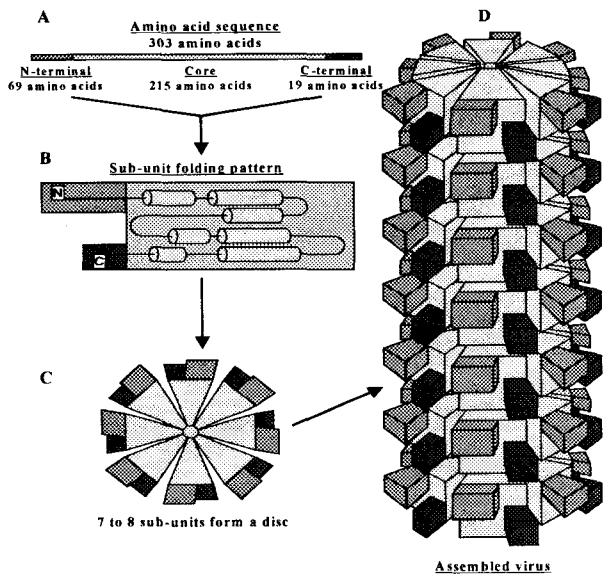

Fig. 1. Schematic drawing of the assembly of the JGMV coat protein. The coat protein sub-unit, the sub-unit folding pattern and the surface-exposed N- and C-termini are shown. A. Primary structure. B. Secondary and tertiary structure. C. and D. Assembled virus. Diagram obtained from Dr. D. D. Shukla (personal communication)

Ward 1992; Shukla 1992a; Shukla 1994b; Handley 1998).

There are four known JGMV strains; the Johnsongrass strain (Jg) (Teakle 1973; Shukla 1987), the Krish-infecting strain (Persley 1987) and the Kansas and Texas strains, originally known as the MDMV-KS1 and MDMVO strains respectively (McDaniel 1985; Tosic 1990; Suranto 1998a). 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 (Kim 2000). 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 (Shukla 1989c; Tosic 1990).

Host range and transmission of JGMV

A number of species belonging to the family *Gramineae* are hosts for JGMV, including maize, sweet corn, grain fodder and sweet sorghums (Gough 1993). *Phaseolus vulgaris* L. appears to be the only dicot host (Teakle 1973). Its hosts include 24 species in 10 genera within the same family (Shukla 1994a). 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 (Teakle 1973).

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* (Shukla 1989e) in a non-persistent manner, and can also be transmitted through seed from infected maize but not from sorghum (Shepherd 1965; Williams 1968; Teakle 1973; Penrose 1974; Persley 1978).

History of the JGMV Krish-infecting strain in Australia

When sorghum containing the Krish resistance gene was introduced from India to Australia in 1959, it seemed to solve Australia's problems in sorghum production. The resistance of Krish sorghum to the type strain of JGMV-Jg was reported by Pritchard in 1964 after field and glasshouse trials conducted during 1959~1970 (Teakle 1971) (Table 6). Following the first planting of Krish sorghums in 1959 at Lawes in Australia, almost all Krish sorghums were free

Table 4. Symptoms, genotype and potential yield losses of sorghum affected by JGMV-Jg (SCMV-Jg)

Reaction	Symptoms	Genotype	Potential yield loss (%)
Mosaic	Mosaic pattern	kk nn RLF RLF	0~30
Red leaf	Necrosis	kk nn/*N-rlf rlf	10~90
Red stripe	Necrotic red or tan stripes	kk N- rlf rlf/*RLF RLF	60
Resistant	NIL	k-	0

Data from Persley *et al.* (1986).

Table 5. Differences in symptoms caused by four viruses in the SCMV subgroup in selected hosts

Viruses	Sorghum cultivars				Johnsongrass	Oat	Sugarcane
	OKY8	SA8735	Rio	Atlas			
JGMV	Necrotic red stripe	Necrotic	Mosaic	Mosaic	Mosaic	Mild mosaic	NI
MDMV	Mosaic	Necrotic	Necrosis	Mosaic	Mosaic	NI	NI
SCMV	Mosaic	Mosaic	Mosaic	Necrotic streaks and stripes	NI	NI	Mosaic
		Mosaic			NI	NI	Mosaic

Adapted from Shukla and Ward (1994). NI represents no-infection.

Table 6. Infection rates in Krish sorghums compared with other sorghum lines following inoculation with strains of JGMV, SCMV, MDMV and SrMV in glasshouse trials

	Virus strain	Infection rate	Reference
Krish sorghum	SCMV-Jg	13%	Teakle &Pritchard, 1971
	SCMV	29%	
	SCMV-Sabi	23%	
	SCMV-BC (Blue-couch)	0%	
Krish sorghum 1	SCMV	25%	Teakle & Pritchard, 1973
Krish sorghum 2	SCMV	25%	
S. bicolor	MDMV	60%	Peterschmitt *et al.*, 1991
S. bicolor NK220Y	JGMV-Jg	60~100%	Karen *et al.*, 1992
S. halepense	MDCV	50%	Gingery & Nault, 1990

of viral symptoms, even though adjacent susceptible sorghum varieties had a very high incidence of mosaic symptoms towards the end of every season. Statistical data collected until 1964 revealed that only about 1.1% of 440 Krish sorghums were infected by JGMV-Jg. Moreover, none of 780 Krish sorghums, planted during 1966 in field trials, was found to be infected by JGMV-Jg although nearby *Sorghum alamum* species were 100% infected. Furthermore, in 1970, 77 Krish sorghums were grown for four years, of which only two individuals were found to develop mosaic symptoms. Thus it was clear that the Krish sorghums were resistant to JGMV-Jg. However, some infection on the Krish sorghums could be obtained with JGMV-Jg if high inoculum concentrations were employed. For example, Teakle and Pritchard (1971) reported that 13% of Krish sorghum seedlings could be infected systematically by JGMV-Jg in a glasshouse trial. This meant that a JGMV Krish-infecting strain can occur in Krish sorghums at anytime and be readily propagated in Krish sorghum lines (Shukla 1994a). In 1985 this apprehension came true when the JGMV Krish-infecting strain was first found in the field in the Mt Tyson-Jondaryan area (Persley 1986). It has become widely dispersed since. The effects on sorghum infected with the JGMV Krish-infecting strain may include delayed flowering, stunting, small seeds, reduced grain quality, panicle necrosis, and reduced yield (Persley 1986; Persley 1990).

Distribution and economic significance of JGMV in Australia

So far, two major strains of JGMV have been found in Australia; JGMV-Jg and the JGMV Krish-infecting strain. JGMV-Jg has a wide distribution in New South Wales, Victoria, Queensland and the Northern Territory (Simmonds 1966; Taylor 1968; Grogan 1969; Teakle 1973; Penrose 1974; Teakle 1978; Persley 1981; Persley 1986). The JGMV Krish-infecting strain is fairly widely spread throughout the major grain sorghum production areas of southern and central Queensland and New South Wales (Persley 1986; Persley 1987; Franzmann 1992), and also in Kununurra in Western Australia and in the Northern Territory (Persley 1986).

