

## Research Article



## Effect of Environmental Factors on Potato Leaf Roll Virus (PLRV) Infecting Potato Varieties and *Myzus persicae* (Sulzer)

Yasir Iftikhar<sup>1</sup>, Mustansar Mubeen<sup>1,2\*</sup>, Waqas Raza<sup>1</sup>, Qaiser Shakeel<sup>3</sup>, Waseem Abbas<sup>1</sup>, Shehzad Iqbal<sup>1</sup> and Ashara Sajid<sup>1</sup>

<sup>1</sup>Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha-40100, Pakistan; <sup>2</sup>State Key Laboratory of Agricultural Microbiology and Provincial Key Laboratory of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, P.R. China; <sup>3</sup>Discipline of Plant Pathology, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

**Abstract** | The Potato leaf roll virus (PLRV), aphid population, and environmental factors are normally used to monitor the disease severity as these factors are interconnected. In the present study, ten potato varieties viz., FSD white, FD 8-1, FD 71-1, Tota 704, Kuruda, FD 74-41, FD 76-24, SH 216-A, N 96-25 and FD 74-50 were evaluated against PLRV. The range of disease incidence was from 15.93% to 64.58%. Moreover, susceptible reaction was observed in five varieties (FD 71-1, Tota-704, FSD white, FD 76-24 and FD 8-1). Two varieties (Kuruda and SH 216 A) were moderately resistant, while three varieties, FD 74-41, FD 74-50 and N 96-25 were found to be moderately susceptible. In susceptible varieties, disease severity was significantly correlated with temperature (max and min), and relative humidity (RH). Whereas, disease severity in moderate resistant varieties also showed a significant correlation with temperature. However, disease severity in the moderate resistant varieties were significantly correlated with the temperature and relative humidity. Highest disease incidence (39.33 %) was recorded at maximum temperature 19°C. However, the disease severity was found to be highest (21.33%) in minimum temperature at 5°C. The disease severity was highest at the highest aphid population. Highest population of aphids 33 was recorded at the 27°C and lowest of 6 at 5°C. Hence, it is concluded that PLRV incidence generally influenced by environmental factors and aphid population. It is proposed that PLRV can be managed through resistant varieties and monitoring of aphid population.

**Received** | July 18, 2019; **Accepted** | March 25, 2020; **Published** | July 10, 2020

\***Correspondence** | Mustansar Mubeen, Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha-40100, Pakistan; **Email:** mustansar01@yahoo.com

**Citation** | Iftikhar, Y., M. Mubeen, W. Raza, Q. Shakeel, W. Abbas and A. Sajid. 2020. Effect of environmental factors on potato leaf roll virus (PLRV) infecting potato varieties and *Myzus persicae* (Sulzer). *Pakistan Journal of Agricultural Research*, 33(3): 473-479.

**DOI** | <http://dx.doi.org/10.17582/journal.pjar/2020/33.3.473.479>

**Keywords** | PLRV, Aphid, DAS-ELISA, Symptomology, Environmental factors

### Introduction

Potato (*Solanum tuberosum* L.) family *Solanaceae* and the most economical important vegetable crops all over the world. It is cultivated in tropical and subtropical zones. More than 100 billion people depend upon potatoes for their survival. It contains water (78 %), protein (2 %), vitamins (1%), starch

(18 %), carbohydrate, and several elements in traces (Haase, 2008) show its nutritive value. In Pakistan during 2016-17, it was cultivated on 179.3 thousand hectares with 3849.5 thousand tons an annual production (Economic Survey of Pakistan, 2016-17). Potato is attacked by many biotic and a-biotic diseases (Khan and Abbas, 2008). Among the biotic factors, potato crop is infected by more than 40

