Plant Res. 1(2) 92~97(1998)

Comparison and Sequence Analysis of the 3' -terminal Regions of RNA 1 of Barley Yellow Mosaic Virus

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ABSTRACT

An isolate of barley yellow mosaic virus(BaYMV-HN) obtained from Haenam, Korea was compared with two BaYMV strains, BaYMV-Ⅱ-1 from Japan and BaYMV-G from Germany. The sequence of the 3'-terminal 3817nucleotides[excluding the poly (A) tail] of RNA 1 of BaYMV-HN was determined to start within a long open reading frame coding for a part of the NIa-VPg polymerase(26 amino acids), NIa-Pro polymerase (343 amino acids), NIb polymerase(528 amino acids) and the entire capsid protein(297 amino acids), which is followed by a noncoding region(NCR) of 235 nucelotides. In the partial ORFs, BaYMV-HN shows higher sequence homology with BaYMV-Ⅱ-1(99.5%) than BaYMV-G(92.7%). The 3' non-coding regions of BaYMV-HN(235nt) shows higher nucleotide sequence homology with BaYMV-G(235nt) (99.6%) than BaYMV-Ⅱ-1(231nt) (97.0%). The 3' NIa-Pro protein sequence of BaYMV-HN shows higher amino acid sequence homology with BaYMV-Ⅱ-1(95.0%) than BaYMV-G 93.6%), but, NIb protein sequence of BaYMV-HN shows same all amino acid sequence. The capsid protein sequence of BaYMV-HN(297aa) shows same with BaYMV-Ⅱ-1, and shows higher nucleotide sequence homology with BaYMV-UK (from United Kingdom) (97.3%) than BaYMV-G(96.9%) and G2(96.9%). Difference of capsid protein amino acid were 0-9 between the Japan, United Kingdom and Germany and were 2-6 between all Korean isolates. Many of the amino acid differences are located in the N-terminal regions of the capsid proteins from 1 to 74 amino acid positions.

Key words: bymovirus, BaYMV, capsid protein, strain, RNA 1.

INTRODUCTION

Barley yellow mosaic virus(BaYMV) and barley mild mosaic virus(BaMMV) bymoviruses are responsible for the economically important yellow mosaic disease of winter barley in East Asia and Europe(Huth and Adams, 1984). Both viruses are transmitted in soil the root-infecting fungus *Polymyxa graminis* Led.(Adams etc., 1989). In korea, the two viruses are widespread, and as no resistant barley cultivars are available, they cause severe damage to barley crops. BaYMV has a bipartite genome comprising two 3' -polyadenylated ssRNA molecules of 7.6kb(RNA 1) and 3.7kb (RNA 2) (Huth etc., 1984, Kashiwazaki etc., 1989b). Complete nucleotide sequences have been determined for Japanese(Kashiwazaki etc., 1990, Kashiwazaki etc., 1991) and German(Peerenboom etc., 1992) isolates of BaYMV. However, little is known about the properties of BaYMV occurring in Korea(Lee etc., 1998).

We determines here the nucleotide sequence of the 3' -terminal regions of RNA 1 of a Korean isolate(BaYMV-HN) and compared with reported sequences of other BaYMV strains. We determined and compared the sequences of the BaYMV capsid protein genes from field samples collected at different sites in Korea.

MATERIALS AND METHODS

Virus isolation and propagation.

BaYMV-HN was isolated from a naturally infected barley

Table 1. Samples used for sequence analysis of the BaYMV capsid protein genes

No. of site	Location	Barley cultivar or type	ELISA detection BaMMV	BaYMV
1	Haenam	Doosan 22 (two-rowed)	+*	+
2	Jangheung	Two-rowed barley	+	+
3	Yeongam	Baegdong (six-rowed, naked)	+	+
4	Naju	Naehanssalbori (six-rowed, naked)	+	+
5	Hampyung	Six-rowed barley	+	+
6	Yeongkwang	Baegdong (six-rowed, naked)	+	+
7	Iksan(Iri)	Baegdong (six-rowed, naked)	+	+
8	Suwon	Six-rowed barley	+	+
9	Samcheok	Six-rowed barley	-	+
10	Sacheon	Two-rowed barley	+	+
11	Koseong	Two-rowed barley	+	+
12	Bukuk	Six-rowed barley	+	+

*BaMMV was detected (+) or not detected (-) by ELISA.

plant(cv. Doosan 22) collected in Haenam, Korea and propagated in barley cv. New Golden by mechanical inoculation as described previously(Kashiwazaki etc., 1989a).

