Genetic Characterization of Differential Reactions Among Host Group 3 Common Bean Cultivars to NL-3 K Strain of *Bean common mosaic necrosis virus*

C. A. Strausbaugh, P. N. Miklas, S. P. Singh, J. R. Myers, and R. L. Forster

First, third, and fifth authors: Department of Plant, Soil, and Entomological Sciences, University of Idaho, Kimberly Research and Extension Center, 3793 N. 3600 E., Kimberly 83341; second author: Vegetable and Forage Crop Production Research, U.S. Department of Agriculture-Agricultural Research Service, 24106 N. Bunn Rd., Prosser, WA 99350; and fourth author: Department of Horticulture, Oregon State University, Corvallis 97331.

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ABSTRACT

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A previously unrecognized recessive resistance gene (or allele) was identified in three host group (HG) 3 common bean (*Phaseolus vulgaris*) cvs. Olathe, Victor, and UI 37, based on genetic analysis of plants from five populations screened with the NL-3 K strain of *Bean common mosaic necrosis virus* (BCMNV). The gene (or allele) was associated with resistance to leaf stunting and deformity and reduction in plant height. The gene (or allele) provides similar, but slightly better resistance than the *bc-1²* gene that is characteristic of HG 3 cultivars. Traditional HG 3 cultivars like Redlands Greenleaf B with *bc-1²* are susceptible to

NL-3 K, whereas this newly identified gene (or allele) conditions resistance to NL-3 K. Other slight variations in disease reaction pattern to a wide array of bean common mosaic (BCM)-inducing strains were noted among HG 3 differentials, indicating that additional resistance to BCM exists in common bean that remains to be exploited. To gauge the full breeding value of this newly identified gene (or allele), allelism tests with existing genes, namely *bc-1²*, and further characterization of responses to all *Bean common mosaic virus* (BCMV) and BCMNV strains need to be conducted. Meanwhile, breeders should consider introgressing this more effective gene (or allele) into susceptible cultivars while plant pathologists continue to decipher the genetic variability present among HG 3 differential cultivars.

Additional keyword: potyvirus.

Bean common mosaic is one of the world's most serious and widespread diseases of common bean (*Phaseolus vulgaris* L.). In nature, the pathogens *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) mainly infect *Phaseolus* spp., with *P. vulgaris* as the predominant host (23). The virus spreads through seed, pollen, and by numerous aphid species in a nonpersistent manner (23). However, little or no seed transmission occurs if a plant becomes infected after flowering (24).

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. The value of this resistance has been evident whenever growers have tried to produce pathogen-free seed of susceptible cultivars. For example, in recent years, both Idaho and Washington State Seed Certification programs have been unsuccessful in producing certified seed of Maverick (9), a pinto bean cultivar lacking resistance to endemic BCMV strains. In the past, BCMV strain US-10 was detected in seed lots originating from the Pacific Northwest region (7). Since 1989, strains of BCMNV (NL-8 and NL-3) also have been sporadically present in the western United States (7,10). Recent taxonomic revisions of BCMV and closely related potyviruses placed former serotype A BCMV strains (NL-3, NL-5, and NL-8) into a new species named BCMNV (17,22). Strains belonging to serotype B were retained under the species name BCMV. Control still is achieved primarily through seed certification programs and

resistant cultivars; however, the most economical control will be to develop cultivars with durable resistance by pyramiding resistance genes with broad effect against both BCMV and BCMNV strains (15). The development of genotypes with pyramided resistance is facilitated by the use of marker-assisted selection in combination with direct disease screenings (13,14,18–20,29).

In 1950, Ali (2) discovered the *I* gene, which conditions either an immune or temperature-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. *I* gene dosage also can affect resistance responses (4). The linkage of the *I* gene with color-intensifying genes at the B locus has hampered the deployment of this gene into red- or yellow-seeded market classes (14,15).

Drijfhout (5,6) discovered six *bc* genes located at four loci, which is consistent with the identification of recessive genes (12) conditioning strain-specific resistance to other potyviruses in pea (*Pisum sativum* L.). Of the four loci, *bc-u* and *bc-1* recently were shown to be loosely linked (29). Furthermore, 10 BCMV pathogenicity and 11 differential host groups were recognized (5). In this system, *bc-1*, *bc-1²*, *bc-2*, *bc-2²*, and *bc-3* were strain-specific genes. The *bc-u* gene was not strain specific, but was necessary for the expression of the other *bc* genes unless the *I* gene was present. In the presence of the *I* gene, the *bc-1*, *bc-1²*, and *bc-3* genes will confer resistance without *bc-u* (14,20,26). In the *I* + *bc-1* genotype, the NL-8 BCMNV strains will result in only local lesions and veinal necrosis on the inoculated leaf while the NL-3 and NL-5 strains will result in top necrosis (20). The *I* + *bc-1²* genotypes exhibit only local lesions and veinal necrosis on the

Corresponding author: C. A. Strausbaugh; E-mail address: carl@uidaho.edu

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inoculated leaf when challenged by the NL-8 and NL-3 BCMNV strains, while the NL-5 strain will express top necrosis but with delayed symptom development (20). Miklas et al. (20) also showed that *bc-l*² was dominant to *bc-l* in conferring resistance to top necrosis in the presence of the *I* gene. The *I* + *bc-u* + *bc-l*² gene combination challenged by BCMNV strains will result in only pinpoint local lesions on the inoculated leaf (14). No reactions will occur on the inoculated leaf with BCMV or BCMNV strains if *bc-u* + *bc-l*² are present, regardless of the presence or absence of the *I* gene (5,14). The host groups were established by screening cultivars or lines against what were considered to be representative strains of BCMV (5,6). Based on this screening, differential cultivars were assigned to host groups (5,6).

