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Variation for pathogenicity among isolates of bean common mosaic virus in Africa and a reinterpretation of the genetic relationship between cultivars of *Phaseolus vulgaris* and pathotypes of BCMV

N.J. SPENCE *and* D.G.A. WALKEY
Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

Bean common mosaic virus (BCMV) isolates were collected from crops of *Phaseolus vulgaris* (bean) and from wild legume species in 13 African countries. Isolates of pathotype VIa from both beans and wild legume species were predominant in central, eastern and southern Africa. Isolates of pathotypes I, III, IVa, IVb and Va were also found. Some isolates did not conform to previously published pathotypes, and therefore represent records of novel pathotypes. The susceptibility of various wild legume species to BCMV was investigated and isolates of the virus obtained from *Crotalaria incana*, *Rhynchosia* sp., *Macroptilium atropurpureum* and *Cassia occidentalis* (synonym *Senna occidentalis*) were aphid-transmitted both from *P. vulgaris* to their original host species and to *P. vulgaris*. Isolates of BCMV from wild legume species were seed-transmitted in bean and in several other legume species. The natural occurrence of BCMV in wild legume species in Africa is probably a significant factor in the ecology and epidemiology of the virus and possibly the evolution of isolates of the 'A' serotype which induce necrotic reactions in cultivars carrying the *I* gene for resistance. The occurrence of viruses other than BCMV from *P. vulgaris* and other legume hosts is also reported. The gene-for-gene model described by Drijfhout (1978) is reinterpreted to explain the variation for pathogenicity, and it is proposed that there may be genes which control the temperature sensitivity of necrosis in combination with the *I* gene.

INTRODUCTION

Bean common mosaic potyvirus (BCMV) has a worldwide distribution and is economically important throughout Africa, Asia, Europe and North, Central and South America. The virus can cause severe crop losses, and its severity is usually dependent on the incidence of aphid vectors (Morales & Bos, 1987). BCMV is the most important virus disease of *Phaseolus vulgaris* (beans) in Africa. However, other viruses have been isolated from this host species, including bean yellow mosaic potyvirus (BYMV), cucumber mosaic cucumovirus (CMV), peanut mottle potyvirus (PeMoV), cowpea mild mottle carlavirus (CMMV) and blackeye cowpea mosaic potyvirus (BlCMV) (Vetten & Allen, 1991).

Natural hosts of BCMV are mainly restricted to *Phaseolus* spp., especially *P. vulgaris* (Drijfhout, 1978). However, BCMV has been isolated from other leguminous hosts, including *Vigna unguiculata* (Zaumeyer & Thomas, 1957), *Vigna radiata* (Kaiser & Mossahebi, 1974), *Crotalaria juncea* (Singh & Singh, 1977), *Crotalaria striata* (Sarkar & Kulshreshtha, 1978), *Rhynchosia minima* (Meiners *et al.*, 1978) and *Lupinus luteus* (Frencel & Pospieszny, 1979).

BCMV causes two types of symptom in *P. vulgaris*: common mosaic (Fig. 1), which may severely reduce yield, and systemic necrosis ('black root') (Fig. 2), which can cause plant death. Black root develops as a consequence of a hypersensitive reaction to so-called necrotic strains of BCMV, but only in cultivars possessing the dominant *I* gene which confers resistance to mosaic-inducing strains (Drijfhout, 1978). Necrotic strains were first identified in Europe (Hubbeling, 1963; Drijfhout & Bos, 1977), and BCMV epidemics caused by necrotic strains have since been reported in the USA (Kelly *et al.*, 1984; Provvidenti *et al.*, 1984; Myers *et al.*, 1990). Recent surveys in Africa suggest that necrotic strains predominate over much of eastern and southern Africa, causing severe economic losses

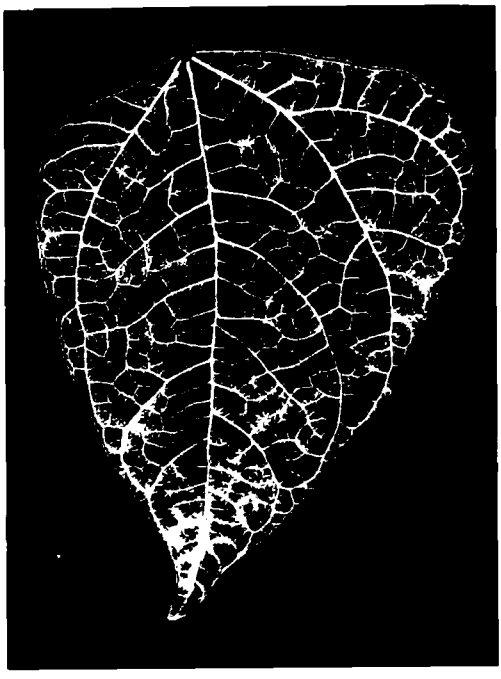

Fig. 1. Mosaic symptom in *Phaseolus vulgaris* caused by bean common mosaic virus.

Fig. 2. Necrosis symptom in a *Phaseolus vulgaris* cultivar carrying the *I* gene caused by bean common mosaic virus.

(Mink, 1985; Vetten & Allen, 1991; Spence & Walkey, 1994).

Several sources of resistance to BCMV are available, but it is essential for plant breeders to have knowledge of the distribution of and variation among BCMV isolates in the region where new cultivars are to be deployed. Isolates of BCMV are identified by their pathogenicity on a set of differential bean cultivars. To date, 10 pathogenicity groups of BCMV (referred to as pathotypes in this manuscript) have been identified on the basis of differential host responses (Drijfhout, 1978) (Table 1). Monoclonal antibodies differentiate BCMV isolates into two serotypes, and there is a correlation between pathotype and serotype where isolates belonging to pathotypes III, VIa and VIb also belong to the A serotype, while isolates of all other pathotypes belong to the B serotype (Wang *et al.* 1984).

The aim of the present study was to investigate the pathogenicity of BCMV isolates obtained from samples of infected beans and wild legume species in central, southern and eastern Africa. The identity of any other viruses isolated was also established. The data obtained were also used to reinterpret the gene-for-gene model described by Drijfhout (1978).

MATERIALS AND METHODS

Surveys

Field surveys were carried out in collaboration with personnel from Centro Internacional de Agricultura Tropical (CIAT) and National Bean Programmes in Lesotho, Malawi, Swaziland, Uganda and Zimbabwe (January 1990), Ethiopia (September 1990), Morocco (November 1990), Rwanda, Tanzania, Uganda and Zaire (May 1991), Burundi, Kenya and Rwanda (November 1991). In addition, samples were received at Wellesbourne directly from Africa.

Host plants, virus isolates, inoculations and maintenance of cultures

Seeds of test plants were germinated in plastic boxes on damp cellulose wadding by incubating them for 4 days at 25 °C. The germinated seeds were planted in M2 Levington compost (Fisons, UK) in 7-cm plastic pots. Plants were grown in an insect-proof glasshouse at 26 °C, from October

Table 1. Differentiation and grouping of bean common mosaic virus pathotypes (Drijfhout, 1978)

