Tanzanian Strain of Bean Common Mosaic Virus

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ABSTRACT

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A strain of bean common mosaic virus (BCMV) isolated from seed of *Phaseolus vulgaris* produced in Tanzania was found to be similar pathogenically and serologically to temperature-insensitive (TI) strains found initially in Europe and recently in the United States. This is the first report of a strain of BCMV from Tanzania. The Tanzanian strain (TN-1) induced typical mosaic mottle on cultivars with the recessive *ii* gene but caused lethal systemic vascular necrosis (black root) on many resistant cultivars with the dominant *II* gene at normal (23–27 C) growing temperatures. The combination of the dominant *II* gene and unidentified presumed-recessive genes gave complete protection to both the mosaic mottle and the systemic necrosis phases of TN-1.

Until recently, the few strains of bean common mosaic virus (BCMV) known in the United States (21) were controlled by recessive strain-specific genes (3,15,17) or by the dominant *II* inhibitor gene (1,3). The dominant *II* inhibitor gene prevents seed transmission and has been effective against all known strains of BCMV in the United States for about 40 yr. Dominant *II* gene resistance is due to a hypersensitive host reaction at normal growing temperatures (23–27 C) expressed as necrotic local lesions or restricted spreading veinal necrosis. When infected plants are exposed to temperatures higher than 30 C for a prolonged period, certain *II* gene cultivars develop a lethal systemic vascular necrosis, commonly referred to as "black root" (6).

New strains of BCMV reported in Europe in 1963 and 1977 (4,9) and in the United States in 1982, 1983, and 1984 (8,10,11,14) can induce lethal systemic vascular necrosis on some dominant *II* genotypes at temperatures as low as 20 C. These new necrotic strains of BCMV are referred to as the temperature-insensitive (TI) strains and represent a liability for the "unprotected" *II* gene cultivars used in this country.

Drijfhout (3) described the gene-for-gene relationships between seven host genes for resistance and three BCMV genes for pathogenicity. He found that dominant *II* geneotypes are not killed by TI strains if the dominant *II* gene is "protected" by a complementary, recessive, strain-specific gene identified as $bc2^2$ $bc2^2$. The two-gene combination of resistance factors (*II*, $bc2^2$ $bc2^2$) responds to inoculation with TI strains with necrotic local lesions and restricted, spreading, veinal necrosis. There is no systemic vascular necrosis (black root) or mosaic mottle in response to any known strain of BCMV, and the virus cannot be seedborne.

During the evaluation of Tanzanian bean germ plasm at Prosser, a seedborne isolate (82-Tanzanian Accession 15-2) of BCMV was recovered from a common bean land race, EAI 4853, collected by G. Minja in the Arusha area of northern Tanzania. This is the first known documentation of a specific strain of BCMV from Tanzania. A preliminary report of this work was presented earlier (16).

MATERIALS AND METHODS

We evaluated isolate TN-1 according to the standardized methods recommended for identifying strains of BCMV (5). The isolate was maintained on susceptible Sanilac or Stringless Green Refugee (SGR). The host range included 111 bean cultivars (available on request), 16 of which represented the international standard set of BCMV differentials (plus Black Turtle 1 selection [13]), which were produced from screenhouse-grown single-plant selections to ensure uniform host reactions. Cultivars of host group (HG) 7 and 11 were not available. Susceptible SGR or Sutter Pink were used as assay hosts. The known strains of BCMV (NL-3, NL-4, NL-5, US-1, US-2, US-9, and Sanilac) used for comparisons were maintained at Prosser.

Enzyme-linked immunosorbent assay (ELISA) was also used for partial viral strain determination and assays. Antisera to BCMV strains US-1, US-2, US-9, NL-3, and NL-4 had been prepared for a previous study (20). Double-sandwich (direct) ELISA was conducted by the methods of Clark and Adams (2) and Voller et al (19). Indirect ELISA was done by the method of Koenig (12).

All plant tests were conducted in an insect-free greenhouse at 23–27 C. Care was taken to avoid contamination by other strains of BCMV or other viruses.

