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Kennedya Yellow Mosaic Virus: Another Tymovirus

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Abstract

A virus has been isolated from an Australian native legume, *Kennedya rubicunda*. The virus, which we call *Kennedya* yellow mosaic virus (KYMV), is sap-transmissible to a limited number of other plant species. Purified preparations of KYMV particles contain two types of rounded isometric particles 28 nm in diameter. Particles of one type are infectious nucleoprotein with a sedimentation coefficient of 110 S and particles of the other type are non-infections protein shells with a sedimentation coefficient of 54 S. Nucleoprotein particles of KYMV contain 34% single-stranded ribonucleic acid with a molar base content of guanine 15%, adenine 22%, cytosine 37%, and uracil 25%. The present cryptogram of KYMV is R/1 : */34 : S/S : S/*.

Introduction

Kennedya rubicunda (Schneev), commonly known as red Kennedy pea or dusky coral pea, is a member of a legume genus that is confined to Australia. Plants of this species growing on the foreshore of Murrays Beach, Jervis Bay, A.C.T., had a conspicuous yellow mosaic of their leaves. A virus, which we call *Kennedya* yellow mosaic virus (KYMV), was isolated from these plants (Dale *et al.* 1975). Some properties of this virus, which we have shown to be a tymovirus (Harrison *et al.* 1971), are reported in this paper.

Materials and Methods

Virus Culture and Assay

The original isolate of KYMV was used for all experiments, and this is called KYMV-JB. It was kept by serial passage in *K. rubicunda* and *Pisum sativum* L. cv. Greenfeast, and the latter was used as a source of virus particles for purification and as an assay plant; no local lesion host was found.

For host range studies, at least 20 plants of each species were manually inoculated, and sap from the inoculated and the tip leaves tested by manual inoculation to Greenfeast pea plants 5 weeks later. The properties of the virus in sap were determined using the methods of Bos *et al.* (1960).

Virus Purification

The method used to purify KYMV was similar to that used by Gibbs *et al.* (1966) for other tymoviruses. Infected Greenfeast pea plants were harvested 3 weeks after inoculation and homogenized in two volumes of 100 mM potassium phosphate buffer, pH 7·0, containing 50 mM ascorbic acid. The extract was filtered and one half volume each of chloroform and n-butanol was added and the mixture was triturated for 2 min to form an emulsion. The emulsion was centrifuged at 4000 *g* for 20 min and the virus-containing aqueous phase was collected. The virus particles were purified by two cycles of differential centrifugation (120 min at 100 000 *g* and 10 min at 8000 *g*), the pellet being resuspended each time in 30 mM phosphate buffer, pH 7·0. The final supernatant

was layered on a 10–40% sucrose density gradient in 30 mM phosphate buffer, pH 7·0, and centrifuged for 3 h at 82 000 *g*. The gradients were fractionated, and appropriate fractions pooled and dialysed overnight against 30 mM phosphate buffer, pH 7·0.

Physical Measurements

The ultraviolet absorbance of samples was measured in an Unicam SP 1800 Ultraviolet Spectrophotometer. Purified preparations were examined in a Phillips 300 electron microscope after mixing with 2% sodium phosphotungstate, pH 7·0. The sedimentation coefficients of the virus particles were determined using a Spinco Model E analytical ultracentrifuge equipped with Schlieren optics.

Chemical Analyses

Nucleic acid was extracted from the virus particles by incubating them in 1 M HCl at room temperature overnight. The precipitated protein was removed by centrifugation and the nucleic acid-containing supernatant was sealed in lyophilization ampoules and heated at 100°C for 1 h The hydrolysate was dried at room temperature and resuspended in an appropriate volume of 0·1 M HCl. The purines and the pyrimidine nucleotides were separated by ascending chromatography on thin layer cellulose plates (0·25 mm thick) using an isopropanol–HCl–water (70 : 7 : 23) solvent. Spots were removed with a razor blade, eluted in 1 ml 0·1 M HCl for 12 h, and centrifuged to remove the cellulose. Molar ratios were determined spectrophotometrically (Markham and Smith 1951).

