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A REVIEW PAPER ON POTATO LEAF ROLL VIRUS (PLRV) OF POTATO IN PAKISTAN

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ABSTRACT

Potato leaf roll virus (PLRV) is one of the most important viruses infecting members of Solanaceae family. Among the members of Solanaceae family, potato crop is the most significant host of PLRV. The PLRV belongs to genus *Polerovirus* in the family *Luteoviridae*. It causes variety of symptoms depending on the viral strain, time of infection, host tolerance and environmental factors. The symptoms of PLRV are stunting and dwarfing of infected potato crops, reddening or yellowing and rolling of their leaves. It also reduces the size as well as number of potato tubers with annual global yield losses up to 20 million tonnes. In Pakistan PLRV has caused severe yield losses. It contains positive sense single stranded RNA ((+)ssRNA) of 5.9 kb genome. The virus can be transmitted by aphid vectors in circulative, non-propagative manner and experimentally by grafting. Among the aphid vectors, *Myzus persicae* is known to be its most efficient vector. There are various management strategies but most economic and environmentally satisfactory way of managing is breeding of resistant potato cultivars with effective vector control. Other approaches are thermotherapy, tissue culture, pathogen derived resistance and seed potato certification programs. Since PLRV is responsible for significant yield loss in potato crops of Pakistan, so understanding its biology and developing an efficient management strategy is very important.
Keywords: PLRV, occurrence, distribution, symptoms, transmission, control

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most significant food and vegetable crop of the world and rank fourth in production after cereals such as maize, wheat and rice. Potato is having high nutritive value which contains water (78 %), starch (18 %), protein (2%), vitamins (1%) and several trace elements (Hasse, 2008). The origin of potato is mountainous regions of South America and then it spreads to other countries of the world (Beukema and Eanderzaag, 1990).

Potato is infected by various viruses along with other pathogens (Khan *et al.*, 2008). More than forty viruses are known to infect potato crop (Valkonen, 2007). In Pakistan potato is grown in spring and autumn season in plain areas and as summer crop in hilly areas (Abbas *et al.*, 2012). Plant viruses such as *Potato virus* Y, *Potato leaf roll virus* (PLRV), *Potato virus* A (PVA), *Potato virus* S (PVS), *Potato virus* M (PVM), and *Potato virus* X (PVX) had been reported in these potato growing areas. Among potato viruses PLRV has been considered as major threat to potato production (Peter *et al.*, 2000; Zhang et al., 2010). PLRV is the type species of genus *Polerovirus* in the family *Luteoviridae* (Robert and Lemaire, 1999). The virus particles are isometric, 24nm in diameter (Harrison, 1984). PLRV is single stranded positive sense RNA of about 5.9 kb genome (Miller *et al.*, 1997).

Various aphid species are responsible for natural transmission of PLRV. Among these aphid species green peach aphid (*Myzus persicae*) is the most efficient vector which transmits PLRV through persistent, circulative and non-propagative manner. The virus is a phloem limited virus and is also experimentally transmissible by grafting (Harrison, 1984). The prevalence of *M. persicae* in Pakistan was first reported in 1978 (Mirza, 1978). PLRV has been an emerging problem and widely prevalent in all potato growing areas of Pakistan (Gul *et al*., 2013). PLRV causes rolling and yellowing of potato leaves that later become dry, stiff, leathery, crisp and papery to touch. PLRV also causes net necrosis in potato tubers and inferior crop quality (Harrison, 1984; Radcliffe and Ragsdale, 2002). In Pakistan the yield reduction as high as 90% have been reported for PLRV (Bhutta and Bhatti, 2002). PLRV can be managed by procuring virus free certified seeds, eradicating volunteers and weed hosts and early roughing of infected plants. The management of aphids is also an important strategy to minimize the spread of PLRV

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(Alani *et al*., 2002). Efforts have been made to defend potato crop against viruses by using thermotherapy, tissue culture and micro-propagation. Implementation of strict quarantine measures for seed certification schemes and field spray of insecticides to control vectors were used successfully to manage crop against PLRV (Oosterveld, 1987). This review will focus on occurrence and distribution, management strategies as well as biological, molecular, serological and physical properties of PLRV.

