Plant Viruses in Gilgit-Baltistan (GB) Pakistan: Potential Future Research Direction

Aqleem Abbas1, Muhammad Aamir Sohail1, Mustansar Mubeen1, Mohammad Murtaza Alami2, Muhammad Umer1 and Shahid Ullah Khan3

1State Key Laboratory of Agricultural Microbiology and Provincial Key Laboratory of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, P.R. China, 2College of Plant Science and Technology, Department of Crop Cultivation and Farming System, Huazhong Agricultural University, P.R. China, 3College of Plant Sciences and Technology, National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, P.R. China

ABSTRACT

Potato is becoming the most economically important crop in GB because its production is expanding more rapidly than that of most other crops primarily because it generates huge incomes to the farming community. The disease resistant potato cultivars were replaced by the high yielding varieties, consequently the potato crops has become vulnerable to numerous diseases. Among the diseases viral diseases may play a major role in reducing the yield of potato crops. There are no proper regulatory policies intended to prevent introduction of plant viruses, introduction has still occurred through the potato tubers from the other provinces of Pakistan. Furthermore, the vectors such as aphid’s population have increased. These plant viruses have considerable effects on potato crops range from mere curling and chlorosis of leaves to demise of entire potato crops from the fields. These plant viruses may be major yield limiting factor and therefore there is need to identify the viruses on farms and to determine the severity as well as their impacts on potato yield. Theses plant viruses are major future threats to GB’s food security. There are no well-established plant pathology laboratories as a result their identification and estimation of losses to potato crops, they pose still remain a challenge. In conclusion, the viruses are the future constraints to food security in GB, this review paper uses some plant viruses as a case study to illustrate their key influences on the yield of potato crop.

Keywords: Gilgit-Baltistan (GB); Potato; Food security; Potential plant viruses

INTRODUCTION

Potato (Solanum tuberosum L.) is very economically important crop of family Solanaceae. This family mostly contains herbaceous plant species. Potato is also nutritionally important crops contains 70% water, 18% starch, 2% protein while 1% vitamins, minerals and trace element have been recorded [1]. The potato crop is cultivated almost all countries [2]. Potato crop is among the world’s leading food and vegetable crops. It originated in South American and now as become an important crop in almost all countries including India and Pakistan [3]. It is an important food crop of the world ranking fourth after cereals. About 40% of the world potatoes are grown in Europe, 35% in developed countries and 25% in rest of the world [4].

Europe produced 90% of the world potato before World War II. Currently most of the potato is grown in Asia [1]. According to Harris, When Portuguese traders set food in the subcontinents, they also introduced potato tubers. Over the years, potato has become an imperative crop for the people of India and Pakistan. Moreover, potato crop ranks fourth most significant crop by volume of production and important cash crop due to its high yielding and high nutritive value property. The potato crop gives more profits to farming community as compared to other crops [1].
LITERATURE REVIEW

Pakistan has very unique climate in the world for the cultivation of potato crop. Irrigation system combined with climatic condition allowing the cultivation of three crops round the year in various agro-ecological zones from sea level to 3000m altitude. Autumn crop in plains and in southern Punjab, plains of Balochistan and Sindhb, spring crop is cultivated in the plains and lower hills of Balochistan, NorthWest-Frontier Province; and one summer crop is cultivated in the high hilly northern areas (Gilgit and Skardu), North Western Frontier Province and Azad Jammu Kashmir [5,6].

Potato is among the most significant vegetable and cash crops in Pakistan. It is cultivated over an area of 159.4 thousand hectares with a total production of 3491.70 thousand tons and about 21.90 tons average yield per hectares are obtained [7-9]. Potato is grown in autumn and spring in the plains however in the mountainous region potato is grown only in summer [2] Gilgit-Baltistan (GB) is northern most mountainous region of crop where potato crops is cultivated only in summers season. Potato is grown on an area of 8526 ha with an annual production of 134031 metric ton. Production and area wise potato is ranked first followed by wheat and maize [7]. Though potato crop is known as high yielding crops however crop is vulnerable to diseases caused by fungi, bacteria, viruses and nematodes [10-14].

Among these diseases, viral diseases along with the potato late blight pathogen (Phytophtora infestans) and the early blight diseases have considerable effects on potato crops in GB. However proper diagnosis, effects of these pathogens on potato yield and severity has not yet been conducted. Potato crop has been continuously suffering from plant viruses. Potatoes are susceptible to about 40 viruses and two viroids. However in Pakistan, the most economically important viruses of potato are potato virus M (PVM), potato virus S (PVS), potato virus X (PVX), potato virus A (PVA), potato virus Y (PVY) and potato leaf roll virus (PLRV). Among these viruses, PVX, PVY and PLRV are considered to be the most destructive. Yield reduction in potato by these viruses may reach up to 80% in susceptible cultivars, but an even greater loss might be incurred when PLRV occurs in mixed infection with PVX or PVY [14,15].

