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NEW RECORDS

First report of *Turnip mosaic virus* occurrence in cole crops (*Brssica* spp) from Arunachal Pradesh, India

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Abstract The occurrence of *Turnip mosaic virus* (TuMV) in cole crops (*Brassica* spp) grown in Basar, Arunachal Pradesh, India was confirmed by symptomatology, transmission electron microscopy, reverse transcription-polymerase chain reaction and partial characterization of cytoplasmic inclusion protein and coat protein (CP) domains. Phylogenetic analysis of the partial CP sequences of the new TuMV isolates from Indian mustard (AR-IndM), broad leaved mustard (AR-BrLM) and broccoli (AR-Broc) revealed their closest relationship with members of the World-B genogroup of TuMV. This is the first molecular evidence of TuMV infection in *Brassica* spp from India.

Keywords Cole crops · *Turnip mosaic virus* · RT-PCR · Cylindrical inclusion protein · Coat protein · Phylogeny

Cole crops are an important part of daily diet in India. During 2013–2015, virus-like symptoms including mosaic, mottling, interveinal chlorosis, irregular chlorotic patches and puckering were observed on Indian mustard [*Brassica juncea* (L.) Czern & Coss.], broad leaved mustard (*B. juncea* var. *rugosa*) and broccoli (*B. oleracea* var. *italica*) (Fig. 1a) growing in the experimental farm of ICAR Research Complex for NEH Region, Arunachal Pradesh Centre as well as in backyard vegetable gardens of Basar region, Arunachal Pradesh, India. Severely affected plants showed stunted growth. Maximum mean disease incidence was noticed in case of serrated type broad leaved mustard (63.6 %), followed by Indian mustard (40.4 %) and broccoli (36.2 %). The symptomatic leaf samples were collected from field and examined under electron microscope (EM) following the leaf dip method using 2 % aqueous uranyl acetate (UA). EM analysis revealed the presence of flexuous filamentous virus particle (Fig. 1b). The EM observation indicated the possibility of potyvirus infection in the cole crops [2]. Therefore, attempt was made to identify and characterize the virus species applying reverse transcription-polymerase chain reaction (RT-PCR) based method.

Total RNA extracts (RNeasy Plant Mini Kit, Qiagen Inc., Valencia, CA) from symptomatic, as well as non-symptomatic samples from each *Brassica* sp were subjected to reverse transcription (RT)-PCR assays using One-Step RT-PCR kit (Qiagen Inc., Valencia, CA). RT-PCR assay was carried out using a potyvirus specific degenerate primer (CIFor/CIRev) reported to amplify a ~700 bp region of cylindrical inclusion protein (CI) domain [4]. All the symptomatic leaf samples from each *Brassica* sp showed virus-specific amplification of ~700 bp (Fig. 1c). The RT-PCR amplicons from three samples (Indian mustard, broad leaved mustard and broccoli) were gel purified (GeneJET, Fermentas, India) and each fragment was sequenced bi-directionally (Biolinkk, New Delhi, India). The partial sequences were assembled and submitted in National Centre for Biotechnology Information (NCBI) GenBank designating partial CI domain (KP876499-KP876501) for isolate AR-IndM (Indian mustard),

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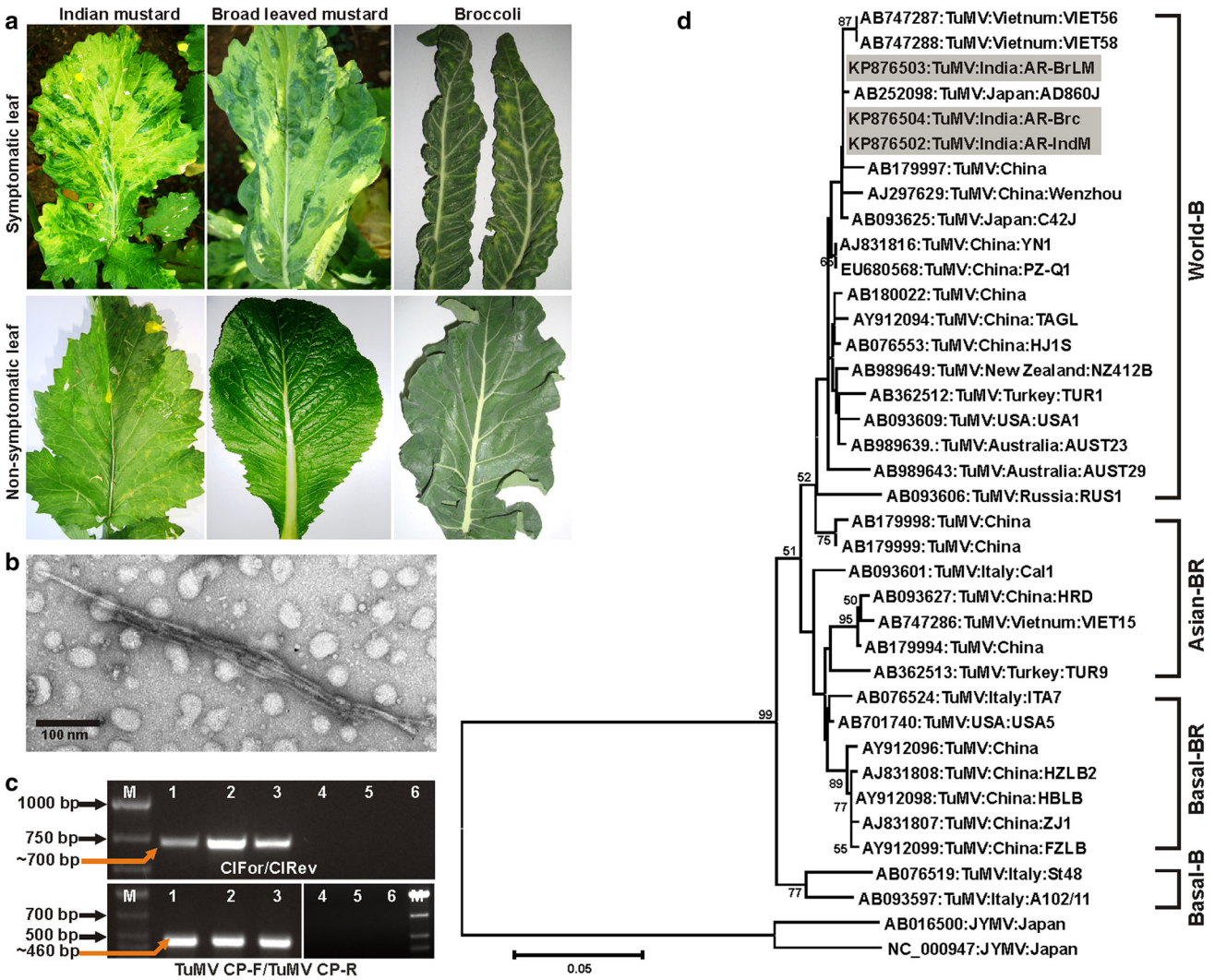

