BEAN COMMON MOSAIC

Screening for Disease Resistance

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Cover photo:
Typical common mosaic symptoms induced by bean common mosaic virus in *Phaseolus vulgaris*, showing the characteristic vein-banding mosaic.

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Contents

Introduction

The dry or common bean, *Phaseolus vulgaris* L., is one of the most important food legumes grown worldwide. In Africa and Latin America, beans are an important protein source in the diet of hundreds of low-income rural and urban communities. The socioeconomic importance of bean production is thus recognized by most national agricultural research institutes, which devote considerable time and effort to the study and control of the various biotic and abiotic constraints of this crop. Among the numerous biotic constraints reported so far, bean common mosaic is undoubtedly the most ubiquitous limiting factor for bean production. This fact has led the CIAT Bean Program to select the common mosaic resistance trait as a prerequisite for the genetic improvement of bean germplasm. The purpose of this publication is to describe the methodology implemented at CIAT to select common mosaic-resistant bean genotypes.

Identification of Bean Common Mosaic Virus

Symptomatology

Bean common mosaic virus (BCMV) may induce two general types of symptoms, depending on the susceptible bean genotype and virus strain.

Common mosaic. Symptom expression is the result of the interaction among a plant genotype, a pathogen, and the environment. Consequently, common mosaic symptoms may vary according to the bean genotype, BCMV strain, and predominant environmental conditions. Nevertheless, most common mosaic-susceptible bean cultivars express characteristic symptoms, particularly during the late stages of the viral infection

process. Typical common mosaic symptoms consist of well-defined dark green areas of foliar tissue against a lighter green background of the rest of the affected leaf lamina (Figure 1*). Mosaic-affected leaves usually show downward curling and, in some instances, severe foliar distortion or blisters (Figure 2). When plants develop from virus-infected seed, common mosaic symptoms are usually evident on the primary (cotyledonary) leaves (Figure 3).

Black root. Some bean genotypes possess hypersensitive, monogenic-dominant resistance to the mosaic-inducing strains of BCMV. However, there are other strains of BCMV, called "necrotic," which can challenge this dominant resistance, inducing the plant to react with a systemic necrosis known as "black root." This necrotic reaction begins in the youngest trifoliate leaves (Figure 4), and descends rapidly down the entire vascular system of the plant, including the pods (Figure 5). Plants affected by black root initially exhibit wilting and later die (Figure 6).

Transmission

Seed transmission. The ability of BCMV to infect the seed embryo and thus be transmitted in susceptible bean plants via the seed is the most important epidemiological factor responsible for the worldwide distribution of this virus. On the average, one can expect 10%-30% BCMV transmission to plants grown from seed produced by a systemically infected bean plant. The incidence of BCMV seed transmission, however, may vary considerably depending on the infected bean genotype and the BCMV strain. It is usually expected that bean plants infected after their flowering stage will not produce a high proportion of BCMV-infected seed.

The seed transmissibility of BCMV is another diagnostic characteristic, since not all bean viruses are seed-borne. Bean southern mosaic and cucumber mosaic viruses are also seed-

* Figures 1-16 can be found in the middle of the book.

transmitted in *P. vulgaris*, but they induce different symptoms in seed-infected bean seedlings.

Insect transmission. BCMV is also transmitted by several aphid species, such as *Myzus persicae* and *Aphis fabae*. BCMV is transmitted by aphids in a "nonpersistent" manner, which means it can be acquired and transmitted from infected to healthy bean plants in a matter of seconds. In Latin America, where aphids seldom colonize beans, BCMV is transmitted by winged aphid vectors alighting on bean fields from different crops. In eastern Africa, where *Aphis fabae* is a serious bean pest, BCMV can be more efficiently transmitted within bean plantings.

For transmission experiments, *Myzus persicae* can be reared on pepper, radish, or another suitable host. Nymphs are preferred to winged individuals since they do not fly away during the critical virus acquisition and transmission periods. Aphids can be handled with a small brush moistened with tap water. In most cases, five aphids per plant should be able to transmit BCMV to individual bean seedlings (preferably at the cotyledonary leaf stage). Under controlled conditions, one or two aphids should transmit BCMV, following 30- to 60-second virus acquisition and transmission probes.

Manual transmission. BCMV is readily transmitted by manual or mechanical means. The inoculum may be obtained by grinding the first and second trifoliate leaves of 15-day-old bean plants previously inoculated with BCMV at the cotyledonary leaf stage (approximately 8-day-old seedlings). The extracted sap can be diluted ten times with water or 0.01 M KPO_4 buffer (pH 7.5) to prepare the inoculum. The diluted sap containing the virus should be maintained cold until it is rubbed on test plants, using a small piece of cheesecloth or gauze. The use of abrasive powders, such as carborundum, is not necessary since the cheesecloth pad causes the necessary abrasion. The inoculum and the cheesecloth pads should be changed frequently to avoid possible contamination of the inoculum with other seed-borne viruses often found in test plants. Common mosaic symptoms should develop within 14 days in the inoculated plants.

Responses of differential bean cultivars to BCMV

Four basic plant responses may be expected in bean genotypes inoculated with BCMV: mosaic; systemic necrosis or restricted vein necrosis; local lesions; and immunity (no symptoms).

Mosaic. This symptom is the result of the systemic infection of a susceptible cultivar by BCMV. This "chronic" infection indicates that the inoculated bean genotype does not possess the dominant necrosis (I) gene. Mosaic-affected genotypes may or may not possess strain-specific recessive genes which confer resistance to selected BCMV strains.

