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NUCLEOTIDE EVIDENCE OF CAPSID PROTEIN GENE OF POTATO VIRUS Y FROM A PAKISTANI ISOLATE

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Potato virus Y (PVY) is an emerging problem in main potato growing areas of Pakistan and new molecular tools are necessary for confirmation of PVY. Serological detection of PVY was achieved through Enzyme Linked Immunosorbant Assay (ELISA) and biologically authentication was accomplished on susceptible indicator host plants (*Nicotiana tabacum cv. Samsun NN*, *N. rustica*, *N. benthamiana* and *Chenopodium album*). Appropriate sense and antisense primers for Capsid Protein (CP) gene were used for molecular diagnosis of PVY in Polymerase Chain Reaction (PCR) and sequence of Pakistani isolate (JQ518267) exhibited 795 nucleotides with maximum composition of adenine followed by guanine, thymine or uracil and cytosine respectively. Physical and chemical properties of CP gene were computed and different bioinformatics tools clearly indicate that amplified gene is hydrophilic, non-allergen and highly conserved region. The final sequence was further compared with ordinary, common, necrotic and recombinant strains of PVY. The phylogenetic tree clearly indicates four different clusters and Pakistani isolate falls in first cluster exhibiting the high degree of nucleotide homology (99.98%) with previously reported isolates of Pakistan.
Keyword: Capsid protein, potato diseases, PVY strains, potyvirus, phylogenetic analysis

INTRODUCTION

Eight edible potato species are being grown in more than 140 countries and it is source of food for about one billion people (Jamal *et al*., 2012). In Pakistan, annually three potato crops provide 3726.5 thousand tons which it is very low as compared to other potato growing countries of the world (GOP, 2013). It produces more per acre yield (12-15 times) as compared to cereals crops (wheat, rice and maize) and different pests and pathogenic diseases reduced the yield of potato crop in Pakistan (Nosheen *et al*., 2013; Abbas *et al*., 2013). In Pakistan, many taxonomic groups of plant viruses are reported which are responsible for the reduction of yield in different crops including potato (Mubin *et al*., 2009). Smith (1931) first time reported PVY and it belongs to Potyvirus group (Kerlan and Moury, 2008). PVY is placed at 5th position among top ten most economically damaging plant viruses in the world and it consists of a wide host range including *Leguminosae, Solanaceae and Chenopodiaceae* (Gray *et al*., 2010). It causes premature plant death, mottling, leaf drop, chlorosis and necrosis. Application of insecticides in potato crop to control vector of potyviruses is not effective and yield losses vary from 50% to 80% (Alam *et al*., 2013). It has +ve sense RNA genome (10 kb) with VPg protein (5'), poly-A (3') and short open reading frame has been reported within the previous large open reading frame (Chung *et al.,* 2008). Different strains (ordinary, common, necrotic and recombinant) have been reported from genomic recombination in PVY (Ali *et al*., 2010). Necrotic strain was first reported in Poland and now it is common in most of the potato fields (Ali *et al*., 2007). It is common and destructive potato virus in the world and is getting an alarming threat in main potato growing areas of Pakistan (Abbas *et al*., 2012). It can cause significant yield and quality loss in potato tubers when either present alone or in combination with other potato viruses (Singh, 1999) and for every 1% increase in its infection reduces the tuber yield of 0.1805 t/ha (1.6 cwt per ac) (Nolte *et al*., 2004). A large number of pests and pathogens can be carried from one generation to the next through vegetative propagation material. Sensitive, appropriative and reliable methods should be developed and adopted for proper management of this virus. Serological methods (enzyme linked immunosorbant assay) screen the potato samples effectively with high concentration but these methods are time consuming and unable to detect the virus in dormant potato tubers (Singh and Singh, 1996). New molecular techniques like Reverse Transcription Polymerase Chain Reaction (RT-PCR) have been introduced for detection of virus at low titer (Peiman and Xie, 2006). PCR has already revolutionized research in molecular biology and is being intensively applied in most of the molecular biology laboratories. The importance of PCR depends on its ability to amplify specific cDNA (PVX, PLRV and PVY) sequence from as short as 50bp to over 10000 bp in length more than million fold in 2-4 hours (Abdel-Hamid *et al*., 2003). The aim of this study was to detect CP gene of PVY at biological, molecular and serological level and provide the nucleotide evidence, physical and chemical properties of local isolate. Biological, molecular and serological properties of local isolate of PVY

may play a vital role to help the experts working on this virus (Singh *et al*., 2008).

