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# CHARACTERIZATION OF COAT PROTEIN (CP) GENE OF *SUGARCANE MOSAICVIRUS* (SCMV) FROM ISOLATES OF PAKISTAN AND ITS PHYLOGENETIC RELATIONSHIPS

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## ABSTRACT

Sugarcane (*Saccharum officinarium*, family *Poaceae*) is a widely grown cash crop in Pakistan. Pakistan is the fifth largest country in the world in terms of area under sugarcane cultivation, 11th by production and 60th in yield. Mosaic is amongst the major diseases of sugarcane in the country, which causes decline in cane yield in the range of 10-32% and in sugar yield in the range of 6-10%. Thirty two (32) sugarcane leaf samples, showing mosaic symptoms, and five asymptomatic leaf samples were collected from Punjab and Khyber Pakhtun Khwa (KPK) provinces of Pakistan during 2013-2014. RNA was extracted and subjected to PCR amplification using a universal set of coat protein (CP) gene primers. An expected size band (~1.2kb) was obtained from all symptomatic leaf samples. Amplified products were T/A cloned and sequenced in both orientations. The available CP sequences of 20 samples were ranged between 890-938bp in length which showed 95-100% nucleotide sequence identities among them and 85-100% nucleotide sequence identity with the rest of CP sequences from world while shared highest nucleotide sequence identity (98% over the stretch of ~900bp) to an isolate of *Sugarcane mosaic virus* (SCMV; accession number DQ648195). The sequence analysis of present study CP isolates and their phylogenetic relationships with the rest of world are discussed in the manuscript.

**Keywords:** *Potyviridae*, *Sugarcane mosaic virus*, Coat protein, Phylogenetic, Diversity.

## INTRODUCTION

Sugarcane (*Saccharum officinarium*) is an important cash crop belonging to the family *Poaceae*, genus *Saccharum* and to tribe Andropogoneae Daniels and Roach (1987). It is cultivated in tropical and subtropical areas with diverse temperature, ranging from hot dry to cool and moist conditions. It is primarily used for the sugar production but also a source of energy production in the form of biofuels such as cellulosic ethanol (Hashmi and Suhail, 2013). Pakistan is the fifth largest country in the world in terms of area under sugarcane cultivation, 11th by production and 60th in yield. Approximately, 1.5 million employees (either directly or indirectly) contribute to 1.9% share in GDP in about 86 sugar mills all over the country. The Punjab, Sindh and Khyber Pakhtun Khwa (KPK) provinces are the major sugarcane-growing regions of Pakistan with a total annual sugar production of 3,172,408 tonnes per annum from Punjab, 1,547,547 tonnes per annum from Sindh and 310,174 tonnes per annum from KPK (http://edu.par.com.pk/wiki/sugarcane/#punjab). In Pakistan, sugarcane production reached maximum during the year 2013-2014 which was about 67,460 tonnes / hectare. However, significant crop losses of expected level were observed every year because of numerous diseases caused by variety of different biological agents such as bacteria, fungi and viruses (Bock and Baily, 1989).

Mosaic, caused by *Sugarcane mosaic virus*, is amongst the major diseases of sugarcane in the country, causing recorded decline in cane yield from 10-32% and in sugar yield from 6-10% (Anwar, 2005). High infection rate (~75%) of SCMV was observed in KPK Province while ~55% infection was detected in sugarcane grown in Punjab Province during the year 2010 (Yasmin *et al*., 2011). Mosaic causes extensive yield loses in the country when its infectivity rate reaches 50% (Zia-ul-Husnnain and Afghan, 2004). Typical symptoms of SCMV include greenish or yellowish chlorotic areas on leaves with different level of necrosis and reddening.

SCMV is non-enveloped, filamentous and flexuous particles. It is approximately 680-900 nm in length and 11 nm in width and consists of positive sense RNA genome of about 10 kb in length. Polyphagous aphids (*Rhopalosiphium paidi* and *Rhopalosiphum maidis*) are the main biological vector responsible for its transmission in non-persistent and non-circulative manner but it is also transmitted mechanically through sap (Zhang *et al*., 2008). Multiple copies of coat protein (around 2000) protect the genome, which are arranged in the helical manner around the genome (Shukla and Ward, 1989). CP plays an important role in virus movement

from one cell to another as it contains DAG motif near N terminus which is crucial for aphid mediated viral transmission (Dolja *et al*., 1994). This multifunctional protein is also involved in local and systemic movement of virus inside the infected plant, coating and multiplication of viral genome (Shukla *et al*., 1991, Urcuqui-Inchima *et al*., 2001). CP gene is also important for the classification of strains and species (Adam *et al*., 2011).