Systemic or local necrosis. The appearance of necrosis indicates that the inoculated bean plant possesses the necrosis (I) gene. The necrotic reaction may be localized (restricted vein necrosis) or systemic (black root). These "hypersensitive" bean genotypes may or may not possess BCMV strain-specific recessive genes.

Local lesions. The induction of *ring-shaped local lesions* on the mechanically inoculated primary (cotyledonary) leaves of the bean cultivar "Monroe" is a complementary diagnostic feature for BCMV (Figure 7). These lesions are best observed when the environmental temperature is within a 16-28 ^{0}C range, since at lower or higher temperatures this type of local lesion may not be induced. It is important to bear in mind that these lesions can also be caused by related viruses, such as soybean mosaic virus. Genotypes showing this type of ring-shaped lesion are not supposed to carry dominant (I gene) BCMV resistance. The appearance of *pin-point local lesions*, on the other hand, suggests that the genotype has the I gene, protected by a recessive gene (Figure 8).

Immunity. The absence of mosaic or necrosis symptoms in a BCMV-inoculated bean genotype may suggest different plant-virus interactions: the plant is truly immune; the plant escaped infection; and the plant is infected but remains symptomless.

For diagnostic purposes, the value of these plant responses is that all BCMV strains should induce common mosaic symptoms in highly sensitive bean genotypes such as Dubbele Witte,

Bountiful, or Stringless Green Refugee. Conversely, no mosaic symptoms should occur in common mosaic-resistant cultivars such as Topcrop, Black Turtle Soup, Jubila, etc. If mosaic symptoms do occur in this latter group of cultivars, it would suggest that the causal virus is not BCMV.

Cytology

Light microscopy. It is possible to observe the presence of the large protein inclusions induced by BCMV in the cytoplasm of infected bean cells, using a light microscope (Figure 9). This cytological technique (Christie and Edwardson, 1977) requires the selection of common mosaic-affected leaf tissue, preferably showing advanced symptoms. Using a pair of fine, straight-tipped tweezers a small (5 x 3 mm) strip of tissue, preferably from the large leaf veins on the lower side of the leaf, is obtained by inserting the tweezers under the epidermis. The epidermal strip is then treated with 5% Triton X-100 for 5 minutes to destroy cytoplasmic organelles, such as plastids, which may look like virus-induced inclusions to the untrained eye. The excess Triton can be removed by placing the treated plant tissue on a piece of blotting paper. The tissue is then treated for 15 minutes with a mixture containing water, Calcomine orange 2 RS, and "Luxol" brilliant green at a 1:1:8 volume proportion, respectively. Once stained, the epidermal strip is treated with absolute ethanol (for 15-30 seconds) prior to mounting on a glass slide, in Euparal or Canada balsam. The inclusions are observed with the oil immersion lens or at a lower magnification. For further information on this technique, you can write to Mr. R. Christie, Agronomy Department, University of Florida, Gainesville, FL 32611, U.S.A.

Electron microscopy

Infected plant extracts. The transmission electron microscope makes possible the detection of filamentous viruses, such as BCMV, within minutes. BCMV has flexuous particles 700-800 nm (1 meter x 10^{-9}) long and 12-15 nm in diameter (Figure 10),

typical of all the members of the potyvirus group. To obtain a sample for electron microscopy, a 1-cm² tissue section is obtained, preferably from a mosaic-affected leaf area, and gently slashed with a razor blade in a few drops of 2% phosphotungstic acid or uranyl acetate. Once the sap has diffused into the stain, a drop is transferred by means of a Pasteur pipette onto an electron microscope sample grid. The excess sample stain is removed with a piece of filter paper and the grid, once dry, is observed with the electron microscope.

Infected plant tissue. Bean tissue showing mosaic symptoms can be prepared for cytology with the aid of an ultramicrotome, for observation in a transmission electron microscope. As mentioned before, BCMV induces the formation of large (*1* µm) protein inclusions in the cytoplasm of infected cells. Thanks to the high resolution of the electron microscope, one can see that these inclusions have a cylindrical morphology. These inclusions, known as "pinwheels" (Figure 11), are characteristic of the potyvirus group. The implementation of this technique obviously requires special equipment and materials.

Purification and serology of BCMV

Most BCMV strains can be isolated by following the purification procedure outlined in the Appendix. Some *necrosis*-inducing strains, such as BCMV NL3, are isolated in larger quantities than the type strain. This purification procedure, however, requires a well-equipped virology laboratory if highly purified virus is required to produce a specific antiserum.

In the absence of an ultracentrifuge, it is possible to obtain a partially purified virus preparation by omitting the high speed centrifugation steps. These partially purified virus preparations can be used to immunize laboratory animals, but the resulting antiserum will obviously react with extracts from virus-free plants. Nevertheless, these contaminated (host-plant proteins) antisera can be useful for serological tests such as the immunosorbent electron microscopy (ISEM) test, described below.

Serological tests. Although there are several serological assays, the most adequate for BCMV are the Ouchterlony test (double diffusion in agar), the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent electron microscopy (ISEM) test.

