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# Molecular Detection and Characterization of Virus Causing Yellow Mosaic Disease of Redgram (*Cajanus cajan* L. Millsp) in Karnataka

**Manjunatha[1]*, N., Noorulla Haveri[2], B. Anjaneya Reddy[3], Archana[4], S. and Manjunath, S. Hurakadli[5]**

[1]Division of Seed Technology, IGFRI, Jhansi-284003

[2]KrishiVignana Kendra, Kolar-563103

[3]Department of Plant Pathology, College of Horticulture, UHS Campus, GKVK Post, Bengaluru-560065

[4]Department of Plant Pathology, College of Agriculture, UAS (B), Mandya

[5]Department of Plant Pathology, UAS, GKVK, Bengaluru-560065

*Corresponding Author E-mail: manju.ars@rediffmail.com

## ABSTRACT

*A roving survey of different redgramfields for begomovirus incidence was conducted in the Kolar district of Karnataka during kharif 2014-15. Results indicated that disease incidence was ranged from 1-5 percent.The infected plants initially showed, small yellow speck on the newly developed leaves later, these specks coalesced and formed larger yellow patches. The infected plants showedstunted growth and symptoms and there was no production of any fruits. PCR product of 520bp of coat protein region was amplified using deng primers with infected samples of redgram. The nucleotide sequences of viral CP shared maximum nucleotide identity (98%) with previously identified horse gram yellow mosaic virus (HYMV) strains of southern India followed by 87% identity with Mung bean yellow mosaic virus (MYMV). Phylogenetic analysis of begomoviruse's CP nucleotide sequences grouped into HYMV infecting legumes and MYMV infecting legumes which are previously described as members of begomoviuses from different geographical locations. The virus infecting redgram in Karnataka formed a separate cluster with begomoviruses (HYMV and MYMV) infecting legumes indicated that, virus associated with redgram yellow mosaic disease is a closest relative of HYMV and MYMV.*

***Keywords:*** *Redgram, Begomovirus, Disease incidence, Sequencing, Phylogeny*

## INTRODUCTION

Redgram (*Cajanuscajan* L. Millsp) is an important drought resistant leguminous food crop, used both for dhal and also vegetable purpose. The crop is being grown extensively in the Indian subcontinent in an area of about 3.8mha with the production of 3.0 mt. The crop is known to be affected by more than 50 diseases[19.] Occurrence of yellow mosaic disease of pigeon pea was first described by Williams *et al.*[31] Later Nene *et a.l*[20] reported that the yellow mosaic of pigeon pea was caused by mungbean yellow mosaic virus (MYMV) on the basis of white fly (*Bemisiatabaci*) transmission and symptomatology. In Karnataka, the yellow mosaic disease of pigeon pea has been reported by Muniyappa and Veeresh[16]. The virus was detected in the naturally infected pigeon pea plants and the geminate particles were measuring 15-18 X 30nm[17].

The detection and diagnosis of viruses is most important to devise a management strategy. Begomoviruses have been detected in plants or insects by tools, such as visualization of nuclear inclusion bodies by light microscopy, ultrastructural localization of virions in plant cell by transmission electron

microscopy, serological assays[24,23,9,6], DNA hybridization assays[10], Polymerase Chain Reaction (PCR)[2,14,3,10,23,26], immunocapture PCR[23]. Molecular cloning and DNA sequencing of viral genomes have become the tools of choice, allowing virus identification and comparing relationships with other virus isolates[27,21,22,1.]

All begomoviruses code for coat protein which act as the protective coat of the virus particle and determine vector transmissibility of the viruses by whitefly vector *B. tabaci*. Thus, the CP gene is highly conserved among begomoviruses originating from the same geographical region and adapted to transmission by local vector populations[13,11.] Smaller fragments comprising the core Coat Protein gene (core CP), a partial 575-579bp sequence of the Coat Protein gene[1], or the complete CP sequence have also been used to establish provisional species identification owing to the highly conserved nature of the viral CP sequence. The core CP primers have been illustrated to amplify a fragment for most, if not all, begomoviruses irrespective of Old or New World origin, making possible the rapid detection followed by prediction of provisional species affiliation by comparing with reference begomovirus core CP sequences[1,5,32].

In Karnataka, the redgram yellow mosaic disease was not reported in the field conditions for large number of years and disease has reappeared in the *kharif* season of 2014-15 in the newly developed cultivars. Hence, an effort has been made for the diagnosis of the virus. In this paper we are reporting symptoms, detection and molecular characterization of the begomovirus associated with redgram from Karnataka.

