Int.J.Curr.Microbiol.App.Sci (2019) *8*(9): 1644-1657

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 09 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.809.187

Characterization of Recombinant Bean Yellow Mosaic Virus belongs to Group-IV Infecting Gladiolus

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Keywords

Bean yellow mosaic virus, Gladiolus,Phylogenetic analysis, RT-PCR and Recombination.

Article Info

Accepted:
18 August 2019
Available Online:
10 September 2019

A B S T R A C T

Gladiolus is an important vegetatively propagated ornamental plant cultivated in different parts of the world. The major constrains for production quality and quantity of flowers in gladiolus is due to infection of different viruses. During survey, the gladiolus plants (Ten samples) with mosaic symptoms were collected from different farmer fields in Bangalore rural district and were confirmed for Bean yellow mosaic virus (BYMV) infection by ELISA and PCR using specific primers. Further compete genome of BYMV that infects gladiolus was amplified cloned and sequenced. The analysis showed that the genome of BYMV shared maximum nucleotide identity 92-97.2% with BYMV isolates belonging to the group IV infecting different crops in India, Japan, USA and Taiwan. Further recombination analysis showed that most part of the genome was derived from BYMV isolates from the phylogenic group of IV and I to emerge as a new variant of BYMV infecting gladiolus. The significance of these findings is discussed.

Introduction

The genus Gladiolus belongs to the family Iridaceae. It consists of more then 150 species originated from Africa, Asia, South Europe and few from Mediterranean area. The cultivars of gladiolus exhibit more diversity in shape, size, flower colour, time, bulbing and dormancy (Kaur *et al.*, 2015). Gladiolus is an important ornamental plant grown

commercially for different purposes in worldwide. The major constrains for production of quality and quantity of flowers and corms are viruses, which are causing extensive crop yield losses.

The most conspicuous viral disease symptoms observed on the infected gladiolus plantings are stunting, flower color breaking, distortion and reduced cormel production. On the leaves severe mosaic appearance and stunted growth of the plants are common. Gladiolus is natural host for many viruses (Arneodo *et al.,* 2005). The important viruses documented in different parts of the world are Arabis mosaic virus (ArMV), Bean yellow mosaic virus (BYMV), Broad bean wilt virus (BBWV), Cucumber mosaic virus (CMV),Soybean mosaic virus (SMV), Strawberry latent ring spot virus (SLRSV), Tobacco mosaic virus (TMV), Tobacco rings pot virus (TRSV),Tomato black ring virus (ToBRV), Tomato ringspot virus (ToRSV), Tobacco rattle virus (TRV), Tomato spotted wilt virus (TSWV), Tobacco streak virus (TSV) (Katoch *et al.,* 2003;Dubey *et al.,* 2010). These viruses are spread by vegetative propagation and by insect vectors.

The genus BYMV is a member of the Potyvirus (Shukla *et al.,* 1994) infecting different leguminous and ornamental crop plants (Sasaya *et al.,* 1998; Sutic *et al.,* 1999). BYMV is made up of 750 ηm long flexuous particles, induces cylindrical inclusions bodies in host cells, and is transmitted by aphids in a non persistent manner (Edwardsons & Christie 1986; Milne 1988).

BYMV can be readily detected in the infected gladiolus plants (Zettler & Abo el-nil 1977) by ELISA or RT-PCR but cannot be readily detected in corm tissue (Vunsh *et al.,* 1991). The detection ofvirus in gladiolus corms is difficult (Katoch *et al.,* 2003) due low titer of the virus in the corms or cormlets. Limited work has been carried in India on virus morphology and serological detection of BYMV (Srivastava *et al.,* 1983): CMV (Raj *et al.,* 2002, Singh, *et al.,* 2007).

Considering the above fact with high disease incidence of BYMV on gladiolus, the characterization and identification of recombinant BYMV infecting gladiolus was conducted with a long term goal to contain the disease in gladiolus.

Materials and Methods

Collection of Gladiolus virus infected samples

The roving survey was conducted for collection of symptomatic (Mosaic like symptoms on leaves and sepals, colour breaking in flowers) and asymptomatic gladiolus samples from commercially cultivated famer's fields in Bangalore rural areas and also experimental plots at ICAR-Indian Institute of Horticultural Research, Bangalore India. Total 10 fields were surveyed; from each field 2 samples (ten symptomatic and ten asymptomatic samples) were collected and used for analysis. Symptomatic and asymptomatic leaf samples collected were used for transmission and characterization and the remaining samples was stored at -80° for further studies.

