Review Article

# Advanced Strategies for Detection and Diagnosis of Potato Viruses: Harnessing Molecular Innovations and Digital Tools for Precision Agriculture

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**Abstract** |Potatoes (*Solanum tuberosum* L.) rank fifth in global human consumption and fourth among food crops, surpassing cereals in yield and calorie production. However, potato production faces growing threats from various abiotic factors, pests, and pathogens, including bacteria, nematodes, viruses, viroids, fungi, and phytoplasmas. In Pakistan, significant viral infections, particularly PVY, PMTV, PLRV, PVM, PVS, PVA, and PVX, are of serious concern, with alarmingly high prevalence of PLRV, PVX, and PVY in key potato-growing regions. Effective management of these viral threats is challenging due to the lack of direct chemical controls, underscoring the importance of prevention as the primary strategy. Rapid and precise detection methods are essential for controlling the onset, spread, and progression of potato viruses, which are host-specific and detectable only through transmission electron microscopy. This review highlights various diagnostic techniques for potato viruses, with a focus on emerging molecular diagnostic tools. While traditional methods such as biological indexing, serological testing, and electron microscopy remain crucial for epidemiological research, molecular techniques provide a promising approach to producing virus-free seed potatoes. Implementing robust viral detection strategies will significantly contribute to sustainable agriculture, improve potato health monitoring, and equip researchers with vital tools to combat viral infections. This knowledge is critical for facilitating the safe transfer of potato germplasm globally, in response to increasing concerns from national quarantine agencies.

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## Introduction

After wheat, rice, and maize the cultivated potato (*Solanum tuberosum* L) is the most important vegetable and staple food crop in the world and is rated fourth in terms of output (Degebasa, 2020). In 2017, global potato production reached 388MT across 19-19.30Mha, with an average yield of 20-21T/ha. It produces fifteen times more calories/unit area as compared to grains. With its high dry matter content, protein synthesis, and resilience to varying temperatures, the potato has potential as a critical food source for the world's growing population (Sapakhova *et al*., 2023). The rapid rise in population raises concerns about future food security, necessitating increases in both production and productivity. However, this also intensifies disease pressures. Potatoes are affected by over 36 plant viruses. The microscopic plant pathogen is made of nucleic acid and coat proteins called viruses, while the smallest pathogen of plants having only RNA is called Viroids (Gergerich and Dolja, 2024). Increased viral incidence, particularly with early or severe infection, can drastically reduce tuber yield. Although exact figures are lacking, potato viruses are known to cause significant economic losses, with preliminary estimates suggesting reductions in tuber production by up to 50%. Pakistan's climate and soil are ideal for fall, spring, and summer potato crops, but bacterial, fungal, nematode, viral, and biotic factors remain major challenges (Abbas *et al*., 2023).

ELISA is commonly used in plant diagnostics for its automation, sensitivity, and ability to process a wide range of samples (Hema and Konakalla, 2021). Nucleic acid-based research has revolutionized plant virus diagnostics, particularly through molecular hybridization and PCR techniques. Immunocapture PCR (IC-PCR), which merges serology with PCR, is more sensitive and cost-effective than traditional PCR, as it bypasses the need for viral RNA purification (Rollin, 2023). Print capture and Direct binding are also effective for detecting viruses of potato. For instance, cDNA libraries of coat protein genes for PLRV, PVX, and PVY were created using sense and antisense primers (Serçe and Ayyaz, 2020). The PVX CP gene (613 bp) was cloned and sequenced from a Pakistani isolate (HE577130), showing the closest genetic similarity to PVX strains from the USSR, marking the first report of PVX in Pakistan. A recombinant PVY strain was identified in two Pakistani isolates based on CP gene of nucleotide evidence, biological and serological (Wani *et al*., 2021).

Modern diagnostic tools offer improved flexibility, sensitivity, and specificity, facilitating rapid virus identification. This is essential for virus resistant breeding program, post entry quarantine, surveillance of disease, epidemiological studies, and seed potato certification (Jones *et al*., 2021). However, applying the above technologies to combat potato viral infections requires sufficient research infrastructure and expertise. The following sections review potato virus detection and diagnostic techniques, with an emphasis on how scientists in developing countries like India and Pakistan can implement them. This paper explores recent molecular detection strategies and diagnoses of potato viruses and viroids, emphasizing their significance. Effective virus management strategies focus on reliable and cost-effective methods for sustainable agriculture.