OCCURRENCE AND DISTRIBUTION

PLRV damages potato tubers, fresh markets and processing potatoes of the world. PLRV cause stunting of potato crop and reduction in tuber number as well as tuber size consequently farmers get low yield. PLRV is also responsible for internal net necrosis that makes the tubers unfit for processing and consumption (Radcliffe and Ragsdale, 2002). PLRV was primarily reported from Germany and Denmark in 1905 and now its distribution is worldwide. In Pakistan, PLRV is the most disparaging virus of potato crop (Ahmad and Ahmad, 1995). It was first reported from Punjab followed by Khyber Pakhtunkhwa, Sindh and Baluchistan (Khan and Abbas, 2008). PLRV has severely infected potato growing districts of Khyber Pakhtunkhawa province with the percent incidence as 9.44%, 9.45%, 14.33%, 14.43% and 17.68% in Swat, Dir, Abbotabad and Mansehra respectively. Halim, (1999) reported 60% reduction in yield of potatoes grown from PLRV infected tubers (Bhutta and Bhatti, 2002). Lack of resistance in potato cultivars against PLRV indicate that inoculums level of the PLRV virus is building up and may cause serious effects on yield of potato crop (Khan and Abbas, 2008). PLRV has severely devastated potato crops in plains of Punjab and Sindh with incidence 20-60% (Batool *et al.,* 2011).

Abbas *et al.,* (2012) recorded 52.3 % average incidence of *Potato virus* A (PVA), *Potato virus* X (PVX), *Potato virus* S (PVS), *Potato virus* M (PVM) and *Potato leaf roll virus* (PLRV) from Rawalpindi, Faisalabad, Islamabad and Sahiwal

BIOLOGICAL PROPERTIES

Characteristics symptoms caused by PLRV include chlorosis, reddening, rolling and leathering of leaves, stunting of potato crop and net necrosis of potato tubers (Alani *et al.,* 2002). Symptoms caused by PLRV are categorized into two major types i.e. primary symptoms and secondary symptoms. Primary symptoms are also considered as primary infection which is caused by current season aphid transmitted viruses. The primary symptoms include pallor or reddening of leaf tips of upper or young leaves, after ward these young leaves become roll and erect. Secondary symptoms appear on the potato crops which are grown from the infected tubers. These symptoms consist of stunting of shoots and rolling of oldest or lower leaves upward. Secondary symptoms turned out to be more severe as compared to primary symptoms with leathery texture and overall roll of leaves and with stunted growth and net necrosis. Plants exhibiting primary infection the virus can be transmitted through a variable portion of tubers but in plants with secondary infection all tubers will remain viruliferous (Harrison, 1984). The plants which are infected early in the growing season will be dwarfed and if virus infects the plants late in the growing season even foliar symptoms may not be exhibited. With the age the potato plants are shown resistance to foliar infection consequently no foliar symptoms but there would be virus particles within the host cell (Erik *et al.,* 1993). Infection caused by PLRV can be observed in a circular pattern in the potato fields, generally surrounding the source of virus inoculums, an infected seed piece. Aphids are responsible for direct damage and even kill potato plants technically called aphid's hole in the field (Robert *et al.,* 2000). PLRV translocated through the phloem of the plant into tubers. Afterward the virus reduces size of potato tubers and causes net necrosis. Net necrosis or tuber necrosis is actually manifestations of darkening or browning of the vascular bundles which are extended throughout potato tubers. Potato tubers with symptoms like net necrosis are undesirable for processing into chips and fries (Scagliusi, 2000).

The members of Solanaceae family are the major hosts of PLRV Non-*solanaceous* hosts belong to nine plant families have also been considered as hosts of PLRV. These nine plant families are as;

Chenopodiaceae, Brassicaceae, Malvaceae, Asteraceae, Cucurbitaceae, Lamiaceae, and

Portulacracea (Tamada *et al.,* 1984). *Datura spp* and *Physalis floridana* are considered most excellent diagnostic and propagative host respectively (Harrison, 1984).