Culture maintenance

The field collected symptomatic (Mosaic like symptoms on leaves and sepals, color breaking in flowers) gladiolus plants were initially screened with DAC-ELISA using polyclonal antibodies of Bean yellow mosaic virus (BYMV).

The virus positive gladiolus plants samples were mechanically transmitted to Beans cv. Anup by sap inoculation and maintained under insect proof glass house for further use.

Mechanical transmission

The methodology of inoculation involved maceration of 1.0g of symptomatic leaf tissue of gladiolus in 10ml of ice cold 0.05M potassium phosphate buffer (pH 7.5) using a sterile pestle and mortar on ice. The crushed sap was filtered through double layered muslin cloth and filtrate was mixed with celite powder (600mesh at 0.025 g per ml). Then sap was used for mechanical inoculation on the leaves of Beans cv. Anup. The inoculated plants were kept under insect proof glass for symptoms expression. The development of local and systemic symptoms was recorded on host plants for a period of 30 days after inoculation. After that the infected young leaves were harvested and analyzed for presence of virus using DAS-ELISA and RT-PCR.

Partial Purification and Electron Microscopy

The virus was partially purified from infected gladiolus sample showing mosaic symptoms collected from experimental plot at Indian institute of Horticultural Institute according to the methodology described by Kaur *et al.*,(2015). The partially purified virus particles were transferred to carbon coated copper grid and the excess buffer on grid was washed with 10 mM phosphate buffer (pH 7.0) followed by sterile water and negative-stain with 2% uranyl acetate. The morphology of partially purified virus particles was visualized in JEOL 100s electron microscope at 80kv.

Viral cDNA synthesis, PCR amplification and cloning

Total genomic RNAs was extracted from infected gladiolus samples and plants maintained at Plant Virology Laboratory, ICAR-Indian Institute of Horticultural Research, Bangalore, by RNeasy Plant Mini Kit (Qiagen), following the manufacturers protocol. The integrity and quality of the total RNA were checked on 1% agarose gel and also quantified by nanodrop (Thermo Fisher Scientific, USA).

The First strand viral cDNA synthesis was carried out with 5µg total RNA that was denatured along with 1.0µl reverse primer (20pmol/µl) at 72^0C for 5 min, followed by addition of 4µl of 5X first strand buffer, 0.2µl ribonuclease inhibitor (40 U/µl), 2 µl of 10mM dNTPs and 01µ1 MMLV-RT (200 U/µl) (Fermentas) in a total reaction of 25µl. Reaction was performed at 42^0C for 60 min followed by incubation at 75^0C for 5 min.

PCR amplification was performed using sets of reverse and forward primers designed to amplify complete genome of the BYMV. PCR reactions were carried out in a GeneAmp PCR system 9700 (PE Applied Biosystems, Foster City, CA) thermocycler. PCR reactions were carried out in a volume of 25µL containing 100ng of DNA template 0.5U *Taq* DNA polymerase (Fermentas, Germany), 25mM $MgCl_2$ (Fermentas, Germany), 2 mMdNTPs (Fermentas, Germany) and 25 pmol of each primer. The thermo cycler was set for 35 cycles of denaturation at 94^oC for 1 min, annealing at 58^oC to 62^oC for 45 seconds and extension at 72^oC for 90 seconds with final extension at 72^0C for 20 min. PCR products were electrophoresed on 0.8% agarose gels stained with ethidium bromide (10mg/mL) and were viewed in a gel documentation system (Alpha Innotech, USA).

The amplified PCR products of different genome fragments of BYMV were purified from agarose gels following standard protocols and ligated into pTZ57R/T vector using InsTAclone PCR product cloning kit (Fermentas, city Germany) according to the manufactures instructions. The ligated product was transformed into *Escherichia coli* DH5α

competent cells (Invitro gen Bioservices India Pvt. Ltd., Bengaluru, India). Bacterial colonies bearing recombinant plasmids carrying apparent monomeric full length viral inserts of three clones from sample were selected for sequencing by automated DNA sequencer ABI PRISM 3730 (Applied Biosystems) at from Medauxin Sequencing Services, Bangalore, Karnataka, India.

