First Report of Garlic Virus B Infecting Garlic in India

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Garlic (*Allium sativum* L.) is an economically important spice and vegetable crop grown throughout the world. Garlic viral disease complex caused by multiple virus infections is an important constraint in exploiting the potential yield of garlic. Among these viral pathogens, *Allexivirus* (family *Alphaflexiviridae*) is the genus of viruses known for their degenerative effect on garlic yield. Their coexistence with other viruses, particularly potyviruses, has an adverse effect on garlic yield and quality (Perotto et al. 2010). During September 2018, while screening garlic germplasm accessions for the presence of allexiviruses, symptoms like foliar mosaic and curling were observed on accession G-204, planted at an experimental plot of Indian Council of Agricultural Research–Directorate of Onion and Garlic Research, Pune, India. A total of five samples comprising five randomly selected G-204 garlic plants were collected from the experimental plot. Each sample contained leaves from the top, middle, and bottom portion of the individual garlic plants. These samples were subjected to RNA extraction using the RNeasy Plant Mini Kit (Qiagen, Germany) followed by reverse transcription (RT) using the Transcriptor cDNA synthesis kit (Roche Diagnostics, Germany). The extracted RNA was then tested for allexiviruses such as *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), *Garlic virus D* (GarV-D), and *Garlic virus X* (GarV-X) by polymerase chain reaction (PCR) (Baranwal et al. 2011; Gawande et al. 2015; Gieck et al. 2009; Roylawar et al. 2019). Leaf samples tested through RT-PCR were found positive for garlic viruses

GarV-A, GarV-B, GarV-C, GarV-D, and GarV-X. Allexiviruses other than GarV-B had been previously reported in India, and hence further tests were conducted to confirm GarV-B infection. RT-PCR using primers CF (5'-ATGGGAGACAGGTCGCAA-3') and CR (5'-CTAAATGTAAGCAT-GAGCGGT-3') designed specific to the coat protein yielded a 735-bp amplicon from all five G-204 plants. The amplified product was purified using QIAquick PCR Purification Kit (Qiagen) and cloned in pJET1.2 vector (Thermo Scientific, Lithuania). Two clones containing the CP gene were bidirectionally sequenced, and a consensus sequence was submitted to GenBank (MN650206). BLASTn results indicated that this consensus sequence showed 97.96% nucleotide (KP657919.1) and 100% amino acid sequence (AKN19940.1) identity with the CP sequence of GarV-B isolate from Poland. The presence of GarV-B was confirmed by enzyme-linked immunosorbent assay (ELISA) using a double-antibody sandwich ELISA kit (Arsh Biotech, India) as per the manufacturer's protocol. An absorbance of reaction was read using a microplate reader at 405 nm. The mean optical density (OD) values of negative and positive controls were 0.034 and 0.373, respectively. The OD values of five samples tested ranged from 0.210 to 0.296, indicating a positive reaction for GarV-B. To assess the presence of GarV-B in the available genetic stock, we tested 30 garlic germplasm accessions for GarV-B using RT-PCR. Out of these, 17 accessions were found positive for GarV-B. GarV-B has been reported from many countries (Gieck et al. 2009). This is the first report of GarV-B from India. Globally, allexiviruses are known for their adverse impact on garlic production (Oliveira et al. 2014). GarV-B together with other viruses can be a potential threat to garlic production in India. Further, detailed evaluations are needed to study the impact of GarV-B on garlic production in India.

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