PLRV acquired and transmitted through aphid vectors and mechanisms associated with PLRV are circulative, non-propagative and persistent manner (Thomas, 1987). PLRV is restricted in phloem cells therefore it required more time to acquire by aphids (Ragsdale *et al.,* 2001).

PHYSICAL AND MOLECULAR PROPERTIES

PLRV particles are isometric and 25 nanometer in diameter (Harrison, 1984). The genome of PLRV is a single-stranded, positive-sense RNA(5.8kb) with a molecular weight of 2 × 10^6 daltons (, with a small protein covalently linked to the 5' end of no 3' poly (A) tail (Casper, 1988). The positive sense RNA of PLRV has neither a 5'cap nor 3' poly (A) tail but carries a special protein called VPg and an OH group at the 3' end. The protein VPg stands for viral genome linked protein at the 5' end of positive sense RNA of PLRV (Taliansky *et al.,* 2003). Nucleic acid of PLRV is 30% of particle weight and its molecular weight is 2.0 x 10^6 daltons (Harrison, 1984). Genome sequencing has revealed the presence of six open reading frames (ORFs) alienated by a small non coding RNA (Priifer *et al.* 1992). These ORFs are responsible for encoding of various important viral proteins (Prfifer *et al.* 1992). Small ORFs is followed by large ORFs 2a and 2b at the 5'end which may code for 72 and 68 kDa proteins and also contains motifs which are characteristics of enzymes such as helicases and RNA polymerases (Prfifer *et al.* 1992). The genes which are present in highly conserved region in 3' half are translated from a 2.4kb sub genomic RNA (Tacke *et al*. 1990). The subgenomic RNA is not encapsidated into proteins (Smith and Harris 1990). One of the genes in the sub-genomic RNA encodes the coat protein of approximately 23 kDa (Tacke *et al.* 1991).

There is also a single ORF for 17 kDa protein which has a regulatory role during replication of PLRV (Tacke *et al.* 1991). The coat protein of luteoviruses is responsible for transmission specificity (Vanden Heuvel *et al.* 1990) and serological properties (Massalski and Harrison 1987). It is assumed that the read through protein coupled with the coat protein at its C terminal plays a significant role in the transmission of virus (Mayo *et al.* 1993). PLRV consist of single stranded non-polyadnylated RNA molecule with plus orientation that encodes six open reading frames (ORFs). Among these six ORFs the three (ORFs) are located near the 3' ends and encode sub genomic RNA molecules, the 23 kDa coat protein (CP) (Vanderwilk *et al.,* 1989). Sokolova *et al.* (1997) concluded that the given three open reading frames encode a 17 kDa fluolimetric movement protein. Chayca *et al.,* (1996) reported that a 56 kDa protein plays major role in the virus or virus vector interaction. Awan *et al.* (2010) demonstrated molecular detection of PLRV through reverse transcription polymerase chain reactions (RT-PCR) in potato tubers. It was concluded that expected nucleotide sequence of amplified PLRV-CP gene shows homology of 94 to 97 % when compared to the sequences already reported in Gen-Bank database. Jeevalatha *et al.,* (2013) reported that genome of PLRV harbors nine open reading frames (ORFs). These nine ORFs are numbered from 0 to 8 coding for proteins (P0-P7) and Rap1 respectively. Three 5' proximal ORFs encode the proteins (P0, P1 and P2). Five other ORFs are expressed from two subgenomic RNAs (sgRNAs) namely sgRNA1 and sgRNA2. The proteins P3, P5 and P4 are encoded by sgRNA1 where as P6 and P7 which are 3' –proximal proteins are encoded by the sgRNA2. The proteins P3 and P5 are the structural proteins. The remaining ORFs, the ORF1 contains a small ORF whereas ORF 8 encodes Rap 1. The functions of various proteins harbored by ORFs of PLRV are shown in Table 1

