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MAJOR POTATO VIRUSES IN POTATO CROP OF PAKISTAN: A BRIEF OVERVIEW

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ABSTRACT

Potato is one of the main cash crops for the farmer and also contributes a considerable amount in Gross Domestic Product of Pakistan because it is cultivated as a garden vegetable and also on commercial scale. In high altitude valley, potatoes are grown as summer crop while in the plains and low valleys three consecutive crops can be grown per year. Potato crop provides 12-15 times more yield and calories production is also higher than wheat maize and rice while the average yield of potato crop in Pakistan is low as compared to other potato growing countries. Potato growers introduced high yielding foreign potato varieties which have significantly enhanced the yield of potato crop along with new viral problems and among these, Potato Virus A (PVA), Potato Virus M (PVM), Potato Virus S (PVS), Potato Virus X (PVX), Potato Virus Y (PVY), Potato Leaf Roll Virus (PLRV) and Potato Mop-top Virus (PMTV) have been reported in spring, summer and autumn potato crop along with 83% yield losses. In Pakistan, a large number of potato germ-plasms were certified through Enzyme Linked Immunosorbant Assay (ELISA) which is unable to detect the virus at initial stage of infection and new molecular tools like Polymerase Chain Reaction (PCR) assay were introduced for successful, sensitive and more reliable confirmation of viruses. Different percentage incidence of PVA and PVM was reported from main potato growing areas of Pakistan and no molecular optimization along with nucleotide evidence of these two viruses was reported from Pakistan. PCR assay were developed for molecular detection of PVX, PVY, PMTV, PVS and PLRV while no nucleotide evidence of PVS is still reported from Pakistani while coat protein (CP) gene sequence of PVX, PVY and PLRV from Pakistani isolates is available at the data bank of National Center for Biotechnology Information. The increasing incidence of PVY is getting an alarming position in potato crop of Pakistan and nucleotide sequence of CP gene reveals the presence of new strain in Pakistani potato while the nucleotide evidence of CP gene of PVX from a Pakistani isolate exhibiting the maxim homology with USSR isolate.

Key word: Potato Virus A (PVA), Potato Virus M (PVM), Potato Virus S (PVS), Potato Virus X (PVX), Potato Virus Y (PVY), Potato Leaf Roll Virus (PLRV) and Potato Mop-top Virus (PMTV), Pakistan

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's leading vegetable crop along with staple food and is placed at fourth position after rice, wheat and maize respectively (Rauscher *et al.*, 2006). Potato has two compensations over the other crops i.e. its production and calories per unit area are higher than wheat and rice while in term of total production potato provides 12-15 times more yield per hectare as compared with cereals. Potato tubers are excellent source of carbohydrates, protein, and vitamins. Potato contains 77.8% water, 2.0g protein, 13g calcium, 0.06g riboflavin, 25mg/100g vitamin C, 12mg ascorbic acid, 0.11mg mythiamin, 1.18mg niacin and per 100g energy of edible protein is 85 calories (MacGillivary, 1953). Potato is one of the main cash crops for the farmer and also contributes a considerable amount in Gross Domestic Product (GDP) of Pakistan because potatoes are cultivated both as garden vegetable and also on commercial scale. It is growing on an area of 149.1 thousand hectares with an annual production of 2542.7 thousand tones and the average yields rose from around 9 in 1947 to 20 MT/ha in 2006. Presently, it is estimated that the total annual domestic production amounts to around 1.9MT, of which 0.28MT is used as seed and 1.8MT is available for consumption after post harvest losses and per capita consumption of 9.3kg (GOP, 2011). In high altitude valley potato is grown as summer crop and in the plains and low valleys three consecutive crops can be grown per year. Pakistan can be separated into eight agro ecological zones for the production of potato which are: (I) Southern Balochistan and Punjab and Irrigated plains of Sindh; (II) Irrigated plains of South East Khyber Pakhtunwa and Central Punjab; (III) Irrigated and rain fed plains of Khyber Pakhtunkhwah and Northern Punjab; (IV) Irrigated lower valleys of Khyber Pakhtunwa; (V) Rain fed high valleys and hill sides of Khyber Pakhtunkhwah, Azad Kashmir and Northern Punjab; (VI) Irrigated high valleys of Khyber Pakhtunwa, Northern Areas around Azad Kashmir and Chillas; (VII) Irrigated high valleys of Northern Areas and Khyber Pakhtunkhwah around Mastuj; (VIII) Irrigated high valleys of Balochistan and South and North Waziristan. These eight agro ecological zones are different in altitude, longitude, Latitude, topography, climate, soil and irrigation infrastructure (Zanoni, 1991). Northern Pakistan is the key for seed producing and supplies the seed to the rest of the country.