MATERIALS AND METHODS

***Serological and biological confirmation*:** Potato plants exhibiting typical symptoms of PVY were subjected to ELISA using monoclonal and enzyme alkaline phosphatase conjugated Immunoglobulin of potato virus Y (BIOREBA AG kit) as described by Clark and Adam (1977). For biological confirmation, it was maintained on susceptible indicator host plants. Physical and chemical (molecular weight, theoretical pI, number of negatively and positively charged residue, extinction coefficient, instability and aliphatic index, grand average of hydopathicitiy and atomic composition) properties of CP gene were determined using secondary structure of protein and amino acid sequence was further checked with AllerHunger tool to determined allergencity or non-allergens nature (Muh *et al*., 2009; Gasteiger *et al*., 2005).

***Molecular detection*:** Total RNA was isolated with the help of TRI Reagent. *Moloney murine leukemia virus reverse transcriptase* was used for cDNA synthesis (Revert Aid First Strand cDNA Synthesis Kit) and polymerase chain reaction components (50µl) were comprised of cDNA (2µl), reaction buffer (5µl), magnesium chloride (3µl), sense (5´-ATG ACA CAA TTG ATG CAG G-3´) and antisense (5´-TCA CAT GTT CTT GAC TCC-3´) each primer (5µl), *Taq DNA polymerase* (1µl) and mix dNTPs (5µl). PCR mixture was initially heated at 94°C for 3 minutes followed by 35 threshold cycle of denaturation at 94°C for 2 minutes, annealing 51°C for 90 seconds and extension 72°C for 1 minute. At the end, final extension was performed at 72°C for 10 minutes and size of amplified product was determined with 1kb DNA ladder on agarose gel (1%).

***Sequencing and sequence analysis*:** The amplified product was further purified **(**Thermo Scientific Gene JET PCR Purification Kit) and send to Ibc (Korea) for sequencing in sense and antisense directions. Molecular evolution for genetic analysis was used for amino acid translation of CP gene and analyses were conducted using the maximum composite likelihood model (Tamura *et al.,* 2011). CP gene sequences of twenty nine different isolates comprising all strains of PVY were downloaded from NCBI and compared with the final sequence of Pakistani isolate. Phyre 2 tool was used to determine mutation sensitivity and 3D structure of capsid protein gene of local isolate (Behjati *et al*., 2012).

RESULTS

Shortening of leaves, stunting, mosaic, mottling and vein-banding were observed on suspected potato leaves and yellow color confirmed PVY while white color indicated the absence of virus (Fig. 1A). In response to PVY infection, susceptible indicator host plants (*Nicotiana tabacum cv. Samsun NN*, *N. rustica*, *N. benthamiana* and *Chenopodium album*) produced necrotic whitish lesion, mottling and leaf spot. CP gene specific primers of PVY successfully amplified 795 bp fragment and no such amplification was observed in negative control (Fig. 1B). The final sequence of a Pakistani isolate was submitted to NCBI and got the GenBank accession number JQ518267. The length of CP gene comprised of 795 nucleotides and the nucleotide composition of Pakistani isolates acquired the highest contents of adenine (35%) followed by guanine (24%), thymine or uracil (21%) and cytosine (20%). Several