Populations of plant viruses tend to be heterogeneous and capable of evolving (8,27) into new strains. In 1995, a novel pathotype was found in Africa that induced necrosis in host group (HG) 8 cultivars even though the strains were serotype B (28). In this work, new pathogenicity gene symbols *Px* and *Px*² were proposed (28). Other novel pathotypes originating from Africa associated with strains TN-2 and TN-3 have been proposed (25).

Recently, in the United States, a more virulent strain of NL-3, designated NL-3 K (K = Kimberly), has been described along with differential common bean cultivars (19,21). We observed differential reactions to NL-3 K among Olathe, Ouray, Sapphire, UI

37, and Victor, all putative cultivars within HG 3 (7,30). Drijfhout (5) also had noted variability within host groups and lumped together susceptible and tolerant reactions in HG 2 differentials inoculated with the NL-7 strain and in HG 3 differentials inoculated with NL-3 and NL-5 strains, indicating the presence of additional yet uncharacterized pathogenicity and resistance genes. The primary objective of this study was to characterize, genetically, the differential reactions, ranging from partially resistant to highly susceptible, among HG 3 cultivars to the NL-3 K strain of BCMNV.

MATERIALS AND METHODS

Parental selection and population development. Pure lines cvs. Olathe, Ouray, Sapphire, UI 37, and Victor were developed via single-seed descent from single plant selections that were selfed for two generations. These cultivars were determined (7) to possess the *bc-u* and *bc-l*² genes based on resistance to pathogroups 3 (NL-8 strain) and 5 (NY-15 strain) and susceptibility to pathogroups 6 (NL-3 D; D = Drijfhout strain) and 7 (US-6), which places them within HG 3 (5,6). These cultivars, however, varied from partially resistant (Olathe, Victor, and UI 37) to susceptible (Ouray) and highly susceptible (Sapphire) for reaction to the NL-3 K strain of BCMNV. To examine this differential response, five

TABLE 1. Summary of reactions for cultivars and pure lines inoculated with strains of *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV)

Cultivar or line ^a	Resistance genes ^b	SCAR markers ^c		BCMV and BCMNV strains ^d													
		SW 13 ₆₉₀	SBD 5 ₁₃₀₀	US-1	NL-7	NL-8 ID	US-5	Western	NY-15 Z ^e	NY-15 Z ^f	NY-15 P	NY-15 S	NL-2	NL-3 K	NL-3 D	NL-5	US-6
Differential cultivars																	
DW	None	—	—	S	S	S	S	S	S	S	S	S	S	S	S	S	S
RGC	<i>bc-u</i> , <i>bc-l</i>	*	—	R	R	R	S	S	R	P	P	P	R	S	S	S	S
RGC exp.	<i>bc-u</i> , <i>bc-l</i>	—	—	R	S	R	S	S	S	S	S	S	S	S	S	S	S
RGB	<i>bc-u</i> , <i>bc-l</i> ²	*	+	R	R	R	S	S	R	P	P	P	R	S ^g	S	P	S
RGB exp.	<i>bc-u</i> , <i>bc-l</i> ²	—	+	R	R	R	S	S	R	R	R	R	R	S	S	S	S
UI 123	<i>bc-u</i> , <i>bc-l</i> ²	—	+	*	*	*	*	*	*	P	P	R	R	*	*	*	*
UI 59	<i>bc-u</i> , <i>bc-l</i> ²	*	+	*	*	*	*	*	*	R	R	R	R	*	*	*	*
Sanilac	<i>bc-u</i> , <i>bc-l</i> ²	*	—	R	R	S	R	R	R	*	*	*	*	S	S	*	R
UI 31	<i>bc-u</i> , <i>bc-l</i> ² , <i>bc-l</i> ²	*	±	*	*	*	*	*	*	R	R	R	R	*	*	*	*
Monroe	<i>bc-u</i> , <i>bc-l</i> ² , <i>bc-l</i> ²	*	+	R	R	R	R	V	R	*	*	*	*	R	R	R	S
IVT 7214	<i>bc-u</i> , <i>bc-l</i> ² , <i>bc-l</i> ²	*	—	*	*	*	*	*	*	*	*	*	*	R	R	R	*
BTS 1	<i>I</i>	+	—	R	R	T	R	R	R	R	R	R	V	T	T	T	R
Brasil-2	<i>I</i>	+	+	*	*	*	*	*	*	R	R	R	R	T	T	T	*
Jubila	<i>I</i> , <i>bc-l</i>	*	—	*	*	*	*	*	*	R	R	R	R	R ^h	*	*	*
Top Crop	<i>I</i> , <i>bc-l</i>	*	—	*	*	*	*	*	*	R	R	R	T	T	*	*	*
Amanda	<i>I</i> , <i>bc-l</i> ²	*	*	*	*	*	*	*	*	*	*	*	*	R	*	*	*
Redcloud	<i>I</i> , <i>bc-l</i> ²	+	+	*	*	*	*	*	*	R	R	R	R	*	*	*	*
Kodiak	<i>I</i> , <i>bc-l</i> ²	+	+	*	*	*	*	*	*	*	*	*	*	R	R	P	*
IVT 7233	<i>I</i> , <i>bc-l</i> ² , <i>bc-l</i> ²	*	—	*	*	*	*	*	*	R	R	R	R	R	*	*	*
Parental lines																	
Sapphire	<i>bc-u</i> , <i>bc-l</i> ²	—	+	R	S	R	S	S	R	V	*	R	S	S	S	S	S
Ouray	<i>bc-u</i> , <i>bc-l</i> ²	—	+	V	S	R	S	S	R	V	S	R	S	S	S	S	S
UI 37	<i>bc-u</i> , <i>bc-l</i> ² , ?	—	+	R	V	R	S	S	R	R	R	R	R	P	R	V	S
Victor	<i>bc-u</i> , <i>bc-l</i> ² , ?	—	+	R	V	R	S	S	R	P	S	R	S	P	P	S	S
Olathe	<i>bc-u</i> , <i>bc-l</i> ² , ?	—	+	R	V	R	S	S	R	R	R	R	R	P	R	S	S