Sequence Analysis

The sequence similarity searches were performed by comparing the full length genome sequence of BYMV to all available sequences in GenBank using BLASTn (Altschul*et al.,* 1990). The Genbanksequence showing highest scores with the present isolate were obtained from database (Table 1) and aligned using SEAVIEW program (Galtier *et al.,* 1996). The open reading frames (ORFs) located in the genome and their putative proteins were analyzed by ORF Finder (www.ncbi.nlm.nih.gov/projects/gorf/) and ExPasy translation tools (http://www.expasy. org/resources/ search/keywords: translation), respectively. The sequence identity matrixes for the BYMV infecting gladiolus were generated using Bioedit Sequence Alignment Editor (version 5.0.9) (Hall, 1999) and phylogenetic tree was generated by MEGA 7 software (Kumar *et al.,* 2016) using the neighbor joining method with 1000 bootstrapped replications. The evidence for recombination in BYMV infecting gladiolus were analyzed by Splits-Tree version 4.3 using the neighbor-Net method (Huson and Bryant, 2006) by the alignment of selected BYMV and other potyviruses sequences. The method depicts the conflicting phylogenetic signals caused by recombination as cycles within the untreated bifurcating tree. Further recombination analysis was carried using Recombination detection program (RDP), to detect recombination in the BYMV genome with default RDP settings (Martin *et al.,* 2015).

Results and Discussion

Symptomatology and Viral Incidence

During survey it was observed that naturally BYMV infected gladiolus plants in farmers field showed symptoms viz; mild to severe mosaic patterns on leaves, stem, and inflorescence; color breaking in floret petals and reduction of number of corms (Fig I). Apart from this, in the infected plants it was observed that the plant height, number of tillers perplant, length of spike and florets per plant were also reduced. The incidence of disease varied from field to field and recoded on the basis of visual symptoms of infected plants over healthy plants by crossing the rows of the plants. The disease incidence in gladiolus field varied from 26.8 to 80% in years 2014 to 2017.

Virus transmission

The inoculated beans (cv. Anup) plants using crude sap obtained from infected leaf tissue of diseased gladiolus plants (showing severe mosaic symptoms) induced necrotic local lesions on leaves at 25-30 days post inoculation (dpi) (Fig II). Further the sap also induced systemic mosaic symptoms on healthy gladiolus plantlets (obtained from Division of Ornamental and Medicinal Crops ICAR-IIHR-Bangalore) at 25-30 dpi which were similar to those of naturally infected gladiolus collected from fields. The infection confirmed for presence of virus using DAS-ELISA and RT-PCR.

Virus particle morphology and Transmission Electron Microscopy

The numerous flexuous rod particles of virus measured about 720 nm x11 nm was observed in partially purified and negatively stained preparation (Fig III). The size and shape of the virus particles observed TEM was similar

other reported potyviruses so far (Katoch *et al.*, 2002).

Detection of BYMV infecting gladiolus and corms

Total genomic RNA isolated from the naturally infected ten gladiolus samples was confirmed BYMV infection through PCR using potyvirus degenerate primer pairs. The resulted PCR amplicon of ~1.2 kb was obtained in ten infected gladiolus samples. The partial amplified genome (1.2 kb) of BYMV was cloned and sequenced. The sequence data obtained had 99-100% nucleotide identity with each other and 89-92% with other BYMV isolates reported worldwide. Based sequence data the isolates was identified isolate of BYMV from gladiolus. Therefore one BYMV isolate was selected (OV65) for complete genome characterization (Fauquet *et al.,* 2005) using degenerate primer pairs (Table 1) from the infected gladiolus plant.

Molecular characterization of BYMV by complete genome sequence analysis

The complete genome of BYMV infecting gladiolus was amplified by PCR using eight primer pairs, which are overlapping four region of the viral genome (Fig IV). The expected size amplicons of ~1.2 kb in size were obtained in all pairs of primers from infected gladiolus plants. The PCR amplified different overlapping fragments were cloned sequenced and assemble using different bioinformatics programs and the complete genome sequence data were submitted to GenBank under the accession: MK131270.

