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Progress of plant disease research: Rig veda to molecular plant pathology

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Abstract
Much earlier than the time of Theophrastus (300 BC), evidence about natural havoc in crop plants is found in the ancient Greek civilization and in our Epics and Vedas (3700 BC). Ever since human beings started growing plants, plant pathogens have also started competing with humans to obtain their share of food from plants which resulted into disease in the plants. The quest to know the causes of plant disease and measure to control them had begun with the advent of civilization several decades before the birth of Christ. Existence of microbial organisms, although unseen at that time was indirectly recognized by the ancient Greek civilization (3400 BC), ancient Hebrews and the ancient Hindu culture (1500 BC). Ancient India not only had a medical science for humans (Ayurveda) but also for the plants i.e. Vrikshyayurveda. Surapala wrote Vrikshyayurveda, an ancient Indian science of plant life which is the starting point of systematic plant protection in Indian agricultural history, where he mentioned about the ailments of plant diseases. As status and importance of various disease have changed over the years, more sophisticated technologies have to be carried out in the future. RNA interference has emerged as a powerful tool for battling some of the most challenging disease caused by viruses, bacteria and fungi. Nanoscale science and nanotechnologies are visualized to have the potential to revolutionize agriculture and food systems and has given birth to the new era of Agronanotechnology. Society, consumers and growers will only be able to continue to benefit from plant pathology if the discipline can evolve appropriate disease management schemes that can respond to the significant changes in agricultural practices in India; the ultimate goal being to produce more and safer food in sustainable agricultural systems that conserves natural resources and the environment.

Keywords: Surapala, Vrikshyayurveda, RNAi, Agronanotechnology

Introduction
History of science is science itself - Pasteur.
By the study of history of science, we get a better perspective of the subject, we come to know the contributions of that field, the problems that are encountered and the manner in which they were tackled. The ultimate aim of crop husbandry is to cultivate crops and get maximum returns out of it. But, ever since human beings started growing plants to meet their requirements of food and various products, the plant pathogens have also started competing with human to obtain their share of food from plants which as a result cause disease. The crop losses due to pests, disease and weeds are approximately assessed to be ranging between 10 to 30 per cent of crop production. If we consider an average crop loss of 20 per cent and the present gross value of agricultural produce as Rs.7 lakh crore, the loss comes to Rs.1,40,000 crores which is a very high loss for us. Even if we could save 50 per cent by using plant protection measures, it will add Rs.70,000 crores additional income to the farmer. Mention about natural havoc in crop plants is found in the ancient Greek civilization and in our ancient Epics and Vedas (Kautilya in 3700 BC). Existence of microorganisms was indirectly recognized by the ancient Greek civilization (3400 BC), ancient Hebrews and the ancient Hindu culture (1500 BC).

History of Plant Disease Research
Ancient Era: Ancient to 5th Century (476 A.D)
They were aware of the living beings (*krimi*) that caused disease in man, cattle and plants. Two facts mentioned in the Vedas are: Visible and invisible creatures entered the body and caused diseases and the sun heat and fire kill these poisonous creatures. 'Ayurveda' comes from the two Sanskrit words "Ayush" means life and "Vedas" means wisdom or science.

The ancient India not only had a medical science for humans but also for the plants i.e. "VRIKSH- AYURVEDA". References to plant protection are found starting from: Vedas (Rigveda c.3700 BC, Atharvaveda c.2000 BC), Kautilya's Artha-sastra (c.300 BC), Buddhist literature (c.200 BC), Krishi Parashar (c.100 BC), Sangam literature of Tamils (200 BC-100 AD), Agnipuran (c.400 AD), Brhat Samhita of Varahamira (c.600 AD), Kashyapiyakrisukti (c. 800-900 AD), Surapala's Vrikshayurveda (c.1000 AD), Someshwara Deva's Manasollasa (1100 AD) etc.

Krishi Parashar is the oldest text on Indian agriculture written by Parashar. Here, plant protection is mentioned directly only in one verse that mentions powdery mildew, rust, insects and larger animals as enemies of crops and involves the Wind God to move away from the field. Then, Varahamira in his Brhat Samhita included about fungi (mushroom) and algae in his writings. Surapala's Vrikshayurveda was the starting point of systematic plant protection in Indian agricultural history.

