SUMMARY

China rose (Hibiscus rosa-sinensis) is a perennial ornamental plant grown throughout the tropics and subtropics. China rose plants with severe vein thickening/greening, leaf curling, and enations on the lower leaf surface were found near cotton fields at the Nuclear Institute for Agriculture and Biology (NIAB) in Faisalabad (Pakistan). These symptoms were very much similar to those of cotton leaf curl disease (CLCuD). DNA was extracted from 10 naturally infected symptomatic China rose plants and subjected to PCR using begomovirus- and betasatellite-specific primers. The expected products of 2.8 kb and 1.4 kb for begomoviruses and betasatellites, respectively, were amplified. The begomovirus and its cognate betasatellite isolated from China rose were sequenced and submitted to GenBank with accession Nos. HG003876 and HG003877. Owing to a 99% nucleotide sequence identity, the virus under study was identified as an isolate of Cotton leaf curl Burewala virus (CLCuBuV). The sequenced betasatellite showed 96% nucleotide sequence identity with Cotton leaf curl Multan betasatellite (CLCuMuB). Induction of CLCuD symptom upon indexing in LRA-5166, a CLCuBuV susceptible cotton genotype that resists CLCuD symptom upon indexing in LRA-5166, a CLCuBuV susceptible cotton genotype that resists Cotton leaf curl Multan virus (CLCuMuV), further confirmed the presence of CLCuBuV in China rose. To our knowledge, this is the first report of CLCuBuV and its cognate betasatellite in China rose.

Key words: Hibiscus rosa-sinensis, CLCuBuV, betasatellite, PCR, sequencing, whitefly transmission.

Cotton leaf curl disease (CLCuD) is a serious disorder of several plant species in the family Malvaceae, the most important of which is cotton (Akhtar et al., 2013; Sattar et al., 2010). The disease occurs in Africa, China and Pakistan/north-western India, where its causative agent has been successfully characterized and found to consist of a complex of monopartite begomoviruses and a small symptom-modulating, single-stranded DNA betasatellite transmitted by whitefly Bemisia tabaci (Azhar et al., 2010; Sattar et al., 2013). Distinct begomovirus-betasatellite complexes were found to cause leaf curl disease of cotton in Asia and Africa. The complex in Pakistan and India during the 1990s was shown to consists of multiple begomovirus species (often occurring as multiple infections) supporting a disease specific betasatellite (Cotton leaf curl Multan betasatellite [CLCuMuB]) as well as an alphasatellite (Tahir et al., 2011). In 2001, a begomovirus strain that overcame existing resistance was reported in Pakistan (Akhtar et al., 2010). Recent sequence analysis of the DNA-A component of this strain showed that only a single begomovirus type is prevalent in Pakistan, contrasting earlier reports (Amrao et al., 2010). This virus is a recombinant consisting of sequences derived from Cotton leaf curl Multan virus (CLCuMuV) and Cotton leaf curl Kokhran virus (CLCuKoV). The recombinant virus, currently denoted Cotton leaf curl Burewala virus (CLCuBuV), is associated with a single CLCuMuBbur, a recombinant betasatellite of CLCuMuBmul having some of the sequences derived from Tomato leaf curl betasatellite (ToLCB) (Amrao et al., 2010; Tahir et al., 2011). Recently, CLCuBuV has been found in most of the cotton fields in India (Kumar et al., 2011; Rajagopalan et al., 2012).

China rose (Hibiscus rosa-sinensis) is a popular ornamental and hedge plant (Parrilla et al., 2012) widely present in cotton-growing areas of Pakistan and India. During May 2009, China rose plants showing vein thickening and greening (Fig. 1), leaf curling, enations and stunting were observed on the premises of the Nuclear Institute for Agriculture and Biology (NIAB) at Faisalabad (Punjab, Pakistan), a hot spot for CLCuBuV. Similar symptoms had previously been reported in China rose in relation to an old begomovirus complex (before the appearance of resistance breaking strain, CLCuBuV, in 2001) (Navaz-ul-Rehman et al., 2012). Based on the recent prevalence of a single CLCuBuV and its cognate betasatellite in most of the cotton-growing areas of Pakistan, it was feared that a recombinant CLCuBuV might be involved in the infection of China rose. Therefore, the present investigations were

SHORT COMMUNICATION

CHINA ROSE (HIBISCUS ROSA-SINENSIS): A NEW NATURAL HOST OF COTTON LEAF CURL BUREWALA VIRUS IN PAKISTAN

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Edizioni ETS Pisa, 2014

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carried out for determining the cause of the disorder in question.

Fresh leaves from 10 naturally infected China rose plants showing typical leaf curl disease were collected in 2009 from the fields at NIAB. Total genomic DNA was extracted using the CTAB method (Doyle and Doyle, 1990), suspended in water and maintained at −20°C. Virus and betasatellite were PCR-amplified using begomovirus-specific (Shahid et al., 2007) and universal betasatellite-specific primers (Briddon et al., 2002) which are theoretically capable of amplifying all of the begomoviruses and betasatellites, respectively. The full length PCR-amplified products of virus and betasatellite were cloned in pTZ57R/T (Fermentas) and sequenced (Macrogen, Korea).