Complete BYMV genome sequence analysis

The complete genome sequence of BYMV obtained in the present study was compared with 39 BYMV isolates infecting different crops and other potyvirus obtained from GenBank. The result showed that the BYMV isolate, isolated from the infected gladiolus plant showed highest nucleotide identity ranged from 92-97.2% with BYMV isolates (CK-GL2, G1, GDD, CKGL5, GB2, MB4, Lisianthus, Gla, MBGP, Vfaba2) infecting different crops and belonged to the group IV reported from India, Japan, USA and Taiwan (Table 2).The BYMV isolate infecting gladiolus also sheared 92.5 to 92.5% nucleotide identity with BYMV isolates (M11, Ib) infecting different crops that belonged to the group III reported from Japan. Further BYMV isolate sheared 86.8 to 87.1% sequence similarity with BYMV isolates (AR87C, ES55C, MD7, SW9, SW3.2 and LMBNN) infecting different crops of belong the group II reported from Australia. Similarly BYMV isolate sheared 86.6 to 86.7% nucleotide identity with group I BYMV isolates (SP1, PN83A, GB17A, Fr, PN80A, KP2, KP2, NG1) infecting different crops reported from Australia. The BYMV isolate showed less than 85% sequence similarity with BYMV isolates (921, S, LP, LPexFB, 902, FB, WLMV, CS) infecting different crops that belonged to different groups viz; V, VI, VII, VIII and IX respectively (Table 2). The alignment analysis of deduced amino acid residues of various proteins translated within the long polypeptide of BYMV isolate showed similar variable sequence identity with individual proteins to other phylogenetic groups (Data not shown).

phylogenetic analysis

The phylogenetic analysis was done using complete genome of BYMV isolate infecting gladiolus under study with the selected thirty nine BYMV isolates along with other potyviruses, macluravirus and ipomovirus sequences (Fig. V).

Table.1 List of eight degenerative primer pairs used in current study

S. No.	Primer Name	Sequence (5'-3')
1	BYMF20 BYMR1282	5' CAAGACAAYACAAGACAWAACG 3' 5' GAACACRCTTGCATTRTYAAATC 3'
2	BYMF1147 BYMR2326	5' GTGGDTCAGTCATGGCKCTKT 3' 5' CAACYCTATAAWAMTTCAGTTCAG 3'
3	BYMF2302 BYMR3596	5' GCAGTYCTTGACTCATATGGTTC 3' 5' CACAATCRCTCCTYTCAGCATC 3'
4	BYMF3495 BYMR4610	5' CGAGTAACAGCAYTGRTACTCATG 3' 5' CAAATCTARYTCTGGYACCAC 3'
5	BYMF4557 BYMR5754	5' ATGGTGTAACGCTTGACATTGAAG 3' 5' GTACCTYTMACYTTTCCYETCTTTG 3'
6	BYMF5612 BYMR6862	5' ATTGCAGCGGGAGTGCTCGG 3' 5' CAAYTTCCARCCACAACACCAG 3'
7	BYMF6769 BYMR7948	5' CAGGTGAYCTYAATGTGTTCAC 3' 5' CATGAGYGTGTTRTCAACCACTG 3'
8	M4TR WEICNF	5' CCAGTGGCTCTTTTTTTTTTTTTTT 3' 5' TGCTCYATHCTMAAYCGMACNAG 3'

Table.2 Pair wise sequence similarity (%) of complete genome of BYMV infecting *Gladiolus sp.* and with other potyviruses reported in worldwide

Viruses	Acc. number	Host species	Isolate	Place	Group	Identity (%)
BYMV	HG970851	*L. angustifolius*	SP1:	Australia	**I**	86.6
BYMV	HG970860	*L. angustifolius*	PN83A	Australia	**I**	86.6
BYMV	HG970852	*L. angustifolius*	GB17A	Australia	**I**	86.7
BYMV	FJ492961	*Freesia sp.*	Fr	S.Korea	**I**	86.5
BYMV	HG970861	*Freesia sp.*	PN80A	Australia	**I**	86.6
BYMV	JX173278	*D. magnifica*	KP2	Australia	**I**	86.4
BYMV	HG970865	*L. angustifolius*	KP2	Australia	**I**	86.6
BYMV	HG970869	*L. angustifolius*	NG1	Australia	**I**	86.6
BYMV	HG970863	*L. angustifolius*	AR87C	Australia	**II**	86.8
BYMV	HG970858	*L. angustifolius*	ES55C	Australia	**II**	86.7
BYMV	HG970850	*Lupinus cosentinii*	MD7	Australia	**II**	86.9
BYMV	KF632713	Diuris sp	SW9	Australia	-	86.9
BYMV	JX156423	*Diuris sp.*	SW3.2	Australia	**II**	87.1
BYMV	HG970855	*L. angustifolius*	LMBNN	Australia	**II**	87.0
BYMV	AB079886	*L. pilosus*	M11	Japan	**III**	92.5
BYMV	AB079887	*G. hybrida*	Ib	Japan	**III**	92.3
BYMV	KM114059	*Gladiolus sp.*	CK-GL2	India	**IV**	94.1
BYMV	AB439730	*G. hybrida*	G1	Japan	**IV**	97.2
BYMV	AY192568	*Gladiolus sp.*	GDD	USA	**IV**	96.9
BYMV	KF155420	*Gladiolus sp.*	CKGL5	India	-	95.5
BYMV	AB079888	-	GB2	Japan	**IV**	94.7
BYMV	NC003492	-	MB4	Japan	**IV**	96.8