Virus purification.

BaYMV-HN was purified from infected barley tissues as described by Usugi and Saito(1976).

cDNA synthesis and cloning.

Viral RNA was isolated from purified virus particles as described by Kashiwazaki *et al.*(1989b). Oligo(dT)-primed cDNA was synthesized using the Amersham cDNA synthesis kit. Double-stranded cDNA was size-selected by agarose gel electrophoresis and then ligated into *Sma*I-cut, dephospholylated pBluescript Ⅱ KS+(Stratagene). The ligated cDNA was used to transform competent *Escherichia coli* NM 522 cells. Recombinatant plasmids were screened for large cDNA inserts using the Screen Test Recombinant Screening kit(Stratagene). Two large cDNA clones, pByHN40(3.8Kb) and pByHN8(3.8Kb), were selected and hybridized to Northern blots of viral RNA using the ECL gene detection kit(Amersham).

DNA sequencing.

Subclones were obtained from pByHN40 using nested deletions generated by exonuclease Ⅲ and were sequenced by the dideoxynucleotide chain termination reaction using an automated DNA sequencer(377, Applied Biosystems). All parts of the cDNA were sequenced in both orientations. Sequence data were compiled and analyzed using the DNASIS system (Hitachi Software Engineering).

Field samples.

Barley plants showing yellow mosaic fields at twelve different sites in Korea in the March, 1997(Table 1).

RT-PCR.

Barley leaves with symptoms were examined by enzyme-linked immunosorbent assay using antiserum coating BaYMV were used for RT-PCR. Leaf samples and RT-PCR was performed as described by Kashiwazaki and Hibino(1996), with a change of annealing temperature in the PCR programs from 65℃ to 55℃. To amplify a 891bp fragment containg the capsid protein gene, a revers primer S14(5' AAA*GGATCC*GTGGGTGATGTATAAGGC3', complementary to 3599-3616 nucelotides of BaYMV-HN RNA 1, including) site which is underlined) and a forward primer S13(5' CCC*TCTAGA*TGATGAAA TTTGGCTGC3', complementary to 2671-2689 nucelotides of BaYMV-HN RNA 1, including) were designed. Amplified fragments were digested with BamH I and Xba I, and were cloned into compatible sites of pBlueScript Ⅱ KS(+). The fragments were sequenced from both orientations.

RESULTS

From the oligo(dT)-primed cDNA library prepared from the BaYMV-HN, we selected two large clones pByHN40 and pByHN8, which hybridized specifically to RNA 1 and RNA 2, respectively. The sequence of 3' terminal 3817 nucleotides upstream of the poly(A) tail, determined by analysis of pByHN40, is given in Fig. 1. It contains one large open reading frame(ORF) which is open at the 5' end and terminates with a UAA stop codon at position 3583. The partial ORF codes for a polypeptides of 1194 amino acids, which correspondings to the C-terminal part of the single large polyprotein encoded by BaYMV RNA 1(Kashiwazaki etc., 1990). This ORF product has EA(26-27), QA(369-370) and QA(898-899) dipeptides which are thought to be the cleavage site between the NIb and capsid proteins, and the NIb and NIa-pro, and the NIa-pro and NIa-VPg as reported for RNA 1 of other BaYMV isolates(Kashiwazaki etc., 1990). Thus, it contains a part of the NIa-VPg protein(26 amino acid), NIa-pro protein(343 amino acid), NIb protein (528 amino acids)