		Pathotype of the virus and representative strains									
		I NL1 US1	II NL7	III NL8	IVa US5 FLA NVRS	IVb NL6 US3 US4	Va US2 NY15	Vb NL2	VIa NL3	VIb NL5	VII NL4 US6
		Pathogenicity genes									
		P0	*P0* *P1*	*P0* *P2*	*P0* *P1* *P1*^{2}	*P0* *P1* *P1*^{2}	*P0* *P1* *P2*	*P0* *P1* *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*	*P0* *P1* *P1*^{2} *P2*^{2}
Differential hosts											
Group	Cultivar										
1	Double White The Prince Stringless Green Refugee (SGR) Common Red Mexican (CRM)	+[a]	+	+	+	+	+	+	+	+	+
2	Redlands Greenleaf C (RGC) Puregold Wax (PGW) Imuna	−[b]	+t[c]	−	+	+	+t	+	+t	+	+
3	Redlands Greenleaf B (RGB) GN UI 59 (GN59) GN UI 123 (GN123)	−	−	−	+	+	−	−	+t	+t	+
4	Michelite Sanilac Pinto III (P111) Red Mexican 34 (RM34)	−	−	+	−	−	+	+	+	+	−
5	Pinto 114 (P114)	−	−	−	−	−	+	+	+	+	−
6	Monroe Red Mexican 35 (RM35) GN UI 31 (GN31)	−	−	−	−	−	−	−	−	−	+
7	IVT 7214	−	−	−	−	−	−	−	−	−	−
8	Black Turtle Soup (BTS) Widusa	−	−	N[d]	−	n[e]	−	−	N	N	−
9a	Jubila	−	−	−	−	N	−	n	N	N	−
9b	Improved Tendergreen (ITG) Topcrop (TC)	−	−	−	−	n	−	n	N	N	−
10	Amanda	−	−	−	−	−	−	−	−	N	−
11	IVT 7233	−	−	−	−	−	−	−	−	−	−

[a] +, host group susceptible to systemic infection.
[b] −, host group resistant to systemic infection.
[c] +t, symptomless host but systemic infection detected by ELISA.
[d] N, systemic necrosis at 26°C and 32°C.
[e] n, systemic necrosis at 32°C, but not at 26°C.

to March supplementary lighting was provided to give a daylength of 16 h. Seeds of the genera *Chenopodium* and *Nicotiana* were sown directly in sifted peat compost and germinated seedlings were pricked out into individual pots.

Infected plant material was collected as leaves. If samples other than bean were collected, additional herbarium material was preserved in a flower press and photographs were taken to aid identification. For each sample, records were

Table 2. Standard viruses and virus strains used

Virus strain	Source	Propagation host	Country of origin
BCMV NL1	E. Drijfhout	*P. vulgaris*[a]	The Netherlands
BCMV NL3	E. Drijfhout	*P. vulgaris*	The Netherlands
BCMV NL4	E. Drijfhout	*P. vulgaris*	The Netherlands
BCMV NL5	H.J. Vetten	*P. vulgaris*	Germany
BCMV NL6	E. Drijfhout	*P. vulgaris*	The Netherlands
BCMV NL8	E. Drijfhout	*P. vulgaris*	The Netherlands
BCMV NVRS	D.G.A.Walkey	*P. vulgaris*	UK
BCMV NY15	H.J. Vetten	*P. vulgaris*	Germany
BlCMV NR	H.J. Vetten	*N. clevelandii*	Germany
CABMV	H.J. Vetten	*N. clevelandii*	Germany
PeMoV	H.J. Vetten	*N. clevelandii*	Germany
AMV	D.G.A.Walkey	*N. clevelandii*	Yemen

[a] cv. Double White.

taken of location, altitude, host species, cultivar (if known) and disease severity. Leaf samples were placed between two sheets of filter paper (Whatman No.1), lightly moistened with water and placed in a polythene bag. The samples were stored in cool bags in the field and later refrigerated at 4–5°C until they reached the laboratory (usually within 2–3 weeks). Additional similar leaf samples were immediately dried in plastic scintillation vials containing approximately 10 g of coarse $CaCl_3$.

Samples (fresh or dried) were homogenized in 1% K_2HPO_4 solution containing 0·1% Na_2SO_3 (1 g/ml) and used for sap transmission to appropriate test seedlings, including ***P. vulgaris*** cv. Double White (Dubbele Witte), ***Chenopodium quinoa***, ***Nicotiana benthamiana***, ***N. clevelandii***, *Vigna unguiculata* and *V. radiata*. Sap inoculum for sub-culturing was prepared in the same way. Some virus isolates were obtained from infected seedlings grown from seed samples collected in Africa.

BCMV strains representing pathotypes I (NL1), III (NL8), IVa (NVRS), IVb (NL6), Va (NY15), VIa (NL3), VIb (NL5) and VII (NL4) (Table 1) were maintained in bean cv. Double

Table 3. Antibodies used in ELISA

Name	Type	Source
BCMV NL1	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL3	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL4	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL5	Pab	H.J. Vetten, Germany
BCMV NL6	Pab	N.J. Spence, HRI Wellesbourne
BCMV NL8	Pab	N.J. Spence, HRI Wellesbourne
BCMV NY15	Pab	H.J. Vetten, Germany
BCMV NVRS	Pab	D.G.A. Walkey, HRI Wellesbourne
BlCMV-NR	Pab	H.J. Vetten, Germany
CABMV	Pab	H.J. Vetten, Germany
PeMoV	Pab	H.J. Vetten, Germany
bc-1-3	Mab	H.J. Vetten, Germany
bc-1-1A4	Mab	H.J. Vetten, Germany
12	Mab	G.I. Mink, Washington, USA
197	Mab	G.I. Mink, Washington, USA

Pab, polyclonal antiserum; **Mab**, monoclonal antibody.

Table 4. Number of isolates of bean common mosaic virus derived from *Phaseolus vulgaris* samples only from countries in Africa and assigned to particular pathotypes

Country	Number of samples collected	BCMV pathotypes												Approximate percentage of samples infected
		I	II	III	IVa	IVb	Va	Vb	VIa	VIb	VII	Novel	Total	
Burundi	58	1	0	8	0	2	0	0	10	0	0	5	26	45
Ethiopia	103	1	0	0	0	1	0	0	0	0	0	3	5	5
Kenya	69	0	0	0	0	0	1	0	7	0	0	7	15	22
Lesotho	31	0	0	0	0	0	0	0	2	0	0	1	3	10
Malawi	42	0	0	0	0	1	0	0	7	0	0	6	14	33
Rwanda	78	0	0	4	0	2	0	0	22	0	0	3	31	40
Swaziland	36	0	0	0	0	1	0	0	1	0	0	1	3	8
Tanzania	58	1	0	2	0	0	0	0	6	0	0	6	15	26
Uganda	43	1	0	0	0	2	0	0	11	0	0	0	14	32
Zaire	25	0	0	0	0	0	0	0	1	0	0	3	4	2
Zambia	14	0	0	0	0	1	0	0	4	0	0	1	6	43
Zimbabwe	52	0	0	0	1	2	0	0	8	0	0	2	13	25
Total	609	4	0	14	1	12	1	0	79	0	0	38	149	
Approximate percentage of each pathotype		3	0	9	1	8	1	0	53	0	0	25	100	

White and are referred to as 'standard' strains to be used for comparison with unidentified isolates. Cultures of BlCMV, cowpea aphid-borne mosaic potyvirus (CABMV), PeMoV and alfalfa mosaic virus (AMV) were also maintained for comparison (Table 2).

Differential bean cultivars

Original seed was obtained from E. Drijfhout (Agricultural Research University, Wageningen, The Netherlands) and multiplied at Wellesbourne in insect-proof polythene tunnels. Up to 22 cultivars were used to characterize BCMV isolates on the basis of systemic infection (Drijfhout, 1978). A minimum of two cultivars per host resistance group were used in each test, and four seedlings of each cultivar were inoculated as soon as the primary leaves unfolded. In addition, two further plants of cultivars in resistance groups 8, 9a, 9b and 10 (which possess the dominant *I* gene) were inoculated with each isolate and maintained in a controlled environment cabinet at 32°C in order to identify those isolates which induced systemic necrosis only at this higher temperature.

Each plant was scored for symptoms at intervals up to 4 weeks after inoculation, differential cultivars that developed questionable symptoms were back-inoculated to plants of cv. Double White to check for virus infection, or the suspect plants were tested for infection by ELISA. Potentially mixed infections were back-inoculated from individual cultivars to a new set of differential cultivars. Isolates were assigned to pathotypes according to the reaction patterns of the differential host cultivars. The reaction pattern on the differential host cultivars following inoculation with the standard BCMV strains was investigated in order to confirm the original descriptions by Drijfhout (1978).