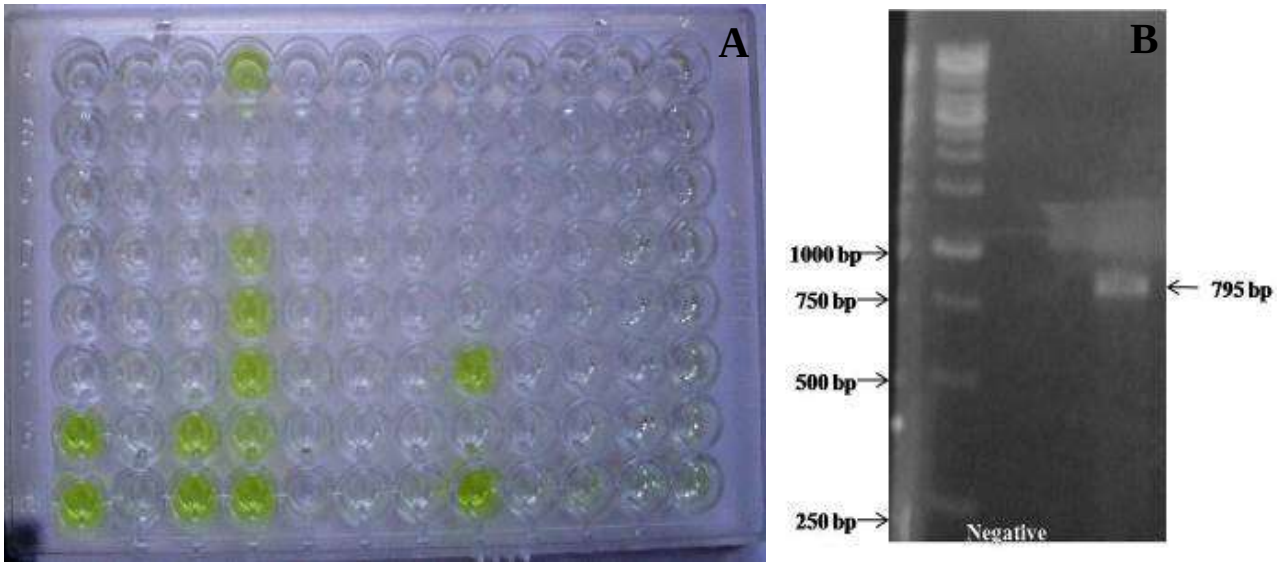

Figure 1. Seriological (A) and molecular (B) confirmation of PVY.