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CCAGAGCUUCGGAGAGCCUAUGAAAAGAGACCAUACUUCAAUUUCUACGACCUUCAAACAGAUAGCAACAUUCUGGAAGCUAUUUUCUACACCACUGAAGGCGAUGAGUUUUUCCGAACA   120
  P  E  L  R  R  A  Y  E  K  R  P  Y  F  N  F  Y  D  L  Q  T  D  S  N  I  L  E/A  I  F  Y  T  T  E  G  D  E  F  F  R  T    40
GCAGAUCCCAACAAGGACAUGAACUUGGUUGCUGACAAACUACGCUCUUUUCUCGACACAAAGGUUGUUGUUGGACACCACCAGCGGCAGAUGCUAGAAGAAACAGCCAAGGUUGUCAUC   240
  A  D  P  N  K  D  M  N  L  V  A  D  K  L  R  S  F  L  D  T  K  L  V  V  G  H  H  Q  R  Q  M  L  E  E  T  A  K  V  V  I    80
AAAGAUACGAAGGGAACUGCGCACCACACAUGGACAUUUCACAGCAUGAUCCAGACCAUCUAAAGCAGAAUGGGUCCGGCAAAAUUGGAUAUCCAGAACACCGGGGGCAGUUCCGACAGGAA   360
  K  D  T  K  G  T  A  H  H  M  D  I  S  Q  H  D  P  D  H  L  K  Q  N  G  S  G  K  I  G  Y  P  E  H  R  G  Q  F  R  Q  E   120
GGACCUGCCAAAACAGCGGAUUACGAUCCUUGGAGUUGAGUUUGGCACCGACACCGAUGACAUCACGCUCGAAGCCUCUACUGGGAUAUUAUUGUCACAAGUUGGGGUCGACGUCGCAACC   480
  G  P  A  K  T  A  D  Y  D  L  G  V  E  F  G  T  D  T  D  D  I  T  L  E  A  S  T  G  I  L  L  S  Q  V  G  V  D  V  A  T   160
CGAGUUGGAAGAAUUUGCAUUGGUACUUUCAACAUGAACUGUUACUCCUACAGCGACUGGAUUCUGGUUCCAGGACACCUGCAAGACAGAUCCGGUAACGUGACAAUUCAAUUUCCUGAC   600
  R  V  G  R  I  C  I  G  T  F  N  M  N  C  Y  F  Y  S  D  W  I  L  V  P  G  H  L  Q  D  R  S  G  N  V  T  I  Q  F  P  D   200
CAAACAGUGCAAACCACAACUGAUGCACUCAACGCAAAUGGUGUGAAACGAUUCUAUGGAUUAGAUGUGAUAGCAAUUCGGCGCCCUGCUACUUUGAGACCUCGCACCAAGUUGGUCAAA   720
  Q  T  V  Q  T  T  T  D  A  L  N  A  N  G  V  K  R  F  Y  G  L  D  V  I  A  I  R  R  P  A  I  L  R  P  R  T  K  L  V  K   240
GCUUUGGCUAUUGAGGAACCAGUUAUCGCACAAAUGGUCUUUGUUGACGCACAAGGAGUUCGUAAGUGUACUCAAUCUGAUUGGGCAGAAAGGAGGAGAAUUCCGGACGUUGGUCACAU   840
  A  F  A  I  E  E  P  V  I  A  Q  M  V  F  V  D  A  Q  G  V  R  K  C  T  Q  S  D  W  A  R  K  E  E  N  S  G  R  W  S  H   280
AAGAUUUCCACUGUUCUUGGCAUGUGUGGAUGCCCAGUUCUGGACGUUGGAAAGAACAGAUUGAUAGGAAUCCAUGUUGCCACAAACUACACAAAGAAGCGCAACGAGUUCCAACCCUUC   960
  K  I  S  T  V  L  G  M  C  G  C  P  V  L  D  V  G  K  N  R  L  I  G  I  H  V  A  T  N  Y  T  K  K  R  N  E  F  Q  P  F   320
