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DETERMINATION OF RESISTANCE TO BCMV IN DRY EDIBLE BEAN CULTIVARS AND BREEDING LINES

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INTRODUCTION

Bean common mosaic virus (BCMV) in beans (*Phaseolus vulgaris*) is controlled primarily through resistant cultivars and seed certification programs. Numerous BCMV strains have been identified, and no commercially available cultivars are resistant to all strains of the pathogen. Drijfhout has identified sources of resistance to BCMV strains and developed a set of cultivars to differentiate the strains (1, 2). Based on his study, seven pathogenicity groups ("pathogroups") and 11 host resistance groups were identified.

Different resistance genes result in different symptomatic responses to BCMV in beans. The dominant *I* gene confers resistance to all strains, whereas the recessive *bc* genes confer resistance to one or more specific strains. The strain specific genes also require the presence of the nonspecific *bc-u* gene to be active. Dominant *I* gene resistance takes the form of immunity or a hypersensitive response depending on environment/strain combinations. The hypersensitive response is known as "black root" and usually results in plant death. However, those cultivars possessing *I* gene and one or more of the recessive *bc* genes will not exhibit black root if the *bc* gene(s) confers resistance to the infecting strain of BCMV. This is termed "protected *I* gene" resistance.

Dry edible bean breeding programs in the Pacific Northwest have successfully relied on recessive gene resistance to develop cultivars in the pinto, pink, great northern, and red Mexican market classes which were resistant to the commonly occurring BCMV strains in the area (i.e., Type, NY-15, and Western strains). However, during the years 1989-1991, strain NL-8, in pathogroup 3, occurred frequently in Idaho (3). A second strain (US 10), in pathogroup 7 which was not previously known to occur in the United States, was also detected in several seedlots from Idaho and Washington (M.J. Silbernagel, unpublished data). These recent developments have prompted an enhanced effort in breeding for resistance.

The appearance of strains in pathogroups 3 and 7 is cause for concern. Strains in pathogroups 3 and 6 cause black root at moderate temperatures (i.e., temperature insensitive necrosis) in bean cultivars possessing the unprotected *I* gene, and several bean fields in southern Idaho sustained severe losses due to black root in 1991 (3). Since strains in pathogroup 7 were not known to occur in the United States, very few commercially available cultivars are resistant to strains in that pathogroup.

The objective of the present study was to determine which recessive resistance genes occur in dry edible bean cultivars and breeding lines not carrying the dominant *I* gene. The results of this study are currently being used in selecting resistant parents in the University of Idaho bean breeding program.

MATERIALS AND METHODS

BCMV strains - A modified pathogen screen using four BCMV strains [NL-3, NL-8 (Idaho), NY-15 (Zaumeyer), and Mexican] was employed. Strains were obtained from M.J. Silbernagel (Prosser, WA) and maintained on cultivar Dubbele Witte in separate aphid-proof cages.

Inoculation of host cultivars - Ten plants each of 37 cultivars and breeding lines were seeded into potting mix in 10 cm plastic pots (two seeds/pot) and inoculated with each of the BCMV strains. Four additional cultivars (Ember, Othello, UI 35, and UI 196) which possess *bc-2*^{2} were not included, because they had been tested extensively previously (1; M.J. Silbernagel, personal communication). We attempted to obtain seed of the cultivars from sources as close to the original release as possible. Ten uninoculated plants from each cultivar or breeding line served as negative checks, while inoculated plants of Redlands Greenleaf C, Monroe, and Black Turtle Soup served as positive checks. When the unifoliate leaves were fully expanded (9-11 days after seeding), plants were mechanically inoculated with a triturate of infected tissue and sodium phosphate buffer (pH 7) with a small amount of abrasive (Carborundum, 600 mesh) powder. A new cotton-tipped stick was used to inoculate each plant. After inoculation, plants were placed in the greenhouse at 30 C day/18 C night temperatures under natural light supplemented with artificial light from metal halide lamps to extend the photoperiod to 12 hours.

All plants were visually evaluated 21 days after inoculation for systemic mosaic and other foliar symptoms. In addition, leaf samples of the second trifoliolate leaf above the inoculated leaf were collected from a subset of the inoculated plants for serological verification by ELISA. Tests were conducted by G. Mink at the ELISA Laboratory at Prosser, WA using the broad spectrum monoclonal antibody (MAb) 197 and the serotype A specific MAb I-2.

