Comparison of Barley Stripe Mosaic Virus Strains

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Z. Naturforsch. **63c**, 271–276 (2008); received April 23/September 4, 2007

BSMV (barley stripe mosaic virus) particles were obtained in a pure state from infected host plant tissues of *Hordeum vulgare*. The three genomic parities (α, β and γ) were amplified by PCR using specific primers for each particle; each was cloned. Partial sequence of the α, β and γ segments was determined for the Egyptian isolate of barley stripe mosaic virus (BSMV AE1). Alignment of nucleotide sequences with that of other known strains of the virus, BSMV type strains (CV17, ND18 and China), and the generation of phylogenetic trees was performed. A low level of homology was detected comparing 467 bp of the α and 643 bp of the segments to that of the other strains, and thus BSMV α and β segments were in separate clusters. However, 1154 bp of the γ segments of BSMV AE1 showed a high level of homology especially to strain BSMV ND18, as they both formed a distinct cluster. Northern blotting of pure BSMV AE1 virus and *H. vulgare*-infected tissue were compared using an α ND18 specific probe. Western blotting using antibodies specific for the coat protein (CP) and the triple gene block 1 (TGB1) protein, which are both encoded by the β ND18 segment, still indicated a high level of similarity between proteins produced by BSMV ND18 and AE1. We suggest that the BSMV AE1 isolate is a distinct strain of BSMV which reflects the genetic evolutionary divergence among BSMV strains and members of the *Hordeivirus* group.

Key words: Hordeiviruses, Egyptian BSMV AE1, Barely Stripe Mosaic Virus

Introduction

Barley stripe mosaic virus (BSMV) is the type species of the genus *Hordeivirus* (Lawrence and Jackson, 1998; Lawrence *et al*., 2000). The genome of BSMV consists of three separately encapsidated RNA species (Brakke and Palmor, 1976) designated α, β and γ in order of decreasing molecular weight (Jackson *et al*., 1983a). The BSMV genome has been completely sequenced, and most of its functions have been mapped to distinct gene products (Gustafson *et al*., 1989; Jackson *et al*., 1989; Donald *et al*., 1995). The genome is divided among three positive-stranded RNA molecules designated as RNAs α (3.8 kb), β (3.3 kb), and γ (2.8 or 3.2 kb), depending on the virus strain (Gustafson *et al*., 1987; Solovyev *et al*., 1996). BSMV RNA α codes for a 130-kDa protein that contains the methyltransferase and NTPase-helicase dominase and is required for virus replication (Gustafson *et al*., 1989; Koonin and Dolja, 1993; Petty *et al*., 1990). RNA γ contains a gene of importance for virus replication (Petty *et al*., 1990). γ a contains the RNA-dependent RNA polymerase sequence motifs (Gustafson *et al*., 1987; Morozov *et al*., 1989; Koonin and Dolja, 1993), and the 3′-proximal ORF γ b codes for a cysteine-rich protein with the putative "zinc finger" motif (Gustafson *et al*., 1987; Morozov *et al*., 1989; Agranovsky *et al*., 1992). γ b has been also implicated in seed transmissibility of BSMV.

BSMV rod-shaped virions primarily infect members of the Graminae. However, several dicot species can also act as hosts (Jackson and Lane, 1981). Several isolates have been reported: Type strain, North Dakota (ND18), Norwich (NW), CV42, CV17, China, Russian and Argentina mild (Lane, 1974; Gustafson *et al*., 1982). Incidence of BSMV infection in Egypt has been reported (Zein and Aboul-Ata, 2004). An Egyptian isolate of the virus, BSMV AEl, has been isolated, purified and partially characterized.

We report the partial sequence of BSMV AE1 RNA α, β and γ genome components and their degree of homology in comparison to other reported isolates of the virus.

Materials and Methods

Virus isolation and RNA purification

BSMV isolate AE1 isolated from infected wheat hosts in Egypt was maintained, propagated and isolated from "Black Hulless" barley (NSSL PI 24849 MD, USA) grown in controlled climate chambers. Viral RNA was isolated from purified virus as previously described (Gustafson *et al.*, 1981).

Synthesis and isolation of cDNA clones

First-strand cDNA was synthesized using murine leukemia virus reverse moloney transcriptase (Fermentase Inc., MD, USA) in the presence of the random hexamer primer (Promega Corp., WI, USA) according to standard procedures (Sambrook and Russell, 2001). The second step of PCR was carried out using specific synthetic oligonucleotide primers for each α, β and γ genome component (Sambrook and Russel, 2001). The α, β and γ amplicons (BSMV AE1) were cloned using the TOPO TA cloning kit (Invitrogen, USA) according to the manufacturer's instructions. Plasmids miniprep for the inserts α, β and γ DNAs were performed using the minilysates boiling method (Sambrook and Russel, 2001). Small aliquots from each were analyzed on 1% agarose gel in TBE buffer.