Studies related to genetic variation of virus are key parameters to developing techniques for virus control and its spread (Moreno *et al*., 2004). Several studies have been performed on the diversity and characterization of SCMV all over the world in the previous decades but very little molecular data about SCMV is available from Pakistan. The present study is aimed at determining the disease incidence based on coat protein gene detection of SCMV and its phylogenetic relationships with the rest of the world.

## MATERIALS AND METHODS

**Sample collection and maintenance:** Young leaves of sugarcane showing typical mosaic pattern (Supplementary Fig. 1) and asymptomatic samples were collected from sugarcane growing areas of Punjab and KPK, Pakistan during the year 2013-2014 (Supplementary Table 1). Samples were kept in plastic zippers, well labeled and stored at -80ºC for further utilization.

**Total RNA extraction and quantification of RNA:** Total Ribonucleic acid (RNA) was extracted using TRIzol reagent (Invitrogen). Approximately 0.1 gram of sugarcane leaf sample was ground into fine powder in a mortar and pestle using liquid nitrogen. Extracted RNA was analyzed on 1.5% agarose gel and quantified using spectrophotometer.

**RT-PCR for amplification of coat protein of SCMV:** Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was used for qualitative detection of CP gene by reverse transcribing RNA in to complementary DNA (cDNA) (ThermoFisher, Scientific).

**Complementary DNA (cDNA) synthesis:** Complementary DNA synthesis was achieved through two incubation steps. In the first step, 10 pmol of CP gene specific reverse primer (CTGAAATAGTAAATAC GAGG) was incubated with 2 µg of total RNA at 70 ºC for 10 minutes. Second incubation step was performed at 42 ºC for 1 hour after adding 200U of M-MLV reverse transcriptase, 10 mM dNTPs and 100U of RNAse inhibitor. Synthesized cDNA was used for amplification of coat protein gene.

**PCR amplification:** A 50 µL PCR reaction mixture consisted of 10X Taq buffer, 10 pM each of primers (Forward: 5'-TTACAACGAAGATGTTTTCC-3' and Reverse: 5'-CTGAAATAGTAAATACGAGG-3') (Souza *et al*., 2012), 5 Units of *Taq* polymerase and 5µL cDNA. PCR reaction mixture was incubated in thermal cycler and reaction was set as: preheating at 94 ºC for 1 min, followed by 40 cycles of denaturation at 94ºC for 30 sec, annealing at 51ºC for 30 sec and extension at 68ºC for 1 min. A final extension was done at 68ºC for 7 min. PCR product was loaded on 1.5% agarose gel.

Expected size DNA band was cleaned from gel by using silica beads DNA gel elution kit according to manufacturer's instructions.

**Cloning of PCR product in to pTZ57R/T vector:** Purified DNA was ligated into pTZ57R/T vector according to manufacturer's instruction (InsTAclone™ PCR cloning kit, Fermentas) and transformed intoDH5α competent cells. Plasmid DNA was isolated using Qiagen kit by following manufacturer's protocol.

**DNA sequencing of plasmids:** Pure plasmids were initially confirmed through restriction digestion with *Eco*RI and *Hin*dIII and finally confirmed through DNA sequencing. 100 ng of purified plasmids were sent to Macrogen, Korea for sequencing using M13 Forward and M13 Reverse primers.

**Sequence comparison and bioinformatics analysis:** DNA sequences obtained were trimmed through Sequencher (version 4.9) software. To investigate whether trimmed sequences were similar to SCMV CP sequences, they were analyzed initially through BLAST software (http:// www.ncbi.nlm.nih.gov/BLAST/) which determined the percentage identity with the relevant nucleotide bases already stored in data base. Sequences were aligned with ClustalW (Thompson *et al*., 1994) program and phylogenetic tree was constructed with MEGA6 (Tamura *et al*., 2013) software using neighbor joining method. 1000 Bootstrap replicates were applied on the tree for the reliability of tree topology. However, Pairwise distance values between sequences were determined through Species Demarcation Tool software (Muhire *et al*., 2014).

