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Biosensors for Plant Viruses

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Plant diseases result in severe economic loss in the agriculture economy around the globe. In most of the crops, the fungal and bacterial pathogens have been effectively controlled by chemical treatment methods, however, no such effective treatment is available for plant virus diseases once established. The most preferred strategy to manage the losses by viral diseases are through preventive measures, for which detection of specific viruses associated with the plant at the early stage of infection is indispensable. Various detection methods have been developed for plant viruses including symptomatology, electron microscopy, ELISA, PCR, microarrays and next-generation sequencing. These approaches have ability to detect viruses, however, they have limitations in terms of field use. Recently, biosensors have been proposed as easy-to-use and point-of-care highly selective and sensitive diagnostic devices that allow real-time monitoring in the field.

Point-of-Care biosensing devices are fast, inexpensive, easy to handle and does not require trained manpower. Although the use of biosensors in healthcare is well known and has revolutionized the POC diagnostics but the use of biosensors in agriculture is new and is still in a primitive stage. Most of the work in this area has attracted attention of agricultural scientists in the last decade only.

A biosensor is an electronic device that uses biological elements as the analyte sensor, such as antibodies, enzymes, receptor proteins, nucleic acids, cells, or tissue sections, and couples the biological element to a transducer for signal detection, which is then displayed on a panel after processing and amplification. Biosensors combine biological molecules' excellent selectivity with the processing power of current microelectronics and optoelectronics to create powerful analytical tools. A transducer detects and measures the physiochemical change caused by specific interactions between the target analyte and the bio recognition element. A biological signal is subsequently converted to an electronic signal, which is ultimately processed into an analogue or digital format by the transducer. The amount of signal created is directly proportional to the concentration of an analyte, allowing for quantitative and qualitative measurements.

The biosensors comprise of three main components: (i) Biomolecular recognition element, (ii) Immobilization matrix and (iii) Transducer. Biosensors are designed to sense a biologically specific material, for example, antibodies, proteins, enzymes, immunological molecules and so on.

(i) Biomolecular recognition element: It is responsible for specificity of the biosensor and has the potential of recognizing a target analyte. Enzymes, antibodies, DNA, and cells are the most frequently used bioreceptors.

(ii) Immobilization matrix: A solid support to hold the sensing molecule is known as a matrix. The enzyme immobilisation matrix might be organic or inorganic. It is preferable for a material to make films and be adaptable to varied settings in order to form an appropriate matrix. It should be able to withstand a wide range of physiological pH, temperature, ionic strength, and chemical composition, among other things.


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(iii) Transducer: It is used to convert a biological signal into the electronic signal received from a biochemical reaction. Different types of transducers have been used depending upon the type of signal received such as electrochemical, optical, thermal or piezoelectric. The signal received from a transducer is recognized by the detector and is translated in the digital form corresponding to the analyte concentration.

Immunosensors are standard detection technologies used in clinical laboratories for disease diagnosis, food safety testing, and environmental pollution monitoring. In an Immunosensor biological recognition element is an antigen or antibody, depending upon the type of target/analyte. There are three major types of Immunosensor detection devices:

Optical, Piezoelectric, and Electrochemical (potentiometric, amperometric & conductimetric). Various biosensors developed for pathogen detection are listed in Table 1.

Table 1. Details of various biosensors developed for plant pathogens

S. No.	Application	Nanotechnology system	References
1.	Cucumber mosaic virus (CMV)	Electrochemical enzyme-linked immunoassay (ECEIA)	Jiao *et al.* (2000)
2.	Cucumber mosaic virus (CMV)	Electrochemical immunosensor	Sun *et al.* (2001)
3.	Cymbidium mosaic potexvirus (CymMV) and Odontoglossum ringspot virus (ORSV)	Quartz Crystal Microbalance (QCM) immunosensor	Eun *et al.* (2002)
4.	Tobacco mosaic viruses (TMV)	Bio-imprinted QCM sensors	Dickert *et al.* (2004)
5.	Cucumber green mottle mosaic virus (CGMMV	Biosensor employing membrane engineering of fibroblast cells	Moschopoulou *et al.* (2008)
6.	Plum pox virus (PPV)	Electrochemical impedance spectroscopy (EIS) immunosensor	Jarocka *et al.* (2011)
7.	Potato virus *Y* (PVY), cucumber mosaic virus (CMV) and tobacco rattle virus (TRV)	Membrane-engineering of Vero cells.	Perdikaris *et al.* (2011)
8.	Tobacco mosaic virus (TMV) and cherry leaf roll virus (CLRV)	Escherichia coli (XL-1Blue MRF) cell-based sensor	Gramberg *et al.*, 2012
9.	Cucumber mosaic virus (CMV)	Label-free chemiresistive sensor	Chartuprayoon *et al.* (2013)
10.	Maize chlorotic mottle virus (MCMV)	Surface Plasmon Resonance (SPR)	Zeng *et al.* (2013)
11.	Prunus necrotic ringspot virus (PNRSV)	Impedimetric immunosensor	Jarocka *et al.* (2013)
12.	Papaya ringspot virus (PRSV)	Electrochemical impedance spectroscopy-based immuno-sensor	Valekunja *et al.* (2016)
13.	Maize chlorotic mottle virus (MCMV)	Quartz crystal microbalance (QCM) immunosensor	Huang *et al.*, 2014
14.	Cymbidium mosaic virus (CymMV) and Odontoglossum ringspot virus (ORSV)	Fiber optic particle plasmon resonance (FOPPR) immunosensor	Lin *et al.*, 2014
15.	T7 bacteriophage	One-step colorimetric sensor	Lesniewski *et al.* (2014)
16.	Tomato mosaic virus (ToMV) and turnip yellow mosaic virus (TYMV)	Electrical impedance sensor	Ambrico *et al.* (2016)
17.	Capsicum chlorosis virus (CaCV)	Nano-Au/C-MWCNT based label-free amperometric immunosensor	Sharma *et al.* (2017)
18.	Citrus Tristeza Virus (CTV)	Quantum Dot nanobiosensor based on the fluorescence emission of cadmium telluride quantum dots(CdTe-QDs)	Safarnejad *et al.* (2017)
19.	Plum pox virus (PPV)	electrolyte-gated organic field-effect transistor (EGOFET) based biosensor	Berto *et al.* (2018)
20.	Citrus Tristeza Virus (CTV)	Label-free impedimetric biosensor	Khater *et al.* (2018)
21.	Groundnut bud necrosis virus (GBNV)	Graphene oxide based electrochemical immunosensor	Chaudhary et al 2021

The use of nanomaterials or nanoparticles in biosensors allows for the development and application of new signal detection assay. For a particular detection between target phytopathogenic cells and bio-functionalized nanomaterials, many techniques such as antibody-antigen, adhesion-receptor, antibiotic, and complementary DNA sequence recognitions have been developed. Nano sensors will help us to detect specific plant pathogens before symptoms development, allowing us with its proper management. The advantages include a low detection limit, reduced time, cost, biocompatibility, precision, and accuracy. Because the technology is smaller, faster, and more sensitive, it offers various advantages over traditional pathogen detection methods such as culture techniques, PCR (polymerase chain reaction), ELISA (Enzyme Linked Immunosorbant Assay), Flow cytometry, and so on.

Nano-scale devices with unique features have potential to create smart agricultural systems in the near future. These nanodevices may be employed to detect plant health issues before they become sever. Such gadgets may be capable of reacting to different circumstances, diagnosing the problem, and helps in the necessary disease management. Their uses include the detection of analytes such as chemicals, metabolites and pathogens. Their portability makes them suitable for field use, and they can also be used in the laboratory.

Implementing nano smart devices will serve as a protective and early warning system. Plant pathogens might be accurately tracked, detected, and diagnosed using specific nanodevices in the early stages of disease. The use of nano biosensors in diagnostics could be a game-changer, filling a gap in the field of pathogen detection technologies. Although this concept has not been fully implemented yet, the use of nanotechnology, minute sensors and monitoring system will have a revolutionary impact on farming methods in near future.

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Diagnosis and management of seed borne tobamoviruses in vegetable crops