Nucleotide sequencing and analysis of sequence

α, β and γ clones were subjected to partial sequencing (University of California, Plant and Microbial Biology Department, Berkeley, USA). Each reaction mixture of 13 μl total volume consisted of 1 μl DNA, 1 μl primer (1:10) and 11 μl H_2O. Pairwise and multiple DNA sequence alignment were carried out using clustal W (1.83) (http://www2.ebi.ac.uk/clustalw; Thompson *et al.*, 1994). A bootstrap neighbour-joining tree was generated using MEGA3 (Kumar *et al.*, 2004) from clustal W alignments.

Northern blotting for the detection of BSMV AE1

Northern blotting was performed according to Sambrook and Russel (2001). The PCR amplicone obtained from purified virus (Egyptian isolate BSMV AE1) as well as from the infected *Hordeum vulgare* leaf tissue (at 10 d post inoculation) were blotted onto a nylon membrane. The BSMV ND18 was used as specific radio-labeled RNA probe (^{32}P-labeled UTP) targeting the 3′-end of α, β and γ ND18 BSMV RNA. The hybridized nylon membrane was finally exposed to a phosphoimager screen (Typhoon 8600, Amersham Biosciences, Piscataway, NJ, USA) for the detection of ^{32}P signals.

Western blot

Western blotting for the detection of BSMV in *Hordeum vulgare*-infected tissue 7 and 15 d post inoculation (dpi) was performed using BSMV strain ND18 antibodies specific for the coat protein (CP). Samples of leaf extracts were electrophoresed in 12% SDS-PAGE, and then electroblotted onto a Hy bond TM ECL nitrocellulose membrane. The membrane was subsequently incubated with antibody preparations as described by Torrance *et al.* (2006).

Results and Discussion

cDNA synthesis and cloning

To prime the cDNA synthesis of BSMV isolate AE1 α, β and γ genomic RNA components, specific synthetic oligonucleotides were used. Following reverse transcription into first-strand cDNA and by the use of the specific primers polymerase chain reaction was used to amplify each of the 3

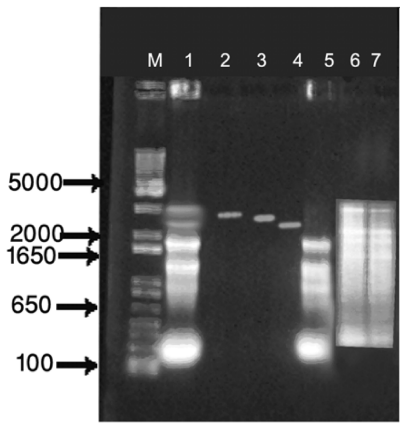

Fig. 1. Electrophoretic mobility of BSMV AE1 RNA extracted from infected *Hordeum vulgare* L. cultivar "Black Hulless" tissue on 1% agarose gel. Lane M, molecular weight standard 1 kb plus DNA ladder (GIBCO BRL); lane 1, total RNA; lane 2, α RNA transcript; lane 3, β RNA transcript; lane 4, γ RNA transcript; lane 5, total RNA extracted from healthy control; lane 6, total RNA extracted from BSMV AE1-infected leaf 1; lane 7, total RNA extracted from BSMV AE1-infected leaf 2.

(a)

1 tattcttgaa aaaaaggtta aataaatttt atccaangcg gaaagacact agtattctta
61 ttagtgcatt ttttttacc acttcacagt atgcctaacg tttctttgac tgctaagggt
121 ggaggacact acaacgagga tcaatgggat acacaagttg tggaagccgg agtatttgac
181 gattggtggg tcccggagg ggccagtaat acgtggatat ttccggtact atttagacca
241 gtatagaatc gcacctctca tgcaccagaa cctggatcaa gctctttgtt tagatgtgga
301 ctaaatgcta atatcttatg gaggttatca agtgtcagat gattaactga agtaaattct
361 ctggagttaa aaacgatccc acaaagacaa gataaatccc cggctacttg tccggttaat
421 tccactaggt ttcacgatgt ccacgggcta ctattgatac gaaacacatt ggagcggatg
481 tttctgattt aaaactaaag ctacgtgcgt cttcaggacc tgatgcacat aagctatcag
541 aagatcctgt gctttgggca gtgcgtcaca acaatcgaca gggttgttcc ttttcctgca
601 gtgcgggggc ccacttgtaa ttcttatttc attcttaatg tac

