Bean Common Mosaic Virus Strains Associated with Bean Mosaic Epidemics in the Northwestern United States

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ABSTRACT

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Several bean (*Phaseolus vulgaris*) cultivars considered to be resistant to indigenous strains of bean common mosaic virus (BCMV) were attacked in varying degrees during a bean common mosaic epidemic throughout the northwestern United States in 1977. These cultivars included Black Turtle, Columbia Pinto, Great Northern UI 31, Great Northern UI 1140, Montcalm Red Kidney, Pinto UI 114, Red Mexican NW 59, Red Mexican NW 63, Rufus, and Viva. A milder epidemic, which was primarily restricted to Pinto UI 114, recurred in 1981. Using bean cultivars standard for differentiating BCMV strains, we identified the strains associated with these epidemics as members of strain groups II, III, and IV; one strain approximated strain group VII (~VII). Two of these strain groups, III and ~VII, were previously unknown in the United States. Strain group I (type strain) was isolated only from bean cultivars lacking any BCMV resistance. Only strain group V (NY-15 strain) was detected in Pinto UI 114 in the 1981 epidemic. Greenhouse isolates of each strain tended to be somewhat less virulent than the field cultures from which they originated. At least part of this difference appeared to result from a reduction in innate infective capacity of the virus during cultural subtransfers. Three BCMV isolates were readily seed-transmitted in the most susceptible cultivars, but cultivar susceptibility to infection did not predetermine susceptibility to seed transmission of BCMV.

Bean common mosaic virus (BCMV), the principal seedborne virus attacking bean (*Phaseolus vulgaris* L.) crops, occurs worldwide (7). During 1977, a major bean common mosaic (BCM) epidemic occurred in the northwestern United States as well as in western Europe. Another milder epidemic recurred in the United States in 1981. The spectrum of bean cultivars being attacked in the 1977 epidemic suggested the presence of unusual strains of BCMV.

The definitive work of Drijfhout et al (1–3) in 1977–1978 facilitated the identification of BCMV strains causing such epidemics. Bean cultivars selected as hosts for differentiating BCMV strains were used both to examine the virulence of field cultures and the strain identities of derived BCMV isolates.

MATERIALS AND METHODS

Tissue samples were collected in 1977 and 1981 in Washington, Idaho, and Montana from diverse field-infected bean cultivars showing typical systemic mosaic symptoms. To facilitate isolation of unusual BCMV strains, we selected most samples from cultivars generally considered resistant to the type and NY-15 strains of BCMV. These cultivars included Black Turtle, Columbia Pinto, Great Northern UI 31, Great Northern UI 1140, Montcalm Red Kidney, Pinto UI 114, Red Mexican NW 59, Red Mexican NW 63, Rufus, and Viva. Tissue samples were triturated fresh in 0.02 M phosphate buffer, pH 7.0, and applied by rub-inoculation to Carborundum-dusted plants of bean cultivar Sutter Pink. These and other test plants were grown in greenhouses under 14-hr photoperiod (solar illumination supplemented with fluorescent lights, 2,500 ft-c), at 16–22 C. At the same time, sampled tissues were quick-desiccated and placed in sealed vials at −30 C for reference storage.

Cultures that induced typical BCM symptoms in inoculated Sutter Pink plants were tested for pathogenicity to *Vicia faba* var. *minor* (Peterm.) Beck, which is immune to BCMV but broadly susceptible to other legume viruses (4). Cultures inducing no infection in *V. faba* var. *minor* and tentatively considered to be free of other viruses were examined by electron microscopy for potyvirus particles. Each culture was tested with appropriate controls by sodium dodecyl sulfate (SDS) immunodiffusion (5) against BCMV antiserum provided by D. Maat, Plant Protection Institute, Wageningen, The Netherlands. Cultures identified as BCMV were transferred from infected plants of Sutter Pink to BCMV-strain-differentiating bean cultivars comprising resistance groups (RG) 1–4 and host group 6 (3), hereafter referred to as differential cultivars, for preliminary tests of virulence. Greenhouse virulence tests of BCMV cultures were conducted to provide estimates of their virulence in the field.