Surapala's VRIKSHAYURVEDA" (1000 AD)

No definite facts are known about Surapala and his time. It was just known that he was a renowned and highly respected physician in the court of Bhimpala, the last Rajput king and he lived in the Gangetic plains i.e. at present day UP/Bihar region. His book "Vrikshyayurveda" is an ancient Indian science of plant life, a body of knowledge that has been systematically compiled in the form of 325 Sanskrit slokas approximately 1000 years ago. Asian Agri-History Foundation (AAF) of Andhra Pradesh is doing great service to the history of Indian agriculture by bringing out authentic translations of ancient texts. The hopes of tracing any independent text of Vrikshayurveda were given up by scholars, till Y L Nene (Chairman, Asian Agri-History Foundation) procured a manuscript of Vrikshayurveda of Surapala from the Bodleian Library, Oxford, UK. On Nene's request, Nalini Sadhale (Proff & Head of Deptt of Sanskrit at the Osmania University at Hyderabad) translated the text from Sanskrit to English in 1994 and it was released in 1996 and then itself Surapala's work became known to the modern world. After that Dr. S.L. Choudhary translated it in Hindi and released it in 2003.

Ailments described by Surapala

Diseases of all kinds of trees are stated to be of two types: internal and external. It is unfortunate that all textbooks on plant pathology give credit to the French botanist, Tournefort (1705 AD) for classifying diseases as internal and external. This was more than 700 years after Surapala had already done such classification. For the 'internal disorder of plants he borrowed the "Tridosha principle of Ayurveda". He classified "internal causes as the imbalance of humors, vata, kapha and pitta; and external ones are caused by insects, cold weather/frost, scorching heat, water stress etc. Among these the diseases caused by vata are due to the land that becomes arid on account of excessive supply of dry and pungent matter which leads to thinness and crookedness of trunk, appearance of knots on trunk and trees, and the fruits become hard with less juice and sweetness. The possible causes are underground mechanical barrier, leaf galling insects, root infecting fungi or nematodes, viruses, saline/alkaline soils. The diseases of kafa type occur in winter and spring if the trees are extensively watered with materials which are sweet, sour, salty, or cold. Affected trees take long time to bear fruits, show paleness, dwarfing of leaves, tastelessness, and ripe prematurely, oozing without wounds. The possible causes are fungal gummosis/rot, nutrient deficiencies or toxicities, excessive watering. The diseases of pitta type occur at the end of summer if trees are extensively watered with materials which are bitter, sour, salty, and strong. These diseases are characterized by yellowness of leaves, dropping of fruits, dryness, paleness of flowers and fruits, and decay. The possible causes are viruses, salinity in irrigation water, pre disposal of blossoms blight and fruit decays due to fungal or bacterial infection.

Treatments of ailments suggested by Surapala:

Seed procurement: Seeds sprinkled with milk and dried for five days, then smoked with mustard (*Brassica juncea* L.) seeds with vidanga (*Embelia ribes* Burm. F). Milk is sprinkled to protect the seeds from viral infection and mustard is having anti –insect properties due to presence of sinalbin, nematicidal properties due to glucosinolates and antifungal activity due to allyl isothiocyanate. Vidanga is antibacterial and insecticidal due to presence of embelin (benxaquinone) which is effective against stored grain pests. These treatments induces disease resistance after germination of seeds.

Insect removal: To remove insects both from the roots and branches of the trees, water the trees with cold water for seven days. Insects on the leaves destroyed by sprinkling the powder of ashes and dusts.

Wound treatment: Anointing with the paste of bark of nyagrodha (*Ficus bengalensis*) and udumbara (F. *glomerata*), cow dung, honey and ghee.

Vrikshayurveda Organic Farming - An Overview in India

KUNAPA JAL, which is a natural pesticide made from fish and animal waste increses fertility of the soil by return of earthworms, pesticide residue eliminated from soil when continuously used for 4-6 months. It is made in large quantities and applied in the tea gardens of Assam, Darjeeling, the Nilgiris and in the coffee estates of Karnataka. This controlled red spider mite and helopeltis within a few months.

Other liquid manures used are: SASYAGAVYA uses green weeds and cowdung; AMRITAPANI uses cowdung and jaggery; BHASMAPANI uses wood ash and cow urine; JAIVIK TIKA uses cowdung and cow urine; AGNIHOTRA BHASMA is the ash obtained after performing agnihotra havan. Sir Albert Howard in his book "An Agricultural Testament" has admitted the superiority of traditional Indian methods of agriculture over European methods.

Dr. Anjali Pathak, a naturopath, writer and organic farming consultant who has worked with the growers and planters of NE and the nilgiris, uses the indegineous methods including those of Vrikshyayurveda methods all over India upon invitation. Her book, 'Annam brahma'- Organic Food in India (released in 2009) contains much information on Organic Farming.