Gilgit-Baltistan has two divisions Gilgit and Baltistan. These divisions are further divided into districts. Skardu, Shigar, Kharmang and Ghanche are districts of Baltistan division while Gilgit, Ghizer, Diamer, Astore, Hunza and Nagar are major districts of Gilgit division [1-3]. Main crops are wheat, mazie, potatoes, fruits mostly include dry fruits and Main vegetable includes potatoes, tomatoes, Turnips, Carrots. Over the last fifteen years, potato production has increased dramatically and now is major cash crops in all the districts of GB. GB is a mountainous region and its high elevated valleys are more suitable for potato production due to the availability of ideal climatic conditions. In summer hot days and cool nights make these valleys more conducive for the production of potato crop. Potato crop is sown during March to April months and harvested in August-October as a summer crop. The per hectare potato yield is 23.64 ton as compared to rest of the country’s potato growing area (17.7 ton) which is declining over the period of time due to lack of crop rotation, lack of proper technology, timely availability of inputs, rising production costs, pests and plant diseases [16].

Twenty years back potato crop was cultivated on a subsistence base. With little or none existence of trade, lack of proper roads to markets and unawareness about the economic importance of potato crops, the people of GB were doing subsistence farming. At the time wheat was considered as important food crops as there were no proper supplies of wheat from other provinces. Further wheat crops were devastated by rust and smut diseases. These diseases and high value potato commodity propel GB farmers to accept potato crop as major cash crops. Since then the potato production has considerably increased. The area is considered rich basket of producing quality seed potato. Unfortunately, in the recent years, potato production seems to be decline and this could be due to biotic and abiotic constraints. Biotic constraints include diseases, insect pests and weeds. Among the diseases, the viruses (PLRV, PVY, PVX) and fungi (early blight, late blight, black scurf) might be major biotic constraints. Till now there are no publish reports, about the losses caused by these diseases. Though there are local reports about the severity of these diseases but there is a need to verify these diseases and severities on molecular as well as serological basis. There is a report on black scurf disease of potato which is a very serious problem in all potato growing valleys of Gilgit-Baltistan but further verification through molecular basis is required [17]. Among the viral diseases, potato leaf roll virus (PLRV), potato virus Y (PVY), potato mop top virus (PMTV), potato virus X (PVX), potato virus S (PVS) and potato virus A (PVA) could be the major viral pathogens in Gilgit Baltistan.

The reason is as the potato tubers were introduced with the other provinces of Pakistan and as a result the infected potato tuber with latent pathogens might have also entered into GB. The damages posed by plant viruses on potato crops are more than other plant diseases. Because viruses reduce both the yield and quality as a result market value of potato crops drastically reduces. Moreover, the severity of individual viral diseases may vary with the locality, virus species, stage of infection and crop variety [18].

Growers are unaware of the diseases and unintentionally introduced superficially seem to be healthy but cryptic diseased potato cultivars to GB. As the potato crop is major cash crop therefore Growers are struggling to high yielding potato cultivars as a result viral disease are also introduced. According to one study these viruses can be responsible for 83% yield losses to potato crop [4]. Among the potential plant viruses in GB, Potato leaf roll virus (PLRV) and PVY can be ranked first as personal observation based on symptomology, potato crops leaves in several areas of GB seem to yellowing, curling and rolling and potato tubers have shown necrotic areas. PLRV is the type species of the genus Polerovirus, family Luteoviridae [19,20]. The virus particles are isometric, 24 nm in diameter [21]. The genome of PLRV consists of a positive-sense single-stranded RNA of about 5.9 kb [22,23]. Potato virus Y (PVY) is the most dangerous plant pathogenic virus belongs to largest genus of Potyvirus of plant viruses and in the family Potyviridae. Furthermore, PVY has been classified into several isolates based on extensive biological, serological and molecular studies.
Furthermore, PVY is one of the major emerging diseases of potato crop in Pakistan [22,23]. However, both viruses PLRV and PVY are transmitted by various aphid species. Aphids (Myzus persicae) (family: Aphididae) are the most efficient vectors which transmit virus through persistent, non-propagative manner. Among the aphids, the Green peach aphid is known to be most important vector of both viruses. For the first time, in Pakistan the prevalence of Myzus persicae was reported in 1978 [24]. A research was carried out to explore aphid fauna of Gilgit-Baltistan of Pakistan the collection surveys were carried out yielding 15 species in 10 genera and 2 families. Among these genus Myzus and species of Aphis were also reported [25]. Furthermore, the transmission of the viruses by insect vectors is also a complicated process that includes the interactions between the virus, vector and plant [26]. Virus transmission also depends on many factors including the aphid biotype, species, clones, morphs, genotype, virus isolates and environmental factors [27,28]. Both viruses i.e., PLRV and PVY are also transmitted through infected tubers from one growing season to another, while during the season; they are transmitted from infected plants within the crop and from plants in the surroundings (source of infection) by various species of aphids [29,30].

According to previous reports from other provinces of Pakistan, PLRV has considerably affected the potato production [27-31]. About 70% yield losses due to PLRV has been reported from Pakistan [31]. In some potato production areas of Pakistan, the yield losses have been recorded up to 90% [18]. Aphids are mainly responsible for rapid spread of PLRV especially spring potato crop [10].