Cultures found to be free of other viruses were examined for strain mixtures by assessing the homogeneity of subcultures transferred from strain-selective cultivars (1) back to differential cultivars. When infectivity by subcultures was the same as or only slightly different from that of the parent culture, the parent culture was considered a single strain. At the same time, attempts were made to obtain BCMV isolates from differential cultivars with maximum virulence. BCMV isolates were also derived from selected cultures by passage through seed of one or more susceptible cultivars.

With few exceptions, terms used herein relating to the BCMV-*Phaseolus* interaction correspond to those of Drijfhout et al (1–3). Strain groups, assigned numbers I–VII in ascending order of virulence, are the same. The term "resistant," used in lieu of immunity, indicates an absence of systemic infection; "tolerant" indicates systemic infection with few or no symptoms; "susceptible" indicates systemic infection with marked symptoms, generally with severe effects on the host. The term "resistance group" replaces the term "host group" and denotes a known genetic resistance to BCMV. Specifically, cultivars of RG 1–7 contain the recessive alleles, *i i*, of the BCMV-inhibitor gene; cultivars of RG 8–11 contain the dominant alleles, *I I*, of that gene. BCMV-resistance in cultivars with *i i* alleles is usually conditioned by additional single-factor, strain-specific genes (1); cultivars with *I I* alleles are resistant to all known strains but develop systemic necrosis when infected by some BCMV strains under certain conditions. The comprehensive study of Drijfhout (1) was foundational to our use of the term "resistance group."

Bean cultivars constituting RG 1–4 and 6 were, respectively, RG-1, Dubbele Witte, Sutter Pink, and Stringless Green

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Refugee; RG-2, Redlands Greenleaf C, Purefold, and Immuna; RG-3, Redlands Greenleaf B, and Great Northern UI 123; RG-4, Sanilac, Red Mexican UI 34, and Michelite 62; RG-6, Monroe, Great Northern UI 31, and Red Mexican UI 35. RG 5 and 7–9 were deemed unnecessary to our purposes and were not included in this study.

Isolates derived from two field cultures (54 and 60), both typed as strain group IV, were purified by calcium phosphate clarification, polyethylene glycol (mol wt 6,000) precipitation, and density gradient centrifugation as described for carlaviruses by Veerisetty and Brakke (6). Purified preparations of each isolate reacted specifically in SDS immunodiffusion tests (5) with the BCMV antiserum of Maat. On this basis, antiserum against both isolates was produced by a series of 10 weekly intramuscular injections of 1 mg of virus with incomplete adjuvant into rabbits. Microprecipitin titers of resultant antisera were 32–64, with no reaction to extracts from healthy bean plants. Desiccated tissue samples from field-infected plants and from derived isolates were subsequently tested against one or both of these antisera.

RESULTS

Infected bean cultivars and BCM symptoms. Numerous bean cultivars reported to be resistant (some incorrectly) to the type and NY-15 strains of BCMV became infected during the 1977 BCM epidemic. Examples of these cultivars, their previously reported (1) resistance group classifications, and types of symptoms exhibited when inoculum samples were taken are presented in Table 1. Symptoms that were considered indicative of BCMV infection generally conformed to one of four generalized types described by Drijfhout (1). Reproducibility of these symptom types during cultural subtransfers in the greenhouse depended primarily on the differential cultivars inoculated. Induction of vein chlorosis or the very striking interveinal chlorotic mosaic was reproducible in some BCMV strain and bean cultivar combinations. All field cultures found to be free of strain or virus mixtures produced typical, severe BCM symptoms in cultivars of RG 1.

After inoculations from field cultures to Sutter Pink, *V. faba* var. *minor*, or subsequently to differential bean cultivars, 37 of 53 cultures were eliminated from further study as probable mixtures of BCMV strains or other viruses.

