Toward The Molecular Characterization of Bean Common Mosaic Strains: The Cloning and Nucleotide Sequence of the NL-3 Strain of BCMV

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Bean common mosaic potyvirus (BCMV) is distributed worldwide because of its efficient seedborne dissemination. In fields where secondary spread occurs by aphid vectors, BCMV incidence can reach high levels and result in yield losses and reduced quality. Numerous strains of BCMV exist, but two major groups of strains can be identified: those that are unable to infect bean genotypes possessing the dominant inhibitor gene I (non-necrotic or soft strains) and those that induce the hypersensitive or 'black root' response on these genotypes (necrotic strains). These two major BCMV subgroups can also be distinguished serologically and are referred as A serotype (necrotic strains) and B serotype (non-necrotic strains). The necrotic strains of BCMV are believed to have originated in Africa, where they are the prevalent, but are also found in parts of Latin America and more recently in bean growing regions of the United States and Canada. BCMV has been considered a minor pathogen in the United States for the past few decades, because of effective seed certification programs and the use of dominant I gene resistance, which provided resistance against the non-necrotic strains prevalent in the US. However, recent outbreaks of necrotic strains in Michigan, New York, Idaho, and Ontario, Canada, and of possibly different non-necrotic strains in Washington, have led to increased concern about the reemergence of BCMV as an important pathogen, particularly in areas where bean genotypes are grown without I gene resistance, leading to the establishment and spread of new BCMV strains.

The present method for differentiating BCMV strains is through the use of differential bean cultivars, which possess different combinations of I gene and recessive resistance genes. However, this is a time-consuming, expensive, and laborious procedure, and is not always unambiguous. This is because some BCMV stains cause mild to almost symptomless infections in certain differential cultivars, necessitating back-inoculations to highly susceptible cultivars.

A BCMV project has been initiated at the University of California-Davis through the support of the California Crop Improvement Association and the USAID Bean/Cowpea CRSP project with the objectives of:

1) the molecular characterization and detection of BCMV strains
2) the evaluation of BCMV resistance in important bean genotypes in California and Malawi and incorporation of BCMV resistance into selected genotypes
3) the study of BCMV evolution and ecology in California and Malawi

The NL-3 strain of BCMV was initially selected for characterization because it is the most widely distributed necrotic strain, it is the BCMV strain that we have identified in Malawi, and it is a strain we routinely use for screening purposes. The NL-3 strain used was an isolate from Michigan, kindly provided by Dr. J. D. Kelly. The strain designation was confirmed by inoculation of differential varieties. The virus was purified and the presence of virus particles confirmed by electron microscopy. Virus RNA was extracted from the purified virus preparation using a phenol/chloroform method, and the integrity of the RNA confirmed by agarose gel electrophoresis. A single RNA band was observed that was of high molecular weight and was presumably the genomic RNA of BCMV (~10 kilobases). This RNA was used to make cDNA clones using standard methods. The cloning strategy, which involves the use of a polythymine (polyT) oligonucleotide primer, will generate clones starting at the 3' polyadenylated end of the RNA molecule, which is where the potyvirus coat protein is located. A number of clones were

obtained, and the sizes of the insert DNA determined. One of the largest clones, pBC29, that was approximately 4.5 kilobase pairs was selected for further characterization. The 3' terminal 1500 nucleotides were determined and the putative BCMV NL-3 coat protein amino acid sequence and 3' non-coding region were determined. The predicted NL-3 coat protein consisted of 261 amino acid residues having a predicted molecular weight of 29.7 kilodaltons. A putative glutamine-serine cleavage site was identified, where the coat protein could be cleaved away from the potyvirus polyprotein. The triple amino acid residue sequence, DAG (aspartic acid, alanine and glycine), associated with aphid transmissibility of potyviruses was identified toward the amino terminus of the coat protein. A number of other highly conserved amino acid residues in other potyvirus coat proteins were also identified in the NL-3 coat protein sequence. These results suggest that the BCMV NL-3 coat protein is a typical potyvirus coat protein gene.

The amino acid sequence of the BCMV NL-3 coat protein was compared to previously determined coat protein sequences of other potyviruses, including BCMV strains NL-8 (necrotic) and NL-4 (non-necrotic), bean yellow mosaic potyvirus (BYMV), peanut stripe potyvirus (PStV), and two strains of watermelon mosaic virus 2 (WMV2). The results of these comparisons showed that the NL-3 and NL-8 strains of BCMV were closely related (97% amino acid similarity), but were considerably different from the non-necrotic NL-4 strain (67% similarity). Based on this analysis, the NL-4 strain of BCMV was more closely related to PStV than to the necrotic strains of BCMV. The NL-3 strain of BCMV was only distantly related to BYMV or clover yellow vein potyvirus (CYVV). Thus, based on these results, the necrotic BCMV strains appear to be quite different than the non-necrotic strain, possibly different enough to be considered different potyviruses. This finding is also consistent with the existence of two distinct BCMV serotypes. With the current high level of interest in potyvirus taxonomy, it is likely that the taxonomy of BCMV strains will be reevaluated. It is very important that such a reevaluation takes into consideration the biological and practical aspects of BCMV before changing the present BCMV nomenclature.

The NL-3 coat protein clone was used as a DNA probe to detect BCMV in infected plants using a modified squash blot hybridization method. The technique was modified from that used for geminiviruses to include a pretreatment of membranes with proteinase K, which inactivates RNAase that can degrade viral RNA. Under low stringency conditions, the probe detected both necrotic and non-necrotic BCMV strains, but under high stringency conditions it detected only necrotic strains. This technique provides an additional detection method for BCMV, and we plan to evaluate this method for BCMV detection in large numbers of samples during a BCMV survey in Malawi in 1992. Our ultimate goal is to develop a BCMV detection procedure based on the polymerase chain reaction (PCR), and to use general and specific PCR primers for detection of any BCMV strain or for rapid and specific detection of individual BCMV strains.

The NL-3 coat protein was expressed in *Escherichia coli* using standard protein expression technologies. The expressed protein was detected in protein extracts from *E. coli* cells by Western blot analysis using monoclonal antisera specific for the necrotic strain of BCMV. The possibility of using the coat protein gene in transgenic plants as an additional BCMV resistance gene is being evaluated.

The considerable interest in BCMV that has been generated by outbreaks of new BCMV strains and by the development of molecular techniques for analysis of BCMV strains will lead to new insight into this important virus. This will hopefully result in new and rapid methods for strain detection and identification as well as incorporation of available BCMV resistance genes into presently susceptible genotypes.