A Quantitative Method to Screen Common Bean Plants for Resistance to *Bean common mosaic necrosis virus*

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ABSTRACT

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A quantitative method to screen common bean (*Phaseolus vulgaris*) plants for resistance to *Bean common mosaic necrosis virus* (BCMNV) is described. Four parameters were assessed in developing the quantitative method: symptoms associated with systemic virus movement, plant vigor, virus titer, and plant dry weight. Based on these parameters, two rating systems (V and VV rating) were established. Plants from 21 recombinant inbred lines (RILs) from a Sierra (susceptible) × Olathe (partially resistant) cross inoculated with the BCMNV-NL-3 K strain were used to evaluate this quantitative approach. In all, 11 RILs exhibited very susceptible reactions and 10 RILs expressed partially resistant reactions, thus fitting a 1:1 susceptible/partially resistant ratio ($\chi^2 = 0.048$, $P = 0.827$) and suggesting that the response is mediated by a single gene.

Using the classical qualitative approach based only on symptom expression, the RILs were difficult to separate into phenotypic groups because of a continuum of responses. By plotting mean percent reduction in either V (based on visual symptoms) or VV (based on visual symptoms and vigor) rating versus enzyme-linked immunosorbent assay (ELISA) absorbance values, RILs could be separated clearly into different phenotypic groups. The utility of this quantitative approach also was evaluated on plants from 12 cultivars or pure lines inoculated with one of three strains of BCMNV. Using the mean VV rating and ELISA absorbance values, significant differences were established not only in cultivar and pure line comparisons but also in virus strain comparisons. This quantitative system should be particularly useful for the evaluation of the independent action of *bc* genes, the discovery of new genes associated with partial resistance, and assessing virulence of virus strains.

Additional keyword: Potyvirus.

Bean common mosaic virus (BCMV) is one of the world's most serious and widespread bean pathogens. BCMV has been documented throughout the world in bean-producing areas since the beginning of the 20th century (2,7,10,11). In nature, BCMV mainly is found infecting *Phaseolus* spp., with *P. vulgaris* as the predominant host (9). However, certain strains of BCMV are adapted to non-*Phaseolus* hosts (*Vigna*, *Arachis*, *Glycine*, *Passiflora*, and *Dendrobium*). BCMV is spread through seed and pollen, and by numerous aphid species in a nonpersistent manner (9). Revisions of BCMV and closely related potyviruses changed the taxonomic status of some strains (8). For example, former serotype A BCMV strains now compose a new species named *Bean common mosaic necrosis virus* (BCMNV) (8).

Bean common mosaic epidemics occurred frequently in the United States in the early 1900s but have been sporadic since the development of resistant cultivars in the 1930s. Currently, control is achieved primarily through seed certification programs and resistant cultivars, the most economical control. Cultivars with durable resistance can be developed by pyramiding the strain-non-specific *I* gene with strain-specific *bc* resistance genes (6). Plant breeders have focused on incorporating durable resistance into germ plasm and cultivars, but resistant phenotypes are difficult and expensive to evaluate because of epistatic interactions and the influence of environmental factors. To avoid problems with epistatic interactions, marker-assisted selection (3,4,6) can be used to pyramid genes into resistant lines. However, not all the *bc* alleles

have markers, some markers are gene pool specific, some markers have reproducibility problems, and not all bean researchers have this technology available.

Common bean plants still are screened using the classical qualitative approach (1) based on symptom development associated with systemic virus movement. This approach is inadequate when a continuum of symptom expression exists, such as in the case of separating partially resistant genotypes from resistant and susceptible genotypes. Thus, an objective system that generates numeric values suitable for statistical analysis and does not rely solely on subjective ratings and variable symptom expression would be useful. The objective of this study was to establish the utility of the quantitative rating system through characterizing resistance to the BCMNV strains in recombinant inbred lines (RILs), pure lines, and cultivars. The RILs from the Olathe/Sierra population also have been utilized in research work to establish random amplified polymorphic DNA (RAPD) markers for *bc-u* and *bc-l²* (13). Preliminary work with this quantitative rating system has been published (14).

MATERIALS AND METHODS

Experimental design for RILs. All 21 RILs from the Olathe (*bc-u bc-u bc-l² bc-l²*, partially resistant)/Sierra (*Bc-u Bc-u Bc-l Bc-l*, susceptible) population were developed using single-seed descent from the F₂ plants to the F₅. Of the 21 RILs, 11 were evaluated in experiment 9602, and 10 others were evaluated in experiment 9606. All 21 RILs were evaluated a second time in experiment 9611. For each RIL, eight seed per treatment were planted into potting mix (EKO compost: 80% compost + 20% vermiculite by volume; Organix Supply, Inc., Missoula, MT) in 10-cm plastic pots. Two seed were planted per pot and served as

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the experimental unit. The pots were arranged in a randomized complete block design with four blocks and two treatments. The treatments were plants inoculated with BCMNV-NL-3 K strain and an uninoculated check. The BCMNV-NL-3 K strain also was inoculated into plants of differential cultivars (Stringless Green Refugee, Dubbele Witte, Black Turtle Soup I, Monroe, and Redlands Greenleaf B) and the parental cultivars to verify strain purity, identity, and reactions. The differential cv. Monroe was resistant to the BCMNV-NL-3 K strain, whereas other differentials tested were susceptible, as expected. Plants of the differential Black Turtle Soup I exhibited local lesions on the inoculated leaf and systemic necrosis throughout.