### *Virus Identification and Detection in Potatoes*

Chemical approaches are an excellent way to manage diseases caused by bacteria, fungi, and nematodes, but there are currently no chemicals that can be used to directly control viral diseases in potato crops in the field. The most efficient and trustworthy way to stop a viral infection in its natural state is to eradicate contaminated seed tubers and validate the presence of the virus in seed stock in a sensitive and trustworthy manner. Plant viruses have a protective protein sheath around their nucleic acid, and detection techniques have developed from these two elements (Bhat and Rao, 2020). Plant viruses were previously identified using serological techniques such as ELISA, which were proven to be successful in the initial phases of detection, based on the protein component of the virus (Varma and Singh, 2020). However, only two to six percent genetic material of the viral genome is present in the protein coat, making these methods insufficient for capturing the entire genome.

Serological techniques cannot confirm the presence of a viroid due to the lack of a protein coat. Viroid can be identified by using polyacrylamide gel electrophoresis and Bioassays, though these methods may not be practical for large-scale sampling (Singh, 2007). In plant virology laboratories, recombinant DNA technology and molecular hybridization are employed to more accurately detect viruses and viroids (Sastry, 2013).

### *Immunosorbent Electron Microscopy*

The form and size of a specific virus may be found using (electron microscopy), and these fundamental procedures are essential to the identification of virus-

es. Electron microscopy is also used to analyze viruses in simple extracts from affected plants and may be utilized to gather information on viral morphology within minutes of sampling a diseased plant (Bhat and Rao, 2020). The very sensitive method known as immunosorbent electron microscopy (ISEM) was created by fusing electron microscopy with serology, and it was first used to identify plant viruses (Bhardwaj and Kulshrestha, 2020). For certain viruses, ISEM is 1,000 times more sensitive than conventional electron microscopy and more sensitive than ELISA. PLRV is a phloem-restricted virus that has a low titer that makes it difficult to identify with ELISA and standard electron microscopy (Reddy and Rao, 2021).

*Immunocapture PCR*

Serological and molecular methods differ in the viral component they target, as well as in their sensitivity, specificity, and automation potential. IC-PCR combines both molecular and serological method by capturing virus particles through their protective protein coat and then viral nucleic acid amplification using a Polymerase chain reaction (Trippa *et al*., 2024). This method is about 250-260 times more effective than conventional polymerase chain reaction and eliminates the need for viral purification to remove plant cell components that could interfere with PCR procedures. IC-PCR has proven highly effective in detecting PLRV (Mehetre *et al*., 2021).

*Molecular Hybridization*

The molecular hybridization technique is employed to confirm both viruses and viroids. These methods depend on the bond between pyrimidines and purines to form a stable hybrid between the target and probe sequences (Jain *et al*., 2024). Hydrophobic, electrostatic forces, and hydrogen bond quantity influence these hybrids' stability. Electrostatic forces are generated by phosphate molecules in the nucleic acid backbone, while the staggered bases contribute to hydrophobic interactions. The most used technique is dot-blot hybridization, which utilizes specific probes and target nucleic acids (Bhat *et al*., 2020). Non-radioactive riboprobes have been successfully used to confirm PVS, PVX, PVY, and PLRV. For molecular hybridization analysis, it depends on the specific probe, virus, and host used there are no universal methods (Singhal *et al*., 2021).

*Serology*

Monoclonal antibodies (Mabs) are produced by hybrid cells (hybridomas) created by fusing the myeloma cell of mouse with a B lymphocyte. The first Mabs were generated against the Tobacco mosaic virus (Nessa *et al*., 2020). Early immunological tests included chloroplast agglutination, microprecipitation assays, and gel immunodiffusion. To enhance serological test sensitivity, solid-phase adsorption of antibodies or antigens, followed by antibody enzyme conjugate detection (ELISA), became a key method of detecting viruses in plants and it gained popularity due to its simplicity, versatility, speed, sensitivity, and accuracy in detecting plant viruses (Minic and Zivkovic, 2020). The most common format typically plastic on a solid phase is double antibody sandwich. Specific antibodies capture plant viruses, and an enzyme labeled antibody (conjugate) is added. Later on the application of enzyme-substrate, viral presence is evaluated visually (by color change) or spectrophotometrically. Adding the primary antiviral antibody and coating plates from a species of animal is an indirect ELISA method (Makio, 2023).

The Crop Disease Research Institute, NARC Islamabad, Pakistan uses ELISA to screen thousands of potato samples for viruses (Khan). Swiss certification labs, ELISA is used to screen around 20,000-25,000 seed potato tubers daily for PVY and PLRV. ELISA's minimal detection of viruses is 2-3 ng/ml, but it cannot detect viruses at the early infection stage (low titer) (Yadegari *et al*., 2023). Serologically negative samples can still show symptoms later. A low-titer virus can multiply to billions of copies in days, infecting healthy potato plants via mechanical inoculation or insect vectors in open fields. Dot-ELISA is a versatile method for detecting PVX, PVS, PVY, and PLRV by binding antibodies or antigens to nitrocellulose or nylon membranes. For samples of the field, it is suitable. Crude sap from a suspected potato sample is spotted on a membrane and air-dried at room temperature. Bovine serum albumin (BSA) is used to block the membrane surface before adding viral antibodies. After applying the secondary antibody-enzyme conjugate and substrate, the reaction produces an insoluble colored product at the reaction site (Mehraj et al., 2020).