^a DW = Dubbele Witte ; RGC = Redlands Greenleaf C; RGB = Redlands Greenleaf B; RGC exp. and RGB exp. indicate the reactions expected for RGC and RGB based on Drijfhout's work (5); BTS 1 = Black Turtle Soup 1.

^b Bean common mosaic resistance genes. The symbol ? is a generic symbol used to represent the recessive resistance gene (or allele) found in the pure lines Olathe, Victor, and UI 37. The gene designation for Brasil-2 is based on phenotypic reaction to BCMNV strains.

^c Symbols: + or — represent the presence or absence of the marker, respectively, and ± indicates that a genotype was heterogeneous for the marker. The marker data shown represents a summary of results from this study and those reported by Miklas et al. (20).

^d S = susceptible, R = resistant, P = partially resistant, V = variable (reactions ranged from susceptible to resistant), T = top necrosis, and * = no data.

^e NY-15 Z (Zaunmeyer strain) data from study at Kimberly, ID.

^f NY-15 Z data from study at Prosser, WA. The data for NY-15 P (Provvidenti strain), NY-15 S (Shellenberger strain), and NL-2 came from studies conducted at Prosser, WA.

^g Some RGB plants were partially resistant when tested.

^h Jubila was resistant 21 days after inoculation; however, some plants had developed isolated spots of veinal necrosis. If the experiment had been run longer, the other plants may have developed symptoms and, potentially, top necrosis.

sets of populations derived from crosses among the HG 3 cultivars were developed using the hook technique of pollination and selfing in a greenhouse (3). Three types of crosses were conducted: susceptible $\times$ partially resistant (populations 1 = Sapphire/Olathe, 2 = Sapphire/Victor, and 4 = Sapphire/UI 37), partially resistant $\times$ partially resistant (population 3 = Olathe/Victor), and susceptible $\times$ susceptible (population 5 = Sapphire/Ouray). Each set of populations comprised the parents, F_1 , F_2 , and backcrosses of the F_1 to both parents. The F_3 progeny obtained from inoculated F_2 plants that produced seed also were examined for reaction to NL-3 K.

Experimental design. To compare disease reactions of the parental cultivars used to develop the genetic populations along with representative cultivars of the differential host groups, 4 to 12 seed of each cultivar per virus strain were planted individually into 10-cm plastic pots containing Basic 2 Sunshine mix (70 to 80% Canadian sphagnum peat moss, perlite, dolomitic limestone gypsum, and wetting agent; Sungro Horticulture, Smithfield, UT) and challenged with 13 strains of BCMV or BCMNV. The differential cultivars (Table 1 for genotypes) tested included: Dubbele Witte (HG 1), Redlands Greenleaf C (RGC; HG 2), Redlands Greenleaf B (RGB; HG 3), Sanilac (HG 4), UI 31 (HG 6), Monroe (HG 6), IVT 7214 (HG 7), Black Turtle Soup I (HG 8), Jubila and Top Crop (HG 9), and IVT 7233 (HG 11). Additional

HG 3 differential cultivars tested included UI 123 and UI 59, and Kodiak was tested as a putative HG 10 cultivar (16). Individual pots served as the experimental unit and were arranged in a randomized complete block design with four replicates. The 13 virus strains inoculated onto separate plants included: US-1 (pathogroup 1), NL-7 (pathogroup 2), NL-8 ID (pathogroup 3; ID = Idaho strain), US-5 (pathogroup 4a), Western (pathogroup 4b), NY-15 (pathogroup 5a; Z = Zaumeyer strain, P = Provvidenti strain, and S = Shellenberger strain), NL-2 (pathogroup 5b), NL-3 (pathogroup 6a; K = Kimberly strain and D = Drijfhout strain), NL-5 (pathogroup 6b), and US-6 (pathogroup 7).

To further examine the differential response of the putative HG 3 cultivars, seed from parents, differential cultivars (Dubbele Witte, RGC, RGB, Sanilac, Monroe, IVT 7214, Black Turtle Soup I, Jubila, Topcrop, Amanda [HG 10], and IVT 7233), and five sets of populations (Table 2) were planted individually into 10-cm plastic pots containing Basic 2 Sunshine mix. Emerged plants were inoculated with BCMNV strain NL-3 K and subsequently evaluated for disease reaction. Individual pots served as the experimental unit and were arranged in a randomized complete block design with four replicates. Noninoculated plants of parents and differential cultivars also were grown for comparison with inoculated plants.