(b)

1 atacctgcag ggattccttt atccgtattg cgaggctttt ctgtcggaat cattaatgat
61 gtgccgtaac caatctacga cctttattgg cgtgcaagta tactgacttc ggatggaatc
121 ccactcgcct gttgatgggc ctgtacagat atgaaccatg tgatctaact ctttatttgg
181 gactcctagc cgcttatcac ccggtagatc ccttcccact cacgcatctt cacagttcat
241 atggcacggg gtcggctgcc gaacagttga cattactgtc agataccttc tcggccatat
301 gtgtgtgaga gcctaaaatt tggatctatt ttccaacagc acctctacac tatttatctc
361 gcccattagt acacttctca tctcgacgaa aaactgggcg gtttggtgag ccacaacgca
421 ttgattcctc cgccgacccc caacttgaag ggtctcaaag acctagg

(c)

1 cttgtagctt gagcattacc gtcgtgtaat tgcaacactt ggcttgccaa ataacgctaa
61 agcgttacga aacaaacaac acttcggcat ggatgttgtg aagaaattcg ccgtcatgtc
121 agtgactgta gtagcaggtc ccgtccttac gctttcatca cctgtggtgg tgacgtttgg
181 aacaggctta attgccgtat ctttggtgaa acggttgcta caggaacaac cccgtgtaat
241 tgctcacgat cacgaacatt acccaggtgg ttctgagagc agttctagct cttgtgctac
301 cgcgcctatt ttacgtaatc tttcgcgaga tcagtgcgat tcagagaata ttggatgcag
361 ttctagcgcc tgttctccgt ctgaaattgt gaaagttaca aggcaggtag tgggagttga
421 acgtggtctt taccgggaca tttttcagga caacgaaatc ccatcagtca tggaagagaa
481 actgcagaaa ctcctttact ctgagggtga gaagattcga agacgttgcc aatttgaagc
541 atcaacgatg cactcacgca aagtaaaggt tccggaggta ggtactatcc cagatatcca
601 aacttggttc gatgctacgt ttcctggtaa ctccgttagg ttttctgatt tcgacggtta
661 tactgttgct acggaggaca ttaacatgga tgttcaggat tgtagactta agttcgggaa
721 gacttttcga ccttatgaat ttaaggaatc actgaaacca gtactgagga cagcaatgcc
781 agaaaaacga cagggtagtt tgattgaaag tgtgctggcc tttcgtaaaa gaaatttggc
841 tgcgcccaga ttacaaagga gctttgaatg aatggcacac aattgagaat gtgctaacga
901 aggcgttaag gtattcttct ttgaagattt aattgatcga acggatcact gcacttacga
961 tcagcgctca atggtgggat aaacaatcag tgacagctcg agcgcacctc gggcggaaca
1021 acggaaggtt tgtgatgtac tccccgactt taacttctga aaaaatgatg taagccaagt
1081 taaatctaac ccccagttga atatgcactt tgcaaacttt taccgnaaaa aacaaggctt
1141 tcnttggccc aaca

Fig. 2. Partial sequencing for the α, β and γ segments of BSMVA E1. (a) Barley stripe mosaic virus isolate Egyptian segment beta (forward), partial sequence (643 bp), accession number DQ418755. (b) Barley stripe mosaic virus isolate Egyptian segment alpha, partial sequence (467 bp), accession number DQ418753. (c) Barley stripe mosaic virus isolate Egyptian segment gamma, partial sequence (1154 bp), accession number EF027005.

components. Fig. 1. illustrates the electrophoretic mobility of the Egyptian isolate α, β and γ BSMV AE1 genomic components with molecular weights estimated at 3, 2.8 and 2.2 kb. This estimate is in reasonable agreement with the previous electrophoretic estimates (Hunter *et al.*, 1989; Gustafson *et al.*, 1981; Dolja *et al.*, 1991; Jackson *et al.*, 1983b, 1989). The sizes of the α, β and γ RNAs are reported to be similar to different strains of BSMV, whilst RNA γ varies in size. The ND18 RNA γ is 2.8 kb, while that of the type strain is 3.2 kb. The Argentin mild strain contains mixtures of RNA γ species of 3.2, 2.8 and 2.6 kb.

Amplified α, β and γ cDNAs were cloned separately into the plasmid vector (PCR 2.1-TOPO). The resultant clones were subjected to amplifica-

tion by PCR using the specific primer for each particle. The results indicated that the clones contain the full length of each particle.