Its natural host range is mainly restricted to a few solanaceous plants, including Physalis floridana Rydb., Datura stramonium L. and Lycopersicon esculentum in addition to the cultivated potato [32]. PLRV multiplies largely in phloem tissue and disease symptoms reflect this localization. Infection, especially from infected seed potato (secondary infection) causes leaf rolling and stunting. Symptoms of primary infection are usually less severe unless plants become infected early in the season. In tubers, virus infection results in net necrosis which is expressed as darkening of vascular bundles. Replication probably occurs in phloem companion cells [32]. PLRV can be effectively managed by employing four methods viz., obtaining virus-free certified seed, killing volunteers, weed hosts, controlling aphid species and early rouging of infected plants. Since PLRV is not mechanically transmissible, aphids are solely responsible for the in-season spread of the virus. Therefore, the management of aphids is a key for the management of PLRV [33].

Efforts have been made to protect crop plants against viruses including potato by producing virus free seed tubers stock using thermotherapy, tissue culture and micropropagation. 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 [34].

More than 40 species of aphids are transmitting PVY in natural conditions [34,35]. Myzus persicae alone is a vector of more than 150 types of viruses [36-38]. It is the most efficient aphid vector of PVY [35-38]. Mineral oils are widely used to reduce transmission of PVY, because these oils change aphid’s feeding behavior. Moreover aphid’s styllet penetrations in a plant host could also be late when plants are treated with mineral oil [38]. Moreover, mineral oil had been revealed for having a repulsive effect on aphids, however the repulsive effect remains only for a short period of time (30 min after treatment) [38].

Pesticides are used to control aphids, but many aphid species have become resistant to various chemical compounds. Systemic insecticides and/or accurately timed foliar insecticide applications are useful to reduce within field spread of PLRV and PVY, especially if colonizing aphids are virus free on arrival [39,40]. PLRV was eliminated from diseased tubers of several potato cultivars by hot air as well as hot water treatments [40]. Continuous application of insecticides contaminates the environment, increase the cost of production and as well as increases resistance in aphids against such insecticides. In comparison to insecticides, mineral oils are less harmful to environment and more significant in reduction of PVY transmission by aphids. The need to promote more rational use of pesticides has been a great incentive to manage aphids as well as viruses. PVY and PLRV are considered to be the most destructive. Yield reduction in potato by these viruses may reach upto 80% in susceptible cultivars, but an even greater loss might be incurred when PLRV occurs in mixed infection with PVX or PVY [41].

The incidence of six potato viruses (PLRV, PVX, PVY, PVS, PVA and PVM) was surveyed in spring, summer and autumn crops of Khyber Pakhtunkhwa. A total of 1338 samples from 76 fields were tested by dot-immunobinding assay. Two major aphid-borne viruses, PLRV and PVY were frequently detected in potato with incidence ranging from 0-14.7 percent in spring crops, 1.8-45.5 percent in summer crops and 0-71 percent in autumn crop [14]. Potato growing areas in Punjab, AJK, Khyber Pakhtunkhwa, Baluchistan and Sindh have been adequately surveyed and potato diseases were monitored and identified [6-18]. However no substantial work seems to have been done on potato diseases in Gilgit-Baltistan (GB). The incidence and distribution of potato diseases have been increased in Gilgit-Baltistan to a point where successful seed and ware potato production in some areas is impossible. It is therefore required that detailed studies on some of the most important and emerging potato diseases in the region could be made for the management of seed and ware potatoes against such disease problems. The variation of the altitudes and other weather conditions in Gilgit-Baltistan (GB) and the effect of such conditions on potato diseases also need to be exploited. Furthermore, it is required to look new pockets for seed potato production in Gilgit-Baltistan (GB) and the effect of vector and weather conditions on potato diseases are also need to be studied.

**Occurrence, symptoms and distribution**

Potato has been successfully grown in Pakistan, both in plains and hilly areas, depending upon season. Potato is generally a high yielding crop, but crop is vulnerable to the attack of a number of diseases that are caused by fungi, bacteria, viruses...
Potato leaf roll virus (PLRV) is one of the most detrimental viruses of seed potatoes, processing and fresh market potatoes of the world. Potatoes infection with PLRV cause yield losses through stunting of plants and reduction in tuber number as well as size. In addition, infection may lead to internal net necrosis, resulting in tubers being inappropriate for processing [42]. PLRV was primarily reported from Germany and Denmark in 1905 and now its distribution is worldwide [34]. In Pakistan, PLRV is the most disparaging virus of potato crop [37]. It was first reported from Punjab [43-45], Khyber Pakhtunkhwa [46] and Sindh and Baluchistan [47]. PLRV is widely distributed in major potato growing districts of Khyber Pakhtunkhwa Province with the incidence as 8.44%, 8.45%, 13.33%, 13.43% and 16.68% in Swat, Dir, Abbottabad and Manshera, respectively. Hamm and Hane [48] reported 60% reduction in yield of potatoes grown from PLRV infected tubers. In Pakistan yield losses due to PLRV have been recorded up to 90% [18]. 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 [42]. Fifteen potato advance lines were screened against Potato Leaf Roll Virus (PLRV), Potato Virus X (PVX), Potato Virus Y (PVY) under favorable natural field conditions and in vitro by using double antibody sandwich ELISA (DAS-ELISA). On the basis of symptomatology, only two lines FD70-1 (PRI RED), SL15-26 were found free of symptoms to PLRV. No one genotype was found to be highly resistant against PVX and PVY. Environmental factors had a great influence on disease severity. In ELISA screening no one advance line was highly resistance against six potato viruses. Three lines namely viz; FD70-1 (PRI RED), SL15-24 and SL15-26 were found resistant, five moderately susceptible and seven susceptible in case of PLRV. In PVY, two advance lines i.e., FD70-1 (PRI RED) and SL1405 were found resistant. In PVX, only one line SL15-26 was found to be resistant. In PVA, four resistant advance lines, eight moderately susceptible and three were found as susceptible. In PVY, only one resistant genotype, six moderately susceptible and eight were found as susceptible. Likewise in PVM there is no genotype as a resistant, eight moderately susceptible and seven were found as susceptible. The resistance germplasm of potato screened under field conditions and by ELISA of different potato viruses may be used to produce the virus free germplasm in the next breeding programme [49].