BYMV	AM884180	*E. russellianum*	Lisianthus	Taiwan	**IV**	96.0
BYMV	AB439729	*G. hybrida*	Gla	Japan	**IV**	92.0
BYMV	D83749	-	MBGP	Japan	**IV**	96.8
BYMV	JN692500	*V. faba*	Vfaba2	India	**IV**	93.9
BYMV	KT934334	sunflower	BYSun	Iran	-	85.4
BYMV	KF155419	gladiolus	CK-GL4	India	-	94.0
BYMV	KF155414	gladiolus	CK-GL3	India	-	96.8
BYMV	KF155409	gladiolus	CK-GL1	India	-	94.4
BYMV	MG600297	*Trifolium pratense*	PV2	Czech Republic	-	72.0
BYMV	AB439732	*Trifolium pratense*	921	Japan	**V**	84.3
BYMV	U47033	*V. faba*	S	Australia	**V**	85.5
BYMV	HG970866	*L. pilosus*	LP	Australia	**VI**	82.2
BYMV	HG970868	*V. faba*	LPexFB	Australia	**VI**	81.2
BYMV	AB439731	*V. faba*	902	Japan	**VII**	80.2
BYMV	HG970867	*V. faba*	FB	Australia	**VII**	79.7
WLMV	DQ641248	*L. albus*	WLMV	USA	**VIII**	78.1
BYMV	AB373203	*Pisum sativum*	CS	Japan	**IX**	75.5
CYVV	NC_003536	*Phaseolus vulgaris*	CYVV	Japan	-	65.4
CYVV	HG970870	*T. repens*	CYVV	Australia	-	64.4
OrMV	NC_019409	-	OrMV	Australia	-	52.4
CYNMV	NC_018455	*D. opposita*	-	-	-	39.9
SVYV	NC_010521	Watermelon	-	USA	-	42.8

BYMV= Bean yellow mosaic virus, CYVV= Clover yellow mosaic virus, WLMV=White lupin mosaic virus, OrMV=Ornithogalum mosaic virus, SVYV=Squash vein yellowing virus and CYNMV=Chinese yam necrotic mosaic virus.

Figure.1 Symptoms of BYMV in gladiolus

Table.3 Breakpoint analysis of BYMV infecting gladiolus and their putative parental sequences

BYMV	Break point begin-end	Major Parent	Minor parent	P-Values					
				RDP	GENECOV	Max Chi	Chimera	Si Scan	3Seq
RNA	16-3123 46-3242 3977-8417 6500-6624 6625-8519	BYMV-KP2:Australia[I]-HG970865 BYMV-Fr:S.Korea[I]FJ492961 BYMV-S:Australia[V]-U47033 BYMV-Fr:S.Korea[I]FJ492961 BYMV-Vfaba2:India[IV)]-JN692500	BYMV-India[IV]-KF155414 BYMV-India[IV]-KF155414 BYMV-India[IV]-KT934334 BYMV-India[IV]-KT934334 BYMV-Fr:S.Korea[I]FJ492961	$1.777X10^{-17}$ $1.73X10^{-26}$ $5.509X10-^{40}$ $9.423X10^{-7}$ $1.360X10^{-38}$	$2.049X10^{-17}$ $1.445X10^{-24}$ $4.472X10^{-26}$ $1.208X10^{-4}$ $1.290X10^{-26}$	$1.307X10^{-6}$ $4.940X10^{-21}$ $3.335X10^{-10}$ $4.479X10^{-2}$ $5.25X10^{-16}$	$2.605X10^{-5}$ $2.O57X10^{-57}$ $6.603X10^{-11}$ $2.369X10^{-2}$ $1.423X10^{-16}$	$4.2X10^{-45}$ - $1.165X10^{-38}$ - $1.493X10^{-35}$	$7.401X10^{-13}$ $1.2286X10^{-72}$ $2.501X10^{-12}$ $5.755X10^{-5}$ $1.470X10^{-12}$