The method of Laemmli (1970) for sodium dodecyl sulphate–polyacrylamide gel electrophoresis was used to estimate the number and sizes of proteins in purified virus preparations. Acrylamide separating gels, 12% with 2% stacking gels, were used. The protein standards used were bovine serum albumin (molecular weight 68 000), aldolase (molecular weight 40 000), trypsin (molecular weight 23 000) and myglobin (molecular weight 17 200). The composition of the coat protein was determined by acid hydrolysis and column chromatography in an automatic analyser; KYMV coat protein was precipitated from virus preparations with 1 M HCl, washed first with distilled water and then acetone and finally dried in a dessicator; the protein samples (each 1 mg) were hydrolysed for 24 h with constant-boiling HCl although under these conditions cysteine and tryptophan were destroyed.

Results

Host Range and Symptoms

K. rubicunda is the only species we have found naturally infected with KYMV. The virus has a limited experimental host range, and infected only seven species (in two families) of the 25 species (in six families) tested. The symptoms shown by naturally infected *K. rubicunda* vary throughout the year; leaves produced during late winter and the spring show a bright yellow mosaic (Fig. 1) and may be slightly distorted, but the mosaic becomes less obvious in the summer and leaves produced at that time may show no symptoms. Symptoms in *K. rubicunda* grown and inoculated in the glasshouse first appear about 14 days after inoculation as a vein clearing of the first trifoliate leaves. The vein clearing fades and subsequent growth usually remains symptomless for up to 4 weeks. Leaves produced later show a mild mosaic with diffuse chlorotic areas. These leaves are not distorted. *K. coccinea* plants grown and inoculated under the same glasshouse conditions show similar but more conspicuous symptoms than *K. rubicunda* plants.

In *P. sativum* cv. Greenfeast, a conspicuous vein yellowing of the third to seventh leaves developed about 15 days after inoculation. Leaves produced later showed some yellow mottling. Symptoms in *Glycine max* L. cv. Dare appeared 10 days after inoculation. Plants were stunted, with leaves epinastic and showing a bright yellow mottle. In *Phaseolus lathyroides*, KYMV induced a yellow mosaic and distorted leaves. KYMV was also recovered from the inoculated leaves of *Datura stramonium* L. and *Nicotiana glutinosa* L., and these were usually symptomless but sometimes

showed faint chlorotic lesions. KYMV was not recovered from the following inoculated species: Amaranthaceae: *Gomphrena globosa* L.; Chenopodiaceae: *Chenopodium amaranticolor* Coste and Reyn., *Chenopodium quinoa* Willd.; Cruciferae: *Brassica nigra* (L.) Koch; Cucurbitaceae: *Cucumis sativus* L.; Leguminoseae: *Centrosema pubescens* Benth., *Dolichos lablab* L., *Glycine wightii* Verdc. cv. Tinaroo, *Medicago sativa* L. cv. Hunter River, *Phaseolus vulgaris* L. cv. Redlands Greenleaf, *Trifolium incarnatum* L., *Trifolium repens* L., *Trifolium subterraneum* L. cv. Geralton, *Vicia faba* L. cv. Coles Dwarf; Solanaceae: *Lycopersicon esculentum* L. cv. Gros Lisse, *Nicotiana clevelandi* Gray.