Potato Virus X (PVX) causes significant damage to plants of the families of Amaranthaceae, Cruciferae, Solanaceae and some member of Leguminosae. Most important damaged crop plants are potato (Solanum tuberosum), tobacco (Nicotiana tabacum), brassica (Brassica campestris ssp. rapa), Lycopersicon esculentum). The damage becomes more significant when PVX attack with association of other viruses like PVY and PLRV. PVX is often a latent virus i.e., the symptoms are not clearly visible to the naked eye. It may show symptoms ranging from a mild mottling of the leaf to a severe mottling of the plant with roughening and reduced leaflet size. Mottling may be more visible in the cloudy weather, and may not be existent after a few days of sunny weather. The overall growth of plant may be stunted with small leaves. In some cases, the tips of the plant may die [50] The PVX is distributed worldwide in potato grown areas. It is transmitted mechanically by plant to plant contact (leaves, shoots and roots), cutting tools and animals. Chewing insects such as grasshoppers have also been suspected as a means of spreading the disease. There must be wounding and an exchange of plant sap for infection to occur [41].

Potato Virus Y (PVY) causes significant yield loss in variety of crops of solanaceous family including potato (Solanum tuberosum L.), tobacco (Nicotiana tabacum L.), tomato (Lycopersicon esculentum) and pepper (Capsicum spp. L.), wherever they are cultivated [51]. The yearly transfer is mainly via potato tubers. Different strains show different symptoms. Primary infection with PVYO and PVYAC strain group isolates induces necrosis, motting or yellowing, necrotic leaf spots or rings, leaf drop and premature death of stems. Sometimes necrosis in leaf, dwarfing and crinkling symptoms causes in potato by secondary infection of PVYO and PVYAC [52-55]. PVYN produces milder form of leaf motting. Plants infected with PVYO, PVYC and PVYN produces the tubers with no symptoms. However, with PVYNNTN produces tubers with irregular brownish colored rings on skin, which forming necrotic arc in the flesh and cracking the skin at the surface. Isolates of PVY are placed in different strain groups. This division is based on the mosaic symptoms (PVYO, PVYC and PVYZ) or necrotic symptoms (PVYN) induced in tobacco and potato [56].

PVYN has a subgroup of isolates, designated as PVYNNTN that includes those isolates causing necrotic ring spot in the tubers. 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 potatoes germplasms were certified through Enzyme Linked Immunosorbent 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 PVY has been confirmed in different potato growing areas of Punjab through ELISA in Toba TekSingh (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.

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 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 [4].

PVY has been confirmed in different potato growing areas of Punjab through ELISA in Toba TekSingh (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 PVYO strain is reported serologically. 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 reveal a new strain of PVY in Pakistan which was not previously known [3].

Six varieties/advance lines viz TPS-9801, 394017-45, TPS-9620, 304509-129, TPS-9804 and 391202-103 were selected to study the relationship of environmental conditions (maximum and minimum temperature, relative humidity, wind velocity, clouds, pan evaporation and wind direction) with Potato Virus Y (PVY) disease. Maximum PVY severity was recorded at 24-28°C and 9-12°C as maximum and minimum temperatures, respectively. There was an increasing trend of PVY disease development at maximum temperature i.e., 13-31°C and at 5-13°C minimum temperature. Relative humidity of 78-84% showed increasing trend with higher r values (0.98). Disease severity was recorded at 1.7-2.5 mm pan evaporation as explained by higher r values (0.98). None of the variety had significant correlation with clouds, wind velocity and wind direction [57-60].