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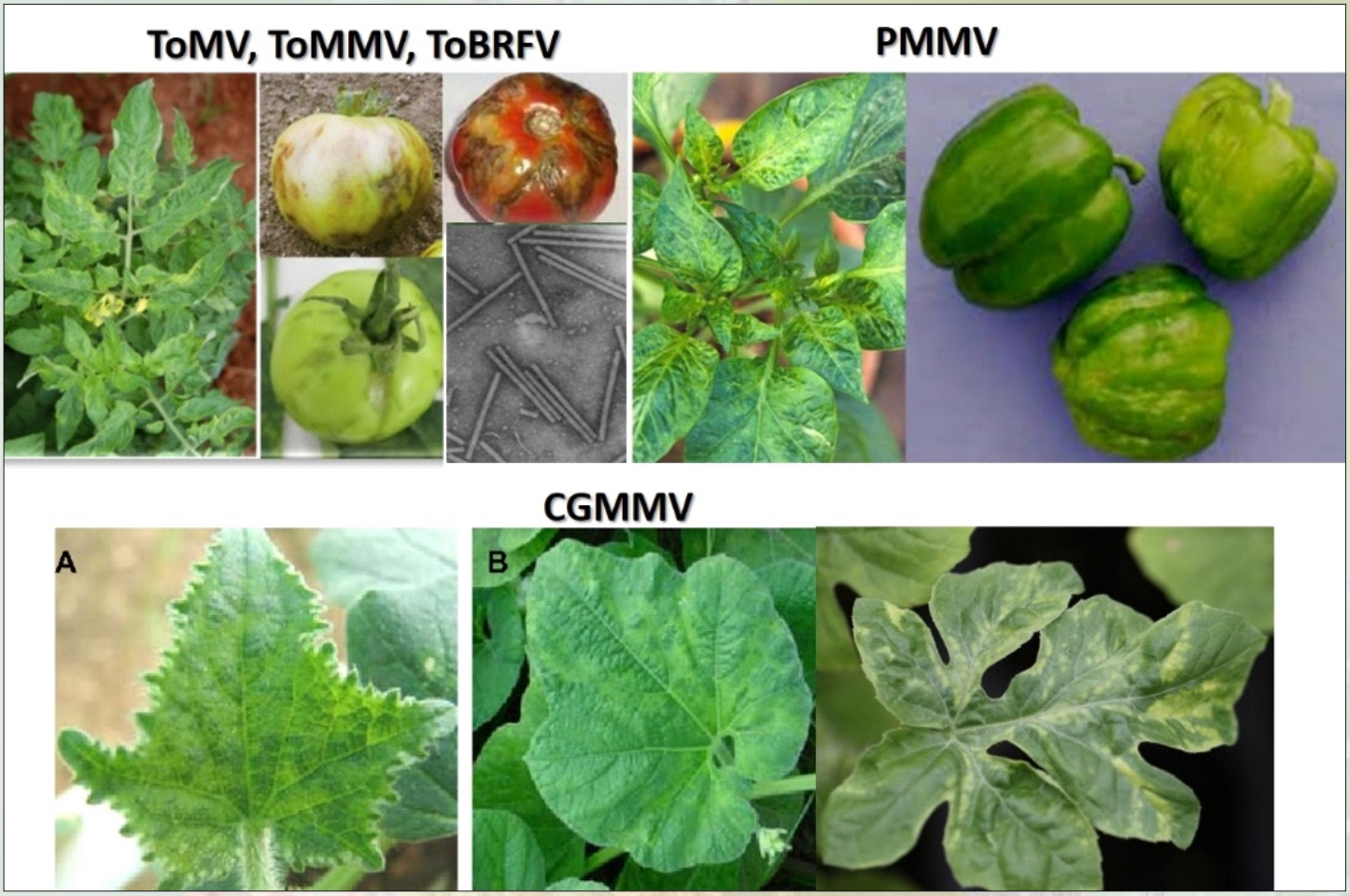
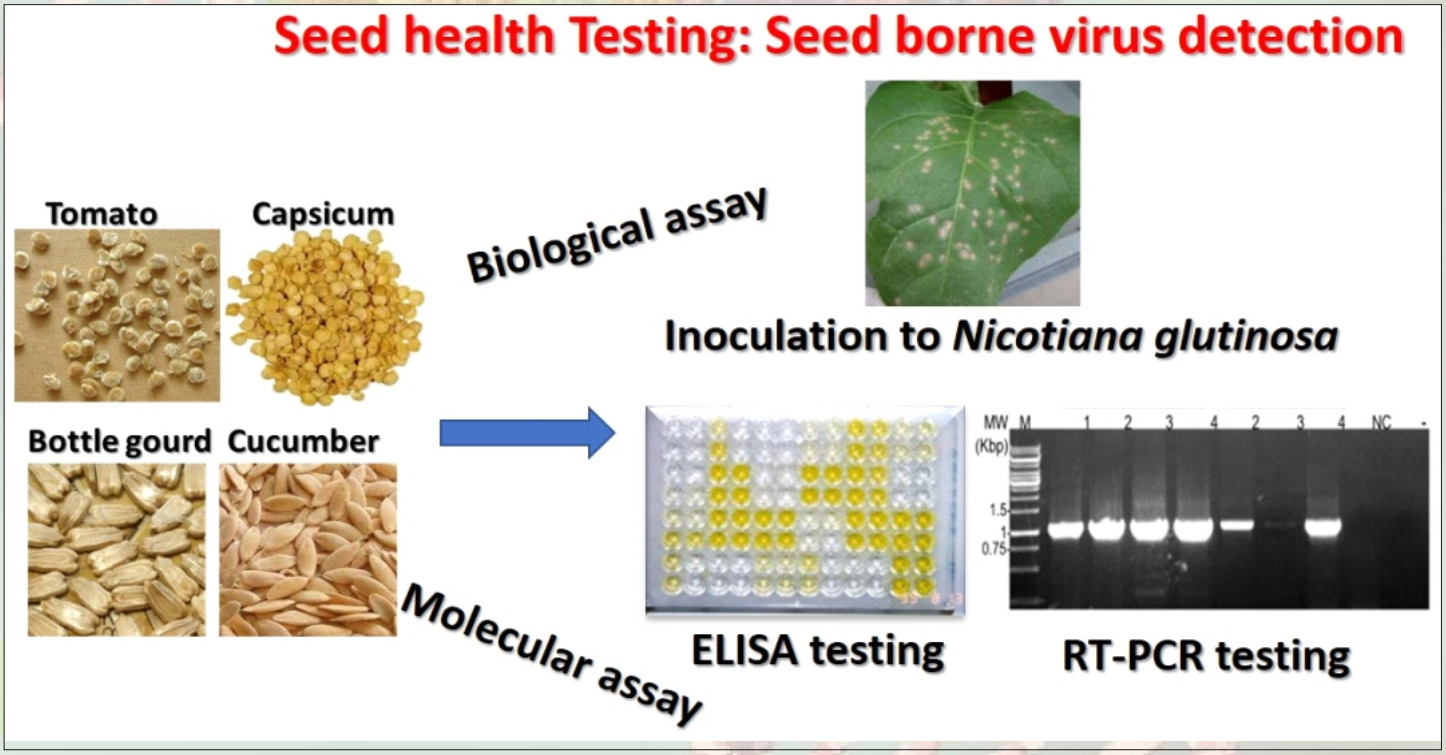
Global seed trade contributed to development and improvement of world agriculture. Seed transmission of plant viruses has a great epidemiological significance causing disease outbreaks worldwide. Seed trade globalization has opened new pathways for the mobilization of crop produce between continents and countries, which reflected in disease outbreaks in new growing areas. Seed Transmission is of considerable epidemiological significance and has a special bearing on international transfer of genetic resources, now being stimulated for crop diversification and crop improvement. Seed Transmission of plant viruses known to play a very important role in the ecology of the crop virus diseases. Seed borne inoculum was of considerable importance in the overwintering of the disease and as a primary inoculum source. Plants developed from infected seed may themselves suffer from infection.

Seed-borne plant viruses are a threat to world agriculture. Among them, species that belong to the genus Tobamovirus are considered a major peril to a range of cultivars especially to those belonging to the Solanaceae and Cucurbitaceae (cucurbit) families. For more than a century, tobacco, tomato, and pepper plants that belong to the Solanaceae family are infected by the Tobacco mosaic virus (TMV), Tomato mosaic virus (ToMV), Tomato mottle mosaic virus (ToMMV), Tomato brown rugose fruit virus (ToBRFV) is a new Tobamovirus isolated from tomato plants grown in greenhouses and Pepper mild mottle virus (PMMoV). Among the main cucurbit-infecting tobamoviruses, Cucumber green mottle mosaic virus (CGMMV) is the most economically important and currently considered a significant threat for the production of bottle gourd, cucumber, melon, watermelon, gherkin, and pumpkin. The virus species of Tobamovirus genus have worldwide distribution and can occur in different environments, in tropical as well as in temperate climates.



The tobamoviruses are seed-borne, mechanically transmitted stable viruses. Infectious particles are primarily attached to the seed coat. Indeed, viral inoculum is efficiently transmitted when it enters the embryo, and viruses attached to the seed coat may not survive germination when seed coat separates from the seedlings. However, in nursery seedlings, the Tobamovirus-contaminated seed coat may affect the wounded roots, which occur upon transplantation. Furthermore, low transmission rate to seedlings does occur when tobamoviruses contaminate the seeds. Seeds or seedlings are used in large scale continuously in regular farming. Therefore, even a low percentage of contaminated seeds can cause a multitude of infection foci. Consequently, the primary infectious source can be spread rapidly by mechanical contacts, workers' hands, tools, greenhouse structure, and trellising ropes and the tractor path in open fields. The infectivity of tobamoviruses is preserved in plant debris and in the contaminated soil and clay for months up to years



High titer of Tobamovirus species accumulates in reproductive organs leading to viral particles adsorbed to seed coat, which potentially establish a primary infectious source. Tobamovirus-contaminated seeds show very low virus transmission in grow-out experiments as detected by enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) analysis. Interestingly, *in situ* immunofluorescence analysis of Cucumber green mottle mosaic virus (CGMMV) reveals that the perisperm-endosperm envelope (PEE) is contaminated as well.

The current ishi-veg method for detecting Tobamoviruses in tomato seed, an industry standard, is a local lesion assay that provides conclusive evidence of presence of viable and infectious. Whereas ELISA and RT-PCR detects proteins and nucleic acids that are specific to the target virus but does not demonstrate the presence of infectious virus. The International Seed Federation (ISF) http://www.worldseed.org/, the International Seed Testing Association (ISTA) https://www.seedtest.org/en/seed-health-methods-_content---1--1452.html, and International Seed Health Initiative for Vegetable Crops (ISHI-Veg) http://www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg/#protocols adapted, developed, and validated protocols for pathogen diagnosis in seed stock materials. For the tobamoviruses such as TMV, ToMV, ToMMV, ToBFRV and PMMoV testing requires 12 batches of 250 seeds each (3000 seeds in total), with detection threshold of 1:249 (1 infected seed/249 healthy seeds). For detection of cucurbit-infecting viruses such as the CGMMV, 20 batches of 100 seeds each (2000 seeds in total) are required.