Enzyme-linked immunosorbent assay (ELISA)

A number of monoclonal antibodies and polyclonal antisera (Table 3) were used in ELISA to aid the identification of the BCMV isolates and other viruses (Clark & Adams, 1977). Monoclonal antibodies bc-1-3 (BC3) and 197 were broad-spectrum, reacting in plate-trapped antigen ELISA (PTA-ELISA) with all BCMV strains and other potyviruses including BlCMV, CABMV and PeMoV. In contrast, monoclonal antibodies bc-1-1A4 (BC1) and I2 were highly specific to BCMV strains of the 'A' serotype only. Each survey sample was tested with these monoclonal antibodies by PTA-ELISA in duplicate wells. To aid identification, samples were also tested in direct double-antibody sandwich ELISA (DAS-ELISA) with a number of polyclonal

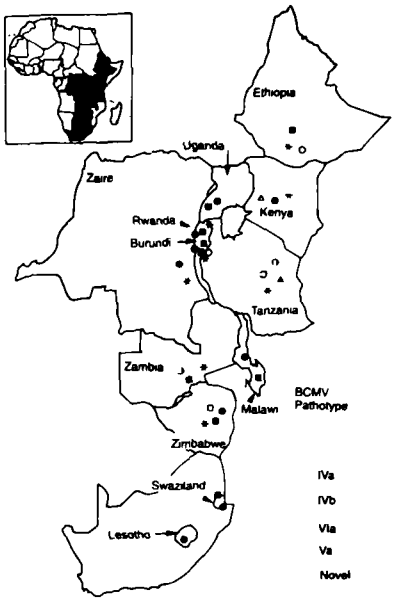

Fig. 3. Distribution of pathotypes of bean common mosiac virus obtained from *Phaseolus vulgaris* growing in Africa.

antisera raised to specific BCMV strains and other potyviruses.

Aphid transmission

Myzus persicae was used for aphid transmission experiments. Aphids were reared on healthy plants of *Brassica juncea* cv. Tendergreen Mustard and starved for 2 h before an access feeding period of *c.* 5 min on infected leaves. Ten or more aphids were then removed with a paintbrush, placed on healthy seedlings and killed 24 h later by spraying with heptenophos (Hostaquick).

Host range and seed transmission

Seeds of 29 wild legume species were tested for susceptibility to BCMV. Seeds were soaked in distilled water for 24 h before being nicked with a scalpel blade to aid germination; seedling production was as described for beans. Five seedlings of each species were sap-inoculated with the following standard BCMV strains: NL1, NL3, NL4, NL6 and NL8, and isolate 30 from *Crotalaria incana* L. Symptoms were recorded weekly for 4 weeks, at which time all seedlings were tested by ELISA for systemic infection.

Plants of the 29 wild legume species which had been inoculated with BCMV isolates were repotted into 18-cm pots 8 weeks after inoculation. Flowering was induced by growing the plants under short-day conditions (8-h photoperiod). Plants were either allowed to self-pollinate or were hand-pollinated with a paintbrush. The resultant seeds were harvested and subsequently germinated. Four weeks after sowing, virus symptoms were recorded and leaf samples from every plant were tested by ELISA for the presence of virus.

The seed transmission of virus isolates from wild legumes in *P. vulgaris* was tested by inoculating four plants of the bean cvs Double White and/or Sutter Pink with each isolate. At flowering, *c.* 3 weeks after sowing, the plants were repotted into 18-cm pots. Plants were allowed to self-pollinate and the resultant seeds were collected and subsequently tested for seed-transmitted virus by visually recording virus symptoms in germinated seedlings, and by testing for the presence of virus by ELISA.

RESULTS

BCMV isolates from *P. vulgaris*

In general, the reaction patterns observed following inoculation of the differential bean cultivars with the standard strains of BCMV were consistent with those described by Drijfhout (1978). The small differences that were observed can probably be attributed to environmental variation (particularly temperature).

BCMV was isolated from 149 of the 609 samples of *P. vulgaris* collected in Africa (Table 4). From the remaining samples either no virus was detected, or BCMV was detected by ELISA but was not isolated. Viruses other than BCMV were isolated from some samples or detected by ELISA, but not isolated (Spence & Walkey, 1994). The variation in the reaction patterns observed on differential cultivars following inoculation with the BCMV isolates from Africa was considerable, but the isolates have been assigned, wherever possible, to the pathotype to which their reaction patterns most closely conformed. In some cases, assignment to a pathotype was clear-cut, as reaction patterns were identical to those of the standard strain representative of the pathotype. In other cases there were small but distinct and consistent differences between the reaction phenotype observed and that of standard strains. This

Table 5. The reactions of differential host cultivars to isolates of bean common mosaic virus expressing novel pathogenicity phenotypes compared with NL3

Differential hosts		NL3	100	874	191	3515	3507	161	162
Group	Cultivar		S	B	Z	Za	Za	M	M
1	The Prince	+[a]	+	+	+	+	+	+	+
	SGR	+	nt[f]	nt	nt	+	+	nt	nt
	Double White	+	nt	+	nt	+	+	nt	nt
	Sutter Pink	+	nt	+	nt	nt	nt	nt	nt
	CRM	+	+	nt	+	+	+	+	+
2	PGW	+	−[b]	−	−	−	−	−	+
	RGC	+	+t[c]	−	−	−	−	+	+
3	RGB	+	−	−	−	−	−	+	+
4	Michelite	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	−	+	+
6	Monroe	−	−	nt	−	−	−	−	−
	RM 35	−	−	−	−	−	−	−	−
	GN 31	−	nt	−	nt	−	−	nt	nt
8	BTS	N[d]	N	N	N	N	n[e]	N	N
	Widusa	N	N	N	N	N	n	n	N
9a	Jubila	N		n	n	n	n	n	
9b	ITG	N	N	N	N	N	N	"	
	TC	N	n	N	N	N	N	−	n
10	Amanda	−	n	−	−	−	−	−	−
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
		P1	*P1*	*P1*	*P1*	*P1*		*P1*	*P1*
		$P1^2$						$P1^2$	$P1^2$
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*

[a]+, host susceptible to systemic infection.
[b]−, host resistant to systemic infection.
[c]+t, symptomless host but systemic infection detected by ELISA.
[d]N, systemic necrosis at 26°C and 32°C.
[e]n, systemic necrosis only at 32°C.
[f]nt, not tested.
Origin of isolates: B, Burundi; M, Malawi; S, Swaziland; Z, Zimbabwe; Za, Zaire.

variation included the failure of an isolate to infect one particular cultivar from a particular host resistance group even though other cultivars of the same group were infected. Isolates whose reaction phenotype deviated from that of the standard strain by variations such as this were still assigned to the pathotype they most closely resembled. Isolates whose reaction patterns did not conform to any previously described pathotype were considered to represent novel pathotypes.

Approximately 53% of BCMV isolates from *P. vulgaris* were assigned to pathotype VIa, the reaction phenotypes on differential bean cultivars being similar to NL3. Isolates of pathotype VIa occurred widely in all the areas surveyed except Ethiopia. Isolates assigned to pathotype III, similar to NL8, had a more limited distribution, being found only in Burundi, Rwanda and Tanzania. Isolates assigned to pathotype I, similar to NL1, were found occasionally in Burundi, Ethiopia, Tanzania

Table 6. The reactions of differential host cultivars to isolates of bean common mosaic virus expressing novel pathogenicity phenotypes compared with NL3 and NL8