viruses (Valkonen, 2007). Mughal et al. (1988) was reported eight potato viruses in Pakistan. The PLRV, PVY, and PVX are widely distributed in Pakistan. Ahmad et al. (2003) found in his survey of 169 fields that PVY, PVX, PLRV, and PVS were major viruses of potato. Bhutta and Bhatti (2002) concluded that the potato leaf roll virus (PLRV) was most important virus and causes heavy losses (90%) in susceptible varieties. The PLRV having positive sense single stranded RNA genome size 5.9 kb, genus *Polerovirus* and family *Luteoviridae* (Robert and Lemaire, 1999). Aphid was identified the main vector for this disease (Botjes, 1920). Among different species, *Myzus persicae* (green peach aphid) is the efficient vector that transmits PLRV by persistent and circulative manner (Alvarez et al., 2013). The PLRV incidence was recorded as 43% in general, 12% in certified seed, and 52% in uncertified seed (Ragsdale et al., 2001). The PLRV symptoms in primary infection upper leaf rolling and in secondary infection lower leaf rolling, young buds are yellow and purplish, erect growth, and stunting of plant. The PLRV became emergent disease in Pakistan due to its high incidence (Batool et al., 2011). The aphid *M. persicae* has a key role in PLRV transmission as it results in causes severe losses up to 90% (Alvarez et al., 2013). So, it is essential to check the PLRV incidence in relation to population of *M. persicae* for disease management in future. Environmental factors (maximum, minimum temperature, and relative humidity) performed dynamic role in disease incidence and vector population (Mubeen et al., 2017). Keeping in view the above status of PLRV, this experiment was aimed to monitor the disease incidence and disease severity of PLRV and to establish a correlation between virus, vector, and environmental factors.

## Materials and Methods

### Monitoring of PLRV disease and insect vector

The research trial was performed in research field of College of Agriculture, University of Sargodha. Ten varieties were checked against PLRV according to Qamar and Khan (2003) disease rating scale with slightly modification as follows. Samples were collected from infected plants. Symptomology was the basic criteria. The samples were confirmed through indirect ELISA (Clark and Adam, 1977). Yellow color sticky traps were installed in the field for the collection of aphids (*Myzus persicae*). The data was collected on weekly basis.

### Disease rating scale and level of resistance/ susceptibility.

0	No visible symptom	Immune
1	Mosaic pattern starts on leaves (25% leaves showing symptoms)	Resistance
2	Mosaic and Mottling (50% leaves have symptoms)	Moderately resistance
3	Dwarfing, rigidity and mottling of leaves (75% leaves affected)	Moderately susceptible
4	Leaf drooping severe mosaic and mottling (100)	Susceptible

### Correlation of environmental factors

Environmental factors were also recorded on weekly basis for the months of October to January from In-service Agriculture Institute, Sargodha. Environmental factors were included maximum, minimum temperatures and relative humidity.

### Statistical analysis

Randomized Complete Block Design (RCBD) was used to carry out the present experiment. The data was analyzed to check the level of significance (<0.05) for disease development (Steel et al., 1997). The correlation coefficients were also estimated according to Dewey and Lu (1959) formula.

## Results and Discussion

### Incidence of potato leaf roll virus in Sargodha

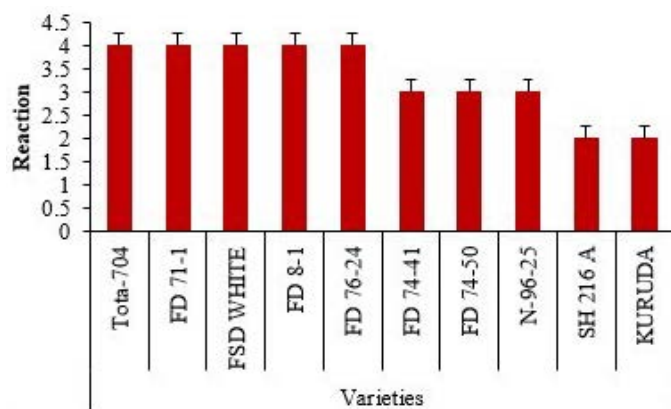
Symptomology was the basic criteria during the screening of ten varieties against PLRV (Figure 1). Highest incidence of 64.58% was recorded in Variety FSD WHITE. The disease incidence in range from 15.93% to 64.58% (Table 1). Varieties were kept in three groups; Susceptible, Moderately susceptible and moderately resistant according to disease rating scales (Figure 2). The five varieties were susceptible, three were Moderate susceptible and two were Moderate resistant for PLRV (Table 1). The ELISA confirmed the presence of virus in samples (Figure 3).