TABLE 2. Reactions and segregation ratios for populations 1 through 5 where the plants were inoculated with the NL-3 K strain of *Bean common mosaic necrosis virus*

Pedigree	Number of plants ^a		Expected ratio	χ^2	P
	PR	S			
Population 1					
Olathe	10	0	1:0	...	...
Sapphire	0	10	0:1	...	...
Sapphire/Olathe (F ₁)	0	10	0:1	...	...
Sapphire/Olathe (F ₂)	15	42	1:3	0.0526	81.86
Olathe//Sapphire/Olathe	8	17	1:1	3.2400	7.19
Sapphire//Sapphire/Olathe	0	23	0:1	0.0000	100.00
F ₃ from par. resist. F ₂	75 ^b	0	...	...	...
F ₃ from susceptible F ₂	6	33	...	...	...
Population 2					
Victor	10	0	1:0	...	...
Sapphire	0	10	0:1	...	...
Sapphire/Victor (F ₁)	0	9	0:1	...	...
Sapphire/Victor (F ₂)	21	72	1:3	0.2903	59.00
Victor//Sapphire/Victor	ND	ND	...	...	...
Sapphire//Sapphire/Victor	0	18	0:1	0.0000	100.00
F ₃ from par. resist. F ₂	41	0	...	...	...
F ₃ from susceptible F ₂	25	49	...	...	...
Population 3					
Victor	10	0	1:0	...	...
Olathe	10	0	1:0	...	...
Olathe/Victor (F ₁)	10	0	1:0	...	...
Olathe/Victor (F ₂)	96	0	1:0	0.0000	100.00
Victor//Olathe/Victor	25	0	1:0	0.0000	100.00
Olathe//Olathe/Victor	25	0	1:0	0.0000	100.00
Population 4					
UI 37	5	0	1:0	...	...
Sapphire	0	10	0:1	...	...
Sapphire/UI 37 (F ₁)	0	10	0:1	...	...
Sapphire/UI 37 (F ₂)	31	67	1:3	2.2993	12.94
UI 37//Sapphire/UI 37	11	14	1:1	0.3600	54.85
Sapphire//Sapphire/UI 37	ND	ND	...	...	...
F ₃ from par. resist. F ₂	71	0	...	...	...
F ₃ from susceptible F ₂	13	35	...	...	...
Population 5					
Ouray	0	8	0:1	...	...
Sapphire	0	10	0:1	...	...
Sapphire/Ouray (F ₁)	0	10	0:1	...	...
Sapphire/Ouray (F ₂)	6	80	1:15	0.0775	78.07
Ouray//Sapphire/Ouray	ND	ND	...	...	...
Sapphire//Sapphire/Ouray	0	24	0:1	0.0000	100.00

^a PR = partially resistant plants that have no deformed leaves, S = susceptible plants with deformed leaves, and ND = no data.

^b F_3 seed was generated by allowing the inoculated F_2 plants to produce seed. The F_2 population was subjected to selection pressure; therefore, the progeny cannot be used for testing genetic ratios. However, the F_3 population clearly shows which phenotypes in the F_2 contained genotypes that were homozygous or heterozygous.

In a subsequent experiment, the F_3 progeny derived from inoculated F_2 plants of populations 1, 2, and 4 that produced seed were screened with the NL-3 K strain. A total of 355 F_3 seed representative of all F_2 phenotypes except the top death phenotype were screened using methods described for the earlier generations. Seed from the top death phenotype were not included because these plants did not produce seed. The top death phenotype represents plants that had the terminal die but lacked systemic necrosis symptomatology associated with top necrosis of unprotected *I* gene cultivars. The parents and differential cultivars (the same set utilized with the earlier generation screening) also were included with this screening. The experiment was arranged in a randomized complete block design with five replicates.

Virus inoculations. All virus strains had been obtained from the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) and Washington State University (WSU) at Prosser and maintained for long-term storage as freeze-dried material at -20°C . All fresh inoculum was maintained on Dubbele Witte plants except for the NL-3 K strain, which was maintained in plants of the Ouray pure line in aphid-proof cages in the greenhouse. A triturate of infected tissue was prepared by macerating young trifoliolate leaves exhibiting strong mosaic symptoms in 50 mM sodium phosphate buffer (pH 7; 1 g of tissue per 10 ml of buffer) with a mortar and pestle and adding 600-mesh Carborundum powder (0.2 g per 10 ml of buffer). One primary leaf per plant was mechanically inoculated with the triturate when the primary leaves were 50 to 75% expanded (7 to 10 days after seeding). 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 $^{\circ}\text{C}$, a mean minimum of 20 $^{\circ}\text{C}$, and a mean maximum of 33 $^{\circ}\text{C}$. Supplemental lighting from sodium halide lamps was used to extend the photoperiod to 13 h.