## RESULTS

**Detection of *Sugarcane mosaic virus* through RT-PCR:** Universal primers were used for detection and amplification of CP gene of *Sugarcane mosaic virus*. Asymptomatic leaf samples did not produce any amplification product while an amplification product of expected size (~1.2kb) corresponding to CP gene of SCMV, was obtained from symptomatic leaf samples (Fig. 1). All the symptomatic leaves samples of present study were positive for CP gene of SCMV.

An expected size band of ~1.2 kb was also detected from sugarcane leaf sample (CPSG-27-NARC) showing streak symptoms. The amplification products were subsequently cloned and subjected to DNA sequencing.

**DNA sequencing and sequences analysis:** The available CP sequences of 20 samples were ranged between 890-938bp in length and are available in the database under accession numbers given in Supplementary table 1. These sequences showed 95-100% nucleotide sequence identities among them (Table 1) while shared highest nucleotide sequence identity (98% over the stretch of ~900bp) to an isolate of *Sugarcane mosaic virus* (SCMV; accession number DQ648195). In pairwise nucleotide sequence identity (in percentage) to compare the isolates of present study with the sequences available in the databases shared a maximum identity between 85-100% to SCMV (Table 1) and nucleotide sequence identity between the isolates of SCMV in the current study is mentioned in the Table 2.

The results obtained indicate the 85-100% nucleotide identity among the sequences analyzed in this study and 95-100% nucleotide identities between all isolates studied from Pakistan (Table 1). Studied sequences showed maximum nucleotide similarity (98%) with the accession number DQ648195 while minimum nucleotide similarity (~91%) was recorded with the accession number JX237869. According to species demarcation criteria of *Potyviridae* published in 9$^{Th}$ report of International Committee on Taxonomy of Virus (ICTV), nucleotide sequence identity either in coat protein or with in entire genome should be less than 76% and CP amino-acid sequence identity must be less than 80% (Adam *et al*., 2011). Since all the twenty CP sequences of present study shared a maximum nucleotide sequence identity between 95-100% these isolates are believed to be derived from the same SCMV species. However, amino-acid sequences of all the twenty sequences were determined to be (209-317) in length and shared a maximum amino-acid sequence identity of 99% with the CP gene product of SCMV.

**Investigation of SCMV evolutionary history through viral coat protein gene:** A phylogenetic tree was constructed using DNA sequences of present study and sequences retrieved from databases based on high sequence identity in BLAST (Basic Local Alignment Search Tool) search (Fig. 2). These sequences were aligned using ClustalW and phylogenetic tree was constructed using neighbor joining method of MEGA6 software. The tree was rooted on coat protein gene sequence of *Potato virus* Y (Argentina; accession no. X14136). All CP sequences in the present study formed a single clade, depicting that virus infecting sugarcane crop in different areas of Pakistan have identical sequence and may have been originated from same source. Phylogenetic analysis of SCMV CP sequences of the isolates in the present study and the selected sequences available in the database could be categorized into three clusters. SCMV CP sequences from Pakistan, Australia, China, France, Argentina, South Africa and Iran grouped together in cluster I; while SCMV CP sequences from India make a distinct cluster II and situated between cluster I and III. Cluster III contains SCMV CP sequences obtained from China, Spain, Germany, Mexico and Thailand and showed divergence from the clusters I and II.

**Analysis of conserved amino-acids sequences of CP:** Aligned nucleotide sequences were translated into amino-acid through MEGA6 software. Deduced amino-acids sequences contain conserved DAG (Asp-Ala-Gly) motif near N terminus of coat protein (Fig. 3), DAG motif is required for transmission of virus.

