Development of a strand-specific RT-PCR to detect the positive sense replicative strand of Soybean blotchy mosaic virus

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Highlights

- A positive-strand specific RT-PCR detecting the intermediate replicative strand of Soybean blotchy mosaic virus (SbBMV) was developed.
- cDNA synthesis using a tagged reverse transcription primer, PCR amplification using a tag-specific primer and removal of residual reverse transcription primer ensures specificity of the assay.
- This assay can be used to study the replication of SbBMV in its insect vector in future.

Abstract

Soybean blotchy mosaic virus (SbBMV), a plant virus of the genus *Cytorhabdovirus* is an economically important virus of soybean reported only from the warmer, lower-lying soybean production areas in South Africa. The virus consistently appears in soybean crops annually in spite of the absence of soybean plants in winter. One possible reason for this may be that the virus replicates and hence persists in the SbBMV vector, a leafhopper, *Peragallia caboverdensis*. RNA viruses with antisense genomes as inferred for SbBMV produce positive sense RNAs as intermediate replicative forms during replication in their hosts, and detection of the positive strand in the plant host or vector is evidence of virus replication. In this study, a positive-strand specific RT-PCR (pss-RT-PCR) was developed to detect the positive RNA strand of SbBMV and validated on nine SbBMV isolates from soybean. The effect of tagged reverse transcription (RT) primers for cDNA synthesis, coupled with PCR using a tag-specific primer, as well as removal of unincorporated RT primers following cDNA synthesis was assessed. The positive RNA strand of SbBMV in infected plants was successfully detected following this protocol. Reverse transcription with forward and unmodified reverse primers confirmed that the assay was not able to detect the genomic sense RNA or self-primed cDNAs, lacking the non-viral tag, respectively. However, Exonuclease I (ExoI) treatment of cDNA was required to eliminate false-positive results during PCR amplification.

Keywords: replication; rhabdovirus; positive strand-specific RT-PCR