Experimental design for cultivar, line, or virus strain comparison (experiments 9921 and 0015). Plants from differential cultivars (Black Turtle Soup I, IVT7214, Monroe, Redlands Greenleaf B, Redlands Greenleaf C, and Sanilac), single-seed-decent pure lines (Olathe-1, Ouray-4, Sapphire-5, UI37-1, and Victor-4), and cv. Kodiak were evaluated against three strains of BCMNV (NL-3 D, NL-3 K, and NL-5) inoculated separately. The virus strains were obtained from the Washington State University (WSU)–United States Department of Agriculture Experiment Station at Prosser. Four seed from each cultivar or pure line were planted into separate 10-cm plastic pots containing potting mix (Sunshine Mix No. 2: composition is 70 to 80% Canadian Sphagnum peat moss, perlite, dolomitic limestone for pH adjustment, gypsum, and a wetting agent, no fertilizer; Sunagro Horticulture, Smithfield, UT). The pots served as the experimental unit and were arranged in a randomized complete block design with four blocks. The cultivars or lines and virus strains served as treatments producing a 12-by-3 factorial within each block. The experiment was repeated once.

Virus inoculations. When the primary leaves were 50 to 75% expanded (7 to 10 days after seeding), one primary leaf per plant was mechanically inoculated with a triturate of infected tissue in 50 mM sodium phosphate buffer (pH 7) with a small amount of 600-mesh Carborundum powder (0.2 g per 10 ml of buffer). The BCMNV-NL-3 D (D = Drijfhout strain) and BCMNV-NL-5 strains were maintained in Dubbele Witte plants, and the BCMNV-NL-3 K (K = Kimberly strain) strain was maintained in Ouray-4 plants in separate aphid-proof cages. The triturate was prepared by

macerating young, symptomatic trifoliolate leaves in buffer (1 g of tissue per 10 ml of buffer) with a mortar and pestle. A separate, sterile, cotton-tipped stick was used to inoculate each plant. After inoculation, plants were placed on a greenhouse bench with mean daily temperatures between 23 and 28°C and mean minimum and maximum temperatures of 20 and 33°C, respectively. Supplemental lighting from sodium halide lamps was used to extend the photoperiod to 13 h.

The BCMNV-NL-3 K strain arose as a variant of the BCMNV-NL-3 D that originated from the virus collection at Prosser, WA. The BCMNV-NL-3 D strain should be the same or similar strain to the BCMNV-NL-3 that Drijfhout (1) utilized in his research. Over the course of 10 years, the BCMNV-NL-3 D strain became more virulent than the original. We now refer to this variant as BCMNV-NL-3 K strain. Research currently is being conducted at Prosser, WA to investigate the difference between the BCMNV-NL-3 D and BCMNV-NL-3 K strains. The BCMNV-NL-3 D strain can be maintained on Dubbele Witte plants but the BCMNV-NL-3 K strain is troublesome to maintain on this cultivar. BCMNV-NL-3 K severely stunts or even kills Dubbele Witte plants. Thus, we maintain the BCMNV-NL-3 K strain on Ouray-4 plants.

Disease evaluations. All plants were evaluated 21 days after inoculation for symptoms and vigor (leaf area and plant height) to establish quantitative ratings (V or VV), top dry weight (not determined in experiments 9921 and 0015), and virus titer (based on enzyme-linked immunosorbent assay [ELISA]). The V Rating was based only on visual symptoms (vigor was not utilized; Table 1). The symptom with the lowest corresponding value on the rating scale was given precedence in establishing a value from 0 to 10. Clarification on how to use these rating systems is provided in the three examples in the first footnote of Table 1. The VV rating was based on both symptoms and vigor (leaf area reduction, plant height reduction in a visual comparison to uninoculated plants, or both). For the VV rating, the symptom or vigor level with the lowest corresponding value on the rating scale was given precedence in assigning a value from 0 to 10. The numbers on the rating scale are only guidelines. Therefore, if a plant appeared to be slightly different from others, a decimal number between the whole numbers was utilized. Thus, the scale should be considered continuous between 0 and 10 and not categorical. Top dry weights were deter-

TABLE 1. Description of the V and VV rating systems based on symptoms or symptoms plus vigor, respectively, used to evaluate the responses of bean plants inoculated with *Bean common mosaic necrosis virus* strains^y