Disease evaluations. All plants were evaluated 21 days after inoculation using a traditional approach of phenotyping (the following responses were recorded: leaf size and plant height compared with noninoculated controls, leaf curling and other deformities, rugose symptoms on the first trifoliolate leaf, isolated spots of veinal necrosis, veinal necrosis, mosaic, dead patches, collapsing leaves, top death, top necrosis, local lesions, and chlorosis). The phenotype separation between partially resistant plants and susceptible plants was based on leaf stunting and deformity

and plant height. The plants also were characterized with a quantitative rating system (30) using the VV rating which is based on a scale of 0 to 10 (0 = dead plant and 10 = healthy plant) and takes into account symptoms and plant vigor. In addition, the second trifoliolate leaf was collected 28 days after inoculation for testing by enzyme-linked immunosorbent assay (ELISA) to evaluate the plants for the presence of virus. ELISA assays were conducted by K. Eastwell (WSU) using the general potyvirus antibody. Statistical analyses were performed using SAS (version 8.2; SAS Institute, Cary, NC).

DNA extraction. DNA extractions were performed using a modified "mini-prep" method developed by Afanador et al. (1). The first trifoliolate leaf was detached as it began to open. Tissue was placed immediately in a 1.5-ml Eppendorf tube and frozen for at least 30 min at -80°C in a freezer. The tissue was then immersed in 0.75 ml of extraction buffer (2%, wt/vol cetyltrimethylammonium bromide, 1.4 M NaCl, 0.02 M EDTA, 0.1 M Tris-HCl, and 1% 2-mercaptoethanol at 65 $^{\circ}\text{C}$), macerated with a micropestle (Kontes KT749520-0090), and placed in a 65 $^{\circ}\text{C}$ water bath for 30 min. If tissue had not been completely macerated, a second maceration was performed. Then, 0.75 ml of Tris-buffered phenol/chloroform/isoamyl alcohol (25:24:1), pH 8.0, was added to the mixture. The tubes were gently inverted five times, and the mixture was centrifuged at $16,000 \times g$ for 5 min at 21 $^{\circ}\text{C}$. Approximately 700 μl of the top aqueous layer was transferred to a different 1.5-ml Eppendorf tube containing 400 μl of chloroform/isoamyl alcohol (24:1). The tubes were gently inverted five times and the mixture was centrifuged at $16,000 \times g$ for 5 min. Approximately 550 μl of the top aqueous layer was transferred to a different 1.5 ml Eppendorf tube containing 550 μl of ice-cold isopropanol and placed at -20°C for 15 min. This mixture was centrifuged at $16,000 \times g$ for 5 min, the top liquid layer was discarded, and the pellet was allowed to air dry for 20 min in a laminar hood. The pellet was resuspended in 100 μl of TE buffer (10 mM Tris, pH 8.0, and 1 mM EDTA) and 200 μl of ice-cold ethyl alcohol/ammonium acetate solution (9 ml of 200-proof ethyl alcohol/1 ml of 5 M ammonium acetate) was added and the mixture was placed at -20°C for 15 min. The mixture was centrifuged at $16,000 \times g$ for 15 min, the supernatant discarded, and the pellet air dried for 20 min in the laminar hood. The pellet then was resuspended in 200 μl of TE buffer and placed in a water bath at 65 $^{\circ}\text{C}$ for 1 h. The mixture was brought to room temperature (21 $^{\circ}\text{C}$). Then, 20 μl

Fig. 1. Amplicons from a multiplexed reaction generated with SBD5 (1,300-bp SCAR marker for *bc-12* gene) and SW13 (690-bp SCAR marker for *I* gene) primers. The molecular weight standard (lane M) is a 100-bp ladder (Life Technologies, Rockville, MD; the three double intensity bands are 2,072, 1,500, and 600 bp, respectively). No bands were present in the mastermix check with no DNA (lane 1) and Dubbele Witte (lane 4). Putative host group (HG) 3 check and pure line cultivars containing only the *bc-12* marker included UI 123 (lane 2), Sapphire (lane 9), Olathe (lane 10), Ouray (lane 11), UI 37 (lane 12), and Victor (lane 13). HG 10 cultivars with both markers were Beryl (lane 3), Redkloud (lane 6), and Kodiak (lane 7). The putative HG 8 cv. Brasil-2 (lane 8) also had both markers. The cultivar with only the *I* gene marker was Black Turtle Soup I (lane 5).

of RNase A solution, consisting of 1 µl of RNase A (R 4642; Sigma-Aldrich, St. Louis) per 19 µl of cell culture water (W 3500; Sigma-Aldrich) was added and left at room temperature for 30 min. DNA was quantified using an Eppendorf BioPhotometer (Brinkmann Instruments, Westbury, NY) and diluted to 25 ng/µl with cell culture water. The DNA samples were stored at -20°C until they were needed.

Polymerase chain reaction amplification. DNA from parents and differential cultivars Dubbele Witte, Black Turtle Soup I, UI 123, Kodiak, Beryl (HG 10), and Redcloud (HG 10) were probed with the SCAR markers SW13 (detects the presence of *I* gene) and SBD5 (detects the presence of *bc-l²*) via polymerase chain reactions. The reactions were performed oil-free in volumes of 25 µl which contained: 3.3 µl of cell culture water, 2.5 µl of 10× Stoffel buffer (Applied Biosystems, Foster City, CA), 2 µl of 10 mM dNTPs (Applied Biosystems), 2.5 µl for each of four 10 µM primers (multiplexed reaction with forward and reverse primers for the SW13 and SBD5 SCAR markers), 0.2 µl of AmpliTaq DNA polymerase (Stoffel fragment; Applied Biosystems), 5 µl of 25 mM MgCl₂, and 2 µl of DNA template (=50 ng of total DNA). Reagents and reactions were held at 4°C until the thermal cycler program was initiated. The primers for the *I* gene-linked SW13 SCAR marker (F: CACAGCGACATTAATTTTCCTTTC, R: CACAGCGACAGGAGGAGCTTATTA) were developed by Melotto et al. (18). The primers for the *bc-l²* gene-linked SBD5 SCAR marker (F: GTGCGGAGAGGCCATCCATTGGTG, R: GTGCGGAGAGTTTCAGTGTTGACA) were developed by Miklas et al. (20). Amplifications were performed using a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA). The cycler was programmed for 34 cycles of 10 s at 94°C, 40 s at 66°C, and 2 min at 72°C followed by 1 cycle of 5 min at 72°C.