NS- Recombination Non-significance

Figure II: Sap transmission of BYMV on beans

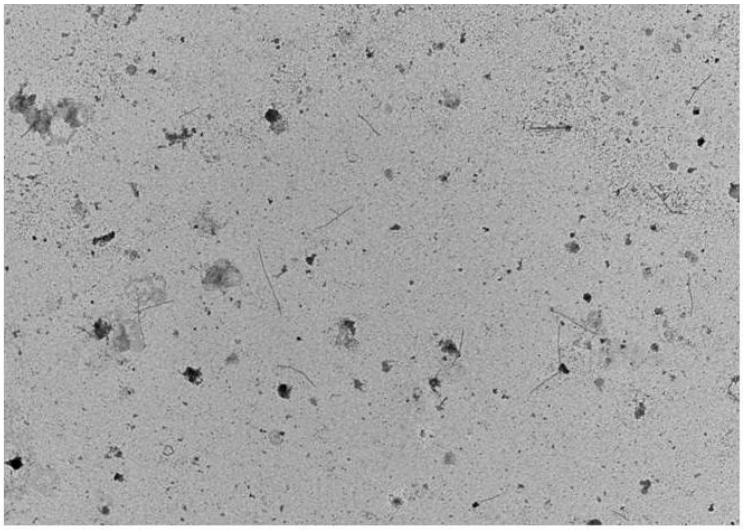

Figure III: TEM showing BYMV virus particles

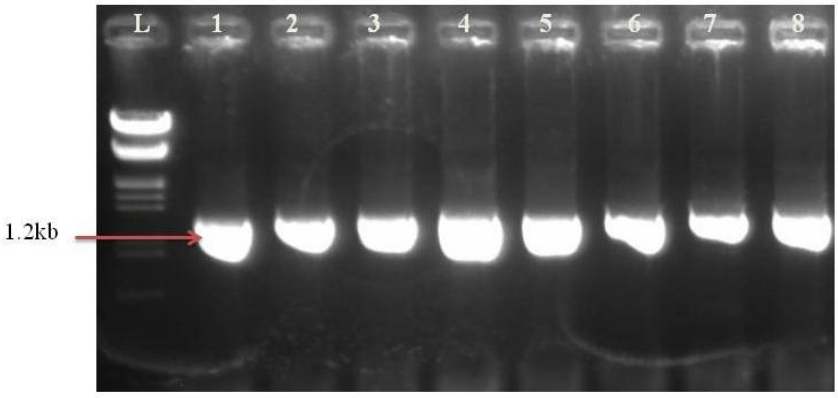

Figure IV: Amplification of full genome of BYMV using eight sets of overlapping primers (L-Ladder (EcoRI+HindIII marker), 1-8 primer sets).

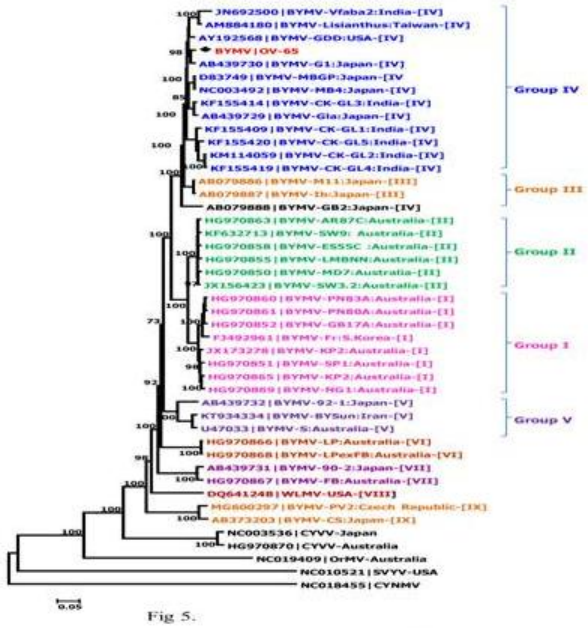

Fig 5.