These viruses have caused the greatest yield reduction in maize and sorghum (Table 4) with their severity depending on the cultivar, time of infection and the environmental conditions. The effect of the JGMV Krish-infecting strain on sorghum growth is influenced by the sorghum cultivar, the degree of disease incidence, and the time of infection (Persley 1986). Early infection during growth will affect the cultivars by necrosis and will result in greater loss of yield (Henzell 1979; Persley 1986). A high incidence of early infection causes severe stunting or death, resulting in an enormous reduction in yield, particularly in susceptible cultivars exhibiting the red stripe and red leaf symptoms (Mayers 1978; Persley 1978; Henzell 1979; Henzell 1982). Under prolonged periods of cool and overcast conditions, leaves can redden and cause a reduction in yield up to 90%, while red stripe symptoms can result in a yield loss of about 60%. Certain susceptible sorghum lines, such as KL9 and KP131, naturally infected with JGMV-Jg, can exhibit infection rates ranging from 33% to 99% (Persley 1981). However in grain sorghum infection rates are lower, commonly 20~30% on the average (Persley 1978). In maize, the situation in the past was more severe as total failures of inbred lines have been recorded, together with about 50% of yield loss in susceptible hybrids (Persley 1976), occasionally accompanied by a 100% infection of the crop. A 100% infection rate in forage sorghum was also recorded over a growing season (Teakle 1978) although the infection rate is more commonly about 30~40% at early stages of growth (Persley 1986). Teakle and Moore (1972) observed that the incidence of JGMV-Jg infection appears to be higher in mosaic-producing cultivars than in those showing necrotic symptoms. In 1986 the JGMV Krish-infecting strain caused about 40% infection in Krish resistant hybrid crops of sorghum (Persley 1987).

Symptomatology and cytopathology

Symptom development on infected plants depends on the host genotype, virus strain and environmental conditions (Persley 1977; Naderi 1997). 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 (Shukla 1989c). 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 (Table 5). Table 7 shows the responses of 14 sorghum cultivars infected by the JGMV Krish-infecting strain. Of these, only cultivar QL11 was resistant.

The appearance of mosaic or necrotic symptoms in JGMV infected sorghum plants is controlled by a single dominant gene *N* (*n*) in the host plant, which is independently inherited (Teakle 1970). The presence of the dominant allele (*N*) results in systemic necrotic symptoms, while the residence of the homozygous recessive *nn* causes systemic mosaic symptoms. The necrotic lesions first appear locally on the leaf inoculated by aphids or inoculated by hand with infected leaf sap. The necrotic symptoms then move systemically towards the basal portions of the inoculated leaf, which then spread to other leaves to form severe yellow mosaic symptoms on mature plants (Teakle 1970). Mosaic symptoms typified by mild mottling, and mosaic symptoms in young sorghum leaves, consist of broken light and dark green lines between the leaf veins, which can cause leaves to become necrotic and red at low temperatures (Persley 1970; Persley 1977). The mosaic symptom may decrease in intensity or disappear entirely on mature plants, making it difficult to distinguish these symptoms in the field from the normal spotting or mottling caused by aphids.

Necrosis can be further subdivided into either red or tan colours, which are under the control of a single gene, *P* (purple) or *p* (tan). However, *P* and *N* genes do not appear to be closely linked (Teakle 1971). Local necrotic symptoms, generally formed at low temperatures (below 15°C), can develop into red necrosis (or red stripes) or tan necrosis (or tan stripes) depending on the host genotype (Teakle 1970). The red leaf symptoms characterised by leaf reddening and the formation of interveinal necrotic red spots and streaks, are known to occur as a result of the interaction between low temperature and the virus (Bockholt 1968). Persley and his colleagues (1977) demonstrated that the necrotic red leaf and red stripe symptoms were independently inherited and controlled by a gene *RLF* (*rlf*) under both low (15.5°C) and high temperatures (23.8~26.6°C). They claimed that after manual inoculation the necrotic red leaf symptom was expressed in the presence of a homozygous recessive allele *rfl* and the dominant allele *N* at low temperatures, while the mosaic symptom was expressed by the presence of a dominant allele *RLF* and a homozygous recessive allele *nn*.

In field trials Teakle and Moore (1972) showed that sorghums with necrotic symptoms were infected at lower rates than the sorghums exhibiting the mosaic symptoms, explaining that the necrotic regions probably contained a lower concentration of the virus than the mosaic regions. Consequently, aphid vectors transmit the virus at a proportionately lower rate within plots exhibiting necrotic symptoms than within plots having the mosaic symptoms. However, it should be borne in mind that necrosis is a much more severe disease as far as individual plants are concerned.

JGMV-Jg infected plants showing systemic symptom developments possess cylindrical cytoplasmic inclusions (CI), but not the nuclear and amorphous inclusions found in plants infected with some other potyviruses such as TEV(tobacco etch virus) (Gough 1993). The virus particles and the pinwheel inclusions are found in the cytoplasm of cells in tissues infected with JGMV (Shukla 1989c). The distribution of JGMV virions in infected tissues differs for sorghum and maize. In sorghum, the particles appear to be equally distributed in the chlorotic and green regions of infected leaves, while in maize they are largely confined to the chlorotic areas (Shukla 1989a). Potyvirus particles are usually scattered throughout the cytoplasm of infected cells.

JGMV genome structure

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 (Gough 1993). The potyvirus genome consists of a single-stranded positive sense RNA (~ 10 kb, Mr 3.0~3.5 $\times 10^6$) 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

Table 7. Responses of 14 sorghum genotypes to natural infection by the Australian JGMV Krish-infecting virus strain

Line	Plants affected* (%)	Symptom**	Line	Plants affected* (%)	Symptom**
Rio	100	M	BT×623	72	M
Atlas	96	M/RL	YEF×SSK	51	RS
RT×430	92	M	OKY8	38	RS
BT×3197	90	M/RL	QL19***	16	M/RL
NM31	86	M/RL	SC0097-14E	15	M
BT×398	81	M	Q75339	1	M
KS4	80	M/RL	QL11	0	Nil

Data taken from Persley *et al.*, 1986.

*Data from three replicates taken 52 days after planting and 35 days after inoculation of sweet corn inoculum spreader rows.

** Symptoms: M=Mosaic, RL=necrotic red leaf, RS=necrotic red stripe. The red leaf (RL) response develops following cold temperatures.

*** *QL=KS4×Q75339.

3 *cis*- or *trans*-acting proteinases (P1, HC-Pro and NIa-Pro) to yield at least 8 functional proteins (Table 8 and 9) (Dougherty 1988; Shukla 1991; Riechmann 1992; Gough 1993; Shukla 1994a). 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 (Beclin 1998; Kasschau 1998; Voinnet 1999; Llave 2000; Marathe 2000; Voinnet 2000; Mallory 2001). Jenner *et al.* (2000) recently reported that the CI of TuMV determines the virulence of the virus in *Brassica napus TuRB01* that possesses a dominant resistance allele.

Table 8. Putative cleavage sites in the JGMV-Jg polyprotein

Protein	Amino acid sequences of the cleavage sites in JGMV-Jg polyprotein
P1/HC-Pro	K-Q-I-C-H-Y ↓ S-F
HC-Pro/P3	K-E-Y-I-V-G ↓ G-D
P3/6K1	T-E-V-E-H-E ↓ R-K
6K1/CI	Q-E-V-K-H-E ↓ G-S
CI/6K2	E-N-C-V-K-E ↓ L-D
6K2/VPg	T-E-V-E-H-E ↓ G-K
VPg/NIa-Pro	P-E-V-E-H-E ↓ G-T
NIa-Pro/NIb	E-R-I-S-N-E ↓ S-A
NIb/CP	V-D-V-E-H-Q ↓ S-G

Adapted from Gough and Shukla (1993). Cleavage positions are marked by an arrow.

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, and were sequenced by Gough and Shukla (1993). The 3' UTR of JGMV is considered to be the longest non-coding region amongst the potyviruses (Shukla 1994a).