Differential hosts																				
Group	Cultivar	NL3	NL8	820 R	550 Za	437 T	444 T	450 T	521 R	528 R	532 R	836 R	587 B	277 E	286 E	319 E	50 L	830 R	28 U	30 U	38 U
1	The Prince	+[a]	+	+	+	−[b]	+	−	+	+	−	+t[c]	+	+	+	+	+	+	−	−	−
	SGR	+	+	nt[f]	+	+	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
	D. White	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	nt	+	+	+	+
	Sutter Pink	+	+	+	nt	nt	nt	+	+	+	+	−	nt	+	+	+	nt	+	+	+	+
	CRM	+	+	nt	nt	+	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	+	nt	nt	+	+
2	PGW	+	−	−	−	+	+		+t	+t	−	−	+	+	−	−	−	−	−	−	−
	RGC	+	−	+t	−	−	+		−	−	−	−	+	+	−	−	−	−	−	−	−
3	RGB	+	−	+t	+	−	−	−	−	−	−	+t	+	−	+t	+t	−	−	−	−	−
4	Michelite	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+
5	Pinto 114	+	−	−	+	−	−	+	−	−	+	−	+	−	−	−	−	−	−	−	−
6	Monroe	−	−	nt	−	−	−	−	nt	nt	−	nt	nt	nt	nt	nt	−	nt	−	−	−
	RM 35	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	GN 31	−	nt	−	nt	nt	nt	nt	−	−	nt	−	nt	−	−	−	nt	−	−	−	−
8	BTS	N[c]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	n[d]	N	n	n	n
	Widusa	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		N	n	n	n
9a	Jubila	N	−	n	−	−	−	−	−	n	−	−	−	−	−	−	−	−	−	−	−
9b	ITG	N	−	N	−	−	−	−	−	−	−	−	−	−	−	n	−	n	−	−	−
	TC	N	−	N	−	−	−	−	−	−	−	−	−	−	−	−	−	N	−	−	−
10	Amanda	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
		P1		*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*		*P1*	*P1*							
		P1[2]		*P1*[2]	*P1*[2]							*P1*[2]	*P1*[2]	*P1*[2]	*P1*[2]						
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*					*P2*	*P2*	*P2*	*P2*

[a] +, host susceptible to systemic infection.
[b] −, host resistant to systemic infection.
[c] N, systemic necrosis at 26°C and 32°C.
[d] n, systemic necrosis only at 32°C.
[e] +t, symptomless host but virus detected by ELISA.
[f] nt, not tested.

Origin of isolates: B, Burundi; E, Ethiopia; L, Lesotho; R, Rwanda; T, Tanzania; U, Uganda; Za, Zaire. Isolates 820, 830 and 836 were isolated from *Cassia sophera*; isolates 28 and 30 from *Crotalaria incana*; isolate 38 from *Glycine max*; all other isolates were obtained from *P. vulgaris*.

Table 7. The reactions of differential host cultivars to isolates of bean common mosaic virus expressing novel pathogenicity phenotypes compared with NL6

Differential hosts		NL6	127	403	404	887	149	178	407	1008	179	151	853
Group	Cultivar		Z	T	T	B	M	M	T	Zm	M	M	B
1	The Prince	+[a]	+	+	+	+	+	+	+	+	+	+	+
	SGR	+	nt[f]	nt	+	nt	nt	nt	nt	nt	nt	nt	nt
	Double White	+	nt	+	+	+	nt	nt	+	+	nt	nt	+
	Sutter Pink	+	nt	+	nt	+	nt	nt	+	+	nt	nt	+
	CRM	+	+	nt	+	nt	+	+	nt	nt	+	+	nt
2	PGW	+	−[b]	−	−	−	−	−	+	+	−	−	−
	RGC	+	−	−	−	−	−	−	+	+	+t[e]	+	+
3	RGB	+	−	−	−	−	−	−	−	−	−	−	−
	GN 59	+	−	nt	−	nt	−	−	nt	nt	−	nt	nt
4	Michelite	−	−	−	−	−	−	−	−	−	−	−	−
	Sanilac	−	−	−	−	−	−	−	−	−	−	−	−
5	Pinto 114	−	−	−	−	−	−	−	−	−	−	−	+
6	Monroe	−	−	−	−	nt	−	−	−	nt	−	−	nt
	RM 35	−	−	−	−	−	−	−	−	−	−	−	−
	GN 31	nt	nt	nt	−	−	nt	nt	nt	−	nt	nt	−
8	BTS	n[d]	N[c]	n	n	n	n	n	N	n	N	N	n
	Widusa	n	N	n	n	n	n	n	N	n	N	n	n
9a	Jubila	n	n	−	n	−	n	n	−	−	−	n	n
9b	ITG	n	n	n	n	n	n	n	n	n	n	N	n
	TC	n	n	n	n	n	n	n	−	n	n	N	n
10	Amanda	−	−	−	−	−	−	−	−	−	−	−	−
Pathogenicity genes		*P0* *P1* *P1*[2]	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0* *P1*	*P0* *P1*	*P0* *P1*	*P0* *P1*	*P0* *P1* *P2*

[a] +, host susceptible to systemic infection.
[b] −, host resistant to systemic infection.
[c] N, systemic necrosis at 26°C and 32°C.
[d] n, systemic necrosis at 32°C but not at 26°C.
[e] +t, symptomless host but virus detected by ELISA.
[f] nt, not tested.
Origin of isolates: B, Burundi; M, Malawi; R, Rwanda; T, Tanzania; Z, Zimbabwe; Zm, Zambia.

and Uganda, and an isolate assigned to pathotype IVa, similar to US5, was found in only one sample from Zimbabwe. The distribution of isolates assigned to pathotype IVb, similar to NL6, was more widespread and they were frequently isolated from infected seeds. The single isolate in the survey which was assigned to pathotype Va, similar to NY15, was found in western Kenya, whilst all other isolates obtained in Kenya were assigned to pathotype VIa (Fig. 3). Isolates with apparently novel pathotypes were found infecting *P. vulgaris* in all of the countries surveyed except Uganda. The details of the pathogenicity phenotype of some of these isolates are given in Tables 5–8, together with an indication of the pathogenicity genes of the isolate.

In Ethiopia there was a much lower incidence of BCMV than in the other areas surveyed, and the absence of isolates of the 'A' serotype is significant. When infection by BCMV was found, the symptoms were generally mild. Only two

Table 8. The reactions of differential host cultivars to isolates of bean common mosaic virus expressing novel pathogenicity phenotypes compared with NY15

Differential hosts											
Group	Cultivar	NY15	961 K	963 K	964 K	967 K	970 K	959 K	953 K	973 K	847 B
1	The Prince	+[a]	+	+	+	+	+	+	+	+	+
	Double White	+	+	+	+	+	+	+	+	+	+
	Sutter Pink	+	+	+	+	+	+	+	+	−	+
2	PGW	+t[c]	+	−[b]	+	−	−	−	−	−	−
	RGC	+	+	+t	−	+t	−	−	−	−	−
3	RGB	−	+	+t	+	+t	+t	+	−	−	−
4	Michelite	+	+	+	+	+	+	+	+	+	+
	Sanilac	+	+	+	+	+	+	+	+	+	+
5	Pinto 114	+	+	+	+	+	+	+	+	+	−
6	Monroe	−	−	−	nt[d]	nt	nt	−	nt	nt	nt
	RM 35	−	−	−	−	−	−	−	−	−	−
	GN 31	nt	−	−	−	−	−	−	−	−	−
8	BTS	−	−	−	−	−	−	−	−	−	−
	Widusa	−	−	−	−	−	−	−	−	−	−
9a	Jubila	−	−	−	−	−	−	−	−	−	−
9b	ITG	−	−	−	−	−	−	−	−	−	−
	TC	−	−	−	−	−	−	−	−	−	−
10	Amanda	−	−	−	−	−	−	−	−	−	−
Pathogenicity genes		*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*	*P0*
		P1	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	*P1*	
			$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$	$P1^2$			
		P2	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*	*P2*

[a] +, host susceptible to systemic infection.
[b] −, host resistant to systemic infection.
[c] +t, symptomless host but systemic infection detected by ELISA, tolerant reaction.
[d] nt, not tested.
Origin of isolates: K, Kenya; B, Burundi. Isolate 963 was obtained from *Crotalaria comanestiana*; all other isolates were obtained from *P. vulgaris*.

isolates of BCMV were obtained which were both of the 'B' serotype, and these were assigned to pathotypes I and IVb. In addition, three isolates (277, 286 and 319) of an apparently novel pathotype were found at different locations in the Rift Valley; these were confirmed to be of the 'B' serotype of BCMV (Table 6).