Figure V: The phylogenetic analysis of gladiolus BYMV with the selected thirty nine BYMV isolates

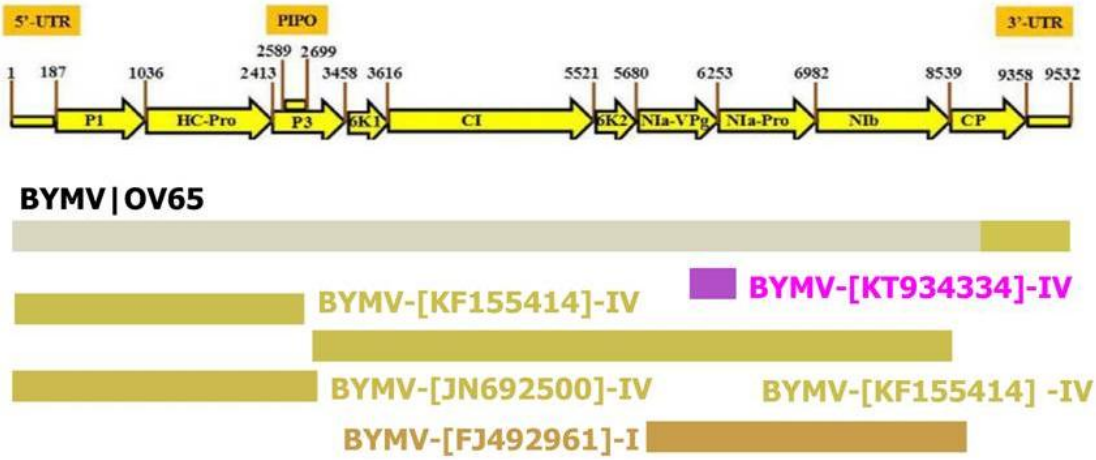

Figure VI: Neighbor-network of gladiolus BYMV using the Splits-Tree program

The complete genome of BYMV isolate infecting gladiolus is closely clustered with group IV of BYMV isolates (CK-GL2, G1, GDD, CKGL5, GB2, MB4, Lisianthus, Gla, MBGP, Vfaba2) infecting different crops reported from India, Japan, USA and Taiwan respectively. Other BYMV isolates (Australia, Japan, South Korea and USA) reported from different parts of world clustered in I-IX different phylogenetic groups, the other two closely related CYVV isolates grouped in a separate cluster, while OrMV (NC019409),

macluravirus (NC018455) and ipomovirus (NC010521) were taken as out group. Pairwise sequence similarity and phylogenetic relationship analysis showed that the BYMV isolate infecting gladiolus in South India was closely related potyvirus isolates belonging to group IV.

Neighbor-net and Recombination analysis

A neighbor-network (using the program Splits-Tree version 4.11.3) was constructed using the sequences of different thirty nine BYMV isolates (I - IX groups) along with other potyviruses, macluravirus and ipomovirus sequences obtained from NCBI database along with the sequence of the BYMV isolate characterized in the present study. The analysis showed a reticulated network like structure indicative of phylogenetic incongruence suggesting parts of the sequences have different origins due to recombination (data not shown). The pairwise homoplasy index PHI test also strongly supported the presence of recombination BYMV isolate characterized in the present study ($P < 0.001$). Further recombination break point analysis done using RDP4, indicated the evidence of intra and inter-recombination group recombination in the BYMV genome infecting gladiolus and its most of the genome is derived from BYMV isolates from the phylogenic group of IV and I to emerge as a new variant of BYMV infecting gladiolus(Fig VI, Table 3). The recombination was detected with parental phylogenetic groups: IV and I. Most of the recombination was observed within the 5'-UTR, a large ORF, P1 proteinase; helper component proteinase (HC-Pro); P3N-PIPO; P3 protein; 6K1 protein; cylindrical inclusion (CI) protein; 6K2 protein; nuclear inclusion a (NIa) protein and viral protein genome linked (VPg); the NIa proteinase (NIa-Pro); nuclear inclusion b (NIb) protein; viral RNA dependent RNA polymerase and coat protein (CP) and no recombination was detected in the 3'-UTR, of BYMV isolate (Table 3). The overall recombination analysis showed that the genome BYMV infecting gladiolus is derived from phylogenetic groups of IV and I of BYMV infecting different crops reported from India, Japan, USA, Taiwan and Australia(Table 3).