Fig. 1 **a** Leaf symptom of *Turnip mosaic virus* (TuMV) infection in Indian mustard, broad leaved mustard and broccoli along with non-symptomatic healthy leaf, **b** transmission electron micrograph of flexuous filamentous *Potyvirus* particle in infected leaf tissue, **c** RT-PCR detection of *Potyvirus* using degenerate primer CIFor/CIRev and revalidation of TuMV infection through RT-PCR using TuMV CP specific primer; M = 1 kb DNA ladder; *lane 1* template from symptomatic Indain mustard; *lane 2* template from symptomatic broad leaved mustard; *lane 3* template from symptomatic broccoli; *lanes 4, 5, 6* template from non-symptomatic plants, **d** phylogenetic tree based on nucleotide sequences of CP domain of three newly sequenced isolates of TuMV (in *box*) from India along with reported TuMV and JYMV as out group member. The analyses were conducted in MEGA6 using neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (shown only when >50 %). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. Each sequence is labelled with the GenBank accession number followed by virus name, origin and isolate name

AR-BrLM (broad leaved mustard) and AR-Broc (broccoli), respectively. The partial sequences of CI (547 bp) domains of three isolates from Arunachal Pradesh (Ar-IndM, AR-BrLM and AR-Broc) shared 97.4–100.0 % identity both at nucleotide and amino acid level. Thus, the three isolates belonged to the same species, as the threshold of 85.0 % nucleotide sequence identity proposing to differentiate species within the genus *Potyvirus* [2]. The initial BLAST analysis showed that the partial CI domain of the new isolates shared 91–95 % nucleotide identity with previously reported *Turnip mosaic virus* (TuMV) isolates available in GenBank. However, the maximum nucleotide identity of 95 % was shared with TuMV isolate ZH1 from China (KF246570). The corresponding protein identity was 98.9–99.5 % with the same isolate (protein id AGX26124). We revalidated our findings by screening the same samples with TuMV coat protein (CP) specific primers TuMV CP-F/TuMV CP-R [7]. Only the infected samples gave specific

amplicon of ~460 bp (Fig. 1c). Further, the direct sequencing of the eluted amplicons (368 bp) generated from TuMV CP specific primers (GenBank Accession Nos. KP876502: AR-IndM, KP876503: AR-BrLM, KP876504: AR-Brc) showed 100 % identity with previously reported TuMV isolates both at nucleotide and protein level. Thus, TuMV was identified as the causal agent of mosaic disease in *Brassica* spp grown in Basar region of Arunachal Pradesh, India.

Previous studies have shown that TuMV isolates had been phylogenetically classified into four genogroups called basal-*Brassica* (basal-B), basal-*Brassica/Raphanus* (basal-BR), Asian-*Brassica/Raphanus* (Asian-BR) and world-*Brassica* (world-B) [8]. Thus to investigate the phylogenetic relationship of newly obtained TuMV isolates from India, the partial CP sequences was compared with 33 TuMV isolates representing all genogroups of TuMV. Two *Japanese yam mosaic virus* (JYMV) isolates were used as out group member. A total of 38 sequences were aligned using ClustalW algorithm of MEGA6 (www.megasoftware.net) and the phylogenetic tree was constructed on the matrices of aligned sequences with 1000 bootstrap replicates following neighbour-joining phylogeny of MEGA6. The tree comprised of four major clades, consistent with previously reported TuMV genogroups (Fig. 1d). New TuMV isolates (Ar-IndM, AR-BrLM and AR-Broc) from India grouped with reported isolates of World-B group (Fig. 1d). The World-B group is mostly comprised of B-pathotype (infects only *Brassica* sp) isolates of TuMV from brassicas distributed all over the world. While, the basal-B cluster is considered as the ancestral TuMV population consisting of B-pathotype isolates from both brassicas and non-brassicas distributed throughout southwest and central Eurasia [8]. The BR pathotype (infects both *Brassica* sp and *Raphanus* sp) isolates have evolved from the B pathotype and the basal-BR and Asian-BR group are only of BR pathotype isolates [8].

In India, incidence of TuMV infection has been reported in various cole crops from different locations [1, 3, 5, 6]. However, all those reports were mainly based on host range, transmission, physical, biological and serological properties. This study first time reported partial characterization of TuMV (from brassicas) from Arunachal Pradesh, India based on CI and CP domains and indicated existence of World-B group. To the best of our knowledge, this is the first molecular evidence of TuMV infection in cole crops from North-east India.

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