In most countries there was little difference between the strains found at or near research stations and those found in farmers' fields. In Malawi, however, isolates assigned to pathotype VIa were only found near the Bunda College and Makapwa Research Stations, while isolates assigned to pathotype IVb were only found in farmers' fields. The recovery of a single isolate conforming to pathotype IVa from the Harare Research Station, Zimbabwe, may have originated from imported virus-infected seed, because no other isolates of this type were found in Zimbabwe.

The 'A' serotype of BCMV was detected by ELISA in several bean plants collected during a very limited survey in Morocco. However, BCMV was always present in a mixed infection with alfalfa mosaic virus (AMV), and as a result the reaction of differential bean cultivars to these isolates was not determined.

Full details of all BCMV isolations made during the survey have been documented by Spence & Walkey (1994).

Table 9. Bean common mosaic virus isolates obtained from wild legume species in Africa

Isolate number	Host species	Country of origin	BCMV serotype[a]	BCMV pathotype
28	*Crotalaria incana*	Uganda	A	Novel
30	*Crotalaria incana*	Uganda	A	Novel
38	*Glycine max*	Uganda	A	Novel
145	*Rhynchosia* sp.	Malawi	B	IVb
465	*Cassia hirsuta*	Uganda	B	I
499	*Macroptilium atropurpureum*	Uganda	A	VIa
531	*Vigna unguiculata*	Rwanda	A	VIa
820	*Cassia sophera*	Rwanda	A	Novel
830	*Cassia sophera*	Rwanda	A	Novel
836	*Cassia sophera*	Rwanda	A	Novel
956	*Vigna vexillata*	Kenya	B	Va
963	*Crotalaria comanestiana*	Kenya	B	Novel

[a] Serotype determined using monoclonal antibodies 197 and 12.

BCMV isolates from wild legume species

BCMV was isolated from several wild legume species in Kenya, Malawi, Rwanda and Uganda (Table 9). Isolates 28 and 30 from *Crotalaria incana* and isolate 38 from *Glycine max* obtained from Nakabango, Uganda were all of the 'A' serotype and produced a similar set of reaction patterns on differential host cultivars. Systemic veinal necrosis was observed in cultivars of host resistance groups 1 and 4, but this reaction was not the typical hypersensitive reaction associated with the action of the *I* gene. The phenotypic reaction pattern was not similar to any previously described BCMV pathotype, and the isolates were therefore considered to represent a novel pathotype (Table 5). Isolate 465 from *Cassia hirsuta* (synonym *Senna hirsuta*) obtained at Bukalasa, Uganda was of the 'B' serotype, and following inoculation of differential host cultivars the reaction pattern conformed to pathotype I. An isolate of the 'A' serotype (499) from *Macroptilium atropurpureum* obtained at Mubuku, Uganda conformed to pathotype VIa following inoculation of differential bean cultivars.

An isolate of the 'A' serotype (531) from *Vigna unguiculata* growing as a weed in a bean plot in Rwanda conformed to pathotype VIa, while three other isolates of the 'A' serotype (820, 830 and 836) from *Cassia sophera* (synonym *Senna sophera*) obtained from different sites in Rwanda displayed a novel set of reactions patterns on differential cultivars, and were considered to be previously undescribed pathotypes (Table 6).

Isolate 145 from a *Rhynchosia* sp. (not in flower) growing at Champhira, Malawi was of the 'B' serotype. This isolate conformed to pathotype IVb following inoculation of differential bean cultivars.

At Nyangusu, near Kisii, Kenya an isolate (956) of the 'B' serotype was obtained from *Vigna vexillata*, and the reaction pattern on differential host cultivars assigned it to pathotype Va. At Nyangena, *c.* 30 km from the Nyangusu site, an isolate (963) of the 'B' serotype obtained from *Crotalaria comanestiana* yielded a novel reaction pattern and was therefore considered to represent a novel pathotype (Table 8).

The survey indicated that herbaceous wild legume species were numerous in Burundi, Malawi, Rwanda, Uganda and the wetter parts of Ethiopia and Kenya. Fewer herbaceous legumes were observed in other areas, and no samples were collected from wild legumes in Lesotho, Swaziland, Zaire or Zimbabwe, as none were observed to have virus symptoms.

Full details of all BCMV isolations made from wild legumes in the different countries have been documented by Spence & Walkey (1994).

Isolation of viruses other than BCMV

BCMV was the virus most commonly isolated from *P. vulgaris* in Africa, but PeMoV and AMV were also found infecting *P. vulgaris*, and CMV was isolated from *P. lunatus* in Ethiopia. PeMoV was also isolated from wild legume species collected in bean-growing areas. Several isolates

Table 10. Summary of viruses other than bean common mosaic virus isolated from legume hosts in Africa

Isolate	Country	Location	Host species	Virus isolated
31	Uganda	Nakabango	*Arachis hypogea*	Peanut mottle potyvirus
225	Ethiopia	Jima	*Cassia sophera*	Peanut mottle potyvirus
228	Ethiopia	Jima	*Cassia sophera*	Peanut mottle potyvirus
242	Ethiopia	Bonga	*Cassia sophera*	Peanut mottle potyvirus
250	Ethiopia	Diri	*Phaseolus lunatus*	Cucumber mosaic cucumovirus
313	Ethiopia	Lake Shalla	*Phaseolus vulgaris*	Peanut mottle potyvirus
314	Ethiopia	Lake Shalla	*Phaseolus vulgaris*	Peanut mottle potyvirus
316	Ethiopia	Nazreth	*Cassia occidentalis*	Cassia severe mosaic potyvirus[a]
336	Ethiopia	Melkassa	*Cassia laburnifolium*	Unidentified potyvirus
338	Ethiopia	Melkassa	*Cassia laburnifolium*	Unidentified potyvirus (as 336)
344	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
347	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
348	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
366	Morocco	Beni Mellal	*Phaseolus vulgaris*	Alfalfa mosaic virus
428	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
429	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
430	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
434	Tanzania	Moshi	*Phaseolus vulgaris*	Unidentified potyvirus
435	Tanzania	Masasani	*Phaseolus vulgaris*	Unidentified potyvirus
522	Rwanda	Musambira	*Vigna* sp.	Unidentified isometric virus
945	Kenya	Meru	*Cassia* sp.	Peanut mottle potyvirus

[a] Tentative name.

of other potyviruses unrelated to BCMV or any other known potyvirus were obtained from *P. vulgaris* and various wild legume species during the survey. An isolate from *Cassia occidentalis* (synonym *Senna occidentalis*) obtained in Ethiopia may be a previously undescribed potyvirus, and has been tentatively named cassia severe mosaic virus (Walkey *et al.*, 1994) (Table 10).

Host range

A total of 29 legume species were tested for their susceptibility to standard strains of BCMV. Five species, namely *Cassia didymobotrya*, *Crotalaria laburnifolia*, *Desmodium heterocarpon*, *D. triflorum* and *Rhynchosia sublobata*, were apparently resistant to infection by all strains tested. A further five species, namely *Centrosema pubescens*, *Crotalaria anagyroides*, *C. lanceolata*, *C. ochroleuca* and *Rhynchosia minima*, were susceptible to infection by five of the BCMV strains tested (Table 11). Twenty-two species were apparently resistant to NL1, but many species were susceptible to the other four BCMV strains, particularly NL3. Only *Crotalaria anagyroides*, *C. incana* and *Vigna angularis* were susceptible to isolate 30, an isolate of a novel pathotype derived from *C. incana* growing in Uganda. In many cases infection was symptomless and only detected by ELISA.

Aphid transmission

The aphid transmission of isolates 28, 30 and 38 of a novel pathotype from *Crotalaria incana* (propagated in *Chenopodium quinoa*), isolate 145 (pathotype IVb) from a *Rhynchosia* species (propagated in *P. vulgaris*) and isolate 499 (pathotype VIa) from *Macroptilium atropurpureum* (propagated in *P. vulgaris*) was investigated, using short acquisition and inoculation access feeding times, to *Nicotiana clevelandii*, *Chenopodium quinoa*, *P. vulgaris* cvs The Prince and Double White, *Crotalaria incana*, *Cassia occidentalis* and *C. sophera* (Table 12).

