

## FIRST REPORT OF BEAN COMMON MOSAIC VIRUS (BCMV) INFECTING COWPEA *VIGNA UNGUICULATA* IN IRAQ

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### **Abstract**

Bean common mosaic virus (BCMV) was investigated in a cowpea field at Al-Jadriya/Baghdad. Sixteen leaf samples were collected from symptomatic plants and screened for BCMV infection using nested RT-PCR. About 500 bp DNA fragments of expected size were amplified from 2 samples only indicating they were BCMV infected. Sequence analysis confirmed BCMV detection when sequences obtained shared 98% maximum nucleotide identity with the equivalent GenBank sequence from China (MN786956). To the best of our Knowledge this is the first report of BCMV on cowpea in Iraq.

**Keywords:** Bean Common Mosaic Virus, BCMV, cowpea, *Vigna unguiculata*

### **Introduction**

Cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous crop belonging to the genus *Vigna* within the family Fabaceae (Doumbia, 2012). It can tolerate high temperatures and poor soil conditions (Baidu and Munxia, 2014) Cowpea is grown in Iraq as a food source, during the summer season to score the local food demand when other crops are in shortage (AL-Sahaf and others, 2012). In addition, the roots provide soil protection from erosion and their high ability to fix nitrogen in the soil (Radhi and Al-Assaf, 2012). In 20110, the Iraqi production of cowpea was 31064 tons (Central Statistical Organization, 2020). Cowpea can be infected by several pests, limiting its production. About 33 viruses belonging to 16 genera and 11 families including were reported to infect cowpeas, worldwide ( Hema et al., 2014; Dai et al., 2011; Hampton et al., 2003). ). Bean common mosaic virus (BCMV) is amongst the most widespread and destructive viruses, impacting a range of cultivated and wild legumes (Morales, 2006). This virus can decrease production up to 100%, depending on strain, legume variety/species, plant growth stage and vector activity (Damayanti et al., 2008; Li et al., 2014). BCMV belongs to the genus Potyvirus within the family Potyviridae (ICTV, 2022). Symptoms caused by BCMV on legumes include stunting, yellowing, and leaf curling (Flores-Este ´vez et al., 2003). So far, limited molecular studies investigating potyviruses infecting legumes have been done in Iraq (Al-Kuwaiti et al., 2016). Most studies were based on biological and serological approaches (e.g. El-Muadhidi et al., 2001), but not confirmed yet by molecular methods (Alkuwaiti, 2013). This study, therefore, aimed at investigating Bean common mosaic virus on cowpea based on molecular approaches.

### **Materials and Methods**

Sixteen cowpea samples were collected from symptomatic plants in a field in College of Agriculture, University of Baghdad, in Al-Jadriya district (Fig 1A). Total RNA was extracted from

plants samples by using AccuZol™ Total RNA extraction kit (Bioneer, Korea) according to manufacturer's instructions. RNA quantity and quality were measured using Nanodrop spectrophotometer at 260/280 nm. cDNA was synthesized using (AccuPower RocketScript™ RT PreMix) company following the manufacturer's standard protocol. Nested PCR was performed using two primer sets designed in this study (Table 1). The N-PCR products was analyzed by agarose gel electrophoresis. Nested PCR products of BCMV were sent for sequencing (Macrogen, S Korea) Sequences analysis was performed using (MEGA 6.0 version) software. Sequences obtained were submitted into NCBI Genbank and accession numbers (OP785723-OP785724) were assigned.

**Table 1: Primer sets designed in this study**

Primer	Sequence 5'-3'		Amplicon
BCMV-PCR External primers	F	ACCAGAGCGTGTGGTTTCAA	718bp
	R	ATCCGGTGAAGTGCCATTGT	
BCMV-Nested PCR Internal primers	F	GCAGCTAATGGAGGAGCTCC	511bp
	R	AGCCGTTTCATCACAATTGACA	

## Results

N-PCR could detect BCMV in 2 out of 16 cowpea samples DNA fragments of expected size when ~500 bp were amplified indicating the association of BCMV with symptomatic plants (Fig.1B). Sequence comparison confirmed the detection of Bean common mosaic virus when shared 98.64-98.80% maximum identities with equivalent gene bank sequences.

The phylogenetic tree genetic relationship analysis showed that Bean common mosaic virus IQ-Baghdad isolates were closely related to BCMV isolates from China (MN786956) suggesting a common origin (Fig. 2). In Iraq, Bean common mosaic virus was reported for the first time on bean *Phaseolus vulgaris* based on biology and electron microscopy in 1980s (Al-Fadhil and Al-Ani, 1987). The current study confirmed the identification BCMV in cowpea and provided the first molecular data

