



Fig. 2. Amplification products from (A) RACE-PCR reaction using first-strand cDNA from purified viral RNA (lane 1) or water as a negative control (lane 4). Further negative-control PCR reactions are shown using the products of a mock first-strand cDNA reaction on viral RNA without reverse transcriptase (lane 2) or without RNA (lane 3). (B) Shows the amplification product from (A, lane 1) digested with either *Hind*III (lane 1) or *Dra*I (lane 2). (C) shows the amplification product of a PCR reaction on clone pBM-217 using primers BAM-3 and BMCP-1. DNA size markers (M_r) are shown (100 bp DNA ladder, Gibco- BRL).

which contains an ATG start codon linked to the sequence of the first 18 nucleotides of the CP gene (4).

BAM-3: 5'-CGC GGATCC AACA ATG GCA GGG CAT GAG GAA CCA -3'
M A G H E E P

The ATG codon is in a favorable plant translation context (5) and a *Bam*HI restriction site (underlined) was included to facilitate subsequent cloning steps. Since restriction enzymes do not cleave sites close to the end of DNA strands very efficiently, the *Bam*HI site is preceded by a CGC triplet (Note 6).

1. A 25- μ L PCR reaction is performed on clone pBM-217, which contains the BaMMV CP gene as an *Eco*RI fragment in pUC 13 (Fig. 1C). The 5' end of the CP gene is amplified using primer BAM-3 and a second primer BMCP-1 complementary to a region within the gene (see Fig. 1A).

BMCP-1: 5'-GGAATAACAGCGGAAGA-3'

The reaction contains 2.5 μ L of 10X PCR buffer (see above), 1.5 μ L of 25 mM $MgCl_2$, 4 μ L of 1.25 mM dNTPs, 0.2 μ L of 5 U/ μ L *Taq* polymerase (Advanced Biotechnologies), 2.5 μ L of each primer (25 μ g/mL stock), 1 μ L of template DNA