**Table 1. Pairwise nucleotide sequence identity (in percentage) for the comparative study between isolates (number of isolates used for analysis are mentioned in the parenthesis) of different countries and reported sequences from Pakistan (In Red).**

| | Spain (3) | Germany (1) | Mexico (2) | South Africa (2) | Iran (2) | Australia (6) | France (8) | Thailand (4) | China (6) | Argentina (13) | Pakistan (22) | USA (2) | India (6) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **India** | 87-88 | 88-89 | 88-90 | 95-97 | 95-97 | 96-97 | 95-97 | 87-100 | 87-97 | 95-98 | 94-96 | 87-94 | 95-100 |
| **USA** | 87-94 | 88-92 | 89-92 | 89-94 | 89-94 | 89-95 | 88-94 | 86-90 | 87-94 | 89-94 | 87-94 | 90-100 | |
| **Pakistan** | 87-88 | 87-89 | 88-89 | 95-97 | 95-96 | 95-97 | 95-97 | 85-87 | 86-96 | 95-97 | **95-100** | | |
| **Argentina** | 87-88 | 88-89 | 89-90 | 97-98 | 96-97 | 97-99 | 96-97 | 86-88 | 87-97 | 97-100 | | | |
| **China** | 88-97 | 89-94 | 90-94 | 87-98 | 88-98 | 87-99 | 87-97 | 86-97 | 86-100 | | | | |
| **Thailand** | 90-91 | 89-91 | 89-90 | 86-88 | 87-88 | 86-88 | 87-89 | 92-100 | | | | | |
| **France** | 88-89 | 89-90 | 90 | 96-97 | 96-97 | 97-98 | 98-100 | | | | | | |
| **Australia** | 88 | 89 | 89-90 | 97-98 | 97-98 | 98-100 | | | | | | | |
| **Iran** | 89 | 89 | 90 | 97 | 97-100 | | | | | | | | |
| **South Africa** | 88 | 89 | 89-90 | 98-100 | | | | | | | | | |
| **Mexico** | 94 | 96 | 100 | | | | | | | | | | |
| **Germany** | 93 | 100 | | | | | | | | | | | |
| **Spain** | 100 | | | | | | | | | | | | |

## Table 2. Percentage nucleotide sequence identity between the isolates of SCMV studied in the current study.

| | KU 557287 | KU 557291 | KU 557288 | KU 557289 | KU 557290 | KU 557292 | KU 557293 | KU 557294 | KU 557295 | KU 557296 | KU 557297 | KU 557298 | KU 557299 | KU 557300 | KU 557301 | KU 557302 | KU 557303 | KU 557304 | LM 645013 | LM 645014 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **KU557287** | | 98 | 96 | 99 | 99 | 99 | 99 | 99 | 99 | 96 | 96 | 99 | 98 | 99 | 96 | 99 | 98 | 99 | 99 | 96 |
| **KU557291** | 98 | | 95 | 98 | 98 | 98 | 98 | 98 | 98 | 95 | 95 | 98 | 100 | 98 | 95 | 98 | 97 | 99 | 99 | 95 |
| **KU557288** | 96 | 95 | | 95 | 95 | 95 | 95 | 95 | 95 | 99 | 100 | 95 | 95 | 95 | 100 | 95 | 97 | 96 | 96 | 100 |
| **KU557289** | 99 | 98 | 95 | | 100 | 100 | 100 | 100 | 100 | 95 | 95 | 100 | 98 | 100 | 95 | 100 | 98 | 99 | 99 | 96 |
| **KU557290** | 99 | 98 | 95 | 100 | | 100 | 100 | 100 | 100 | 95 | 95 | 100 | 98 | 100 | 95 | 100 | 97 | 99 | 99 | 95 |
| **KU557292** | 99 | 98 | 95 | 100 | 100 | | 100 | 100 | 100 | 95 | 95 | 100 | 98 | 100 | 95 | 100 | 98 | 99 | 99 | 96 |
| **KU557293** | 99 | 98 | 95 | 100 | 100 | 100 | | 100 | 100 | 95 | 95 | 100 | 98 | 100 | 95 | 100 | 98 | 99 | 99 | 96 |
| **KU557294** | 99 | 98 | 95 | 100 | 100 | 100 | 100 | | 100 | 95 | 95 | 100 | 98 | 100 | 95 | 100 | 98 | 99 | 99 | 96 |
| **KU557295** | 99 | 98 | 95 | 100 | 100 | 100 | 100 | 100 | | 95 | 95 | 100 | 98 | 100 | 95 | 100 | 97 | 99 | 99 | 95 |
| **KU557296** | 96 | 95 | 99 | 95 | 95 | 95 | 95 | 95 | 95 | | 100 | 95 | 95 | 95 | 100 | 95 | 97 | 96 | 96 | 100 |
| **KU557297** | 96 | 95 | 100 | 95 | 95 | 95 | 95 | 95 | 95 | 100 | | 95 | 95 | 95 | 100 | 95 | 97 | 96 | 96 | 100 |
| **KU557298** | 99 | 98 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 95 | | 98 | 100 | 95 | 100 | 97 | 99 | 99 | 95 |
| **KU557299** | 98 | 100 | 95 | 98 | 98 | 98 | 98 | 98 | 98 | 95 | 95 | 98 | | 98 | 95 | 98 | 97 | 98 | 99 | 95 |
| **KU557300** | 99 | 98 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 95 | 100 | 98 | | 95 | 100 | 98 | 99 | 99 | 96 |
| **KU557301** | 96 | 95 | 100 | 95 | 95 | 95 | 95 | 95 | 95 | 100 | 100 | 95 | 95 | 95 | | 95 | 97 | 96 | 96 | 100 |
| **KU557302** | 99 | 98 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 95 | 100 | 98 | 100 | 95 | | 98 | 99 | 99 | 96 |
| **KU557303** | 98 | 97 | 97 | 98 | 97 | 98 | 98 | 98 | 97 | 97 | 97 | 97 | 97 | 98 | 97 | 98 | | 98 | 98 | 98 |
| **KU557304** | 99 | 99 | 96 | 99 | 99 | 99 | 99 | 99 | 99 | 96 | 96 | 99 | 98 | 99 | 96 | 99 | 98 | | 99 | 96 |
| **LM645013** | 99 | 99 | 96 | 99 | 99 | 99 | 99 | 99 | 99 | 96 | 96 | 99 | 99 | 99 | 96 | 99 | 98 | 99 | | 96 |
| **LM645014** | 96 | 95 | 100 | 96 | 95 | 96 | 96 | 96 | 95 | 100 | 100 | 95 | 95 | 96 | 100 | 96 | 98 | 96 | 96 | |