RESULTS AND DISCUSSION

The interpretation of the data is based on Table 1 which is abbreviated from Drijfhout's Tables 6 and 31 (1). With pathogroups 3, 5, 6, and 7, it is possible to distinguish all presently known recessive resistance genes. Since we did not use the full set of pathogroups, there may be novel resistance reactions that were not observed. Such is the case for Fiesta where our results differ from the novel reaction observed by Silbernagel who used BCMV strains from all seven pathogroups (unpublished data). In addition, certain resistance genes may be epistatic to others (*bc-2*^{2} masks expression of *bc-1* and *bc-1*^{2}).

About one quarter of the entries produced results which clearly identified the recessive resistance gene(s) which were present. UI 126, for example, was homogeneous for disease reactions to the four strains and possessed *bc-1*^{2}. Gene *bc-1*^{2} occurred most commonly in the cultivars tested, either alone or in combination with *bc-2*.

The remainder of the entries had ambiguous results, that is, for a given cultivar/strain combination, some plants were resistant while others were susceptible. Ouray, for example, had homogeneous reactions to NL-3, NL-8, and NY-15, but had mixed results with the Mexican strain. Those Ouray plants which were susceptible to the Mexican strain possessed *bc-1*^{2}, whereas those that were resistant possessed *bc-1*^{2} *bc-2*. Spinel and ISB 462 are also heterogeneous, and some plants of both entries also appear to possess *bc-1*^{2} *bc-2*. UI 129 was homogeneous for disease reactions for all four virus strains and also possesses *bc-1*^{2} *bc-2*.

In some cultivars such as UI 31, mixed disease reactions occurred for all four virus strains making a determination of which resistance genes are present virtually impossible. Whenever mixed disease reactions occurred, the cultivar/strain combination was retested, usually with similar mixed results. Some

heterogeneous responses may have been due to escapes; however, we feel confident that our level of escapes was very low as indicated by the minimal heterogeneity found for resistance/susceptibility to the Mexican strain (Table 2).

The cultivars and breeding lines in this test were not known to possess the dominant *I* gene and would not be expected to develop black root. However, in a preliminary test with a mixture of NL-3 and an unidentified strain, two plants out of ten of cultivar UI 425 developed black root indicating the presence of the dominant *I* gene. Four other plants exhibited veinal necrosis or partial stem necrosis. Further tests are planned to verify this result and determine the incidence of the *I* gene in the population.

Another significant discovery occurred when the results of symptom evaluations were compared to the ELISA results. In general, the results were very similar. A positive mosaic reading almost always had a positive ELISA reading. Conversely, a negative mosaic reading usually had a negative ELISA reading, but occasionally a positive ELISA reading was obtained in the absence of symptoms. We rationalized that some inoculated plants were infected but did not show symptoms. This was most often found for cultivars possessing *bc-12*, and coincides with an observation of a tolerant reaction of some host group 3 cultivars when inoculated with pathogroup 6 strains (1,2).

In the few cases where mosaic symptoms were present and ELISA results were negative, we judged the plants to be susceptible. In cases where mosaic symptoms were absent and ELISA results were positive, we also rated the plants as susceptible but tolerant. Since most recessive *i* beans are not immune, they are technically susceptible to BCMV, at least in the inoculated leaf, since the virus replicates in that leaf and can usually be recovered (G. Mink, personal communication). From a plant breeding point of view, we and others make an arbitrary, but practical, distinction between resistance and susceptibility based on the uninoculated leaf symptoms and the ELISA reaction of the second trifoliolate leaf above the inoculated leaf at a defined period (21 days in our case) after inoculation.

It is possible that we may err in rejecting a germ plasm from further consideration in a breeding program by calling it susceptible or tolerant based on a positive ELISA result and negative mosaic symptom result. There may be an undescribed resistance gene or factor which renders the plant symptomless even though the virus is present in the tissue. These kinds of anomalous reactions may have resulted from a number of possible causes which need to be investigated.

The importance of verifying negative visual evaluations with ELISA readings cannot be overemphasized. For example, when NW 59 was inoculated with NL-3, all 10 plants showed no foliar mosaic symptoms, but all 10 plants were positive by ELISA. If the gene determination were based on visual symptoms, one would conclude that NW 59 possessed *bc-2²*; however, when the ELISA results are considered, *bc-12* is the obvious conclusion. Back inoculation to a susceptible cultivar could be used instead of ELISA, but ELISA is more rapid, and requires less greenhouse space.

