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Genetic variability in coat protein gene of sugarcane mosaic virus in Pakistan and its relationship to other strains

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Sugarcane mosaic virus (SCMV) is one of the three causative viruses of mosaic in sugarcane, a sugar crop widely grown under tropical and subtropical conditions worldwide. Although molecular characterization of SCMV strains was reported from many countries, strain occurring in Pakistan, a major sugarcane producer has not been reported so far. One hundred and two (102) sugarcane samples from foremost sugarcane growing districts in Pakistan were transcribed by reverse-transcription polymerase chain reaction (RT-PCR) by means of a pair of primers. All of them were found infected with SCMV. The sequences of SCMV coat protein gene amplified in this study varied between 406 and 457 nt. The sequence assessment of 12 Pakistani SCMV isolates revealed extensive range of sequence resemblances, 82-100% nucleotide (nt) and 5.0-95% amino acid (aa) respectively. A deletion of aa residues was observed in the amplified segments of the Pakistani amplicons. 50 SCMV CP sequences (12 from Pakistan and 38 from other sugarcane growing countries) were subjected to phylogenetic and *in-silico* restriction analyses grouped the isolates mostly in order of their geographical origin. The 12 Pakistani SCMV isolates were included in one group. Nearly 97.0% of isolates from Pakistan have no signs for close association with earlier categorized sugarcane mosaic virus strains SCMV-A, SCMV-B, SCMV-D, SCMV-E, and SCMV-SC described from various countries. Our studies discovered that the sugarcane mosaic in Pakistan is instigated by a new group/strain SCMV-PAK. It is the first representation on the diversity and existence of novel SCMV population in Pakistan.

Key words: Sugarcane, sugarcane mosaic virus, geographical, Pakistan, reverse transcriptase polymerase chain reaction (RT-PCR).

INTRODUCTION

Mosaic of sugarcane is a significant disease in sugarcane which is nearly a couple of centuries ancient and disseminated worldwide (Koike and Gillespie, 1989). In Pakistan, the occurrence of mosaic disease in sugarcane

was validated by Jensen and Hall (1993), and since then it broadly prolonged its acclimatization in all the sugarcane growing areas of Pakistan (Yasmin et al., 2011). Sugarcane mosaic is considered as least problematic in some regions but it affects considerable crop damages in many countries by its extreme epidemics. Epidemics of mosaic of sugarcane is about 100% in the world (Mandahar, 1987) and is a major threat to gigantic area under sugarcane farming, which causes damages up to 46% (Singh, 1971; Mandahar, 1987).

The sugarcane mosaic virus can also infect other economic crops such as corn and sorghum (Teakle et al., 1989). Four distinct potyviruses, sugarcane mosaic virus (SCMV), Johnson grass mosaic virus (JGMV), sorghum mosaic virus (SrMV) and maize dwarf mosaic virus (MDMV) are accepted as SCMV subgroups (Teakle et al., 1989; Shukla et al., 1992). Previously, mosaic in sugarcane was recognized to only a Potyvirus named as sugarcane mosaic virus (SCMV) possessing several strains (Shukla et al., 1989; Koike et al., 1989). Presently, sugarcane mosaic virus subgroup from the genus Potyvirus comprises of seven various species SCMV, MDMV, SrMV, JGMV (Shukla et al., 1989; McKern et al., 1991), Zea mosaic virus (ZeMV) (Seifers et al., 2000), Cocksfoot streak virus (CSV) (Gotz and Maiss, 2002), and Pennisetum mosaic virus (PenMV) (Fan et al., 2003a, b). Amongst these viruses, only SCMV and SrMV are considered to be infectious in sugarcane naturally, hence it is a major causative organism of mosaic in sugarcane (Grisham et al., 2000). Five strains of SCMV, SCMV-A, SCMV-B, SCMV-D, SCMV-E and SCMV-SC, and three strains of SrMV, SrMV-SCH, SrMV-SCI and SrMV-SCM have been categorized on the basis of sequencing of coat protein gene from United States and Australia (Shukla et al., 1992, 1994; Frenkel et al., 1991; Yang and Mirkov, 1997). On the basis of differences in mosaic pattern on sugarcane, 12 SCMV strains: SCMV-A, SCMV-B, SCMV-C, SCMV-D, SCMV-E, SCMV-F, SCMV-H, SCMV-I, SCMV-J, SCMV-K, SCMV-M and SCMV-N were described from India (Farrag and Kandaswamy, 1979; Kondaiah and Nayudu, 1984a, b, 1985; Rishi and Rishi, 1985; Gopal and Reddy, 1988; Gopal et al., 1991). SCMV is transmitted by several aphid species, particularly *Rhopalosiphum maidis*. A very important secondary dissemination method is vegetative propagation of sugarcane through infected setts (Chen and Adams, 2002; Li et al., 2013).

Nucleotide and amino acid sequences of coat protein gene have been used as molecular marker to differentiate eight strains of SCMV but they symbolize only United States of America, Australia and India amongst the countries growing sugarcane. Based on serological and differential host interactions, dissemination of different SCMV strains in Pakistan has been described and minute exertions have been tried up to now to detect the prevailing strains of SCMV in Pakistan

using molecular biology techniques. The present study of SCMV was carried out against different sugarcane cultivars grown in Pakistan.

MATERIALS AND METHODS

RNA isolation from infected varieties/cultivars of sugarcane

Isolation of RNA was carried out from 102 samples of the nine different cultivars/varieties of sugarcane (SPF-234, NSG-555, NSG-646, HSF-240, BF-162, HSF-245, HSF-242, SP-98-133 and SPF-213) from 18 districts of Punjab Province, Pakistan. All samples were collected on the basis of symptoms of mosaic disease and were selected based on the following conditions: (i) SCMV dissemination pattern in *Saccharum* spp. (ii) detection of the widespread variants of SCMV strains; and (iii) assessment of degree of discrimination among the variants of the strains and certain isolates prevailing globally.

Primer designing

A pair of primers SCMV-F454 (5'-GAGCAACCAGAGAGGAGTTTG-3') and SCMV-R454 (5'-CCAGACCGAACAATCGTGTG-3') was synthesized for the amplification of ~456 bp of coat protein from core region based on reported sequences of viral strains SCMV-A, SCMV-B, SCMV-D, SCMV-E (Yang and Mirkov, 1997), and SCMV-SC (Frenkel et al., 1991), and isolates conveyed earlier from School of Biological Sciences, University of the Punjab (GenBank Acc. AM040436; DQ648195) (Haider et al., 2011).

RT-PCR

RNAs were subjected to reverse transcription by the enzyme M-*MuLV* H-minus reverse transcriptase, using Oligo(dT) primer. cDNA were subjected to PCR using *Taq* DNA polymerase (1.0 U) (Enzymology Lab. National Centre of Excellence in Molecular Biology, University of the Punjab Lahore, Pakistan), 1.0x reaction buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), primers (10 pmol each), and 0.2 mM of each dNTP in a 20 µl reaction. Polymerase chain reaction was carried out by heating for 4 min at 94°C, following 35 cycles (94°C for 1 min, 55°C for 1 min and 72°C for 1 min), and finally a single cycle of final extension at 72°C for 10 min. DNA fragments were separated on 1.5% agarose gel, stained with 100 ng/ml ethidium bromide and visualized under UV light.