**Supplementary Table 1. List of infected sugarcane leaves samples, sample codes, accession numbers, area of collection, sugarcane variety original locality in parenthesis and symptoms.**

| No. | Sample Code | Accession No. | Sample Name | Area of collection | Symptoms |
|---|---|---|---|---|---|
| **1.** | 8ShA | LM645013 | US-640 | NARC (Faisalabad) | Mosaic |
| **2.** | 9ShA | LM645014 | CSSG-212 | NARC (Jhang) | Mosaic |
| **3.** | 10ShA | KU557287 | CPSG-27 | NARC (Jhang) | Streak |
| **4.** | 12ShA | KU557291 | HSF-240 | NARC (D.I. Khan) | Mosaic |
| **5.** | 15ShA | KU557288 | AUS-375 | NARC (Australia) | Mosaic |
| **6.** | 16ShA | KU557289 | HOSG-33 | NARC | Mosaic |
| **7.** | 17ShA | KU557290 | NARC-I | NARC | Mosaic |
| **8.** | 18ShA | KU557292 | US-104 | NARC (USA) | Mosaic |
| **9.** | 20ShA | KU557293 | CP-77-400 | NARC (KPK) | Mosaic |
| **10.** | 21ShA | KU557294 | US-469 | NARC (Faisalabad) | Mosaic |
| **11.** | 22ShA | KU557295 | MARDAN-1 | Mardan | Mosaic |
| **12.** | 23ShA | KU557296 | MARDAN-2 | Mardan | Mosaic |
| **13.** | 24ShA | KU557297 | MARDAN-3 | Mardan | Mosaic |
| **14.** | 26ShA | KU557298 | CSSG-239 | Shakarganj (Jhang) | Mosaic |
| **15.** | 27ShA | KU557299 | SPF-213 | Shakarganj | Mosaic |
| **16.** | 28ShA | KU557300 | NSG-19 | Shakarganj | Mosaic |
| **17.** | 29ShA | KU557301 | CPSG-2923 | Shakarganj(Jhang) | Mosaic |
| **18.** | 30ShA | KU557302 | CPSG-437 | Shakarganj(Jhang) | Mosaic |
| **19.** | 31ShA | KU557303 | US-127 | Shakarganj (Faisalabad) | Mosaic |
| **20.** | 32ShA | KU557304 | CSSG-2453 | Shakarganj (Jhang) | Mosaic |