Double immunodiffusion (Ouchterlony) test. The double immunodiffusion test is a standard serological method in most virology or microbiology laboratories. However, it requires an antiserum of medium-to-high specificity and special reagents of good quality. The test for BCMV is conducted as described by Purcifull and Batchelor (1977). Basically, healthy and infected plant extracts are placed in small peripheral wells made in an agar medium, around a central well which generally contains the antiserum. If present, viruses diffuse in the agar toward the antibodies, which also diffuse in the agar in all directions from the central well. Once the virus and its specific antibodies come into contact, they produce a visible precipitin line, best seen by placing the serological plates under a diffuse light source (Figure 12). The use of virus-free bean tissue extracts as controls is critical in this test to verify that the antiserum is not contaminated with antibodies against normal proteins. Bean seeds usually produce a strong nonspecific precipitin reaction; therefore, this test is not suitable to detect viruses in bean seed.

Enzyme-linked immunosorbent assay. The enzyme-linked immunosorbent assay (ELISA) is a highly sensitive serological test based on the conjugation of the virus-specific antibody with an enzyme. After the antibody-enzyme complex attaches to the virus, a suitable substrate for the selected enzyme is added to produce a colorimetric reaction which can be recorded visually or spectrophotometrically. The advantage of this technique is that a considerably lower number of antibodies (less antiserum) are required to produce a visual reaction, due to the high sensitivity of the enzyme (attached to the antibody) to its substrate.

The disadvantages of the ELISA technique are the cost of the reagents and materials required, relative to the gel diffusion methods, and the high purity of the virus required to produce suitable antibodies (without host-plant contaminants).

The ELISA technique, however, is the indicated serological test to detect viruses in bean seed, as discussed later in this publication. For technical details and application for the detection of BCMV in seed, the reader is referred to the publications by Clark and Adams (1977), and Jafarpour and Shepherd (1978), respectively.

Immunosorbent electron microscopy. The immunosorbent electron microscopy (ISEM) or serologically specific electron microscopy (SSEM) technique permits simultaneous trapping and identification of virus particles on electron microscope grids by prior treatment of the grids with virus-specific antibodies (Derrick, 1973).

The advantage of this serological technique is the direct observation of several virus particles with the electron microscope, and the rapid identification of the virus, based on the higher number of virus particles "trapped" (relative to an untreated grid in which only one or very few virus particles may be seen). The main disadvantage, of course, is the requirement of an electron microscope.

BCMV strain characterization

Once the virus has been diagnosed as BCMV, or as a simultaneous process, a set of 20 bean cultivars can be inoculated to characterize BCMV at the strain level (Drijfhout, 1978). The expected reactions of these differential cultivars to the known strains of BCMV are described in Table 1. It is recommended that differential cultivars in groups 7 to 9 be maintained within a 26-30 ^{0}C range to detect temperature-dependent, necrosis-inducing BCMV strains.

Those cultivars which remain symptomless should be tested for the presence of the virus by back-inoculation to a highly sensitive bean cultivar, such as Dubbele Witte, or by any other suitable virus detection method, such as serology or electron microscopy.

Once a BCMV strain has been identified, it is advisable to maintain it in the seed of its respective differential cultivars. For instance, BCMV NL4 could be maintained in seed of group 6

cultivars. However, the strains should also be maintained in the seed of a cultivar belonging to group 1, since some genotypes do not efficiently transmit BCMV through the seed (Morales and Castaño, 1987). Obviously, the necrosis-inducing strains must be maintained in mosaic-susceptible genotypes, since mosaic-resistant bean genotypes do not transmit BCMV in the seed.

Table 1. Classification of bean common mosaic virus strains.

Cultivar group	Differential cultivar	BCMV strain[a]									
		Type	Fla	NY15	NL2	NL3	NL4	NL5	NL6	NL7	NL8
Cultivars possessing recessive (I^+I^+) resistance											
1	Dubbele Witte	+	+	+	+	+	+	+	+	+	+
	Stringless Green Refugee	+	+	+	+	+	+	+	+	+	+
2	Redlands Greenleaf C		+	+v	+	+v	+	+	+	+	
	Puregold Wax		+	+v	+	+v	+	+	+	+	
	Imuna		+	+v	+	+v	+	+	+	+v	
3	Redlands Greenleaf B		+			+	+	+	+		
	Great Northern 123		+			+	+	+v	+		
4	Sanilac			+	+	+		+			+
	Michelite 61			+	+	+		+			+
	Red Mexican 34			+	+	+		+			+
5	Pinto 114			+	+	+		+			
6	Monroe						+				
	Great Northern 31						+				
	Red Mexican 35						+				
Cultivars possessing dominant (II) resistance											
7	Widusa					+		+	+t		+
	Black Turtle Soup					+		+	+t		+
8a	Jubila				+t	+		+	+		
8b	Topcrop				+t	+		+	+t		
	Improved Tendergreen				+t	+		+	+t		
9	Amanda							+			

a. Reactions to BCMV strains: + = mosaic in I^+I^+ cultivars or systemic necrosis in II cultivars; +v = variable and mild symptoms; +t = systemic necrosis at high temperatures; blank spaces = no reaction.

SOURCE: Drijfhout, 1978.

Genetic Improvement of Bean Cultivars for Their Resistance to BCMV

Since producing virus-free bean seed or controlling migrant aphid vectors in bean plantings is a difficult task seldom accomplished, the incorporation of genetic resistance to BCMV has been the most recommended and widely practiced control method.

Sources of BCMV resistance

According to Drijfhout (1978), resistance to BCMV in *Phaseolus vulgaris* is conditioned by nonspecific recessive genes (bc-u), strain-specific recessive genes (bc-1 to 3), and a dominant "necrosis" (I) gene. The genotype of the various groups of differential cultivars is shown in Table 2, together with the pathogenicity genes of the known BCMV strains (Drijfhout, 1978).