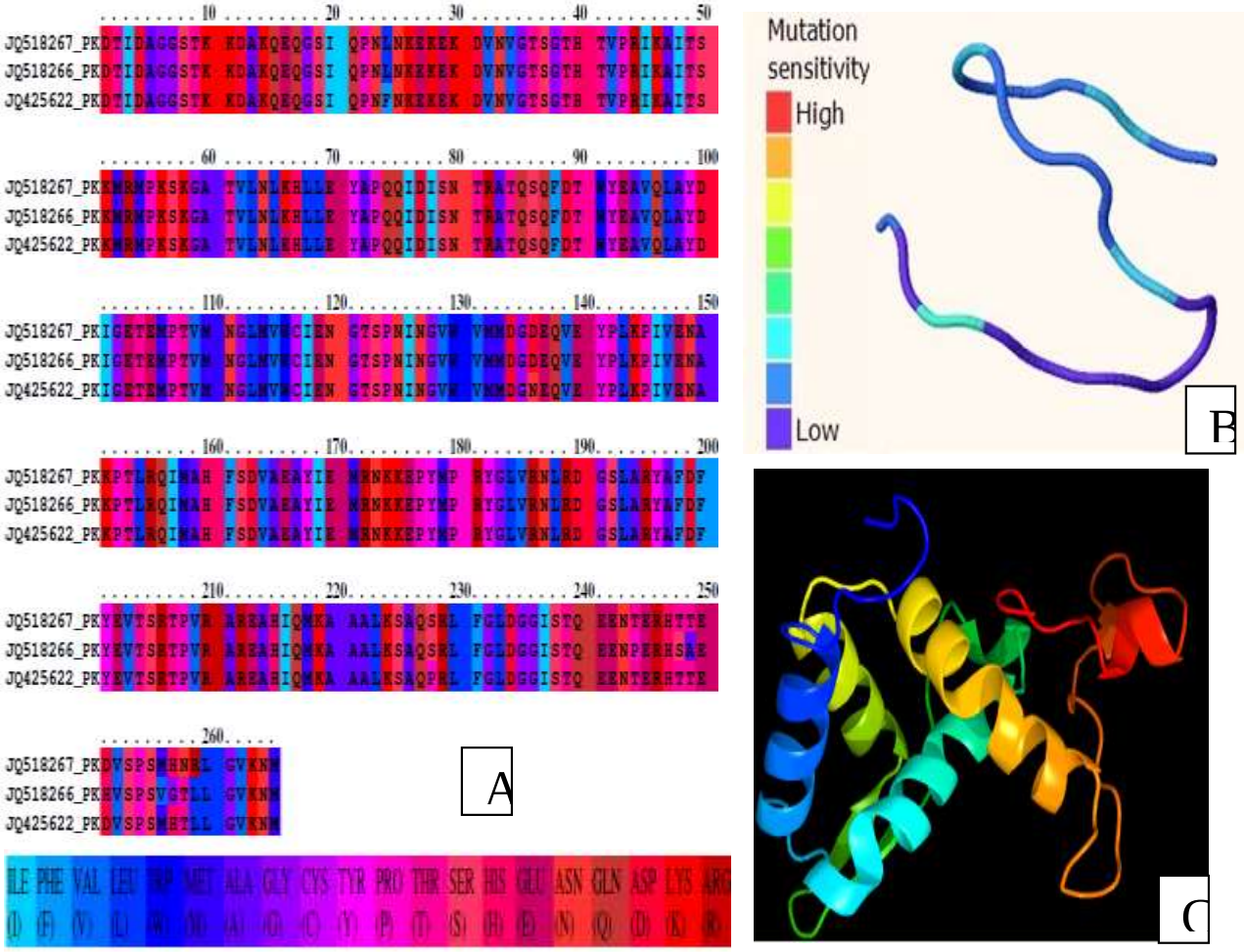

Figure 2. Amino acid comparison (A), mutation sensitivity (B) and 3D structure (C) of PVY-CP gene from a Pakistani isolate.

parameters including molecular weight (29782.7), theoretical pI (6.86), number of negatively (Asp+Glu = 34) and positively (Arg+Lys = 33) charged residue, extinction coefficient (29910), instability (47.19) and alphatic index (70.30), grand average of hydopathicitiy (-0.643) and atomic composition ($C_{1294}H_{2067}N_{371}O_{407}S_{14}$) were determined. According to AllerHunger, no sequence homology with contiguous amino acids revealed that this gene of is non-allergenic in nature and mutation sensitivity and 3D structure revel that it has low mutation sensitivity (Fig. 2B). The final sequence of Pakistani isolate was further compared with isolates reported from Pakistan (JQ425622 and JQ518266), China (AY792597.1, HQ631374.1 and JN635310.1), UK (EU161658.1), Syria (AB295477.1 and AB295479.1), South Africa (GQ853665.1 and GQ853667.1), Italy (AB025415.1), India (JN034046) and some other countries (AB025417.1, AF012026.1, AF012027.1, AJ133454.1, AY061994.1, AY319647.1, EF027859.1, EF027861.1, EF027863.1, EF027869.1, EF027879.1, EF027881.1, EF027886.1, EF027891.1, EF027897.1, EF027898.1 and EF027901.1). All the downloaded sequences were found to be comprised of ordinary, common, necrotic, recombinant and new strains of PVY. The phylogenetic tree clearly indicates four different groups of PVY strains and Pakistani isolate falls in first group along with Syria, China, United Kingdom and Pakistani isolates (Fig. 3).

