GENETIC VARIABILITY OF ISOLATES OF *BEET SOILBORNE MOSAIC VIRUS*

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ABSTRACT

Beet soilborne mosaic virus (BSBMV) is a soilborne virus and member of the genus Benyvirus that infects sugar beets (*Beta vulgaris*). BSBMV is a rigid rod-shaped virus with a divided genome consisting of four polyadenylated single stranded RNAs. This virus has been identified in the central and western sugar beet growing regions of the United States and is a close relative of beet necrotic yellow vein virus (BNYVV) that causes rhizomania. The objective of this study was to determine the genetic diversity in BSBMV isolates from different sugar beet growing areas in the United States. Soil samples of sugar beets with foliar symptoms were collected from sugar beet fields in Colorado, Minnesota, North Dakota, and Texas. Soil samples were biossayed. Total RNA from harvested sugar beet roots was extracted and first strand cDNA was made from each RNA extract. Based on the previously determined nucleotide sequence of BSBMV, specific primers were developed for six ORF's of RNA2 and different regions of RNA3. On RNA 2 the greatest genetic variability between isolates was detected in the ORF 2, 75 kDa readthrough protein. A high degree of variability was also found within RNA 3. No variability was found in the 21kDa, 42kDa, 14kDa and 15kDa. Th

INTRODUCTION

Beet soilborne mosaic virus (BSBMV) was first identified in Texas as a complex virus associated with rhizomania disease (Lui, H.Y. & Duffus, J.E., 1988). Both BSBMV and BNYVV possess a divided genome consisting of four polyadenylated, single-stranded RNAs (Rush, C.M. & Heidel, G.B., 1995). The genomic organization of BSBMV is identical to BNYVV: a single open reading frame (ORF) on RNA 1, six putative ORF's on RNA 2, and single ORF's on RNA 3 and RNA 4 (Lee, L., et al., 2001). In some strains of BNYVV, the presence of an additional RNA 5 has been reported (Richard, K.E. & Tamada, T., 1992). BNYVV is a very stable organism and recently there have been three major strain groups reported: A, B and P types (Koenig, R. & Lennefors, B.L., 2000). In contrast, evidence of higher variability has been reported (Brewton, R. et al., 1999) for BSBMV for RNA 2 and RNA 3. The objective of this study was to determine the genetic diversity in BSBMV isolates from different sugar beet growing areas in the United States.

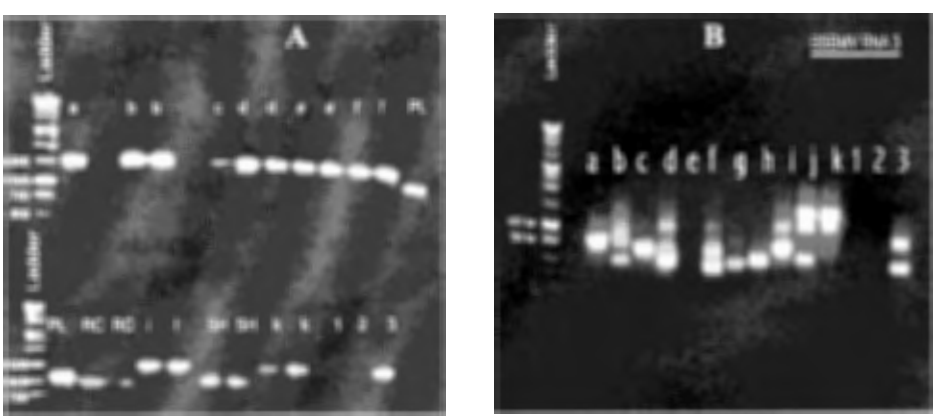
MATERIAL AND METHODS

Rhizosphere soil from sugar beet with foliar symptoms of BSBMV was collected from sugar beets fields in Colorado (KM, RC, EA), Minnesota (BS, LN, S), North Dakota (Fargo, SH) and Texas (SS, PL, HK). Soil samples were bioassayed in the green house. Roots were harvested and viral presence was confirmed by (DAS)-ELISA as described by Heidel, G.B. et al., 1997. Total RNA from roots testing negative for BNYVV and positive for BSBMV was obtained with the commercial FastRNA® kit green according to the manufacturer's protocol (QBiogen, Carlsbad, CA). First strand cDNA was made from total RNA using the commercial kit SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen™ Life technologies, Carlsbad, CA) following the manufacturer's protocol. PCR amplification was performed using specific primers for BSBMV RNA 2. Cycling parameters were 94°C for 30 sec., 56 °C for 30 sec., and 72 °C for I min. for 35cycles. PCR products were analyzed for variations by agarose gel electrophoresis and sequenced by Gene Technologies Laboratory (Texas A&M University, College Station, TX). Sequence data was compared with the BSBMV RNA 2 sequence in GenBank (Accession #AF061869) (Lee, L., et al., 2001).