The coat protein (CP)

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 (Figure 1), 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 (Kashiwazaki 1989; Jagadish 1991; Ward 1991; Jagadish 1993). 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 (Shukla 1988c; Yu 1989; Shukla 1989c; Frenkel 1991; Gough 1992). The N-terminus of the CP is

Table 9. Genome organisation of JGMV-Jg and putative functions of gene products. Proposed functions are largely based on comparison with other potyviruses

	JGMV-Jg			
Protein	Amino acid	Nucleotide	MW(kD)	Gene products and functions
5 UTR		1~135		
P1	1~237	136~846	28	Aphid transmission, cell-to-cell Proteinase[1)]
HC-Pro	238~698	847~2,229	53	Aphid transmission, cell-to-cell movement[2)], proteinase[1)] and PTGS /VIGS suppression[3)]
P3	699~1,045	2,230~3,270	40	Unknown
6K1	1,046~1,097	3,271~3,426	6	Unknown, genome replication
CI	1,098~1,757	3,427~5,406	74	Genome replication, RNA helicase, membrane and cell-to-cell movement[2)]
6K2	1,758~1,802	5,407~5,541	5	Unknown, genome replication
NIa-VPg	1,803~1,991	5,542	22	Genome replication
NIa-Pro	1,992~2,232	6,109~6,831	27	Proteinase1
NIb	2,233~2,749	6,832~8,382	60	Genome replication, RNA dependent RNA polymerase
CP	2,750~3,052	8,383~9,294	34	RNA encapsidation, aphid transmission, cell-to-cell movement[2)], host specificity[4)]
3 UTR		9,295~9766		

Data adapted from Gough and Shukla (1993), Shukla *et al.* (1994). [1)]Proteinases for polyprotein processing [2)]Perhaps also involved in cell-to-cell movement [3)]Mallory *et al.* (2001) [4)]Suranto (1998b).

immuno-dominant and contains virus-specific epitopes (Shukla 1984; Shukla 1988c; Shukla 1989c; Shukla 1989d; Armstrong 1995; Desbiez 1997). Shukla *et al.* (1989c) reported that JGMV-Jg is serologically close to WMV 2, 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 (Lain 1989; Blanc 1997). Atreya *et al.* (1990 and 1995) were the first to prove that 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. (Blanc 1997). The DAG motif is common to potyviruses but occurs at different positions, generally between 5 to 13 residues from the N-terminus (Shukla 1991).

There is no doubt that the CP has a role in host specificity and virulence. Based on the CP sequences provided by Shukla (1987), Tosic and his colleagues (1990) showed that the grouping of SCMV strains is well correlated with the reactivities of these strains to different sorghum cultivars. Jiao (1993) reported that 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 (Sherwood 1982). Shukla *et al.* (1991) suggested that similarities in the N-terminal region of the coat protein might determine cross-protection. However, transgenic plants expressing the SCMV 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 (Powell 1990). The mechanisms by which protection is achieved in CP-mediated virus resistant transgenic plants are most probably both host and virus specific.

It is well known that symptom development can be determined by a single nucleotide change (Rao 1995). Symptoms on TVMV infected *Nicotiana sylvestris* are controlled by a point mutation in the CP (Knorr 1988). Interestingly, a spontaneous single point mutation within the BMV CP recovers the systemic-spread phenotype and increases virion stability (Flasinski 1997).

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. According to Dolja *et al.* (1994), 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 (Dolja 1995).

In non-potyviruses the CP has also been strongly implicated as a movement protein, with the N- and C-terminals playing a different role to the core region of the CP. It is a general rule, where the CP has been shown to be involved, it appears to be vital for long distance transport. Flasinski *et al.* (1995) showed that the CP of BMV is necessary for systemic translocation (long-distance movement) but not for cell-to-cell movement (short-distance movement). The synthesis and accumulation of the CP has been shown to be required for long-distance movement of RCNMV (Vaewhongs 1995). Schneider *et al.* (1997) reported that CCMV virion formation is not necessary for systemic infection to occur, but that the C-terminus of the CP is necessary for systemic movement of viral RNA, indicating that the CP has at least two functions; to facilitate long-distance movement and virion formation. The CP of CLRV also appears to be required for long-distance movement (Mas 1996). Deletion of the N-terminal 25 amino acids of the CP in BMV has no affect on the accumulation of CP, but encapsidation and systemic infection does not take place (Sacher 1989). CPMV can spread only if the RNA is encapsidated in particles (Wellink 1989), highlighting the crucial role of the CP in systemic spread, but obviously not in all cases.

Infectious full-length transcripts derived from viral cDNAs: Significance and application

Many plant RNA viruses do not have a DNA intermediate in their life cycle (Boyer 1993; Boyer 1994), 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 (Bujarski 1992; Boyer 1994). One of the biggest challenges in preparing a full-length cDNA of a potyvirus is to maintain the 10 kb ORF of the polyprotein.

Despite successful preparations of full-length cDNA's by RT-PCR in some cases (Fakhfakh 1996; Lindberg 1997), 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 (Quillet 1989; Singh 1991; Jakab 1997). Singh and Singh (1991) reported on the instability of a recombinant plasmid containing PVY cDNA in *E. coli*, strain DH5. In a more serious case, Jakab *et al.* (1997) reported that full-length cDNA clones of PVY-N605 could not be maintained in a bacterial vector due to the cytotoxic products associated with the CI gene by internal cryptic prokaryotic promoter elements in the

Table 10. Potyviruses. Properties of infectious *in vitro* and *in vivo* transcripts of full-length cDNA clones. Adapted from Kim (2000)

Viruses	Transcripts		Infection rates (%)		Pro-	Ter-	Cap	Non-viral bases		Poly (A)	Template form	Plant species
	c	*in vivo*	Hand	Bomb	moter	minator		5'	3'	Tail (nts)		
PVA	*in vitro*		49		T7			1	6	22	Linearised (*Pin*AI)	*Nicotiana tabacum*
PPV	*in vitro*		49		T7		yes	1	12	100	Linearised (*Pst*I) or	*Nicotiana clevelandii*, *N. benthamiana*,
	in vitro		0		T7		yes	1	12	100	fragmented	*Chenopodium foetidum* and *C. quinoq*
		in vivo	48		35S	NOS	none	1	12	82	(*Pst*I and *Pvu*II)	
PSbMV	*in vitro*		50~100		T7			1	6		Linearised (*Xba*I)	*Pisum sativum*
		in vivo	100		35S	NOS	yes	1	6			
PStV	*in vitro*		100		SP6			1	6	30	Linearised (*Mlu*I)	*Chenopodium amaranticolor*
PYV-N605	*in vitro*		75~100	30~100	SP6		yes	1	21	100	Linearised (*Kpn*I) or	*Nicotiana tabacum* var. SR1,
	in vitro		0	0	SP6		yes	1	21	100	Intact plasmid	*N. clevelandii*, *N. benthamiana*
		in vivo	25~83	50~100	35S	none	none	0	21	100		and *Solanum tubersum* var. Bintje
PVY-^{NTN}		*in vivo*	62		35S	NOS		0	0	100	PCR fragment	*Nicotiana tabacum* var. Xanthi
TEV	*in vitro*		27		SP6			2	6	70~75	Linearised (*Bgl*II)	Tobacco
TuMV	*in vitro*		3		T7		yes	1	28	56	Linearised (*Spe*I) or	*Arabidopsis thaliana*, *Chenopodium*
		in vivo	29		35S	NOS	yes	0	28	56	intact plasmid	*quinoa* and *C. amaranticolor*
		in vivo	19		35S	35ST		0	28	56		
		in vivo	22		35S	none		0	28	56		
TVMV	*in vitro*		5		T7			1	1	37~96	Linearised (*Sac*I)	*Nicotiana tabacum* var. Xanthi
	in vitro		5		T3		yes	2	1	37~96		
	in vitro		0		T7/T3		yes	1~2	1	37~96		
TVMV-S	*in vitro*		36~100		T7		none	1	1	130	Linearised (*Nco*I)	*Nicotiana tabacum* cv. TN86
ZYMV	*in vitro*		9~11		T7		yes	1	6	66	Linearised (*Asp*718I)	*Cucurbita pepo*, *Cucumis sativus*
	in vitro		0		T7		yes	1	21	66		*Cucumis melo*, *Citrullus lanatus*,
	in vitro			100	T7		none	1	21	66		and *Nicotiana benthamiana*
	in vitro			11	T7		yes	1	21	66		
		in vivo	0	7	35S	NOS	none	127	28	66	Intact plasmid	
		in vivo	19	100	35S	NOS		0	28	66		
		in vivo	0	100	35S	none		0	28	66		
JGMV-Jg	*in vitro*		13.3		T7			1	30	46		*Zea mays* cv. Terrific
	in vitro			18.3	T7		none	1	30	46		
	in vitro		31.6		T7		none	1	30	46	Linearised (*Not*I)	*Sorghum bicolor* cv. Sugardrip
	in vitro		21.6		T7		yes	1	30	46		
	in vitro			38.3	T7		yes	1	30	46		
	in vitro			26.6	T7		yes	1	30	46		
	in vitro		45.0		T7		yes	1	30	46		
	in vitro		20.0		T7		yes	1	30	46		
				26.6	T7		none	1	30	46		
		in vivo	7.5		35S	35ST	none	none	1	46	Intact plasmid	
		in vivo	5.0		35S	35ST		none	none	46		
		in vivo	10.0		35S	35ST		none	1	46		
		in vivo	2.5		35S	35ST		none	none	46		
		in vivo		12.5	35S	35ST		none	1	46		
		in vivo		7.5	35S	35ST		none	none	46		
		in vivo		12.5	35S	35ST		none	1	46		
		in vivo		5.0	35S	35ST		none	none	46		