BCMV field cultures and isolates. From 53 field cultures, 10 originating from bean cultivars of RG 1–4 and 6 were selected as BCMV isolates for detailed study. These cultures induced typical systemic BCM symptoms in the differential cultivars, consisted of flexuous particles 750–780 nm long without other observable virus-like particles, reacted to

Table 1. Examples of bean cultivars infected in the 1977 bean common mosaic (BCM) epidemic in the northwestern United States (Washington, Idaho, Montana)

Cultivar[a]	Resistance group[b]	BCMV strains infective[c]	Field symptoms[d]
Black Turtle	Mixture[e]	u	BCM
Bunsi	u	u	IvChM
Cascade	u	u	Mm
Chief	3	IV, VI, VII	IvChM
Contender	u	u	Mm
Columbia Pinto	3	IV, VI, VII	BCM
Cranberry UI 51	u	u	IvChM
Great Northern UI 31	6	VII	BCM
Great Northern UI 1140	3	IV, VI, VII	BCM
Improved Cranberry	u	u	BCM
Montcalm Red Kidney	3	IV, V, VI, VII	IvChM
Peabean 119	u	u	VCh,LR
Peabean 395	u	u	BCM
Pinto UI 111	4	III, V, VI	BCM
Pinto UI 114	5	III, V, VI	BCM
Red Mexican UI 34	4	III, V, VI	IvChM
Red Mexican NW 59	u	IV, VI, VII	BCM
Red Mexican NW 63	u	IV, VI, VII	BCM
Rufus	u	III, IV, VI	N
Viva	u	IV, VI, VII	BCM

[a] Cultivars known to be susceptible to standard bean common mosaic virus (BCMV) strain not listed.
[b] Resistance groups of cultivars (levels of BCMV resistance) determined by Drijfhout (1); u = group unknown.
[c] BCMV strains known to be infective to affected bean cultivars; u = strain unknown.
[d] Symptoms in field-infected bean cultivars: BCM = typical bean common mosaic as illustrated (1) by that of NL1 on cultivar Dubbele Witte; IvChM = interveinal bright chlorotic mosaic as illustrated (1) by that of NL5 on Redlands Greenleaf B; Mm = very mild BCM-like symptoms with no leaf distortion; VCh,LR = vein chlorosis as illustrated (1) by that of NL2 on Dubbele Witte, with marked leaf rolling; N = vein necrosis, tip necrosis, and whole plant necrosis as illustrated (1) by that of strain NL8 on Widusa.
[e] See text for comments on genotypic mixtures in seed lots of cultivar Black Turtle.

Table 2. Virulence of bean common mosaic virus (BCMV) field cultures and strain identities of BCMV isolates[a] derived from them

Culture/isolate (I)		Resistance group[c]					Strain group simulated by field culture	Strain identity, subisolate	Pathogenicity to resistance relationship[d]	
No.	Source[b]	1	2	3	4	6			RG	P
49	Col Pinto	S	T	T	S	R	VI	···	3[e]	Yes
I-49	Isolate	S	R	R	S	R	···	III	···	No
51	Viva	S	S	S	R	R	IV	···	u	···
I-51A	Isolate	S	S	S	R	R	···	IV	···	Yes
I-51B	Isolate	S	S	S	R	T	···	VII	···	Yes
54	R M UI 36	S	S	S	R	R	IV	···	2	Yes
ST-54	Isolate	S	S	R	R	R	···	II	···	Yes
57	P UI 111	S	S	T	S	R	VI	···	4	Yes
I-57	Isolate	S	R	R	S	R	···	III	···	Yes
59	G N UI 61	S	S	S	R	R	IV	···	2	Yes
ST-59	Isolate	S	S	S	R	R	···	IV	···	Yes
60	G N US 1140	S	T	S	R	T	VII	···	3	Yes
I-60A	Isolate	S	S	S	R	T	···	VII	···	Yes
I-60B	Isolate	S	S	S	R	R	···	IV	···	Yes
80	G N UI 31	S	T	T	S	R	VI	···	6	No
ST-80	Isolate	S	R	R	S	R	···	III	···	No
96	Roma	S	R	R	R	R	I	···	1	Yes
110	Earliwax	S	T	R	S	R	V	···	1	Yes
1-41	P UI 114	S	T	R	S	R	V	···	5	Yes