Nucleotide sequence of BSMV AE1 α, β and γ

Partial sequencing for 467 bp of the α segment, 643 bp of the β segment and 1154 bp of the γ segment were obtained in respective manner. Fig. 2. illustrates the obtained nucleotide sequences. The nucleotide sequence data reported have been deposited in the Genbank under accession numbers DQ418753 (BSMV isolate Egyptian segment alpha), DQ418755 (BSMV isolate Egyptian segment beta), and EF027005 (BSMV isolate Egyptian segment gamma). Computer-based sequence comparison of the α, β and γ DNA sequence was carried out by sequence alignment using clustal W (1.83). Alignment was performed between the Egyptian AE1 isolate sequences and those of four BSMV strains, namely the type strain, CV14, ND18 and China strain. A phylogenetic tree generated using MEGA3 from clustal W alignments is illustrated for each genomic component, α, β and γ, in Fig. 3. A low degree of sequence homology between the nucleotide sequences, obtained for 467 bp and 643 bp sequenced from the α and β segments of the Egyptian isolate, placed them in a separate cluster distinct from other strains, Figs. 3a and b. However, the phylogenetic tree constructed for the γ segment (1154 bp) of BSMV, Fig. 3c, placed the Egyptian isolate in the same cluster with the BSMV ND18 strain. The cluster algorithms obtained showed clearly distinct variations in nucleotide sequences between various strains of BSMV.

BSMV Egyptian isolate could therefore be regarded as a possible distinct strain of the virus. Comparison of the BSMV genome to that of other tripartite viruses showed no homology between some BSMV proteins (β c and γ b) and polypeptides encoded by other viruses. Although many BSMV genes encode proteins which are related to other viruses, the way in which BSMV genes are coupled has been reported to be completely distinct (Solovyev *et al.*, 1996; Gustafson *et al.*, 1989). This suggests that both divergent evolution and RNA recombination have contributed to the structure of the BSMV genome.

The genus *Hordeivirus* consists of BSMV (type member) and *Poa Semilatent Virus* (PSLV), *Lychis Ringspot Virus* (LRSV), and *Anthoxanthum Blanching Latent Virus* (Atabekov and Dolja, 1986; Jackson *et al.*, 1989). Similarity between BSMV and PSLL was confirmed by partial sequence comparisons of their genomes (Agronovsky *et al.*, 1992). LRSV RNA β, however, contained an internal poly (A) sequence that is absent from PSLV RNA (Solovyev *et al.*, 1996). Thus diverse variations in degrees of homology within the *Hordeiviruses* genome were not uncommon.

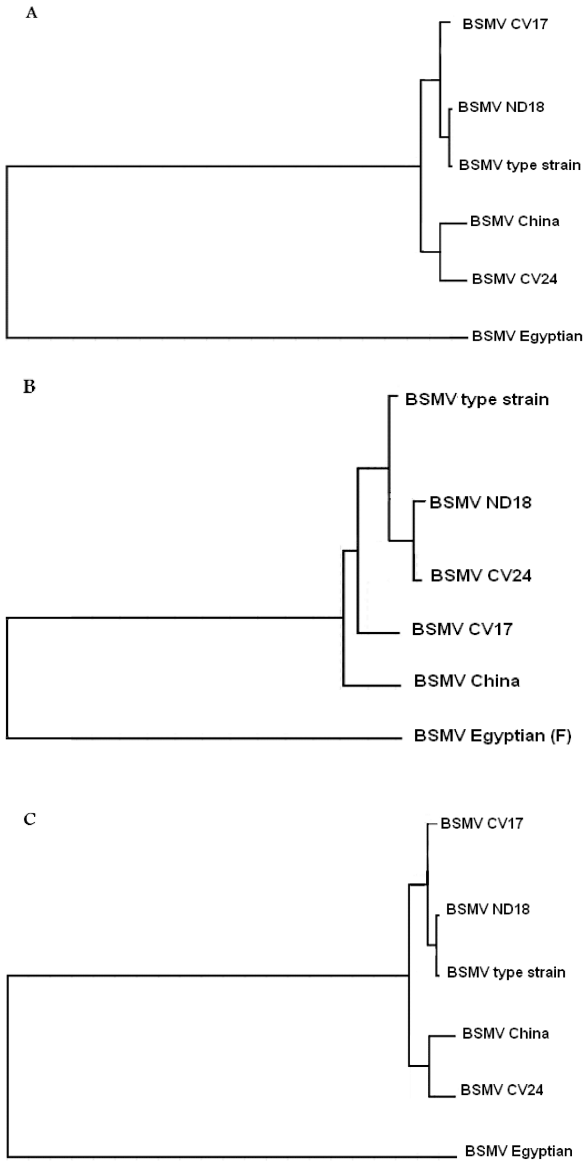

Fig. 3. Phylogenetic trees generated using MEGA3 clustal W alignments for (A) α, (B) β, and (C) γ.