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RESULTS

Reactions of differential bean cultivars. Among the *ii* genotype differential bean cultivars tested (Table 1), TN-1 is pathogenic on HG 1, 2, 3, 4, and 5 differentials but not on HG 6 cultivars. This places TN-1 in pathogroup VI of Drijfhout et al (5). Subcultures from susceptible hosts behaved similarly to the original isolate; this suggests the original isolate is a single biological strain (5). Reactions produced by the TN-1 strain of BCMV most closely resemble those of the NL-3 strain with minor exceptions. The TN-1 strain induced a clear-cut mosaic mottle on HG 2 cultivars, whereas NL-3 usually produced mild symptoms or a tolerant reaction on these cultivars. In this respect, TN-1 resembled the NL-5 strain reaction (not shown); however, the NL-5 strain induced a lethal systemic necrosis on Amanda at 23–27 C, whereas TN-1 induced only necrotic local lesions and restricted veinal necrosis on primary leaves of Amanda. The TN-1 strain also differed from NL-3 by the failure to induce a lethal systemic necrosis on Jubila at 23–27 C.

The Sanilac Mosaic strain from Michigan (10,11) differed from TN-1 by inducing only tolerant reactions in cultivars of HG 2 and 3 and in not inducing a lethal top necrosis in any *II* genotypes except those in HG 8 at 23–27 C.

Table 1. Comparison of three strains of bean common mosaic virus (BCMV) on differential bean (*Phaseolus vulgaris*) cultivars at greenhouse temperatures of 23–27 C

Host groups[a]	Differential cultivars and their systems	BCMV strain TN-1	NL-3	Sanilac	Control
	Recessive *ii* gene cultivars (mosaic mottle reactions)				
1	Dubble Witte	+[b]	+	+	–
	Stringless Green Refugee	+	+	+	–
2	Redlands Greenleaf C	+	+t	+t	–
	Puregold Wax	+	+t	+t	–
3	Redlands Greenleaf B	+	+	+t	–
	Great Northern UI 123	+t	+t	+t	–
4	Sanilac	+	+	+	–
	Red Mexican UI 34	+	+	+	–
5	Pinto UI 114	+	+	+	–
6	Great Northern UI 31	–	–	–	–
	Red Mexican UI 35	–	–	–	–
	Dominant *II* gene cultivars (systemic necrosis reactions)				
8	Black Turtle Soup 1	+n	+n	+n	–
	Widusa	+n	+n	+n	–
9	Topcrop	+n	+n	±n	–
	Jubila	±n	+n	±n	–
10	Amanda	–	–	–	–

[a] Host groups 7 (IVT 7214) and 11 (IVT 7233) not available (3).
[b] + = Susceptible, sensitive, mosaic mottle; +t = susceptible but tolerant, weak, or questionable symptoms, virus recoverable by sap assay from new growth to Stringless Green Refugee; – = resistant, no systemic symptoms, virus not recoverable by sap assay from new growth to Stringless Green Refugee; +n = susceptible to lethal systemic vascular necrosis at 23–27 C; and ±n = susceptible to localized systemic necrosis at 23–27 C but may succumb to lethal systemic vascular necrosis at 30 C.

Table 2. Absorbance values (A_{405nm}) obtained in double-sandwich (direct) enzyme-linked immunosorbent assay with rabbit antisera prepared against five bean common mosaic virus (BCMV) strains

BCMV strain	Antiserum against US-1	US-2	US-9	NL-4	NL-3
TN-1[a]	0.02	0.05	0	0.24	2.74
Healthy sap	0.10	0.04	0	0.20	0.35
Homologous strain[b]	0.93	1.80	1.21	2.74	2.74

[a] Tanzanian-1 strain of BCMV on cultivar Sanilac.
[b] On cultivar Sutter Pink.

Table 3. Biological and enzyme-linked immunosorbent assay (ELISA) indexing of differential cultivars inoculated or not inoculated with bean common mosaic virus strain Tanzanian-1 (TN-1)

Host group	Differential cultivars	Sap assay[a] to Sutter Pink	ELISA[a] TN-1 (inoc. plants) (antiserum) US-2	NL-3	Healthy check (uninoc. plants) (antiserum) US-2	NL-3
1	Stringless Green Refugee	S[b]	++[c]	++++	–	–
2	Redlands Greenleaf C	S	+	++	–	–
	Puregold Wax	S	++	++++	–	–
3	Redlands Greenleaf B	S	++++	++++	–	–
	Great Northern UI 123	S	–	–	–	–
5	Pinto UI 114	S	+	+	–	–
6	Great Northern UI 31	R	–	–	–	–
	Red Mexican UI 35	R	–	–	–	–
	Vicia faba var. *minor*	R	–	–	–	–

[a] Indexed new growth.
[b] S = respective differential cultivar susceptible to TN-1 as indicated by mosaic mottle on Sutter Pink; R = respective differential cultivar resistant to TN-1 as indicated by lack of symptoms on Sutter Pink.
[c] Relative strength of serological reactions: – = no reaction, + = weak positive reaction, and ++++ = strongest positive reaction.