Cloning and sequencing of CP genes

The amplified segments of SCMV CP gene from all positive were eluted from the high melting agarose gel using QIAquick Gel Extraction Kit (QIAGEN) and following manufacturer's instructions. The purified PCR fragments were cloned into the pCR®2.1 vector using the TA Cloning® Kit (invitrogen™) and transformation was carried out into the *Escherichia coli* DH5α competent cells. Using GeneJET Plasmid Miniprep Kit (Ferments, USA) the plasmids having inserts of estimated size were purified. Two cloned inserts, for each virus isolate, were subjected to complete sequencing on both strands by DNA Sequencing Lab. National Centre of Excellence in Molecular Biology, Lahore, Pakistan. The amplicons with 1.0% sequence differences was assumed as distinct isolate.

Phylogenetic analysis and sequence comparison

Sequences of nucleotide and amino acid of the coat protein from amplified region of 50 SCMV isolates, 12 from this study and 38

from worldwide locations including Pakistan (two sequences) were aligned separately using ClustalW (Thompson et al., 1994) and matched themselves. The trimming of 5'-terminal and 3'-terminal regions was carried out in accordance with the isolate SCMV-CEMB-5 evenly by BioEdit version 7.2.0.0 with built in Treeview software (Hall, 1999). Phylogenetic tree was produced and envisaged, and grouping patterns were compared. Similarities were calculated by creating similarity index and converting it into percent similarities using the same alignment (Table 2).

Genetic diversity and *in-silico* restriction analysis

Strain differentiation among potyviruses was reported in sugarcane mosaic virus and sorghum mosaic virus based on restriction profile (Yang and Mirkov, 1997) using a group of restriction enzymes. Using the enzymes *Alu*I, *Bst*MWI, *Cvi*JI, *Eco*RI, *Hinf*I, *Hpy*CH4V and *Taq*I (Table 3), the restriction analysis of nucleotide sequences were executed.

RESULTS AND DISCUSSION

All the 102 samples of the nine different cultivars/varieties of sugarcane were observed with different intensity of mosaic symptoms. All the samples under investigation were found infected with SCMV using RT-PCR (Singh et al., 2009). On the other hand, Hema et al. (1999) stated that SCMV subgroup viruses are not the cause of mosaic in sugarcane, it is due to SCSMV.

In the same way, in many Asian countries which grow sugarcane, at least two strains of SCSMV are the major cause of mosaic in sugarcane (Chatenet et al., 2005). Conversely, Xu et al. (2008) collected 164 field samples from Guangdong, Guangxi, Hainan and Yunnan provinces of China and reported that all of them were infected either with sugarcane mosaic virus or sorghum mosaic virus alone or with both viruses and SCSMV was not the cause of sugarcane mosaic except a few germplasm from India.

In Louisiana, Grisham and Pan (2007) failed to categorize SrMV or sugarcane mosaic virus in sugarcane presenting symptoms of mosaic. In the same way, the primers used to detect SrMV and SCMV could not amplify the virus genes in the mosaic diseased sugarcane in Argentina (Perara et al., 2007). Hence, it can be concluded that sugarcane mosaic virus is the main cause of mosaic in sugarcane worldwide. Either sugarcane mosaic virus or sugarcane streak mosaic virus alone or their combinations are the cause of sugarcane mosaic in Pakistan (Yasmin et al., 2011; Li et al., 2013).

Using a pair of primers (SCMV-F and SCMV-R), we observed a noteworthy difference among the lengths of the amplified sequences (406-457 nt) used in this study. The amplified sequences varied from 406 (SCMV-CEMB-2) to 457 (SCMV-CEMB-5) nt for different isolates of SCMV (Table 1). The variation in sequence segments of SCMV amplified from sugarcane varieties from Pakistan predicted the genetic variation among different SCMV strains going through different levels of mutations. The

diversity in the CP sequence is inevitable in the hyper variable region (Oertel et al., 1997) in order to meet the selection pressure, possibly when it is exposed to the insects and the environment.

The overall nt similarity in the core region (CR) of the CP gene of sugarcane mosaic virus isolates occurring worldwide varied between 26 and 100% (mean 84.6%) while aa identity ranged between 1.0 and 100% (average 53.5%) (Table 2). The lowest range of resemblances was observed with SCMV-VN/SC1 and SCMV-VN/SC2; isolates from Veitnam (Ha et al., 2008) present the greater inconsistency.

Though, the isolate SCMV-CEMB-9 (GenBank Acc. KC249914) of this study expressed the lowest nt (59-99%) and aa (4.0-100%), sequence identities in the CR of the CP were compared with the strains from all over the world, and it exhibited extreme nucleotide identity of 100% with Indian isolate SCMV-CB89003-4 (GenBank Acc. EF 655890) and amino acid similarity of 92% with SCMV-CEMB-6 (GenBank Acc. KC249911).

The overall genetic similarities among our 12 SCMV isolates, and the isolate cssg-668 (GenBank Acc. DQ648195) reported from Pakistan (Haider et al., 2011), ranged between 82 and 100% at nucleotide and between 6.0 and 90% at amino acid levels respectively in the sequenced region (Table 2). The corresponding resemblances among the isolates prevailing worldwide (except Pakistani isolates) fluctuated between 26 to 100% and from 1.0 to 100%. The mean of the sequence identity level of isolates from all over the world was 84.6 and 53.5% at nt and aa. The isolates SCMV-CEMB-4 (GenBank Acc. KC249909), SCMV-CEMB-7 (GenBank Acc. KC249912) and SCMV-CEMB-8 (GenBank Acc. KC249913) of this study presented the least nt (27%) and aa (4.0%) identities with the strains from USA and Veitnam.

Recurrence of short peptide motifs was observed in the isolates from Pakistan. They are recurring in a similar fashion as perceived by Frenkel et al. (1991) and Xiao et al. (1993) in SCMV-SC and USA strains (Yang and Mirkov, 1997). The sequence form ''EQPERSLIGGMKP'' (Figure 1) has accurately recurred in 4 of 12 (33.3%) Pakistani isolates. Similarly, the sequence patterns "MDGDEQRVF-PLKPVI" and "ENASPTFRQIMHHFSDAAEAYIEYRNSTERYMPRYGL QR" have exactly been repeated in 6 of 12 (50%). Among the isolates/type strains from worldwide included in this study, the conserved motif (EQPERSLIGGMKP) is repeated exactly in 18 of 38 (47%) and the motif "HDMDFSEISPTIA" has been repeated in 21 of 38 (55.25%) isolates. Several other amino acid sequence motives have repeats with residual variation of 1 or 2 aa, which range between 40 to 60%.

Phylogenetic tree was built from the nucleotide sequences of our 12 and 38 selected isolates from all over the world (Figure 2). In the phylogenetic tree, most of the virus isolates were grouped with respect to their

Table 1. Details of SCMV-CEMB isolates and other SCMV types strains/isolates used in the comparison study.