Viral cDNA clones were linearised or fragmented by suitable restriction enzymes (brackets) prior to *in vitro* transcription reactions. *Bgl*II, *Kpn*I, *Pst*I and *Sac*I produce a 3' overhang, while *Asp*718I, *Cla*I, *Mlu*I, *Nco*I, *Pin*AI, *Spe*I and *Xba*I generate a 5 overhang and *Pvu*II a blunt end at the 3' end. Only the 3' overhang residues are polished by T4 DNA polymerase. Transcripts were mechanically inoculated by hand (Hand) or by bombardment (Bomb). Transcripts from the *in vitro* transcription reaction mixture were not purified. Terminators are NOS and 35ST, the 35S transcription terminator. Blank spaces indicate no experiment recorded. PVA, Puurand *et al.*, (1996); PPV, Riechmann *et al.* (1990) and Maiss *et al.*, (1992); PSbMV, (Johansen. I. E., 1996); PStV, Flasinski *et al.*, (1996); PVY-N605, Jakab *et al.* (1997); PYV^{NTN}, Fakhfakh *et al.* (1996); TEV, Dolja *et al.* (1992); TuMV, Sanchez *et al.*, (1998); TVMV, Domier *et al.* (1989); TVMV-S, Nicolas *et al.*, (1996); ZYMV, Gal-On *et al.* (1991 and 1995) ; and JGMV-Jg, Kim (2000) and Suranto (2000).

Table 11. Non-poty viruses. Properties of infectious *in vitro* and *in vivo* transcripts of cDNA clones. Adapted from Kim (2000).

Viruses	Transcripts		Infection (%)	Pro-moter	Ter-minator	Cap	Non-viral bases		Poly (A) tail (nts)	Template form	Plant species
	in vitro	*in vivo*					5'	3'			
BaMMV		*in vivo*	10	35S[2]	none					Linearised (*Hin*dIII or *Cla*I)	*Hordeum vulgare*
BMV[1)]	*in vitro*		20~44	T7		yes	0	6		Linearised (*Eco*RI)	*Hordeum vulgare*
	in vitro		23~54	T7		yes	1	6			
	in vitro		0~7	T7		yes	7~16	6			
	in vitro		20~44	T7		yes	1~13	6		Linearised (*Eco*RI)	*Hordeum vulgare* L. cv. Goseshikoku and
BMV[2)]		*in vivo*	0.5~1*	35S	NOS		0~12	6			*Chenopodium hybridum*
						yes					
BNYVV[1)]	*in vitro*		5~30**	T7			2	12~28		Linearised (*Hin*dIII)	*Chenopodium quinoa* and *Tetragonia expansa*
BNYVV[2)]		*in vivo*	20~60	35S	NOS		25~40	several hundred		Intact plasmid	*Chenopodium quinoa* and *Tetragonia expansa*
BSMV	*in vitro*		35	T7		yes	1	1		Linearised (*Spe*I)	*Hordeum vulgare*, *Chenopodium*
	in vitro		0	T7		none	1	1			*amaranticolor* and *Nicotiana benthamiana*
BWYV	*in vitro*			T7		yes	2	32	26	Linearised (*Sal*I)	*Chenopodium quinoa* protoplasts
CMV		*in vivo*	10~50	35S	NOS					Linearised (*Kpn*I)	*Nicotiana glutinosa*
										Fragmented (*Bam*HI and *Kpn*I)	
										Intact plasmid	
CMV-Q	*in vitro*		5~90	T7		yes	1~2	16		Linearised (*Bam*HI)	*Nicotiana tabacum* cv. Samsun
CPMV		*in vivo*	100	35S	none		0	0~7	none	Linearised (*Eco*RI and	*Vigna unguiculata* L.
				T7		yes	1			*Mlu*I)	
CTLV	*in vitro*			T7		none	1			Linearised (*Nco*I)	*Chenopodium quinoa*
PVX	*in vitro*		0.2*	T7		yes	1	13	16	Linearised (*Sac*I)	*Chenopodium amaranticolor*
RCNMV	*in vitro*		43~97	T7		yes	1~2	3		Linearised (*Sma*I)	*Nicotiana benthamiana*,
			77.9			none					*N. cleveladii*
TAV		*in vivo*	10~90	35S[2]	35ST					Fragmented (*Hae*II and *Pvu*II)	*Nicotiana glutinosa* and *N. clevelandii*
TCV	*in vitro*		15~54**		T7/Pm	yes	1~2	5		Linearised (*Xba*I)	*Nicotiana benthamiana, Brassica campestris*
			10~12**				14	5			*Chenopodium amaranticolor*
						none	1~2	5			
TMV[1)]	*in vitro*		23~416**	Pm		yes	0	6		Linearised (*Mlu*I) or	*Nicotiana tabacum* var. Xanthi
	in vitro		3**	Pm		none	0			intact plasmid	
TMV[2)]	*in vitro*		16.7~100	Pm		yes	0~6	0~6		Linearised (*Pst*I)	*Nicotiana tabacum* L. var. Xanthi
TYMV	*in vitro*			T7		yes	1	2~25	50	Linearised (*Age*I)	*Phaseolus vulgaris* L. rapeseed protoplast

Viral cDNA clones were linearised or fragmented by suitable restriction enzymes (brackets) prior to *in vitro* or *in vivo* transcription. *Bgl*II, *Hae*II, *Kpn*I, *Pst*I and *Sac*I produce a 3' overhang, while *Age*I, *Bam*HI, *Cla*I, *Eco*RI, *Hin*dIII, *Mlu*I, *Nco*I, *Not*I, *Pin*AI, *Spe*I and *Xba*I generate a 5' overhang and *Pvu*II a blunt end at the 3' end. Only the 3' overhang residues are polished by T4 DNA polymerase. Transcripts were mechanically inoculated by hand. Blank spaces indicate no experiment recorded. The infectivity of PVX and BMV[2)] transcripts (marked as *) is relative infectivity as compared to that of authentic viral RNAs. BNYVV, TMV and TCV infectivities (marked **) are numbers of local lesions on infected leaves. The promoters are 35S[2], the CaMV 35S promoter with double enhancer, Pm for *E. coli* RNA polymerase, and T7. Terminators are NOS and 35ST, the 35S transcription terminator. BaMMV, Meyer and Dessens (1997); BMV, Janda *et al.* (1987)[1)] and Mori, *et al.* (1991)[2)]; BNYVV, Quillet *et al.* (1989)[1)] and Commandeur *et al.* (1991) [2)]; BSMV, Petty *et al.* (1989); BWYV, Veidt *et al.* (1992); CMV, Ding *et al.* (1995); CMV-Q, Hayes and Buck, (1990); CPMV, Dessens and Lomonossoff (1993); CTLV, Ohira *et al.* (1995); PVX, Hemenway *et al.* (1990); RCNMV, Xiong and Lommel, (1991); TAV, Shi *et al.* (1997); TCV, Heaton *et al.* (1989); TMV, Meshi *et al.* (1986)[1)] and Dawson *et al.* (1986)[2)] and TYMV, Boyer *et al.* (1993).

viral genome. They also observed an instability of viral cDNA clones in *E. coli* strains SURE and DH10B. Hence, the PVY cDNA was always maintained in two subclones and ligated before infection experiments with *in vivo* or *in vitro* transcripts. Many of these difficulties can be circumvented by changing the bacterial strain used for cloning.