Tissue blotting, similar to ELISA, is a reliable method for detecting PVX and PVY from tubers in the field. Freshly sliced tissue is pressed onto a nitrocellulose membrane to create blots, and enzyme-labeled probes are used to detect antigens. This technique is vital for

large-scale plant virus diagnosis due to its speed, simplicity and accuracy (Jain *et al*., 2024).

### *Conventional Methods of Diagnosing Potato Viruses*

To produce safe seed potatoes, maintain quarantine, and implement certification programs, traditional methods of diagnosing and detecting viral diseases such as serology, biological indexing, electron microscopy, symptomatology, transmission studies, and electron microscopy were foundational (Mehetre *et al*., 2021). Virus reactions on indicator hosts allow for easy initial detection of infections. Biological indexing was successfully used for identifying and detecting PVA. However, there remained a gap in accurately identifying viruses, even though the symptoms appear on host plant susceptible indicator were sometimes considered adequate. Because of complexities of symptom development, host range studies became critical for identifying unknown viruses and their strains. Various indicator hosts are known, including *Nicotiana tabacum, Nicotiana clevelandii, Nicotiana glutinosa, Phaseolus vulgaris, Nicotiana debneyi, Solanum tuberosum, Chenopodium species like C. amaranticolor, C. hybridum, C. ambrosioides, C. murale, C. quinoa, C. opulifolium, C. polyspermum, C. rubrum, and C. urbicum, Physalis floridana, Datura stramonium, D. metel, Lycopersicon esculentum, and Gomphrena globosa*. Other diagnostic hosts include *Cyamopsis tetragonoloba* and *Cucumis sativus* for localized or systemic viral infections (Kumar and Singh, 2023). Though not ideal for testing large samples and considered time-consuming, these plants can be grown in vitro for experimental purposes. The primary challenge of this diagnostic approach lies in the varying symptom development across different indicator hosts infected by the same virus or strain.

Serological techniques gained popularity for diagnosing potato viruses following the successful production of antibodies against TMV through hybridoma technology (Shukla *et al*., 2023). Techniques such as gel immunodiffusion, micro-precipitation tests, and chloroplast agglutination revolutionized the virus indexing process. Subsequently, using these methods 50 distinct viruses of plant, including significant viruses of potato like PLRV, PVA, PVS, PVY, PVX, and PVM, were identified using these methods. Solid-phase ELISA was later developed to enhance the sensitivity of serological techniques, ability for simultaneous analysis of multiple samples, high sensitivity, and gaining prominence because easy to use (Ahsan, 2022). The minimum viral titer detectable by ELISA is around 2 ng/ml. ELISA was also adapted to detect PLRV in single aphids, and PVY was successfully identified using DAS-ELISA (Singhal *et al*., 2021).

### *Potato Viruses Diagnosing by Nucleic Acid Based Method*

To develop healthy seed potatoes and manage viral infections, easy to use, reliable, a highly specific, cost effective, sensitive, and reliable method is needed (Kreuze *et al*., 2020). Because of sensitivity, specificity and high precision polymerase chain reaction offers significant advantages in detecting viruses of potato, thereby addressing the limitations of earlier nucleic acid on based approaches. RT PCR and PCR have become increasingly popular in detecting and identifying viruses in potatoes (Abd El-Aziz, 2020). For RNA viruses, reverse transcriptase (RT) is used to generate complementary cDNA. RT-PCR, known for its high sensitivity and specificity, is regarded as the "gold standard" molecular technique for detecting potato viruses, researchers found thousand times more sensitive than ELISA (Raigond *et al*., 2022). Using antibodies, followed by PCR amplification PLRV is captured by ICPR. PVY has been detected through Print capture and direct binding PCR eliminates the need for sample grinding without affecting sensitivity. Using this method PLRV, PVY, and To LCNDV were detected. Nested PCR, a highly specific PCR variant, effectively detects multiple potato viruses. These methods are more reliable, specific, sensitive, and cost-effective than conventional ones (Baranwal *et al*., 2021).