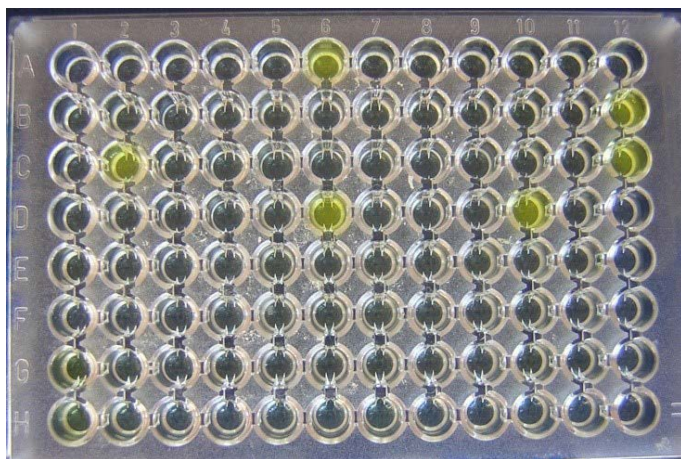
Figure 1: Potato leaves showing symptoms of PLRV.

**Table 1: Incidence and reaction of PLRV.**

Varieties	PLRV incidence (%)			Reaction
	Total plants observed	Infected plants	Incidence(%)	
Tota-704	30	11	38.44	Susceptible
FD 71-1	30	12	39.58	Susceptible
FSD white	30	19	64.58	Susceptible
FD 8-1	30	14	48.19	Susceptible
FD 76-24	30	18	58.47	Susceptible
FD 74-41	30	15	49.30	Moderate susceptible
FD 74-50	30	13	42.08	Moderate susceptible
N-96-25	30	13	44.44	Moderate susceptible
SH 216 A	30	5	15.93	Moderate resistant
KURUDA	30	6	19.58	Moderate resistant



**Figure 2: Resistance level/susceptibility in varieties against PLRV according to disease reaction.**

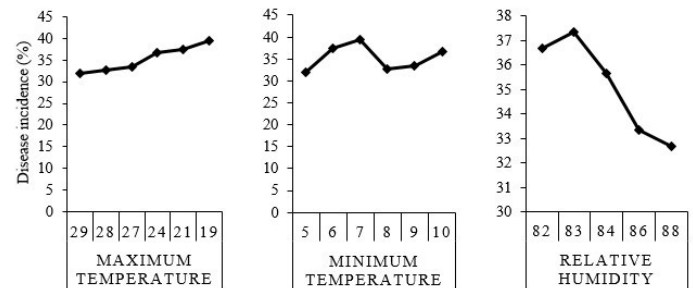


**Figure 3: Yellow color showing the confirmation of virus in the samples.**

*Effect of environmental factors on the susceptible varieties against PLRV*

According to Qamar and Khan (2003) scale, Tota 704, FD 71-1, FSD white, FD 76-24 and FD 8-1 were susceptible for PLRV. These varieties showed significant correlation between environmental factors and disease severity. Disease severity in susceptible varieties was maximum. In the maximum temperature,

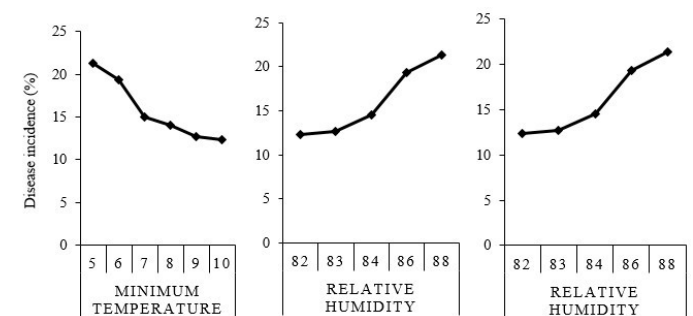
maximum incidence (39.33 %) was recorded at temperature 19°C. Disease severity was started decreasing at gradually as the maximum temperature increases and minimum in disease severity (32.00 %) was recorded at 29°C. Disease severity (32.00 %) was highest at minimum temperature (5°C) as the increase in minimum temperature decreased the severity index of disease. Relative humidity 82% was favorable for disease severity as relative humidity increases; disease incidence decreases gradually (Figure 4).