ACUCAGGAGGUCGUUGAUUUCAUAAAUGGACCUGGAACAAAAAUCCCCUCACUGCCCAUGGGUAUUCGAUAGACCGGCUUGUGGGCAUGCAUCCCACACAGCUUUGUUCGAGAAACCAACC  1080
  T  Q  E  V  V  D  F  I  N  G  P  G  T  K  I  P  Y  C  P  W  V  F  D  R  P  A  C  G  Y  A  S  H  T  A  L  F  E  K  P  T   360
ACACUAACUGACAUCAUUCACAUGGCAAGCUUCUGAUGGUUUGCACAACAUCAACAAUGGCAUUGAGGGUUUCUGGAGUUCACUGAGGGGUCAGCUCGUGUCACCACCUACUGAAUCCACC  1200
  T  L  T  D  I  I  H  M  Q  A  S  D  G  L  H  N  I  N  N  A  I  E  G  F  G  S  S  L  R  G  Q  L  V  S  P  P  T  E  S  T   400
AGGCAACGUUUCGAUAAGCUGUUUGGCAGUGGCAGCUUUGAACUCAUUGGGCAAAUGAACAAAGGCUUAAUCGACAAACACGCAAUUGUUGGGGAAAAUGAUGAUGUUCAUGACUUCAUG  1320
  R  Q  R  F  D  K  L  F  G  S  G  S  F  E  L  I  G  Q  M  N  K  G  L  I  D  K  H  V  I  V  G  E  N  D  D  V  H  D  F  M   440
CGCGAGCACCCAACAUUUACUUGGUCGAAAGAUUUCAUGAAUGAGUAUGGCGCUAGUGUUUUGUCUUACUCUGCUUACUACAAAGACCUUUGCAAGUAUAAUCGUGCAAAACAUGUGCUA  1440
  R  E  H  P  T  F  T  W  L  K  D  F  M  N  E  Y  A  P  S  V  L  S  Y  S  A  Y  Y  K  D  L  C  K  Y  N  R  A  K  H  V  L   480
ACUUACAACUCGGAAGAGCUCCCAUUAUGCAACGAAAGGACUGAUCAAGAUGUUGGAAGACGCUGGCUUGACACAAGGCAGUGUGAGGACACCCCAACAAGUCAUUUCAGACAUCCAAUGG  1560
  T  Y  N  S  E  E  L  H  Y  A  T  K  G  L  I  K  M  L  E  D  A  G  L  T  Q  G  S  V  R  T  P  Q  Q  V  I  S  D  I  Q  W   520
AACACUCCCGCUGGACCUAGCUAUCAAGGGAAGAAACGCGACCUCCUGCGCCCAUUUGAGUGAUGAUGAGGUGCUGCAUCUUGCUGAGGUGUGUCGCCAACAAUUUCUCCGAAGGCAAGUCA  1680
  N  T  S  A  G  P  S  Y  Q  G  K  K  R  D  L  C  A  H  L  S  D  D  E  V  L  H  L  A  E  V  C  R  Q  Q  F  L  E  G  K  S   560
ACUGGAGUGUGGAAUGGUUCUUUGAAAGCUGAGUUGAGAACCAUUGAGAAAGUUGAGGCGGAGAAAACGCGUGUUUUUCACAGCCUCUCCCAUAACAAGCUUGUUUGCCAUGAAAUCUCAC  1800
  T  G  V  W  N  G  S  L  K  A  E  L  R  T  I  E  K  V  E  A  E  K  T  R  V  F  T  A  S  P  I  T  S  L  F  A  M  K  F  Y   600
GUUGACGAUUUUAACAAGAAGUUCUACGCCACCAAUCUGAAAGCCCCCCAUACUGUUGGCAUCAAUAAGUUCGGUAGGGGAUGGGAAAAAUUGCAUGAUAAGCUUAAUCGCCUUGGAUGG  1920
  V  D  D  F  N  K  K  F  Y  A  T  N  L  K  A  P  H  T  V  G  I  N  K  F  G  R  G  W  E  K  L  H  D  K  L  N  R  P  G  W   640