Serological evaluations of TN-1. Rabbit antisera prepared against five BCMV strains (20) were tested in double-sandwich ELISA against TN-1 grown in Sanilac. TN-1 reacted strongly with NL-3 antiserum but not with antisera developed against BCMV strains US-1 (type), US-2 (NY-15), US-9, and NL-4 (Table 2). TN-1 therefore belongs in the same serogroup as the TI strains (NL-3, NL-5, and NL-8) reported by Wang et al (20) and Provvidenti et al (14).

Differential cultivars in several key host groups were assayed for susceptibility to TN-1 biologically by mechanical transfer to Sutter Pink and serologically by using antisera prepared against US-2 and NL-3 (Table 3). Biologically, the virus was recovered onto Sutter Pink indicator plants from all differential bean cultivars except those in HG 6 (Great Northern UI-31 and Red Mexican UI-35). This places TN-1 in pathogroup VI (3,5) along with NL-3 and NL-5.

TN-1 was not able to infect *Vicia faba* L. var. *minor* (Table 3), and no strains of BCMV are known to infect this host; however, *V. faba* var. *minor* (= broad bean or bell bean) is a known host to 26/38 mechanically transmissible legume viruses (7). Thus, the failure of TN-1 to infect *V. faba* var. *minor* is further evidence supporting its identity and purity.

Indirect ELISA was used with US-2 antiserum to detect the broadest range of BCMV isolates. Double-sandwich (direct) ELISA was used with NL-3 antiserum to detect only the narrow spectrum of the TI serogroup (20). As expected, TN-1 was detected with US-2 antiserum by indirect ELISA after passage through several susceptible cultivars. However, the cultivar through which the virus was passed seemed to affect the strength of the serological reactions observed against both antisera. These differences in susceptible host sensitivity may represent different levels of host

resistance, which are important to breeding programs and epidemiological studies. No serological reactions were obtained with Great Northern UI-123, even though the virus was recovered by back-assay from new growth to susceptible Sutter Pink.

The double-sandwich ELISA with NL-3 antiserum showed a host-cultivar interaction similar to the US-2 antiserum reaction obtained by indirect ELISA with the same cultivars. In general, the degree of relatedness indicated by strength of the serological reactions reflected the severity (or lack) of mosaic mottle symptoms expressed by the particular host cultivar. For example, Pinto UI-114 usually shows mild symptoms after a long incubation period, whereas Great Northern UI-123 often is quite tolerant, showing few or no mosaic mottle symptoms (after a long incubation period), although the virus is recoverable by bioassay.

DISCUSSION

This first report of a specific strain of BCMV from Tanzania is especially interesting in that it is a highly virulent strain in pathogroup VI that has three genes for pathogenicity (*P1*, $P1^2$, and *P2*) plus genes for TI lethal systemic necrosis (3). This high level of pathogenicity in a Tanzanian cultivar that has no resistance genes to any strains of BCMV seems inconsistent with Vanderplank's (18) theory that unnecessary pathogenicity genes tend to be eliminated from host populations with no resistance genes. In a separate study, we tested the resistance of 10 indigenous land races or cultivars collected from Kenya, Tanzania, and Malawi against all seven BCMV pathotypes and found no resistance.

Thus, it appears that these new strains are not only present in susceptible indigenous land races but also that the "excess" genes for pathogenicity may not be detrimental to fitness for strain survival. Further studies of BCMV strains throughout East Africa are needed to determine if this is the rule or the exception.

The failure to detect TN-1 serologically in Great Northern UI-123, even though the virus was recovered by back-assay from new growth to susceptible Sutter Pink (Table 3), is a clear warning that serology is only one of many diagnostic tools and cannot be the sole criterion on which to base judgments about cultivar susceptibility. More work is required to establish the relationships between the bean host symptoms, biological virus titer, and serological detectability at different stages of growth and seed transmission.