Methods that are used in large-scale commercial seed production are mostly based on various chemical treatments such as 1–9% hydrochloric acid HCl, 1–5% calcium hypochlorite $CaOCl_2$ 1–3% sodium hypochlorite NaOCl, tetramethylthiuram disulfide (TMTD) $(CH_3)_2NCSS_2CSN(CH_3)_2$, and the most commonly used in commercial seed production 10% trisodium phosphate (TSP) Na_3PO_4, which have been reported to provide satisfactory control of tobamoviruses in cucurbit and solanaceous seeds. Physical seed treatment such as heat treatmentsincluding hot water, hot air, aerated steam, and radiation were also used.In addition to the chemical treatments, several heat treatment protocols at various temperature conditions ranging from 72 to 76°C for a minimum of 12 h up to 72 h are also applied in large-scale production.

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CRISPER/Cas9: third generation genome editing tool to develop plant viral resistance

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Protecting crops against viral infections is a serious concern for agriculture. Many crops suffered upto 90 percent economic losses as a result of viral infection, such as BBTV, CMV, PLRV, PVX, ToMV, TSWV, and TYLCV. Disease management methods rely heavily on resistant cultivars which is often extremely successful once deployed. Pesticides used in the field are often not particularly selective; when killing infections, they might also impact other beneficial species, disrupting the ecological balance. Disease-resistant crop varieties are being developed as part of an integrated agricultural programme that is both efficient and ecologically friendly. Recently, a novel technique to genome editing known as clustered regularly interspaced short palindromic repeats and CRISPR associated protein 9 (CRISPR/Cas9) has been employed to create virus-resistant cultivars. Oweing to its unique characteristics, such multiple-gene editing, simplicity, consistent accuracy, reduced off-target and higher output, CRISPR/Cas9 is recognized as a very promising genome-editing technology in crops. This process invades foreign DNA pieces of virus particles, allowing them to recognise and destroy the DNA or RNA sequences in anticipation for subsequent invasion. CRISPR/Cas9 technology manipulates plant viral defence mechanisms by recognising and deleting harmful genes that infiltrate them. It may also be used to create crop cultivars with greater resistance to certain plant viruses. Because of its sequence-specific nuclease capacity, this method has transformed viral resistance research.

Several researchers have used this technique to successfully combat virus infection in plants by targeting the viral genome. So far, the CRISPR/Cas9 gene editing technology has been used in over 20 crop species for a variety of features such as yield enhancement and biotic and abiotic stress management. Many of the published publications are thought to be proof-of-concept studies since they detail the use of the CRISPR/Cas9 system to knock out certain reported genes that play a vital role in abiotic or biotic stress tolerance systems. The CRISPR/Cas9 system has the potential to transform plant improvement methods in a revolutionary way, and it is a flexible tool for genome editing in which many genes may be targeted using short RNA guidance.

CRISPER/Cas9 and Virus resistance mechanism

The CRISPR/Cas9 technology finds and locate a pathogen's genetic material using three steps. These are

i) acquisition, ii) expression, and iii) interference. The first stage, acquisition, involves inserting foreign DNA of viruses or plasmids as a spacer. CRISPR loci have been replicated and utilised to generate short RNA (crRNA), directing effector endonuclease genes to target viral components via simple complementarity. The PAM (protospacer adjacent motif) is a brief (2–5 bp) length of retained nucleotides that receives the DNA fragment (spacer) for identification. Any mutation in PAM sequence or viral genomes renders this CRISPR-mediated defence ineffective. DNA interference in the CRISPR/Cas9 technique relies on a single protein known as CRISPR associated protein or Cas9. The crRNA protein guide the Cas9 protein to the target foreign DNA location to be broken down during the interference stage, providing defence against pathogen assaults. Cas9 is a large protein complex comprising two short RNA segments, crRNA and trans-activating crRNA, as well as numerous domains (RuvC domain at the amino terminus and HNH nuclease domain positioned centrally) (tracrRNA).

Cas9 promotes adaptability and process pre-crRNA into crRNA, and introduces DNA double-strand breaks (DSBs) guided by tracrRNA and RNAse III-specific double-stranded RNA. The design and development of CRISPR/Cas9 structures is generally easy, inexpensive, and free of intellectual property issues. The CRISPR/Cas9 tool, crRNA, and tracrRNA components may be combined to form the sgRNA, which directs Cas9 to target particular DSBs. The design of sgRNAs is relatively simple, which makes genome editing more appealing. Initially, the approach was intended to cleave DNA *in vitro* at different locations. This approach has recently been used to modify bacterial, fungal and yeast genomes.

Developing Plant Virus-Resistance Strategies

This method has been adopted to develop resistance by interrupting the function of the susceptible (S) gene(s), altering the plant–viral communication leading to lower viral activity in the host plant. Another strategy employs introducing InDels in promoter region of a gene rather than the coding region. CRISPR-mediated promoter disruption affects total gene expression and the effector-binding site of susceptible plants by abolishing connection between viral effector and the promoter. Deleting chromosomal segments next to S gene clusters may result in long-term viral resistance in many hosts. Apart from viral resistance, CRISPR-mediated gene insertion provides the gateway to studying key S genes. Recent research has identified many resistance (R) gene(s) in wild species and demonstrated effective resistance transfer in cultivated crop species.Using homology directed repair (HDR) methods, this approach may replace incorrect and non performing R genes with a functioning R gene. It is preferable to introduce just certain mutations linked with viral resistance features rather than altering the entire gene.

Specific examples of key resistance techniques by CRISPR/Cas9 were used. For instance, Pyott *et al*. (2016) developed considerable genetic resistance against TuMV in *A. thaliana* plants by deleting a known host component (eIF(iso)4E) required for viral survival using this technique.

Successful delivery of the Cas9-sgRNA complex via CRISPR/Cas9 technology demonstrated its viability for segregation of the transgenes originated by induced mutation at the targeted eIF(iso)4E location, initially to generate stable and heritable mutations, with the exception of any persistent transgene.This strategy is projected as the reason why the recessive gene allele eIF(iso)4E displays more lasting resistance to TuMV infection; the existence of VPg polymorphisms acting through eIF(iso)4E is a separate mechanism.

Zhang *et al*. (2018) demonstrated selection of CaMV CP gene target sites using standard Cas9 protein.Linear arrays of Arabidopsis U6 promoter: sgRNA units were developed and constructed. Cas9 and sgRNAs are constantly expressed in cells when viruses are controlled using the CRISPR-Cas9 system. Cas9 recruitment to viral DNA is dependent on the availability and quantity of sgRNAs. However, because sgRNAs contain folded dsRNA domains, siRNAs can be generated to encapsulate the foreign RNAs.

Tripathi *et al*. (2019)- designed the gRNAs to target the sequences of BSOLV and eBSOLV. Three gRNAs were inserted into the triploid Musa genome based on their specificity to their target location (targeting sequences S1, S2, and S3 from ORF1, ORF2, and ORF3, respectively) and low potential off-targets. The OsU6 promoter followed two BbsI restriction sites and a tracer RNA scaffold in the gRNA cassette, amplified from the pZKOsU6-gRNA plasmid and cloned into pENTR-D/Topo. In order to generate the gRNA modules, one gRNA from each ORF of the BSV genomic sequence was custom synthesised and cloned into pMR185. The Cas9 endonuclease was used in an Arabidopsis plasmid that was codon-optimized and driven by the parsley ubiquitin promoter (PcUbi).

Pramanik *et al*. (2021) employed Agrobacterium-mediated transfer DNA (T-DNA) modification to produce sgRNA cassettes via the U6-26s promoter and Cas9 protein via the CaMV-35S promoter in *N. benthamiana* plants. The U6-sgRNA cassette and Cas9 protein were cloned in a binary vector and transferred to *N. benthamiana* leaf discs using *Agrobacterium tumefaciens*.

Their findings depict the potential of CRISPR/Cas9 technology to target infectious TYLCV strains in the CP, IR, and Rep sequences of transgenic *N. benthamiana* plants.

Overall, genome editing has known to be an efficient technique for both molecular plant–microbe interactions and disease resistance breeding. We will undoubtedly see further uses of genome editing in the development of plants resistant to various infections and in the acceleration of breeding for robust and broad-spectrum resistance. These advances in genome editing will undoubtedly improve ecologically friendly agriculture.

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Virus pandemics haunting the human beings and their origin

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The year 2020 will be remembered in the history for the catastrophe of the deadly Coronavirus (COVID-19) which virtually halted the world. The invisible enemy with diameters of approximately 125 nm caged the entire population of the globe to the periphery of their homes for months together. These invisible enemies continued haunting the human beings despite of our enlarged knowledge and technological advancement.

Pandemics are large-scale outbreaks of infectious disease that can greatly increase morbidity and mortality over a wide geographic area and cause significant economic, social, and political disruption. Evidence suggests that the likelihood of pandemics has increased over the past century because of increased global travel and integration, urbanization, changes in land use, and greater exploitation of the natural environment. Pandemics have afflicted civilizations throughout human history, with the earliest known outbreak occurring in 430 BC in the ancient Greece during the Peloponnesian War fought between Athens and Sparta in ancient Greece which went to war with each other from 431 to 405 B.C. Another pandemic struck during the reign of Justinian I, the emperor of the Byzantine Empire- Roman Empire then situated midway between the Mediterranean and the Black Sea in the 6th century. Now known as the Plague of Justinian, this pandemic is thought to have killed between 30 – 50 million people, perhaps equal to as much as half of the world's population at the time.

As per the records, pandemic of the pathogens also hit Europe in 1347. It is believed that it entered through the Crimean town of Caffa where it was brought by the besieging Mongol army. Then, fleeing merchants unwittingly carried it back to Italy and from there, it spread to France, Spain and England and within six years, the Black Death had engulfed the continent. Due to the scant records of the 14th century, it is difficult to know the true toll, but it is estimated that somewhere between 5 to 14% of the entire world's people were killed. European population levels took over 200 years to return to return to their level before the pandemic. This pandemic killed greater numbers in Asia, especially China, where it is thought to have originated. In this man verses pathogen conflict, Europeans introduced a number of new diseases when they first arrived in the continent of the Americas in 1492. One of these was smallpox and this disease claimed the lives of approximately 20 million people, close to 90% of the population, in the Americas.