Rating scale	Symptoms	Vigor
10	Healthy plant	0% LAR, PHR, or both
9	Slight chlorotic spots or general chlorosis	5–10% LAR, PHR, or both
8	Chlorotic spots or ring spots or vein clearing	20% LAR, PHR, or both
7	Spotty veinal necrosis or local lesions or questionable mosaic or small dead patches ^z	30% LAR, PHR, or both
6	Spotty mosaic or vein banding	40% LAR, PHR, or both
5	Mosaic or vein banding on half of plant or widespread veinal necrosis	50% LAR, PHR, or both
4	Mosaic throughout plant	60% LAR, PHR, or both
3	Strong mosaic	70% LAR, PHR, or both
2	Strong mosaic plus slight systemic necrosis	80% LAR, PHR, or both
1	Strong systemic necrosis (black root) but plant not dead	90% LAR, PHR, or both
0	Dead plant	Dead plant

^y V rating was based only on visual symptoms (vigor was not utilized). The symptom with the lowest corresponding value on the rating scale was given precedence in establishing a value from 0 to 10. The VV rating was based on both symptoms and vigor (LAR = leaf area reduction and PHR = plant height reduction). For the VV rating, the symptom or vigor level with the lowest corresponding value on the rating scale were given precedence in assigning a value from 0 to 10. The numbers on the rating scale were only guidelines. Therefore, if a plant appeared to be slightly different from others, a decimal number between the whole numbers was utilized. Thus, the scale should be considered continuous between 0 and 10 and not categorical. Three examples are given to clarify how the rating systems work. Example 1: A plant with local lesions and 20% LAR or PHR would rate a 7 in the V rating system because the symptom “local lesions” corresponds to a 7 on the rating scale. With the VV rating system, the reduction of leaf area and plant height also would be considered but the rating of 7 would remain the same. The 20% reduction in leaf area or plant height has a corresponding value of 8, which is higher than the value of 7 associated with the local lesion symptoms. Thus, the lower value associated with the symptoms takes precedence and a rating of 7 would be given to this plant. Example 2: A plant with spotty mosaic and 50% LAR or PHR would rate a 6 in the V rating system. The same plant in the VV rating system would rate a 5, because the 50% reduction in leaf area or plant height has a lower corresponding value of 5 than the value of 6 associated with spotty mosaic. Example 3: If a plant appears to be intermediate between plants assigned values of 6 and 7, an intermediate value of 6.5 can be assigned to this plant.

^z Small dead patches appear when the leaf collapses in small interveinal areas. The areas are most likely cut off by the collapse or clogging of veins associated with these areas. Veinal necrosis is sometimes associated with these dead patches. These patches should not be confused with local lesions, which are darker in color and much smaller.

mined by cutting the plants at soil level 21 days after inoculation and drying them in an oven at 90°C for 5 days. The values from the V rating, VV rating, and dry weight measurements then were compared with values from uninoculated check plants to determine the percent reduction in the ratings for experiments 9602, 9606, and 9611 (Table 2). In experiments 9921 and 0015 (Tables 3 and 4), the mean rating value was used instead of taking the additional step of calculating percent reduction in rating values for inoculated versus uninoculated plants. This change demonstrates that good separation can be achieved without resorting to the additional work of calculating percent reduction in the rating value.

ELISA absorbance values at 405 nm were the fourth quantitative measure used to evaluate the bean genotypes. Plants were tested with indirect ELISA by collecting the second trifoliolate leaf above the inoculated leaf 21 days after inoculation. The plant tissue then was weighed and ground in carbonate buffer (pH 9.6) in a ratio of 1 g of fresh tissue per 10 ml of buffer. The ELISA assays for experiments 9602, 9606, and 9611 were conducted by G. Mink (WSU, Prosser) using the broad-spectrum monoclonal antibody bc-197 (15). The ELISA assays for experiments 9921 and 0015 were conducted by Ken Eastwell (WSU, Prosser) using the general *Potyvirus* antibody (Agdia, Inc., Elkhart, IN). The plants with absorbance values of ≤ 0.20 were listed as resistant because they never show symptoms. This cutoff level also allows for some inherent background detection, which normally varies between 0.0 and 0.1. Plants with absorbance values between 0.20 and 0.30 may or may not show symptoms. If they do show symptoms, they will be very mild. Plants with values of 0.40 or higher typically will have symptoms.

Statistical analyses were performed using SAS (SAS Institute, Cary, NC). Data from each experiment were considered separately, because statistical analysis indicated significant differences ($P \leq 0.05$) were present between experiments.

RESULTS

RIL results. Individual plants from RILs exhibited disease reactions ranging from highly susceptible to nearly resistant when inoculated with the BCMNV-NL-3 K strain (Table 2). Partially resistant plants were characterized by systemic virus movement in isolated areas (based on symptoms and ELISA testing), nearly normal growth, and small chlorotic patches in some trifoliolates. The most susceptible plants had extensive virus movement, distinct mosaic symptoms on numerous trifoliolates, and obvious stunting and deformity (Table 2). The RILs were difficult to separate into phenotypic groups using the standard symptom-based qualitative method (1) because of a continuum of symptom expression. With the quantitative approach, the RILs segregated into two phenotypic groups (Figs. 1 and 2). Based on data from all three experiments (Table 2; Figs. 1 and 2), the phenotypic groups fit a 1:1 susceptible/partially resistant ratio, because there were 11 susceptible and 10 partially resistant RILs ($\chi^2 = 0.048$, $P = 0.827$). This segregation is consistent with the presence of one gene conditioning partial resistance to BCMNV-NL-3 K. The mean values for the two segregating phenotypes were found to be significantly different using all four parameters (ELISA absorbance, top dry weight, VV rating, and V rating) (Table 2).