SCMV isolates/strains	Size (bp)	Source/variety	State/Country	GenBank Acc. No.	Reference
SCMV-CEMB-1	455	Saccharum hybrid cultivar	Punjab, Pakistan	KC200152	This study
SCMV-CEMB-2	406	Saccharum hybrid cultivar	Punjab, Pakistan	KC249907	This study
SCMV-CEMB-3	421	Saccharum hybrid cultivar	Punjab, Pakistan	KC249908	This study
SCMV-CEMB-4	455	Saccharum hybrid cultivar	Punjab, Pakistan	KC249909	This study
SCMV-CEMB-5	457	Saccharum hybrid cultivar	Punjab, Pakistan	KC249910	This study
SCMV-CEMB-6	456	Saccharum hybrid cultivar	Punjab, Pakistan	KC249911	This study
SCMV-CEMB-7	456	Saccharum hybrid cultivar	Punjab, Pakistan	KC249912	This study
SCMV-CEMB-8	455	Saccharum hybrid cultivar	Punjab, Pakistan	KC249913	This study
SCMV-CEMB-9	432	Saccharum hybrid cultivar	Punjab, Pakistan	KC249914	This study
SCMV-CEMB-10	431	Saccharum hybrid cultivar	Punjab, Pakistan	KC249915	This study
SCMV-CEMB-11	422	Saccharum hybrid cultivar	Punjab, Pakistan	KC249916	This study
SCMV-CEMB-12	423	Saccharum hybrid cultivar	Punjab, Pakistan	KC249917	This study
mosIAC	360	Sugarcane	Brazil	JF699509	Sawazaki et al., 2013
AP1	852	Sugarcane	India	GQ386846	Reddy et al., 2011
SCMV-Mfc	286	Maize	France	HM014060	Marie-Jeanne et al., 2011
Q86	700	NA	Iran	AY648298	Ghasemi et al., 2005
SCMV-VN/SC1	939	Sugarcane(*Saccharum officinarum*)	Yenbai, Vietnam	DQ925431	Ha et al., 2008
SCMV-VN/SC2	939	Sugarcane (*Saccharum officinarum*)	Hoabinh, Vietnam	DQ925427	Ha et al., 2008
CB44-101	866	*Saccharum officinarum* cv. CP-44-101	India	EF655894	Viswanathan et al., 2008
KhzL66	939	Sugarcane (*Saccharum officinarum*)	Iran	DQ369960	Ghasemi et al., 2005.
GX-1	888	Saccharum sp.	China	DQ227694	Zhou et al., 2007
KhzQ86	939	Sugarcane	Iran	DQ438949	Masumi et al., 2006
PIR-2	886	NA	Brazil	AY819718	Goncalves et al., 2012
CSSG 676	888	Sugarcane	Punjab, Pakistan	AM040436	Haider et al., 2011
cssg-668	888	Sugarcane	Pakistan	DQ648195	Haider et al., 2011
SC	889	NA	Sichun, China	AJ421468	Jiang and Zhou 2002
ZAF 53-1	852	Saccharum hybrid cultivar	South Africa	AJ491973	Alegria et al., 2003
USA Flo 36-1	852	Saccharum hybrid cultivar	Florida, USA	AJ491971	Alegria et al., 2003
USA Lou 40-1	828	Saccharum hybrid cultivar	Louisiana, USA	AJ491965	Alegria et al., 2003
EGY7-1	852	Saccharum hybrid cultivar	Egypt	AJ491963	Alegria et al., 2003
CON98-1	852	Saccharum hybrid cultivar	Republic of Congo	AJ491961	Alegria et al., 2003
CAM94-1	852	Saccharum hybrid cultivar	Cameroon	AJ491939	Alegria et al., 2003
ZJ1	802	NA	Zhejiang, China	AJ421465	Jiang and Zhou 2002
NJ1	802	NA	Jiangsu, China	AJ421463	Jiang and Zhou 2002
SCMV-Seehausen/S26	942	NA	Germany	X98165	Oertel, et al., 1997
SCMV-CB72-1	898	Saccharum hybrid cultivar BO 72	Bihar, India	DQ842502	Viswanathan et al., 2008

Table 1. Contd.

SCMV-CB617	818	Saccharum hybrid Co 617	Tamil Nadu, India	EU089686	Viswanathan et al., 2008.
SCMV-CB89003-4	898	*Saccharum officinarum* cv. Co 89003	Haryana, India	EF655890	Viswanathan et al., 2008
SCMV-CBA7701	871	*Saccharum officinarum* cv. CoA7701	Andhra Pradesh, India	EF655899	Viswanathan et al., 2008
SCMV-CB84213-3	898	*Saccharum officinarum* cv. Co Pant 84213	Uttaranchal, India	EF655889	Viswanathan et al., 2008
PB-CoJ85	899	NA	Punjab, India	DQ866746	Singh et al., 2009
KL-Co86032	899	NA	Kerala, India	DQ866744	Singh et al., 2009
IND	898	NA	India	AY241923	Gaur et al., 2003
TUC-1C	900	Sugarcane	Argentina	EU196423	Perera et al., 2007
E	939	NA	USA	U57357	Yang and Mirkov 1997
D	927	NA	USA	U57356	Yang and Mirkov 1997
A	915	NA	USA	U57354	Yang and Mirkov 1997
B	927	NA	USA	U57355	Yang and Mirkov 1997
SC	939	NA	Australia	D00948	Frenkel et al., 1991
BRIS	942	NA	Australia	AF006734	Handley et al., 1998

Table 2. Percent nucleotide (above diagonal), amino acid (below diagonal) sequence similarity of Pakistani SCMV isolates, strains, and other isolates from worldwide at core region of the coat protein spanning 457 nt, corresponding to SCMV-CEMB-5 (GenBank Acc. KC249910).

Acc. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
AJ421465	100	100	94	59	92	89	89	89	69	89	89	89	70	91	91	91	89	89	90	89	90	89	88	83	88
AJ421463	99	100	93	59	92	89	89	89	69	89	89	89	71	91	91	91	89	89	90	89	90	89	88	83	88
X98165	82	81	100	58	91	88	89	88	69	89	89	89	69	90	89	89	89	89	90	90	89	88	88	83	88
HM014060	5	5	5	100	6	57	57	57	53	56	56	57	70	56	56	57	56	56	57	57	57	57	56	59	56
AJ421468	76	76	73	6	100	89	90	89	69	88	89	89	70	89	89	89	88	88	89	89	89	89	88	83	88
DQ866746	69	69	67	5	69	100	100	98	75	95	95	95	74	95	95	96	95	95	96	96	95	95	95	90	95
DQ866744	68	69	67	5	70	99	100	98	75	95	96	95	74	95	95	96	95	95	96	96	95	95	95	90	95
GQ386846	68	69	67	4	69	94	94	100	75	95	95	96	74	94	95	96	94	94	95	95	95	95	95	90	95
JF699509	51	52	52	4	52	68	69	68	100	75	75	75	53	75	75	75	74	74	75	76	76	75	75	79	75
AJ491971	68	69	69	6	68	87	87	86	71	100	99	96	74	95	95	96	95	95	96	97	96	96	95	91	95
U57357	69	70	69	6	69	87	88	87	71	99	100	97	74	96	96	97	96	96	97	97	96	97	96	91	96
AJ491973	66	66	67	6	67	85	85	86	70	91	91	100	75	87	96	97	96	96	97	97	97	96	97	91	97
AY648298	6	6	6	4	7	6	7	7	5	7	7	7	100	77	76	76	74	74	75	76	75	75	75	70	75
DQ369960	73	73	71	5	69	83	84	81	69	89	90	87	6	100	97	97	96	96	97	97	96	96	96	91	96
DQ438949	74	75	69	6	69	84	85	85	69	89	90	87	6	90	100	99	95	95	96	96	96	96	96	91	96
AJ491963	73	73	69	6	69	87	88	87	70	92	93	90	7	90	96	100	96	96	97	98	97	97	97	92	97
EF655890	69	70	69	6	64	84	85	82	67	89	90	87	7	89	87	89	100	100	98	98	97	96	96	91	96

Table 2. Contd.