**Biological properties**

Characteristics symptoms caused by PLRV include leaf rolling, chlorosis, reddening, leathering of leaves, phloem necrosis and stunting of potato crop [11]. Symptoms of PLRV through primary infection also called current season transmission by aphids in potato plants include pallor or reddening of leaf tips of upper leaves, which may roll and become erect. Secondary symptoms build up in plants which are grown from infected potato tubers including stunting of shoots and leaflets rolling upwards, starting with the oldest leaves or lower leaves. In plants with primary infection the virus is transmitted through a variable portion of tubers, whereas all tubers on plants with secondary infection are viruliferous [32]. Plants infected early in the growing season may also be dwarfed but if virus infection occurs late in the growing season foliar symptoms may not be exhibited. With the age potato plants show resistance to foliar infection [24]. Many times, infection can be observed with a circular pattern in the field, frequently surrounding the original source of virus inoculums, an infected seed piece. Aphids are responsible for direct damage and even kill potato plants producing what are referred to as an ‘aphid hole’ in the field [61-64]. PLRV translocated through the phloem of the plant into tubers, reducing size and causing net necrosis. Net necrosis causes browning of the vascular system extending throughout the entire tuber. Tubers with net necrosis are undesirable for processing into chips and fries, causing serious losses to growers and potato processors [65-66].

Most of hosts of PLRV belong to the Solanaceae family. Non-solanaceous hosts have been reported from a few species belonging to nine plant families. These include the Chenopodiaceae, Brassicaceae, Malvaceae, Asteraceae, Cucurbitaceae, Lamiaceae, and Portulacaceae [67-71]. Datura spp and Physalis floridana are considered most excellent diagnostic and propagative host respectively [32].

PLRV acquired and transmitted through aphid vectors and mechanisms associated with PLRV are persistent, circulative and non-propagative [71-74]. As PLRV is restricted in phloem cells therefore it required more time to acquire by aphids [62]. In a study conducted in Plant Pathology Department, University of Agriculture, Faisalabad twenty-nine potato varieties/lines were screened against potato leaf roll virus (PLRV) under natural field conditions favorable to induce maximum virus infection of PLRV. Nineteen varieties/lines were considered as moderately susceptible and six were susceptible to PLRV. ELISA test was carried out to identify resistant source and confirm the presence of virus. The results showed that only 25 varieties/lines were ELISA positive. This proves that ELISA is more reliable and sensitive serological assay to detect the resistant virus free source.

**DISCUSSION**

PVX 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 [74-78]. 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 [38]. PVX is distributed throughout potato growing areas of Pakistan ranging infection between 1.56-2% 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 [21]. 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%), Toha Tek Singh (2.77%), Rawalpindi (16%), Islamabad (23%) and Faisalabad (36%) [54]. OCEANIA, FSD-RED and FD3713 showed resistant, FD3-10, FD3-9 and 393574-61 were highly resistant and Mirrato, Arterix and Desiree were moderately susceptible against PVX [8]. Among the environmental factors, temperature (25-28°C) 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 28°C and the disease severity decreased [60]. Serological confirmation of PVX was used for screening purpose but coat protein gene specific sense (GGCGCAACTCCTGCCACAGC) and antisense (TTTGTGTCTCCAGTGATACGA) 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 [37].
Influence of environmental factors

Potato virus X (PVX) and Y (PVY) disease severities had significant correlation with maximum and minimum temperatures, relative humidity and pan evaporation. PVX had non-significant correlations with wind direction, while PVY with clouds, wind velocity and wind direction. Out of the twenty-one lines, none of the variety had significant correlation with clouds, wind velocity and wind direction for the causation of PVX and PVY [75-82]. Eighteen and six varieties, 18 and four, 18 and one, 18 and three varieties had negative, significant but negative significant and positive significant but negative correlations with maximum temperature (15-31°C), minimum temperature (5-13°C), relative humidity (72-88%) and pan evaporation (0.5-2.8 mm), respectively with PVX and PVY [53]. Correlation studies between environmental factors (temperatures and relative humidity, rainfall, aphid) and PVX and PVY infection in selected lines revealed negative and significant interaction in case of maximum and minimum temperature but in case of relative humidity rainfall the interaction was positive and significant and the relationship of aphid in case of PVY was also positive and significant [50].

CONCLUSION

The effects of potato viruses have been exacerbated and these viruses are emerging as future threats to potato crops and thus to food security of GB. Proper monitoring of potato fields and detection of viruses through serological and molecular methods is very important. Furthermore, guidance to farmers regarding usage of cultural practices and aphid control through biocides is needed. Furthermore, advanced cost-effective technologies such as drones-based disease detection, image based, volatile profiling based and next generation sequencing technologies (NGS) can be employed to monitor the health and diseases of potato crops.