Incorporation of BCMV resistance in mosaic-susceptible bean cultivars

There are two basic methods to incorporate genetic resistance into mosaic-susceptible bean genotypes: by introducing recessive genes specific to a particular BCMV strain or group of strains of the virus, and by incorporating the I gene, which precludes the chronic systemic infection of bean genotypes by any of the known BCMV strains.

Resistance to selected BCMV strains. This breeding objective first requires a thorough knowledge of all the BCMV strains present in a given geographical area and, second, a strict control of the phytosanitary quality of imported bean seed to exclude the possibility of introducing new BCMV strains. In case some of the mosaic-inducing strains of BCMV are not present in the area, the appropriate mosaic-resistant genotypes could be selected as parental materials among cultivar groups 2 to 6, possessing BCMV strain-specific recessive resistance genes (Table 2).

Photographs

Figure 1. Bean common mosaic symptoms.

Figure 2. Foliar malformation.

Figure 3. Common mosaic in primary leaves.

Figure 4. Early black-root symptoms.

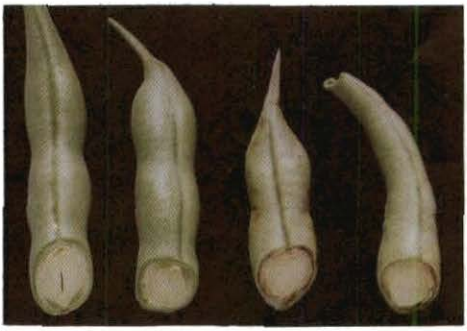

Figure 5. Black-root symptoms in pods.

Figure 6. Advanced black-root symptoms.

Figure 7. Ring-shaped local lesions.

Figure 8. Pin-point local lesions.

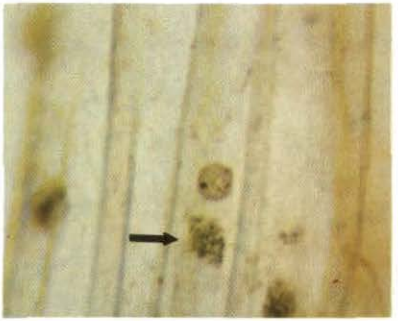

Figure 9. Cytoplasmic virus-induced inclusions.

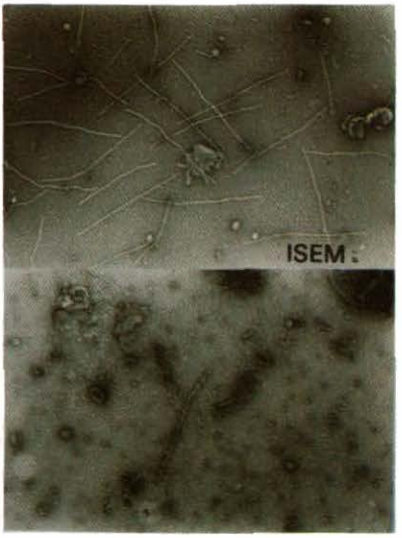

Figure 10. Virus particle.

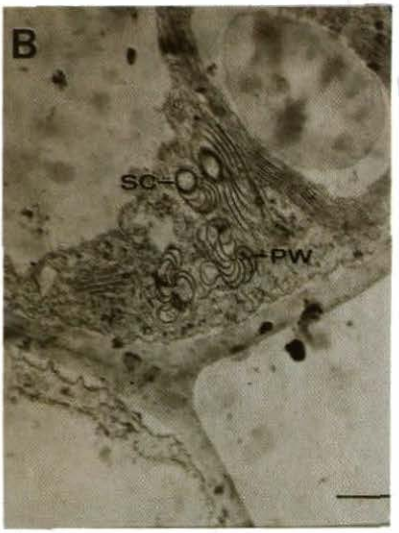

Figure 11. Cylindrical virus-induced inclusions.

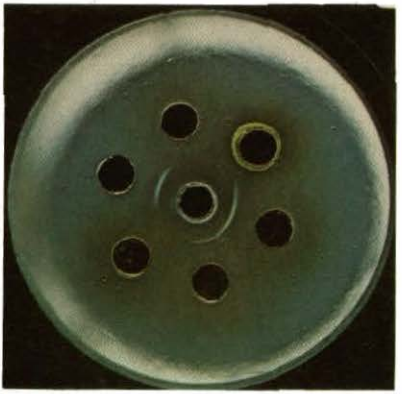

Figure 12. Double immunodiffusion test.

Figure 13. Artificial inoculation progeny test in trays.

Figure 14. Necrosis test in moist chamber.

Figure 15. Enlarged local lesions.

Figure 16. Restricted vein necrosis.

Table 2. Genetic interaction between selected *Phaseolus vulgaris* cultivars and bean common mosaic virus strains.

