First report of barley yellow dwarf virus PAV in uncultivated grasses of Pakistan

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Barley yellow dwarf virus PAV [BYDV PAV; icosahedral virions; ss(+)RNA genome; genus *Luteovirus*; family *Luteoviridae*] is a phytopathogen causing yellow dwarf disease, which is epiphytotic to almost all cereal growing regions (Ali et al. 2018). Apparently asymptomatic and symptomatic (reddening, yellowing and stunted plant growth) leaf samples attributed to luteovirid infection were collected in winter (November, 2011) from uncultivated grasses [Johnsongrass (*Sorghum halepense*) and Italian ryegrass (*Lolium multiflorum*)] from Pothohar region near federal capital area of Pakistan. Along with the presence of symptoms, Enzyme-Linked Immunosorbent Assay (ELISA) with a BYDV-PAV reagent set (Agdia, Elkhart, IN, USA), according to the manufacturer's protocol, confirmed BYDV-PAV infection. ELISA results showed 70% disease incidence on symptomatic plants, while 30% were negative for BYDV-PAV. To further confirm the infection, one ELISA positive samples from each of *S. halepense* and *L. multiflorum* were used for total RNA extraction by the TRIzol method. The Thermo Scientific RevertAid Reverse transcriptase (RT) kit for cDNA synthesis was used while for polymerase chain reaction (PCR) Thermo Scientific DreamTaq DNA Polymerase kit was used with coat protein gene specific primers (sense; 5'-ATGAATTCAGTAGGCCGTAGA-3', anti-sense; 5'-CTATTTGGCCGTCATCAAAC-3') which were designed from the sequences of BYDV-PAV available from GenBank. The amplification program was set as 30 cycles of

94 °C for 1 min; 50 °C for 1 min; and 72 °C for 1 min, followed by a final step of 10 min at 72 °C. The RT-PCR yielded amplicons of 0.6 kb, which were then sequenced in both orientations. The sequences were then submitted to the GenBank as JX473287 and JX473288. The sequences were further aligned with other available isolates using ClustalW and a phylogenetic tree was deduced using the neighbor joining (NJ) method implemented in molecular evolutionary genetics analysis (MEGA, version 6.0) (Tamura et al. 2013). Nucleotide sequence percent identities showed that the two isolates (JX473287 and JX473288) shared 99% identity, a maximum of 97.0% identity with a US isolate (DQ285671), and a minimum of 87.8% identity with a Chinese isolate (FJ875303). BYDV-PAV was first reported in Pakistan with serological confirmation in 1997, and was found to the most prevalent strain in the cereal crop (Bashir et al., 1997). In the absence of crop plants the virus may be maintained in the wild relatives and therefore uncultivated grasses may serve as inoculum sources for the next season crops, where the virus may reduce yield up to 80% in some cases (Simon and Roger, 2005). To our knowledge this is the first report of BYDV infecting uncultivated grasses in Pakistan.

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