EF655889	67	68	69	6	66	84	85	84	69	91	91	89	9	90	87	90	93	100	98	98	97	96	96	91	96
DQ842502	70	70	71	6	67	87	88	85	70	92	93	90	7	91	89	91	96	96	100	99	98	97	97	92	97
EU089686	69	70	70	6	67	87	88	87	71	94	94	91	7	92	90	93	94	96	97	100	99	98	97	92	97
EF655899	70	71	70	5	69	87	88	87	71	93	94	91	7	91	91	94	94	95	96	98	100	97	96	91	96
EU196423	68	68	67	6	69	85	86	85	70	93	94	89	7	89	87	90	90	91	93	94	64	100	96	91	96
KC249911	65	66	66	6	66	85	86	85	69	91	91	90	7	88	89	91	89	91	91	92	91	89	100	94	100
KC249914	59	60	59	6	60	79	80	79	73	86	86	84	7	82	81	84	82	86	85	86	86	84	92	100	94
KC200152	12	12	12	44	13	15	15	15	14	16	16	14	5	14	16	16	16	16	17	16	16	16	16	16	100
KC249910	14	14	16	3	16	18	19	18	18	20	20	20	79	18	18	20	19	22	21	20	20	20	21	20	17
KC249907	8	8	8	4	8	8	8	8	7	9	9	9	87	8	8	8	8	10	9	9	9	9	9	8	6
KC249908	7	7	7	5	8	7	7	7	6	8	8	7	77	7	7	7	7	9	8	8	8	8	8	7	19
KC249916	6	6	5	48	7	5	5	5	4	7	7	5	6	5	6	6	6	6	7	6	6	6	5	5	88
KC249917	8	8	6	47	8	6	6	6	4	7	8	5	5	6	8	8	8	8	8	8	8	8	7	5	86
KC249912	8	8	9	3	9	9	10	10	9	11	10	11	71	9	9	10	9	11	10	10	10	10	10	11	8
KC249915	7	7	7	4	9	7	8	8	7	9	9	9	80	7	7	8	8	9	9	9	9	9	9	8	7
KC249913	58	59	56	6	59	77	78	77	69	83	83	81	7	79	79	82	79	83	82	84	83	82	89	90	16
KC249909	11	11	12	3	12	13	14	14	13	15	15	16	71	13	13	14	14	16	15	15	15	15	15	14	12
AM040436	67	68	67	6	67	87	88	87	71	92	93	91	7	90	90	93	90	93	93	94	94	91	98	91	16
DQ648195	68	68	66	6	68	85	86	86	69	90	91	91	7	88	88	91	88	91	91	92	93	89	96	89	15
AY819718	68	67	67	5	66	85	86	86	71	91	92	90	8	88	89	91	89	91	92	94	94	90	90	84	17
AF006734	67	68	66	6	66	87	88	87	71	94	94	91	7	91	90	93	91	94	94	96	95	90	94	88	16
AJ491965	68	69	70	6	66	85	86	85	70	93	94	89	7	89	88	91	91	94	94	94	93	92	91	86	16
U57354	69	70	71	6	66	84	85	84	69	92	93	89	7	89	87	90	90	93	93	93	92	91	91	85	16
AY241923	67	68	65	6	64	87	86	82	67	89	90	87	7	89	86	89	89	90	91	91	91	70	89	84	16
D00948	68	69	66	6	65	88	87	84	69	91	91	89	7	90	87	90	90	91	93	93	92	91	91	85	16
U57356	68	68	68	6	67	89	89	87	71	95	96	91	7	91	90	93	91	94	94	96	95	94	94	88	16
U57355	68	68	68	6	63	85	86	83	70	91	92	88	7	91	88	91	89	91	92	92	91	91	91	86	16
AJ491961	67	68	68	6	66	87	88	87	70	93	94	89	8	87	89	91	89	90	92	93	92	89	89	84	16
AJ491939	69	69	69	5	65	85	86	85	69	91	91	87	8	87	85	88	87	89	89	91	90	91	90	84	16
DQ227694	67	68	65	5	65	84	84	84	67	87	88	85	9	84	84	87	84	87	87	88	89	87	87	82	16
EF655894	69	70	69	6	64	84	85	82	67	89	90	87	7	89	87	89	100	93	96	94	94	91	89	82	16
DQ925431	4	4	5	5	1	4	4	4	5	3	3	4	2	3	3	3	4	4	4	4	4	4	4	4	3
DQ925427	1	1	3	5	1	4	4	4	5	3	3	4	5	1	2	3	4	4	4	4	4	4	4	4	4

place of isolation, except the isolates IND (GenBank Acc. AY241923) and AP1 (GenBank Acc. GQ386846), isolated from India and the strain type GX-1 (GenBank Acc. DQ227694) from China. The hyper variable region (HVR) of the CP gene undergoes much variations due to

Table 2. Contd.