Differential cultivar	Resistance genes	BCMV strain and pathogenicity genes[a]						
		Type PO	Fla-NL6 P1.1²	NY15-NL2 P1.2	NL3-NL5 P1.1²2	NL4 P1.1².2²	NL7 P1	NL8 P2
Dubbele Witte	I^+	+	++	++	++	+	+	+
Imuna	I^+bc-u bc-1		++	++	++	+	+	
RG-B	I^+bc-u bc-1²		++		++	+		
Michelite	I^+bc-u bc-2			++	++			+
Pinto 114	I^+bc-u bc-1 bc-2			++	++			
Great Northern 31	I^+bc-u bc-1² bc-2²					+		
Widusa	I		+		++			+
Jubila	I bc-1		+	+	++			
Topcrop	I bc-1		+	+	++			
Amanda	I bc-1²				+			

a. Reaction to BCMV: + = mosaic in I^+I^+ cultivars or systemic necrosis in II cultivars; blank spaces = no reaction.

SOURCE: Drijfhout, 1978.

Unfortunately, although some BCMV strain characterization efforts have been made in several countries, very few countries have adequate quarantine facilities to prevent the introduction of new BCMV strains. Thus, these limitations often prevent the implementation of common mosaic resistance breeding projects based solely on strain-specific recessive genes.

Resistance to chronic systemic infection caused by BCMV. The dominant necrosis (I) gene prevents the establishment of a chronic systemic infection (mosaic) by any of the known strains of BCMV. Consequently, seed transmission of BCMV is not possible in any plant that possesses the I gene. It is thus easy to understand the emphasis most bean virologists and breeders have given so far to the incorporation of the I gene in commercial bean cultivars. Furthermore, considering the very narrow host range of BCMV outside the genus *Phaseolus*, the monoculture of I-gene

cultivars should theoretically lead to the eradication of bean common mosaic.

Unfortunately, the monoculture of I-gene cultivars is seldom possible due to the persistent cultivation of traditional common mosaic-susceptible cultivars by small farmers in almost every bean-growing region. These BCMV-susceptible cultivars thus act as sources of inoculum for "necrotic" or necrosis-inducing BCMV strains, which can challenge the I gene, inducing the necrotic or "black root" (hypersensitive) reaction.

The occurrence of systemic necrosis is fortunately not a frequent event in most production regions where I-gene bean cultivars are grown. In Latin America, for instance, several I-gene bean cultivars have grown for decades without ever having suffered a black-root epidemic. In East Africa, however, the predominance of necrosis-inducing strains of BCMV has provoked some isolated but devastating cases of black root which necessitated the adoption of different strategies in breeding for common mosaic resistance.

Multiple resistance to mosaic- and necrosis-inducing strains of BCMV

It is possible to produce bean genotypes which are resistant to both bean common mosaic and black root. To this end, the dominant necrosis (I) gene has to be "protected" by a recessive gene which is not attacked by the known necrotic strains of BCMV. For example, a "protective" recessive gene (bc-2^2), found in group 6 cultivars, can only be attacked by the mosaic-inducing BCMV NL4 strain. The first experimental material, IVT 7233, possessing the gene combination I bc-2^2, proved resistant to all known strains of BCMV, necrotic and non-necrotic alike. This genetic combination is being incorporated at CIAT in improved germplasm for East Africa.

Another approach has been the incorporation of a recessive gene, bc-3, which by itself or in combination with the dominant I gene confers resistance to all known BCMV strains, since the bc-3 gene does not seem to have a corresponding pathogenicity gene in

any of the known BCMV strains. This gene has already been introduced into tropically adapted bean genotypes, since its original source (an experimental breeding line developed in the Netherlands) had a very poor adaptation to tropical environments.

Evaluation of bean germplasm for bean common mosaic resistance

Screening of germplasm bank accessions. The local germplasm bank is the logical place to start a BCMV-screening project. Possessing information on the response of bean landraces to BCMV is of considerable value for plant improvement programs, since at least one of the parents selected for crossing should be resistant to BCMV.

The inoculation and subsequent evaluation of germplasm, however, depends on the pathogenic characteristics of the local BCMV strains. Once the local BCMV strains have been characterized, those with a broad spectrum of pathogenicity should be selected for germplasm screening. Finally, data obtained from these evaluations are reported to breeders, along with information on the BCMV strain used to screen the germplasm, to avoid misunderstandings regarding the possible resistance genes present in the evaluated germplasm.

Screening for common mosaic resistance. "Common mosaic" refers to the various symptoms associated with the chronic systemic infection of bean genotypes devoid of the dominant I gene.

The objective here is to select the most pathogenic local BCMV strain capable of causing mosaic or chronic systemic infection in as many of the bean cultivar differentials of groups 1 to 6 as possible. Ideally, a combination of the New York 15 and NL4 strains of BCMV suffices to detect all bean genotypes possessing recessive genes for BCMV resistance (with the exception of the bc-3 gene). The incidence of BCMV NY15 and NL4 strains, however, is low in most tropical countries. Consequently, they may have to be replaced by the most pathogenic mosaic-inducing

BCMV strain(s) available, hopefully capable of attacking at least the first three groups of mosaic-susceptible bean cultivars.

When the presence of a necrosis-inducing strain has been detected in the country, the first step is to characterize it. Some "necrotic" BCMV strains have rather limited pathogenicity ranges in *P. vulgaris*, while others, such as BCMV NL3, have a broad pathogenicity spectrum in most bean cultivars possessing recessive or dominant resistance. In eastern Africa and in some Latin American countries, BCMV NL3 seems to be the most widely distributed necrosis-inducing strain. This strain can infect 8 out of the 10 groups of differential bean cultivars, inducing either mosaic or black-root symptoms, according to the bean genotype attacked. The BCMV NL3 strain is thus the most appropriate BCMV strain to use for screening purposes, since it can simultaneously detect the presence of both the dominant necrosis (I) gene and most of the recessive genes. The NL3 strain can also detect the presence of some dominant and recessive gene combinations through induction of local necrotic lesions or restricted vein necrosis.