Plotting ELISA mean absorbance value versus mean percent reduction in V or VV rating for RILs (Fig. 1 and 2) enabled clear separation of individual RILs into two phenotypic classes. This separation was noted only when V or VV data were related to virus content. In Figure 2, the VV rating appears to give slightly better separation of the RILs than the V rating. The dry weight data were more variable (Table 2) and did not allow for as clear a separation (data not shown). Likewise, the three other comparisons (VV rating versus V rating, VV rating versus dry weight, and V rating versus dry weight) did not allow for as clear a separation of the RILs into phenotypes (data not shown).

TABLE 2. Summary of disease reaction data from the inoculation of plants from Olathe/Sierra recombinant inbred lines (RILs) with the NL-3 K strain of *Bean common mosaic necrosis virus*

Experiment, RIL phenotype and parents ^w	Mean absorbance at 405 nm ^x	Mean percent reduction in ^v		
		Top dry weight	V rating ^y	VV rating ^z
9602				
Sierra	No data	83 a	100 a	100 a
Susceptible RILs	1.19 a	57 ab	76 b	80 b
Partially resistant RILs	0.59 b	30 b	40 c	36 c
Olathe	0.72 b	38 b	36 c	36 c
$P > F$	<0.0001	0.0039	<0.0001	<0.0001
LSD	0.26	27	13	13
9606				
Sierra	2.98 a	68 a	62 b	81 a
Susceptible RILs	2.93 a	76 a	77 a	85 a
Partially resistant RILs	0.88 b	17 b	42 c	30 b
Olathe	1.45 b	16 b	4 d	27 b
$P > F$	<0.0001	<0.0001	<0.0001	<0.0001
LSD	1.13	32	13	14
9611				
Sierra	0.56 ab	39 ab	82 a	87 a
Susceptible RILs	0.83 a	56 a	63 b	67 b
Partially resistant RILs	0.24 bc	21 b	31 c	18 c
Olathe	0.00 c	-5 c	21 c	10 c
$P > F$	<0.0001	<0.0001	<0.0001	<0.0001
LSD	0.49	26	12	17

^v Percent reduction in comparison to the uninoculated checks. For the quantitative ratings, the V or VV rating for the parents or RILs was compared with the V or VV rating for uninoculated plants to establish percent reduction. Thus, even though the rating scales are based on a 0-to-10 scale, because we are calculating percent reduction in rating for inoculated plants in comparison to the rating for uninoculated plants, the values in the table range from 0 to 100. Numbers in a column within an experiment that are followed by the same letter are not significantly different according to analysis of variance performed on the data, and Fisher's protected least significant difference (LSD) was calculated. $P > F$ was the probability associated with the F value.

^w Sierra = susceptible parent, Olathe = partially resistant parent. Data in experiments 9602, 9606, and 9611 were significantly different ($P \leq 0.05$) and, therefore, not combined for analysis.

^x Enzyme-linked immunosorbent assay absorbance values obtained using the bc-197 monoclonal antibody.

^y The V rating was based on symptoms.

^z The VV rating was based on symptoms and plant vigor.

Cultivar and line or strain comparisons. The data in experiments 9921 (Table 3; Fig. 3) and 0015 (Table 4) were significantly different ($P \leq 0.05$) and, thus, were not combined for analysis. In both experiments, significant differences were found between cultivars and lines and between virus strains. Plants of the differential Black Turtle Soup I had local lesions on the inoculated leaf and systemic necrosis. Plants of IVT7214 were resistant to all three virus strains (Table 4). Kodiak plants were resistant to both the BCMNV-NL-3 D and BCMNV-NL-3 K strains but susceptible to the BCMNV-NL-5 strain. In experiment 9921, Kodiak plants were susceptible to BCMNV-NL-5 because the mean VV rating was quite low and the mean absorbance was well above our cutoff (ab-

sorbance of 0.20) for resistant plants. In experiment 0015, the VV rating again indicated that BCMNV-NL-5 was having a negative influence on Kodiak plants, but not nearly as dramatic an effect as in the first experiment. The mean absorbance for Kodiak plants in experiment 0015 was not different from a resistant reaction. Monroe plants were resistant to all three virus strains in both experiments. Olathe-1 plants were resistant to the BCMNV-NL-3 D strain in both experiments. Olathe-1 plants were resistant to BCMNV-NL-3 K in experiment 9921 but only partially resistant in experiment 0015. Olathe-1 plants were susceptible but not highly susceptible to BCMNV-NL-5. Ouray-4 plants were susceptible to all three virus strains. Redlands Greenleaf B and Redlands Greenleaf C plants