**Figure 4: Incidence of PLRV in susceptible variety under maximum and minimum temperature and relative humidity.**

*Effect of environmental factors on the moderate susceptible varieties against PLRV*

The varieties, N 96-25, FD 74-50 and FD 74-41 were moderately susceptible against PLRV. The disease severity was maximum (21.33 %) at maximum temperature of 19 °C. As the maximum temperature increased up to 29 °C, the severity index was decreased (12.33 %). The disease severity was maximum (21.33%) in minimum temperature at 5 °C and the minimum disease severity was (12.33%) at 10 °C. Disease incidence was maximum at relative humidity 88% and intensity of disease decreases as humidity decreases. Among three variables only minimum and maximum temperature were high significant and have negative correlation with disease progression, while relative humidity showed positive correlation with disease incidence and highly significant (Figure 5).



**Figure 5: Incidence of PLRV in moderately susceptible varieties under maximum and minimum temperature and relative humidity. Effect of environmental factors on the moderate resistant varieties against PLRV.**

The moderately resistant showed in SH 216-A and KURUDA. In maximum temperature at 19 °C, the disease severity was maximum (65.00%) and increase in maximum temperature decreased disease severity, and minimum disease intensity (11.66 %) was recorded at maximum temperature of 29 °C. For minimum temperature disease severity was maximum (65%) at 5 °C. Whereas, minimum disease severity (11.66%) was recorded at 10 °C. Disease incidence was maximum at relative humidity of 83% as relative humidity increases the disease incidence was decreased (Figure 6). Among three variables, minimum, maximum temperature, and relative humidity were highly significant and have negative correlation with disease progression.

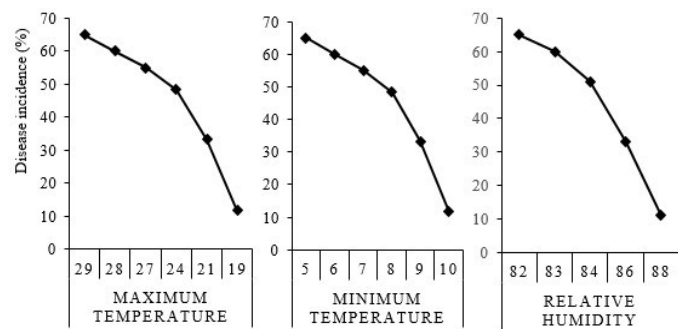


Figure 6: Incidence of PLRV in moderately resistant varieties under maximum and minimum temperature and relative humidity.

*Correlation between aphid population with environmental factors*

Aphid plays very important role in spreading of viral diseases. The aphid (*Myzus persicae*) which transmit *Potato leaf roll virus* in non-persistently. Among the environmental variables, relative humidity, and minimum temperature were highly significant and negatively correlated with insect population while maximum temperature had no significant correlation with vector population. Aphid population in the field was recorded at weekly interval throughout the research and correlated with environmental factors. Maximum aphids 33 population was observed at the 27 °C and lowest of 6 at 5 °C. The insect population gradually decreases when the temperature becomes low. In case of relative humidity, Maximum number of insect catches (30) at the 84% (Figure 7).

*Correlation of aphid population with PLRV incidence*

Aphid population was examined after seven days' interval and correlated with disease. Maximum disease incidence (50.33%) was recorded when aphid population was 19 per plant, while minimum disease incidence (10%) recorded at aphid population of

4-11 per plant. The disease incidence was gradually increased with the increase in the population of aphid. The vector population was significant correlated with disease incidence (Figure 8).

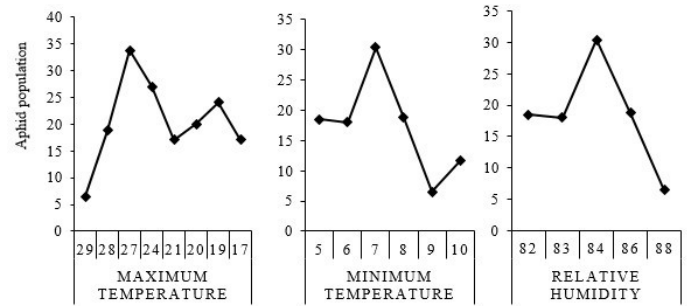


Figure 7: Aphid population under maximum and minimum temperature and relative humidity.