An often-encountered problem is the occurrence of spontaneous mutations in the viral cDNA during cloning or mutations caused by polymerase errors during cDNA synthesis. In some cases viral functions such as systemic or non-systemic symptom development, and weak infectivity or none at all, were associated with mutations in the cDNA (Dolja 1993; Rao 1995; Nicolas 1997). Consequently the full-length cDNA of the virus must match the genomic sequences for meaningful studies to be carried out. In terms of general cloning strategies, a long poly (C) tract (50~250 bp) has been found to interfere with plasmid replication. A short tract of at least 32 C residues is required for stability (Deng 1981; Duke 1989; Zibert 1990). A number of other parameters may also be important in determining the infectivity of viral transcripts, such as the transcript cap structure, the presence of non-viral nucleotides at both ends and the nature of the promoter used.

Once a full-length infectious cDNA 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. The use of viral cDNA clones for the synthesis of infectious *in vitro* RNA transcripts was first reported for BMV by Ahlquist and Janda (1984). Three cDNAs 1 (3.2 kb from RNA 1), 2 (2.8 kb from RNA 2) and 3 (2.1 kb from RNA 3), representing the complete genome of BMV, were placed in tandem in the transcriptional vector pM1. The *in vitro* derived transcripts (prepared by using *E. coli* RNA polymerase) were infectious. Greater yields of infectious BMV transcripts were obtained with the use of T7 RNA polymerase (Janda 1987). Since then, other full-length infectious viral cDNAs have been reported (Table 10 and 11). The tables summarise the data for potyviruses and non-potyviruses separately. The infectivity of viral transcripts, however, varies considerably from virus to virus as well as from host to host. Nevertheless, the infectivity of *in vitro* transcripts can be much less (sometimes 1% or less) than that of authentic viral RNAs when compared with an equivalent amount of RNA inoculum (Hemenway 1990; Riechmann 1990).

Since the first publication of the complete nucleotide sequences of TVMV (Domier 1986) and TEV (Allison 1986), many other potyviruses have been sequenced (PPV, Lan et al., 1989 and Maiss et al., 1989; PVY-N, Robaglia et al., 1989; PSbMV, Johansen et al., 1991; PepMoV, Vance et al., 1992; SMV, Jayaram et al., 1992; PRSV, Yeh et al., 1992; TuMV, Nicolas and Lalibert, 1992; JGMV, Gough and Shukla, 1993; PVY-H, Thole et al., 1993; PStV, Gunasinghe et al., 1994; PVA, Puurand et al., 1994; BCMV-NL3, Fang et al., 1995, and BYMV-S, Guyatt et al., 1996; TVMV-S, Nicolas et al., 1996). However, despite the fact that the potyvirus group is the largest plant virus group with a large number of fully sequenced potyviruses, so far only 11 potyvirus cDNA clones, including JGMV-Jg, have been prepared and reported to be infectious (Gough 1993; Kim 2000) (Table 10). Except for JGMV-Jg and BYMV-S, all of their hosts are dicots.

A classical method for virus inoculation has been to mechanically inoculate plants by hand (Suranto 1998, Kim 2002). The inoculum can be 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. 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. However, the infectivity

Table 12. Studies on potyviral genomic functions using infectious cDNA clones in dicot hosts. Adapted from Kim (2000)

Polyprotein regions examined	References
5 UTR	Riechmann *et al.* (1991); Simón-Buela *et al.* (1997)
P1	Verchot and Carrington (1995a and b); Verchot *et al.* (1991 and 1992)
HC-Pro	Oh and Carrington (1989); Carrington *et al.* (1990); Kasschau and Carrington (1995); Cronin *et al.* (1995); Peng *et al.* (1998); Mallory *et al.* (2001)
P3	Riechmann *et al.* (1995)
$6K_1$ and $6K_2$	Riechmann *et al.* (1995); Restrepo-Hartwig and Carrington (1994)
NIa-VPg and	Restrepo-Hartwig and Carrington (1992); Nicolas *et al.* (1996 and 1997); Murphy *et al.* (1996)
NIa-Pro	Schaad and Carrington (1996); Schaad *et al.* (1997)
NIb	Li *et al.* (1997)
CP	Atreya *et al.* (1990 and 1995); Dolja *et al.* (1994 and 1995); Desbiez *et al.* (1997); Schaad and Carrington (1996); Schaad *et al.* (1997); López-Moya and Pirone (1998); Suranto et al. (1998); Suranto (2000)
3 UTR	Rodríguez-Cerezo *et al.* (1991)

s ems not to be greatly different between *in vivo* and *in v tro* transcripts (Tables 10 and 11).

Infectious *in vivo* and *in vitro* RNA transcripts derived fi om full-length viral cDNA clones have proved to be p owerful and efficient tools for the study of RNA viruses. I addition, reporter genes such as green fluorescent protein (G FP) and -glucuronidase (GUS) have been used to study v rious aspects of viral gene functions by inserting them a fusion products into infectious cDNA clones, as has been d ne for TEV and PVX (Dolja 1992; Angell 1995; Oparka 1 96). Infectious transcripts have been used for the clarifi cation of gene(s) or region(s) responsible for breaking the r sistance of plants against the potyvirus TVMV-W (Nicolas e al., 1996 and 1997). Using infectious cDNA clones, the p tyviral genomic functions relating to various aspects such a infectivity, virus movement, aphid transmission and p lyprotein processing have been studied extensively (Table 1). Table 12 shows a brief summary of potyviral gene fi nctions determined experimentally using infectious transs ripts of full-length cDNA clones. However, with one e ception, these achievements have been made on dicot i fecting potyviruses: Suranto (1998) investigated the role o CP sequences in the monocot sorghum.

References

. Allison, R., Johnston, R. E. and Dougherty, W. G. 1986. The nucleotide sequence of the coding region of tobacco etch virus genomic RNA: evidence for the synthesis of a single polyprotein. *Virology* **154**, 9-20.

. Angell, S. M. a. B., D. C. 1995. Cell-to-cell movement of potato virus X revealed by microinjection of a viral vector tagged with the beta-glucuronidase gene. *Plant J.* **7**, 135-140.

. Beclin, C., Berthome, R., Palauqui, J.-C., Tepfer, M. and Vaucheret, H. 1998. Infection of tobacco or *arabidopsis* plants by CMV counteracts systemic post-transcriptional silencing of non-viral (trans) genes. *Virology* **252**, 313-317.

. Blanc, S., Lopez-Moya, J.-J., Wang, R., Garcia-Lampasona, S., Thornbury, D. W. and Pirone, T. P. 1997. A specific interaction between coat protein and helper components correlates with aphid transmission of a potyvirus. *Virology* **231**, 141-147.

. Bockholt, A. J. a. T., W. G. 1968. Effect of maize dwarf mosaic on grain sorghum. *Prog. Rep. Tex. Agric. Exp. Stn.*, No. PR-2509.