UUGCAUGGUAGUGGAGAUGGUUCUAGGUUCGAUAGCUCCAUCGAUCCUUUCUCCUUCGACGUUGUGAAAACUAUCCGCAAGCACUUUCUCCCUUCUGAGCAUCAUAAGGCCAUUGAUCUC  2040
  L  H  G  S  G  D  G  S  R  F  D  S  S  I  D  P  F  F  D  V  V  K  T  I  R  K  H  F  L  P  S  E  H  H  K  A  I  D  L   680
AUAUACGAUGAGAUCUUCAACACAACCAUCUGCCUGGCUAAUGGGACUGAUCAUCAAGAAGAAUGUUGGGAACAACAGCGGGCAGCCUAGCACUGUUGUCGAUAACACACCUGUGCUCCAUG  2160
  I  Y  D  E  I  L  N  T  T  I  C  L  A  N  G  M  I  I  K  K  N  V  G  N  N  S  G  Q  P  S  T  V  V  D  N  T  L  V  L  M   720
ACUGCAUUCUCUCACGCAUACAUCCACAAAACAGGAGACCGUGAAUUGGCCUUGCUAAACGAGCGUUUCAUCUUUGUUUGCAAUGGCGAUGACAACAAGUUUGCCAUAUCUCCGCAGUUU  2280
  T  A  F  L  Y  A  Y  I  H  K  T  G  D  R  E  L  A  L  L  N  E  R  F  I  F  V  C  N  G  D  D  N  K  F  A  I  S  P  Q  F   760
GACGAAGAGUUCUGGGCAUGAUUCUCUCCCAGAACUUGUUGAACUUCGGCUACGAACGAAUUGAUGACAUCACUAGCGACAUUUGCGAGAACUCCUUCAUAGUCCCUCCACCAUGGUGAAA  2400
  D  E  E  F  G  H  D  F  S  P  E  L  V  E  L  G  L  T  Y  E  F  D  D  I  T  S  D  I  C  E  N  P  Y  M  S  L  T  M  V  K   800
ACCCCUUUUGGCGUUGGUUUUCUCUUCCCCGUGGAAAGAAUCAUAGCUAUCAUGCAAUGGUCCAAGAAAAGGGGGAGUUCCUGCACUCAUAUUUGGCUGGCAUUCUCCGCAAUUUACGAAUCC  2520
  T  P  F  G  V  G  F  S  L  P  V  E  R  I  I  A  I  M  Q  W  S  K  K  G  G  V  L  H  S  Y  L  A  G  I  S  A  I  Y  E  S   840
UUCAACACACCAAAACUUUCAAAUCAAUCUAUGCGUAUCUUCUUUGGCUCACUGAGGAGCACGAGGCAGAAAUUCUCGCAGCCAUGACUCAAUCCUCAACUGCUCUUCCCAUACCAUCA  2640
  F  N  T  P  K  L  F  K  S  I  Y  A  Y  L  L  W  L  T  E  H  E  A  E  I  L  A  A  M  T  Q  S  S  T  A  L  P  I  P  S   880
AUGCUCGAUGUUUAUCGGUUGCAUUAUGGCGAUGAUGAAAUUUGGCUGCAAGCUGCUGAUCCUCUCACUGAUGCACAAAAAGAAGAUGCACGCAUUGCUGCAGCUGAUGGAGCACGCUUC  2760
  M  L  D  V  Y  R  L  H  Y  G  D  D  E  I  W  L  Q/A  A  D  P  L  T  D  A  Q  K  E  D  A  R  I  A  A  A  D  G  A  R  F   920
GAACUAGCCGAUGCUGAUCGUAGACGUAAAGUUGAGGCUGACCGUGUGGAAGCGGCAAGAGUCAAAAAGGCAGCUGAUGCUGCACUAAAACCAGUUAACCUCACAGCAACACGCACGCCC  2880
  E  L  A  D  A  D  R  R  R  K  V  E  A  D  R  V  E  A  A  R  V  K  K  A  A  D  A  A  L  K  P  V  N  L  T  A  T  R  T  P   960