## MATERIAL AND METHODS

### Virus source and Total DNA extraction

A roving survey was conducted in the different redgram fields in the Kolar district of Karnataka state during *kharif* 2014-15 and the information on variety, its age and the percent disease incidence and symptoms was recorded. The total DNA was extracted from the infected field samples, following standard DNA extraction protocols of Cetyltrimethyl ammonium bromide method (CTAB)[11]. The DNA was amplified using begomovirus coat protein (CP) specific primers[2].

### PCR amplification and Sequence analysis

The total DNA extracted used for PCR amplification. Further, the Polymerase Chain reactions was carried out in 25 µl of reaction mixture containing DNA template - 2.0 µl, nuclease free water-14.4 µl, 10X PCR buffer-2.5 µl, 2.5mM dNTPs -2.0µl, 2.5MmMgCl$_2$.1.5µl and primers- Forward primer (5'TAATATTACCKGWKGVCCSC3')-1.25µl, Reverse primer (TGGACYTTRCAWGGBCCTTCACA) -1.25µl and Taq DNA polymerase (1.5 U/µl) - 0.1µl.The PCR amplification was carried out in a thermal cycler with initial-denaturation at 94 $^0$C for 1 min. followed by 35 cycles each consisting of denaturation at 94 $^0$C for 1 min., annealing at 60$^0$C for 30 sec. followed by extension at 72 $^0$C for 1.30 min. with final extension at 72 $^0$C for 10 min. Amplified DNA fragments were electrophoresised in 0.8 per cent agarose gel and documented.

After successful confirmation of the specific amplification the amplified product was sequenced. For sequence comparison, published nucleotides of related begomovirusesobtained from NCBI Gen bank throughBLAST.The nucleotide sequences aligned and phylogenetic tree was constructed by CLUSTALW.

## RESULTS AND DISCUSSION

### Virus source and Symptomatology

A roving survey conducted in the different pigeon pea fields in the Kolar district of Karnataka state during *kharif* 2014-15 revealed that disease incidence was ranged from 1-5 percent and the symptoms were observed before flowering. Initially, the symptoms appeared as yellow speck on the newly developed leaves. These specks coalesced and formed yellow patches against the green background of the lamina (Fig.1). The infected plants were remained to be stunted and symptoms were seen throughout the plant on leaves and there was no production of any fruits. The similar symptoms were reported on pigeon pea[31,16]. The symptoms of pigeon pea yellow mosaic virus was closely resemble the symptoms of other yellow mosaic legume viruses, though the symptoms varied with the host and susceptibility. This is in accordance with the reports of Nene[18], Muniyappa*et al.*[15] Sudhakar Rao[29], Singh *et al.*[28] and JavariaQazi *et al.*[7]

**PCR amplification and sequence analysis**

Coat protein (CP) region of about 520bp from begomovirus infected sample DNA was amplified (Fig. 2) by using deng primers.The DNA obtained from asymptomatic plants was not amplified. This clearly indicates association of begomovirus with redgram yellow mosaic disease. Deng primers have been employed by several workers for the detection and association of begamoviruses with bendi yellow vein mosaic (BYVMV) (Nagaraja, *et al*., 2005), Jatropa mosaic virus (Rangaswamy*et al*., 2005 and Ashwathnarayana*et al*., 2006), moth bean yellow mosaic virus (Divya, 2008).

The DNA sequence of redgrambegomovirus CP gene was analyzed in BLAST on NCBI database and information on sequence similarity was obtained (Table 1). The results revealed that sequence from symptomatic redgram plant shared maximum 95-98% identify with HYMV of different pulses reported from southern India, followed by MYMV (87%). The nucleotide sequences of redgram virus CP shared maximum nucleotide identity (98%) with previously identified HYMV strains of southern India followed by MYMV (87%).

Phylogenetic analysis of begomoviruse's (Fig.3) CP nucleotide sequences grouped into HYMV infecting legumes and MYMV infecting legumes which are previously described as members of begomoviuses. Interestingly MYMV infecting legumes formed into two sub clusters. The mungbean infecting viruses formed as one sub-cluster which were reported from Vietnam and Cambodia. Whereas, MYMV associated with legumes in India clustered separately, indicated that virus specificity depends on host and geographical location. The virus infecting redgram in Karnataka formed a separate cluster with begomoviruses (HYMV and MYMV) infecting legumes. The results indicated that virus associated with redgram yellow mosaic is a closest relative of HYMV and MYMV.