The relevance of vrikshayurveda for the farmers of northeast India

Arijit Bhuyan and Babul Lahkar of Golaghat, small tea growers, brought fish and meat waste from the local market, learnt how to make kunapa jal from the author and applied it to their gardens and fields in 2006. They obtained glorious result in a very short period of time and yield increased. These farmer today produce a good income and also they are getting appreciation from foreign buyers for their unique natural flavour.

This was also tried in paddy fields in Golaghat area by Dr. Padmeswar Gogoi, a retired botanist and he has also praised these vrikshayurveda manures when he saw their wonderful field results in paddy and tea. He has now become a champion of Vrikshayurveda in Assam. Their gardens and farms are now totally organic and some have been certified as organic.

History of Plant Pathology

Theophrastus (300 BC), a Greek philosopher first noted plant diseases in his two books: "Enquiry into Plants" and "On the Causes of Plants" and suggested some remedies to control them. He is considered as "Father of Botany". According to him, plant diseases were severe in lowlands than hilly areas and some diseases like rust was more common in cereals than legumes. However, in ancient time, people believed that diseases were caused by some power, religious beliefs, superstitions, effect of stars and moon, bad wind, wrath of God etc. The Romans thought that it was due to wrath of God on people who continued to do sin. In order to get rid of these diseases, they started creating a rust God "Robigo" and offered special sacrifices. This continued for many decades and at last it was proved that diseases are caused by microbes only after the invention of microscope. The history of microscope begins with the invention of the first compound microscope in 1590s by two Dutch spectacles makers. They put several lenses in a tube and would get an enlarged image but blur. This was more a novelty than a scientific tool. Then after them, Anton Von Leewenhoek became the first man to use a real microscope. He invented the simple microscope in late 17th century. He also discovered Bacteria in 1675 and named as "animalcules" or "little animals". His works were verified and further developed by Robert Hooke (1665) who published that plant tissues are made up of cells. He is known as "Father of cell theory". P.A. Michelli also proved that if these spores were placed on a piece of fruit, they grew into new thallus of the fungus. This was not universally accepted at that time. Mathieu Tillet, a Frenh scientist proved that the Bunt disease caused by *Tilletia tritici* and T. *foetida* were contagious plant diseases. However, he believed that it was the poisonous or toxic substance that continued in the smut dust that caused the disease rather than the microorganism. Prevost (1807), another French scientist showed that the black powder contained in the wheat seeds was the fungus spores and these spores caused the disease. But French Academy of Science did not accept his contention, as scientist throughout the world still believed that microorganism and their spores were the result and not the cause of the disease.

Plant pathology in 19th century

One of the most tragic events in human history is the Potato Famine or the Irish Famine (1845- 1846), failure of the potato crop led to mass hunger in late 19th century. It swept the whole of Europe and USA but it was catastrophic in Ireland. Potato was the staple food of Ireland. Due to this, the potatoes became small, mushy and impossible to eat. Over one million people died and one and half million migrated to North America which resulted in a drastic fall of population due to the famine. The battle against the spontaneous origin of disease ended and the science of plant pathology evolving for a long time was born when a German scientist, Anton De Bary in 1861 did detail study on the disease and he established that it was caused by a fungus *Phytophthora infestans* and he elucidated its life cycle. For his excellent contribution, he was honoured as the "Founding Founder of Plant Pathology" and also as "The Father of Modern Mycology".

Spontaneous generation VS Germ theory of disease

Many scientists like Van Helmont, John Needham etc were proponents of Spontaneous generation i.e. they believed in the spontaneous formation of living being from non-living matter. Louis Pasteur (1864) disproved the spontaneous theory by demonstrating the germ theory of disease by his famous Swan neck experiment. Later, Robert Koch confirmed the theory by putting forward a hypothesis known as "Koch's Postulates" in 1875 which is used to confirm the pathogenecity of a particular microorganism. He confirmed causal agents of anthrax (*Bacillus anthracis*) and tuberculosis (*Mycobacterium tuberculosis*). He is known as "Father of Microbial Techniques".