The "Spanish" flu of 1918 was one of the deadliest pandemics in human history. In a matter of months, a third of the world's population was infected and the global economy shrank by 5 per cent. This pandemic killed an estimated 50 million people worldwide, including an estimated 675,000 people in the United States. An unusual characteristic of this virus was the high death rate it caused among healthy adults of 15 to 34 years of age. The pandemic lowered the average life expectancy in the United States by more than 12 years. A comparable death rate has not been observed during any of the known pandemics that have occurred either prior to or following the 1918 pandemic. The virus that caused the 1918 influenza pandemic probably sprang from North American domestic and wild birds. Origins of influenza A virus has been traced to its evolution and flow through different animal hosts over two centuries. Since 1918, the world has experienced four additional pandemics, in 1957, 1968, 2009 and most recently in 2019-20. These subsequent pandemics were less severe and caused considerably lower mortality rates than the 1918 pandemic. The 1957 H2N2 pandemic and the 1968 H3N2 pandemic each resulted in an estimated 1 million global deaths, while the 2009 H1N1 pandemic resulted in fewer than 0.3 million deaths in its first year.

One reason the pandemics in last few decades might be the extensive interface between human beings, domestic poultry, and wild waterfowl, which is generated by the high human population density, the high density of domestic poultry, and ample opportunities for domestic birds to be exposed to wild waterfowl in some regions of Asia. Hidden history of human disease for centuries is illuminated by genetic evidence. The family tree of the measles virus, for example, is telling, revealing that today's measles is descended from a rodent disease that jumped to cattle and subsequently to humans. Recovery of lung tissue from victims of the 1918 pandemic has allowed the isolation of viral RNA and the reconstruction of the complete 1918 pandemic virus in the laboratory. These experiments support the hypothesis that the 1918 H1N1 virus was of avian origin and adapted to human infection and transmission. In contrast, the influenza viruses that caused the 1957 and 1968 pandemics are human-avian reassortant viruses, and this difference may be relevant to the severity of the 1918 pandemic.

The SARS outbreak originated from bats of the genus Rhinolophus and its human emergence is believed to have been facilitated through intermediate hosts in the wet markets of southern China. The H1N1 epidemic appears to have arisen in North America primarily through the reassortment of viruses of swine origin. The swine-origin influenza A (H1N1) virus that appeared in 2009 and was first found in human beings in Mexico, is a reassortant with at least three parents. Six of the genes are closest in sequence to those of H1N2 'triple-reassortant' influenza viruses isolated from pigs in North America around 1999-2000. Its other two genes are from different Eurasian 'avian-like' viruses of pigs; the NA gene is closest to H1N1 viruses isolated in Europe in 1991-1993, and the MP gene is closest to H3N2 viruses isolated in Asia in 1999-2000. The sequences of these genes do not directly reveal the immediate source of the virus as the closest were from isolates collected more than a decade before the human pandemic started. Many of the emerging influenza viruses that are deemed to have pandemic potential, including H5N1, H5N6, H6N1, H7N9, and H10N8, have crossed the species barrier from animals to human beings in Asia. Increased surveillance in swine has revealed that human-to-swine transmission actually occurs far more frequently than the reverse. Swine-to-human transmission occurs periodically and can trigger pandemics, as in 2009. But swine are not necessary to mediate the establishment of avian viruses in humans, which invites new perspectives on the evolutionary processes underlying pandemic emergence. Coronavirus is believed to have originated in bats and it enters the body through the nose, mouth or eyes, then attaches to cells in the airway that produce a protein called ACE2. The virus infects the cell by fusing its oily membrane with the membrane of the cell. Once inside, the coronavirus releases a snippet of genetic material called RNA. The infected cell reads the RNA and begins making proteins that will keep the immune system at bay and help assemble new copies of the virus.

The full history of humanity of at least 200,000 years is testimony to the fact that the chance of human extinction from natural catastrophes of any kind must have been very low for most of this time. In all, humans are afflicted by dozens of respiratory viruses that have evolved to specialize in the exploitation of us. Current coronavirus pandemic is part of our unique trajectory as a species.

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The Race for Viral Vaccines to beat the Next Pandemic

Anil Handa, Ajay Brakta and Bhupesh Gupta

Deadly influenza strains leapt from birds to humans in Hong Kong and the Netherlands in 2003 and ominous dark clouds started gathering overhead posing serious threats of emerging infectious diseases. In the background, a new coronavirus was spreading around the world causing a mysterious illness that became to be known as severe acute respiratory syndrome (SARS) and leading virologists feared the start of a global pandemic. After the SARS threat subsided, interest on this new virus evaporated and the world paid the price. Another warning shot came in 2012 when Middle East respiratory syndrome (MERS) caused by another relative of SARS-CoV-2 started spreading through a handful of countries.

Dr Anil Handa

RNA vaccines

Aside from one qualified success in remdesivir, a therapy originally developed to treat hepatitis C and Ebola, there were practically no strong antiviral drug candidates to quickly test and deploy against SARS-CoV-2. New initiatives to create that arsenal are on the horizon. The US National Institutes of Health (NIH) is planning a major programme to develop therapeutics against SARS-CoV-2 variants and other viruses with pandemic potential. A new industry-backed coalition is also taking aim at influenza viruses and coronaviruses and a few groups hope to create antivirals for more distantly related pathogens that pose a pandemic risk. The past year has seen a large number of SARS-CoV-2 centred drug discovery efforts. Since viruses in general are not good at catching genetic mistakes, nucleoside analogues based therapies often work across viral families. Antiviral drugs bind enzymes directly and block their function which is not a typical broad activity expressed by the vast majority of antivirals. In principle, scientists could design drugs that work in many viruses by going after the most highly conserved regions of target proteins though conventionally it has always been a *One Bug One Drug* approach. This philosophy has served the pharmaceutical industry well when it comes to making new medicines for HIV or hepatitis C but it' has proven inefficient in terms of rapidly addressing epidemics or pandemics.

Mechanism

Viral vaccines are medications that help the body ward off certain viruses that can cause disease. Antiviral drugs are also preventive and can protect us from getting viral infections or spreading a virus to others and can ease symptoms and shorten the length of a viral infection. Viral vaccines work differently depending on the drug and virus type. These vaccines can block receptors to prevent viruses for preventing binding to and enter healthy cells; boost the immune system to help it fight off a viral infection and lower the viral load in the body. In nature, most viruses clear up without antiviral medications however, healthcare providers prescribe antivirals to treat chronic or life-threatening viral infections including Coronaviruses

like COVID-19, Ebola, flu, H1N1 (swine flu), Genital herpes, Hepatitis B and C besides Human immunodeficiency virus (HIV).

Antiviral drugs can ease symptoms and shorten the illness period with viral infections like the flu and Ebola and can rid the body of these viruses. On the contrary, viral infections like HIV, hepatitis and herpes are chronic and antivirals cannot get rid of these viruses. However, antiviral medicines can render the virus latent to suppress the symptoms or. Symptoms that develop while you take antivirals may be less severe or go away faster. Most antivirals are oral drugs that you swallow. But you may also receive antiviral medications as eye drops, inhaled powder, injection (shot) into a muscle, IV into a vein or topical (skin) ointments or creams. Skipping doses or starting and stopping an antiviral medicine can allow a virus to change/adapt so that the antiviral is no longer effective and the virus develops resistance against the vaccine. Prolonged use of antivirals can also result in the development of resistance against viral vaccines.

Viruses- hard nuts to crack

In many ways, the narrow activity of existing antivirals boils down to the nature of viruses themselves. Other types of pathogen like bacteria, fungi and parasites are more easily contained because their cellular properties offer an abundance of targets for drug activity as in case of penicillin which blocks cell wall synthesis or azole antifungals which disrupt the cell membrane. With their compact genomes and lack of cellular anatomy, viruses offer fewer targets. High rate of replication a typical SARS-CoV-2 infection, for instance, is thought to produce more than one million virions per person per day coupled with an inherent genetic mutability, and it's no wonder that most existing antivirals proved useless for COVID-19. The plasticity of viruses means that a drug with activity against one virus is unlikely to make a dent against another virus. Need of the hour is to develop inhibitors that work on an entire *Coronaviridae* family thus making the best case scenario a pan-coronavirus inhibitor. But a more reasonable goal might be developing a drug for a subset of coronaviruses such as alphacoronaviruses that currently cause non-lethal infections in humans and have a different drug for betacoronaviruses, the group responsible for SARS, MERS, and COVID-19.

The researchers need to find 'druggable pockets' on the surface of essential enzymes that are conserved between related viruses and can be used to design active molecules if the drug is directed at the virus itself. Some drug researchers instead aim to interfere with human pathways that a broad array of viruses commandeer for their own purposes. Efforts are on to develop a drug that blocks a fat-regulating enzyme used by many viruses to promote cellular entry and replication. By inhibiting this enzyme, the virus is deprived access to a host function upon which it depends. In yet another host-directed antiviral strategy, small peptide drugs are developed that poke holes in the lipid wrappings found around enveloped viruses. These lipids come from the membrane surface of human cells. But the peptides penetrate only lipids that encase viruses and not cells because of differences in the size and bending ability of the membrane structure. The lipid coating is the common denominator of all enveloped viruses, a group that includes flaviviruses, alphaviruses, coronaviruses, filoviruses, retroviruses and more and no other shared feature exists broadly across all these diverse viruses. It leads to the speculation that host-targeted antivirals might have greater potential as pandemic-preparedness tools. Human biology also offers many more potential targets than do viruses. Additionally, viruses are less able to develop resistance against host-targeted antivirals. A viral protein might need just a mutation or two to thwart drug binding whereas a host-targeted therapy could force the virus to exploit entirely different cellular processes.