Isolates 28, 30 and 38 were aphid-transmitted in a non-persistent manner to *Crotalaria incana*, *Cassia occidentalis*, bean cv. Double White and *C. quinoa*. Isolates 28 and 30 were non-persistently aphid-transmitted to *Cassia sophera*. Isolates 145 and 499 were only tested for aphid transmission to cv. Double White, and they were both non-persistently aphid-transmitted to this cultivar (Table 12).

Table 11. Reactions of wild legume species to inoculation with bean common mosaic virus strains and isolate 30

Host species	Seed source	NL1	NL3	NL4	NL6	NL8	30
Cassia didymobotrya	Ethiopia	0	0	nt	0	0	0
Cassia hirsuta	Uganda	mos	mos/E	E	mos/E	0	0
Cassia occidentalis	Ethiopia	0	mos/E	nt	0	mos/E	0
Cassia sophera	Ethiopia	0	0/E	nt	0/E	0/E	0
Cajanus cajan	Yemen	0	0/E	0	0/E	0	0
Centrosema plumieri	Colombia	0	0	0/E	0	0/E	0
Centrosema pubescens	Colombia	mos	mos	mos	mos	mos	0
Crotalaria anagyroides	Colombia	vc/E	vc/E	vc/E	vc	vc/E	vc
Crotalaria goreensis	Colombia	0	0	0/E	0	0	0
Crotalaria incana	Colombia	0	vc/E	0	cl	vc/E	vc/E
Crotalaria juncea	Colombia	0	0/E	0	0	0	0
Crotalaria laburnifolia	Colombia	0	0	nt	0	0	0
Crotalaria lanceolata	Colombia	0/E	vc/E	0/E	0/E	0/E	0
Crotalaria ochroleuca	Colombia	mos	mos/E	0/E	0/E	0/E	0
Crotalaria retusa	Colombia	0	0	0/E	0	0	0
Crotalaria verrucosa	Colombia	0	0/E	0	0/E	0	0
Desmodium heterocarpon	Colombia	0	0	0	0	0	0
Desmodium triflorum	Colombia	0	0	0	0	0	0
Glycine max	USA	0	cl/E	0/E	0	0/E	0
Glycine tomentella	Colombia	0	0/E	0	0	0 E	0
Indigofera hirsuta	Uganda	0	0/E	0/E	0/E	0/E	0
Macroptilium atropurpureum	Colombia	0	0	0	0	mos	0
Rhynchosia diversifolia	Colombia	0	0/E	0	0	0/E	0
Rhynchosia edulis	Colombia	0	0/E	0	0	0	0
Rhynchosia minima	Colombia	mos	n/E	n/E	n/E	n/E	0
Rhynchosia sublobata	Colombia	0	0	0	0	0	0
Vigna angularis	Yemen	mot	0/E	0/E	mot/E	0	mot/E
Vigna radiata	Yemen	0	mot	0	mot/E	0	0
Vigna unguiculata	Yemen	0	0/E	0	0/E	0/E	0

0, no symptoms; mos, systemic mosaic; vc, systemic vein clearing; cl, systemic chlorosis; mot, systemic mottle; n, systemic necrosis; /E, virus detected by ELISA using the monoclonal antibody 197; nt, not tested.

Seed transmission

Of the 29 legume species inoculated with the standard BCMV strains (Table 11), only seven produced seed. Some plants failed to flower, and others flowered but either fruits were not produced or they contained no seed. Of the plants which did produce seed, only a low percentage germinated in some cases to produce relatively few seedlings. If many seedlings were produced, fifteen were tested by ELISA for seed-transmitted virus; for species where fewer seedlings were produced all of them were tested for the presence of virus. Plants of *Crotalaria ochroleuca*, *Cassia occidentalis* and *C. sophera* that produced seed were those inoculated with NL3 and NL8; in each case a proportion of the resulting seedlings was infected (Table 13). All infected plants of *Crotalaria incana* produced seed, and a proportion of the resulting seedlings from plants infected with NL1, NL3, NL4, NL6, NL8 and isolate 30 was infected (Table 13).

All the BCMV strains tested were seed-transmitted to a proportion of seedlings of *Macroptilium atropurpureum*, although during host-range studies only NL8 was found to infect *M. atropurpureum* as demonstrated by testing with ELISA (Table 11). This could be explained by the concentration of virus in the parent plants being too low to be detected by ELISA.

In some cases, symptoms in bean cvs. Double White and Sutter Pink were so severe that no seed was produced. Alternatively, seed was produced by one cultivar but not the other (Table 14). There was no seed production by either cultivar infected with isolates 28, 30, 38, 499 or 963. In contrast, both cultivars produced seed when infected with isolates 820 and 830. In the case of the latter isolates, seed from cv. Sutter Pink was not infected but a proportion of the

Table 12. Aphid transmission of virus isolates from wild legume species to various host species

Host species	Isolate				
	28	30	38	145	499
Cassia occidentalis	2/2 m	2/2m	1/2m	nt	nt
Cassia sophera	1/2v	1/2v	0/2	nt	nt
Chenopodium quinoa	2/2c	2/2c	2/2c	nt	nt
Crotalaria incana	2/2v	2/2v	2/2v	nt	nt
Nicotiana clevelandii	0/2	0/2	0/2	nt	nt
P. vulgaris cv. The Prince	0/2	0/2	0/2	nt	nt
P. vulgaris cv. Double White	2/2n	2/2n	2/2n	1/4m	2/4

Nominator = number of seedlings developing symptoms; denominator = total number of seedlings tested.
m, mosaic; v, vein clearing; c, chlorosis; n, necrosis; nt, not tested.

seed from cv. Double White was infected. Virus was not transmitted in seed from plants of cv. Double White infected with isolates 465 or 956. Isolate 145 was seed-transmitted in cv. Sutter Pink, but no seed was produced by plants of cv. Double White infected with this isolate.

DISCUSSION

Geographical distribution of BCMV

BCMV isolates were assigned to pathotypes according to their pathogenicity phenotype on differential host cultivars, and could readily be classified as belonging to either the 'A' or 'B' serotype by ELISA. Pathotype and serotype were strongly correlated. The results clearly demonstrated the widespread occurrence and predominance of the 'A' serotype in central, eastern and southern Africa; this serotype was completely correlated with the occurrence of a temperature-independent necrotic response by cultivars carrying the *I* gene.

The widespread occurrence of isolates similar to NL3 and NL8, and of other isolates of the 'A' serotype with novel pathogenicity phenotypes, emphasizes the importance of incorporating resistance to these so-called necrotic strains into new cultivars. Many cultivars that have been introduced into bean improvement programmes in Africa carry only the dominant *I* resistance gene and are therefore susceptible to 'black root' when infected by any isolate that provokes necrosis in the presence of this gene. In areas where necrosis-inducing isolates exist, it is essential to incorporate additional resistance genes such as $bc2^2$ or *bc*3 into breeding programmes. Exceptionally, necrosis-inducing isolates of the 'A' serotype were not located in Ethiopia. However, three isolates of a novel pathotype were found that did induce necrosis in host group 8 cultivars even though they were of the 'B' serotype. In Ethiopia, cultivars carrying the *I* gene alone may be sufficient to confer resistance against BCMV in most areas, but plant breeders need to be aware of the existence of this novel pathotype, which could influence the durability of any newly introduced resistant bean cultivars.

The occurrence of non-necrosis-inducing isolates of the 'B' serotype in both beans and wild legume species, over a relatively wide geographical area around Kisii and Kakamega in western Kenya, is of interest. One isolate expressed a phenotypic reaction pattern identical to pathotype Va, while others expressed novel phenotypes. NY15 is the type strain of pathotype Va which until now had only been recorded in North America.