Towards the later part of 19th century

T.J. Burill (1878-1882) first proved the association of bacterium with plant disease. He showed that *Erwina amylovora* causes Fire Blight of apple and pear. This was the first bacterial disease to be recognized. But at that time no one believed that it was bacteria that caused the disease. Then comes the important contribution of E.F. Smith who is known as "Father of Phytobacteriology" due to his contribution towards identification of many bacterial diseases of plants, particularly in bacterial wilts of cucurbits, solanaceous crops and crucifers. He gave the final proof that bacteria are incitants of plant diseases. He also resolved the controversy with Alfred Fisher, a German Bacteriologist who did not think that bacteria are primary cause of plant diseases. He was also the one to notice crown gall disease.

Discovery of Bordeaux Mixture

The discovery of Bordeaux mixture led to the emergence of fungicidal era. It was discovered by Pierre Marie Alexis Millardet in 1885 against Downy Mildew of grapes (*Plasmopara viticola*) which causes outbreaks in the vineyards at that time. He was a Botany professor at the University of Bordeaux and he studied the disease of vineyards of the Bordeaux region. One day on invitation by a farmer for survey in his field, he observed that the vines closest to the roads did not show downy mildew while all other vines were affected. After enquiry he found that the owner i.e. the farmer sprayed a mixture of $CuSO4$ and hydrated lime to make it less attractive as the passer by used to eat the grapes when they ripe. This gave the idea of Bordeaux mixture that saved the French and European wine industries. Bordeaux mixture is a mixture of copper sulphate and slaked lime.

Discovery of antibiotics

The first chemotheraputically active antibiotic was discovered by Alexander Fleming in 1929, a British bacteriologist who had long been interested in treatment of wound infections on returning from a vacation, he noticed a pile of petridish on his lab, the one that was streaked with culture of *Staphylococcus aureus* was contaminated by a single colony of mold. He observed the plate and saw that the colonies immediately surrounding the mold were transparent and appeared undergoing lysis. He reasoned that the mold was excreting some chemicals which kills the bacterial colony. Then from that plate he isolated the mold and proved it to be a species of *Penicillium* and established that the culture contains anti-

bacterial substance which he named “Penicillin” which is effective against Gram positive bacteria.
In 1940, Second antibiotic Streptomycin, a broad-spectrum antibiotic which was effective against Gram -ve bacteria and *Mycobacterium tuberculosis* was discovered by A. Schatz and S. Waksman.

Virology enters molecular era!!!
By the mid 1800’s, the reality of bacterial diseases of man, animals and plants hadbeen established and generally accepted. Virus diseases of plants like the Tulipomania (colour break in tulip flower in 1576) were known long before the discovery of bacteria. But there was a controversy at the beginning of the discovery of virus. Adolf Mayer in 1886 first pointed out that Tobacco Mosaic disease is sap transmissible and infectious. He believed bacteria to be the cause of the disease but he failed to isolate any bacteria and firmly believed some “minute bacteria” to be associated with the cause. Iwanowskii in 1892 confirmed that the causal agent was not bacteria by demonstrating that TMV could pass through filters that retained even the smallest bacterial cells. At last in 1896, Martinus Willem Beijerinck, a Dutch microbiologist and botanist proved that the Tobacco Mosaic disease did not involve any microbial agent but it was a contagious living fluid "contagium vivum fluidum" which was actually responsible for the cause. He used the word “virus” for the contagious fluid. He is considered as the “Father of Virology”. Virology enters molecular era!!!

Gene -for- gene relationship
Discovered by Harold Henry Flor in rust of flax in 1955. He showed that the inheritance of both resistance in the host and parasite ability to cause disease is controlled by pairs of matching genes. One is a plant gene called the resistance gene (R) gene. The other is a parasite gene called the avirulence (Avr) gene. R gene is resistant towards a pathogen that produces the corresponding Avr gene product. Gene-for-gene relationships are a widespread and very important aspect of plant disease resistance.

Plant Pathology in the 21st century
In India, initiatives have been taken by ICAR, SAUs, and National Institute of Plant Health Management GOI Hyderabad has initiated a Plant Health Newsletter to focus and strengthen sustainable agriculture as a new trend for plant health and disease management to reduce the pesticide load by 40% by 2018 compared with the load level of 2011. Some of the important aspects initiated by R&D are: Biopesticides, RNAi, Nanotechnology etc.