Electrophoresis. Amplification products were electrophoresed through agarose gels (1.5% wt/vol) using TBE buffer (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA) for 5 h at 80 mA constant current. The gels were stained for 20 min with 40 µl of ethidium bromide solution (10 mg/ml of water) per 400 ml of TBE buffer and visualized with a DigiDoc-It System (UVP, Inc., Upland, CA).

RESULTS

The presence of the SBD5 SCAR marker suggests that all the putative HG 3 parental pure lines possessed the *bc-l²* gene (Fig. 1). Markers also indicated that Brasil-2 carried both *I* gene and *bc-l²*. However, disease reaction of Brasil-2 to NL-3 K and NL-5 strains indicated the presence of *I* gene only, because *I* gene was not protected. Similarly, Miklas et al. (20) observed the presence of SBD5 marker in kidney and cranberry lines that expressed only *I* gene reaction. The other differential cultivars had band patterns that were expected (20).

An overall summary of disease reaction of all HG differential and parental cultivars versus all BCMV and BCMNV strains is given in Table 1. A more detailed analysis of the differentials and parents screened along with the genetic populations is presented in Figure 2. Strains US-1, NL-8 ID, US-5, Western, NL-3 D, NL-5, and US-6 performed as expected when compared with Drijfhout's results (5). NL-7 performed as expected except for its resistant reaction on RGC, which made it similar to US-1. However, Sapphire and Ouray were susceptible to NL-7, indicating that this strain was different from US-1. The NY-15 strains performed normally except for reactions on RGB and RGC. RGC was resistant to the NY-15 Z strain and NL-2 when we expected susceptible reactions. We expected a resistant response to NY-15 on RGB but observed a partially resistant response with some strains. With NL-3 K, we observed resistant responses on most Jubila plants when we expected top necrosis. Some isolated spots of veinal necrosis did begin to appear on some Jubila plants by 21 days after inoculation.

When testing the putative HG 3 parents, we found that all were susceptible to US-5, Western, NL-5, and US-6 (Table 1). All parents were resistant to US-1, NL-8 ID, and some NY-15 strains. The parental lines Sapphire and Ouray differed from the other parental lines by giving a susceptible or more susceptible response to NL-7, NL-3 K, and NL-3 D. Ouray and Victor were both susceptible to NY-15 P and NL-2.

The differential cvs. Dubbele Witte, RGC, RGB, and Sanilac were susceptible to the NL-3 K strain of BCMNV based on

Fig. 2. Differential cultivars and parental pure lines were screened with the NL-3 K strain of *Bean common mosaic necrosis virus* and evaluated with a quantitative rating system (30) to generate the VV rating (a rating system based on symptoms and plant vigor where 0 = dead plant and 10 = healthy plant) and the general potyvirus antibody via an enzyme-linked immunosorbent assay (ELISA) to get absorbance values. Black Turtle Soup I, Top Crop, and Brasil-2 plants developed top necrosis and, therefore, were not sampled for ELISA.

ELISA results (Fig. 2). The quantitative rating indicated that RGC and RGB were partially resistant, with mean VV ratings of 7.75 and 7.55, whereas Dubbele Witte and Sanilac were susceptible, with mean VV ratings of 4.0 and 2.03. Black Turtle Soup I and Topcrop both had necrotic local lesions on the inoculated leaf followed by top necrosis (black root reaction) in response to NL-3 K. However, the top necrosis in Topcrop did not occur until $\approx$ 21 days after inoculation. The inoculated leaf on Jubila had local lesions but did not succumb to top necrosis as mentioned previously. Monroe and IVT 7214 did not have local lesions on the inoculated leaf and were resistant to NL-3 K, as expected. Amanda had localized vein necrosis lesions and IVT 7233 had only pinpoint local lesions on the inoculated leaf in response to NL-3 K.

Highly susceptible to partially resistant responses to NL-3 K were observed for the putative HG 3 parents (Fig. 2). The susceptible Sapphire plants were severely stunted, had deformed leaves with strong mosaic symptoms, no isolated spots of veinal necrosis, high ELISA values, and, eventually, top death. Although the terminal died, top death lacked systemic necrosis symptomology associated with top necrosis of unprotected *I* gene cultivars. The plants with top death had only the terminals collapse (no necrosis) beginning $\approx$ 8 days after inoculation and the severely stunted trifoliolates produced prior to terminal death remained alive and grew slightly over the remaining weeks. Veinal necrosis, ring spots, and dead patches (areas where the leaf collapsed but was not necrotic) on the inoculated primary leaves also were characteristic of plants with top death. Ouray plants also were stunted but not as severely as Sapphire, had deformed leaves with strong mosaic symptoms, no isolated spots of veinal necrosis, and exceptionally high ELISA values, which makes this line an excellent choice for generating inoculum for work with NL-3 K. The partially resistant Olathe, Victor, and UI 37 pure lines had normally shaped leaves and plant height, isolated spots of veinal necrosis in an occasional trifoliolate leaf, no mosaic, and occasionally had virus detectable by ELISA in the second trifoliolate leaf.