DISCUSSION

Mirza (1978) first reported PVY in Pakistan and it has dispersed in eight agro-ecological zones of Pakistan with 83% yield losses (Abbas *et al*., 2013). Different incidence percentage of PVY was recorded in Toba Tek Singh (52.77%), Jhang (28.20%), Sialkot (27.83%), Chiniot (18.72%), Gujranwala (14.37%), Okara (12.72%) and Sahiwal (52%) (Abbas *et al*., 2012: Ahmad *et al*., 1995). Farmers use different insecticides to control the virus but

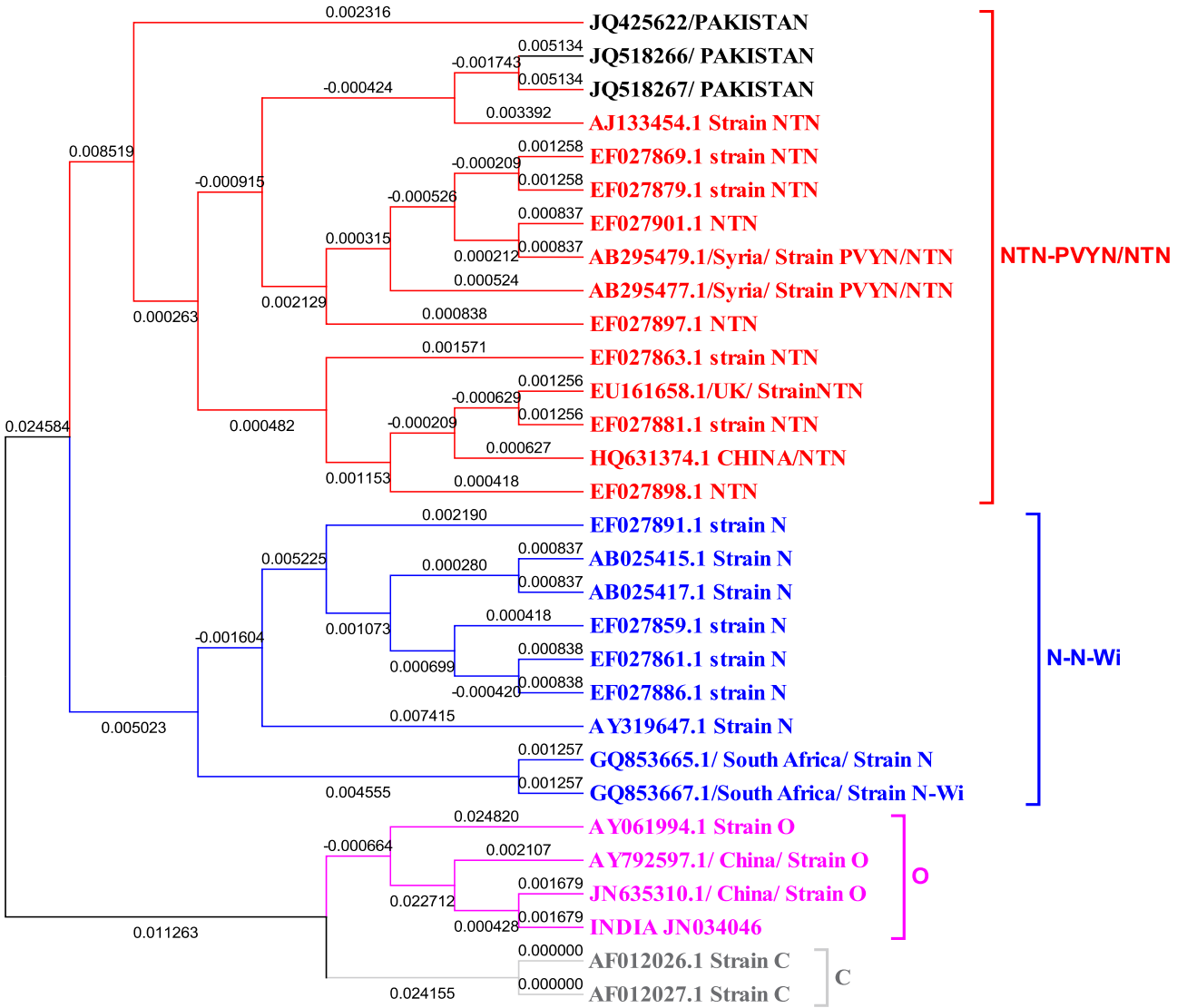

Figure 3. Nucleotide sequence analysis of PVY-CP gene of Pakistani isolate along with different strains of PVY.