Sequences were assembled and analysed using Lasergene (DNASTAR, USA), then compared with those available in the databases (http://www.ncbi.nlm.nih.gov/BLAST/) using sequence similarity searches (BLAST). Multiple sequence alignments were performed using MegAlign (Lasergene) and ClustalX2 (Larkin et al., 2007). Using the Neighbour-joining algorithm of ClustalX2 phylogenetic dendograms were shaped and viewed using Treeview (Page, 1996).

The extracted DNA samples from China rose plants subjected to PCR using begomovirus and universal betasatellite specific primers yielded 2.8 kb and 1.4 kb amplicons, respectively. The begomovirus amplified from China rose was completely sequenced and submitted to GenBank as accession No. HG003877. Analysis of the betasatellite using ORF finder (NCBI) revealed a typical genome of a monopartite begomovirus. BLAST analysis showed that the sequence corresponded to CLCuD-causing monopartite begomoviruses in the Indian sub-continent. However, detailed pairwise and multiple sequence alignment of the whole genome of this virus showed 99% identity with CLCuBuV isolates, followed by CLCuShV, CLCuKoV and CLCuMuV, negating any possible chances of a major recombination (results not shown). Phylogeny, based on the complete alignment of this virus with the selected sequences of monopartite begomoviruses, showed its segregation with different isolates of CLCuBuV followed by CLCuShV, CLCuKoV and CLCuMuV (Fig. 2A).

The betasatellite isolated from China rose was also sequenced and submitted to GenBank as accession No. HG003877. Analysis of the betasatellite using ORF finder (NCBI) showed a typical genome organization possessing a single complementary sense BC1 gene encoding a 118 amino acid (aa) long protein, an adenine rich (A-rich) region and a satellite conserved region (SCR) (Briddon et al., 2003). Detailed pairwise and multiple sequence alignment of this betasatellite showed 96% identity to CLCuMuB occurring in the Indian sub-continent. Thus, this betasatellite is an isolate of CLCuMuB. Phylogenetic analysis confirmed its segregation with CLCuD-associated CLCuMuB from Pakistan (Fig. 2B).

The presence of CLCuBuV was confirmed by back-indexing. For this purpose infected China rose shoots were grafted onto healthy greenhouse-grown China rose plants. After symptom development, 100 healthy whiteflies were subjected to a 72 h acquisition access period, followed by a 72 h inoculation access period on five 5-week-old potted plants of cotton genotype LRA-5166 (resistant to CLCuMuV and susceptible to CLCuBuV) under insect proof cages in a greenhouse. Whiteflies were killed by insecticide spraying and plants were observed daily for disease development.

All the plants of cotton genotype LRA-5166 (resistant to CLCuMuV) inoculated with CLCuBuV using whiteflies after a 72 h acquisition access period on infected China rose plants exhibited disease symptoms 7-9 days post inoculation (dpi) and symptoms became severe 13 dpi.

CLCuBuV is a recombinant virus and one of the nine begomoviruses associated with CLCuD that has been reported to cause severe crop losses in the Punjab province of Pakistan and in some cotton-growing areas of northern India (Sattar et al., 2013; Rajagopalan et al., 2012; Kumar et al., 2013). Recently CLCuBuV was found in Sonchus arvensis (Mubin et al., 2010), Gossypium robinsonii (Azhar et al., 2011), Ricinus communis (Fareed et al., 2012), Xanthium strumarium (Mubin et al., 2012) and Luffa cylindrica (Ziaur-Rehman et al., 2013). The identification of CLCuBuV and its cognate betasatellite for the first time in China rose expands the range of natural hosts of this virus.

China rose is esteemed for its aesthetic value (Rajeshwari et al., 2005) and is cultivated widely as a perennial ornamental flowering plant in cotton-growing areas of Pakistan, thus the association CLCuBuV-China rose may impact cotton production in this country. Bemisia tabaci,
Fig. 2. Phylogenetic relationships of (A) the complete genome sequence of the new variant of Cotton leaf curl Burewala virus (CLCuBuV) from China rose (boxed in bold font) and old world begomoviruses and (B) the complete sequence of Cotton leaf curl Multan betasatellite (CLCuMuB) from China rose (boxed in bold font) and selected CLCuMuB sequences from GenBank using the neighbor-joining method. Tomato leaf curl Ghana virus (ToLCuGV) and Cotton leaf curl disease (CLCuD) associated alphasatellite were used as outgroups in (A) and (B), respectively.
the vector of CLCuBuV, can feed and multiply on many diverse cultivated and non-cultivated plant species (Ling et al., 2011), including China rose (Attique et al., 2003). Thus, once China rose gets infected with CLCuBuV, it could serve as a potential reservoir where CLCuBuV over-winters thus providing the primary source of infection for subsequent cotton crops.

ACKNOWLEDGEMENTS

The authors are thankful to Mr. Muhammad Tanvir Elahi, (RA), NIAB, for his appreciated assistance, the Pakistan Atomic Energy Commission (work done at NIAB, Faisalabad) and the Higher Education Commission Pakistan (work done at NIBGE, Faisalabad) for providing the financial support.

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Received October 6, 2013
Accepted October 21, 2013