The F_1 between the partially resistant Olathe and susceptible Sapphire was susceptible (Table 2), indicating that partial resistance was a recessive trait. However, although Sapphire plants had top death and no rugose symptoms in the first trifoliolate leaf, the F_1 plants did not have top death but did have rugose symptoms in the first trifoliolate. The F_2 segregation into a partially resistant/susceptible 1:3 ratio suggests that partial resistance was controlled by one recessive gene (or allele). The phenotype separation was based on plant stunting and leaf stunting and curling. In the F_2 , 15 plants were partially resistant, which indicates that they all had normal plant height and leaf shape. The susceptible plants all had a very distinct mosaic, stunted deformed leaves, and stunted plant height. The backcross to Olathe segregated into a partially resistant/susceptible 1:1 ratio. The backcross to Sapphire was all susceptible. Data from the F_3 progeny test showed that the partially resistant F_2 plants were homozygous for disease reaction, whereas some susceptible plants were heterozygous. These F_3 data further support that the partial resistance segregating in the F_2 and backcrosses was controlled by a single recessive gene (or allele). The reactions of the parents and differential cultivars screened along with the F_3 progeny are summarized in Table 1.

As was the case with population 1, the F_1 between partially resistant Victor and susceptible Sapphire was susceptible, indicating that inheritance of resistance was recessive (Table 2). As in population 1, the F_1 did not have any top death and did have rugose symptoms in the first trifoliolate. The phenotype separation and segregation of plants in the F_2 , backcrosses, and F_3 was the same as in population 1, confirming the presence of a single recessive gene (or allele) for partial resistance to NL-3 K in Victor.

In population 3, plants in all the generations (parents, F_1 , F_2 , and backcrosses) were partially resistant (Table 2). The lack of segregation for susceptible plants in the F_2 and backcrosses indi-

cates that both Olathe and Victor contained a recessive resistance gene (or allele) for partial resistance to NL-3 K at the same locus. However, 54 of the plants in the F_2 had isolated spots of pinpoint veinal necrosis, which was different from the isolated spots of veinal necrosis noted in the plants of populations 1 and 2. An additional 24 plants in the F_2 had isolated spots of dead patches in some of the trifoliolates, which was another symptom not observed in plants in the first two populations.

As with populations 1 and 2, the F_1 between partially resistant UI 37 and susceptible Sapphire of population 4 was susceptible, indicating that resistance was inherited in a recessive manner (Table 2). As in populations 1 and 2, the F_1 did not have any top death and did have rugose symptoms in the first trifoliolate. The phenotype separation and segregation of plants in the F_2 , backcrosses, and F_3 was the same as in populations 1 and 2, again confirming that resistance to NL-3 K was associated with one recessive gene (or allele).

For population 5, two susceptible parents, Sapphire and Ouray, were crossed. Sapphire plants had more severe stunting and leaf deformation than those from Ouray, but did not have as high an ELISA absorbance value (Fig. 2). The F_1 plants had strong mosaic symptoms and severe stunting but no top death. The mean VV ratings for Sapphire, Ouray, and F_1 were 2.03, 4.45, and 3.22, respectively, indicating that the F_1 response was intermediate compared with the parents. In the F_2 , 6 individuals with partial resistance and 80 susceptibles were observed. This segregation fits a two-recessive-gene hypothesis, indicating that the two parents contained different complimentary recessive genes that imparted partial resistance to NL-3 K. However, phenotype separation in population 5 was difficult because symptoms from partially resistant to highly susceptible were continuous. Therefore, the phenotype separation for population 5 was based solely on the quantitative system. Six individuals were given a rating of six in the quantitative system (30), and another six had a rating of seven, which was the threshold in determining partial resistance. Of the six partially resistant plants, two had mild mosaic and four had isolated spots of veinal necrosis, including the two plants with mosaic. Furthermore, there were too few plants in the backcrosses to confirm the two-gene hypothesis.

DISCUSSION

Differential reactions to NL-3 K among the putative HG 3 cultivars were due to a recessive gene (or allele) in the partially resistant parents Olathe, Victor, and UI 37 that was absent in the susceptible parents Sapphire and Ouray. This recessive resistance was associated with the elimination of leaf stunting and deformity and plant height reduction. The presence of this gene (or allele) could explain why Drijfhout (5) found both susceptible and tolerant responses to NL-3 among the HG 3 differential cultivars RGB and UI 123. Drijfhout showed that RGB and UI 123 both contain *bc-u* and *bc-I²*; therefore, this new gene (or allele) could explain why UI 123 was tolerant to NL-3 and RGB was not.