**Supplementary Fig. 1. Typical mosaic symptoms on sugarcane leaves (A) Asymptomatic leaves (B).**

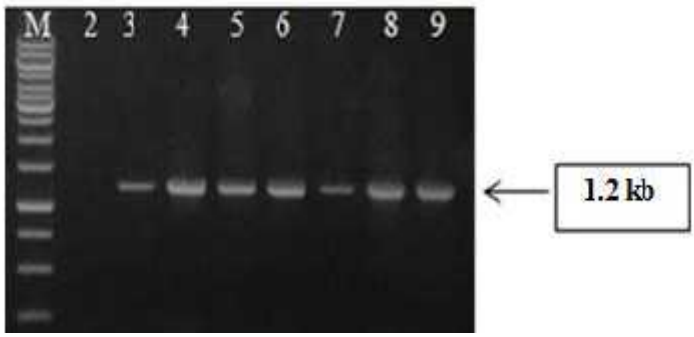


**Fig. 1. Agarose gel electrophoresis of PCR product. Lane M: 1kb Marker, Lane 2: Asymptomatic sample, Lane 3-9: Amplified CP product from symptomatic samples of about 1.2 kb.**

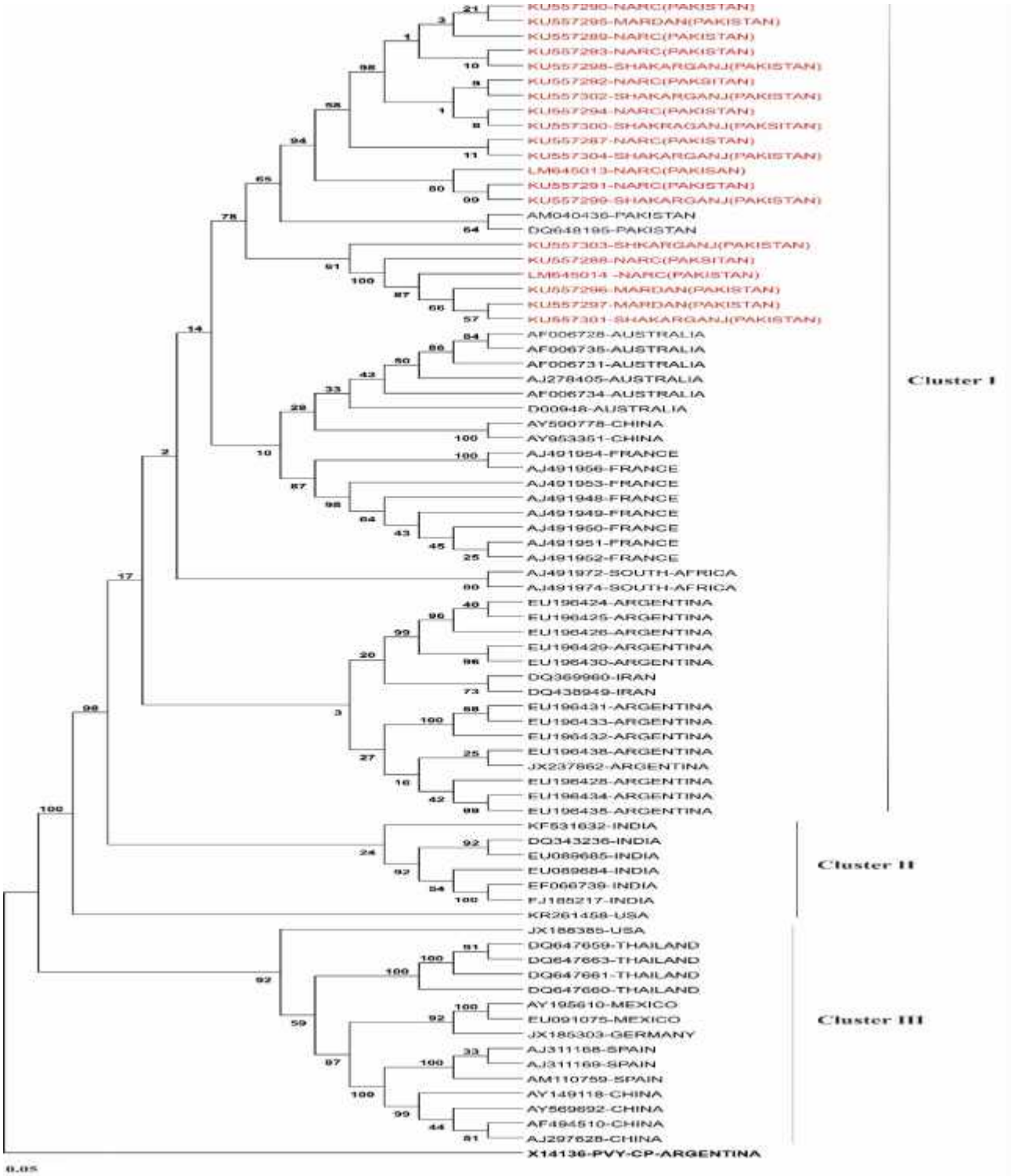


**Fig. 2. Phylogenetic relationship based on CP sequences of SCMV detected in the leaves of *Saccharium officinarium* collected from KPK and Punjab (Pakistan). Dendrogram was built through multiple sequence alignment (using ClustalW) of current study (In Red) with the sequences retrieved from GenBank database. Phylogenetic tree was inferred through Neighbour joining method using 1000 Bootstrap value. Branch length is drawn to the scale; indicating the 0.05 rate of nucleotide substitution per site. As outgroup, *Potato virus Y* (PVY) isolate was used (Accession number X14136).**