CP sequence analyses and phylogenetic tree construction provides a prediction of relatedness among yellow mosaic causing viruses. It is highly similar to information obtained from alignment of begomovirus CP gene sequences[21,12]. CP is highly conserved region interrelated with variable bases in begomoviruses. These properties useful in providing molecular tool for identification of begomoviruses associated with legumes[21,1.] However complete dependency on CP gene may complicate identification of begomoviruses that otherwise can be done by conserved region[8].

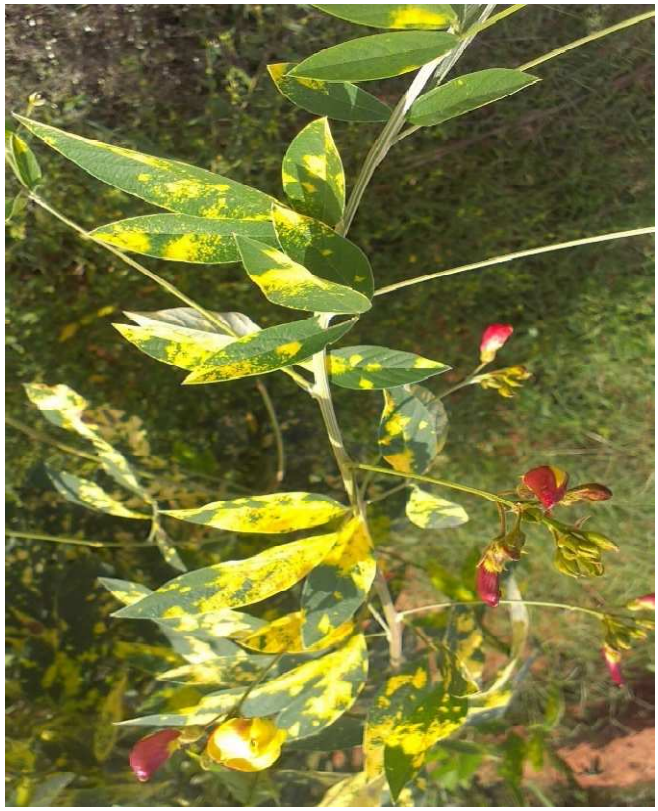

**Fig. 1: Naturally infected redgram plants showing yellow mosaic symptoms on leaves**

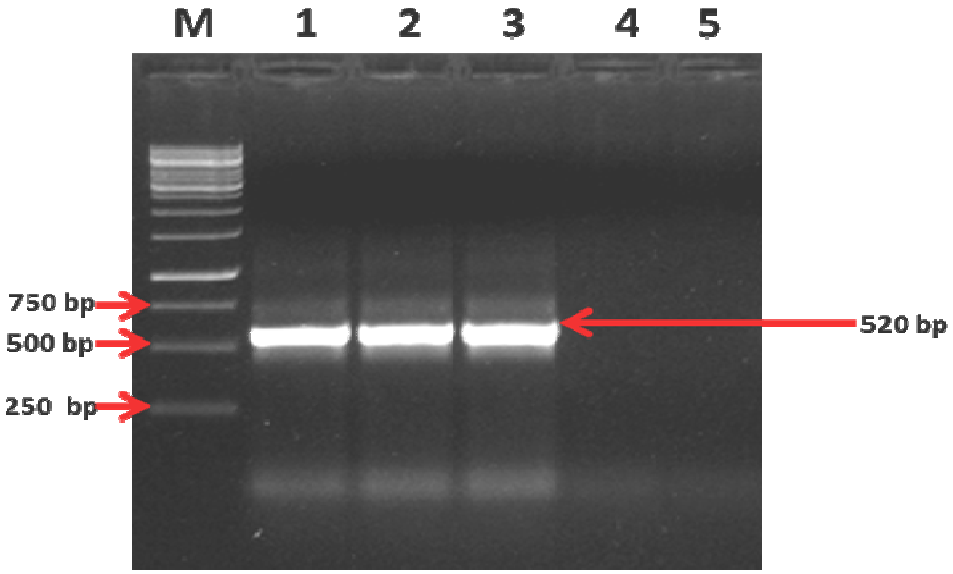


**Fig. 2:** Agarose gel electrophoresis of the PCR products (lanes 1–3) of coat protein gene amplified (~520 bp). Lane M: 1kb Marker; Lane 1: positive control (Horse gram): Lane 2 &3: Begomovirus infected Redgram leaf sample; Lane 4: Healthy redgram leaf sample; Lane 5:Negative Control