Based on differential reactions among all of the HG 3 cultivars, it would appear that the new gene (or allele) allows for reactions very similar to that of *bc-I²* expressed by RGB, except that it conditions partial resistance to NL-3 strains, whereas RGB is moderately susceptible. RGB was the differential used by Drijfhout (5) to characterize and name *bc-I²*. Like RGB, the recessive gene (or allele) in Olathe and UI 37 gives complete resistance to NL-2 and NL-7. The susceptibility of Victor to NL-2 and NY-15 P, however, suggests it may possess yet a different gene (or allele) for resistance than that of Olathe and UI 37. All progeny in population 3 (Olathe X Victor) were partially resistant to NL-3 K, indicating that gene complementation occurred. However, the F_2 symptoms in population 3 of isolated spots of pinpoint veinal necrosis and dead patches did not occur in populations 1 and 2. These different symptoms, along with the differential responses to NL-2 and NY-

15 P, provide further evidence that Olathe and Victor may possess different genes (or alleles).

The putative HG 3 cultivars Ouray and Sapphire apparently lack the same *bc-1²* gene (or allele) found in RGB because they are susceptible to NL-2, NY-15 P, and NL-7. Within HG 3 differentials, UI 123 also differed slightly from the other cultivars for reactions to the different NY-15 strains. Thus, cultivars and differentials categorized as HG 3 are variable for reaction to different BCMV and BCMNV strains. In fact, no two HG 3 cultivars (putative or otherwise) had the same reaction profile to all strains (Table 1). Such variability could be due to different but similar alleles at a common locus such as *bc-1²*. A precedent for allelism at *bc-1* already has been established by Drijfhout (5) with the naming of *bc-1²*. Although *bc-1* and *bc-1²* have been described, perhaps other alleles at this locus with less differential effects are being detected in this study. This argument is confounded by the presence of the *bc-1²*-linked SBD5 marker in all HG 3 cultivars. However, if a complex locus or block of genes exists, then the marker could still be present while portions of the gene block are lost or experience change in function due to recombination or some other chromosomal rearrangement. For example, a marker linked with the *Ur-5* gene block (11) exists in many different lines that express similar yet variable resistance when challenged by a wide array of bean rust (*Uromyces appendiculatus* (Pers.:Pers.) Unger) strains (P. Miklas, unpublished data). The possible existence of different alleles at the *bc-u* locus which could interact differently with the *bc-1* locus also should be considered in future studies involving HG 3 differential cultivars.

The high level of resistance to NL-3 K in UI 59 (data not shown) is the same response observed in Olathe, UI 37, and Victor. UI 59 was selected directly from the Great Northern landrace. Olathe, UI 37, and Victor all have pedigree connections to the Great Northern landrace. Thus, this "new" source of recessive resistance to NL-3 K most likely derives from the Great Northern landrace, from which *bc-1²* also is derived. As *bc-1²* was being transferred from these original sources to newly developed cultivars, portions of the gene complex may have been lost, leading to susceptibility to more strains (RGB, Ouray, and Sapphire), but not enough to classify them as new genes or host groups due to the lack of strain differentials. The slight variability observed among HG 2 cvs. Puregold Wax, Immuna, and RGC when challenged by a wide array of virus strains (P. N. Miklas, unpublished data) further supports the above supposition for a complex locus at *bc-1*. Slight differential reactions to NL-3 strains also are observed between *I* + *bc-1* differentials Top Crop and Jubila.

Variability shown in Table 1 for the pathogroup 5 strains (NY-15 and NL-2) was greater than previously described. Numerous variations occurred between cultivars and lines containing *bc-1* and *bc-1²* across these strains. RGC was resistant to NL-7 when susceptibility was expected. Responses among parental lines also were not uniform to NL-7, indicating that unknown genes may be present. These varying responses among cultivars and lines possessing *bc-1* and *bc-1²* to the NY-15 strains and NL-2 indicate that at least one unknown gene (or allele) was present.

In population 5, the parents Sapphire and Ouray were susceptible to NL3-K (Fig. 2), but responded differently. Plants from Ouray were less severely stunted and did not suffer top death like plants from Sapphire. However, Ouray plants had much higher ELISA absorbance values. Although both parents were susceptible, the occurrence of 6 out of 86 F₂ plants with weak partial resistance to NL-3 K in population 5 indicated that Sapphire and Ouray each carried a complementary recessive resistance gene. The responses among the plants in population 5 represented a continuum from partially resistant to highly susceptible; therefore, the phenotype separation had to be based only on a quantitative rating system. This approach to phenotype separation is unproven; thus, we should proceed with caution in determining the number of genes responsible for this response. Nevertheless, the two suscep-

tible parents were able to complement one another. Additional data from backcrosses or F₃ progenies and tests of allelism with known resistance genes will be required to ascertain whether or not these were previously unrecognized genes.

The recessive gene (or allele) described herein is more effective than *bc-1²* and, thus, should be easily introgressed into bean common mosaic resistance breeding programs, given that it is present already in a wide array of bean market classes, including Pinto (Olathe), Pink (Victor), and Red Mexican (UI 37). An appropriate differential strain such as NL-3 K will need to be used in bean common mosaic screening programs to select new cultivars with this more effective gene (or allele). To name this new source of resistance and realize its full potential in breeding for bean common mosaic resistance, further allelism tests with *bc-1²* derived from RGB and UI 123 and *bc-1* derived from Immuna need to be conducted, independence from *bc-2* and *bc-3* loci needs to be established, and the protection it provides to the *I* gene needs to be examined. Until the relationship of this new gene (or allele) with the *bc-1* locus is fully understood, the use of SBD5 SCAR for marker-assisted selection of *bc-1²* should proceed with caution.

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