. Boyer, J. C., Drugeon, G., Seron, K., Morch-Devignes, M.-D., Agnes, F. and Haenni, A. L. 1993. *In vitro* transcripts of turnip yellow mosaic virus encompassing a long 3' extension of produced from a full-length cDNA clone harbouring a 2 kb-long PCR-amplified segment are infectious. *Res. Virol.* **144**, 339-348.

. Boyer, J. C. a. H., A. L. 1994. Infectious transcripts and cDNA clones of RNA viruses. *Virology* **198**, 415-426.

. Bujarski, J. J. a. M., W. A. 1992. Use of *in vitro* transcription to study gene expression and replication of spherical, positive sense RNA plant viruses. Genetic engineering with plant viruses. T. M. A. W. a. J. W. Davies. Boca Raton, FL, CRC press, 115-147.

9. Conde, B. D., Moore, R. F., Fletcher, D. S., Teakle, D. S. 1976. Inheritance of the resistance of Krish sorghum to sugarcane mosaic virus. *Australian Journal of Agricutural Research* **27**, 45-52.
10. Deng, G. a. W., R. 1981. An improved procedure for utilizing terminal transferase to add homopolymers to the 3' termini of DNA. *Nucleic Acids Research* **9**, 4173-4188.
11. Desbiez, C., Gal-On, A., Raccah, B. and Lecoq, H. 1997. Characterization of epitopes on zucchini yellow mosaic potyvirus coat protein permits studies on the interactions between strains. *Journal of General Virology* **78**, 2073-2076.
12. Dolja, V. V., McBride, H. J. and Carrington, J. C. 1992. Tagging of plant potyvirus replication and movement by insertion of beta-glucuronidase into the viral polyprotein. *Proc. Natl. Acad. Sci.* **89**, 10208-10212.
13. Dolja, V. V., Herndon, K. L., Pirone, T. P. and Carrington, J. C. 1993. Spontaneous mutagenesis of a plant potyvirus genome after insertion of a foreign gene. *Journal of Virology* **67(10)**, 5968-5975.
14. Dolja, V. V., Haldeman-Cahill, R., Montgomery, A. E., Vandenbosch, K. A. and Carrington, J. C. 1995. Capsid protein determinants in cell-to-cell and long distance movement of tobacco etch potyvirus. *Virology* **206**, 1007-1016.
15. Domier, L. L., Franklin, K. M., Shahabuddin, M., Hellmann, G. M., Overmeyer, J. H., Hiremath, S. T., Siaw, M. F. E., Lomonossoff, G. P., Shaw, J. G. and Rhoads, R. E. 1986. The nucleotide sequence of tobacco vein mottling virus RNA. *Nucleic Acids Research* **14**, 5417-5430.
16. Dougherty, W. G. a. C., J. G. 1988. Expression and function of potyviral gene products. *Annu. Rev. Phytopathol.* **26**, 123-143.
17. Duke, G. M. a. P., A. C. 1989. Cloning and synthesis of infectious cardiovirus RNAs containing shor, dicrete poly (C) tracts. *Journal of Virology* **63**, 1822-1826.
18. Edwardson, J. R. 1974. Some properties of the potato virus Y group. *Fla. Agric. Exp. Stn. Monogr.* **4**, 225.
19. Edwardson, J. R. 1992. Inclusion Bodies. Potyvirus Taxonomy. New York, Springer-Verlag, 25-30 (*Arch. Virol. [Suppl] 5*).
20. Fakhfakh, H., Vilaine, F., Makni, M. and Robaglia, C. 1996. Cell-free cloning and biolistic inoculation of an infectious cDNA of potato virus Y. *Journal of General Virology* **77**, 519-523.
21. Flasinski, S., Dzianott, A., Speir, J. A., Johnson, J. E. and Bujarski, J. J. 1997. Structure-based rationale for the rescue of systemic movement of brome mosaic virus by spontaneous second-site mutations in the coat protein gene. *Journal of Virology* **7**, 2500-2504.
22. Franzmann, B. A., Walker, S. R., and Persley, D. M. 1992. Current status and future trends of pest management in grain sorghum in Australia. *AIAS-occasional publication* No. **68**, **1**, 97-110.
23. Frenkel, M. J., Jilka, J. M., McKern, N. M., Strike, P. M., Clark Jr., J. M. and Shukla, D. D. 1991. Unexpected sequence diversity in the amino-terminal ends of the coat proteins of strains of sugarcane mosaic virus. *Journal of General Virology* **72**, 237-242.
24. Gough, K. H., and Shukla, D. D. 1981. Coat protein of potyviruses. Comparison of the four Australian strains of