Incorporation of the dominant necrosis (I) gene

The deployment of monogenic dominant resistance to control bean common mosaic virus is a sound strategy in areas where only bean cultivars possessing this type of resistance are grown. The rationale behind this approach is that since BCMV cannot be transmitted in the seed of bean genotypes possessing monogenic dominant resistance, and this virus has a narrow host range outside *Phaseolus vulgaris*, BCMV could hardly exist and spread in such an environment. Moreover, the monogenic dominant resistance to BCMV has held up for decades in Latin America, even in the presence of necrosis-inducing BCMV strains capable of challenging the dominant I gene. Since some of the "necrotic" strains of BCMV are temperature dependent, the incidence of systemic necrosis (black root) can sometimes be noticeable in bean plots or fields during certain warm and dry periods of the year, which characterize "summer" months in some tropical countries. However, black-root incidence is seldom higher than

5% in affected plant populations, and it mainly occurs under experimental conditions where mosaic-susceptible and mosaic-resistant cultivars and breeding materials are grown together.

Germplasm evaluation sequence. Assuming that the hybrid progenies to be evaluated were derived from a simple cross between a common mosaic-susceptible cultivar and a common mosaic-resistant (dominant I gene) genotype, the F_1 generation should be heterozygous resistant and, as a result, there would be no point in inoculating F_1 plants.

In the F_2 generation of such a simple cross, approximately 25% of the plant population should be homozygous susceptible. However, since 50% of the population would still be heterozygous resistant to common mosaic, and breeders usually generate large F_2 populations, inoculating the F_2 generation could be both a costly and inefficient operation. At CIAT, the whole F_2 generation is planted in the field for observation of desirable agronomic characteristics. Under field conditions, however, it is possible to detect and discard some of the homozygous-susceptible F_2 plants, depending on the presence of viruliferous aphids transmitting BCMV. Planting BCMV-infected seed of a mosaic-susceptible cultivar in the F_2 nurseries helps increase the incidence of the virus, particularly in the dry months of the year.

Once plants are selected in the F_2 nurseries, preferably on an individual basis, a 10-seed sample can be taken from each individual selection for BCMV screening. The 10 seeds are then planted under screenhouse or glasshouse conditions in pots or, preferably, trays (see Figure 13) for mechanical inoculation. Seedlings are best inoculated as soon as primary leaves open (but before the first trifoliate leaf appears), approximately one week after sowing.

Expected plant responses in F_3 plants inoculated with BCMV. In the F_3 progeny we thus expect to find segregating lines derived from individual F_2 selections which were heterozygous for the I gene (these plants behaved as BCMV resistant in the F_2 nurseries). These heterozygous materials can be detected by the presence of a few mosaic-affected plants in the 10 seedlings inoculated, 10 to 14 days after inoculation. If most or all of the

inoculated plants are showing common mosaic symptoms, it means that the individual F_2 selection was homozygous susceptible (it escaped infection or did not express mosaic symptoms in the F_2 nursery). Finally, homozygous-resistant lines should make up approximately 25% of the individual selections tested. In this case, none of the 10 inoculated F_3 seedlings should show mosaic, but, all the plants should show local or systemic necrosis if they were inoculated with a necrosis-inducing BCMV strain, such as NL3.

Based on these results, the breeder can proceed to select the homozygous-resistant F_3 lines planted in the field from the remainder of the seed produced by the individual F_2 selections. It is possible, however, to save plants inoculated with necrosis-inducing BCMV strains by simply detaching the primary leaves to be inoculated and placing them inside a moist chamber (Figure 14). Under these conditions, vein necrosis should develop within a week. Also, in case the inoculation was conducted with a mosaic-inducing strain (one which does not induce necrosis), the symptomless F_3 plants can be transplanted after the evaluation period (it would be advisable to index these plants by an infectivity test to a highly susceptible bean cultivar, to make sure the symptomless plants do not harbor the virus).

The proportion of susceptible, segregating, and homozygous-resistant lines in the F_3 generation obviously may vary considerably, depending upon the presence of recessive BCMV-resistant genes and different pathogenicity genes in the BCMV strain selected for inoculation. Information on the genetics of BCMV resistance of several genotypes commonly used as parents in crossing programs can be requested from the Virology Research Unit of CIAT.

Screening for multiple (mosaic/systemic necrosis) BCMV resistance

Screening for mosaic and black-root-resistant genotypes first requires the use of a necrosis-inducing BCMV strain and, ultimately, the selection of lines which do not show either mosaic or systemic necrosis following their inoculation with a necrotic

BCMV strain, such as NL3 or NL5. For instance, if the bc-3 gene is used as the source of resistance, the homozygous-resistant lines should remain symptomless and, more important, should assay negative for the presence of BCMV in infectivity or other virus detection tests. If the I gene is combined with the bc-2^2 gene, the mosaic and black-root-resistant plants or lines should only develop pin-point local lesions on the inoculated primary leaves.

In some cases, certain genotypes exhibit enlarged local lesions (Figure 15) or a restricted vein necrosis on the inoculated primary leaves (Figure 16), without any further systemic spread of the virus. These plants apparently possess the I gene and one or more recessive genes which condition these reactions. In our experience, the restricted vein necrosis reaction has been effective against most necrotic BCMV strains, with the exception of BCMV-NL5, particularly under high-temperature conditions.