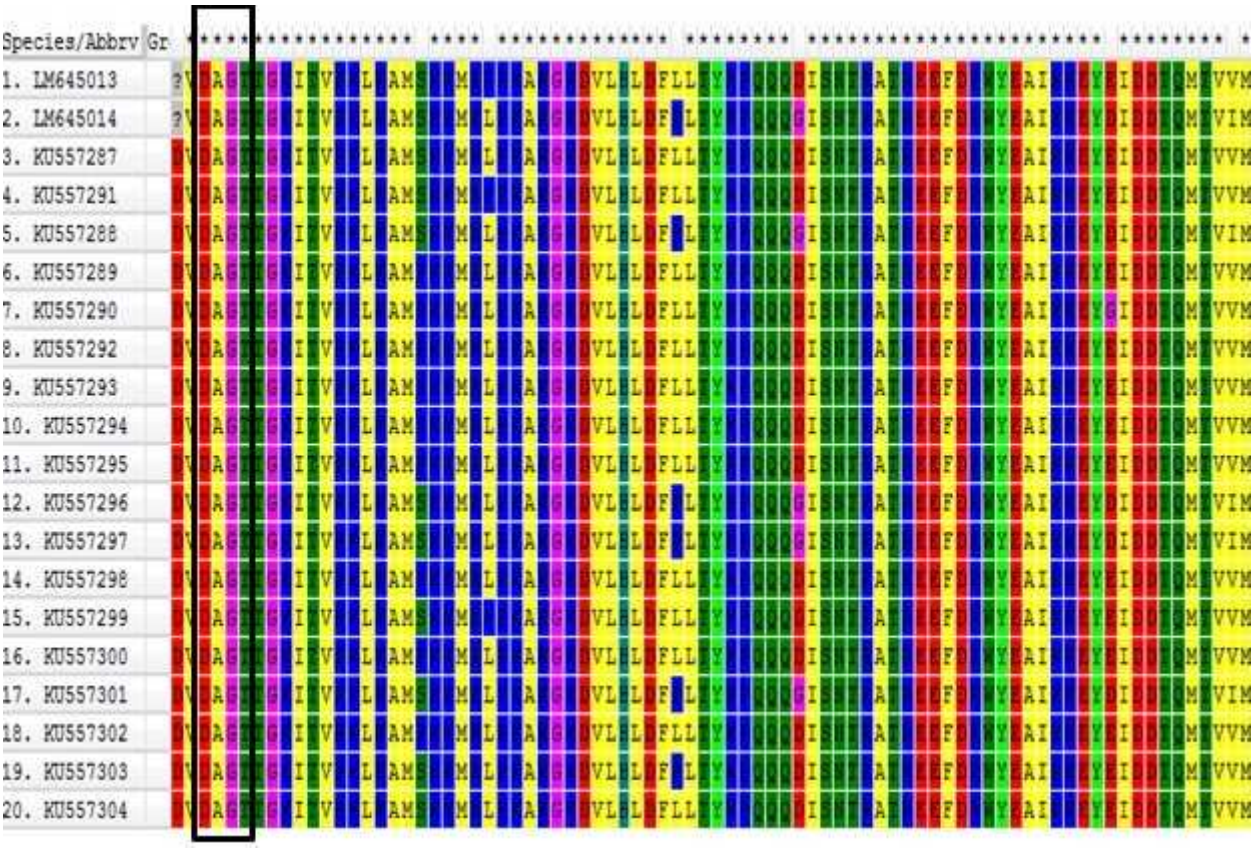


**Fig. 3. Multiple alignment of translated protein sequences of coat protein gene of *Sugarcane mosaic virus* from the isolates of reported Pakistan sequences. Block region show the conserved DAG (Asp-Ala-Gly) motif near N-terminus of all aligned amino-acids sequences.**

## DISCUSSION

High infection rate of SCMV was observed in most of sugarcane growing regions of the Pakistan. The possibility for high incidence of infection may be due to more susceptibility of sugarcane varieties to *Sugarcane mosaic virus* and another possible reason is the presence of more insect vector aphids or their reservoir near sugarcane fields which is responsible for transmission of disease (Luo *et al*., 2003; Zhou *et al*., 2007). Infectivity level is also high probably due to frequent rate of mutations and recombination between the related groups of RNA viruses and particularly due to lacking of RNA-dependent RNA polymerase proof reading activity (Domingo and Holland, 1997, Sztuba-Solinska *et al*., 2011).

All the thirty-two sugarcane leaf samples, showing mosaic and streak symptoms, collected from different sugarcane growing region of the country were positive for CP of SCMV. The available CP sequences of 20 samples shared highest (95-100%) nucleotide sequence identities among them and (85-100%) with the rest of the world. One sugarcane leaf sample showing streak symptoms was also positive for CP gene of SCMV, the possible explanation for which is dual or mixed infection of SCMV and *Sugarcane streak virus* (SSV). It is well known that dual infection is beneficial for viruses for causing local as well as systemic infection (Fraile *et al*., 2008).

Phylogenetic tree depicts the SCMV distribution throughout the world with very minor sequences variations. The SCMV CP sequences from the present study and sequences collected from databases were classified into three clusters I, II, III. SCMV CP sequences from various regions of Pakistan clustered in a single clade, representing very minor sequence variations among them. Previously reported two SCMV CP sequences (AM040436, DQ648195) from Pakistan were also grouped with present study isolates together with the sequences obtained from