The widespread occurrence and predominance of pathotypes which induce necrosis in cultivars carrying the *I* gene and which are of the 'A' serotype raises questions about the geographical origin of these pathotypes. NL3 and NL5 were isolated from cultivars carrying the *I* gene in bean variety trials conducted by a commercial company which at that time was not multiplying bean seed in Africa (Hubbeling, 1963, 1972). However, as cultivars carrying the *I* gene do not transmit virus in their seed (Morales, 1989), they were probably not the source of the virus. It is more likely that the virus was aphid-transmitted from

Table 13. Seed transmission of standard bean common mosaic virus strains and isolate 30 in wild legume species

Host species	Seed source	NL1	NL3	NL4	NL6	NL8	30
Cassia occidentalis	Ethiopia	nt	2/5	nt	nt	2/5	nt
Cassia sophera	Ethiopia	nt	3/5	nt	nt	2/5	nt
Crotalaria incana	Colombia	4/15	2/15	1/15	1/15	3/15	5/15
Crotalaria lanceolata	Colombia	0/14	0/15	nt	0/6	0/6	nt
Crotalaria ochroleuca	Colombia	nt	2/5	nt	nt	2/5	nt
Macroptilium atropurpureum	Colombia	3/15	4/15	9/15	1/7	10/15	nt
Rhynchosia diversifolia	Colombia	5/5	2/2	2/2	nt	4/5	nt

Nominator = number of seedlings developing symptoms; denominator = total number of seedlings tested.
Isolate 30 was obtained from *Crotalaria incana* (Uganda).
nt, not tested as no seed produced.

different cultivars included in the trial from other seed companies. These other companies were known at that time to be multiplying seed in Africa likely to have been included in these trials (L. Bos, personal communication, 1993). NL8 was isolated from the progeny of a breeding experiment in a private breeder's field in Holland (Drijfhout & Bos, 1977). This strain may also have been introduced from Africa via the same route as NL3 and NL5, but there is no further evidence to confirm or discount this possibility.

The geographical origin of isolates has particular significance in view of the recent proposal that the so-called necrotic strains are sufficiently distinct from other forms of BCMV to be regarded as a separate virus designated bean necrosis mosaic virus (BNMV) (McKern *et al.*, 1992a, 1992b; Vetten *et al.*, 1992). Necrotic strains are not thought to occur naturally in South America, the centre of origin of *P. vulgaris*, and when they have been recorded there, or in North America, their occurrence can usually be traced to infected imported seed (F. Morales, personal communication, 1990). The results of this study support the contention that the two serotypes are distinct and may have separate origins. The prevalence of isolates of the 'A' serotype in central and eastern Africa indicates that they may have evolved in this region, whereas the 'B' serotype of BCMV probably has its origin, along with *P. vulgaris* beans, in Central or South America.

The occurrence of the 'A' serotype in wild legume species in Africa, and the observed tolerance (symptomless infection) of some of these species, is a further indication that their geographical origin may be central and southern Africa. The phenotypic and therefore genotypic diversity of pathotypes from this region suggests that the host population imposes a strong selective force for pathotypic variation. Potyviruses are known to produce new variants by recombination among heterogeneous RNA populations within infected plants (Goldbach, 1992; Lecoq & Purcifull, 1992). In this context, the recovery of isolates expressing a previously unrecorded pathogenicity phenotype is of interest. The majority of the isolates expressing novel phenotypes induced a temperature-independent necrotic reaction in cultivars carrying the *I* gene, and were of the 'A' serotype. The pathogenicity phenotypes of some of these isolates are summarized in Table 6, and compared with those of NL3 and NL8.

A range of wild legume species that commonly occur in Africa, notably of the genera *Cassia*, *Crotalaria*, *Macroptilium*, *Rhynchosia* and *Vigna*, were susceptible to infection by a number of standard strains of BCMV. Some species were particularly susceptible to NL3, which was prevalent in the areas where these genera occur. Infection was often symptomless and only detected by ELISA. Such latent infections imply genetic adaptation by the host as a result of host and pathogen coexistence over a long period of time. This suggests that some pathotypes of BCMV may have originated in this region of the world.

Several BCMV isolates from wild legume species were aphid- and seed-transmitted to other legume species and *P. vulgaris*, and the standard BCMV strains were also seed-transmitted in wild legume species. It is therefore possible that in bean-growing areas in Africa, BCMV could be transmitted from cultivated or wild legume species to beans, or from beans to the other legume species. Infected wild legume species could provide a source of

Table 14. Seed transmission of bean common mosaic virus isolates from wild legume species in *P. vulgaris* cvs Double White and Sutter Pink

	Double White		Sutter Pink	
Isolate	Mosaic[a]	ELISA[b]	Mosaic	ELISA
145	nt[c]	nt	4/8	4/8
465	0/7	0/7	nt	nt
820	3/11	3/11	0/15	0/15
830	1/10	1/10	0/10	0/10
956	0/13	0/13	nt	nt

Nominator = number of infected seedlings; denominator = number of seedlings tested.
[a] Seedlings with mosaic symptoms.
[b] Seedlings which were positive for BCMV in ELISA.
[c] nt, not tested as no seed was produced.

inoculum to infect bean crops, which are grown twice a year in some areas. They might also act as alternative hosts, facilitating the evolution of pathogenic variants of BCMV.

A number of isolates expressing novel pathogenicity phenotypes were also found to induce temperature-dependent necrosis in the differential host cultivars carrying the *I* gene; the pathogenicity phenotypes of these isolates are compared with those of NL6 in Table 7. Fewer isolates with novel pathogenicity phenotypes were of the 'B' serotype and did not induce necrosis, and all but one of these isolates were found in western Kenya. The reactions of differential hosts to these isolates are compared with those of NY15 in Table 8.

The gene-for-gene relationship described by Drijfhout (1978) to explain the genetic relationship between BCMV and differential host cultivars of *P. vulgaris* (Table 15) goes some of the way towards providing a genetic interpretation of the novel pathogenicity phenotypes observed in this study. It does not, however, provide an explanation for the full range of pathogenic variation observed among isolates of the 'A' serotype. Drijfhout (1978) presented the total number (64) of virus genotypes that were theoretically possible based on combinations of pathogenicity genes *P*1, $P1^2$, *P*2 and $P2^2$ (Table 15). It was assumed that all pathogenicity genes occurred at different loci in the virus genome and could therefore, be expressed by a single virus. It should be noted that no isolate carrying the putative pathogenicity gene *P*3 to match host gene *bc*3 has been found to date. At the time of Drijfhout (1978), only seven of the 64 possible theoretical genotypes had been identified, and only three strains (NL3, NL5 and NL8) expressing temperature-independent necrosis on *I* gene cultivars were known.

The strains Drijfhout assigned to pathotype IV were considered to carry identical pathogenicity genes (Drijfhout, 1978), but the pathotype was subdivided into those strains that do not induce necrosis in *I* gene cultivars (IVa) and strains that do (IVb). Pathotypes V and VI were similarly divided. The gene-for-gene model described by Drijfhout does not provide an explanation for these differential reactions on *I* gene cultivars. It is proposed that there may be a virus gene responsible, in combination with the *I* gene, for inducing temperature-independent necrosis (N), designated *Px*, and a gene responsible for inducing temperature-dependent necrosis (n), designated Px^2.