Acc. No.	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
AJ421465	87	78	80	81	81	87	83	87	86	89	89	89	89	89	89	88	89	89	89	89	89	88	89	29	28
AJ421463	87	79	80	81	81	87	83	88	86	89	89	88	89	89	89	88	89	89	89	89	89	88	89	29	28
X98165	87	78	80	81	80	87	82	87	86	89	88	89	89	90	90	88	88	89	89	89	89	87	89	29	27
HM014060	56	62	60	61	60	56	59	56	56	56	56	57	57	57	57	55	56	57	56	56	56	56	56	34	32
AJ421468	87	78	80	81	80	87	82	87	86	89	89	88	88	89	88	87	88	89	88	88	88	87	88	28	27
DQ866746	94	84	86	87	86	94	89	94	93	96	95	95	96	95	95	95	96	96	95	95	95	94	95	28	26
DQ866744	95	84	86	87	87	94	89	95	93	96	95	95	96	95	95	95	96	96	95	96	95	94	95	29	27
GQ386846	94	84	86	87	86	93	88	94	93	95	95	95	96	95	95	94	94	95	94	95	95	94	95	28	26
JF699509	74	64	66	67	67	75	70	75	75	75	75	76	76	75	75	74	75	76	75	75	75	74	75	23	22
AJ491971	95	84	86	87	86	94	89	94	93	96	95	96	96	96	96	95	95	97	96	97	97	95	96	28	27
U57357	95	85	87	88	87	95	89	95	94	97	96	97	97	97	97	96	96	98	96	97	96	95	96	29	27
AJ491973	96	86	88	89	88	95	90	96	95	97	97	97	98	97	97	96	96	97	96	96	96	95	96	29	27
AY648298	75	84	81	81	81	74	78	74	73	75	74	75	76	75	75	74	75	75	75	74	74	73	75	37	35
DQ369960	95	85	88	88	88	95	90	95	94	97	96	96	97	97	96	96	96	97	97	96	95	94	96	30	28
DQ438949	95	85	87	88	87	95	89	95	94	96	96	96	97	96	96	95	95	96	96	96	95	94	95	29	28
AJ491963	96	86	88	89	88	96	90	96	95	98	97	97	98	97	97	96	96	98	97	97	96	95	96	29	28
EF655890	95	84	87	88	87	94	89	95	94	96	95	96	97	97	96	96	96	97	96	96	95	94	97	29	27
EF655889	95	84	87	88	87	94	89	95	94	96	95	96	97	97	96	96	96	97	96	96	95	94	97	29	27
DQ842502	96	85	88	89	88	95	90	96	95	97	96	97	98	98	98	97	97	98	97	97	96	95	98	30	28
EU089686	96	86	88	89	88	96	91	96	95	98	97	98	98	98	98	97	97	98	97	98	97	95	98	30	28
EF655899	96	85	88	88	88	95	90	96	95	97	97	97	98	97	97	96	96	98	96	97	96	96	98	30	28
EU196423	96	85	87	88	88	95	90	95	94	97	96	97	98	97	97	96	96	98	97	97	96	95	97	30	27
KC249911	99	88	90	91	91	98	93	99	97	99	98	97	98	97	97	96	96	98	97	96	96	95	96	30	28
KC249914	93	82	85	86	86	93	89	93	93	94	93	91	93	92	92	91	91	92	92	91	91	90	92	30	28
KC200152	99	88	90	91	91	98	93	99	97	99	98	97	98	97	97	96	96	98	97	96	96	95	96	30	28
KC249910	100	87	90	90	90	97	92	98	97	99	98	96	97	96	96	95	96	97	96	95	96	95	96	30	28
KC249907	84	100	95	95	94	87	91	87	86	88	87	85	86	86	86	85	85	86	86	85	85	84	85	33	31
KC249908	75	79	100	97	96	89	92	90	88	90	89	88	89	88	88	87	88	89	88	87	87	86	88	32	30
KC249916	7	7	21	100	98	90	93	91	89	91	90	89	90	89	89	88	88	89	89	88	88	87	88	32	30
KC249917	6	6	20	95	100	90	94	90	89	91	90	88	89	88	88	87	88	89	88	88	88	86	88	32	30
KC249912	80	80	67	6	6	100	92	98	97	98	97	95	96	96	96	95	95	96	96	95	95	94	95	29	27
KC249915	81	89	76	8	8	82	100	93	91	93	92	90	91	91	91	90	90	91	90	90	90	89	90	31	29
KC249913	20	9	8	6	6	11	9	100	97	98	98	96	97	96	96	95	96	97	96	95	95	94	96	29	27
KC249909	84	79	66	6	6	88	82	14	100	97	96	95	96	95	95	94	95	96	95	94	94	93	95	29	27
AM040436	20	9	8	5	7	10	9	89	15	100	99	97	98	98	98	97	97	98	97	97	96	95	97	30	28
DQ648195	20	9	8	5	7	10	9	87	15	98	100	96	98	97	97	96	96	97	96	96	96	95	96	29	27

Table 2. Contd.

AY819718	20	9	8	7	8	10	8	82	14	92	90	100	98	97	97	96	97	98	97	97	96	95	97	29	27
AF006734	20	8	8	6	8	10	8	85	14	96	94	95	100	98	98	97	98	99	98	97	97	96	98	29	27
AJ491965	20	8	7	6	8	10	8	83	15	94	91	91	95	100	100	97	97	99	98	97	97	96	98	29	27
U57354	19	8	7	6	8	10	8	82	14	93	91	91	94	99	100	96	97	98	97	97	97	96	97	29	27
AY241923	19	8	7	6	8	10	8	81	14	91	89	89	93	91	90	100	100	98	96	96	95	94	96	29	27
D00948	19	8	7	6	8	10	8	82	14	93	91	91	94	92	91	99	100	98	97	96	96	95	96	29	27
U57356	20	9	8	6	8	10	9	85	15	96	94	94	97	96	96	94	95	100	98	98	97	96	98	29	27
U57355	20	8	7	6	8	10	8	84	14	92	90	90	94	93	92	91	92	95	100	96	97	96	96	29	27
AJ491961	20	10	8	6	7	11	9	81	16	92	89	92	93	92	92	89	90	94	91	100	98	96	96	29	27
AJ491939	21	10	8	6	7	11	9	82	16	91	89	90	92	91	91	89	90	94	91	94	100	96	96	29	27
DQ227694	22	10	9	6	7	12	10	79	16	88	88	87	89	89	88	86	87	91	89	88	91	100	95	29	27
EF655894	19	8	7	6	8	9	8	79	14	90	88	89	91	91	90	89	90	91	89	89	87	84	100	29	27
DQ925431	3	4	3	3	3	3	3	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	100	81
DQ925427	5	5	3	4	5	5	5	4	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	51	100

Table 3. *In-silico* restriction mapping and discrimination of SCMV strains/variants (12 CEMB isolates and 38 from all over world).

Isolates/ Acc. No.	Restriction sites						
	AluI	**BstMWI**	**CviJI**	**EcoRI**	**HinfI**	**HpyCH4V**	**TaqI**
KC200152	248, 380, 386	226, 251, 289, 341, 392	36, 248, 380, 386, 395, 413	364	171	229, 245, 288, 403	261
KC249907	199, 331, 337	177, 202, 240, 292, 343	199, 331, 337, 346, 364	315	122	180, 196, 354	212
KC249908	214, 346, 352	192, 217, 255, 307, 358	5, 214, 346, 352, 361, 379	330	137	195, 211, 369	227
KC249909	250, 382, 388	228, 253, 291, 343, 394	37, 250, 382, 388, 397, 415	366	173	231, 247, 405	263
KC249910	250, 382, 388	228, 253, 291, 343, 394	36, 250, 382, 388, 397, 415	366	173	231, 247, 405	263
KC249911	249, 381, 387	227, 252, 290, 342, 393	36, 249, 381, 387, 396, 414	365	172	230, 246, 289, 404	262
KC249912	250, 382, 388	228, 253, 291, 343, 394	37, 250, 382, 388, 397, 415	366	173	231, 247, 405	263
KC249913	249, 381, 387	227, 252, 290, 342, 393	36, 249, 381, 387, 396, 414	365	172	230, 246, 289, 404	262
KC249914	249, 381, 387	227, 252, 290, 342, 393	36, 249, 381, 387, 396, 414	365	172	230, 246, 289, 404	262
KC249915	226, 358, 364	204, 229, 267, 319, 370	16, 226, 358, 364, 373, 391	342	149	207, 223, 381	239
KC249916	215, 347, 353	193, 218, 256, 308, 359	5, 215, 347, 353, 362, 380	331	138	196, 212, 370	228
KC249917	215, 347, 353	193, 218, 256, 308, 359	5, 215, 347, 353, 362, 380	331	138	196, 212, 370	228
JF699509	249, 331	227, 252, 290, 342	36, 249, 331			230, 246	262
GQ386846	249, 331, 381, 387	252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414, 451			230, 246, 404, 417	196, 262
HM014060	131, 213, 263, 269	134, 172, 224, 275	131, 213, 263, 269, 278			86, 112, 128	

Table 3. Contd.