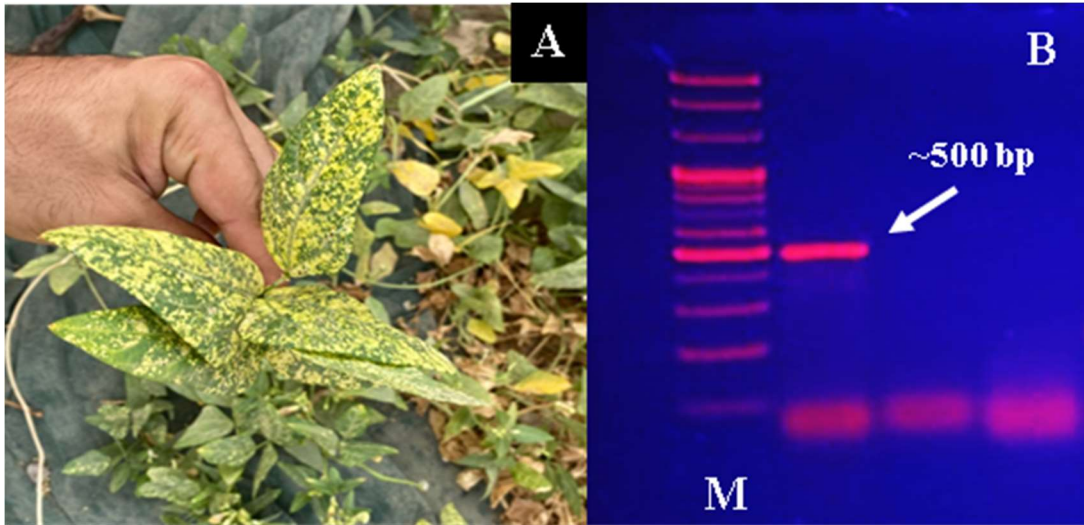


Figure1: A: Naturally infected cowpea plant exhibiting yellow mosaic symptoms detected to be BCMV infected. B: Gel electrophoresis pattern showing ~500 bp DNA fragments amplified from a symptomatic cowpea sample (A), by RT-PCR using nested primer sets targeting BCMV. M: 100 bp DNA markers

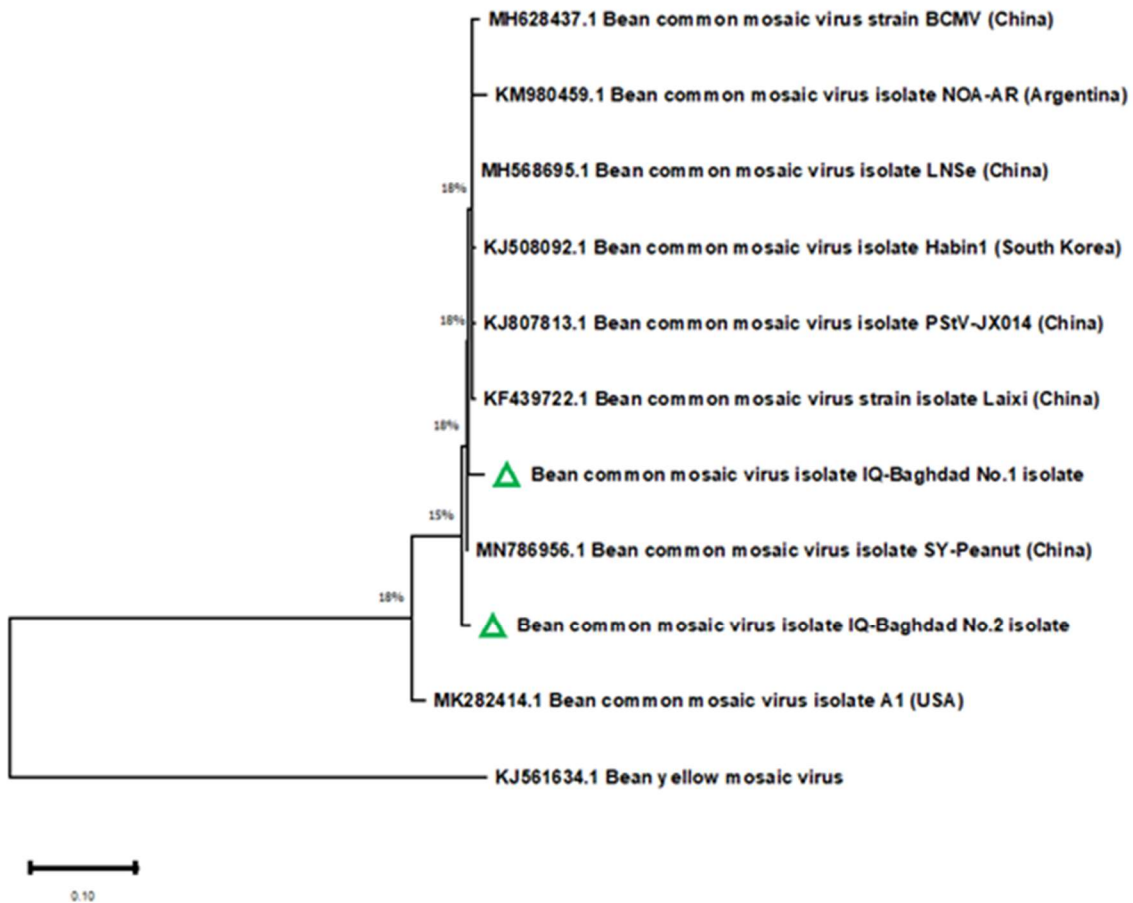


Figure 2: Neighbor-joining phylogenetic trees constructed from BCMV partial nucleotide sequences of Iraq (marked with  $\Delta$ ) isolated from cowpea and equivalent sequences from NCBI. Bean yellow mosaic virus (BYMV) was included as an outgroup comparison.

Regarding this virus from Iraq. Despite the heavy aphid infestation in cow pea field, BCMV showed a low incidence in tested samples (12.5%), indicating they are infected through cowpea seeds (Aishwarya et al., 2020; Adams et al., 2022). Hence, aphid species present in cowpea field from where samples were collected from might be inefficient to transmit BCMV, as aphids may vary in its ability to victories viruses (Wang and Ghabrial, 2002). Mixed infection with other viruses may minimize aphid transmission ability as cowpea samples collected may be infected with other viruses (Pinto et al., 2008). High sequence identity indicated together with the phylogenetic relatedness BCMV may have been introduced to Iraq through cowpea seeds in the near past. This virus may threat cowpea production in Iraq as it causes significant losses in other countries (Manjunatha et al., 2016). A rapid action, therefore, are required to protect cowpea crop in Iraq against BCMV.

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