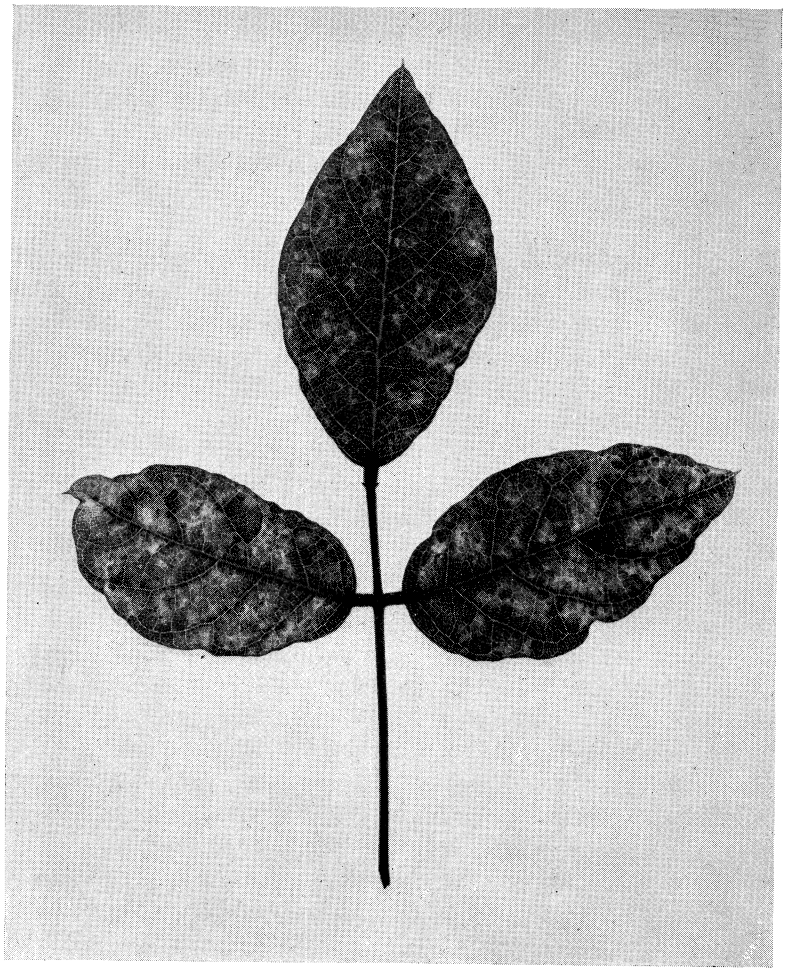

Fig. 1. Leaf of *Kennedya rubicunda* showing the symptoms caused by KYMV.

Properties in Sap

Sap from KYMV-infected plants (*P. sativum*) was still infective after heating for 10 min at 65°C but not after 10 min at 70°C, and it retained some infectivity when diluted 1 in 100 000. Sap stored at room temperature was still infective 10 days after extraction but not after 100 days.

Properties of Purified Preparations of KYMV Particles

When purified preparations were mixed with 2% sodium phosphotungstate, pH 7·0, and examined with an electron microscope before density gradient centrifugation, numerous rounded isometric particles were seen. The particles, 28 ± 2 nm in diameter, were indistinguishable from those of viruses in the tymovirus group, and were of two types: about one third had been penetrated by the stain and the remainder had not.

Unfractionated purified preparations had a u.v. absorption spectrum characteristic of a nucleoprotein with an absorption minimum at 242 nm and a maximum at 261 nm; the ratio A_{260}/A_{280} was usually around 1·63, depending on the proportion of protein shells and nucleoprotein in the preparation.

When purified preparations of KYMV were centrifuged in an analytical centrifuge, two sedimenting boundaries were observed. These two boundaries had sedimentation coefficients ($S_{20,w}$) of 54 S and 110 S. The sedimentation coefficients were independent of virus concentration (a four-fold range of concentrations was analysed). These sedimentation coefficients are very close to those of the major protein shells and nucleoprotein components of other tymoviruses (Bercks *et al.* 1971; Gibbs *et al.* 1966; Matthews 1970).

When KYMV preparations were fractionated by centrifuging in sucrose density gradients, two bands of u.v.-absorbing material were found. The band nearest the top of the gradient had the absorption spectrum of a protein: an absorption minimum at 250 nm, maximum at 275 nm and an A_{260}/A_{280} ratio of 0·81. This top band corresponded to the 54 S component observed in the analytical centrifuge. The lower band had the absorption spectrum of a nucleoprotein: an absorption minimum at 240 nm, maximum at 260 nm, and an A_{260}/A_{280} ratio of 1·69. Samples of the lower band were highly infectious, but those from the upper band were not. Samples of both bands reacted strongly in Ouchterlony gel-diffusion serological tests using an homologous antiserum to give single bands of precipitate that were confluent.