An average yield of potato crop is far less in Pakistan as compared to other potato growing countries of the world because potato crop is affected by many biotic and a biotic factors during its growing season. A biotic factor is surrounded by the zinc deficiency, salinity and high temperature which are the main constraints in potato production. In biotic factors diseases play an important role which includes foliar diseases which are late blight (*Phytophthora infestans*), bacterial wilt, early blight (*Alternaria solani*) and bacterial wilt (*Pseudomonas solanacearum*). Seed-borne diseases include stem canker (*Rhizoctonia*), *Verticillium* wilt (Ashraf *et al.*, 2012), *Fusarium* wilt and common scab (*Streptomyces scabies*) while viral disease causes severe damage in potato crop production (Abbas and Hameed, 2012). Potato growers introduced the high yielding foreign potato varieties in Pakistan which significantly increased the yield of potato crop in main potato growing areas but at the same time result new problems including certain viral disease. Among these viruses, Potato Virus A (PVA), Potato Virus M (PVM), Potato Virus S (PVS), Potato Virus X (PVX), Potato Virus Y (PVY), Potato Leaf Roll Virus (PLRV) and Potato Mop-top Virus (PMTV) have been reported in spring, summer and autumn potato crop of Pakistan (Mughal *et al*., 1986). PVX, PVY and PLRV cause significant yield losses in potato crop of Pakistan (Abbas *et al*., 2012). A large number of potato samples were tested through Enzyme Linked Immunosorbant Assay (ELISA) in certification laboratories but ELISA methods are unable to detect the virus at lower concentration or initial stage of infection. A large number of pest and pathogen can easily transferred from one generation to next through seed propagation material. Certification of potato germ-plasms requires a sufficient sensitive detection method to confirm one viral infected sample in all healthy pooled samples. Few viron (theoretically one) of virus at initial stage of infection in infected host plants multiplied into billion of copies within few days or weeks and virus free potato plants can be infected in the open field by insect vector along with mechanical transmission through viral infected potato plants (Betancourt *et al*., 2008). A detection procedure of virus in infected potato samples must be sensitive, specific, rapid, easy to use, reliable and cost-effective while reverse transcriptase (RT) polymerase chain reaction (PCR) assay technology offers further sensitivity and specificity to detect potato viruses and its strains in potato samples (Rolland *et al*., 2008).

Potato virus A: Potato virus A is a positive sense RNA virus which belongs to family *potyviridae* with approved acronym PVA while other synonyms are Marmor solani, Potato mild mosaic virus, Solanum virus 3 and Potato virus P. In Pakistan, PVA was first isolated in 1978 from Punjab (Anwar and Mirza 1984). Symptom first appear on terminal leaves showing blotchy mottle, mild mottling, slight crinkling, mosaic, necrosis and sever crinkling when infect in combination with PVX or PVY (Mughal *et al.*, 1988). PVA is sap transmissible and stylet borne in persistent manner by insect vector belonging to the family *Aphididae* and helper virus is not required for transmission but *Myzus persica* can transmit potato aucuba mosaic virus when the source plant also contain PVA. The incidence of PVA was 40.7% in Punjab (Abbas *et al.*, 2012) and 31.55% in northern areas (Bhutta *et al*., 2003). Serological confirmation (ELISA) of PVA was used for screening purpose in potato germ-plasms and presence of PVA in tissue culture potato plants of Cardinal, Desiree and Diamant were also reported (Nasir *et al*., 2012) while no molecular technique (PCR) along with nucleotide evidence was reported from Pakistan.