Mode of action and development of viral vaccines

Antiviral drugs are prescription medicines (pills, liquid, an inhaled powder, or an intravenous solution) that fight against viruses in the body. These vaccines are able to enter the cells infected with virus by interfering with viral nucleic acid synthesis and/or regulation wherein some agent interfere with virus ability to bind with cell and other agents stimulate the body's immune system. Antiviral vaccines inhibit viral attachment, prevent genetic copying of virus and prevent viral protein production that are vital for reproduction of virus. The emergence of antivirals is the product of relatively newly acquired knowledge of the genetic and molecular function of organisms that helps in better understanding of the structure and function of viruses, major advances in the techniques for finding new drugs and the pressure placed on the medical profession to deal with the human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS). First experimental antivirals were developed in the 1960s mostly to deal with herpes viruses and were found using traditional trial-and-error drug discovery methods. It was only in the 1980s, when the full genetic sequences of viruses began to be unravelled, did researchers begin to learn how viruses worked in detail and exactly what chemicals were needed to thwart their reproductive cycle.

Future considerations for research

The discovery and development of antiviral drugs, compounds and clinical methods to prevent viral infections is beneficial for global health. The emergence and re-emergence of many viruses is a threatening alarm for both animal and human populations. The best example is that of Zoonotic viruses like Coronavirus which can cause extensive morbidity and mortality. Preventive vaccines that provide protection are available for only a limited number of viruses. It is

crucial to develop new drug therapies combining antivirals to increase efficacy and to avoid the development of drug resistant strains and there is an urgent need to focus research on expanding drug arsenal to address the wide diversity of viruses.

(Department of Plant Pathology, Dr YSP, UHF, Nauni, Solan (HP) 173230)

Pandora Box of Virus evolution and genetics- Unfolding the Mystery

Ajay Brakta, Anil Handa and Bhupesh Gupta

Dr Ajay Brakta

Viruses as pathogens have earned a bad reputation over the years because of pandemics such as Influenza, AIDS, Ebola and SARS. Since time immemorial, "cold" and "flu" viruses have been a part and parcel of our daily lives. With the passage of time, Ebola virus became the hot topic of discussion before Covid-19 made its mark. With the occurrence of Covid-19 pandemic, the term "virus" became a household name for one and all. It is after the appearance of Covid-19 pandemic that our perception towards viral diseases changed as this managed to put the entire world at a standstill. The stark truth lies in the fact that Covid-19 is something one can expect to stay for a long time. The rapid appearance and spread of new variants is indicating how this pandemic can act out over the next several months. There is a continuous apprehension that new mutants and strains may erupt in the coming years. For a better understanding of the epidemics caused by viruses it is crucial to know how the viruses evolved over millions of years and how did viral genetics manipulated their evolution.

Virus evolution

Have you ever wondered why a different strain of seasonal flu comes around every year or why new variants of Covid-19 are spreading? The short answer to these questions is that not only do viruses evolve but they also tend to evolve faster than their hosts such as humans. This makes virus evolution an important topic not just for biologists who study viruses but for all of us who might be exposed to a virus. Viral evolution defines the heritable genetic changes that a virus accumulates during its life time which can arise from adaptations in response to environmental changes or the immune response of the host.

Why do viruses evolve so fast?

Viruses evolve faster than humans. This is because some viruses have a high mutation rate which helps them to evolve quickly by providing more variation as starting material. Two other factors that contribute to the fast evolution of viruses are short generation times and large population size. The bigger the population the higher the odds that it will have a virus with a particular random mutation on which natural selection can act. Also, viruses reproduce quickly so their populations evolve on shorter time scales than those of their hosts. The ability of closely related viruses often infecting different types of organisms points to their very ancient origins. There are three commonly proposed mechanisms for the origins of viruses.

1. **Viruses descended from primitive pre-cellular life forms:** This theory posits that viruses originated and evolved along with the primitive self-replicating molecules that were destined to become cells; the agents we recognize as viruses today were originally self-replicating molecules in the pre-cellular world. If this is correct, it follows that cellular life forms were impacted by "viruses" from their earliest beginnings.
2. **Viruses are "escaped" cellular genetic elements:** This theory posits that viruses evolved after cells. Their origins were cell-associated genetic elements that acquired protein coats allowing for more efficient cell to cell transfer. The DNA genomes of some viruses do resemble plasmids and retroviruses are related to the large group of non viral retro-elements populating cellular genomes.
3. **Retrograde evolution:** The theory of retrograde evolution states that viruses were once complex intracellular parasites that lost the ability for all independent metabolism; they retained only those genes required to manipulate the host cell and produce progeny virions.

None of these theories easily explains the origins of all viruses and it is widely accepted that all viruses did not share a single common ancestor. Instead, distinct lineages of viruses probably evolved by different mechanism. There is also good evidence that viruses have shaped the evolution of their hosts for at least hundreds of millions of years. While some virologists ponder the ancient origins of viruses others examine the mechanisms that drive ongoing virus evolution. Examples of ongoing virus evolution include:**Cross-species jumps that allows virus to find new hosts**:- Bird or swine influenza jumped into humans at the turn of the 20th century and horse influenza virus moved into dogs in 2004. **Decreased or increased virulence in a new host:** Viruses that are well adapted to their hosts often cause little or no disease. When a virus jumps to new host, the virus may cause severe disease, but co-evolution of virus and host often restores more balance. **Emergence of drug resistance:** As antiviral drugs have been developed, drug resistant viruses have emerged. The mutation and replication rates of human immunodeficiency virus (HIV) are so high that successful long-term treatment requires use of drug cocktails, containing three or four different active compounds (to minimize development of drug resistance). **Immune escape mutants:** HIV and influenza viruses have adapted themselves to escape from host immune pressures due to point mutations in certain regions of viral surface proteins. A reason these viruses are closely monitored and vaccines are updated regularly.

Virus evolution is the outcome of two independent events. The first event is mutation of the viral genome. For RNA viruses, this is often a frequent event during genome replication, as most RNA-dependent RNA polymerases have

no proof reading functions (this is why some RNA viruses exist as a quasi-species). DNA damage may play a role in mutation of some DNA virus genomes. Virus genomes can also mutate as the result of recombination or even by capturing genes from the host or from other viruses.

Viral genetics

Viruses are obligate parasites that are completely dependent on the host cell for the replication and transcription of their genomes as well as the translation of the mRNA transcripts into proteins Viral genetics studies the genetic mechanisms that operate during the life cycle of viruses by utilizing biophysical, biological and genetic analysis to study the viral genome and its variation. Disease causing viruses are of particular interest, however geneticists also use viruses to help understand fundamental mechanisms of molecular genetics.

Viruses are continuously changing as a result of genetic selection. They undergo subtle genetic changes through mutation and major genetic changes through recombination. Virus genetics is studied by either investigating genome mutations or exchange of genetic material during the life cycle of the virus. The frequency and types of genetic variations in the virus are influenced by the nature of the viral genome and its structure. Especially important are the type of the nucleic acid that influence the potential for the viral genome to integrate in the host, and the segmentation that influence exchange of genetic information through assortment and recombination.

Mutations in the virus genome could either occur spontaneously or can be induced by physical and chemical means. Spontaneous mutations that arise naturally as a result of viral replication are either due to a defect in the genome replication machinery or due to the incorporation of an analogous base instead of the normal one. Induced virus mutants are obtained by either using chemical mutants like nitrous oxide that acts directly on bases and modify them or by incorporating already modified bases in the virus genome by adding these bases as substrates during virus replication. Physical agents such as ultra-violet light and X-rays can also be used in inducing mutations.

Genotypically, the induced mutations are usually point mutations, deletions and rarely insertions. The phenotype of the induced mutants is usually varied. Some mutants are conditional lethal mutants. These could differ from the wild type virus by being sensitive to high or low temperature. A low temperature mutant would for example grow at 31°C but not at 38°C while the wild type will grow at both temperatures. A mutant could also be obtained that grows better at elevated temperatures than the wild type virus. These mutants are called hot mutants and may be more dangerous for the host because fever which usually slows the growth of wild type virus is ineffective in controlling them. Other mutants that are usually generated are those that show drug resistance, enzyme deficiency or an altered pathogenicity or host range. Some of these mutants cause milder symptoms compared to the parental virulent virus and usually have potential in vaccine development as exemplified by some types of influenza vaccines.

Besides mutation, new genetic variants of viruses also arise through exchange of genetic material by recombination and reassortment. Recombination generally occurs between members of the same virus type (e.g., between two influenza viruses or between two herpes simplex viruses). Classical recombination involves breaking of covalent bonds within the virus nucleic acid and exchange of some DNA segments followed by rejoining of the DNA break. This type of recombination is almost exclusively reserved to DNA viruses and retroviruses. RNA viruses that do not have a DNA phase rarely use this mechanism. Recombination usually enables a virus to pick up genetic material from similar viruses and even from unrelated viruses and the eukaryotic host cells. Exchange of genetic material with the host is especially common with retroviruses.

Re-assortment is a non-classical kind of recombination that occurs when viruses of two different parent strains co-infect the same host cell and interact during replication to generate virus progeny that have some genes from both parents. The resulting progeny virions may get some segments from one parent and some from the other. All known segmented viruses that infect humans are RNA viruses. The process of re-assortment is very efficient in the exchange of genetic material and is used in the generation of viral vaccines especially in the case of influenza live vaccines. Vaccines are also developed through Recombination. Vaccine strains of viruses can be used to create recombinant viruses that carry extra genes coding for a specific immunogen. During viral vaccination, the replicating virus will express the specific immunogen. Specific antibody production will be stimulated, and the host will be protected from the immunogen as well as from the vaccine virus.