TABLE 3. Summary of data for experiment 9921 which compares three strains of *Bean common mosaic necrosis virus* (BCMNV) (NL-3 D, NL-3 K, and NL-5) on plants from differential cultivars and pure lines^v

Cultivar or pure line ^y	Mean VV rating ^w			Mean absorbance at 405 nm ^x		
	NL-3 D	NL-3 K	NL-5	NL-3 D	NL-3 K	NL-5
Black Turtle Soup I	0.1 a	0.0 a	0.0 a	UN	UN	UN
IVT7214	No data	No data	No data	No data	No data	No data
Kodiak	9.2 a	9.8 a	1.5 a	0.00 a	0.02 a	0.54 a
Monroe	8.3 a	9.3 a	7.8 a	0.01 a	0.00 a	0.23 a
Olathe-1	9.2 a	9.1 a	6.0 b	0.05 b	0.01 b	1.60 a
Ouray-4	3.8 a	5.2 a	4.5 a	2.51 a	1.71 b	2.14 ab
Redlands Greenleaf B	4.3 a	5.2 a	4.8 a	1.64 a	1.28 a	1.90 a
Redlands Greenleaf C	4.9 a	5.7 a	4.1 a	2.13 a	1.47 a	2.10 a
Sanilac	3.0 a	1.4 a	3.0 a	1.28 a	0.05 b ^z	1.61 a
Sapphire-5	4.0 b	2.6 b	6.2 a	1.59 a	0.00 b ^z	2.07 a
UI37-1	8.1 a	6.4 b	5.4 b	0.03 b	0.03 b	1.20 a
Victor-4	8.4 a	9.0 a	5.4 b	0.37 b	0.02 b	1.68 a
$P > F$	<0.0001	<0.0001	<0.0001	<0.0001	0.0012	0.0540
LSD	2.8	1.8	1.6	0.72	1.02	1.12

^v Letters allow for in-row comparison of the three BCMNV strains within the VV rating or enzyme-linked immunosorbent assay (ELISA) categories. Means followed by the same letter are not significantly different at $P = 0.05$ using PDIFF to separate least square means in SAS. For cultivar and line comparisons, use the Fisher's protected least significant difference (LSD) value at bottom of table. $P > F$ was the probability associated with the F value. UN = plant material was unavailable for ELISA testing (Black Turtle Soup I plants possess an unprotected *I* gene and die from systemic necrosis when inoculated with BCMNV strains). No data = poor plant emergence led to insufficient plant numbers for testing.

^w The VV rating was based on symptoms and plant vigor. The VV rating can range from 0 to 10 (0 = dead plant and 10 = healthy plant).

^x ELISA absorbance values obtained using the general potyvirus antibody.

^y The pure lines Olathe-1, Ouray-4, UI37-1, Victor-4, and Sapphire-5 were single plant selections from commercial cultivars that were subsequently inbred for two generations.

^z ELISA absorbance values for Sanilac and Sapphire-5 plants were unusually low for having been inoculated with the NL-3 K strain. The plants for these cultivars were almost dead when sampled 21 days after inoculation; thus, the virus titer and absorbance values appear unusually low.

TABLE 4. Summary of virus data for experiment 0015 comparing three strains of *Bean common mosaic necrosis virus* (BCMNV) (NL-3 D, NL-3 K, and NL-5) on plants from differential cultivars and pure lines^w

Cultivar or pure line ^z	Mean VV rating ^x			Mean absorbance at 405 nm ^y		
	NL-3 D	NL-3 K	NL-5	NL-3 D	NL-3 K	NL-5
Black Turtle Soup I	0.7 a	0.0 a	0.0 a	UN	UN	UN
IVT7214	9.1 a	9.5 a	9.4 a	0.00 a	0.30 a	0.00 a
Kodiak	10.0 a	9.8 a	8.2 b	0.00 a	0.00 a	0.05 a
Monroe	10.0 a	10.0 a	9.5 a	0.02 a	0.00 a	0.03 a
Olathe-1	8.9 a	7.0 b	6.5 b	0.19 b	1.18 a	1.34 a
Ouray-4	5.0 a	4.0 b	4.8 ab	1.10 a	1.33 a	1.15 a
Redlands Greenleaf B	7.0 a	7.2 a	7.5 a	1.23 a	1.40 a	1.25 a
Redlands Greenleaf C	6.5 a	6.8 a	7.2 a	1.28 a	1.40 a	1.41 a
Sanilac	4.5 a	3.0 b	No data	1.07 a	1.39 a	No data
Sapphire-5	5.4 a	3.0 b	5.6 a	0.51 a	1.22 b	0.79 ab
UI37-1	9.5 a	7.0 b	7.6 b	0.57 a	1.14 b	1.12 b
Victor-4	9.4 a	7.0 b	7.1 b	1.03 ab	0.64 b	1.44 a
$P > F$	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001
LSD	0.9	0.3	1.5	0.43	0.65	0.60

^w Letters allow for in-row comparison of the three BCMNV strains within the VV rating or enzyme-linked immunosorbent assay (ELISA) categories. Means followed by the same letter are not significantly different at $P = 0.05$ using PDIFF to separate least square means in SAS. For cultivar and line comparisons, use the Fisher's protected least significant difference (LSD) value at bottom of table. $P > F$ was the probability associated with the F value. UN = plant material was unavailable for ELISA testing (Black Turtle Soup I plants possess an unprotected *I* gene and die from systemic necrosis when inoculated with BCMNV strains). No data = poor plant emergence led to insufficient plant numbers for testing.