REFERENCES

1. Abbas FM, Hameed S, Rauf A, Nosheen Q. c. Pak J Phytopathol. 2012; 24: 44-47.
2. Abbas MF, Hameed S. Identification of disease-free potato germplasm against potato viruses and PCR amplification of potato virus X. Int J Biol Biotech. 2012;9:335-39.
3. Abbas MF. Cloning and sequencing of potato virus Y coat protein gene infecting potato crop of Pakistan. M.Sc. (Hons) thesis. Dept Pl Pathol. PMAS, Arid Agriculture University Rawalpindi, Pakistan. 2011;6:45-50.
4. Abbas MF, Aziz-u-din K, Ghani A, Qadir A, Ahmed R. Major potato viruses in potato crop of Pakistan: A brief review. Int J Biol Biotech. 2013;10:425-443.
5. Ashraf A, Rauf A, Abbas MF, Rehman R. Isolation and identification of Verticillium dahliae causes wilt on potato in Pakistan. Pak J Phytopathol. 2012;24:112-116.
6. Khalid S, Iftikhar S, Munir A, Ahmad I. Potato diseases in Pakistan. PARC, Islamabad, Pakistan. 2000;7:164-165.
7. Agriculture Statistics Survey Report. Department of Agriculture Gilgit-Baltistan, Pakistan. 2009;1-20.
8. Ahmad N, Khan MA, Ali S, Khan NA, Binyamin R, Faraz A. Epidemiological studies and Management of Potato Germplasm against PVX and PVY. Pakistan Journal of Phytopathology. 2011;23:159-165.
9. Ahmad N, Khan MA, Khan NA, Binyamin R, Khan MA. Identification of resistance source in potato germplasm against PVX and PVY. Pak J Bot. 2011;43:2745-49.
10. Ahmed M, Ahmed W. Detection of major potato viruses from different potato growing localities of Punjab. Natl. Seminar. R and D. potato. Prod. Pakistan PSPDP/PARC, Islamabad. 1995;175-79.
11. Alani RA, Alesawai UN, Almashaikhy SA. Isolation of proteins from Datura stramonium has ability to inhibition the multiplication of potato virus Y (PVYn). Jerash Journal for Research and Studies. 2002;7:9-21.
12. Alphey TJW, Woodford JAT, Gordon SC. Field and laboratory studies on the Control of nematode and aphid virus vectors in potatoes by pesticides applied as sidebands. Crop Protection. 1986;5:114-21.
13. Arif M, Ravi SJH. Potato Clean Seed Production Manual. International potato center (CIP) and International Center for Agriculture Research in the Dry Areas (ICARDA) 2006;20-30.
14. Arif M, Mughal SM, Khalid S, Hassan S. Some biological, physical and serological properties of PLRV in Pakistan. Pak J Bot. 1995;27:233-41.
15. Asia B, Khan MA, Farooq J, Mughal SM, Iftikhar Y. Elisa-based screening of potato germplasm against potato leaf roll virus. Journal of Agriculture Research. 2011;49:57-63.
16. Beukama HP, Eanderzaag DE. Introduction to Potato Production, Center for Agricultural Publishing and Documentation (PUDOC), Wageningen, UK. 1990;8-13.
17. Bhutta AR, Bhatti MFJ. Seed potato certification in Pakistan. Federal Seed Certification and Registration Department Ministry of Food Agriculture and Livestock, Islamabad, Pakistan. 2002:60-66.
18. Blackman RL, Eastop VE. Aphids on the World’s Crops. (2nd edn), West Sussex, England. 2000;466-70.
19. Bridge J, Page SLJ. Estimation of root-knot nematode infestation levels on roots using a rating chart. Trop Pest Manage. 1980;26;296-98.
20. Burhan M, Khan MA, Irfanullah M, Ishfaq M, Ihsan MJ. Comparison of seed potato from different multiplication sources against PVX, PVY and PVS through Enzyme Linked Immuno Sorbent Assay. J Agric Res. 2006;45:68-71.
21. Burhan M, Khan MA, Irfanullah M, Jamil MR, Ishfaq M. Incidence of Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus S (PVS) on Potato Cultivars in Potato Growing Areas. Journal of Agriculture and Social Sciences. 2007;3:37-38.
22. Chakrabarty PK. Techniques in molecular plant pathology. Training Manual Central Institute of Cotton Research, Nagpur India. 2003;34-36.
23. Erik J, Marianne JH, Ben JCC. Agronomic performance and field resistance of genetically modified, virus-resistant potato plants. Agricultural Research. 1993;4:407-416.
24. Gibbons JD. Nonparametric methods for quantitative analysis (2nd edn), American Sciences Press Inc., Columbus, OH, USA. 1985;21-22.
25. Gomez KA, Gomez AA. Statistical Procedure for Agricultural Research. John Wiley and Sons, New York, USA. 1984;55-60.
26. Gul Z, Khan AA, Khan AUR, Khan Z. Incidence of potato viruses in different districts of Khyber Pakhtunkhawa, Pakistan. J Plant Pathol. 2013;3:32-36.
27. Ganie SA, Ghani MY, Nissar Q, Jabeen N, Anjum Q, Ahanger FA, Ayaz A. Status and symptomatology of early blight (Alternaria solani) of potato (Solanum tuberosum L.) in Kashmir valley. African Journal of Agriculture Research. 2013;5104-115.
28. Gondal, AS, Javed N, Khan SA, Hyder S. Genotypic diversity of potato germplasm against root knot nematode (Meloidogyne incognita) infection in Pakistan. eSci J Plant Pathol. 2012;01:27-38.

29. Haase NU. The nutritional value of potatoes in Canada. J Potatoes Res. 2008;50:415-17.

30. Harris PM. The potato crop: The scientific basis for improvement (2nd edn), London: Chapman and Hall, UK. 1992:900-09.