The theoretical *Px* and Px^2 pathogenicity genes are included in the proposed genotypes of the temperature-independent and temperature-dependent necrosis-inducing BCMV strains: NL8, NL6/US5, NL2/US2 and NL3/NL5 (Table 16). If *Px* and Px^2 are not allelic, the 64 theoretical combinations of *P*1, $P1^2$, *P*2 and $P2^2$ are expanded to result in 256 possible virus genotypes. If this hypothesis is correct, the BCMV isolates expressing novel pathogenicity phenotypes could be carrying the combinations of *P*1, $P1^2$ *P*2, $P2^2$, *Px* or Px^2 pathogenicity genes listed in Table 16. It should be noted that some isolates induce either a temperature-independent necrotic reaction or a temperature-dependent necrotic reaction in all host cultivars of groups 8 and 9. This indicates that the isolates

Table 15. Observed and theoretical genotypes for resistance (host) and pathogenicity (virus) for the strain-specific resistance genes to bean common mosaic virus

Theoretical differential host genotypes				Observed differential cultivars	Theoretical virus pathotypes															Theoretical number of infecting genotypes	Observed number of infecting genotypes
					V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	
					*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	*P*0	
						*P*1				*P*1	*P*1	*P*1				*P*1	*P*1	*P*1		*P*1	
							$P1^2$			$P1^2$			$P1^2$	$P1^2$		$P1^2$	$P1^2$		$P1^2$	$P1^2$	
								*P*2			*P*2		*P*2		*P*2	*P*2		*P*2	*P*2	*P*2	
									$P2^2$			$P2^2$		$P2^2$	$P2^2$		$P2^2$	$P2^2$	$P2^2$	$P2^2$	
					Observed virus pathotypes and representative strains																
					I NL1	II NL7	III NL8		IV NL6 US5	V NL2 US2 NY15						VI NL3 NL5	VII NL4				
H1				Double White	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16	7
H2	*bc*-1			RGC	–	+	–	–	–	+	+	+	–	–	–	+	+	+	–	+	8	5
H3	*bc*-1^2			RGB	–	–	+	–	–	+	–	–	+	+	–	+	+	–	+	+	8	3
H4		*bc*-2		Michelite	–	–	–	+	–	–	+	–	+	–	+	+	–	+	+	+	8	3
H5		*bc*-2^2			–	–	–	–	+	–	–	+	–	+	+	–	+	+	+	+	8	1
H6			*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H7	*bc*-1	*bc*-2		Pinto 114	–	–	–	–	–	–	+	–	–	–	–	+	–	+	–	+	4	2
H8	*bc*-1	*bc*-2^2			–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	+	4	1
H9	*bc*-1		*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H10	*bc*-1^2	*bc*-2		'X'	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	+	4	1
H11	*bc*-1^2	*bc*-2^2		Monroe	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	+	4	1
H12	*bc*-1^2		*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H13		*bc*-2	*bc*-3	IVT7214	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H14		*bc*-2^2	*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H15	*bc*-1	*bc*-2	*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H16	*bc*-1	*bc*-2^2	*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H17	*bc*-1^2	*bc*-2	*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0
H18	*bc*-1^2	*bc*-2^2	*bc*-3		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0

Note that it is assumed that *bc*-1 and *bc*-1^2, and *bc*-2 and *bc*-2^2 are allelic. Table modified from Drijfhout (1978).

Table 16. Observed and theoretical genotypes for resistance (host) and pathogenicity (virus), in which strain-specific resistance genes are in combination with the dominant *I* gene. The observed or expected positive reactions result in systemic necrosis at 30°C; the proposed *Px* and Px^2 genes have been added for the genotypes observed prior to the present survey

	Theoretical differential host genotypes			Observed differential cultivars	Theoretical virus pathotypes																Observed virus pathotypes and representative strains							Theoretical number of infecting genotypes	Observed number of infecting genotypes
					V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	I NL1	II NL7	III NL8	IV NL6 US5	V NL2 US2 NY15			VI NL3 NL5	VII NL4		
8	*I*			Widusa	*P0*	*P0* *P1*	*P0* $P1^2$	*P0* *P2* *Px*	*P0* $P2^2$	*P0* *P1* $P1^2$ Px^2	*P0* *P1* *P2* Px^2	*P0* *P1* $P2^2$	*P0* $P1^2$ *P2*	*P0* $P1^2$ $P2^2$	*P0* *P2* $P2^2$	*P0* *P1* $P1^2$ *P2* *Px*	*P0* *P1* $P1^2$ *P2* $P2^2$	*P0* *P1* *P2* $P2^2$	*P0* *P1* $P1^2$ *P2* $P2^2$	*P0* *P1* $P1^2$ *P2* $P2^2$	–	–	+	+	+	+	+	+	+	+	+	+	+	+	13	4
9	*I*	*bc*-1		Jubila																	–	–	–	–	–	+	+	+	–	+	–	+	6	3
10	*I*	*bc*-1^2		Amanda																	–	–	+	–	–	+	–	–	+	+	+	+	7	2
	I		*bc*-2																		–	–	–	+	–	–	+	–	+	+	+	+	8	3
11	*I*		*bc*-2^2																		–	–	–	–	+	–	–	+	–	+	+	+	7	0
	I			*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I	*bc*-1	*bc*-2																		–	–	–	–	–	+	–	–	–	+	–	+	4	2
	I	*bc*-1	*bc*-2^2																		–	–	–	–	–	–	+	–	–	+	–	+	3	0
	I	*bc*-1		*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I	*bc*-1^2	*bc*-2																		–	–	–	–	–	+	–	+	–	–	+	+	4	1
	I	*bc*-1^2	*bc*-2^2	IVT 7233																	–	–	–	–	–	+	–	+	–	–	+	+	3	0
	I	*bc*-1^2		*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I		*bc*-2	*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I		*bc*-2^2	*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I	*bc*-1	*bc*-2	*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I	*bc*-1	*bc*-2^2	*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I	*bc*-1^2	*bc*-2	*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0
	I	*bc*-1^2	*bc*-2^2	*bc*-3																	–	–	–	–	–	–	–	–	–	–	–	–	0	0

Note that it is assumed that *bc*-1 and *bc*-1^2, and *bc*-2 and *bc*-2^2 are allelic. Table modified from Drijfhout (1978).

Table 17. Theoretical pathotypes of novel isolates of bean common mosaic virus isolated from *Phaseolus vulgaris* and other legume hosts in different countries in Africa

	Number of isolates assigned to each theoretical pathotype of BCMV							
Country	V1[a]	V2	V3	V4	V7	V9	V12	Total
Burundi	1	0	0	1	2	0	1	5
Ethiopia	0	1	2	0	0	0	0	3
Kenya	0	0	0	0	2	0	6	8
Lesotho	1	0	0	0	0	0	0	1
Malawi	2	2	0	0	0	0	2	6
Rwanda	0	0	0	1	3	1	1	6
Swaziland	0	0	0	0	1	0	0	1
Tanzania	2	1	0	0	3	0	0	6
Uganda	0	0	0	3	0	0	0	3
Zaire	0	0	0	1	1	0	1	3
Zambia	0	1	0	0	0	0	0	1
Zimbabwe	1	0	0	0	1	0	0	2
Total	7	5	2	6	13	1	11	45

[a] See Tables 15 and 16 for theoretical virus pathotypes.

carry either *Px* or Px^2 In contrast, other novel isolates appear to induce a temperature-independent necrotic reaction in host group 8 cultivars and a temperature-dependent necrotic reaction in host group 9 cultivars (see isolates 127 and 179 in Table 7). It is proposed that these isolates carry both *Px* and Px^2 The pathogenicity genotypes suggested by the phenotype of novel isolates found in this study can be matched to some of the theoretical genotypes described in Tables 15 and 16 (Table 17).

The results of this survey do not provide proof of the existence of pathogenicity genes *Px* and Px^2 Further genetic studies are required to determine the genetic basis of differential necrotic host reactions. The response of cv. Jubila (group 9a) was frequently inconsistent when it was inoculated with isolates that induce a temperature-independent (N) necrosis in all other group 8 and 9 cultivars (Tables 5–7). This cultivar often responded by developing necrotic symptoms following inoculation with these isolates only at 32°C and not at 26°C. It is possible, therefore, that there may be an additional gene or genes in this cultivar that modifies the development of the necrotic symptoms, or that local fluctuations in temperature may influence gene expression.

All the isolates characterized during this study have been stored in liquid nitrogen and in freeze-dried material at HRI, Wellesbourne; they are available to other workers on request. Many isolates have already been returned to their country of origin for use during selection for resistance in national breeding programmes. Because of the considerable variation that exists between local isolates and standard BCMV strains, the use of local isolates is considered more appropriate for screening local germplasm.

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