Table 1: Functions of various proteins of PLRV

Proteins	Functions
P0	a. Symptom development, suppressor of RNA silencing b. F-Box motifs to overcome PTGS
P1	a. VPg
P2	a. Conserved motifs typical of RNA dependent RNA polymerases (RdRp)
P3, P4 and P5	a. Capsid protein (CP) b. Movement protein (MP) c. Read through domain (RTD)
P5	a. Interaction between virus and vector b. Vector specificity
P6	a. Functions are not known
P7	a. Nucleic acid (NA) binding properties
Rap1	a. Virus replication

SEROLOGICAL PROPERTIES

PLRV virus is serologically associated with *Tobacco necrotic dwarf virus* (TNDV), *Pepper vein yellows virus* (PeVYV), *Barley yellow dwarf virus(*BYDV) and *Beet mild yellowing virus* (BMYV) or *Beet western yellows virus* (BWYV) (Harrison, 1984). Relationships to several members of this group have been detected by gel-diffusion serological tests, immune electron microscopy and by density gradient zone-depletion serological tests The closest relationships were found to be with tobacco necrotic dwarf (SDI = 2), beet western yellows (SDI = 2-5) and bean leaf roll viruses, however, antisera to PLRV react more powerfully with particles of the beet viruses than do antisera to the beet viruses with particles of PLRV (Harrison, 1984).

CROP MANAGEMENT STRATEGIES AGAINST POTATO LEAF ROLL VIRUS

Thermotherapy

Thermotherapy is one of the components of physical method that could be used for the management of PLRV in infected potato tubers. Kassanis, (1950) demonstrated the efficacy of thermotherapy in freeing tubers of PLRV. PLRV was the only virus listed as being inactivated form potato tubers by thermotherapy (Kaiser, 1980). Kaiser, (1980) demonstrated the effectiveness of hot air treatments for eliminating three viruses i.e. alfalfa mosaic virus (AMV), PLRV and tomato black ring viruses (TBRV) from potato tubers. These viruses were eliminated from cultivars of potato by hot air treatment at 37 ^{0}C at various intervals of time. This treatment also eradicated viruses in tubers dually infected with PLRV and TBRV. Viruses could not be detected from these tubers following repeated indexing by mechanical means and by serological methods. Kaiser (1980) report was the first report about thermotherapy on the African continents to free potato tubers of virus infection. Since its introduction in 1949 thermotherapy has been widely used to inactivate PLRV from the potato tubers. However thermotherapy did not inactivate rod-shaped viruses. The reported control of PLRV in tubers of potato by hot water treatment at 50-52 ^{0}C for 17-20 minutes would noticeably reduce the time and space required to liberate tubers of virus infection. Abbas *et al.*, (2016) conducted an experiment to evaluate the effectiveness of hot water treatment to inactivate PLRV from potato tubers. Potato tubers were treated at average 37 °C for various intervals of time. Treatment of potato tubers with hot water at 2 ½ hours were found to be effective in fully or partially elimination of PLRV from potato tubers.

RESISTANT POTATO CULTIVARS AND TRANSGENIC POTATOES

Cultivation of resistant potato cultivars is sustainable strategy for control of PLRV (Beekman, 1987). Unluckily partial resistance against PLRV is present in most of the potato cultivars. Breeders are attempting to produce resistant varieties but hampered by its polygenic inheritance. The remarkable resistance against PLRV so far identified in wild species such as *Solanum brevidens, Solanum demissum, Solanum acaule* or in complex hybrids (Baker *et al.,* 1992). It can be transferred into potato cultivars using the fusion of protoplast. These attempts to incorporate the resistant genes from wild species into potato crop resulted in the release of resistant cultivars but are not immune to PLRV. Since the success of using genes of wild species to increase resistance to PLRV is

limited because the resistance is a polygenetic trait and is controlled by many genes. The trait is also associated with shape and size of potato tubers and yield. Recently transgenic plants have been produced which confers resistance to PLRV. The concept that the expression of viral gene sequences in transgenic plants (Pathogen derived resistance) can protect potato crop against virus infection. The gene sequences include sequences which encode viral coat protein (Coat protein mediated resistance) and replicase related protein (Replicase protein mediated resistance). The other pathogen derived resistance is; movement protein mediated resistance, polymerase mediated resistance and ribozyme mediated resistance (Ruth and Barker, 2001). The other phenomenon to manage PLRV is genetically engineered cross protection which is expression of mild strains in potato crops against severe strains of viruses. The results obtained so far with transgenic potatoes having viral sequences are promising but not fully satisfactory (Syller, 1996).