[a] Field cultures = virus sources consisting of field-infected plants; BCMV isolates were derived from cultures by subtransfers (see Methods) or by seed-transmission (ST) through one or more susceptible bean cultivars. BCMV identity of isolates determined by SDS immunodiffusion.
[b] Bean cultivars from which field cultures originated were, in tabular order, Columbia Pinto, Viva, Red Mexican UI 36, Pinto UI 111, Great Northern UI 61, Great Northern US 1140, Great Northern UI 31, Roma, Earliwax, and Pinto UI 114.
[c] Host groups of BCMV-strain differentials as defined by Drijfhout (1). Reactions of cultivars within host groups were: R = resistant (no infection); T = tolerant (infection with mild or no typical BCM symtoms); S = susceptible (infection with conspicuous BCM symptoms).
[d] Pathogenicity (P) of BCMV field cultures and isolates to bean cultivars relative to the level of BCMV resistance (resistance groups, RG) of cultivars from which field cultures originated; no = BCMV culture or isolate lost ability to infect the resistance group from which it was taken.
[e] Resistance groups of culture-source cultivars primarily determined by Drijfhout (1: see appendix); u = unknown.

BCMV antiserum by SDS immunodiffusion, and were noninfectious to *V. faba* var. *minor*. These cultures, and isolates derived from them through seed transmission or passage through differential hosts, were tested for virulence on differential cultivars comprising five resistance groups (Table 2).

Field cultures 49, 57, and 80 approximated the pathogenicity (ability to infect) but not the virulence (severity of induced symptoms) of BCMV strain group VI, the most virulent strain of BCMV reported to date; and culture 60 approached the pathogenicity but not the virulence of strain VII. Strain identities of BCMV isolates, derived by seed transmission or by repeated transfers through strain selective differential cultivars, included strain groups I–V; isolates I-51B and I-60A were similar to strain VII in their capacity to infect cultivars of RG 6 but not in virulence. In five of nine cases, BCMV isolates were less virulent than the cultures from which they were derived. This difference consisted in most cases of a marginal capacity to infect cultivars of certain resistance groups by the field culture and a corresponding incapacity by the derived isolate. Isolates I-49, I-57, and ST-80 were less virulent than their respective source cultures in precisely the same way: by incapacity to marginally infect cultivars of host groups 2 and 3. Conversely, isolates from cultures 51, 59, and 60 were at least as virulent as their parent cultures.

Strain groups I (type strain), II (R220 strain), IV (Western strain), and V (NY-15 strain) were previously recognized in the United States; however, strain group III and types approximating strain group VII (Mexican strain) were previously unreported in this country.

Lesser virulence of BCMV isolates than parent cultures could be attributable either to separation of strains from a mixture or to a loss of innate infective capacity during inoculum transfers. The latter process, however, is clearly indicated for isolate I-49 and culture 80, each of which was unable to infect cultivars of the resistance groups from which they originated. That is, isolate I-49 (strain group III), having originated from infected plants presumed to be Columbia Pinto, lacked the ability to infect cultivars of host group 3 of which Columbia Pinto is a member (1). Similarly, culture 80, after inoculum transfer through Sutter Pink and isolate ST-80 after seed passage through Sutter Pink, lacked the ability to infect cultivars of RG 6 although both had originated from field plants that were affirmed to be Great Northern UI 31, a member of this group.

Field cultures and isolates presented in Table 2 reacted in SDS immunodiffusion with antisera against BCMV isolates I-60B and I-54B. All BCMV isolates, when compared in adjacent-well combinations against each antiserum, produced single, virus-specific, conterminous precipitin bands, indicating that all were antigenically indistinguishable in these tests. Appropriate control preparations from healthy bean plants produced no visible reactions.

Attempts were made to determine the highest levels of host resistance through which BCMV was seed-transmissible. Accordingly, seeds were harvested from cultivars of RG 3 and 4 infected by selected BCMV cultures and tested for BCMV seed transmission. Although BCMV from neither isolate I-49 nor culture 80 was transmitted in 12 or 17 seeds respectively of Red Mexican UI 34 (RG 4), BCMV from culture 80 was transmitted in two of three seeds and three of 18, respectively, of Sanilac (RG 4). BCMV was transmitted through seeds of Redlands Greenleaf B by neither culture 54 (15 seeds) nor 60 (33 seeds). BCMV from each of these four sources was readily transmitted in seeds of Sutter Pink.

The principal bean cultivar infected during the 1981 BCM epidemic was cultivar Pinto 114 (RG 5). Seven BCMV isolates from this cultivar, from Washington, Idaho, and Montana, were each identified as belonging to strain group V, presumably comprising the familiar NY-15 strain. Culture 1-41 (Table 2) was representative of these isolates. Seedborne BCMV was detectable in only one of 200 seeds harvested from one of the diseased fields in Idaho. Neither seed transmission of BCMV nor yield reduction by BCM appears to be common in Pinto UI 114, although strain NY-15 usually induces symptoms in this cultivar, especially when infected plants are exposed to temperatures >30 C.