^x The VV rating was based on symptoms and plant vigor. The VV rating can range from 0 to 10 (0 = dead plant and 10 = healthy plant).

^y ELISA absorbance values obtained using the general potyvirus antibody.

^z The pure lines Olathe-1, Ouray-4, UI37-1, Victor-4, and Sapphire-5 were single plant selections from commercial cultivars that were subsequently inbred for two generations.

were susceptible to all three virus strains. Sanilac and Sapphire-5 plants were highly susceptible to all three virus strains. UI37-1 plants were resistant to partially resistant when inoculated with BCMNV-NL-3 D. UI37-1 plants were partially resistant to BCMNV-NL-3 K and BCMNV-NL-5 based on symptoms, but absorbance values indicated susceptibility to both strains. Victor-4 plants were resistant to partially resistant to BCMNV-NL-3 D and BCMNV-NL-3 K strains based on symptoms, but absorbance values gave conflicting results between experiments. Plotting the data from experiment 9921 will give clear separation of cultivars that vary in resistance to BCMNV strains (Fig. 3). Using the mean VV rating allowed for clear separation (Fig. 3) without resorting to the additional step of calculating percent reduction (Figs. 1 and 2).

DISCUSSION

Results from the analyses of genetic lines and cultivars tested against strains of BCMNV indicate that a quantitative approach to data collection works well for separating partially resistant lines from susceptible lines. The best separation of RILs into phenotypic groups occurred when mean ELISA absorbance values were graphed against the mean VV rating. The VV rating is a quantitative system that includes assessments of both plant symptoms and vigor. The quantitative data makes graphing and statistical analysis possible.

Fig. 1. Summary of disease reaction data for **A**, experiment 9602 and **B**, experiment 9606 from inoculation of the *Bean common mosaic necrosis virus* NL-3 K strain onto plants from 21 recombinant inbred lines (RILs) from an Olathe (moderately resistant parent; *bc-u bc-u bc-l² bc-l²*) × Sierra (susceptible parent; *Bc-u Bc-u Bc-l Bc-l*) cross. RILs and parents are plotted based on mean enzyme-linked immunosorbent assay absorbance values and mean percent reduction in VV rating (disease rating based on symptoms and plant vigor); the VV rating for the parents or RILs was compared with the VV rating for uninoculated plants to establish percent reduction).

We considered common bean plants inoculated with BCMNV resistant if no systemic virus movement or symptoms occurred on any leaves other than the inoculated primary leaf. Resistant plants were normal in size and appearance except for the inoculated leaf, which at times showed symptoms or even died and fell off. Common bean plants susceptible to BCMNV exhibited symptoms on trifoliolates and high ELISA absorbance values (>1.0), indicating systemic virus movement. The range of symptoms in susceptible plants can vary greatly between adjacent trifoliolates and within trifoliolates and also can be environmentally influenced. The distribution of virus in susceptible plants also can vary. Symptoms in susceptible plants included localized or widespread areas of chlorosis in various patterns or necrosis, mosaic symptoms varying from barely detectable to pronounced, leaf stunting or deformation, and systemic necrosis resulting in plant death (i.e., black-root with cultivars which contain the unprotected *I* gene). Based on these criteria, all the RILs evaluated in this study are susceptible in some degree to the BCMNV-NL-3 K strain. Within this susceptible category, the RILs segregated into partially resistant and susceptible groups due to the presence or absence of a recessive gene (13).

The standard approach to the genetics of resistance to BCMV and BCMNV as outlined by Drijfhout (1) indicates that the *bc-u* must be present for the full expression of resistance from *bc-l²*. The *I* gene by itself will condition either an immune or tempera-

Fig. 2. Summary of disease reaction data for experiment 9611 from inoculation of the *Bean common mosaic necrosis virus* NL-3 K strain onto plants from 21 recombinant inbred lines (RILs) from an Olathe (moderately resistant parent; *bc-u bc-u bc-l² bc-l²*) × Sierra (susceptible parent; *Bc-u Bc-u Bc-l Bc-l*) cross. RILs and parents are plotted based on mean enzyme-linked immunosorbent assay absorbance values and mean percent reduction in **A**, V or **B**, VV rating (disease ratings based on symptoms and symptoms and plant vigor, respectively; the rating for the parents or RILs was compared with the rating for uninoculated plants to establish percent reduction). **B**, The VV rating appears to give slightly better separation of the RILs than **A**, the V rating.