AY648298	154, 236, 286, 292	132, 157, 195, 247, 298	154, 236, 286, 292, 301, 319	270	77	11, 135, 151, 309	14, 167, 254
DQ925431		209	61, 171, 198		217	19, 117, 124, 186	167
DQ925427		206	60, 168, 195		214	49, 114	164
EF655894	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262
DQ369960	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	109, 262, 349
DQ227694	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262
DQ438949	249, 331, 381, 387	252, 290, 342, 393	36, 188, 249, 331, 381, 387, 396, 414	365	172, 314	106, 230, 246, 404	262, 349
AY819718	249, 331, 381, 387	227, 252, 290, 342, 393	36, 91, 249, 331, 381, 387, 396, 414		172	230, 246, 365, 404	262
AM040436	249, 381, 387	227, 252, 290, 342, 393	36, 249, 381, 387, 396, 414	365	172	230, 246, 404	262
DQ648195	249, 381, 387	227, 252, 290, 342, 393	36, 249, 381, 387, 396, 414	365	172	230, 246, 404	262
AJ421468	249, 331, 381, 387	252, 290, 342, 393	249, 331, 381, 387, 396, 414			106, 204, 230, 246, 404	109, 196
AJ491973	249, 381, 387	227, 252, 290, 342, 393	36, 249, 381, 387, 396, 414	365	172	230, 246, 404	262
AJ491971	174, 249, 331, 381, 387	227, 252, 290, 342, 393	36, 174, 249, 331, 381, 387, 396, 414			230, 246, 345, 404	262
AJ491965	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 345, 404	262
AJ491963	249, 331, 381, 387	252, 290, 342, 393	36, 188, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262, 349
AJ491961	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	314	33, 230, 246, 345, 404	262, 349
AJ491939	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365		230, 246, 345, 404	262
AJ421465	249, 331, 381, 387	252, 290, 342, 393	188, 249, 331, 381, 387, 396, 414			106, 230, 246, 404	109, 349
AJ421463	249, 331, 381, 387	252, 290, 342, 393	188, 249, 331, 381, 387, 396, 414			106, 230, 246, 404	109, 349
X98165	249, 331, 387	252, 290, 342, 393	249, 331, 387, 396, 414			106, 230, 246, 345, 404	109
DQ842502	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262
EU089686	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262

Table 3. Contd.

EF655890	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262
EF655899	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262
EF655889	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262
DQ866746	249, 331, 381, 387	252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414, 451			230, 246, 404, 417	196, 262
DQ866744	249, 331, 381, 387	252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414, 451			230, 246, 404, 417	196, 262
AY241923	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365		230, 246, 404	262, 383
EU196423	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365		230, 246, 345, 404	262
U57357	174, 249, 331, 381, 387	227, 252, 290, 342, 393	36, 174, 249, 331, 381, 387, 396, 414			230, 246, 345, 404	262
U57356	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 345, 404	262
U57354	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 345, 404	262
U57355	249, 331, 381, 387	227, 252, 290, 342, 393	36, 249, 331, 381, 387, 396, 414	365	172	230, 246, 345, 404	262, 349
D00948	249, 331, 381, 388	227, 252, 290, 342, 394	36, 249, 331, 381, 387, 396, 415	366		230, 246, 404	262, 383
AF006734	249, 331, 381, 387	227, 252, 290, 342, 393	36, 91, 249, 331, 381, 387, 396, 414	365	172	230, 246, 404	262

addition or deletion mutations, and evolutionary process frequently as it has surface exposure to the environment. Hence, phylogenetic analysis, performed with conserved region (CR) is consistent. Shukla et al. (1994) recommended that conserved region CR is the most reliable marker to discriminate the viral strains in the genus potyvirus. Thus, only the conserved region of coat protein gene of SCMV isolates was amplified and sequenced for phylogenetic analysis. The phylogenetic and restriction analyses resembled in grouping the isolates.

Considering the restriction profile of all 12 isolates in this study, they are identical for *Alu*I, *Bst*MWI, *Eco*RI, *Hin*fI and *Taq*I, but differed for *Cvi*JI and *Hpy*CH4I. Though the sequences from the amplified region resembled highly (nt 82-100% and aa 92%), the level of similarity was not enough to classify them as separate variants, but the phylogenetic analyses and CP sequence assessments did not resemble those of the type strains SCMV-A, SCMV-B, SCMV-D, SCMV-E

and most of the isolates reported from all over the world; possibly it characterizes a novel strain SCMV-PAK. Previously, isolates from India, China and Congo were described as cause of mosaic in the sugarcane under Pakistani environments, based on serology and differential host interactions (Haider et al., 2011). Moreover, the cutting pattern by restriction enzymes varied for other isolates from the world, however they match sequence identities in the reported region of CP gene at high level, perhaps representing a new

Figure 1. Comparison of the aa sequences of the CPs of different SCMV type strains/strains/isolates in the N-terminal region, with reference to the coordinates 1-151 aa of SCMV-CEMB-5 (GenBank Acc. No. KC249910). Sequences were aligned using CLUSTAL W program (Thompson et al., 1994). '-' in the alignment indicates gap introduced for better alignment/deletion mutation undergone by corresponding sequences; C, conserved aa residues; the underlined aa residues are conserved and are repeated twice in 40-60% of the isolates/strains; for accession numbers (see Table 1).

variant, SCMV-PAK.

The cutting pattern of the SCMV-VN/SC1 and SCMV-VN/SC2, isolates from Veitnam (Ha et al., 2008) was entirely different for all the enzymes. They have no

restriction sites for *Alu*I and *Eco*RI. The restriction patterns of *Alu*I, *Bst*MWI, *Cvi*JI, *Hpy*CH4I and *Taq*I, were in a similar fashion as the isolates from Argentina, USA, Cameroon, Republic of Congo and Australia but *Hinf*I and

Figure 1. Contd.

*Eco*RI restriction profile was unique to distinguish them (Table 3).This massive difference in the CR of CP gene concludes its intensity of evolution, subsequently evolving as a compelling virus, extensively widespread in varieties/cultivars of sugarcane in Pakistan.

