Disease Note

FIRST REPORT OF CHILLI RINGSPOT VIRUS ON CHILLI PEPPER IN PAKISTAN

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Viral diseases hamper successful production of solanaceous crops in Pakistan (Ahmad and Ashfaq, 2017; Ashfaq and Ahmad, 2017). In August 2014, 25 chilli pepper leaf samples with symptoms of mottling, interveinal chlorosis and dark green vein-banding were collected from three sites in the Multan district. All diseased samples and two healthy samples were screened against potyvirus infection by indirect PTA-ELISA using "potyvirus group test" kit (Bioreba AG, Switzerland). Only 16 samples were positive for potyvirus infection and were further tested for the presence of specific potyviruses by RT-PCR. Total RNAs extracted using TRIzol reagent (Life Technologies) were subsequently subjected to RT-PCR using primers Poty3 and Oligo(dT)$_{18}$ (Tsai *et al*., 2008). All 16 RT-PCR amplicons (*ca*. 0.8 kb) were purified using QIAquick® PCR purification kit (Qiagen) and sequenced. Sequencing results confirmed the presence of chilli veinal mottle virus (ChiVMV), potato virus Y (PVY) and chilli ringspot virus (ChiRSV) in 11, three and two (*Capsicum annuum* var. *sanam*) samples, respectively. ChiRSV and PVY presence were confirmed in chilli fields in Basti Malook and Basti Islampur, respectively, while ChiVMV presence was recorded in all three sites of Multan district. Aphids were also observed (2-4 aphids per plant) on a few plants in the fields from where ChiRSV positive samples were collected. ChiRSV was further confirmed using ChiRSV-specific forward primer (5'-AAGAAGCTGTACACAGGAGAGGA-3', designed from GenBank Accession No. JQ234922) and Oligo(dT)$_{18}$. A total of 997 nucleotides (nt) were obtained from each amplicon including the full coat protein cistron and 181 nt of the 3' non-translated region, and the sequences deposited in GenBank (KX816566, KX816567); these two sequences shared > 98% nt identity. BLASTn revealed 92-98% sequence identities with chilli (*C. annuum*) isolates of ChiRSV from China (KX258620, KX379001, JN008909) and Vietnam (DQ925438, DQ925439). This virus, first reported in 2008 in Vietnam and China in 2009 from chilli pepper, has not been found elsewhere in the world (Wang *et al*., 2012). To the best of our knowledge, this is the first confirmed report of ChiRSV on chilli pepper in Pakistan.

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Disease Note

FIRST REPORT OF TOMATO SPOTTED WILT VIRUS INFECTING BALLOON FLOWER IN CHINA

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In June 2017, balloon flower (*Platycodon grandiflorus*) plants with leaf mottling, etiolation, and mosaic symptoms were observed in Kunming, Yunnan province of southwestern China. These symptoms were similar to those induced by tomato spotted wilt virus (TSWV) (Fisher, 2013). Suspected TSWV infected plant samples, as well as thrips (*Frankliniella occidentalis*) in flowers were collected. Four of six symptomatic plants were positive using TSWV test strips (Adgen Biotechnology). Total RNA was extracted from four TSWV infected plants and 15 individual thrips and analyzed by two steps RT-PCR (PrimeScript™ II 1st Strand cDNA Synthesis Kit and *LA Taq*, Takara). Three primer sets were used to clone the S-RNA, and five primer pairs to clone the M-RNA as overlapping fragments (Marshall *et al*., 2017). The expected size fragments corresponding to TSWV S-RNA and M-RNA amplification were obtained from 4/4 leaf and 10/15 thrips samples. Amplified fragments from plants and thrips were cloned using pEASY-T1 Cloning Kit, sequenced and assembled, revealing that the S (GenBank accession No. MF688996) and M (MF688997) genome segments of this isolate shared nucleotide identity of > 95% with an isolate of TSWV from China (JF960235, JF960236; Hu *et al*., 2011), indicating that the sampled balloon flower plants were infected with TSWV. To our knowledge, this is the first report of TSWV infecting balloon flower in China. Due to the devastating effects of TSWV and the increase of balloon flower cultivation area in southwestern China, it is necessary to implement management strategies to control this disease and avoid the introduction of the virus into new production areas.

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