Australia, China, France, Argentina, South Africa and Iran. Like China, India is a neighboring country but the SCMV CP sequences obtained from India occupied a distinct position in the phylogenetic tree with high bootstrap value (98). The two SCMV CP sequences obtained from USA occupied independent positions in the tree and do not fit in any cluster. The most probable reason may be their collection from geographically diverge regions of USA.

Correspondingly, Chinese isolates were also found indifferent clusters; with two sequences grouped in cluster I and the rest four sequences segregates with cluster III, indicating their geographically distinct origins.

Further alignment of amino-acids of all studied isolates highlighted the conserved DAG motif. Importance of conserved DAG motif (Asp-Ala-Gly) was previously proven in a study for its responsibility for transmission of *Potyvirus* through its insect vector aphids (Harrison and Robinson, 1988). Despite the highly variable N terminus region, its DAG motif conservation is important for virus transmission while decline in virus spread also co-relates with the alteration of DAG region (Atreya *et al*., 1991). However, the variations in the conserved region were also reported. For instance, isolates of *Pea seed-borne mosaic virus* comprises of DAS conserved amino-acids (Johansen *et al*., 1996) while *Peanut mottle virus* contain DAA motif (Flasinski and Cassidy, 1998) and *Plum pox virus* contain DAL sequences (Lopez-Moya *et al*., 1995). Experiments performed to analyze the mutational effect in DAG motif to the binding efficiency of CP with Hc-pro. Evidences reported indicate that the transmission of virus still occur but with a reduction in transmission efficiency (Blanc *et al*., 1997).

This wide spread of virus transmission is possibly through diseased seeds as SCMV can be transmitted through seeds (Haider *et al*., 2011). In Pakistan, sugarcane varieties probably got infection through import of SCMV from Australia. As Perera *et al*., 2008 reported the similar results of co-relationship of Pakistan and Australian isolates. The present study concluded that no diversity was observed among Pakistani isolates.

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