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Short communication

A novel set of polyvalent primers that detect members of the genera *Bromovirus* and *Cucumovirus*

Jang-Kyun Seo[a], Ye-Ji Lee[a], Mi-Kyeong Kim[a], Su-Heon Lee[b], Kook-Hyung Kim[c], Hong-Soo Choi[a,*]

[a] Crop Protection Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea
[b] School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Republic of Korea
[c] Department of Agricultural Biotechnology and Plant Genomics and Breeding Institute, Seoul National University, Seoul 151-921, Republic of Korea

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A B S T R A C T

Rapid detection and diagnosis of plant virus infection is one of the most important steps in preventing damages caused by viral diseases. Bromoviruses and cucumoviruses belong to the family *Bromoviridae*, which is one of the most important families of plant viruses, and infect a broad range of host plants including various economically important crops. In this study, an RT-PCR assay was developed for the universal detection of bromoviruses and cucumoviruses using a set of primers designed to target the conserved sequences in viral RNA1. The assay detected three species of Cucumovirus (*Cucumber mosaic virus* (CMV), *Peanut stunt virus* (PSV) and *Tomato aspermy virus* (TAV)) and two species of *Bromovirus* (*Brome mosaic virus* (BMV) and *Cowpea chlorotic mottle virus* (CCMV)) with high specificity and sensitivity. The assay developed in this study is predicted to have the potential to detect all major members of the genera *Bromovirus* and *Cucumovirus* and to be used as a routine diagnostic assay.

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Rapid and accurate diagnosis of plant virus infection is important for the preparation of appropriate countermeasures to minimize damages caused by viral diseases. Certain methods including serological methods and reverse transcription-polymerase chain reaction (RT-PCR)-based methods have been used widely for routine diagnosis of plant viruses. To improve cost-efficiency, simplicity, and sensitivity, several approaches have been employed for plant virus detection, including real-time RT-PCR, microarrays, and simultaneous detection of multiple virus species (reviewed in James et al., 2006). While multiplex PCR enables the detection of several viruses in a single reaction by using multiple primer pairs targeting different viruses (Singh et al., 1996; Henegariu et al., 1997), polyvalent PCR using a pair of universal primers allows the amplification of a range of viruses sharing sequence homology in a single PCR (Gibbs and Mackenzie, 1997; Choi et al., 1999; Foissac et al., 2005).

Bromoviruses and cucumoviruses belong to the family *Bromoviridae*, which is one of the most important families of plant viruses (Adams et al., 2014). The most recent report of the International Committee on Taxonomy of Viruses (ICTV) released in 2013 listed 6 viruses as species of genus *Bromovirus* (*Brome mosaic virus* (BMV), *Broad bean mottle virus* (BBMV), *Cowpea chlorotic mottle virus* (CCMV), *Cassia yellow blotch virus* (CYBV), *Melandrium yellow fleck virus* (MYFV), and *Spring beauty latent virus* (SBLV)) and 4 viruses as species of genus *Cucumovirus* (*Cucumber mosaic virus* (CMV), *Peanut stunt virus* (PSV), *Tomato aspermy virus* (TAV), and *Gayfeather mild mottle virus* (GMMV)). The type species of the genus *Bromovirus* and *Cucumovirus* are BMV and CMV, respectively. Like all other genera in the *Bromoviridae*, the genomes of bromoviruses and cucumoviruses consist of three positive-sense single-stranded RNAs (Gunn and Symons, 1980; Palukaitis and Garcia-Arenal, 2003). RNA1 and RNA2 encode the replication proteins 1a (~110 kDa), which contains methyltransferase and helicase motifs, and 2a (~97 kDa), which has an RNA-dependent RNA polymerase activity, respectively. RNA3 is bicistronic and encodes two proteins (movement protein and coat protein) involved in virus movement. Since bromoviruses and cucumoviruses infect a wide range of hosts including various economically important crops, such as wheat, oats, maize, tomato, cucumber, and legume plants (Lane, 1974; Palukaitis and Garcia-Arenal, 2003), developing simple and cost-efficient methods is required for rapid detection of these viruses. This study describes a polyvalent PCR method for simultaneous detection of bromoviruses and cucumoviruses using a set of polyvalent primers designed to target the conserved sequences in viral RNA1.

* Corresponding author. Tel.: +82 31 290 0430; fax: +82 31 290 0406.
E-mail address: hschoi@korea.kr (H.-S. Choi).