Screening for common mosaic/black-root resistance is best initiated in the F_2 generation, where both symptomless plants and plants with local lesions should be selected. However, for the evaluation of large populations, the F_3 generation is again the most convenient stage at which to begin inoculation. As mentioned earlier, all symptomless plant lines should be tested for the presence of a latent virus infection.

Backcrossing

Sometimes, the dominant I-gene resistance cannot be easily transferred to certain BCMV-susceptible genotypes without losing some of the desirable agronomic and/or commercial characteristics of the bean cultivar that is to be improved. In this case, a backcrossing project may be indicated. At CIAT, and in the case of simple crosses involving an I-gene genotype and a BCMV-susceptible cultivar, the backcrossing starts in the F_1 generation, and proceeds to the fifth backcross (F_1 BC^5). During this process, the resulting progenies are inoculated to eliminate the mosaic-susceptible plants (50%). Next, the F_2 (BC^5) generation is obtained and inoculated to eliminate the heterozygous (just like in a regular F_3 progeny test) and homozygous-susceptible plants. Finally, the F_3 (BC^5) generation

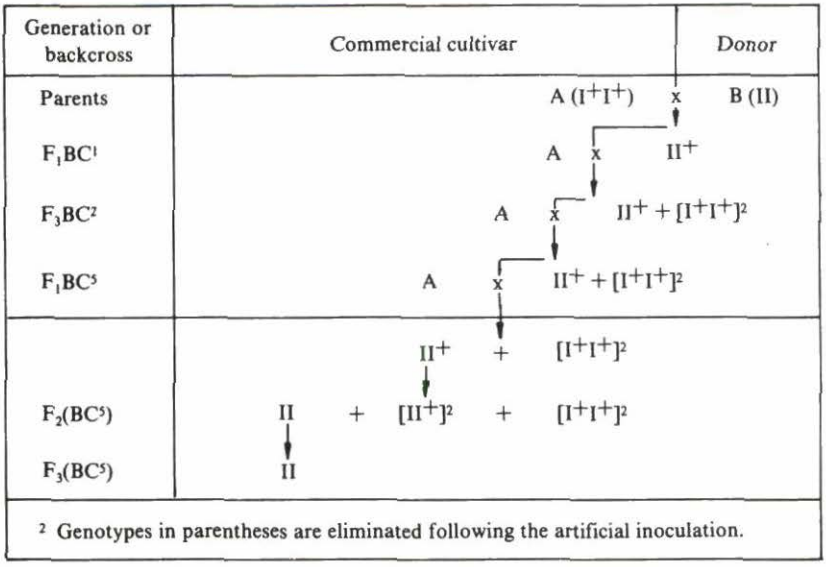

Figure 17. Backcrossing methodology followed at CIAT to incorporate dominant resistance to bean common mosaic virus.

is produced from the homozygous-resistant F_2 BC^5 plants (Figure 17).

The best BCMV strain combination to use for the backcrossing procedure is a mixture of the NY15 and NL4 strains, to eliminate all plants possessing only recessive genes for BCMV resistance. Again, the choice of BCMV strains to be used depends on the nature and availability of local strains. Also, the BCMV-susceptible commercial variety must be either the recurrent progenitor or the mother plant to detect self-pollinated plants.

Production of BCMV-free Seed

The production of BCMV-free seed is necessary for all experimental purposes, as well as for the safe exchange of BCMV-susceptible germplasm.

BCMV is transmitted in the seed of most non-I-gene susceptible bean cultivars. On the average, it is expected that up

to 30% of the seed produced by systemically infected bean plants will produce BCMV-infected plants. Even at levels of seed transmission as low as 1%, there would be 2,500 BCMV-infected plants in a hectare (at a planting density of 250,000 plants/ha), enough for secondary transmission of the virus to occur via aphid vectors.

The production of BCMV-free bean seed was an effective control measure in the U.S.A. for many years. The seed was produced in isolated regions away from other bean plantings and aphid vectors. The best approach, however, is to start with a limited amount of virus-free seed produced under screenhouse or glasshouse conditions. This seed can then be planted in an isolated region, at least some 20 km away from bean fields, without much concern for the possible presence of aphid vectors.

It is important to keep in mind that the roguing of BCMV-infected plants in areas where aphid vectors are present is not recommended due to the significant secondary virus transmission that takes place long before the first BCMV-infected plants can usually be detected.

Also, chemical control of aphids is not recommended in a clean seed production operation since aphids can acquire and transmit BCMV in less than 30 seconds, before any insecticide can effectively kill them.

Detection of BCMV in bean seed

Germination test. This is the simplest test, but it requires some space to grow the plants. A 100-seed sample is recommended since some strains and bean cultivars interact to yield seed-transmission rates below 1%. Typical BCMV symptoms can be observed on the primary leaves in most seed-borne plant infections.

Serological tests. The most recommended test for virus detection in leguminous seeds has been the enzyme-linked immunosorbent assay known as ELISA. Legume seeds usually contain hemagglutinating compounds which may produce nonspecific reactions with the unfractionated antisera used in

most other serological tests. The ELISA test is also highly sensitive, having the possibility to detect infected seed in a lot having low BCMV incidence. Lately, monoclonal antibodies to BCMV are also being used in conjunction with the ELISA test. Some of these monoclonal antibodies should be able to recognize either a single strain, or both mosaic- and necrosis-inducing BCMV strains, making the use of two different antisera unnecessary.

