NEW RECORDS

First report of lily mottle virus on lily (*Lilium* sp.) in southern India

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Abstract Lily samples collected from commercial cut flower growing field at Nilgiris, Tamil Nadu, India were serologically tested through direct antigen coating—ELISA and dot immuno binding assay for the infection of potyvirus through potyvirus group specific polyclonal antibody. The RT-PCR using potyvirus universal degenerate primers (PNIBF1/PCPR1) showed the presence of a potyvirus and the sequencing results showed the association of lily mottle virus (LMoV). Further LMoV was confirmed by RT-PCR amplification using newly designed primer pair covering entire coat protein gene and sequence results showed 100% nucleotide identity with the LMoV reported from South Korean. To our knowledge, this is the first confirmed evidence for the occurrence of LMoV on lily in southern India.

Keywords Lily virus · Potyvirus · RT-PCR · *Lilium* · DAC-ELISA · DIBA

Lily, Genus *Lilium* spp. belongs to family Liliaceae, is a herbaceous flowering plants growing from bulbs for large prominent flowers. We have observed the lily plants showing foliar symptoms consisting of mosaic and

chlorotic streaks on leaves (Fig. 1) in cut flower farms at Nilgiris, Tamil Nadu, India during 2013. The symptomatic leaves were tested for potyvirus through direct antigen coating—enzyme linked immuno sorbant assay and dot immuno binding assay using polyclonal antiserum of potyvirus obtained from ICRISAT. All the five symptomatic leaf showed positive reaction for the potyvirus antiserum. To further confirm the presence of potyvirus, the total RNA was extracted from the symptomatic leaves and healthy leaves of lily, using an RNeasy plant minikit (Qiagen Inc., Valencia, CA, USA) and used in reverse transcription-polymerase chain reaction (RT-PCR). The cDNA was synthesized using Revertaid first strand cDNA synthesis kit (Thermo Scientific, USA) as per manufacturer's instruction. Then PCR was carried out using potyvirus degenerate primer pair, PNIBF1/PCPR1 [1]. A single DNA fragment of ~1100 nucleotides covering the 3' end of the NIB gene and the 5' end of the CP gene was amplified from symptomatic but not healthy leaves (Fig. 2a). The DNA fragment amplified from the symptomatic leaves were cloned into pGEMT vector and sequenced. Two independent clones were sequenced in both the orientations. The pair wise comparison of the sequence showed 100% sequence identity. The nucleotide sequence analysis of NIB and CP genes revealed that it had a maximum identity of 95% with lily mottle virus (LMoV) from South Korea. With the help of the resultant sequences, a primer pair (GKLMoV F: 5'-GAACTTGACAACTCGGTAAAGC-3' and GKLMoV R: 5'-CTACGGAATAAACGA GATAG-3') was designed in order to amplify the entire coat protein region along with partial NIB region. The fragment was amplified with the PCR conditions of 2 min initial incubation at 94 °C followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 90 s with final

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Fig. 1 Lily plant showing mosaic symptoms on leaves

Fig. 2 RT-PCR analysis from LMoV infected lily plants (I1 and I2) with healthy plants (H) using the *Potyvirus* degenerate primer pair PN1bF1 and PCPR1 corresponding to the NIb and coat protein region of potyvirus (a) and using specific primer pair GK LMoV-F/R corresponding to the NIb, coat protein and UTR region of LMoV (b)

extension at 72 °C for 10 min. An amplicon of 1039 bp covering 157 nucleotides at the 3' end of the NIb and 821 nucleotides of entire coat protein and 61 nucleotides from the 5' end of UTR region was amplified (Fig. 2b). The sequence analysis of the full coat protein (KJ561805)100% with LMoV reported from South Korea (JN848600). As this crop is propagated vegetatively through bulbs, if mother plants are infected with viruses, the chances for the virus transmission through the seed materials are more. Since this virus isolate share 100% nucleotide identity with the LMoV of South Korean isolate, this might have been introduced when planting materials are imported. Though this virus has been reported from the India, this is the first confirmed report from Southern India on lily.

Reference

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