Because TI strains of BCMV can induce a lethal systemic necrosis in many popular American cultivars of snap beans and dry beans, they are a threat to the U.S. bean industry. Importers should exercise caution in bringing beans into the United States. Breeders should strive to develop cultivars with stable resistance based on combining the dominant *I* gene for resistance with recessive $bc2^2$.

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LITERATURE CITED

1. Ali, M. A. 1950. Genetics of resistance to the common bean mosaic virus (Bean Virus 1) in the bean (*Phaseolus vulgaris* L.). Phytopathology 40:69-79.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
3. Drijfhout, E. 1978. Genetic Interaction Between *Phaseolus vulgaris* and Bean Common Mosaic Virus, with Implications of Strain Identification and Breeding for Resistance. Agric. Res. Rep. 872, (Centre for Agricultural Publishing and Documentation), Wageningen, Netherlands. 98 pp.
4. Drijfhout, E., and Bos, L. 1977. The identification of two new strains of bean common mosaic virus. Neth. J. Plant Pathol. 83:13-25.
5. Drijfhout, E., Silbernagel, M. J., and Burke, D. W. 1978. Differentiation of strains of bean common mosaic virus. Neth. J. Plant Pathol. 84:13-26.
6. Grogan, R. G., and Walker, J. C. 1948. The relation of common mosaic to black root of bean. J. Agric. Res. 77:315-331.
7. Hampton, R. O., Beczner, L., Hagedorn, D., Bos, L., Inouye, T., Barnett, O., Musil, M., and Meiners, J. 1978. Host reactions of mechanically transmissible legume viruses of the northern temperate zone. Phytopathology 68:989-997.
8. Hampton, R. O., Silbernagel, M. J., and Burke, D. W. 1983. Bean common mosaic virus strains associated with bean mosaic epidemics in the northwestern United States. Plant Dis. 67:658-661.
9. Hubbeling, N. 1963. Complications with the testing of bean varieties for resistance to *Phaseolus virus* 1, as a result of the occurrence of variant virus strains. Commun. Agric. High School Res. Stn. State Gent. 28:1025-1033.
10. Kelly, J. D., Saettler, A. W., and Adams, M. W. 1982. New necrotic strain of bean common mosaic virus in Michigan. Mich. Dry Bean Dig. 7:8-9.
11. Kelly, J. D., Saettler, A. W., and Morales, M. 1984. New necrotic strain of bean common mosaic virus in Michigan. Bean Improv. Coop. Ann. Rep. 27:38-39.
12. Koenig, R. 1981. Indirect ELISA methods for the broad specificity detection of plant viruses. J. Gen. Virol. 55:53-62.
13. Provvidenti, R. 1974. Inheritance of resistance to watermelon mosaic virus 2 in *Phaseolus vulgaris*. Phytopathology 64:1448-1450.
14. Provvidenti, R., Silbernagel, M. J., and Wang, W.-Y. 1984. Local epidemic of NL-8 strain of bean common mosaic virus in bean fields of western New York. Plant Dis. 68:1092-1094.
15. Silbernagel, M. J. 1969. Mexican strain of bean common mosaic virus. Phytopathology 59:1809-1812.
16. Silbernagel, M. J., Mills, L. J., and Wang, W.-Y. 1984. Tanzanian strain (T-1) of bean common mosaic virus. (Abstr.) Phytopathology 74:1141.
17. Silbernagel, M. J., and Zaumeyer, W. J. 1973. Bean chapter in Breeding Plants for Disease Resistance. R. R. Nelson, ed. Pennsylvania State University Press, University Park.
18. Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, New York. 206 pp.
19. Voller, A., Bartlett, A., Bidwell, D. E., Clark, M. F., and Adams, A. N. 1976. The detection of viruses by enzyme-linked immunosorbent assay (ELISA). J. Gen. Virol. 33:165-167.
20. Wang, W.-Y., Mink, G. I., and Silbernagel, M. J. 1982. Comparison of direct and indirect enzyme-linked immunosorbent assay (ELISA) in the detection of bean common mosaic virus. (Abstr.) Phytopathology 72:954.
21. Zaumeyer, M. J., and Thomas, H. R. 1957. A Monographic Study of Bean Diseases and Methods for Their Control. U.S. Dep. Agric. Tech. Bull. 88. 255 pp.