DISCUSSION

The spectrum of bean cultivars attacked in the 1977 BCM epidemic initially suggested that a number of BCMV strains were involved. Our greenhouse and laboratory studies suggest that all known BCMV strains except strain VI were associated with this epidemic.

Although typing of BCMV strains is the intended use of the differential cultivars, we have utilized this same assortment of *Phaseolus* genes to assess the virulence of selected screened BCMV field cultures. Results from this assessment indicated that field cultures, gathered from cultivars resistant to strain groups I and V and considered free of viruses other than BCMV, simulated BCMV strains IV, VI, and VII. Isolates derived from these cultures were identified as strains II–IV and VII. Interestingly, the differences in virulence between cultures 49, 57, and 80 (simulating strain VI) and their strain III derivatives were the same. Whether this difference reflected virulence modification during cultural subtransfers or was caused by separation from initial strain mixtures is conjectural. Nevertheless, our results suggest that at least six strains were isolated from cultivars affected in the 1977 epidemic.

Strain II, present in the United States as "strain 220" (3) and isolated in these studies only from Red Mexican UI 36, presumably played a minor role in the 1977 epidemic.

Strains III and ~VII had not been previously reported in the United States. Preliminary comparisons between our ST-80 and NL-8, the only other reported member of strain group III, indicated that these isolates were distinguishable on Pinto 114; ie, only ST-80 attacked this cultivar. These and other recent results suggest that distinctions among members of BCMV strain groups require only the inclusion of additional bean cultivars into existing *Phaseolus* resistance groups or into new resistance groups. It is also probable that individual isolates of BCMV represent specific points along a continuum of BCMV pathogenicity types of unknown dimensions. Obviously, the numbers of bean cultivars tested to date have not comprehensively described this continuum. Further characterization of both BCMV strain group members and resistance group cultivars is in progress. Results of this study, nevertheless, confirm that the diversity of resistance group cultivars attacked in the two described epidemics was proportional to the diversity of involved BCMV strains.

Bean cultivar Black Turtle is generally considered to be immune to BCMV by virtue of gene *I I*. We assume that the occurrence of BCMV in Black Turtle plants (Table 1) is attributable to the fact that commercial seed stocks of this cultivar frequently consist of a mixture of *i i* and *I I* genotypes. Although BCM usually does not seriously affect this cultivar, presumably because of aphid feeding nonpreference, experimental inoculation of Black Turtle plants with most BCMV strains produces systemic mosaic symptoms in some plants.

Our results suggest that some strains of BCMV may not be generally seed-transmitted in bean cultivars of RG 3–5 and that separate genes may govern susceptibility to plant infection by BCMV and susceptibility to seed transmission of BCMV. Whereas both Sanilac and Red Mexican UI 34 (host group 4) were susceptible to plant infection by isolate ST-80 (strain group III), this isolate was readily seed-transmitted in Sanilac but not (none or limited) in Red Mexican UI 34. Identification of genes for resistance to seed transmission (as in Great Northern UI 31 to all known strains and in Pinto UI 114 to strain NY-15) could be an effective control of BCMV because the primary inoculum reservoir and principal means of long-distance dissemination is almost exclusively by infected seed. Unless both

parents of breeding crosses were resistant to transmission of BCMV through seeds, however, utilization of such genes would necessitate laborious tests of individual progeny plants for BCMV seed transmissibility.

Simultaneous BCM epidemics occurred in the United States and Europe in 1977. Because it was beyond the purpose of this study, we did not attempt analyses of climatic factors that would have affected activities of aphid vectors or expression of BCM symptoms in the two geographic areas. It appears possible that certain BCM outbreaks in both areas could have arisen from common seed-stock inoculum reservoirs because of large-scale exchanges of commercial seed stocks between Europe and the United States. Moreover, the attack of diverse bean cultivars in the northwestern United States in 1977, unprecedented in our experience, suggests that BCMV strains previously reported in Europe may have occurred for the first time in the United States.

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