ACAGAAGAUGAUGGCAAACUUAAAACACCAUCUGGCGCUAGAAUACCAUCAUCAGCUGCAGAUGGUAACUGGAGCGUGCCAGCAACGAAGCAAGUCAACGCUGGUUUGACACUCAAAAUU  3000
  T  E  D  D  G  K  L  K  T  P  S  G  A  R  I  P  S  S  A  A  D  G  N  W  S  V  P  A  T  K  Q  V  N  A  G  L  T  L  K  I  1000
CCCCUGAACUAAACUCAAAAGUGUACCUAAGUCUGUUAUGGAGCAUAACAACUCAGUCGCACUCGAAUCUGAGCUAAAAGCCUGGACUGAUGCUGUCGCAAGUUGGGAUCACAACU  3120
  P  L  N  K  L  K  S  V  P  K  S  V  M  E  H  N  N  S  V  A  L  E  S  E  L  K  A  W  T  D  A  V  R  T  S  L  G  I  T  T  1040
GAUGAGGCAUGGAUCGAUGCCCUCAUUCCCUUCAUCGGAUGGUGCUGCAACAAUGGCACCUCAGACAAGCAUGCUGAGAAUCAAGUAAUGCAAAUUGACAGCGGAAAGGGAGCUGUUACA  3240
  D  E  A  W  I  D  A  L  I  P  F  I  G  W  C  C  N  N  G  T  S  D  K  H  A  E  N  Q  V  M  Q  I  D  S  G  K  G  A  V  T  1080
GAGAUGAGUCCUGUCCCCAUUCAUUGUUCAUGCUCGAAUGAAUGGUGGACUCCGAAGGAUCAUGCGCAACUACAGUGAUGAAACCGUCUUACUCAUCACAAACAACAAACUAGUUGCACAU  3360
  E  M  S  L  S  P  F  I  V  H  A  R  M  N  G  L  R  R  I  M  R  N  Y  S  D  E  T  V  L  L  I  T  N  N  K  L  V  A  H  1120
UGGUCAAUGAAGCAUGGCGCUUCUGCAAACGCAAAAUACGCUUUCGAUUCUUCGUCCCACGAUCAUGGAUGAACCCACAGGACAUCGAAGUGUCAAAGCAAGCGCGCUUAGCAGCACUU  3480
  W  S  M  K  H  G  A  S  A  N  A  K  Y  A  F  D  F  F  V  P  R  S  W  M  N  P  Q  D  I  E  V  S  K  Q  A  R  L  A  A  L  1160
GGAACUGGAACGUACAACACCAUGUUCAACUUCCGACACUACCAAUCUGCGUAAGACAACCAACCACAGGGUUCUGGACUCAGAUGGACACCCAGAACUCAACCUCAAACCAUCACCCCGUGC  3600
  G  T  G  T  Y  N  T  M  L  T  S  D  T  T  N  L  R  K  T  T  N  H  R  V  L  D  S  D  G  H  P  E  L  T  •                1194
CUUAUACAUCACCCACCCCACUCACAUAAUUCAGUCUUUAAAUUAUGUUCGUGACGUCUUUGUAUCAGGCUCGGAAGGUUCUCAUGAUCCAUAUGCCUUUGGCAGUUACAAUGUCAGCAUU  3720
CUCAUUGAGGAGUAUGACAGCGUUUGAUCAGCCGUUUUCAAUCAUCCGUCGUUGGAUGGUGGUGCACUUAGUGCACUCACGAGUACCAGAAGGUAAU(A)n                      3817
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Fig. 1. The nucleotides sequence and deduced amino acid sequence of the 3' -terminal 3817 nucelotides from BaYMV-HN RNA 1. A possible cleavage site between the NIa-Pro, NIb and capside proteins are shown by a slash.