The heterogeneous reactions of many cultivar/strain combinations presents bean breeders with a dilemma. For example, the cultivars Viva and Victor have homogeneous disease reactions to NL-8, NY-15, and Mexican strains, but heterogeneous reactions to NL-3. Does the breeder state the predominant disease reaction ("Viva and Victor are resistant to NL-8 and NY-15 and are susceptible to NL-3 and Mexican") and ignore the small percentage of plants resistant to NL-3? Would reselection based on screening results produce genetically "pure" cultivars with stable, predictable results? We think so.

Similarly, UI 114 pinto, released in 1967, has long been considered resistant to NY-15 under field conditions (i.e., shows only mild, ephemeral foliar symptoms with no seed transmission) and to NL-8; however, our unpublished tests indicate that a small percentage of plants produced from seed deposited in

the National Seed Storage Laboratory in 1966 are susceptible to NY-15 (Zaumeyer) and NL-8 (Idaho) and show strong foliar mosaic symptoms to both strains. The UI 114 (Pinto 114) and UI 31 (Great Northern) used in Drijfhout, et al. and Drijfhout's studies (1, 2) as BCMV differentials were selected as a single plant from bulk populations of UI 114 and UI 31 by Silbernagel (personal communication). This would explain the lack of heterogeneity found in Drijfhout's studies.

Heterogeneity for BCMV resistance may originate from several sources. Cultivar mixtures are one possibility, particularly from seed stocks that have not been recently rejuvenated. Such may be the case with UI 31 where we could not find an original seed stock. Mutation in a homogeneous stock is possible, but most likely would remain at low frequency in the population. Heterogeneity is more likely to result from residual variation from the original cross. It has been a practice for many years to release breeders seed composed of 100-200 phenotypically similar, late generation, single plant selections bulked together. In the case of BCMV, many breeders have, until recently, only tested for resistance to Type and NY-15 strains, while leaving unselected resistance for other strains, because those strains may not have been present at release. Under certain situations, such as component breeding for mixtures, heterogeneity may be desirable.

In summary, resistance gene *bc-12* was most commonly detected in the cultivars and breeding lines tested; it confers resistance to BCMV strains in pathogroups 3 and 5 and susceptibility to strains in pathogroups 6 and 7. Other genes or gene combinations detected included *bc-2*, *bc-2²*, *bc-12bc-2*, and *Ibc-12*. The importance of verifying negative visual mosaic evaluations by ELISA or bioassays is emphasized. Infections by strain NL-3 in pathogroup 6 were occasionally symptomless, especially in cultivars in host group 3 which possess *bc-12*. Cultivar UI 425 was found to be heterogeneous and possessed the dominant *I* gene in a small percentage of plants.

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LITERATURE CITED

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Table 1. Disease reactions induced by four strains of bean common mosaic virus on bean hosts (*Phaseolus vulgaris*) to determine which recessive resistance genes are present[a].

			BCMV strain and pathogroup (PG)[b]			
Host Group	**Differential Cultivar**	**Resistance Gene(s)**	**NL-8 (PG3)**	**NY-15 (PG5)**	**NL-3 (PG6)**	**Mexican (PG7)**
1	Dubbele Witte	*Bc-u* or *bc-u*	+	+	+	+
2	Redlands Greenleaf C	*bc-u bc-1*	-	+	+	+
3	Redlands Greenleaf B	*bc-u bc-1²*	-	-	+	+
4	Sanilac	*bc-u bc-2*	+	+	+	-
5	Pinto 114	*bc-u bc-1 bc-2*	-	+	+	-
6	Monroe	*bc-u bc-2²*	-	-	-	+
7[c]	IVT 7214	*bc-u bc-3*	-	-	-	-
--	--	*bc-u bc-1² bc-2*	-	-	+	-
--	--	(undescribed)	+	-	+	+

[a]Abbreviated from Tables 6 and 31 in Drijfhout, E. 1978. Genetic interaction between Phaseolus vulgaris and bean common mosaic virus, with implications for strain identification and breeding for resistance. Agric. Res. Rep. 872 (Wageningen).