Contemplating the origin of viruses fascinates both virologists and the general public. To date, no clear explanation for the origin of viruses exists but understanding the evolutionary history of viruses is unraveling may secrets about their origin and evolution over the centuries. Perhaps viruses existed long ago and led to the evolution of cellular life. With the ongoing advancements in molecular biology, it might be possible to get the answer one day or may be the answer is even murkier than it now appears.

(Department of Plant Pathology, Dr YSP, UHF, Nauni, Solan (HP) 173230)

Taming of the shrewd- A deep insight

H.R Gautam and Anil Handa

Viruses "*The Smart Pathogens*" have become a tool in the hands of the virologists around the globe who are now using sharp tricks to make viruses do their bidding. The research is drifting towards the use of viruses for combating insect pests or produce vaccines for major human diseases like cervical cancer and chikungunya. A classical example of taming plant viruses is that of Broad bean wilt virus 2 (BBWV2) wherein the researchers circumvented the problem of virulence by carefully engineering the virus with the

aim of inserting the gene sequence for developing transgenics in pepper in addition to study the functionality of genes.

A virus is a small packet of DNA or RNA encapsuled in a protein coat that is unable to do anything other than get itself reproduced by making use of the mini living factories in the cells of plants or animals. Once inside the cell, it makes its way to the nucleus, which is where it needs to be in order to get itself multiplied. A typical scenario is that of a *Baculovirus* that adheres to the leaf surface. Once ingested by a caterpillar, it sheds its protective capsule inside the caterpillar's intestines and the virus starts penetrating one of the intestinal cells. Once inside the cell, it makes its way to the nucleus which is where it needs to be in order to replicate. After inserting its DNA in the nucleus of a caterpillar cell, the caterpillar's enzymes automatically start to read the DNA and synthesise the associated viral proteins misleading the caterpillar to produce more virus particles which in turn penetrate other body cells to repeat the process.

Who is more deceitful- the virus or the researcher?

Viruses are devious but so are the virus researchers. In most of the cases, it is the virus that does the bidding. In the past few decades, virologists have come up with applications wherein baculoviruses can be put to meanigful use. All viruses in the baculovirus family only infect certain insects and leave other organisms alone which makes them ideal for use as biopesticides protecting crops against insect pests. They have been used in this way since the 1940s, for example for combatting caterpillar damage in apples, cotton and sugarcane. In the 1980s, researchers found a way of using these viruses in manipulating insect cell cultures by inserting a new piece of DNA and then using that to infect the artificially cultivated insect cells thereby forcing those cells to produce proteins that can be used as vaccines. This method has big advantages over conventional production methods with genetically manipulated bacteria since bacteria are simple cells that cannot produce all types of proteins. On the contrary, insect cells are much closer to human cells and therefore better able to do this as long as these are supplied with the right bit of DNA which is something that these viruses are skilful at. Moreover, baculoviruses are harmless to humans which renders them an ideal and safe production system for vaccines.

As of now, there are two human vaccines available in the market that are produced in insect cells using manipulated baculoviruses: a vaccine each for cervical cancer and flu. Since we have the first products for application in humans and there is sufficient proof that they work well and are safe, the expectation is that more will follow in near future. Scientists in advanced virology laboratories are now working on a vaccine for the chikungunya virus which causes fever and joint pain and is transmitted from one person to another by mosquitoes. The virus was originally found mainly in Africa and Asia but at present it is a big problem in the Caribbean region. A prototype vaccine has already successfully been tested on mice and is currently being tried out on monkeys and if that turns out to be a success, it will be possible to develop it further by a pharmaceutical company.

How a clever virus kills a very hungry caterpillar?

Virologists all over the world are keen to know as much as possible about baculoviruses owing to the extensive range of applications for these group of viruses. Attention is increasingly being paid to the baculoviruses in their natural conditions and their interaction with the hosts that they infect. Not only does the virus apply a trick to ensure it gets replicated, it also influences the behaviour of its host. The virus is able to make the caterpillar crawl faster and to make it climb upwards. The caterpillar eventually ends up dying from the infection at a place far away from the original leaf where the virus exited the dead body of its previous host. This brings the virus to a new area with caterpillars that are still healthy, whose cells it can use to reproduce further. The further and higher the viruliferous caterpillars crawl, the greater the leaf area gets contaminated with the virus. Caterpillars that end up in the tree tops are also picked up more easily by birds which boost the spread of the virus as well. It is not yet exactly known as to how these viruses influence the movement of caterpillar whether they affect the brain or exert an indirect influence via hormones. Studies are underway to compare the changes in gene expression and protein profiles of caterpillars infected with a normal baculovirus with that of caterpillars infected by a baculovirus in which the gene causing the hyperactivity has been removed.

Headway in virus taming

Besides insect virus research, the focus is on the interactions between the viruses and either their hosts or the vectors, mostly insects, with which viruses can hitch a lift. For instance, some research groups are focusing on arboviruses (including chikungunya) that are spread by mosquitoes and infect humans or livestock besides studying the mechanisms possessed by plants for resisting viruses which is an important consideration in crop production. In marine virology, the efforts are being made on developing a vaccine against viral diseases in salmon. Given the ever-increasing numbers of fish farms, it is only to be expected that there will be all kinds of viral outbreaks as the fishes in these farms live in close proximity and are sometimes under a lot of stress leading to fast spread of viruses. Insects often play a part in the epidemiology of virus diseases and the methods they use to defend themselves are interesting for virologists. The research groups are increasingly working on the fringes of virology because a lot is being done on the virus-host and virus-vector interactions. The current focus in clinical virology is on whether

temperate mosquito species are capable of transmitting viruses that are of tropical origin, such as the West Nile virus and chikungunya. Research efforts in this direction are important because it lets us assess how big a risk there is of those viruses causing havoc worldwide. Different research groups working on vaccine development can benefit from their mutual interconnections thereby leading to positive outcomes.

(Department of Plant Pathology, Dr. YSP, UHF, Solan)

The journey of apple through the eyes of a virologist

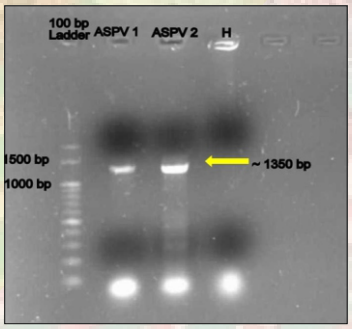
Usha Sharma, Ajay Brakta and Anil Handa

Dr Usha Sharma

Systematic research on virus diseases was initiated in late 1980s in the department of plant pathology and the primary focus was on virus diseases of apple. The major limitation in this aspect was the lack of quick and reliable method of detection as the entire indexing was based on the use of woody indicators (Golden Delicious) and the visual symptoms appeared in 3-5 years. To add to this problem, latent infection resulting in masking of symptoms further adds to the woes. The research in plant virology gained momentum in late 1990s when ELISA was used for the first time for the detection of viruses infecting apple.

Chlorotic and necrotic spots

Severe mosaic symptoms



RT-PCR confirmation of ASPV isolates

Apple chlorotic leafspot (ACLSV) and apple mosaic (ApMV) viruses were detected in the year 2000 through double antibody sandwich (DAS) and direct antigen coating (DAC) enzyme linked immunosorbent assay (ELISA) followed by two new viruses namely apple stem pitting virus (ASPV) and apple stem grooving virus (ASGV) reported for the first time in India in 2007 on the basis of ELISA based detection. Shortly before 2007, a viroid disease of apple (dapple apple or apple scar skin viroid) was reported in 2003 from Shimla district.

The next decade witnessed major shift in detection and diagnosis not only of viruses but also of viroids and phytoplasma with the use of PCR techniques which further streamlined the research on identification and characterization of viruses and related pathogens on molecular basis. These techniques have established the exact identity of 4 major viruses of apple namely ApMV, ACLSV, ASPV and ASGV. Since these techniques were expensive and not readily accessible to the farmers, an alternative approach using woody indicators viz. Virginia crab, *M. platycarpa*, Spy 227, Jay Darling and Russian Clone introduced from Institute of fruit breeding at Holovousy, Czech Republic in 2008 were used for relatively quick and cost effective detection at farmers' field itself. The use of these indicators has gained popularity with the farming community and has become a success story. Prevalence of all four major viruses has been established not only in Himachal Pradesh but also in the neighboring state of Uttarakhand and Union territory of Jammu and Kashmir on the basis of molecular and serological assays. The addition of Electron microscopy and Ultramicrotomy facilities will further strengthen our initiatives to produce plants free from virus and related pathogens.

Establishment of virus-indexed budwood bank

Nuclear stocks of commercial cultivars of apple including rootstocks have been established after serological and molecular indexing. Budwood of virus tested true to type trees of different commercial cultivars namely Starking Delicious, Starkrimson, Golden Delicious, Tydemans' Early Worcester, Well Spur, Oregon Spur, Silver Spur, Red Chief, Vance Delicious and Top Red existing in the University and PCDO plantations of the state Department of Horticulture have been multiplied in isolated nurseries which is further used for developing budwood banks. These budwood banks have been established at Regional Horticultural Research Stations of the University located at Seobagh (Dist. Kullu), Mashobra and Kotkhai (Dist. Shimla) and Sharbo (Dist. Kinnaur) and at the main campus at Nauni. Progeny of virus tested important rootstocks viz. M9, M7, M26, MM106 and MM109 have also been planted and being maintained in these budwood banks. The plants in these budwood banks are subjected to rigorous re-indexing protocols at regular intervals. The plant material imported under the World Bank funded HPHDP project maintained at different PEQ sites is also subjected to serological and molecular indexing against all major apple viruses and phytoplasma to prevent the entry or spread of these pathogens.

The unforeseen threat:

A never ending race for new varieties is going on among farmers of the state leading them to top work their old and senile trees with budwood of new varieties for getting an early crop. The possibility of having these old trees infected with major apple viruses is pretty high as the existing orchards are having upto 60 per cent incidence. The current practice of top working of old orchards with new varieties is going to increase the viral incidence several folds as these budsticks are further used by farmers either in nursery for grafting or being sold to the fellow farmers, thus could aggravate the issues related to viruses in apple in times to come.