ture-dependent hypersensitive resistance to BCMV strains. However, when the unprotected *I* gene is challenged by BCMNV strains, a temperature-independent hypersensitive response called top necrosis or black root results from systemic necrosis and leads to plant death. Recent research, however, demonstrates that *bc-3* and *bc-1²* can confer resistance to some strains of BCMV or BCMNV if the *I* gene is present (1,5,6). Sierra contains no *bc* genes and Olathe contains *bc-u* and *bc-1²*; therefore, we suspect the one gene associated with partial resistance to the BCMNV-NL-3 K strain in our RILs could be the *bc-1²* gene acting independently. The RILs utilized in this study were part of a project in which RAPD markers were found for *bc-u* and *bc-1²* (13). However, results from pure lines Olathe-1, Victor-1, UI37-1, Ouray-4, and Sapphire-5 (all of which contain *bc-u* and *bc-1²*; data not shown) indicate that the partial resistance to BCMNV-NL-3 K appears to be independent of the presence of *bc-u* and *bc-1²* (12). In work by Drijfhout (1), cultivars containing *bc-u* and *bc-1²* were found to be variable in their responses to pathogroup VI virus strains (BCMNV-NL-3 and BCMNV-NL-5). The genes or alleles associated with this variable response may be a previously unrecognized source of resistance. Genetic studies involving crosses between these pure lines have been conducted to verify this hypothesis (12).

Cv. Kodiak reportedly contains the *I* gene protected by *bc-1²* (12). Our results show this cultivar to be resistant to BCMNV-NL-3 D and BCMNV-NL-3 K but susceptible to BCMNV-NL-5 in experiment 9921 (Table 3). When inoculated with BCMNV-NL-5 in experiment 0015 (Table 4), Kodiak's VV rating was significantly reduced compared with the BCMNV-NL-3 strains but ELISA was not able to confirm that virus was present in the second trifoliolate leaves above the primary leaf. Although the greenhouse temperatures and photoperiods were set the same for both experiments, experiment 0015 was started when the weather was cloudy; consequently, the greenhouse struggled to reach peak temperature (set point that initiated cooling) compared with the sunny days when experiment 9921 was started. These differences in temperature response time in the greenhouse may account for the differences we saw between experiments with Kodiak plants inoculated with BCMNV-NL-5. The protection of the *I* gene with *bc-1²* should be investigated further to confirm the extent that the *I* gene is protected from BCMNV strains.

The ELISA absorbance values were considerably different between experiments for certain strains when plants from various cultivars or lines were assayed. These variable reactions were not unexpected where partial resistance was present. The absorbance values for Olathe-1, Victor-4, and UI37-1 plants were expected to be variable for the BCMNV-NL-3 D and BCMNV-NL-3 K strains for which they appear to possess partial resistance. When plants from these three pure lines were inoculated with BCMNV-NL-3 D or BCMNV-NL-3 K, the virus only moved systemically to some of the trifoliolates. Thus, when the second trifoliolate above the inoculated leaf was collected 21 days after inoculation, some trifoliolates were asymptomatic and had no detectable virus by ELISA, whereas other trifoliolates had small chlorotic patches and detectable levels of virus. Thus, the absorbance readings for the second trifoliolate above the inoculated leaf in partially resistant plants can vary from zero to moderate levels. This inherent variability can lead to a continuum of ELISA absorbance values if means are not calculated. By calculating mean values, meaningful separation can be achieved as shown by the figures. Using ELISA to establish virus titer has been the typical approach to assessing plant-virus interactions. However, ELISA results have not always been directly correlated with symptoms. Therefore, this comprehensive quantitative rating system represents a more reliable way to directly measure the effect of virus on the plant.

Variability between experiments also can be related to changing environmental variables beyond greenhouse control and not the result of heterogeneous plant material. Artificial lighting does not compensate completely for the low angle of the sun in the winter

and periods of cloudy or foggy weather. Thus, the intensity of virus expression varies with environmental variables. Plant breeders and researchers can contend with this variability by graphing the mean values for absorbance and a quantitative rating. Being able to handle this variability and clearly separate phenotypic groups are two of the virtues of this quantitative rating system.

Fig. 3. Summary of disease reaction data for experiment 9921 from inoculation of three *Bean common mosaic necrosis virus* (BCMNV) strains (A, NL-3 D, B, NL-3 K, and C, NL-5) onto plants from differential cultivars and pure lines. The reaction of plants from the cultivars and lines are plotted based on mean enzyme-linked immunosorbent assay absorbance (ELISA) values and mean VV rating (disease rating based on symptoms and plant vigor, where 0 = dead plant and 10 = healthy plant). Monroe was the only cultivar or line resistant to all three virus strains, whereas Sapphire-5, Sanilac, Redlands Greenleaf B and C, and Ouray-4 were susceptible to all strains. The absorbance values for Sapphire-5 and Sanilac inoculated with NL-3 K and Kodiak inoculated with NL-5 were unusually low because they were severely infected and, therefore, in very poor condition when sampled for ELISA testing 21 days after inoculation. These plots demonstrate the value of this quantitative approach by clearly separating cultivars with known responses to BCMNV strains. These plots also show that the mean VV rating will give good separation without having to calculate percent reduction in rating.