**Table 1: List of begomoviruses nucleotide sequences (deposited in NCBI, GenBank) used for comparison with CP gene nucleotide sequences of Redgram and construction of phylogenetic tree**

| GenBank Accession No. | Origin | Virus Acronym | Original host | Nucleotide identity (%) |
|---|---|---|---|---|
| AM93245 | Karnataka (India ) | HYMV | French bean | 98 |
| AM93247 | Karnataka (India ) | HYMV | Horse gram | 98 |
| AJ627904 | Tamil Nadu(India) | HYMV | Horse gram | 98 |
| KC019306 | Karnataka (India ) | HYMV | French bean | 98 |
| AM932429 | Karnataka (India ) | HYMV | Lima bean | 97 |
| GU323321 | Srilanka | HYMV | Horse gram | 95 |
| DQ865201 | Tamil Nadu(India) | MYMV | Moth bean | 87 |
| KC911722 | Tamil Nadu(India | MYMV | Black gram | 87 |
| JX44173 | Vietnam | MYMV | Mung bean | 87 |
| AY271892 | Cambodia | MYMV | Mung bean | 87 |
| JX244172 | Vietnam | MYMV | Mung bean | 87 |
| DQ400848 | Tamil Nadu(India) | MYMV | Black gram | 87 |
| ABO17341 | Thailand | MYMV | Mung bean | 87 |
| D14703 | Japan | MYMV | Mung bean | 87 |
| KC911721 | Tamil Nadu(India | MYMV | Black gram | 87 |
| JX244176 | Vietnam | MYMV | Mung bean | 87 |
| AF314530 | Maharashtra (India) | MYMV | Soybean | 87 |
| AJ132575 | Tamil Nadu(India) | MYMV | Mung bean | 87 |
| JX244175 | Vietnam | MYMV | Mung bean | 87 |
| JX244174 | Vietnam | MYMV | Mung bean | 87 |

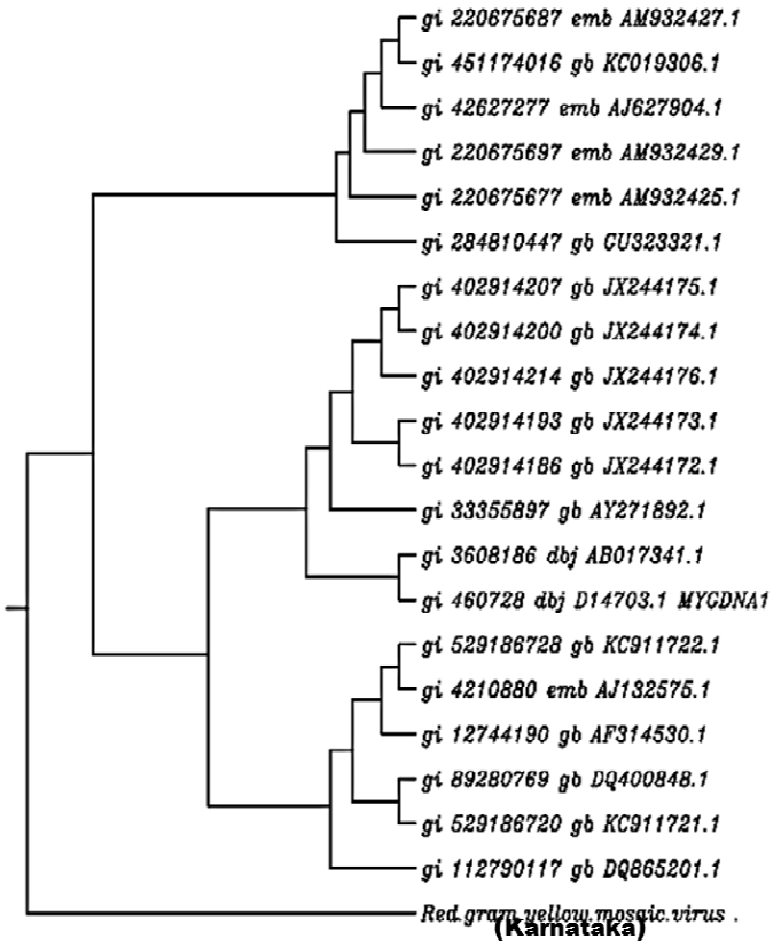


**Fig. 3: The phylogenetic tree constructed for begomoviruses showing identity with CP gene nucleotide sequences of redgram virus**

## CONCLUSION

Present results from the CP gene sequence alignment and construction of phylogenetic tree with other begomovirus nucleotide sequences have clearly established that the virus infecting pigeonpea belongs to begomovirus group.

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