We have shown the molecular basis of genetic varia-

tion of the CP gene which are usually identified for genetic understanding of potyviruses (Shukla et al., 1994; Garcia-Arenal et al., 2003; Frenkel et al., 1989, 1992; Gemechu et al., 2006; Alegria et al., 2003). The 12 Pakistani SCMV isolates expressed a deviation of 0-18 and 0.00-95% at nucleotide and amino acids level

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SCMV-CEMB-4      NLTDYSLARYAFDFYEMNSXTPARAK---------EAPMXMKAAPVRGST----------   146
SCMV-CEMB-7      NLTDYSLARYAFDFYEMNSRTPARAK---------EAHMXMKAAXXRGST----------   146
SCMV-CEMB-3      NLTDYSLARYAFDFYEMNSRTPARAK---------EAHMQMKAAAVRGSN----------   130
SCMV-CEMB-10     NLTDYSLARYAFDFYEMNSRTPARAK---------EAHMQMKAAXVRGSN----------   136
SCMV-CEMB-2      NLTDYSLARYAFDFYEMNSRTPARAK---------EAHMQMKAAAVRGSN----------   128
SCMV-CEMB-5      NLTDYSLARYAFDFYEMNSRTPARAK---------EAHMQMKAAAVRGSN----------   144
AY648298         NLTDYSLARYAFDFYEMNSRTPARAK---------EAHMQMKAAAVRGSN----------   114
DQ925431         AC-------SGVACTSVE-----------P*DGLHWVWLCHLCCESLGERX--------   76
DQ925427         VS-------SGVARTSVE-----------PCHGQHWGWLCHVCCESLGERX--------   75
HM014060         --------------------KLYRAIYAKIRTSAKSHRL*LSTVCF*FL*NDFTHTS*S*   82
SCMV-CEMB-1      --------------------KFYRAMHATIWTSAKSHRL*SSAVCL*LLRNEFKDTS*S*   117
SCMV-CEMB-11     --------------------KFYRAIHATIWTSAKSHRL*SSAVCL*LLRNEFKDTS*S*   107
SCMV-CEMB-12     --------------------KFYRAMHATIWTSAKSHRL*SSAVCL*LLRNEFKDTS*S*   107
AJ491973         IC-------HDMDFSEISPTIAWRGTPLIFTK*IQGHQLELRKPTCR*RPQQF-------   131
SCMV-CEMB-8      AC-------HDMDFSEISPTIV*RGMPLTFTK*IQGHQLELRKPTCX*RPXHS-------   130
SCMV-CEMB-9      AC-------HDMDFSEISPTIV*RGMPLTFTK*IQGHQLELRKPTCR*RPQQF-------   130
DQ648195.1SBS    TC-------HXMDFSEISPTIV*RGMPLIFTK*IQGHQLELRKPTCR*RPQQF-------   130
SCMV-CEMB-6      AC-------HDMDFSEISPTIV*RGMPLTFTK*IQGHQLELRKPTCR*RPQQF-------   130
AM040436         TC-------HDMDFSEISPTIV*RGMPLTFTK*IQGHQLELRKPTCR*RPQQF-------   130
GQ386846         TC-------HDMDFSETSPTIA*RGMPLILTK*LQGHQLELRKPTCR*RLQQF-------   131
DQ866746         TC-------HDMDFSEISPTIA*RGMPLTFTK*LQGHQLELRKPTCR*RLQQF-------   130
DQ866744         TC-------HDMDFSEISPTIA*RGMPLTFTK*LQGHQLELRKPTCR*RLQQF-------   130
DQ369960         TC-------HDMDFSEISPTIA*RGMPSTFTK*IQGHQLELRKPTCR*RPQQS-------   130
EU196423         TC-------HDMDFSEISPTIA*RGMHLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
EF655890         TC-------HDMDFSEISPTIA*RGMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   129
EF655889         TC-------HDMDFSEISPTIA*RGMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   129
EF655894         TC-------HDMDFSEISPTIA*RDMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
DQ842502         TC-------HDMDFSEISPTIA*RGMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
EU089686         TC-------HDMDFSEISPTIA*RGMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
EF655899         TC-------HDMDFSEISPTIA*RGMPLTSTK*IQGHQLELRKPTCR*RPQQS-------   130
DQ438949         TC-------HDMDFSGISPTIA*RGMPSTSTK*IQGHQLELRKPTCR*RPQQF-------   130
AJ491963         TC-------HDMDFSVISPTIA*RGMPSTSTK*IQGHQLELRKPTCR*RPQQF-------   130
DQ227694         TC-------HGMDFSEISPTIA*RGMHLISTK*IQGHQLELRKPACR*RPQQS-------   129
JF699509         TC-------HDMDFSEISPTIA*RGMPLT------------------------------   108
AY241923         TC-------HDMDFSEISPTIA*RGMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
D00948           TC-------HDMDFSEISPTIA*RGMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
AJ491939         TC-------HDMDFSEISPTIA*RGMHLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
U57355           TC-------HDMDFSEISPTIA*RGMHSTFTK*IQEHQLELRKPTCR*RP*QS-------   129
AJ491961         TC-------HDMDFSGISPTIA*RGMHLTFTK*IQGHQLELRKPTCR*RPQQS-------   131
AY819718         TC-------HDMDFSEISPTIA*HGMPLTLKKCIQGHQLELRKPTCR*RPQQS-------   131
AJ491971         IC-------HDMDFSEISPTIA*RGMHLTFTK*PQGHQLELRKPTCR*RPQQS-------   130
U57357           IC-------HDMDFSEISPTIA*RGMHLTFTK*PQGHQLELRKPTCR*RPQQS-------   130
AJ491965         TC-------HDTDFSEISPTIA*RGMHLTFTK*IQGHQLELRKKPTCR*RPQQS------   130
U57354           TC-------HDTDFSEISPTIA*RGMHLTFTK*IQGHQLELRKKPTCR*RPQQS------   130
U57356           TC-------HDMDFSEISPTIA*RGMHLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
AF006734         TC-------HDMDFSEISPTIA*RGMPLTFTK*IQGHQLELRKPTCR*RPQQS-------   130
AJ421468         IC-------QDTDFSEISPTIA*RGMPLISMK*LHAHQLELRKPTCR*KPQQF-------   133
X98165           IC-------QDTDFSVISPTIA*HGMHLISMK*LHAHLLELKKPTCR*KPQRF-------   132
AJ421465         TC-------QDTVFSEISPTIA*HGMLSISMK*LRAHQLELRKPTCR*KPQQF-------   131
AJ421463         TC-------QDTVFSEISPTIA*RGMLSISMK*LRAHQLELRKPTCR*KPQQF-------   131
```

Figure 1. Contd.