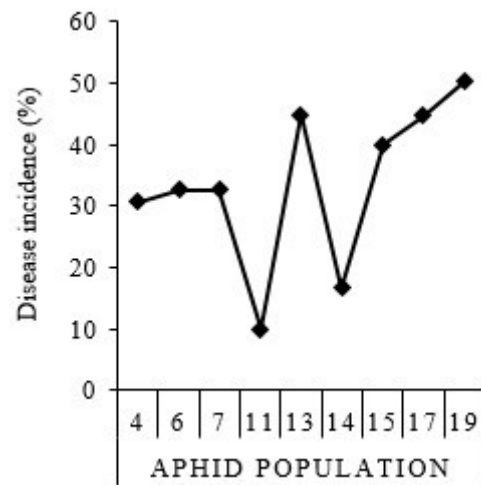
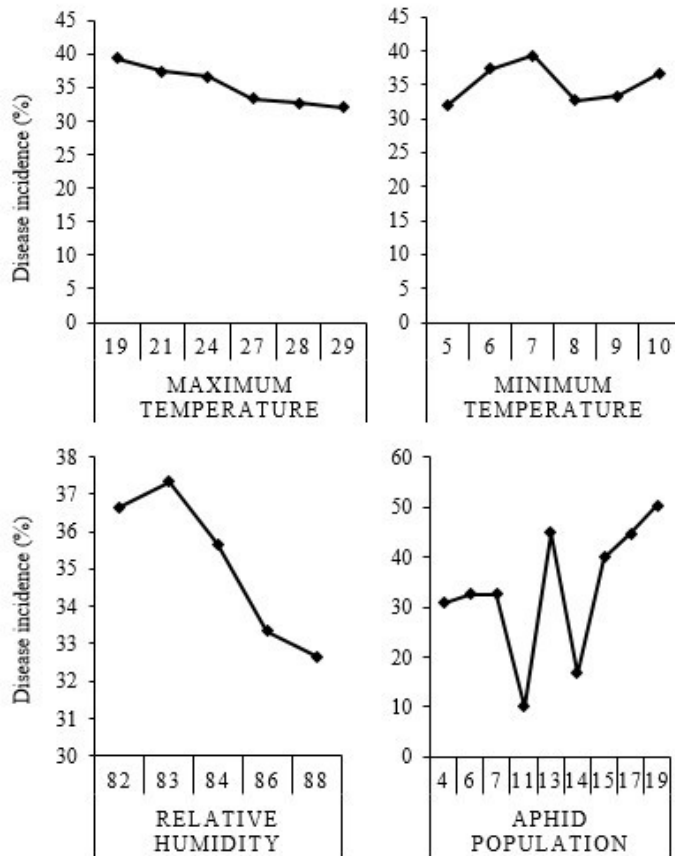


Figure 8: Incidence disease under aphid population per plant.

*Correlation between aphid population, environmental factors and disease incidence*

The aphid population and environmental factors have their role disease development. Environmental factors (maximum, minimum temperatures, and relative humidity) and aphid population favored the disease incidence in potato. The correlation study showed that maximum, minimum temperatures, and relative humidity were highly significantly and negatively correlated with disease incidence while the aphid population showed significant positive correlation with disease incidence. The maximum disease incidence (39.33%) was observed at 19 °C and had declining trend with the increase in temperature up to 29 °C. The maximum disease incidence was recorded at 82% relative humidity. The maximum disease incidence (50.33%) was recorded when the aphid population reached up to 30/plant (Figure 9).

Potato is the fourth-largest and leading vegetable crop after rice, wheat, and maize in the world



**Figure 9:** Incidence of disease under minimum, maximum temperature, relative humidity, and aphid population per plant.