31. Harrison BD. Descriptions of plant viruses CMI/AAB. Potato Leafroll virus (Revised) 1984;36:198-291.

32. Hartman GL, Huang YH. Characteristics of Phytophthora infestans isolates and development of late blight on tomato in Taiwan. Plant Disease. 1995;79:849-52.

33. Hooker WJ. Compendium of potato diseases. American Phytopathological Society. 1981;1-20.

34. Hussain A, Awan MS, Morari F, Iqbal SM, Hassan SN. Spatial analysis of potato black scurf disease distribution using GIS and variability of Rhizoctonia solani isolates in Central Karakoram National Park Gilgit-Baltistan, Pakistan. International Journal of Biosciences. 2014;4:17-27.

35. Islam MU, Muhammad S, Shahbaz M, Javed MA, Hussain N. Screening of potato germplasm against RNA viruses and their identification through ELISA. J Green Physiol Genet Genom. 2013;1:22-31.

36. Jamal A, Nasir IA, Tabassum B, Tariq M, Farooq AM. Incidence and distribution of potato viruses in the upper Kaghan Valley of Pakistan. Pak J Phytopathol. 1995;7:13-16.

37. Jan H, Khan SB. Incidence and distribution of potato viruses in the upper Kaghan Valley of Pakistan. Pakistan J Phytopathol. 1995;7:13-16.

38. Jepson SB. Identification of root-knot nematodes (Meloidogyne species). C.A.B. International, Wallingford, UK. 1987.

39. Kaiser WJ. Use of thermotherapy to free potato tubers of alfalfa mosaic, potato leaf roll and tomato black ring viruses. Journal of Phytopathology. 1980;70:1119-22.

40. Khan A, Tabassum B, Nasir IA, Bilal M, Tariq M. Potato virus X from Pakistan: Coat protein sequence analysis. The Journal of Animal and Plant Sciences. 2015;25:1016-21.

41. Khan NP, Akhtar J. Competitiveness and policy analysis of potato production in different agro-ecological zones of Northern Areas: Implications for Food Security and Poverty Alleviation. The Pakistan Development Review. 2006;45:1137-54.

42. Mehboob S, Khan MA, Rehman A, Idrees M. Role of epidemiological and biochemical factors against early blight of potato. eSci J Plant Pathol. 2013;08-13.

43. Miller WA, Brown CM, Wang S. New punctuation for the genetic code: Luteovirus Gene Expression. J Gen Virol. 1997;8:3-13.

44. Mirza MS. The role of aphids in spreading potato virus diseases in the plains of Pakistan. Potato Research in Pakistan. Shah M.A (ed.), Pak. Agric. Res. Council. 1978;29-32.

45. Mughal SM. Some threatening and emerging plant viral diseases in Pakistan. Proce. 4th Nat. Conf. Plant Pathol. 2003;8:16.

46. Mughal SM, Khalid S. Virus diseases in relation to potato production in Pakistan. In: Potatoes in Pakistan. Pak Agric Res. 1985;154-16.

47. Mughal SM, Khalid S, Gillani TS, Devaux A. Detection of potato viruses in Pakistan. Proc. Asian Potato Assoc. 2nd triennial Conf., June. 12-26, Kunming, P.R. China. 1990;189-90.

48. Monty DH, Livingston CH, Oshima N. Epidemiology of potato early blight in Colorado I. Initial infection, disease development and the influence of environmental factors. American Potato Journal. 1965;279-91.

49. Nadeem A, Khan MA, Ali S, Bhutta NA, Binyamin R. Epidemiological studies and management of potato germplasm against PVX and PVY. Pak J Phytopathol. 2011;23:159-68.

50. Nasir M, Zaidi SSH, Batoel A, Hussain M, Iqbal B, Sajad M, et al. ELISA-based detection of major potato viruses in tissue culture produced potato germplasm. Int J Agric Sci. 2012; 2:75-80.

51. Naveed Q, Khan MA, Rashid A. Correlation of environmental conditions with Potato Virus Y (PVY) Disease Development on Six Varieties / Advanced Lines of Potato. International Journal of Agriculture and Biology. 2003;5:172-74.

52. Naveed Q, Khan MA, Rashid A. Correlation of environmental conditions with Potato Virus (PVX) and Y (PVY) Disease Severities Recorded on 21 Advance Lines/Varieties of Potato (Solanum tuberosum L.). International Journal of Agriculture and Biology. 2003;5:172-84.

53. Nosheen Q. DAS-ELISA and PCR amplification of Potato Virus X coat protein gene. M.Sc. (Hons) Thesis. Dept. Pl. Pathol, PMAS, Arid Agriculture University Rawalpindi, Pakistan. 2011;33-35

54. Oosterweld P. Inspection and grading. In: J.A. de Boks and J.P.H. Van der Wal (Eds) Viruses of potatoes and seed-potato production, Wageningen, The Netherlands: Pudoc. 1987;204-14.

55. Pandey KK, Pandey PK, Kallo G, Banerjee MK. Resistance to early blight of tomato with respect to various parameters of disease epidemics. J Gen Plant Pathol. 2003;69:364-71.

56. Pazcheid JW, Stevenson WR. The critical period for control of early blight (Alternaria solani) of potato. American Potato Journal. 1988;425-38.