and the capsid protein(297 amino acid).

The NIb protein of BaYMV-HN contains the two conserved stretches SGXXXTXXXNT and GDD at positions 706-716 and 749-751, as also found for other BaYMV isolates. These stretches are thoughts to form the core of RNA-dependent RNA polymerases of positive strand RNA viruses. The two conserved blocks NGTS and AFDF reported for the capsid proteins of bymoviruses and other rod-shaped viruses(Kashiwazaki etc., 1990, 1991) are also found in the capsid protein of BaYMV-HN at position 1058-1061 and 1134-1137. The 3' non- coding region(NCR) of BaYMV-HN RNA 1 is 235 nucleotide long.

Difference of capsid protein amino acid were 0-9 between the Japan, United Kingdom and Germany and were 2-6 between all Korean isolates. Its amino acid differences from the four other BaYMV strains vary from 0 to 9(Table 4), and all Korean isolates are differences from the twelve other site BaYMV isolates vary from 2 to 6(Table 4). Many of the amino acid differences are located in the N-terminal regions of the capsid proteins from 1 to 74 amino acid positions. N-terminal of capsid protein of all BaYMV strain and isolate are A, but especially, Samcheok isolate is changed from A to T (position 1).

Table 2. Percentage of identical nucleotides in the 3' -terminal regions of RNA 1 of three BaYMV isolates

(a) Partial ORFs			
	BaYMV-HN	BaYMV-Ⅱ-1	BaYMV-G
BaYMV-HN	-		
BaYMV-Ⅱ-1	99.5	-	
BaYMV-G	92.7	92.6	-
(b) 3' non-coding regions			
	BaYMV-HN	BaYMV-Ⅱ-1	BaYMV-G
BaYMV-HN	-		
BaYMV-Ⅱ-1	97.0	-	
BaYMV-G	99.6	96.6	-

Percent identity was calculated with the DNASIS DNA Homology Search Program which made an optimal alignment of two sequences.

DISCUSSION

In this study, we determined the sequence of the 3' terminal 3817 nucelotides in RNA 1 BaYMV-HN. The sequence share extensive homology with those of the corresponding regions from BaYMV-Ⅱ-1(from Japan)(Kashiwazaki etc., 1990) and BaYMV-G(from Germany)(Peerenboom etc., 1992), which is due to the deletion of the four nucleotides UUAC(3796-3799 in BaYMV-HN RNA 1) from BaYMV-Ⅱ-1 RNA 1.

Table 3. Percentage of in amino acid in putative gene products of four BaYMV isolates

(a) NIa-Pro				
Isolate	BaYMV-HN	BaYMV-Ⅱ-1	BaYMV-G	
BaYMV-HN	-			
BaYMV-Ⅱ-1	95.0	-		
BaYMV-G	93.6	89.0	-	
(b) NIb				
	BaYMV-HN	BaYMV-Ⅱ-1	BaYMV-G	
BaYMV-HN	-			
BaYMV-Ⅱ-1	98.7	-		
BaYMV-G	98.7	98.7	-	
(c) Capsid protein				
	BaYMV-HN	BaYMV-Ⅱ-1	BaYMV-G	BaYMV-G2
BaYMV-HN	-			
BaYMV-Ⅱ-1	100	-		
BaYMV-G	96.9	96.9	-	
BaYMV-G2	96.9	96.9	99.3 -	
BaYMV-UK	97.3	97.3	99.6 99.6	

Percent identity was calculated with the DNASIS DNA Homology Search Program.

Table 4. Amino acid sequence variations in the capsid proteins of twelve Korean and four other isolates of BaYMV