[b] "+" = susceptible; "-" = resistant

[c]Dominant *I* gene may produce an immune reaction with certain strains that is indistinguishable from that produced by *bc-3*. In many but not all cases, inoculation with a necrotic strain will distinguish between *I* and *bc-3*. Pathogroup 3 (NL-8) will produce necrotic local lesions, veinal necrosis, and tip kill (black root) in a completely unprotected *I* genotype, but will only produce necrotic local lesions and/or veinal necrosis of the inoculated leaf if *I* is in the presence of *bc-1*, *bc-1²*, or *bc-2²*. In pathogroup 6, NL-3 will produce necrotic local lesions, veinal necrosis, and tip kill on *I* and *Ibc-1* genotypes, but will not produce black root on *Ibc-1²* or *Ibc-2²*. In contrast, NL-5 which is also in pathogroup 6 differs from NL-3 in that it will cause black root on *Ibc-1²* and only produces restricted necrotic local lesions on the inoculated leaf of *Ibc-2²* genotypes (M.J. Silbernagel, personal communication).

Table 2. Disease reactions and most probable resistance genes of pinto, pink, great northern and red Mexican cultivars and breeding lines inoculated with four strains of bean common mosaic virus.

Cultivar/ Line	BCMV Strain and Pathogroup (PG)								Most Probable Resistance Gene(s)[c]
	NL-8 (ID) (PG 3)		NY-15(Z) (PG 5)		NL-3 (PG 6)		Mexican (PG 7)		
	Mosaic[a]	ELISA[b]	Mosaic	ELISA	Mosaic	ELISA	Mosaic	ELISA	
Agate	10-	1+/8-	10-	5-	9+/2-	8+	10+	2+	*bc-1*^{2}
Bill Z	1+/9-	5+/5-	10-	5+/3-	NT	NT	10+	4+	none
Retest	11-	9+/2-	11-	8+/1-	8+/2-	4+/2-	8+/3-	9+	
Emerson	8-	2+/2-	10-	6+/4-	NT	NT	10+	6+	*bc-1*^{2}
Retest	10-	1+/5-	10-	4-	8+/4-	8+	11+	4+	
Fiesta	10-	10-	10-	1+/9-	1+/10-	8-	10+	10+	*bc-2*^{2}
Retest	NT	NT	12-	4-	NT	NT	NT	NT	
Gala	11-	7-/1+	10-	4-	12-	10-	8+	4+	*bc-2*^{2}
Garnet	10-	4-	10-	4-	12-	3+/5-	10+	4+	*bc-2*^{2}
Gloria	4-	4-	4-	4-	2+/6-	6-	2+/2-	3+/1-	*bc-2*^{2}
Retest	NT	NT	NT	NT	NT	NT	7+	4+	
Harold	10-	5-	10-	5-	3+/6-	7+/2-	10+	4+	*bc-1*^{2}
Harris	8-	6-	8-	1+/3-	7+	4+	8+	4+	*bc-1*^{2}
ISB 462	10-	10-	10-	2+/8-	11+	8+	9-	9-	*bc-1*^{2} *bc-2*
Retest	NT	NT	10-	9-	NT	NT	NT	NT	
Ivory	10-	4-	10-	6-	11-	4+/1-	10+	4+	*bc-1*^{2}
Nodak	1+/9-	2+/8-	2+/8-	4+/6-	NT	NT	10+	10+	none
Retest	10-	2+/2-	9-	3+/4-	8+/2-	6+	12+	4+	
NW 410	7+/3-	8+/2-	10+	10+	12+	4+	10-	10-	*bc-2*
Retest	10-	8+/1-	NT	NT	NT	NT	NT	NT	
NW 59	9-	4-	10-	1+/3-	10-	10+	9+	4+	*bc-1*^{2}
NW 590	2+/7-	2+/7-	1+/8-	1+/8-	NT	NT	7+/3-	7+/3-	Unknown
Retest	8-	6+/2-	9-	4-	8+/2-	10+	6+/4-	6+/1-	
NW 63	10-	4-	10-	4-	1+/11-	12+	10+	4+	*bc-1*^{2}
Olathe	10-	10-	10-	10-	2+/10-	12+	10+	10+	*bc-1*^{2}
Ouray	10-	4-	10-	4-	9+	7+	6+/4-	3+/3-	*bc-1*^{2}
Retest	NT	NT	NT	NT	NT	NT	6+/2-	4+/2-	
Pindak	1+/8-	1+/8-	8-	4+/4-	NT	NT	8-	2+/6-	*bc-1*^{2} *bc-2*
Retest	8-	4-	2+/5-	2+/5-	8+	5+	8-	5-	