Potato virus M: This virus belong to the genus *Carlavirus* with approved acronym PVM while other synonyms are Kartoffel K virus, Kartoffel Rollmosaik virus, Potato Paracrinkle virus, Potato Interveinal mosaic virus, Potato leaf rolling mosaic virus, Potato virus E, Solanum virus 7 and Solanum virus 11 and in Pakistan, first report of its occurrence was by Mughal *et al.* (1988). Symptoms in potato crop range from very slight to very severe and are influenced by virus strain, potato cultivar and environmental condition which are prominent in young plants while older plant may not show symptoms (Khalid *et al*., 2000). An Interveinal mosaic accompanied by clearing of the veins is a common symptom while necrotic spots may develop on the upper leaf surface, accompanied by brown streaks on the veins of the under surface, petioles and stem. PVM is transmitted in nature by insect vectors belonging to the *Aphididae* in a non-persistent manner and it can be transferred through mechanical transmission while seed and pollen transmission is not reported. During the Year 2011-12, the average incidence of PVM was 24 % in Rawalpindi, Islamabad, Faisalabad and Sahiwal while no molecular confirmation and nucleotide evidence is reported from Pakistan (Abbas *et al*., 2012)

Potato Virus S: This virus belongs to the genus *Carlavirus* with approved acronym PVS and it was fist found in Netherland while it is probably distributed all potato growing countries from there. Two strains PVS^A (andean) and PVS^O (ordinary) have been reported while biology of PVS^O isolate is different from PVS^A by not producing systemic infection to *Chenopodium spp*. The main portion of amino acids that is different in PVS^O and PVS^A occurs in coat protein and also in 11 K proteins. This difference might be responsible for divergence in symptomatology and aphid transmissibility. In Pakistan, PVS is reported from Punjab (Burhan *et al*., 2006), Northern areas (Bhutta *et*

al., 2003) and cause 10-20% yield losses with and incidence of 2-12% (Mughal *et al*., 1988) while Mughal and Khalid (1985) characterized PVS infecting potato through host range, symptomology, serology, morphology, and physiochemical properties. The CP gene specific sense and antis sense primer of PVS was developed and these specific primer successfully amplified CP gene form a Pakistani isolate through PCR (Rasheed, 2011).

Potato Virus X: This virus is among the top ten most economically important plant viruses in the world and distributed worldwide along with Pakistan. PVX was also known as Healthy potato virus, Potato latent virus, Potato mild mosaic virus and Solanum virus. Leaves of infected plants show mottling, interveinal, mild and super mild mosaic. Infected plants with mild symptoms in upper leaves may show typical symptoms in the older leaves shaded by the top ones and some viral strains cause rugosity and crinkling of the foliage (Mugal *et al*., 1988). Transmission in nature is without help of a vector and it is easily perpetuated through infected tubers while other ways of transmission are infection sap, field implements and the mechanical contact of roots or leaves. PVX infecting commercially grown potatoes in upper Kaghan valley of Pakistan and average percentage incidence of PVX was 13.18% in seven main potato growing districts of Punjab (Jan and Khan, 1995). PVX is distributed throughout potato growing areas of Pakistan ranging infection between 1.5-6.2% being more in Punjab and even imported seeds have shown 0.7-30% infections indicating the continuous introduction of PVX in the country. Desiree varieties exhibiting 20.8% and 8.33% incidence from Sahiwal, Faisalabad and Pak Pattan respectively while cardinal varieties showed minimum 4.16% infection of PVX (Burhan *et al*., 2006). Different percentage incidence of PVX was reported form Gujrawala (20.62%), Jhang (17.94%), Okara (16.13%), Sahiwal (12.87%), Sialkot (9.79%), Chiniot (12.12%), Toba Tek Singh (2.77%), Rawalpindi (16%), Islamabad (23%) and Faisalabad (36%) (Nosheen, 2011). OCEANIA, FSD-RED and FD3713 showed resistant, FD3-10, FD3-9 and 393574-61 were highly resistance and Mirrato, Arterix and Desiree were moderately susceptible against PVX (Ahmad *et al*., 2011). Among the environmental factors, temperature (25-28ºC) played a critical role in the development of PVX disease on varieties/advance lines of potato while the disease severity of PVX increased when temperature increased above 28ºC and the disease severity decreased (Qamar *et al*., 2003). Serological confirmation of PVX was used for screening purpose but coat protein gene specific sense (GGCGCAACTCCTGCCACAGC) and antisense (TTGTTGTTCCAGTGATACGA) primer amplified 613 bp CP gene fragments from a Pakistani isolate while nucleotide sequence indicates that this isolate exhibiting the maximum genetic homology with USSR isolate of PVX (Jamal *et al*., 2012).