Bibliography

Ball, E. M. 1974. *Serological tests for the identification of plant viruses.* American Phytopathological Society, St. Paul, MN, USA. 31 p.

Bos, L. 1971. Bean common mosaic virus. No. 73. Descriptions of plant viruses. Commonwealth Mycological Institute and Association of Applied Biologists, Kew, Surrey, England. 4 p.

——— and Maat, D. Z. 1974. A strain of cucumber mosaic virus, seed-transmitted in beans. Neth. J. Plant Pathol. 80:113-123.

Christie, R. G. and Edwardson, J. R. 1977. Light and electron microscopy of plant virus inclusions. Monograph series no. 9, Florida Agricultural Experiment Stations, Gainesville, FL, USA. 155 p.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34(3):475-483.

Derrick, K. S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56(2):652-653.

Drijfhout, E. 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands. 98 p.

Fenner, F. 1976. Classification and nomenclature of viruses. Intervirology 7(1-2):4-115.

Hamilton, R. I. and Nichols, C. 1978. Serological methods for detection of pea seed-borne mosaic virus in leaves and seeds of *Pisum sativum*. Phytopathology 68(4):539-543.

Jafarpour, B. and Shepherd, R. J. 1978. Detection of bean common mosaic virus and lettuce mosaic virus by enzyme-linked immunosorbent assay (ELISA). Phytopath. News 12:172. (Abstr.)

Lister, R. M. 1978. Application of the enzyme-linked immunosorbent assay for detecting viruses in soybean seed and plants. Phytopathology 68(10):1393-1400.

Morales, F. J. and Castaño, M. 1987. Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. Plant Dis. 71(1):51-53.

Nelson, R. 1932. Investigations in the mosaic disease of bean. Technical Bulletin 188, Michigan Agricultural Experiment Station, Ann Arbor, MI, USA. 71 p.

Purcifull, D. E. and Batchelor, D. L. 1977. Immunodiffusion tests with sodium dodecyl sulfate (SDS)-treated plant viruses and plant viral inclusions. Bulletin 788, Florida Agricultural Experiment Stations, Gainesville, FL, USA. 39 p.

Shepard, J. F. 1972. Gel-diffusion methods for the serological detection of potato viruses X, S, and M. Bulletin 662, Montana Agricultural Experiment Station, Bozeman, MT, USA. 72 p.

Spragg, F. A. and Down, E. E. 1921. The robust bean. Special Bulletin 108, Michigan Agricultural Experiment Station, Ann Arbor, MI, USA. 9 p.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

Trujillo, G. E. and Saettler, A. W. 1972. Local lesion assay of bean common mosaic virus (BCMV) on 'Monroe' bean. Plant Dis. Rep. 56(8):714-718.

Uyemoto, J. K. and Grogan, R. G. 1977. Southern bean mosaic virus: evidence for seed transmission in bean embryos. Phytopathology 67:1190-1196.

Zaumeyer, W. J. 1969. The origin of resistance to common bean mosaic in snap beans. Seed World 105(4):8-9.

Ziemiecki, A. and Wood, K. R. 1975. Serological demonstration of virus-specified proteins associated with cucumber mosaic virus infection of cucumber cotyledons. Physiol. Plant Pathol. 7(2):171-177.

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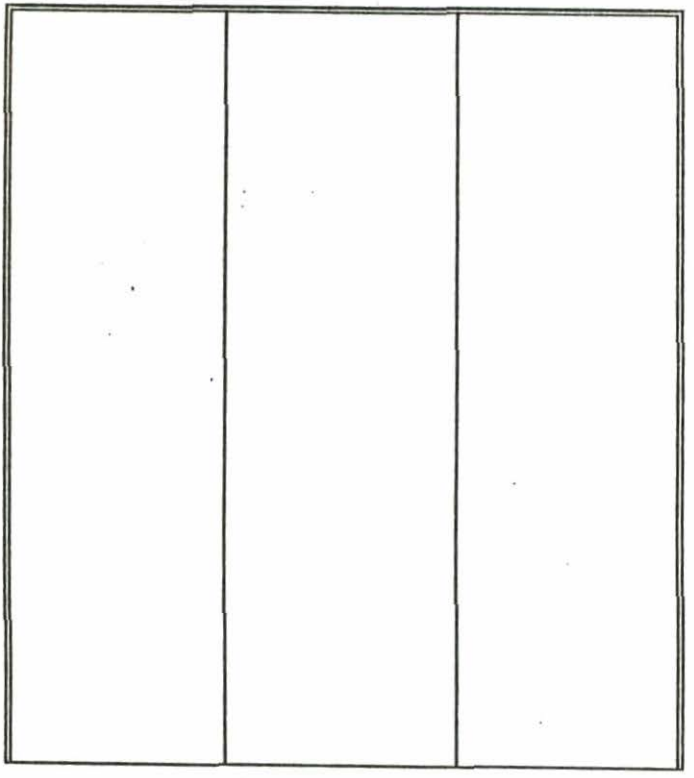

⇩

Density gradient centrifugation
in 30% (w/v) cesium chloride
in 0.05 M KPO_4, pH 7.5

⇩

Recover visible virus band,
dilute in 0.25 M KPO_4, and
precipitate at 84,500 g for 90 min

⇩

Resuspend virus pellet in
0.5 ml 0.02 M Tris-HCl, pH 8.2