RESULTS

Eleven isolates were analyzed with primers designed to amplify five of the six ORF's of BSBMV RNA2: 21 KDa, 75 KDa, 42 KDa, 15 KDa, and 14 KDa. The only size variation observed was in the 75 KDa protein gene amplification from isolates PL from Texas, RC from Colorado and SH from North Dakota (Fig.1A). Sequence results confirmed that the RC isolate has a 459 bp deletion between

Fig. 1. PCR products of the A) BSBMV RNA2 75KDa read-though protein and the B) BSBMV RNA3 47-1352 bp region. cDNA template was from isolates a) BS, b)EA, c) Fargo, d) KM, e)HK, f)LN, g) PL, h)RC, i) S, j) SH, k) SS; controls were 1) Healthy, 2) BNYVV positive, 3) BSBMV positive. The 75 KDa protein PCR product of isolates PL, RC, and SH varied in size compared with the expected 1525 bp. The expected size of the RNA3 region PCR product was 1,306 bp.



positions 1459 to 1918, which results in a +1 nucleotide frame shift. The SH isolate has a 407 bp in-frame deletion from positions 1461 to 1869.The PL Isolate has a 363 bp in-frame deletion from position 1497 to 1860 (Fig. 2). With the exception of the previously mentioned deletions, isolate nucleotide identity with the GenBank sequence was 99.98% to 99.99% (Table 2). RNA 3 also exhibited significant variation in PCR product size (Fig 1B).

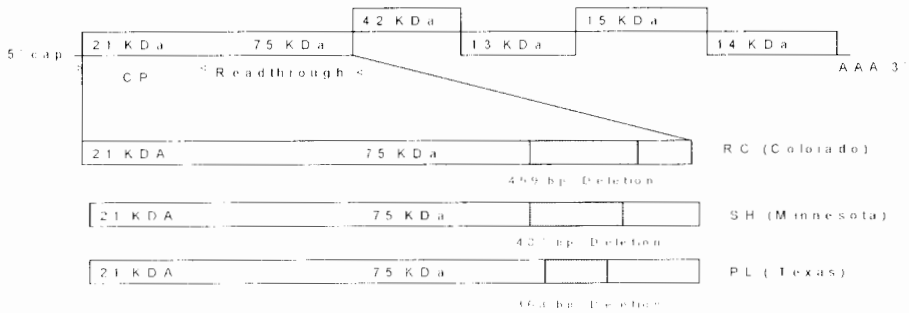


Fig. 2 Schematic of the BSBMV RNA 2; boxes represent the organization of the six ORF's. Enhanced boxes represent coat protein and read-through complex; shaded areas indicate deleted regions for each isolate.

Isolate	%Similarity	Deletion
BS (MN)	99.981	*
Fargo (ND)	99.900	*
KM (CO)	99.985	*
HK (TX)	99.982	*
LN (MN)	99.982	*
PL (TX)	99.996	363 bp
RC (CO)	99.987	460 bp
S (MN)	99.988	*
SH (ND)	99.987	411 bp
SS (TX)	99.991	*

* No deletion found

Table 2. Percent similarities of isolates to the BSBMV RNA 2 sequence in GenBank (Accession # AF061869).

CONCLUSION

Although most of the isolates of BSBMV do in not exhibit variations in the ORF's of RNA 2, we have shown that three isolates, PL from Texas, RC, from Colorado and SH from North Dakota, have deletion sites in the 75 KDa readthrough protein. These deletion sites were found in the same area between 1459 and 1918, although the deletions are different sizes. The BNYVV 75 KDa readthrough protein has been associated with virus transmission; further investigation will be conducted to analyze the effect of these deletions on virus transmission.

BSBMV has exhibited extreme variability in RNA3 PCR products. This observed variability in RNA3 sequences was not surprising considering the variability observed in BSBMV symptom phenotypes. These variations will be sequenced and deletion sites will be analyzed. These findings support reports by Brewton et al., (Brewton, R. et al., 1999) of high genetic variability of BSBMV RNA 3. BSBMV RNA 3 has been associated with symptom expression in BNYVV. It is possible that genetic variability could be responsible for the different phenotypic symptoms found in isolates of BSBMV (Garcia-Arenal, F. et al., 2001).

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