Potato Virus Y: Among viruses, PVY is one of the most economically important and widespread while PVY is among the top five most economically damaging plant viruses in the world and enjoys a promising position among the pathological constrains in the potato crop (Rolland *et al*., 2009). It is the type member of genus *Potyvirus* and selected synonyms of PVY are Potato acropetal virus, Potato leaf drop streak virus, Potato virus 20, Solanum virus 2 and Tobacco veinal necrosis virus (Singh *et al*., 2008). It produced a variety of symptoms depending on the viral strain while helper component is required for transmission and commonly subdivided into different strains groups (Rolland *et al*., 2010). Host range of PVY comprises of up to 9 important families including important crops such as potato, tobacco, tomato, pepper and tomato and also *solanaceous* weeds (Ali *et al*., 2008). It exists as a complex of different strains which are PVY^{O}, PVY^{C}, PVY^{N}, PVY^{E} and PVY^{Z} while potato variants are PVY^{NTN}, PVY^{NW} and $PVY^{N:O}$ (Ali *et al*., 2008). This virus was first reported in Pakistan by Mirza (1978) and it was among the important diseases of potato in the plains of Punjab (Anwar and Mirza, 1984). This virus is scattered throughout the country with a frequency 2-25% (Mughal *et al.*, 1988) and losses caused by PVY in Pakistan were projected 58-83%. Other natural hosts of PVY in Pakistan are tobacco (Mughal *et al.*, 1986), tomato (Hassan, 1995) and chili (Hameed *et al.*, 1995). Ahmad *et al*. (1995) surveyed different potato growing areas of Punjab and confirmed PVY through ELISA in Toba Tek Singh (52.77%), Jhang (28.20%), Sialkot (27.83%), Chiniot (18.72%), Gujranwala (14.37%), Okara (12.72%) and Sahiwal (6.81%). PVY has a wide natural host including some important crops and few weeds. The increasing incidence of PVY is getting an alarming position in the main potato growing areas of Pakistan and only PVY^{O} strain is reported serologically (Abbas *et al*., 2012). CP gene specific forward and reverse primers were developed and molecular confirmation of CP gene fragment along with nucleotide sequence was reported from Pakistan and the nucleotide evidence revel a new strain of PVY in Pakistan which was not previously known (Abbas, 2011).

Potato leaf roll virus: This virus has a great importance in history of potato and it was first found in *Solanum tuberosum* sp. in the Nertherlands and it is distributed worldwide in the potato growing areas (Brunt *et al*., 1990). This virus belong to the genus *Luteovirus* with approved acronym PLRV and other synonyms are Potato phloem necrosis virus, Solanum yellow and Tomato yellow top strain (Harrision, 1984). In nature, PLRV is transmitted to