application of insecticides only controls the insect vector and natural and pathogen derived resistance are more reliable and environment friendly (Hossain *et al*., 2013). In Pakistan, potato samples have been examined serologically and this technique is only reliable with higher concentration of virus (Abbas and Hameed, 2012). Since, the low concentration of the PVY in apparently healthy potato plants is undetectable by serological diagnostic techniques, which may become the source of inoculum for healthy plants through vectors or contaminated agricultural tools (Cai *et al*., 2011).

It is one of the main reasons that serologically negative potato plants produced viral symptoms at later stage of infection. Physical and chemical properties of local isolates can help the scientist working on PVY in Pakistan. Previously, the taxonomy of potyviruses was based on biological and serological methods and it was much more difficult to distinguish between species and strains (Francki *et al*., 1985). Certification requires a sufficient sensitive detection method to confirm one viral infected sample in all pooled healthy samples (Singh *et al*., 2008). A detection procedure of PVY in infected samples must be sensitive, specific, rapid, easy to use, reliable and cost-effective. Molecular tools like PCR detects virus at lower concentration and it will play a pivotal role for the diagnosis of virus (Saiki *et al*., 1985). The high value of instability index (≥40) reveals that CP gene is unstable in test tube and extinction coefficient indicates how much light a protein absorbs at a certain wavelength. In PCR, proper concentration of template, enzyme, magnesium ion, primers and dNTPs are the key factors for successful amplification

from viral nucleic acid (Saiki *et al*., 1985). The nucleotide sequences play pivotal role in developing different types of PCR for diagnosis of several other pathogens along with viruses (potato virus A, S, X, Y and potato leaf roll virus) and virods (Hossain *et al.,* 2013; Abbas and Hameed, 2012; Nie and Singh, 2000; Singh and Singh, 1998) and with the help of nucleotide evidence from an Indian isolate (PVY) CP gene mediated resistance against local isolate was developed. The nucleotide sequence of Pakistani isolate falls in first cluster and high nucleotide percentage homology between Pakistani and other isolates is due to highly conserved sequence of CP gene in complete genome of PVY. Low mutation sensitivity of CP gene also indicates that this gene has more conserved region (Fig. 2A). The range of CP gene nucleotide evidence varies from 38 to 71% among different species and 90 to 99% among strains of the same species (Shukla and Ward, 1988). So it is one of the main reasons that CP gene mediated resistance will be more effective against all type of strains of PVY. World Health Organization and Food and Agriculture Organization have developed criteria to evaluate the sequence alelrgenicity prediction and CP gene is not allergenic in nature. The pathogen derived resistance is more effective for the management of PVY and CP gene mediated resistance in potato plant will not affect the consumer. It has been previously reported that introduction of CP gene in potato plant interferes with the replication process of PVY and it can enhanced the immune system of the host plant (Alam *et al*., 2013). Nucleotide evidence of complete genome and CP gene provide essential information for identifying strains and species in potyviruses (Van der Vlugt *et al*., 1993). Serological, biological and molecular tools have been used to identify different strains of PVY from Belgium, Canada, Czech Republic, France, Europe, Germany, Netherlands, North America, Oregon, Pakistan, Poland, South Africa and Washington (Yin *et al.*, 2012). Korean, Mexican and Egyptian isolates of PVY exhibiting low degree of diversity (1%) with previously reported strain of PVY (Rodriguez *et al*., 2009). Serological, biological, molecular, chemical and physical properties of local isolate will be helpful to develop pathogen derived resistance and thus improve the yield of potato crop.

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