(Scientist, RHRT&S Mashobra, Shimla)

Natural Plant Products- A repository of Virus Inhibitors

Bhupesh Gupta, Ajay Brakta and Anil Handa

Dr Bhupesh Gupta

Viruses are one of the major threats for both humans and animals as they mislead body's metabolism to produce millions of copies of their genome and proteins. Recent outbreaks of viral diseases indicate that virus diseases are difficult to control with the help of currently available antiviral drugs and has forced the scientific community to explore the plants with known antiviral activity. According to a report from the World Health Organization (WHO), infections by viruses affect more than five million patients annually. While limited efficacy and serious adverse effects are the major issues with commonly used antivirals, extracts of plant origin have been in use for medicinal purposes since ancient times and are known for their antiviral properties and very limited if not negligible side effects. Plant based pharmacotherapy may thus be the appropriate alternative for treating viral diseases and various pharmaceutical formulations and delivery systems including micelles, nanoparticles, nanosuspensions, solid dispersions, microspheres and crystals, self-nanoemulsifying and self-microemulsifying drug delivery systems (SNEDDS and SMEDDS) have been developed and used for antiviral delivery of natural products. These diverse technologies offer effective and reliable delivery of medicinal phytochemicals.

Synthetic vs. plant based antiviral drugs

A wide range of synthetic antiviral drugs (moroxydine, ganciclovir, valganciclovir, valaciclovir etc.) are of regular usage for inhibiting the virus replication through different mechanisms. Problems in drug treatment however arises due to their low efficiencies, cytotoxicity and development of viral resistance against them. Vaccination, another antiviral treatment can be put to use but many of the vaccines are still undergoing development phase and suffer from the disadvantage of providing incomplete protection against virus and their reliability needs more research. On the other hand, nature provides more reliable source of antiviral agents and almost 40 percent of currently available drugs are direct or indirect derivatives of plants. A number of ethnobotanical studies have identified potential therapeutic plants for more effective control of health issues demonstrate the importance of plant species in health care system.

Antiviral medicinal plants and phytochemicals

Plants are rich source of phytochemicals like alkaloids, anthocyanins, carotenoids, flavonoids, isoflavones, lignans, monoterpenes, organosulfides, phenolic acids, saponins and many more. These phytochemicals have a proven role for their antimicrobial, antihypertensive, anti-diabetic, antioxidant, hepatoprotective, cardioprotective and other therapeutic activities. Better understanding of natural antiviral agent's mode of action and identification of responsible compounds will be helpful to provide a new insight for the development of new antiviral drugs for more effective viral control.

Where do we go from here now?

Abundant plants are available in nature with massive potential to act as a source of l antiviral compounds. These plants largely target the enzymes involved in replication and integration of virus into host cell. In case of DNA viruses, restricted entry of viral particles into host cell or inhibition of viral replication into the host cells are most frequent mode of actions. Destruction of viral envelop is also one of the identified mode of action against DNA viruses. RT plays a significant role in replication of RNA viruses and most of the plants restrict the activity of RT enzyme of virus. In order to design effective drugs against viruses, enzymes involved in the key metabolic activities (integration, replication) should be focused. A lot of plant population with possible potential is still uncovered as few plants have been studied in detail in order to identify the active phytochemicals against these viruses. More detailed studies in future will help not only to identify the potential antiviral compounds but also in better understanding of their mode of action for more effective control of these lethal viruses.

(Scientist, Department of Plant Pathology, Dr YSP, UHF, Nauni, Solan (HP) 173230)

Strengthening of Virus diagnostic facility in the Department of Plant Pathology

Department of Plant Pathology has completed 56 years of its creation and the rich legacies of 56 years propel us to continuously equip us and strengthen our facilities to effectively address the challenges of effective management of diseases. The department further strengthened its facilities for identification and

detection of virus and related pathogens with the addition of JEOL JEM 1400 Flash Transmission Electron Microscope (TEM) and Leica EM UC7 Ultramicrotome with cryo-sectioning. This facility has been established under the World Bank funded Himachal Pradesh Horticulture Development Project (HPHDP) and was inaugurated by Shri Jai Ram Thakur, Hon'ble Chief minister of Himachal Pradesh on December 01, 2021, the occasion of 37th Foundation Day of the University. The Hon'ble Governor of Himachal Pradesh, Shri Rajendera Vishwanath Arkelar visited the Electron microscopy lab on the occasion of 11th convocation of the University held on December 07, 2021 and was briefed on the working of both the equipments. With these state-of-the art facilities, the University in the elite club of Institutions of prominence in the north India. With such facilities in place, Plant Pathology department will be now able to identify the viruses and phytoplasma associated with crop plants.

Hon'ble Governor breifed on working of transmission electron microscope

Additionally, this facility will immensely help in deep understanding of host-pathogen interaction studies beside use in nanotechnology and material science. The Ultra microtome will be used for cutting specimens into extremely thin slices called ultra-thin section with thickness (50-100 nm) which will then be used in TEM

Hon'ble Governor observing section cutting in ultramicrotome

studies. Beside TEM, Ultramicrotome will be used in flousescence microscopy for the detection of phytoplasma in addition to biological and material science studies. Scientists and students of the University with access to these equipments will have positive bearings on the quality of research in future.

Workshop on Importance of Quarantine Measures in Import of Planting Material

Aiming at creating awareness concerning plant quarantine, the Department of Plant Pathology of Dr. Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan organised a One-day Awareness Workshop on the Importance of Quarantine Measures in Import of Planting Material on 12th November 2021 under the World bank funded Himachal Pradesh Horticulture Development Project (HPHDP). Under HPHDP, elite planting material of apple and other temperate fruit plants has been imported from other countries and more than 20-25 lakh plants of horticultural crops have been imported in State in the last 5 years, majority of them under HPHDP and also by other nursery growers. There are more than 50 PEQ sites in the State where the imported planting material is under the mandatory quarantine period or the sites have been designated for the intended import in 2022. Dr. Parvinder Kaushal, Vice Chancellor of Dr YS Parmar UHF, Nauni, Solan was the Chief Guest on the occasion. He urged the officials to ensure strict adherence to plant

Hon'ble Vice Chancellor addressing the delegates

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Organic Manure

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NPK Bio Fertilizer

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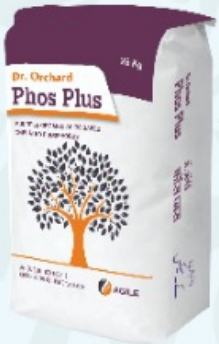
Chelated Potash 26%

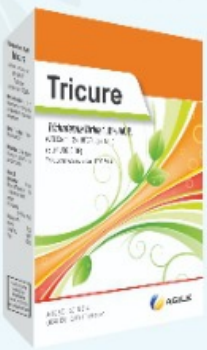


Phos Plus
Chelated Phosphorus 16%

Other Bio Products

Metagold
Metarhizium anisopliae

Prick
Beauveria bassiana



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quarantine guidelines as a small mistake can lead to the entry of viruses and pose a serious risk to the livelihood avenues and adversely affect the economy of the state.

Hon'ble Vice Chancellor releasing book on quarantine during the workshop

Besides scientists of the university, more than 50 officers from the line departments involved in supervision and maintenance of imported planting material at the designated post entry quarantine sites attended the meeting. In addition, farmers engaged in the import of planting material also participated in the workshop. Noted national experts, Dr V K Baranwal, Principal Scientist and Incharge of Advanced Centre for Plant Virology, Division of Plant Pathology, ICAR-IARI delivered a talk on 'Diagnostics of viruses for certification of Quality planting material of clonally propagated temperate fruit crops'. He emphasized the need for establishment of 'virus certification system' as in other countries for apple to contain the spread of viruses and virus like organisms in this important crop.

The Government and private nurseries should be brought in the ambit of such a system and there should be a mandatory protocol for virus free mother stock of plants for multiplication. He further said with early detection of viruses we can get 400 times more benefits in terms of productivity. Dr. Celia Chalam, Head, Plant Quarantine Division ICAR- NBPGR discussed the Importance of Quarantine procedures in the import of planting material. She highlighted various norms to be followed while importing plants. She said that it was important to closely monitor the pests being frequently reported in the imported consignments and the crops that tend to be infected. She exhorted all the importers to follow the norms with precision to avoid severe losses to our crops in future.

Dr HR Gautam, Professor and Head, Plant Pathology, UHF, Nauni emphasized the need for the bar coding of the imported plants on import to track their monitoring and movement to check their unauthorized exit from the quarantine facilities. Dr Anil Handa, organizing secretary of the workshop stressed on the need to have a good certification programme and a network of diagnostics labs in the country. During the discussions in the Workshop, it was impressed upon the importers that quarantine is the first line of defence to check the spread of invasive species coming with the import of planting material of agricultural and horticultural crops. This could be achieved through effective implementation of plant quarantine measures aided with sound scientific and technical principles available to counter such threats. If we invest small amount of money in the beginning for detection of the invasive pests with strict enforcement of the Standard

Progressive farmer interacting with experts

Operating Procedures (SOPs) we can avoid huge losses in our crops in future. The highlight of the workshop was the farmer scientist interaction wherein the queries raised by the progressive growers were addressed by the university scientist, experts and field functionaries of the state department of horticulture. Progressive farmers representing the Plum Growers Forum said that a mechanism needed to be put in place where farmers are compensated in case their infected orchards need to be destroyed. Mr Harish Chauhan took up the issue of certification and the need to educate farmers about the quarantine process.