57. Peter ET, Clift EL, James CZ, Gary LR, Wojciech KK. Extreme resistance to potato leaf roll virus in potato cv. Russet Burbank mediated by the viral replicase gene. Vir Res J. 2000;71:4962.

58. PMID. Pakistan Meteorological Department Report. Islamabad Pakistan. 2015.

59. Qamar N, Khan MA, Rashid A. Screening of potato germplasm against potato virus X (PVX) and potato virus Y (PVY). Pak J Phytopathol. 2003;15:41-45.

60. Radilife EB, Ragsdale DW. Aphid-transmitted potato viruses: The Importance of understanding vector biology. American Journal of Potato Research. 2002;79:352-86.

61. Ragsdale DW, Radilife EB, Difonzo CD. Epidemiology and field control of PVY and PLRV. In: G. Loebenstein, Berger, P.H., Brunt, A.A. and Lawson, R.H., (Eds) Virus and virus-like diseases of potatoes and production of seed-potatoes. Kluwer Academic Publishers, Dordrecht. 2001;237-270.

62. Robert Y, Lemaire O. Epidemiology and control strategies. In: The Luteoviridae Smith HG and Barker H (eds.), CAB Inter., Wallingford UK. 1999;211-279.

63. Robert Y, Wood ford JAT, Ducray-Bourdien DG. Some epidemiological approaches of aphid borne virus diseases in seed potato crops in northern Europe. Virus Res. 2000;71:33-41.

64. Rouze JJ, Terradot L, Pasquier F, Tanguy SG, Ducray DG. The passage of potato leafroll virus through Myzus persicae gut membrane regulates transmission efficiency. J Gen Virol. 2001;82:17-23.

65. Scagliusi SM, Lockhart BEL. Transmission, characterization and serology of a luteovirus associated with yellow leaf syndrome of sugarcane. Journal of Phytopathology. 2000;90:120-124.

66. Shoaib AH, Rafi MA, Javed H, Zia A, Naeem M, Khan I, et al. Aphidoidea (Homoptera) from the Northern areas of Pakistan. Sarhad Journal of Agriculture. 2010;26:1-3.

67. Shoutong W, Tongle HU, Fengqiao Z, Forrer HR, Keiqiang CAO. Screening for plant extracts to control potato late blight. Frontier Agriculture China. 2007;1:43-66.
68. Siddiqui MR, Booth W. Meloidogyne mersa p.n. attacking Sonneratia alba trees in mangrove forest in Brunei Darussalam. Afro-Asian J Nematol. 1991;2:212.

69. Taliansky M, Barker H. Movement of luteoviruses in infected plants. In: The Luteoviridae, Smith, HG and Barker H (eds.), CAB Inter., Wallingford, UK. 1999;69-81.

70. Tamada T, Harrison BD, Roberts IM. Portulacaceae as PLRV hosts Variation among British isolates of potato leaf roll virus. Annals of Applied Biology. 1984;104:107-116.

71. Terradot L, Simon JC, Leterme N, Bourdin D, Wilson ACC, Gauthier JP, et al. Molecular characterization of clones of the Myzus persicae complex (Hemiptera: Aphididae) differing in their ability to transmit the potato leafroll luteovirus (PLRV). Bull Entomol Res. 1999;23-29.

72. Thomas JE. characterization of an Australian isolate of tomato yellow top virus. Annals of Applied Biology. 1984;104:79-86.

73. http://micronet.im.ac.cn/vide/descr644.html.1987;21-30

74. Turechek WW. Non-parametric tests in plant disease epidemiology: Characterizing disease associations. Phytopathology. 2004; 94: 1018-1021.

75. Vakalounakis DJ. Evaluation of tomato cultivars for resistance to Alternaria blight. Ann Appl Biol. 1983;102:138-39.

76. Valkonen, JPT, Hamalainen J, Kekarainen H, Gebhardt T, Watanabe KN. Recessive and dominant genes interfere with the vascular transport of Potato virus A in diploid potatoes. Molecular Plant-Microbe Interactions. 2000;13:402-12.

77. Wang S. Rivers and Human Rights: The Northern Areas, Pakistan’s Forgotten Colony in Jammu and Kashmir. International Journal on Minority and Group Rights. 2004;11:187.

78. Were HK, Kabira JN, Kinyua ZM, Olubayo EM, Karinga JK, Aura J, et al. Occurrence and distribution of potato pests and diseases in Kenya. Potato Research. 2014;8:1-18.

79. Woodford JAT, Jolly CA, Nisbet AJ. Effects of aphid feeding behaviour on the transmission of potato leaf roll virus. Annual Report Scott Crop Res Inst. 1994;155-59.

80. Yvon R, Trefor JAW, Daniele GDB. Some epidemiological approaches to the control of aphid-borne virus diseases in seed potato crops in Northern Europe. Virus Research 2000;71;33-47.

81. Zar JH. Biostatistical analysis, (4th edn). Prentice Hall, Upper Saddle River, NJ, USA. 1999;50-100.

82. Zehnder G. Overview of monitoring and identification techniques for insect pests. Clemson University. Extension, Cooperative Extension System, USA. 2010;1-100.