Gladiolus is an important ornamental crop grown commercially in different parts of the world. The major constrain for cultivation and production quality flowers in gladiolus are viruses, which are harbor in the propagated (corm lets) materials leads to causes significant crop yield loss (Kamo *et al.*, 2005). Gladiolus is also natural host of many RNA viruses (AMV, BYMV, BBWV, CMV, INSPV, OrMV, SLRSV, TRV, TMV, TAV, TRSV and TSWV) belonging to diverse groups reported from different parts of the world (Raj *et al.,* 2002& 2011;Dorrigiv *et al.,* 2013; Duraisamy *et al.,* 2011; Katoch *et al.,* 2003a& 2003b, 2004; Kaur *et al.,* 2011). In the present study the infected gladiolus samples and corms was collected different places of Bangalore rural areas are confirmed BYMV infection through PCR. Further to know the phylogenetic group of BYMV isolate infecting gladiolus, complete genome was characterized using different degenerative overlapping primers. Complete genome analysis of BYVMV isolate infecting gladiolus showed more homology with BYMV isolates (CK-GL2, G1, GDD, CKGL5, GB2, MB4, Lisianthus, Gla, MBGP, Vfaba2) infecting different crops of belong the phylogenetic group IV reported from India, Japan, USA and Taiwan. The genome of BYMV isolate had a length of 9532 nucleotides as depicted by the sequence excluding the poly (A) tract and similar to other BYMV isolates from different crops (Selvarajan *et al.,* 1998). An analysis of the RNA depicts an open reading frame (ORF) having 9171 nucleotides that encode a large

polypeptide that has 3056 amino acids, which has an average mR of 347,571 (Duraisamy *et al.,* 2011). There are nine putative proteolytic cleavage sites, where one of them is cleavage by P1 protease, one by the HC protease, while the remaining seven are cleavage by the Nla protease. The genetic organization of BYMV genome has been found out from the results to be 5'UTR/CP-3'UTR (Selvarajan *et al.,* 1998). A comparison of the amino acid sequences of each of the BYV proteins with the corresponding proteins of other potyviruses showed that the BYMV had a higher homology except the P1.

Based on the CP gene sequence analysis of different BYMV isolates, nine phylogentic groups (I to IX) of BYMV infecting different crops have been proposed so far (Kehoe *et al.,* 2014;Wylie *et al.,* 2008). All the isolates have broad host range infecting monocots and dicot plants. In the present our isolates also showed more sequence homology with BYMV isolates infecting mainly *gladiolushybrida* reported from Japan and recently proposed as BYMV phylogenetic group- IV (Kehoe *et al.,* 2014). The result clearly showed that, introduction of BYMV with infected gladiolus plant material from different locations in different time beings this might be one of the reasons that the isolates from different locations clustered together.

Recombination is one of the key factors for rapid evolution and adaptation of RNA viruses (Gray *et al.,* 2010) has been well documented in potyviruses in different parts other world (Gray *et al.,* 2010,Revers *et al.,* 1996). Recombination is found to play a major role in creating the genetic variability in the vial genome as well as increase it host range (Wylie and Jones, 2009). The recombination analysis revealed that most part of the genome is derived from BYMV isolates with intra recombination with different group (VI and I) BYMV isolates infecting different crops.

The commercial cultivation of gladiolus by most of the growers depends on the corms and corm lets. The occurrence of the mosaic disease on gladiolus gives an alarming signal against utilization of such virus infected planting materials in the crop breeding and improvement program. The technique developed here will be highly useful to detect the virus infection in clonally propagated plants such as gladiolus.

Acknowledgments

The research was supported by the project **"**Consortium platform on Vaccines and diagnostics" funded by Indian Council of Agricultural Research, Government of India, New Delhi, India.

Competing interests

The authors declare that they have no competing interests.

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How to cite this article:

Hemachandra Reddy, P., V. Venkataravanappa, P. Swarnalatha, K. V. Ashwathappa and Krishna Reddy, M. 2019. Characterization of Recombinant Bean Yellow Mosaic Virus belongs to Group-IV Infecting Gladiolus. *Int.J.Curr.Microbiol.App.Sci.* 8(09): 1644-1657.
doi: https://doi.org/10.20546/ijcmas.2019.809.187