Dissemination of Technologies- Kisan Mela Organized

Department of Plant Pathology of Dr Y.S. Parmar University of Horticulture and Forestry, Nauni organized a Kisan Mela on December 28, 2021 under the World Bank funded Himachal Pradesh Horticulture Development Project at Nerwa in Chopal sub-division of district Shimla in which more than 250 farmers participated. As the apple plantation in the State is almost more than five decades old, the senile orchards are resulting in lower productivity; the University has taken several initiatives to infuse new technologies and practices for the enhancement of productivity. Hon'ble Vice Chancellor of the University- Dr Parvinder Kaushal was the Chief Guest on the concluding session of the Kisan Mela. He exhorted the farmers to adopt new

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technologies like high density apple orchards as in the demonstration orchards University has recorded yield of more than 40 tonnes per hectare. He cautioned the farmers about the indiscriminate and overuse of fertilizers and pesticides which has adversely affected our environment and soil health. Soil fertility has also been adversely affected with the overdose of

nitrogenous fertilizers. This Kisan Mela was very different in a context that Dr Kaushal distributed to the farmers a kit of six bio-pesticides and bio-fertilizers which farmers can use in the ensuing season while doing their new plantations and also in their old plantations. Out of the six bio-fertilizers and bio-pesticides distributed, while PGPR and Trichoderma formulations have been developed by the University; four bio-fertilizers like Pusa Bio-Azotobacter, Pusa-Mycorrhizae, Pusa Bio-phosphorus and Pusa Bio-potash were produced by Indian Agricultural Research Institute, New Delhi. Use of these bio-inputs will drastically reduce the consumption of fertilizers and pesticides. As the State Government is giving a lot of emphasis on natural and organic farming, availability of such bio-inputs wil incline farmers for their use. Distribution, use and awareness about the use of such bio-inputs will make our horticulture industry of high value with good quality of fruits. Dr Kaushal also released Apple Spray Schedule for the farmers for 2022 and also released a book on diseases and pests of apple.

The Kisan Mela started with the specialized lectures by the scientists of the University on different topics. Dr Gopal Singh of Department of Fruit Science gave emphasis on the plantation of suitable new varieties on specific rootstocks and proper training and pruning of the plants for getting the higher productivity. Dr Rajesh Kaushal from the Department of Soil Science and Water Management highlighted the role of bio-fertlizers and PGPR for good soil health and sustainable productivity from their orchards. Dr Rakesh Kumar from the Department of Entomology gave a detailed account of important pests in apple and their management. As diseases cause huge losses in apple, Dr HR Gautam, Dr IM Sharma and Dr Bhupesh Gupta all Plant Pathologists made people aware about the symptoms and management practices of scab, premature leaf fall, powdery mildew, canker diseases, white root rot and collar rot diseases. Farmers were advised to adhere to the spray schedule of the apple for effective management of the diseases and pests. Dr Anil Handa, Professor from Department of Plant Pathology made farmers aware about the diseases caused by viruses and viroids and need for virus free and certified apple nursery.

Farmer-scientist interaction was also held to answer the important queries of the farmers related to their apple cultivars . We need to gradually move to such farming practices which are based on organic inputs.

NEWS DESK

New Appointments


Dr Ajay Brakta

Dr Ajay Brakta joined as Assistant Professor (Plant Virology) at College of Horticulture and Forestry, Thunag, in District Mandi, Dr YS Parmar University of Horticulture and Forestry on September 20, 2021. He was born at Jubbal in Shimla district on December 25, 1986 in a farming family. Dr Ajay did his Master's and Doctorate degrees in Plant Pathology with specialization in Plant Virology. He joined BASF India as Field Biologist in 2014 at Chandigarh and worked on development and research activities across herbicides, insecticides and fungicides on major crops grown in north India before moving to BASF global research team in 2016 and worked at one of BASF global Agriculture Research Stations (ARS) based at Pune. In global research, he was engaged in advanced research on fungicides and resistant monitoring studies to develop future strategies for BASF fungicide portfolio according to sensitivity of fungal strains. He has published eight research papers on molecular characterization of plant viruses besides two book chapters. His notable research publications include first reports on Pear stony pit and apple top working disease from India published in Plant Disease (American Phytopathological Society) and New Disease Reports (British Society of Plant Pathology). His work on Molecular Characterization of Apple Stem Grooving Virus was adjudged the best paper award in the 3rd Global Conference on Plant Pathology held at Udaipur in 2012. His work on Woody Indicators of Apple Viruses was also recognized as the best poster by Indian Horticulture Congress in 2012 at PAU, Ludhiana.


Dr. Kaushal Attri

Dr Kaushal Attri joined as Assistant Professor (Plant Pathology) at Dr YS Parmar University of Horticulture and Forestry, Nauni Solan, College of Horticulture and Forestry, Thunag, Distt. Mandi (HP). Before joining this institution he has served as Horticulture Development Officer in Department of Horticulture Govt. of Himachal Pradesh from August 2019 to October 2020 and Subject Matter Specialist (Plant Pathology) at CSKHPKV, KVK Lahaul and Spiti-1 at Kukumseri from October 2020 to August 2021. He did M.Sc. and Ph.D in 2016 and 2020, respectively. Dr Attri has published 6 research papers in national and international journals.

Kiwi imports from Iran Banned

Due to non-conformance to the Indian requirement and due to interception of Quarantine pests in Kiwi consignments imported from Iran, the exportation of fresh Kiwi fruit from lran to India has been suspended by National Plant Protection Organization (NPPO) of India with immediate effect till the revision of Pest Risk Analysis. This action has been prompted due to the continuous non-compliance in imported consignment of Fresh Kiwi fruits from Iran due to interception of the quarantine (*Aspidiotus nerii*) and non-quarantine (*Aonidiella aurontii*) pests. As introduction of the any quarantine pests through import consignments poses threat to Indian bio-security and is dealt under the provisions of Indian regulation of Import into India (PQ Order, 2003) under which stringent actions such as prohibition of the commodity can be imposed. It is pertinent to mention here that *Aonidiella aurontii* is a polyphagus pest and aprt from kiwi it also infects crops like citrus. India imports 4,000 tonnes of Kiwis from various countries, while the domestic production is about 13,000 tonnes.

Combination of Streptomycin + Tetracycline banned in agriculture

The Union Ministry of Agriculture and Farmers Welfare vide the draft order notified on December 17, 2021, on 'Prohibition of Streptomycin + Tetracycline in agriculture', prohibited import, manufacture or

formulation of Streptomycin and Tetracycline for use in agriculture from February 1, 2022. The draft order comes due to growing concerns over antimicrobial resistance observed in various crops, particularly to streptomycin, which is used in the treatment of tuberculosis (TB). According to WHO's 'Report on Surveillance of Antibiotic Consumption', antimicrobial resistance is a major threat to health and human development. It affects the ability to treat a range of infections. Hence, treatments for a growing number of infections have become less effective in many parts of the world due to resistance. Tetracycline antibiotics find application in the treatment of bacterial infections. The order ensures a complete ban on the use of the two antibiotics in agriculture January 1, 2024, onwards. It directed every state government to take all such steps necessary for executing the order in their state.

Students Achievers of the Department

Natasha Kashyap who did her M.Sc. from the Department got selected for Doctoral Programme in the Indian Agricultural Research Institute (IARI) at Pusa New Delhi, India.

Shalaka Ahale, M.Sc. from the Department of Plant Pathology topped the Ph.D. Entrance Examination in discipline of Plant Pathology in PAU, Ludhiana. She has also secured third position in Joint Ph.D. Entrance Examination of State Agricultural Universities of Maharashtra and also secured third position in Ph.D. (Plant Pathology) Entrance Examination conducted by CCS Haryana Agricultural University, Hisar.

FARMING HERO

History of apple cultivation in Himachal Pradesh starts from Kotgarh in district Shimla where American missionary Samuel Evans Stokes first planted the apple saplings in 1916. While still focusing on this majestic village which changed the fortunes of the farmers in the higher hills, we will also remember the contributions of 85 years old Mr Hari Chand Roach for taking this legacy forward with great zeal. He is one of the millionaire orchardists of Kotgarh- the area, where the apple cultivation soared the per capita income of the farmers to new heights. His riches from apple cultivation are due to his tireless hard work he has been doing in his five-acre orchard in Kotgarh and another eight-acre orchard 31 km away in Matiana. He always had a vision and a futuristic approach for the farmers of the area for their sustainable earnings which made him an icon. He is pioneer in taking initiatives way back in 2002 to import new varieties from the US to the area. The imported varieties included Red Delicious, Super Chief, Ace Spur, Fuji, Red Fuji and Sun Fuji. His house in Saroga village is surrounded by some trees from the new varieties; among these is Fuji, a light pink apple from China. His initiative has propelled young people to go for the plantation of these new varieties. But, as now, many people have plunged in to the import of the fruit plants; he is worried about the unauthorized entry of the planting material in the State. He is actively taking these issues with the concerned authorities for invoking and adherence of strict measures to check the entry of the unauthorized planting material of fruit plants in the State. Marketing and better pricing of apple remains his main concern and he wants that technology can play important role in fetching better price. He took active interest to rope in the big industrial houses like Adani to establish cold storage facilities in the State. But, he wants that processing industry should also step in the heart of the key production areas so that the farmers are able to get better price for their low grade apples. Climate change also worries him as he has seen shifting of apple cultivation from the marginal areas. He wants some strategy to deal with the same with change of crops and varieties. He is taking keen interest in some new crops like blueberry as this high priced crop with longer shelf life can provide a better alternative to the farmers. He is actively engaged in the affairs of the farmers' organization like Kisan Sangh to take the issues of the farmers with the State and Central Governments. His age and more than 60 years in apple cultivation do not deter him his daily run to the trees in his orchard to personally observe each and every urgent need of the plant. Agriculture as a whole needs more involvement of youth and they need to emulate this great man for his marvelous apple marathon. The 'Editorial Team' of Technology Notes salutes him for his marvelous journey in this six decade old arduous journey in the tough hilly terrains.

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