Separating plants resistant to BCMV or BCMNV from susceptible plants can be done using tightly linked markers if the markers and facilities are available. In the absence of markers, the conventional qualitative approach (1) and test crosses are used to identify genotypes carrying the recessive *bc* genes. As noted in our results, clearly separating partially resistant plants from susceptible plants is difficult if not impossible when using only the conventional symptom-based qualitative approach to plant evaluation. Thus, we investigated the use of a quantitative system for evaluating plants for resistance to BCMNV strains. Both the mean V and VV rating (Table 1), when graphed versus mean ELISA absorbance values, were shown to be useful in separating RILs with only partial resistance from those that were susceptible. The dry weight data were variable and, consequently, not as useful in separating lines. If lines appear to fall in between phenotypes or appear as outliers, inoculations with other virus strains may be useful in determining which resistance gene or genes is present. The use of the mean VV rating in the strain or cultivar comparison research shows that separation can be achieved without the use of percent reduction. The V rating, a simpler rating system, may be favored over the VV rating if stunting is not evident or stunting from nonvirus related problems is present.

This quantitative approach to rating plants has proven to be useful in the evaluation of resistance in common bean plants inoculated with strains of BCMNV and BCMV (data not shown) on cultivars containing various *bc* genes or the *I* gene. Thus, utilizing this quantitative system to evaluate strains of both BCMNV and BCMV in common bean should be possible. Adapting this quantitative approach to other virus–host systems should be possible after adequate adjustments for differences in symptoms between systems are accommodated. However, these adapted systems would require additional testing to verify their usefulness.

Quantitatively evaluating plants from genetic and breeding material inoculated with strains of BCMNV was found to be useful in handling a continuum of symptom responses. This comprehensive quantitative approach, which includes a variety of factors, provides a novel improved way to measure the outcome of plant–virus interactions. This will be particularly useful for evaluating material for the presence of independent gene action of *bc* genes, the discovery of new genes associated with partial resistance, and assessing virulence differences between virus strains.

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LITERATURE CITED

1. Drijfhout, E. 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. Doctoral thesis. Wageningen, Agric. Res. Rep. 872.
2. 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.
3. Haley, S. D., Afanador, L., and Kelly, J. D. 1994. Identification and application of a random amplified polymorphic DNA marker for the *I* gene (potyvirus resistance) in common bean. *Phytopathology* 84:157-160.
4. Haley, S. D., Afanador, L., and Kelly, J. D. 1994. Selection for monogenic pest resistance traits with coupling- and repulsion-phase RAPD markers. *Crop Sci.* 34:1061-1066.
5. Kelly, J. D. 1997. A review of varietal response to bean common mosaic potyvirus in *Phaseolus vulgaris*. *Plant Var. Seeds* 10:1-6.
6. Kelly, J. D., Afanador, L., and Haley, S. D. 1995. Pyramiding genes for resistance to bean common mosaic virus. *Euphytica* 82:207-212.
7. Klein, R. E., Wyatt, S. D., and Kaiser, W. J. 1988. Incidence of bean common mosaic virus in USDA *Phaseolus* germ plasm collection. *Plant Dis.* 72:301-302.
8. Mink, G. I., Vetten, J., Ward, C. W., Berger, P., Morales, F., Myers, J. R., Silbernagel, M. J., and Barnett, O. W. 1994. Taxonomy and classification of legume infecting potyviruses. A proposal from the Potyviridae Study Group of the Plant Virus Subcommittee of ICTV. *Arch. Virol.* 139:231-235.
9. Morales, F. J., and Bos, L. 1988. Bean common mosaic virus. *AAB Descrip. Plant Viruses* No. 337.
10. Morales, F. J., and Castaño, M. 1987. Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. *Plant Dis.* 71:51-53.
11. Pierce, W. H., and Hungerford, C. W. 1929. Symptomatology, transmission, infection, and control of bean mosaic in Idaho. *Univ. Idaho Agric. Exp. Stn. Res. Bull.* 7.
12. Strausbaugh, C. A., Miklas, P. N., Singh, S. P., Myers, J. R., and Forster, R. L. 2003. Genetic characterization of differential reactions among host group 3 common bean cultivars to NL-3 K strain of *Bean common mosaic necrosis virus*. *Phytopathology* 93:683-690.
13. Strausbaugh, C. A., Myers, J. R., Forster, R. L., and McClean, P. 1999. *Bc-1* and *Bc-u*, two loci controlling bean common mosaic virus resistance in common bean are linked. *J. Am. Soc. Hortic. Sci.* 124:644-648.
14. Strausbaugh, C. A., Myers, J. R., Forster, R. L., and McClean, P. E. 2000. Quantitative method to screen for resistance to bean common mosaic. *Annu. Rep. Bean Improv. Coop.* 43:166-167.
15. Wang, W.-Y., Mink, G. I., and Silbernagel, M. J. 1985. A broad spectrum monoclonal antibody prepared against bean common mosaic virus. (Abstr.) *Phytopathology* 75:1352.