sugarcane mosaic virus. *Virology* **111**, 455-462.
25. Gough, K. H., Azad, A. A., Hanna, P. J., and Shukla, D. D. 1987. Nucleotide sequence of the capsid and nuclear inclusion protein genes from the Johnson grass strain of sugarcane mosaic virus RNA. *Journal of General Virology* **68**, 297-304.
26. Gough, K. H., and Shukla, D. D. 1992. Major sequence variations in the N-terminal region of the capsid protein of a severe strain of passionfruit woodiness potyvirus. *Archives of Virology* **124**, 389-396.
27. Gough, K. H., and Shukla, D. D. 1993. Nucleotide sequence of Johnsongrass mosaic potyvirus genomic RNA. *Intervirology* **36**, 181-192.
28. Grogan, P. W. a. T., D. S. 1969. Resistance of some Australian inbred maize lines to maize dwarf mosaic disease. *Aust. J. Exp. Agric. Anim. Husb.* **9**, 541-544.
29. Handley, J. A., Smith, G. R., Dale, J. L., and Harding, R. M. 1998. Sequence diversity in the coat protein coding region of twelve sugarcane mosaic potyvirus isolates from Australia, USA and South Africa. *Archives of Virology* **143**, 1145-1153.
30. Hemenway, C., Weiss, J., O'connell, K. and Tumer, N. E. 1990. Characterization of infectious transcripts from a potato virus X cDNA clone. *Virology* **175**, 365-371.
31. Henzell, R. G., Persley, D. M., Fletcher, D. S., Greber, R. S. and van Slobbe, L. 1979. The effect of sugarcane mosaic virus on the yield of eleven grain sorghum (*Sorghum bicolor*) cultivars. *Aust. J. Exp. Agric. Anim. Husb.* **19**, 225-232.
32. Henzell, R. G., Persley, D. M., Greber, R. S., Fletcher, D. S., and van Slobbe, L. 1982. Development of grain sorghum lines with resistance to sugarcane mosaic and other sorghum diseases. *Plant Disease* **66**, 900-901.
33. Hollings, M., and Brunt, A. A. 1981a. Potyviruses. Handbook of plant viruse infections: comparative diagnosis. E. Kurstak. Amsterdam, Elservier/North-Holland Biomedical Press, 731-807.
34. Hollings, M., and Brunt, A. A. 1981b. Potyvirus groups. CMI/AAB Descriptions of Plant Viruses No.245.
35. Jagadish, M. N., Ward, C. W., Gough, K. H., Tulloch, P. A., Whittaker, L. A. and Shukla, D. D. 1991. Expression of potyvirus coat protein in Escherichia coli and yeast and its assembly into virus-like particles. *Journal of General Virology* **72**, 1543-1550.
36. Jagadish, M. N., Huang, D. and Ward, C. W. 1993. Site-directed mutagenesis of a potyvirus coat protein and its assembly in *Escherichia coli*. *Journal of General Virology* **74(5)**, 893-896.
37. Jakab, G., Droz, E., Brigneti, G., Baulcombe, D. and Malnoe, P. 1997. Infectious *in vivo* and *in vitro* transcripts from a full-length cDNA clone of PVY-605, a Swiss necrotic isolate of potato virus Y. *Journal of General Virology* **78**, 3141-3145.
38. Janda, M., French, R., and Ahlquist, P. 1987. High efficiency T7 polymerase synthesis of infectious RNA from cloned brome mosaic virus cDNA and effects of 5'extensions on transcript infectivity. *Virology* **258**, 259-262.
39. Kantrong, S., Saunal, H., Briand, J. P. and Sako, N. 1995. A single amino acid substitution at N-terminal region of coat protein of turnip mosaic virus alters antigenicity and aphid Transmissibility. *Archives of Virology* **140**,453-467.
40. Kashiwazaki, S., Hayano, Y., Minobe, Y., Omura, T., Hibino, H. and Tsuchizaki, T. 1989. Nucleotide sequence of the capsid protein gene of barley yellow mosaic virus. *Journal of General Virology* **70**, 3015-3023.
41. Kasschau, K. D. a. C., J. C. 1998. A counterdefensive strategy of plant viruses: Suppression of posttranscriptional gene silencing. *Cell* **95**, 461-470.
42. K-S. Kim, H-Y. Oh, E. Nurhayati, S. Suranto, K. H. Gough, D. D. Shukla and C. K. Pallaghy 2002. Infectivity of *in vitro* transcripts of Johnsongrass mosaic potyvirus full-length cDNA clones in maize and sorghum; Infectious *in vitro* transcripts of JGMV cDNA clones. *Archives of Virology* (accepted August 18, 2002).
43. Knorr, D. A. a. D., W. O. 1988. A point mutation in the tobacco mosaic virus capsid protein gene induces hypersesitivity in Nicotiana sylvestris. *Proc. Natl. Acad. Sci.* **85**, 170-174.
44. Lain, S., Riechmann, J. L. and Garcia, J. A. 1989. The complete nucleotide squence of plum pox potyvirus RNA. *Virus Res.* **13**, 157-172.
45. Lindberg, A. M., Polacek, C. and Johansson, S. 1997. Amplification and cloning of complete enterovirus genomes by long distance PCR. *J. Virol. Methods* **65**, 191-199.
46. Llave, C., Kasschau, K. D. and Carrington, J. C. 2000. Virus-encoded suppressor of posttranscriptional gene silencing targets a maintenance step in the silencing pathway. *Proc. Natl. Acad. Sci.* **97**, 13401-13406.
47. Mallory, A. C., Ely, L., Smith, T. H., Marathe, R., Anandalakshmi, R., Fagard, M., Vaucheret, H., Pruss, G., Bowman, L. and Vance, V. B. 2001. HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. *The Plant Cell* **13**, 571-583.
48. Marathe, R., Anandalakshmi, R., Smith, T. H., Pruss, G. J. and Vance, V. B. 2000. RNA viruses as inducers, suppressors and targets of post-transcriptional gene silencing. *Plant Mol. Biol.* **43**, 295-306.
49. Mas, P. a. P., V. 1996. Long-distance movement of cherry leaf roll virus in infected tobacco plants. *Journal of General Virology* **77**, 531-540.
50. Mayers, P. 1978. Disease control in grain sorghum. Grain Sorghum Workshop. Toowoomba, Australia, 165-174.
51. McDaniel, L. L., and Gordon, D. T. 1985. Identification of a new strain of maize dwarf mosaic virus. *Plant Disease* **69**, 602-607.
52. Naderi, M. a. B., P. H. 1997. Effects of chloroplast targeted potato virus Y coat protein on transgenic plants. *Physiol. Mol. Plant Pathol.* **50**, 67-83.
53. Nicolas, O., Dunnington, S. W., Gotow, L. F., Pirone, T. P. and Hellmann, G. M. 1997. Variation in the VPg protein allow a potyvirus to overcome *va* gene resistance in tobacco. *Virology* **237**, 452-459.
54. Oparka, K. J., Boevink, P. and Cruz, S. S. 1996. Studying the movement of plant viruses using green fluorescent protein. *Trends in Plant Science* **1**, 412-418.
55. Penrose, L. J. 1974. Distribution of a Johnson grass strain of sugarcane mosaic virus in New South Wales and Studies of the host range of the Johnson grass and sugarcane strains. *Australian Journal of Agricutural Research* **25**, 99-104.

56. Persley, D. M. a. G., R. S. 1970. Red leaf of sorghum. Aust. Plant Dis. Rec. **22**, 6.
57. Persley, D. M. 1976. Maize Dwarf Mosaic Disease. Queensland. Agric. J. November-December, 531-532.
58. Persley, D. M., Moore, R. F., and Fletcher, D. S. 1977. The inheritance of the red leaf reaction of grain sorghum to sugar-cane mosaic virus infection. *Aust. J. Agric. Res.* **28**, 853-858.
59. Persley, D. M. 1978. Sugarcane mosaic virus in sorghum. Queensland. Agric. J. May-June, 279-281.
60. Persley, D. M., Martin, I. F. and Greber, R. S. 1981. The resistance of maize inbred lines to sugarcane mosaic virus in Australia. *Australian Journal of Agricutural Research* **32**, 741-748.
61. Persley, D. M., Henzell, R. G., Greber, R. S., and Fletcher, D. S. 1986. Sugarcane mosaic virus in grain sorghum in Australia. Proceeding First Australian Sorghum Conference, Melbourne, Australian Institute of Agriculture Science.
62. Persley, D. M., Greber, R. S., and Henzell, R. G. 1987. Isolates of sugarcane mosaic virus-Johnsongrass strain infecting Krish resistant grain sorghum genotypes in Australia. *Sorghum Newsletter* **30**, 72-73.
63. Persley, D. M., and Syme, J. R. 1990. Field crops and pastures. A disease management guide. Brisbane, Queesland Department of Primary Industries.
64. Powell, P. A., Sanders, P. R., Tumer, N., Fraley, R. T. and Beachy, R. N. 1990. Protection agains tobacco mosaic virus infection in transgenic plants requires accumulation of coat protein rather than coat protein RNA sequences. *Virology* **175**, 124-130.
65. Quillet, L., Guilley, H., Jonard, G. and Richards, K. 1989. *In vitro* synthesis of biologically active beet necrotic yellow vein virus RNA. *Virology* **172**, 293-301.
66. Rao, A. L. N. a. G., G. L. 1995. A spontaneous mutation in the movement protein gene of brome mosaic virus modulates symptom phenotype in *Nicotiana benthamiana*. *Journal of Virology* **69**, 2689-2691.
67. Riechmann, J. S., Sonia, L. and Garcia, J. A. 1990. Infectious *in vitro* transcript from a plum pox potyvirus cDNA clone. *Virology* **177**, 710-716.
68. Riechmann, J. S., Lain, S. and Garcia, J. A. 1992. Highlights and prospects of potyvirus molecular biology. *Journal of General Virology* **73**, 1-16.
69. Sacher, R. a. A., P. 1989. Effects of deletions in the N-terminal basic arm of brome mosaic virus coat protein on RNA packaging and systemic infection. *Journal of Virology* **63**, 4545-4552.
70. Shepherd, R. J., and Holderman, Q. L. 1965. Seed transmission of the Johnson grass strain of sugarcane mosaic virus in corn. *Plant Dis. Reptr.* **49**, 468-469.
71. Sherwood, J. L. a. F., R. W. 1982. The specific involvement of coat protein in tobacco mosaic virus in corn. *Plant Dis. Reptr.* **49**, 468-469.
72. Shukla, D. D., O'Donnell, I. J., and Gough, K. H. 1983. Characteristics of the electroblot radioimmunoassay (EBRIA) in relation to the identification of plant viruses. *Acat. Phytopathol. Acad. Sci. Hung.* **18**, 79-84.
73. Shukla, D. D., and Gough, K. H. 1984. Serological relationships among four Australian strains of sugarcane mosaic virus as determined by immune electron microscopy. *Plant Disease* **68**. 204-206.
74. Shukla, D. D., Gough, K. H., and Ward, C. W. 1987. Coat protein of potyviruses. Comparison of amino acids sequences of the coat proteins of four Australian strains of sugarcane mosaic virus. *Archives of Virology* **96**, 59-74.
75. Shukla, D. D., and Ward, C. W. 1988a. Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. *Journal of General Virology* **69**, 2703-2710.
76. Shukla, D. D., McKern, N. M., and Ward, C. W. 1988b. Coat protein of potyviruses 5.Symptomatology, serology, and coat protein sequences of three strains of passionfruit woodiness virus. *Archives of Virology* **102**, 221-232.
77. Shukla, D. D., Strike, P. M., Tracy, S. L., Gough, K. H., and Ward, C. W. 1988c. The N and C termini of the coat proteins of potyviruses are surface-located and the N terminus contains the major virus-specific Epitopes. *Journal of General Virology* **69**, 1497-1508.
78. Shukla, D. D., Thomas, J. E., McKern, N. M., Tracy, S. L., and Ward, C. W. 1988d. Coat protein of potyviruses 4. Comparison of biological properties, serological relationships, and coat protein amino acids sequences of four strains of potato virus Y. *Archives of Virology* **102**, 207-219.
79. Shukla, D. D., McKern, N. M., Gough, K. H., Tracy, S. L., and Letho, S. G. 1988e. Differentiation of potyviruses and their strains by high performance liquid chromatographic peptide profiling of coat proteins. *Journal of General Virology* **69**, 493-502.
80. Shukla, D. D., and Teakle, D. S. 1989a. Johnsongrass mosaic virus. C.M.I/A.A.B. description of Plant Virus, December. **340**.
81. Shukla, D. D., Jilka, J., Tosic, M., and Ford, R E. 1989b. A novel approach to the serology of potyviruses involving affinity purified polyclonal antibodies directed towards virus-specific N-termini of coat protein. *Journal of General Virology* **70**, 13-23.
82. Shukla, D. D., and Ward, C. W. 1989c. Identification and classification of potyviruses on the basis of coat protein sequence data and serology. *Archives of Virology* **106**, 171-200.
83. Shukla, D. D., and Ward, C. W. 1989d. Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. *Advanced Virus Research* **36**, 273-313.
84. Shukla, D. D., Tosic, M., Jilka, J., Ford, R. E., Toler, R. W., and Langham, M. A. C. 1989e. Taxonomy of potyviruses infecting maize, sorghum, and sugarcane in Australia and the United States as determined by reactivities of polyclonal antibodies directed towards virus-specific N-termini of coat proteins. *Phytopathology* **79**, 223-229.
85. Shukla, D. D., Fenkel, M. J. and Ward C. W. 1991. Structure and function of the potyvirus genome with special reference to the coat protein coding region. Canadian *Journal of Plant Pathology* **13**, 178-191.
86. Shukla, D. D., Frenkel, M. J., McKern, N. M. Ward, C. W., Jilka, J., Tosic, M., and Ford, R. E. 1992a. Present status of the sugarcane mosaic subgroup of potyviruses. Potyvirus Taxonomy. New York, Springer-Verlag. *Archives of Virology*. [Suppl] **5**, 363-373.
87. Shukla, D. D., Lauricella, R., and Ward, C. W. 1992b.