Composition of KYMV Particles

The averages of six estimates of the base composition of KYMV genome RNA extracted from the particles by acid were guanine 15·2%, adenine 21·9%, cytosine 37·4% and uracil 25·4%; it was clear from the R_f values and from the absorption spectra of the separated purines and pyrimidine nucleotides that the fourth base was uracil not thymine. Thus KYMV particles contain a single-stranded RNA with the high-cytosine and low-guanine content that is characteristic of the tymoviruses (Harrison *et al.* 1971).

The amount of RNA in the nucleoprotein particles, calculated from the sedimentation coefficients of the nucleoprotein particles and the protein shells (Reichmann 1965), was 34·1%.

Purified KYMV preparations analysed by polyacrylamide gel electrophoresis (Laemmli 1970) yielded only a single band which had a mean estimated molecular weight of 20 500 ± 300 (mean of 10 estimates from four different virus preparations).

The results of amino acid analyses of two protein samples from different preparations are shown in Table 1, which also lists amino acid analyses for some other tymoviruses. The proportions of different amino acids in KYMV protein are mostly within the ranges reported for other tymoviruses; KYMV coat protein contains no arginine whereas other tymovirus coat proteins contain a small amount of arginine,

also there is less lysine, phenylalanine and valine in KYMV coat protein than in those of other tymoviruses but more histidine and tyrosine.

The size of the KYMV coat protein was estimated from its composition using the FITMOL method (Gibbs and McIntyre 1970). The 'best estimate' obtained by this statistical method was 188 amino acid residues per subunit. This value, which excludes cysteine and tryptophan, is very close to the 189–191 residues reported for other tymoviruses (Symons *et al.* 1963; Harris and Hindley 1965). A subunit of 188 amino acids would have a molecular weight of 19 495, if we assume that KYMV coat protein has the same amounts of cysteine and tryptophan as other tymoviruses, and its molecular weight would be 19 820–20 387 which is close to the 20 500±300 estimated by electrophoresis in polyacrylamide gels.

Table 1. Amino acid composition of the coat proteins of KYMV and some other tymoviruses

Amino acid composition is expressed as mol/100 mol. *N* is the number of amino acid residues in the subunit (estimated total number 188). Name abbreviations: BMV, Belladonna mottle virus; EMV, eggplant mosaic virus; ScrMV, *Scrophularia* mottle virus; TYMV, turnip yellow mosaic virus (type strain); WCuMV, wild cucumber mosaic virus. Sources of information for other tymoviruses: Symons *et al.* (1963), Jankulowa *et al.* (1968), Bercks (1973) and Gibbs and Harrison (1973). n.d., Not determined

Amino acid	Virus							
	KYMV			BMV	EMV	ScrMV	TYMV	WCuMV
	Prep. 1	Prep. 2	*N*					
Ala	11·22	10·86	20	8·5	12·6	6·4	7·9	8·5
Arg	0	0	0	2·5	2·0	3·8	1·6	2·6
Asx	7·03	7·22	14	5·6	8·0	6·8	5·8	7·9
Cys	n.d.	n.d.	n.d.	1·1	0·5	n.d.	2·1	1·1
Glx	6·76	6·44	12	8·8	6·5	7·8	7·9	5·8
Gly	4·99	5·00	9	6·9	3·5	5·8	4·2	4·8
His	2·25	2·15	4	0	1·5	1·7	1·6	1·6
Ile	7·57	7·92	15	9·1	6·5	6·8	7·9	6·9
Leu	11·01	11·74	22	9·5	10·0	8·5	9·5	12·7
Lys	3·18	2·65	5	4·9	3·5	3·7	3·7	4·8
Met	0·71	1·00	2	0·9	1·5	2·2	2·1	0·5
Phe	2·36	2·18	4	2·6	3·1	2·5	2·6	4·2
Pro	9·08	8·99	17	8·3	9·6	10·8	10·6	9·5
Ser	11·59	12·04	23	12·5	10·0	13·3	8·5	13·8
Thr	13·23	12·57	24	8·4	10·0	10·2	13·8	6·9
Trp	n.d.	n.d.	n.d.	0·6	n.d.	0·8	1·1	0·5
Tyr	3·07	3·19	6	2·1	2·0	1·7	1·6	1·6
Val	5·96	6·07	11	7·7	9·0	7·7	7·4	6·3