VECTOR CONTROL THROUGH INSECTICIDES

Vector control consists of several practices (Milosevic, 1996) which often include insecticide treatments as fundamental part since aphids transmit viruses in field during a growing season. Robert *et al*. (2000) applied some epidemiological methods to manage aphid born virus diseases i.e. PLRV has been managed in seed potato crop in Northern Europe. The research has shown that mineral oils interfere with virus retention in the vector (Aphids) mouth parts. Similarly *Cucumber mosaic virus* (CMV) transmission was reduced by foliar applications of mineral oils on pepper plants. All these studies demonstrate that mineral oils are effective tools to control non persistent viruses (Loebstein and Raccah, 1980). Ali *et al*. (2011) evaluated some chemicals against aphids, jassids and white flies in potatoes. Six insecticides were applied against these pests of potato. All these insecticides showed 86 % mortality. Sharp 25WP and 10EC caused the highest 96.5% mortality in aphids. Milosevic *et al*.(2012) study was to analyze a potential use of insecticides in preventing the transmission and spread of PLRV during seed potato production, by controlling aphid vectors. Their studies revealed that Confidor showed the highest mortality in aphids as compared to other insecticides. Olubyo *et al*. (2010) recommended that insecticides in combination with mineral oils could play most important role in reduction of aphids whereas synthetic insecticides were regarded more effective in managing PLRV as compared to mineral oils. Furthermore, the effect of insecticides used to control PLRV transmission has also been reported in the literature (Van Tor and Teulon, 2006). Insecticides cause specific effects in preventing the transmission of PLRV from infected to healthy plants (Van Toor and Teulon, 2006).

Mowry, (2005) reported differences in the efficacy of insecticides in controlling PLRV, emphasizing that the most effective are Imidacloprid and Thiamethoxam. The trial involving experimental transmission of PLRV by the *Myzus persicae* aphid showed high efficacy of insecticides such as Pymetrozin, Imidacloprid and Thiametoxam.

Kotzampigiikis *et al.* (2008) reported higher efficacy of insecticides in preventing the transmission of PLRV by aphids. The researcher further suggested that the most effective insecticides are Imidacloprid and Thiamethoxam in controlling aphids species. Further he concluded that systemic insecticides are efficient foliar insecticide to reduce within field spread of PLRV, especially if colonizing aphids are virus-free on arrival. Ragsdale *et al.* (2001) recommended insecticides Carbamate and Aldicarb to control wingless aphids which are responsible for spread of PLRV within field but doesn't prevent transmission by winged aphids. He also studied that Chloronicotinyl class of insecticides i.e. Imidacloprid and Pymetrozine and revealed that insecticides are appearing to be effective in controlling within-field spread of PLRV. Boiteau and Singh, (1999) studied Confidor and found effective in reducing PLRV spread in the potato crop. *M. persicae* is the most important vector of PLRV so its control is usually focused (Ragsdale *et al.,* 1994). Ragsdale *et al.* (2001) suggested timely application of insecticides for managing PLRV in potato tubers is usually affected by aphid biology and their arrival time relative to age of potato crop. Mowry, (1994) assigned ten aphids per hundred leaves as action thresholds for seed potato crop but such action thresholds are region specific. Weekly monitoring of aphids is essential which provide rapid results

to apply control measures. However intensive practice of using insecticide is expected to contribute resistance in aphids. Cranshaw and Baxendale, (2005) reported that repellants such as azadirachtin(neem) slowed down the spread of PLRV but these may be more effective when applied as oil formulations.

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