Development of duplex RT-PCR for detection of Konjac mosaic virus and Spathiphyllum chlorotic vein banding virus in taro and peace lily

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Konjac mosaic virus (KoMV) and Spathiphyllum chlorotic vein banding virus (SCVbV) infecting aroids in Andhra Pradesh, India have recently been reported as distinct potyviruses. Primers were designed based on their partial genome sequence (GenBank A/C EU979524, GQ421462) to detect above viruses by duplex-reverse transcription-polymerase chain reaction (duplex RT-PCR). Using this method, the two viruses were individually detected in limited number of field/nursery collected Colocasia esculenta (taro) and Spathiphyllum spp. (peace lily). No co-infections with two viruses were traced among the screened samples. However, they were simultaneously detected in the simulated leaf samples of taro and peace lily (1:1, w/w).

Keywords: Duplex-RT-PCR, Konjac mosaic virus, Spathiphyllum chlorotic vein banding virus, peace lily, taro

The Araceae family has at least 100 genera and more than 1500 species that are grown worldwide as edible crops, foliage or flowering ornamentals, and aquarium or medicinal plants. Nearly 25 viruses representing different plant virus taxa have been found naturally infecting aroid plants worldwide. Among these viruses, Dasheen mosaic virus (DsMV), Konjac mosaic virus (KoMV) and Spathiphyllum chlorotic vein banding virus (SCVbV), belonging to the genus Potyvirus, family Potyviridae, were reported to infect aroid tuber crops and foliage ornamentals in India. Mixed potyvirus infections on aroids have also been reported for parallel detection of four taxonomically distinct potyviruses like DsMV, KoMV, ZaMMV and TuMV in calla lily. In India, RT-PCR was developed for detection of DsMV in aroid tuber crops. Further, the same group of researchers has developed a general RT-PCR for detection of potyviruses infecting not only aroid tuber crops but also other tropical root crops. Recently, KoMV and SCVbV were reported as distinct potyviruses naturally associated with infections on aroid plants for the first time from Andhra Pradesh, India. In the present study, authors report the development of duplex-RT-PCR for the simultaneous detection of KoMV and SCVbV in Colocasia esculenta (taro) and Spathiphyllum spp. (peace lily) in India.

The previously collected virus cultures of KoMV-Col-WG-AP and Spathiphyllum chlorotic vein banding virus (SCVbV-)Spa-EG-AP isolates were propagated in the insect proof wire mesh house and used for development of duplex-RT-PCR. The RT-PCR negative taro for KoMV (Fig. 1A) and peace lily for SCVbV (Fig. 1C) infections was used as healthy controls. Taro and peace lily leaf samples exhibiting prominent chlorotic feathery mottling symptoms and chlorotic vein banding symptoms, respectively were collected from fields/nurseries in Chittoor district, Andhra Pradesh state during January 2012 for screening by duplex-RT-PCR. Two sets of virus-specific primers were designed based on the partial genome sequence data of the above two virus isolates (KoMV-Col-WG-AP and SCVbV-Spa-EG-AP (GenBank A/C EU979524, GQ421462, respectively)). The primers for KoMV were 5'-AGAGGAAGAGCGGATAGTCTCAA-3' and 5'-TTCAATCCATAATCCCCTTGT-3'. The primers for SCVbV were 5'-GCAGCCCTCTCAAACGTTAC-3' and 5'-ACGTGCAAAGCACACTAACG-3'. The total RNA was isolated from the healthy and infected leaf samples of laboratory propagated and field/nursery collected aroids and subjected to reverse transcription using the two potyvirus specific reverse primers (SCVbV-R, KoMV-R) and M-MuLV reverse transcriptase (Fermentas, Canada) according to manufacturer’s protocol.
The synthesis of second strand by PCR amplification involved the following components: 5.0 μL of 10× PCR buffer with KCl, 3 mM MgCl₂, 0.2 mM dNTPs, 20 pmol of SCVBV-F-primer, 20 pmol of SCVBV-R-primer, 20 pmol of KoMV-F-Primer, 20 pmol of KoMV-R-Primer, 1.5 U of Taq DNA polymerase (Fermentas), 8.0 μL of 1:10 diluted cDNA in a total reaction volume of 50 μL. The above PCR reaction mix was subjected to the following amplification cycles: initial denaturation 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, 55°C annealing for 45 sec and extension at 72°C for 1 min with a final extension of 72°C for 7 min in a thermal cycler (GeneAMP PCR system 9700, Applied Biosystems, USA). The amplicons resolved by 1% agarose gel electrophoresis in 1× TBE buffer. The gel extracted amplicons were cloned into pTZ57/RT vector (Fermentas) and sequenced by naive commercial sequencing facility (Eurofins Biotech, India). The generated sequences were BLAST analyzed to confirm their identity with KoMV-Col-WG-AP and SCVbV-Spa-EG-AP isolates (GenBank A/C EU979524, GQ421462).

Duplex- or multiplex-RT-PCR tests are nowadays increasingly used for detection of two or more viruses simultaneously in the vegetatively propagated plants. These tests have several advantages especially in certification of plant materials for virus infections. Co-infections with two or more viruses often encountered in field-collected samples. In view of this, KoMV infected taro and SCVbV infected peace lily along with respective healthy controls were used for development of duplex-RT-PCR under the above conditions for cDNA synthesis and subsequent PCR amplification. The virus specific amplicons (KoMV, ~700 bp; SCVbV, ~400 bp) were resolved (Fig. 2). The authenticity of the amplicons was confirmed by sequencing and BLAST analysis (GenBank A/C KoMV-JX289824, SCVbV-JX289825). The exact size of the KoMV amplicons was 670 nucleotides that corresponded to part of Nib and CP genes of KoMV-Col-WG-AP. Whereas SCVbV amplicon was 368 nucleotides in length matching with part of CP and 3’UTR of SCVbV-Spa-EG-AP isolate. From the cDNA mix of these two viruses, amplicons specific to each virus were resolved (Fig. 2). This technique was later applied for the detection of KoMV and SCVbV in the field/nursery collected taro and peace lily samples. All the eight taro samples screened were found to be positive for KoMV infection, whereas out of seven peace lily samples screened, only five are found to be positive (Figs 3 & 4). However, none of these fifteen field/nursery-collected samples were positive for both the viruses. Co-infections involving these viruses on aroid plants in India are unknown so far. In the simulated samples for co-infection, virus specific amplicons were observed, which shows that the developed duplex-RT-PCR is capable to detect both KoMV and SCVbV in field/nursery collected mixed infection samples (Figs 3 & 4). To recognize the natural co-infections involving KoMV and SCVbV, large number of field/nursery collected aroid samples have to be screened by using developed tests.
duplex-RT-PCR. The developed duplex-RT-PCR could be useful for parallel detection of these two viruses in field collected aroid plant samples on a large scale and in certification of aroid germplasms.

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References