Discussion

Some characters are most useful for grouping viruses, others are most useful for distinguishing between viruses (Gibbs 1969). If we compare the 'grouping characters' of KYMV with those of other viruses (Table 2) we find that it classifies with the tymoviruses, and furthermore is a member of the turnip yellow mosaic virus subgroup (Gibbs 1969) or C-subgroup (J. Dale, J. C. Craig and A. J. Gibbs, unpublished data) of the tymoviruses.

Related viruses are usually distinguished from one another by serological tests and by their host ranges and symptoms produced. KYMV is closely serologically related to, but distinct from, turnip yellow mosaic virus, okra mosaic virus and *Clitoria* yellow vein virus (R. Koenig, personal communication). A number of tymoviruses are known to infect members of the Leguminoseae. However, none have been reported to infect *Kennedya rubicunda* probably because the genus *Kennedya* is confined to Australia and Australian native legumes have received little attention.

Table 2. Comparison of some of the characters of KYMV and other tymoviruses

Character	KYMV	Range in tymoviruses
Virus particle		
Shape	Isometric	Isometric
Size (diameter)	28 nm	27–30 nm
Amount of nucleic acid	34·1%	34–38%
Composition of nucleic acid	G 15·2%	15·2–17·5%
	A 21·9%	17·0–22·8%
	C 37·4%	32·2–41·5%
	U 25·4%	21·0–29·4%
Size of protein subunit	20 500	18 500–21 900
Composition of protein	See Table 1	
Sedimentation coefficient	54 S + 110 S	49–56 S + 106–121 S
Type of accessory particle	Protein shell	Protein shell
Thermal inactivation point	65–70°C	60–94°C
Longevity *in vitro*	Between 10 and 100 days	Between 10 and 100 days
Dilution end point	10^{-4}–10^{-5}	10^{-2}–10^{-7}
Virus/Host		
Symptoms in host	Yellow mosaic	Usually yellow mosaic
	No local lesion	Rarely cause local lesions

Table 3. Comparison of hosts of legume-infecting tymoviruses

Name abbreviations: OkMV, okra mosaic virus; OYMV, *Ononis* yellow mosaic virus; DeYMV, *Desmodium* yellow mottle virus; PlMV, *Plantago* mottle virus

Host	Virus				
	OkMV	OYMV	DeYMV	PlMV	KYMV
Glycine max	—	+	—	+	+
Phaseolus vulgaris	—	—	+	—	—
Nicotiana glutinosa	—	—	—	?	+
Nicotiana clevelandii	+	+	?	+	—
Lycopersicon esculentum	—	+	?	—	—
Datura stramonium	—	+	—	—	+

The tymoviruses known to infect legumes are: *Desmodium* yellow mottle virus (Walters and Scott 1972), *Plantago* mottle virus (Granett 1973), okra mosaic virus (Givord and Hirth 1973), *Ononis* yellow mosaic virus (Gibbs *et al.* 1966), *Scrophularia* mottle virus (Hein 1959) and *Clitoria* yellow vein virus (K. Bock, personal communication). KYMV can be distinguished from these viruses, except *Clitoria* yellow vein virus, using a limited number of experimental hosts (Table 3). At present the only sure way to distinguish between KYMV and *Clitoria* yellow vein virus is by sero-

logical tests and by the electrophoretic mobility of their particles (R. Koenig, personal communication), though details of their host ranges are at present being studied.

Thus, to summarize, KYMV is a previously undescribed tymovirus found in a native Australian legume. It has, at present, the cryptogram R/1 : */34 : S/S : S/*.

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