potato only by insect vector in a persistent manner. Symptoms of primary infection results in characteristic rolling of the upper leave at the top of the plants and such plants remain dwarf, upright in habit and their leaves become thick and pale while the leaves of some varieties are often rolled upwards, especially at the base. Infection in late growing season usually let the plants symptomless and yield losses are nominal and tubers from such plants are partially infected. In case of secondary infection, whole plants look erect and may be smaller than the healthy ones. Older plants show rolling of lower leaves and upper leaves are plate while basal leaves are thick, leathery, brittle and crackle when squeezed in hand because of heavy accumulation of starch. A purple pigment at the base of young leaflets may also develop while some cultivars of *S. tuberosum* ssp. *Andigena* and other wild species do not show leaf rolling, but rather stunting and severs chlorosis while infected tubers of some varieties develop internal necrosis, known as net necrosis and other develop thin sprouts from such tubers (Mughal and Khalid 1985). If the potato plant is 100% infected then the yield losses vary between 40-70% (Bhutta and Bhatti, 2002). PLRV is among the important potato viruses in Pakistan and has been reported throughout the country with an incidence of 15-65% (Mughal, 2003) and its prevalence was first reported in 1978 (Mirza, 1978). Different percentages of disease incidence of PLRV was reported from Jhang (12.82%), Sialkot (11.34%), Toba Tek Singh (5.55%), Islamabad (11.7%), Chiniot (4.41%), Okara (3.63%), Rawalpindi (11.7%), Gujranwala (14.37%), Sahiwal (6.81%) and Faisalabad (11.7%) (Abbas *et al*., 2012). High severity of PLRV and lack of resistance to this disease in the majority of varieties/lines indicates that the inoculum level of the virus is increasing and may continue to increase in future while this is mainly due to the presence of diverse virulence of these viruses (Khan *et al.*, 2006). During the year 2011, forty cultivars (varieties/lines/clones) of potato were serologically screened under field conditions to identify resistance against PLRV and seven varieties/lines (Astrix, Mirrato, Oceania, Orla, Hermes, Safreen and 396266-33) were found to be highly resistant, four (FD 7-2, 394021-120, FD 48-4 and FD 49-62) appeared to be resistant and the remaining 19 showed varying degree of susceptibility to PLRV (Umar *et al.*, 2011). During the Year 2010, RT-PCR technique was applied for the detection of PLRV in dormant potato tubers while sense (GCAATGGGGGTCCAACTCAT) and antisense (CGCGCTAACAGAGTTCAGCC) primer was designed from the CP gene encoding fragment of the PLRV genome that amplified a 336 bp product. The amplified product was detected in nucleic acid preparations from leaves and tubers of five cultivars and from purified virions. The specificity of the RT-PCR product was confirmed through southern blot analysis. The primer pair used in the RT-PCR did not produce any nonspecific product from seven other potato viruses. Sensitivity of RT-PCR was confirmed by detecting PLRV from known mixture of PLRV and randomly selected potato virus. Dilution of 1:1000–1:4000 and 1:200–1:1000 were used to detect viral load from foliage and tuber, respectively. RT-PCR efficiently detected PLRV in sprouting tubers as well as dormant tubers stored at 20–25°C for four months (Awan *et al*., 2010).

Potato Mop-top virus: Potato mop-top virus (PMTV) causes an economically important disease of potato while serious yield and quality reductions can occur in some cultivars. The powdery scab fungus (*Spongospora subterranea*) is a soil-borne organism and PMTV survives in the soil within dormant resting spores of its fungal vector that can persist for up to 18 years in the soil. High levels of soil moisture and cool soil temperatures (12-20°C) stimulate resting spores of the fungus to germinate and release virus-carrying zoospores. Zoospores can move only for a very short distance in soil and require free water for movement to occur. Zoospores introduce the virus into the potato plant when they infect the roots, stolons and/or young tubers and systemic movement of the virus within the plant is generally slow and erratic (Kurppa, 1989). When PMTV-infected tubers are planted as seed, the virus is passed on as a secondary infection to only limited numbers of progeny tubers (30-50%). Therefore spread via the obligate vector, powdery scab is the most important means of transmission. Symptoms may occur on the foliage of plants produced from infected tubers and on stems, although not all stems produce clear symptoms. Foliage symptoms develop best at temperatures between 5 and 15°C, particularly during the early stages of plant growth. The most common symptom is the development of aucuba patterns on the stems which consist of bright yellow blotches and ring or line patterns on lower or middle leaves. Less commonly, a second type of symptom may be observed, consisting of pale, V-shaped, chlorotic chevrons, usually on the leaflets of young upper leaves, and ultimately resulting in a distinct mosaic in the upper leaves. A third type of symptom consists of extreme shortening of internodes accompanied by crowding or bunching of foliage, described as a "mop-top". Some of the smaller leaves may have wavy or rolled margins and the overall effect is a dwarfed and bunched growth habit of the infected plants.

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