(Hijmans and Spooner, 2001). Potato leaf roll virus (PLRV) is the most disturbing virus of potato around the world. The PLRV plays vital role in decreasing of yield. PLRV symptoms depends upon the strain of viruses. The most promising symptoms are upward or downward leaf rolling which results in poor photosynthetic rate and poor plant (stunted) growth and therefore result in a significant economic loss. The PLRV is transmitted through green peach aphid. Several studies have documented a host/vector manipulation phenomenon by plant viruses in diverse path systems (Moreno-Delafuente et al., 2013), including in the PLRV/aphid path system (Rajabaskar et al., 2013b). Aphid arrestment and colonization is influenced and often increased by the altered chemical profile and physical appearance in different potato varieties result from PLRV infection (Rajabaskar et al., 2013a). The similar patterns of aphid colonization that we observed suggest that potato could exhibit similar volatile profiles and physical appearances putting at a similar risk not only for PLRV infection but also for becoming potential inoculum sources. The PLRV disease dynamics are complex due to the persistent and circulative manner in which aphids acquire and transmit the virus as well as the presence of alternative host plants for the

virus and aphids (Srinivasan et al., 2010). Disease incidence was verified according to Qamar and Khan (2003) disease rating scale with little modifications. The potato varieties were allocated to three groups i.e. moderately resistant, moderately susceptible and susceptible. Incidence was in range from 57% to 70% in. No variety shows immune or resistance response against PLRV infection. Mean disease incidence was 58% recorded in all varieties. Among them FD 71-1, Tota 704, FSD white, FD 76-24 and FD 8-1 were highly susceptible to PLRV infection with maximum incidence of 38.44%, 39.58%, 64.58%, 48.19%, and 58.47%, respectively. Moderate resistant response was showed by SH 216-A and Kuruda with disease incidence of 15.93% and 19.58, respectively. The N 96-25, FD 74-50 and FD 74-41 were moderately susceptible to PLRV infection showing incidence of 49.30%, 42.08%, and 44.44%, respectively. Our findings were according to the Haq et al. (2016). Similar result was also observed by Gul et al., 2013. They inoculated the potato plant by different doses of PLRV (0, 10, 20, 30, and 100%). Results showed that inoculation of PLRV (20%) significantly enhances disease incidence and decreased the plant height, number and yield of tubers. Similarly, Ragsdale et al. (2001) also reported that plantation of potatoes in field containing 1-4% PLRV infection may also serve as inoculum for the spread of PLRV in these areas compared to weeds. Susceptible Varieties (FD 71-1, Tota 704, FSD white, FD 76-24 and FD 8-1) had very high significant negative correlation with maximum, minimum temperature while relative humidity had significant positive correlation with PLRV infection. Minimum temperature showed very high significant negative correlation with moderately resistant varieties (SH 216-A and Kuruda). Moderately susceptible varieties (FD 74-41, FD 74-50 and N 96-25) showed very high significant negative correlation with maximum and minimum temperature. Our result similar with Qamar et al. (2015, 2016). *Myzus persicae* had significant negative correlation with relative humidity and minimum temperature. Ahmad (2003) studied correlation on different lines/ Varieties. He reported the correlation of PLRV disease incidence with environmental factors. He found significant correlation of environmental conditions with varieties FSD-Red, P-332826 and FSD- white. Relationship between aphid, PLRV and PVY was studied by Kotzampigikis et al. (2010). Aphid movement checked through yellow pan traps. Viral infection was 0- 13.25% (PLRV) and 0.75- 31.8% (PVY) in

seed potato tubers. Virus infection checked through DAS ELISA. Total number of aphid, age corrected aphid intensity, and cumulative aphid intensity have major impact on the proportion of PLRV and PVY. Our results presented in this study were compliance of all the research work conducted by above mention previous researcher.

## Conclusion and Recommendation

The environmental factors are playing a significant role in the epidemiology of the PLRV in combination with the aphids. The aphid population were found to be correlated with the disease severity and disease incidence. When the environmental conditions become favored to aphids the diseases severity and incidence increased. Therefore, it is recommended that study of environmental factors in relation to vector population and disease severity in future will not only be helpful in determining the response against plant diseases but also for the monitoring of the progression of disease.

## Author's Contribution

Yasir Iftikhar and Mustansar Mubeen wrote the manuscript. Yasir Iftikhar and Qaisar Shakeel designed the experiment. Waqas Raza, Waseem Abbas, Ashara Sajid conducted the research and Shehzad Iqbal helped in revision of manuscript.

## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

## References

- Ahmad, S.I., 2003. Correlation of environmental conditions with major potato viruses and their vectors. Proc. 3<sup>rd</sup> Nat. Con. Plant Pathol., NARC, Islamabad, Pakistan. pp. 110-112.
- Alani, R.A., U.N. Alessawi and S.A. Almashaikhy. 2002. Isolation of proteins from *Datura stramonium* has ability to inhibition the multiplication of potato virus Y (PVYn). Jerash J. Res. Stud., 7: 9-21.
- Alvarez, A.E., V.G. Broglia, A.M. Alberti D'Amato, D. Wouters, E. van der Vossen, E. Garzo, W.F. Tjallingii, M. Dicke and B. Vosman. 2013. Comparative analysis of *Solanum stoloniferum* responses to probing by the green peach aphid *Myzus persicae* and the potato aphid *Macrosiphum euphorbiae*. Insect Sci., 20(2): 207-227. <https://doi.org/10.1111/j.1744-7917.2012.01505.x>
- Batool, A., M.A. Khan, J. Farooq, S.M. Mughal and Y. Iftikhar 2011. Elisa based screening of potato germplasm against potato leaf roll virus. J. Agric. Res., 49: 57-63.
- Bhutta, A.R., and M.F.J. Bhatti. 2002. Seed potato certification in Pakistan. Fed. Seed Certification Reg. Dep. Minist. Food Agric. Livest., Islamabad, Pakistan. pp. 60-66.
- Botjes, J.G.O., 1920. De bladrolziekte van de aardappelplant. H. Veeman en Zonen, Wageningen. 8: 1-136.
- Clark, M.F. and A.N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34(3): 475-483. <https://doi.org/10.1099/0022-1317-34-3-475>
- Cranshaw, W.S. and B. Baxendale. 2005. Insect control: Horticultural oils. <http://www.ext.colostate.edu/pubs/insect/05569.html>.
- Dewey, R.D. and K.H. Lu. 1959. A correlation and phenotypic correlation analysis of some quality character and yield of seed cotton in upland cotton (*Gossypium hirsutum* L.). J. Biol. Sci., 1: 235-236. <https://doi.org/10.3923/pjbs.1998.235.236>
- Economic Survey of Pakistan, 2016-2017. Ministry of food, agriculture and livestock, federal berean of statistics Islamabad, Pakistan.
- Gul, Z., A.A. Khan, A.U.R. Khan and Z. Khan. 2013. Incidence of potato viruses in different districts of Khyber Pakhtunkhawa. Pak. J. Plant Pathol. 2: 32-36.
- Haase, N.U., 2008. The nutritional value of potatoes in Canada. J. Potatoes Res., 50: 415-417. <https://doi.org/10.1007/s11540-008-9060-y>
- Haq, A., Y. Iftikhar, M.I. Ullah, M. Mubeen, Q. Shakeel, F. Bakhtawar and I. Bilqees. 2016. Disease progression in potato germplasm from different reaction groups against potato virus Y in relation to environmental factors. Trop Plant Res., 3(3): 600-605. <https://doi.org/10.22271/tpr.2016.v3.i3.079>
- Harrison, B.D., 1984. CMI/AAB Descriptions of Plant Viruses. Potato leaf roll virus 291. [www.dpvweb.net/dpv/showdpv.php?dpvno=291](http://www.dpvweb.net/dpv/showdpv.php?dpvno=291).
- Hijmans, R.J. and D.M. Spooner. 2001. Geographic distribution of wild potato species. Am.