Serology of potyviruses: current problems and some solutions. Potyvirus Taxonomy. New York, Springer-Verlag Wien. *Archives of Virology.* [Suppl] **5**, 57-59.

88. Shukla, D. D., and Ward, C. W. 1994a. Recent development in the identification and classification of viruses comprising the sugarcane mosaic subgroup of potyviruses. *Current Trends in Sugarcane Pathology.* K. S. B. F. e. al. Delhi, International Books and Periodicals Supply Service, 185-198.
89. Shukla, D. D., Ward, W. W., and Brunt, A. A. 1994b. The Potyviridae. Wallingford, Oxon, UK, CAB International.
90. Simmonds, J. H. 1966. Host Index of Plant Diseases in Queensland. Brisbane, Queensland Department of Primary Industries.
91. Singh, M. a. S., R. P. 1991. Propagation of plasmid containing an unstable insert of potato virus Y using STBL2™ competent cells. *Focus* **17**, 72-73.
92. Suranto, S., Gough, K. H., Shukla, D. D., and Pallaghy, C. K. 1998. Coat protein sequence of Krish-infecting strain of Johnsongrass mosaic potyvirus. *Archives of Virology* **143**, 1015-1020.
93. Taylor, R. H., and Pares, R. D. 1968. The relationship between sugra-cane mosaic virus and mosaic viruses of maize and Johnsongrass in Australia. *Australian Journal of Agricutural Research* **19**, 767-773.
95. Teakle, D. S., Moore, R. F., George, D. L. and Byth, D. E. 1970. Inheritance of the necrotic and mosaic reactions in sorghum infected with a "Johnsongrass" strain of sugarcane mosaic virus. *Aust. J. Agric. Res.* **21**, 549-556.
96. Teakle, D. S., and Pritchard, A. J. 1971. Resistance of Krish sorghum to four strains of sugarcane mosaic virus in queensland. *Plant Disease Report* **55**, 465-477.
97. Teakle, D. S., and Grylls, N. E. 1973. Four strains of sugar-cane mosaic virus infecting cereals and other grasses in Australia. *Australian Journal of Agricutural Research* **24**, 465-477.
98. Teakle, D. S. 1978. The cause and control of sorghum viral disease in Australia. Proceedings of the International Workshop on Sorghum Diseases. Hyderabad, India, 409-415.
99. Tosic, M., Ford, R. E., Shukla, D. D., and Jilka, J. 1990. Differentiation of sugarcane, maize dwarf, Johnsongrass, and sorghum mosaic viruses based on reactions of oat and some sorghum cultivars. *Plant Disease* **74**, 549-552.
100. Vaewhongs, A. A. a. L., S. A. 1995. Virion formation is required for the long-distance movement of red clover necrotic mosaic virus in movement protein transgenic plants. *Virology* **212**, 607-613.
101. Voinnet, O., Pinto, Y. M. and Baulcombe, D. C. 1999. Suppression of gene silencing: A general strategy used by diverse DNA and RNA viruses of plants. *Proc. Natl. Acad. Sci.* **96**, 14147-14152.
102. Voinnet, O., Lederer, C. and Baulcombe, D. C. 2000. A viral movement protein prevents spreads of the gene silencing signal in *Nicotiana benthamiana. Cell* **103**, 157-167.
103. Ward, C. W. a. S., D. D. 1991. Taxonomy of potyviruses: Current problems and some solutions. *Intervirology* **32**, 269-296.
104. Ward, C. W., McKern, N. M., Fenkel, M. J., and Shukla, D. D. 1992. Sequence data as the major criterion for potyvirus classification. Potyvirus Taxonomy. O. W. Barnett. New York, Springer-Verlag. *Archives of virology.* [Suppl] **5**, 283-297.
105. Wellink, J. a. K., A. V. 1989. Cell-to-cell transport of cowpea mosaic virus requires both the 58K/48K proteins and the capsid proteins. *Journal of General Virology* **70**, 2279-2286.
106. Williams, L. E., Findley, W. R., Dollinger, E. J., and Ritter, R. M. 1968. Seed transmission studies of maize dwarf mosaic virus in corn. *Plant Dis. Reptr.* **52**, 863-864.
107. Yu, M. H., Frenkel, M. J., McKern, N. M., Shukla, D. D., Strike, P. M. and Ward, C. W. 1989. Coat protein of potyviruses. Amino acid sequences suggest watermelon mosaic virus 2 and soybean mosaic virus-N are strains of the same potyvirus. *Archives of Virology* **105**, 55-64.
108. Zibert, A., Maass, G., Strebel, K., Falk, M. M. and Beck, E. 1990. Infectious foot-and-mouth disease virus derived from a cloned full-length cDNA. *Journal of Virology* **64**, 2467-2473.