Table 2. (continued)

Cultivar/ Line	BCMV Strain and Pathogroup (PG)								Most Probable Resistance Gene(s)[c]
	NL-8 (ID) (PG 3)		NY-15(Z) (PG 5)		NL-3 (PG 6)		Mexican (PG 7)		
	Mosaic[a]	ELISA[b]	Mosaic	ELISA	Mosaic	ELISA	Mosaic	ELISA	
Roza	7+/3-	10+	8+/2-	9+/1-	NT	NT	10+	10+	none
Retest	10-	7+/3-	2+/7-	5+/2-	NT	NT	10+	4+	
Retest	2+/9-	7+/4-	12-	9+/3-	11+	5+	10+	6+	
Rufus	10-	6-	10-	7-	5+/6-	7-	10+	4+	*bc-1*^{2} or *bc-2*^{2}
Sapphire	10-	1+/3-	9-	3+/1-	NT	NT	10+	3+/1-	*bc-1*^{2}
Retest	7-	4-	10-	2+/4-	8+/1-	5+	8+/1-	5+	
Spinel	10-	10-	8-	8-	5+/4-	7+	10-	10-	*bc-1*^{2} *bc-2*
Star	1+/9-	3+/3-	10-	4-	7-	6+/1-	10+	NT	*bc-1*^{2}
Retest	7-	1+/4-	NT	NT	NT	NT	NT	NT	
Topaz	10-	6-	11-	6-	1+/9-	8+/2-	7+/3-	2+/2-	*bc-1*^{2}
Retest	NT	NT	NT	NT	NT	NT	11+	4+	
UI 31	8+/2-	6+/2-	4+/6-	2+/5-	NT	NT	5+/5-	4+/4-	none[d]
Retest	8-	2+/6-	2+/5-	2+/5-	2+/4-	6+/1-	1+/6-	3+/4-	
UI 59	10-	6-	10-	6-	7-	5+/2-	9+/1-	5+/1-	*bc-1*^{2}
Retest	NT	NT	NT	NT	NT	NT	2+/4-	5+/1-	
UI 60	10-	5-	10-	5-	6+/5-	9+	10+	6+	*bc-1*^{2}
UI 126	10-	10-	9-	9-	12+	6+	9+	9+	*bc-1*^{2}
UI 129	10-	10-	10-	10-	12+	8+	10-	10-	*bc-1*^{2} *bc-2*
UI 425	10-	4-	10-	6-	2+/7-[e]	1+/8-	1+/11-	4+/8-	*I bc-1*^{2}
Retest	NT	NT	NT	NT	NT	NT	4+/4-	4+/4-	
UI 537	10-	1+/9-	10-	10-	1+/10-	7+/2-	10+	10+	*bc-1*^{2}
Retest	12-	6-	NT	NT	NT	NT	NT	NT	
Victor	5-	5-	6-	6-	2+/9-	9+/1-	6+	6+	*bc-1*^{2}
Viva	6-	6-	6-	6-	2+/9-	10+/1-	6+	6+	*bc-1*^{2}
WY 166	10+	3+	10+	4+	10+/2-	6+	8-	2+/4-	*bc-2*
Retest	NT	NT	NT	NT	NT	NT	1+/10-	5-	
Yolano	10-	10-	10-	10-	11-	7-	10+	10+	*bc-2*^{2}
6315	10-	10-	10-	1+/9-	3+/8-	9+/2-	9+	9+	*bc-1*^{2}
Retest	NT	NT	10-	4-	NT	NT	NT	NT	

[a]Foliar mosaic symptoms. "+" = symptoms present; "-" = symptoms absent.

[b]ELISA reaction. "+" = positive reaction; "-" = negative reaction. The sum of the "+" and "-" equals the number of plants tested which was usually a subset of the inoculated plants.

Table 2. (continued)

[c]*bc-u* resistance gene is present in combination with indicated gene(s).

[d]According to Drijfhout (Agric. Res. Rep. 872 (Wageningen), 1978), UI 31 possesses $bc\text{-}1^2$ $bc\text{-}2^2$.

[e]A separate test showed 2 plants out of 10 developed black root when inoculated with a mixture of NL-3 and an unidentified strain.

NT=Not tested.