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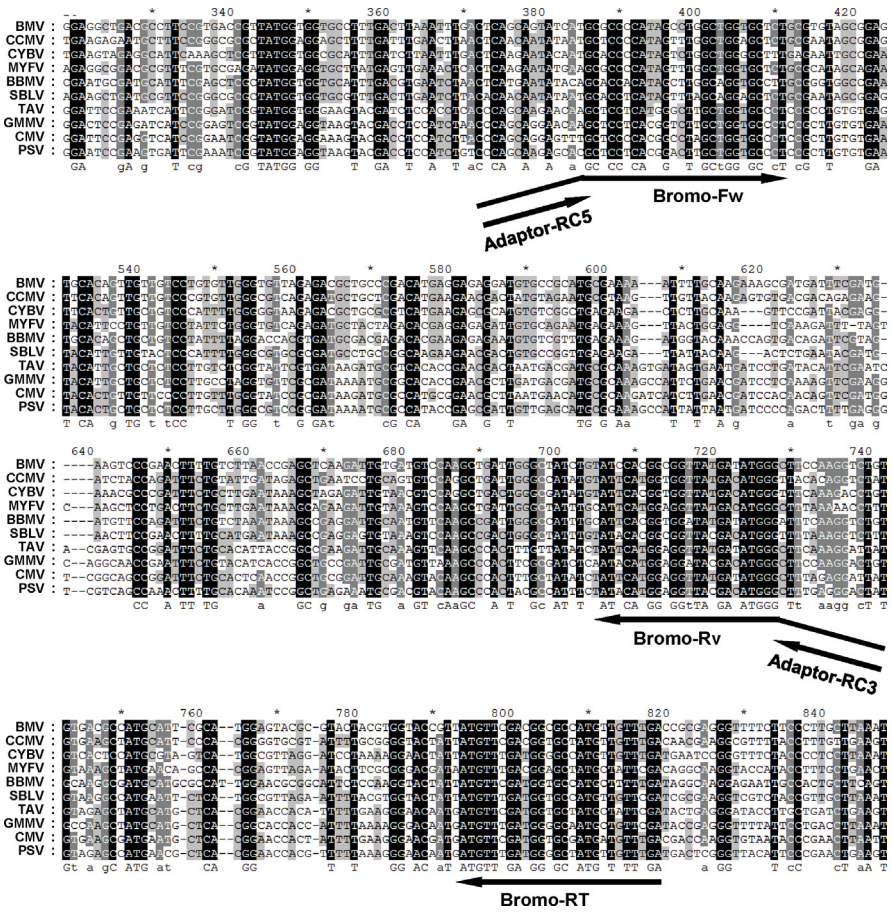

Fig. 1. Alignment of reference nucleotide sequences of RNA1 of bromoviruses and cucumoviruses used for the polyvalent primer design. The positions of the primers were indicated by arrows. *Brome mosaic virus* (BMV; NC002026), *Broad bean mottle virus* (BBMV; NC004008), *Cowpea chlorotic mottle virus* (CCMV; NC003543), *Cassia yellow blotch virus* (CYBV; NC006999), *Melandrium yellow fleck virus* (MYFV; NC013266), *Spring beauty latent virus* (SBLV; NC004120), *Cucumber mosaic virus* (CMV; NC002034), *Peanut stunt virus* (PSV; NC002038), and *Tomato aspermy virus* (TAV; NC003837). Numbers indicate nucleotide positions on the consensus sequence.

The polyvalent primers were designed based on the reference genome sequences retrieved on 9 October 2013 from the Genbank database (http://www.ncbi.nlm.nih.gov). The genome sequences of all 10 species of the genera *Bromovirus* and *Cucumovirus* were aligned using the MEGA 5.1 software (Tamura et al., 2011) and the highly conserved regions among the members were selected in the methyl-transferase motif of RNA1 for polyvalent primer design (Fig. 1). The selected regions were highly conserved among most of the strains and isolates of each species genome sequences available in the GenBank database (data not shown). The degeneracy of the polyvalent primers were minimized by using the consensus sequences and inosine (I), which is able to base pair with all four normal nucleosides (Martin et al., 1985; Kilpatrick et al., 2011; van Boheemen et al., 2012). The inosines were introduced into the primer at positions of more than 3-fold degeneracy. The positions and features of the polyvalent primers are shown in Fig. 1 and Table 1, respectively. The degeneracy of polyvalent primers could result in reduction of specificity and efficiency of the amplification process. Thus, to enhance specificity and efficiency of the assay, adaptor sequences were added at the 5′ ends of the polyvalent primers (Fig. 1). Once the target region was amplified by the polyvalent primers, the primers corresponding to the adaptor sequences (adaptor primers) enhanced the amplification specificity and efficiency, because the adaptor primers contained no degeneracy (Fig. 1 and Table 1).

First, the specificity of the designed polyvalent primers was evaluated. Among the nine virus species used for the primer design, three species of Cucumovirus (CMV, PSV, and TAV) and two species of *Bromovirus* (BMV and CCMV) were selected for primer evaluation. *Potato virus X* (PVX; *Potexvirus*), *Soybean mosaic virus* (SMV; *Potyvirus*), *Soybean dwarf virus* (SbDV; *Luteovirus*), and *Tomato mosaic virus* (ToMV; *Tobamovirus*) were included in the experiments as negative controls. All the virus isolates (except for BMV) used in this study were field isolates collected in Korea. Host

Table 1
A set of polyvalent primers specific for bromoviruses and cucumoviruses.

Primer	Sequence[a]	Position[b]
Bromo-Fw	5′-**CCAACGGAATTCCTCACTAAAC**GCTCCYCAYRGICTKGCTGGTGCYCT-3′	329–354
Bromo-Rv	5′-**CACATCGGAACTCGGTACCT**CCCATRTCATAACCICCATGIAT-3′	644–666
Bromo-RT	5′-RTCRAACAICATIGCICCRTCGAACAT-3′	728–754
Adaptor-RC5	5′-**CCAACGGAATTCCTCACTAAAC**-3′	–
Adaptor-RC3	5′-**CACATCGGAACTCGGTACCT**-3′	–

[a] The non-viral adaptor sequences are shown in boldface.
[b] The positions indicate nucleotide sites in RNA1 of *Cucumber mosaic virus* (NC002034).

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