BaYMV isolate	Number of amino acid differences*	Amino acid position												
		1	12	14	15	20	23	24	28	51	56	62	64	74
Korean isolates														
Haenam	-	A	D	R	I	G	F	E	A	A	N	T	T	S
Jangheung	2	-	-	-	-	R	-	-	-	-	-	M	-	-
Yeongam	2	-	-	-	-	K	-	-	-	-	-	M	-	-
Naju	2	-	-	-	-	K	-	-	-	-	-	M	-	-
Hampyung	2	-	-	-	-	K	-	-	-	-	-	M	-	-
Yeongkwang	2	-	-	-	-	R	-	-	-	-	-	M	-	-
Iksan	2	-	-	-	-	R	-	-	-	-	-	M	-	-
Suwon	2	-	-	-	-	R	-	-	-	-	-	M	-	-
Bukok	2	-	-	-	-	R	-	-	-	-	-	M	-	-
Sacheon	2	-	-	-	-	K	-	-	-	-	-	M	-	-
Koseong	4	-	-	-	-	K	-	-	V	-	-	M	-	T
Samcheok	6	T	A	H	T	R	-	-	-	-	-	M	-	-
Other isolats														
Japan(strain Ⅱ-1)	0	-	-	-	-	-	-	-	-	-	-	-	-	-
United Kingdom(UK)	8	-	A	H	T	R	L	D	-	V	-	M	-	-
Germany(G)	9	-	A	H	T	R	L	D	-	V	T	M	-	-
Germany(G2)	9	-	A	H	T	R	L	D	-	V	-	M	A	-

*Amino acid differences from the BaYMV-HN sequence are indicated.

In the partial ORFs, BaYMV-HN shows higher sequence homology with BaYMV-Ⅱ-1(99.5%) than BaYMV-G(92.7%)(Table 2, a). The 3' NCR of BaYMV-HN consist of 235 nucleotides, but BaYMV-G is 234 nucleotides, having a deletion in nucleotide position 163(U in BaYMV-HN RNA 1) and BaYMV-Ⅱ-1 are 231 nucleotides, having a deletion in nucleotide position 215-218 (UUAC in BaYMV-HN RNA 1). The 3' non-coding region of BaYMV-HN shows higher nucleotide sequence homology with that of BaYMV-G(99.6%) than that of BaYMV-Ⅱ-1(97%) (Table 2, b).

The NIa-Pro protein sequence of BaYMV-HN is identical size(343 amino acid) to BaYMV-G, but BaYMV-Ⅱ-1 was short of one amino acid (Kashiwazaki etc., 1990). The NIb protein sequence of BaYMV-HN shows slightly higher sequence homology with BaYMV-Ⅱ-1(95%) than BaYMV-G (93.6%)(Table 3, a).

The NIb protein sequence of BaYMV-HN is identical size(528 amino acid) to BaYMV-G, but BaYMV-Ⅱ-1 was short of one amino acid (Kashiwazaki etc., 1990). The NIb protein sequence of BaYMV-HN shows slightly higher sequence homology with BaYMV-Ⅱ-1(98.7%) and BaYMV-G(98.7%) (Table 3, b).

The capsid protein sequence of BaYMV-HN is identical size(297 amino acid) to those of other BaYMV strains reported to data: BaYMV-Ⅱ-1 (Kashiwazaki etc., 1990), BaYMV-UK(from United Kingdom)(Shi etc., 1995) and BaYMV-G (Peerenboom etc., 1992). The capsid protein of BaYMV-HN shows higher amino acid sequence homology with BaYMV-Ⅱ-1 (100%) than BaYMV-G and BaYMV-G2(from Germany)(96.9%), and BaYMV-UK (97.3%) (Table 3, c). Its amino acid difference from the four other BaYMV isolates vary from 0 to 9(Table 4). Many of the amino acid differences are located in the N-terminal regions (position 12 to 64) of the capsid protein, which are probably exposed on the surface of the virus particles(Kashiwazaki etc., 1992).

The nucleotide and predicted amino acid sequences of the capsid protein coding region of twelve Korean isolates(Haenam, Jangheung, Yeongam, Naju, Hampyung, Yeongkwang, Iksan, Suwon, Samcheok, Sacheon, Koseong and Bukuk) were determined. Comparisons between theses and those of previously sequenced isolates showed homologies of 98.2-99.9% in nucleotides and 97.6-100% in the

amino acid sequences. The capsid protein coding region of the Samcheok isolate had a one amino acid difference (A to T) from BaYMV-HN at position 1 which did not give rise to any change in the predicted amino acid sequence.

Korean strain(BaYMV-HN) is most closely related to Japan strain (BaYMV- Ⅱ -1) than European strain based on sequence data, but the pathogenicity towards the barley cultivars and the evolution significance remains to be studies.

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