Northern and Western blotting

Due to the low level of sequence homology within the segment of the BSMV AE1 α component and for exploring higher levels of homology within the around 3 kb αRNA molecule, Northern blotting was performed using a specific primer from the 3′-end of BSMV ND18 strain α, β and γ RNA, as shown in Fig. 4. The Egyptian AE1 isolate of BSMV was detected in the form of purified total RNA or in tissue infected by BSMV AE1 at 7 and 15 dpi. Detection of the AE1 isolate indicates that other areas in the α and β molecules share a higher degree of homology between the ND18 and AE1 strain. *Hordeum vulgare* tissue infected with BSMV at 7 and 15 dpi indicated the presence of the 26 kDa protein band characteristic of the BSMV coat protein in both samples, Fig. 5. Thus, indicating that the coat protein gene is expressed in all stages of infection, CP is encoded on β RNAs of BSMV (Lawrence and Jackson, 1998).

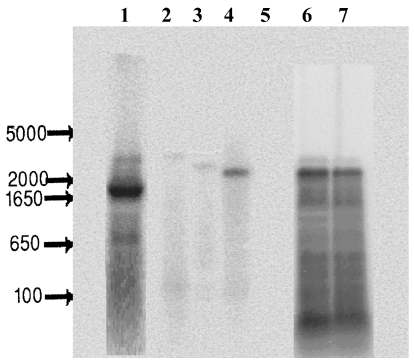

Fig. 4. Northern blot analysis using radioactively labeled RNA probe targeting the 3′-end of α, β and γ RNA to detect the presence of BSMV RNAs in purified RNA and in infected *Hordeum vulgare* L. cultivar "Black Hulless" tissue. Lane 1, total RNA; lane 2, α RNA transcript; lane 3, β RNA transcript; lane 4, γ RNA transcript; lane 5, total RNA extracted from healthy control; lane 6, total RNA extracted from BSMV AE1-infected leaf 1; lane 7, total RNA extracted from BSMV AE1-infected leaf 2.

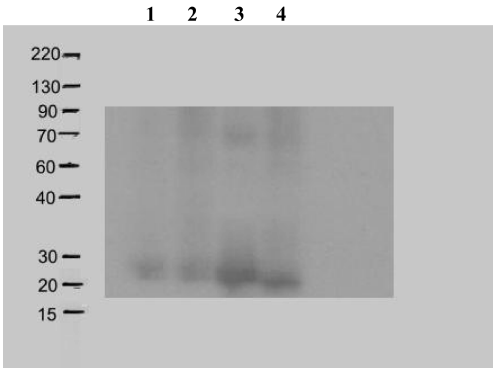

Fig. 5. Western blot analysis to detect the presence of BSMV AE1 coat protein in infected *Hordeum vulgare* L. cultivar "Black Hulless" tissue. Tissue was harvested and extracted between 7 and 15 dpi [leaf 1 and leaf 2 (older, 15 dpi); leaf 3 and leaf 4 (younger, 7 dpi)]. Before separation on 10% SDS-polyacrylamide gels, proteins were boiled in 1× SDS gel-loading buffer for 10 min. Proteins were blotted onto the nitrocellulose membrane and visualized with primary antibody (CP) made to BSMV ND18 and goat anti-mouse horse radish peroxidase (GAM-HRP).

Detection of infection by the AE1 isolate of BSMV confirms its close serological relationship to BSMV ND18. Detection of the coat protein (22 kDa) which is encoded on the RNA β a gene indicates sufficient degrees of homology between the ND18 and AE1 strains of BSMV.

Degrees of difference in nucleotide sequences ensure that AE1 isolate is a distinct strain of the virus not identical to any of the BSMV strains previously reported. It also confirms the genetic divergence of strains of the virus which affects the detailed structure of the BSMV genome.

Acknowledgement

We wish to express our sincere gratitude to Prof. Dr. Andrew O. Jackson and Dr. Jeniffer Bragg at U. C. Berkeley for their valuable assistance and support. This work has been funded by the U. S. Egypt Science and Technology joint fund, Project I. D. code BIO8-001-009.

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