New Record of Association of *Bean yellow mosaic virus* with Mosaic Disease of *Vicia faba* in India

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Faba bean (*Vicia faba* L.) is a species of bean belonging to the family *Fabaceae* extensively cultivated in Africa and Asia for its high nutritional values. In India and Nepal, it is called as “Bakulla”, its young pods are consumed as a green vegetable. It is used as human food in developing countries and as animal feed in industrialized countries. It is rich in L-dopa and used in medicine to treat Parkinson’s disease and to control hypertension (http://en.wikipedia.org/wiki/Vicia_faba).

During a survey in February of 2011 severe mosaic symptoms were observed on about 35 % (38/109) *V. faba* plants growing in kitchen gardens at Lucknow. The symptoms were mild to severe (Fig. 1a) and infected plants were dwarf as compared to healthy plants. Since infection of *Bean yellow mosaic virus* (BYMV) is reported on *V. faba* [6], therefore, infection of potyvirus was suspected.

For the detection of BYMV, total RNA was isolated from leaves of naturally infected *V. faba* plants from two locations using the RaFlex total RNA isolation kit (GeNei, India) and Reverse transcription-polymerase chain reaction (RT-PCR) were performed using a pair of degenerate primers (Pot 1 and Pot 2) capable of amplifying a 1.6–2.1 Kb fragment from 3′ end of the genome (virion protein gene and part of the NIB gene) of 17 species of the

Potyviridae [4]. RT-PCR amplification resulted in the expected size (~1.6 Kb) amplicons from symptomatic *V. faba* (4/5) similar to a positive control (potyvirus infected gladiolus) but not from the healthy one. An additional specific band of ~0.8 Kb was also obtained in all the symptomatic samples. These results indicated presence of potyvirus infection in *V. faba*.

For identification of potyvirus associated with mosaic symptoms of *V. faba*, the amplicons of ~1.6 Kb obtained from two symptomatic samples were sequenced directly and deposited in GenBank under Accession numbers: JF798580 (isolate-1) and JN692500 (isolate-2). BLASTn analysis of isolate-1 from *V. faba* (JF798580) revealed highest sequence identities 87 % with polyprotein gene of various BYMV strains: from Japan (AB097089, AB079782, AB097090); China (AJ311371) and Taiwan (AM884180). It also showed 86 % sequence identities with strains of BYMV: M11 (AB079886) and IbG (AB079887) from Japan and *Vanilla* (AY845011, AY845012) from India. The sequence identity of isolate-2 (JN692500) was highest 86 % with isolates-1 (JF798580) and 85–84 % with BYMV strains (AB079782, AB097089, AY845012, AM884180, AJ311371, AY845011, AB097090, AB079886, AB079887).

The 86–87 % sequence identities of isolates under study with other BYMV strains reported all over the world and in accordance with the species demarcation criteria and identification guidelines for potyviruses [1] suggested the virus isolates of *V. faba* under study as two isolates of BYMV.

Phylogenetic analysis of nucleotide sequence of the BYMV isolates-1 and 2 of *V. faba* (JF798580 and JN692500) with the various BYMV strains of *V. faba* and other host species reported all over the world was performed using MEGA 4.0 tool. The BYMV strains clustered into two clusters, where the isolates under study clustered in cluster 1 along

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Fig. 1 **a** Bean yellow mosaic virus (BYMV) infected *V. faba* leaf showing mosaic symptoms. **b** Phylogenetic analysis of nucleotide sequence of Bean yellow mosaic virus isolate-1 (JF798580) and isolate-2 (JN692500) (highlighted by grey) showing close

relationships with BYMV strains reported on various hosts from all over world. The phylogenetic tree was generated by MEGA 4.0 using the neighbor joining method and viewed by the NJplot. The bootstrap values at the nodes indicate scores calculated in 100 replicates

with Indian BYMV strains of *Vanilla fragrans* (AY845011, AY845012). The analysis of the BYMV isolate-1 (JF798580) and isolate-2 (JN692500) also revealed closest relationships with BYMV strains of *Gentiana scabra* (AB097089, AB079782, AB097090) from Japan; close relationships with BYMV strains of *G. scabra* (AB079886, AB079887) from Japan, *Eustoma russellianum* (AM884180) from Taiwan, AJ311371 from China, *V. fragrans* (AY845011, AY845012) from India and distinct relationship with other BYMV strains of *V. faba* reported from Australia and Japan (Fig. 1b). Based on nucleotide sequence identities and phylogenetic relationships of the virus isolates of *V. faba* with various strains of BYMV, the virus isolates of *V. faba* under study were identified as two BYMV isolates. These isolates did not differ in symptoms on *V. faba* plant.

The occurrence of BYMV on gladiolus in India [5], Japan [6], *V. fragrans* in India [2], *V. faba* in Australia [3], Egypt [7] and Iran [8] were reported previously. However, to our knowledge this is the first report of the natural occurrence of BYMV on *V. faba* in India.

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