- J. Bot. 88(11): 2101-2112. <https://doi.org/10.2307/3558435>
- Kasal, M., 1922. Berichte Ohara Inst. f. Landwirtsch. Forsch. 2: 47-77.
- Khan, M.A., and W. Abbas. 2008. Multiple regression models based upon epidemiological factors to predict *M. persicae* population and PLRV disease incidence. Int. Nat. Conf. Plant Sci., ICPS, pp. 155-168.
- Kotzampigikis, A., D. Hristova and E. Tasheva-Terzieva. 2010. Virus-vector relationship between Potato leaf roll virus PLRV and *Myzus persicae* Sulzer. Bulg. J. Agric. Sci., 16(4): 412-421.
- Moreno-Delafuente, A., E. Garzo, A. Moreno and A. Fereres. 2013. A plant virus manipulates the behavior of its whitefly vector to enhance its transmission efficiency and spread. *PLoS One*, 8(4): 61543. <https://doi.org/10.1371/journal.pone.0061543>
- Mubeen, M., Y. Iftikhar, M.I. Ullah, Q. Shakeel, M. Aatif and I. Bilqees. 2017. Incidence of Okra Yellow Vein Mosaic Disease in Relation to Insect Vector and Environmental Factors. *Environ. Ecol.*, 35(3C): 2215-2220.
- Mughal, S.M., S. Khalid, T.S. Gillani and A. Devaux. 1988. Detection of potato viruses in Pakistan. In Proc. 2<sup>nd</sup> Triennial Conf., pp. 12-26.
- Qamar, M.I., Y. Iftikhar, A. Ali, M.I. Ullah and M. Mubeen. 2016. Disease severity index as it affects responses in Potato Virus X- challenged potato varieties. *Int. J. Veg. Sci.*, 22(5): 471-479. <https://doi.org/10.1080/19315260.2015.1077914>
- Qamar, M.I., Y. Iftikhar, Z. Iqbal and M. Mubeen. 2015. Screening of potato germplasm through ELISA against potato virus X (PVX). *Universal J. Plant Sci.*, 3(2): 21-24.
- Qamar, N. and M.A. Khan. 2003. Relationship of environmental conditions conducive for potato virus X (PVX) disease development on six varieties/advanced lines of potato. *Online J. Biol. Sci.*, 3(2): 247-252. <https://doi.org/10.3923/jbs.2003.247.252>
- Quanjer, H.M., H.A.A. Van Der Lek. and J.O. Botjes. 1961. Meded. Van de Land bouwhooge school 10: 1-138.
- Ragsdale, D.W., E.B. Radcliffe., and C.D. Difonzo. 2001. Epidemiology and field control of PVY and PLRV. In: G. Loebenstein, Berger, P.H., Brunt, A.A. and Lawson, R.H. [ed.], *Virus and virus-like diseases of potatoes and production of seed-potatoes*. Kluwer Acad. Publ., Dordrecht. 237-270. [https://doi.org/10.1007/978-94-007-0842-6\\_22](https://doi.org/10.1007/978-94-007-0842-6_22)
- Rajabaskar, D., H. Ding, Y. Wu and S.D. Eigenbrode. 2013. Different reactions of potato varieties to infection by Potato leaf roll virus, and associated responses by its vector, *Myzus persicae* (Sulzer). *J. Chem. Ecol.* 39(7): 1027-1035. <https://doi.org/10.1007/s10886-013-0311-2>
- Rajabaskar, D., Y. Wu, N.A. Bosque-Pérez and S.D. Eigenbrode. 2013. Dynamics of *Myzus persicae* arrestment by volatiles from Potato leaf roll virus-infected potato plants during disease progression. *Entomol. Exp. Appl.* 148(2): 172-181. <https://doi.org/10.1111/eea.12087>
- Robert, Y., and O. Lemaire. 1999. Epidemiology and control strategies. pp. 211-279. In the *Luteoviridae* Smith, H.G. and H. Barker (eds.), CAB Inter., Wallingford U.K.
- Srinivasan, D.G., B. Fenton, S. Jaubert-Possamai and M. Jaouannet. 2010. Analysis of meiosis and cell cycle genes of the facultatively asexual pea aphid, *Acyrtosiphon pisum* (Hemiptera: Aphididae). *Insect. Mol. Biol.*, 19: 229-239. <https://doi.org/10.1111/j.1365-2583.2009.00960.x>
- Steel, R.G., J.H. Torrie and D.A. Dickey. 1997. *Principal and procedure of statistics: A biometrical approach*. 3<sup>rd</sup> edition. McGraw Hill Book Co., New York.
- Valkonen, J.P.T., 2007. Viruses: Economic losses and biotechnological potential. In *Potato Biology and Biotechnology*. Elsevier Science, Amsterdam. pp. 619-641. <https://doi.org/10.1016/B978-044451018-1/50070-1>