Similarly, biological indexing or bioassays were previously used to detect potato viruses when serological methods were ineffective, especially for viroids with RNA-only genomes (Nie *et al*., 2021). Polyacrylamide gel electrophoresis, a viral diagnostic advance emerged as more reliable, fast method and accurate for viroid detection. Based on different mobility in the electric field PAGE enabled the successful detection of viroids by separating nucleic acids. Using PAGE, PSTVd in potatoes was effectively diagnosed (Kumar *et al*., 2019). However, nucleic acid spot hybridization (NASH) surpassed PAGE with its 1000-fold greater sensitivity. A solid-liquid technique, Dot blot hybridization in which radioactively labeled complementary DNA (cDNA) probes have proven effective for detecting key potato viruses like PVY, PVX, and PLRV. Additionally, non-radioactive, biotinylated RNA and

**Table1:** *Analysis of different types of potato virus diagnostic methods.*

| characteristic | PCR based technique | Hybridization based techniques | Serological techniques |
|---|---|---|---|
| Suitability for rapid identification | often faster, more efficient, and can be completed within one or two days, while requiring fewer samples | Less time-consuming, faster, more accurate, and achievable within a few days, requiring fewer samples | Often faster, but typically takes days to weeks to complete and requires a substantial number of samples |
| Fastness of detecting | Reliable results may take up to 48 hours | It can take a few days to get consistent results | When considering molecular methods, the speed is relatively slow |
| Cost and expertise | Affordable, yet often labor-intensive, requiring specialized equipment and skilled personnel for precise sample processing and results | Highly expensive but labor-efficient; requires advanced specialized equipment and well-trained personnel to meticulously handle samples and interpret findings | Less expensive than other molecular techniques, requires fewer skilled workers, and demands only minimal labor |
| Sensitivity level | PCR methods are more sensitive than serological techniques | Extremely sensitive compared to PCR-based or serological methods | Less sensitive than the molecular methods |
| Level of specificity | Highly selective isolation | Extremely high specificity | Generally beneficial to viruses |
| Accuracy of the Method | PCR methods are more sensitive than serological methods | Very sensitive compared to PCR-based or serological techniques | Less sensitive compared to the molecular approach |

DNA probes have been used to detect PVX and PVS in crude potato extracts (Querci, 1993). PLRV has been identified using dot-blot hybridization, and dot-blot assays have detected PVX, PLRV, PSTVD, and PVY from crude potato extracts. Adopting nucleic acid-based methods has significantly improved viroid detection (Kumar and Singh, 2023).

## Conclusion and Recommendation

Numerous methods for identifying and detecting potato viruses are now available and are employed in productive potato systems based on necessity and practicality. These methods are often useful in epidemiological research, advanced breeding for host plant resistance, seed certification, post entry quarantine systems, and the survey and monitoring of viral infections. Using multiple detection tools enhances sensitivity and specificity, expanding diagnostic applications for managing viral diseases and minimizing the impact of potentially fatal infections. To support the implementation of a global system of standardization and quality assurance, reliable diagnostic procedures must deliver accurate, consistent results. By making kits for diagnosis available to researchers and stakeholders worldwide from a single source, this problem may be addressed. However, these tasks require highly skilled personnel with expertise to perform diagnostic tests across various settings and interpret results accurately.

As molecular methods advance, the demand for diverse detection technologies will continue to grow. The creation of methods for detecting potato viruses that are swift, inexpensive, highly sensitive, and will undoubtedly affect the hygienic state of potatoes and prevent the spread of newly discovered or emerging viruses in an increasingly globalized world. Emerging molecular methods, such as microchips, microarrays, and loop mediated isothermal amplification offer enhanced sensitivity, specificity, and the ability to perform simultaneous testing. A technique capable of detecting multiple viruses at once is essential for testing planting material, especially for quarantined viruses that pose a threat to potato crops. Furthermore, appropriate sampling techniques and careful consideration of sample preparation are vital for each pathogen, plant material, and molecular technology combination. Developing effective detection strategies is an ongoing challenge that requires expertise.

The approach to virus detection is transferring to sophisticated molecular methods from traditional employing many strategies. PCR and serological methods, combined with access to full genome sequences and microarray technologies, will soon allow researchers to determine the functional genomics of most potato viruses. This advancement will result in the discovery of innovative approaches and new diagnostic targets for virus detection. The development of RNA microarrays will facilitate the selection of novel diagnostic markers by enabling gene expression analysis of a wide array of plant virus genes. Host-pathogen interactions will benefit from the insights gained

through these analyses. As new sequences and molecular technologies evolve, the field of genomics will continue to introduce cutting-edge tools for detecting potato viruses.

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## Novelty Statement

This review highlights cutting-edge molecular and digital strategies for detecting and diagnosing potato viruses, emphasizing innovations like CRISPR-based diagnostics and AI-driven tools. By showcasing these advancements, the paper underscores their potential to transform precision agriculture practices, enhancing accuracy, speed, and sustainability in potato virus management.

## Authors Contribution

**Mohsin Raza:** Conceptualized and wrote the manuscript.
**Zeshan Hussain, Fazil Abbas, and Muhammad Atiq/ Ashraf:** Contribute to the investigation and data collection **Talha Riaz and Hadj Henni Imene**: Contribute to reviews of the manuscript.

*Conflict of interest*

The authors have declared no conflict of interest.

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