respectively in the reported sequences as compared with the deviation of 0-73 and 0-96% at nucleotide and amino acids level respectively in the compared region (data based on Table 2), noticed amongst the isolates of SCMV from sugarcane cultivated all over the world. This huge divergence may be based on the addition anddeletion mutations in the CP gene of SCMV isolates/variants. Though, more than 12 strains of SCMV causing mosaic in sugarcane have been described worldwide, but only five strains SCMV-A, SCMV-B, SCMV-D, SCMV-E from USA (Yang and Mirkov, 1997) and SCMV-SC from Australia (Frenkel et al., 1991) have been characterized on molecular level. Serological and host interaction based approaches (Haider et al., 2011;

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SCMV-CEMB-4        -------HXCRSG---------      152
SCMV-CEMB-7        -------HDXSVW---------      152
SCMV-CEMB-3        -------TRLFGLX---------     137
SCMV-CEMB-10       -------TRLVRS---------      142
SCMV-CEMB-2        -------TRLFGLX---------     135
SCMV-CEMB-5        -------TRLFGLX---------     151
AY648298           -------TRLFGLX---------     121
DQ925431           --------------------        76
DQ925427           --------------------        75
HM014060           GSPHX---------------        87
SCMV-CEMB-1        GSPHADEGRSSSWFKHTIVRSG      139
SCMV-CEMB-11       GSPHADEGRSSSWFKHTIVRSG      129
SCMV-CEMB-12       GSPHADEGRSSSWFKHTIVXVW      129
AJ491973           ---VVQTHDCSVW---------      141
SCMV-CEMB-8        ---WFKHTIVRSG---------      140
SCMV-CEMB-9        ---GQ---------------        132
DQ648195.1SBS      ---VVQTHDCSVW---------      140
SCMV-CEMB-6        ---VVQTHDCSVW---------      140
AM040436           ---VVQTHDCSVW---------      140
GQ386846           ---VVQTHDCSAWX---------     142
DQ866746           ---VVQTHDCSAWX---------     141
DQ866744           ---VVQTHDCSAWX---------     141
DQ369960           ---VVQTHDCSVW---------      140
EU196423           ---VVQTHGCSVW---------      140
EF655890           ---VVQTHDCSVW---------      139
EF655889           ---VVQTHDCSVW---------      139
EF655894           ---AVQTHDCSVW---------      140
DQ842502           ---VVQTHDCSVW---------      140
EU089686           ---VVQTHDCSVW---------      140
EF655899           ---VVQTHDCSVW---------      140
DQ438949           ---VVQTHDCSVW---------      140
AJ491963           ---VVQTHDCSVW---------      140
DQ227694           ---VVQTHDCSVWX---------     140
JF699509           --------------------        108
AY241923           ---VVQTHDCSVW---------      140
D00948             ---VVQTHDCSVW---------      140
AJ491939           ---VVQTHDCSVWX---------     141
U57355             ---VVQTHDCSVW---------      139
AJ491961           ---VVQTHDCSVWX---------     142
AY819718           ---VVQTHDCSVW---------      141
AJ491971           ---VVQTHDCSV+X---------     140
U57357             ---VVQTHDCSVW---------      140
AJ491965           ---VVQTHDCSVW---------      140
U57354             ---VVQTHDCSVW---------      140
U57356             ---VVQTHDCSVW---------      140
AF006734           ---VVQTHDCSVW---------      140
AJ421468           ---VVQTHVCSVW---------      143
X98165             ---VVQTHDCSVW---------      142
AJ421465           ---VVQTHVCSVW---------      141
AJ421463           ---VVQTHVCSVW---------      141
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Figure 1. Contd.

Mansoor et al., 2003) have been used to identify the strains SCMV-A, SCMV-B, SCMV-D and SCMV-E in Pakistan earlier; the SCMV isolates characterized in this study are genetically different. This effort presents the detection of population of SCMV in Pakistan by molecular methodologies and it proved thought-provoking results on
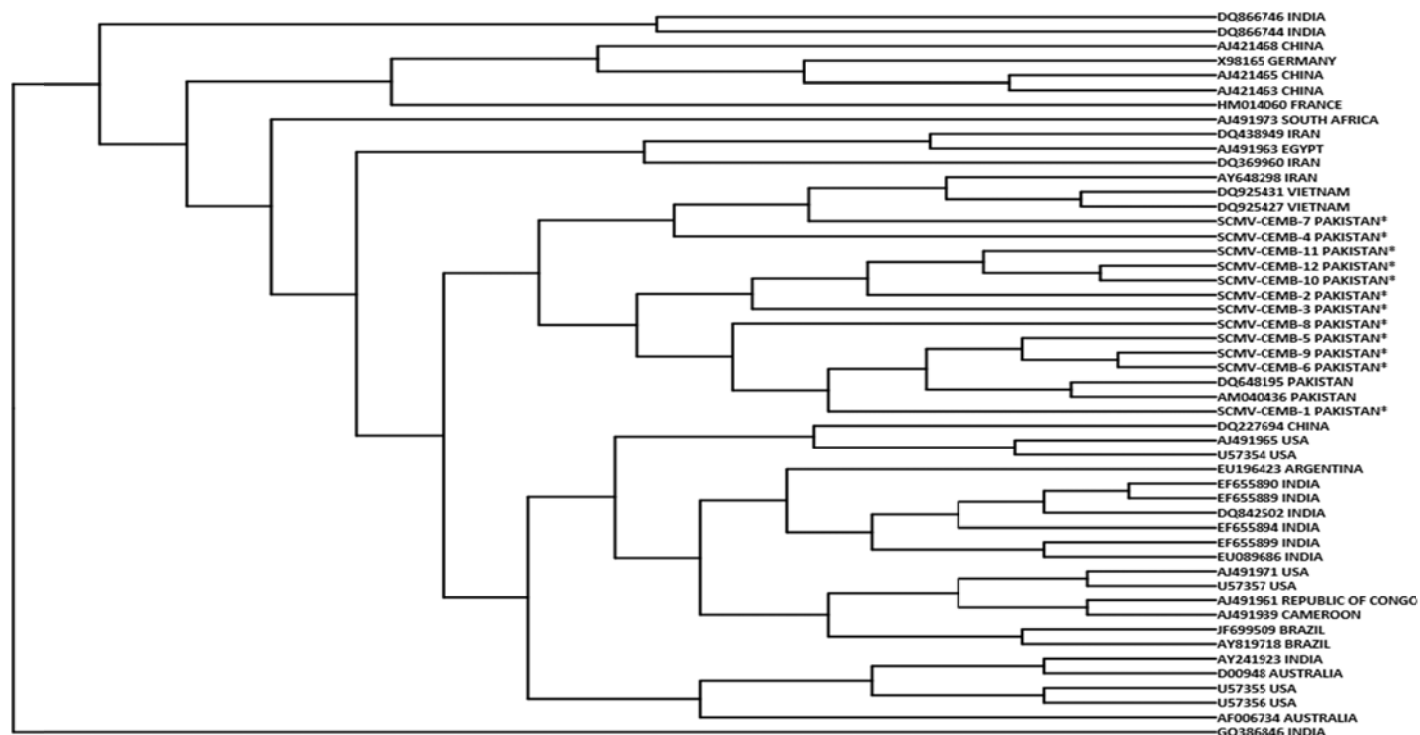
Figure 2. Phylogenetic relatedness of Pakistani SCMV isolates with the SCMV type strain/strains and other SCMV isolates from sugarcane reported from worldwide. Phylogram presented is derived from DNA Maximum Likelihood program with molecular clock, based on a nucleotide sequence alignment of core region in the coat protein coding sequence corresponding to nt 1-457 of SCMV-CEMB-5 (GenBank Acc. No. KC249910) in the CP coding sequence. A 97.0% sequence identity limit and position in the phylogenetic tree was used to assign the virus isolates to different phylogenetic groups. *The isolates from this study. The details of the sequences and their accession numbers are given in Table 1.

the incidence of new sugarcane mosaic virus population. Further investigations regarding the sequencing of complete genome of certain variants are under way which might be helpful to understand and characterize strainal variations more precisely.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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