Biopesticides
According to US Environmental protection Agency (EPA), Biopesticides includes the naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs”. Microbial pesticides contain the microorganisms as the main active ingredient that function as biological control agents, affecting the pathogen directly or indirectly through the compounds they produce or by stimulating specific plant responses. Plant-incorporated protectants (PIPs) basically GM crops are the pesticidal substances produced by plants that contain genetic material added to the plant often through genetic engineering. Biochemical pesticides or botanicals are the substances that control diseases which include potassium bicarbonate, hydrogen oxide, phosphorus acids, plant extracts and botanical oils. In terms of active ingredient, microbial biopesticides dominated the global biopesticide market with over 63% share. *Bacillus thuringiensis* and *Trichoderma viride* – most broadly used in the biopesticides market. The global biopesticide market was expected to grow from $2.1 billion in 2014 to $2.4 billion in 2015 at a growth rate of 15.4%. In addition, the market is expected to grow at the fastest CAGR of 16.7% to reach $5.3 billion in 2020.

Genetically modified plants
The provision of sufficient food to feed an estimated 9.7 billion people by 2050 (United Nations Department of Economic and Social Affairs, 2015) [13] and approximately 11.0 billion by 2100 (James 2015) [6] is one of the major challenges of this century. Genetically modified (GM) crops provide an opportunity to increase food and feed production efficiently by generating plants with higher yields and greater nutritional benefits in reasonably short times (The Gaurdian 2016). The most widely accepted genetically modified traits in GM crops are herbicide tolerance and insect resistance. GM soybean, maize, canola, and cotton are the most common examples of these crops in the market. The Flavr Savr tomato was introduced as the first genetically engineered whole food in 1994 (Krieger *et al.*, 2008) [7]. In 1996, GM crops were grown at 1.7 million hectares and in 2014 at 181.5 million hectares (James, 2014) [5]. Common genetically modified foods are maize, canola, Bt cotton, golden rice, alfalfa, soyabean, potato, tomato.
Advantages of GM crops is that the food production increases, thus improving food availability at the local and global levels; food and food quality; and economic and social impact of farming communities (James, 2014) [5].

Rna interference (RNAi) technology
RNA interference (RNAi) has emerged as a powerful tool for battling some of the most challenging disease caused by viruses, bacteria and fungi (Wani *et al*., 2010) [14]. It blocks gene function by inserting short sequences of RNA that match part of the target gene’s sequence, no proteins are produced. Gene expression is suppressed at transcription or post transcriptional level. Science magazine named it as “Breakthrough of the Year” and Fortune magazine hailed it as “Biotech’s billion dollar breakthrough” in 2003. RNAi has significantly gained prominence as the precise, efficient and a stable method of choice for researchers. Gene silencing was first used to develop plant varieties resistant to viruses. First discovered by Crag C. Mello and Andrew Z. Fire in the nematode worm *Caenorhabditis elegans* and they published in 1998. They got Nobel Prize in Medicine or Physiology 2006 for their discovery of RNA interference-gene silencing by double stranded RNA.

Some examples of novel plant traits engineered through RNAi

Trait	Target gene	Host	Application
Enhanced nutrient content	Lyc	Tomato	Increased concentration of lycopene (carotenoid antioxidant)
Reduced alkaloid production	CYP82E4	Tobacco	Reduced levels of the carcinogen nornicotine in cured leaves
Heavy metal accumulation	ACR2	Arabidiopsis	Arsenic hyperaccumulation for phytoremediation
Reduced polyphenol production	s-cadinene synthase gene	Cotton	Lower gossypol levels in cotton seeds, for safe consumption
Ethylene sensitivity	ACC oxidase gene	Tomato	Longer shelf life because of slow ripening

Management of plant pathogens using RNAi

Fungi- *Neurospora crassa, Magneporthe oryzae, Cladosporium falvum, Saccharomyces cerevisiae, Aspergillus nidulans* etc.

Bacteria- crown gall disease management

Viruses- Vigna mungo yellow mosaic virus (VMYMV), bean golden mosaic virus (BGMV).

Nanotechnology

Nanoscale science and nanotechnologies are visualized to have the potential to revolutionize agriculture and food systems (Norman and Hongda, 2013) [11] and has given birth to the new era of Agronanotechnology. Nanotechnology refers to controlling, building, and restructuring materials and devices on the scale of atoms and molecules. The term 'nanotechnology' was coined by the physicist Norio Taniguchi in 1974.

"What would happen if we could arrange the atoms one by one the way we want them?"

This was a question asked by Richard Feynman, a well-known American physicist, in his speech entitled '"There's Plenty of Room at the bottom", during the meeting of the American Physical Society, on 29th December, 1959. This idea eventually became a research field, known as Nanotechnology.

Applications of nanotechnology in agriculture and allied science: Food Technology, Water Management, Weed Management, Plant Disease Diagnosis, Nanofertilizer for Balanced Crop Nutrition, Seed Technology, Biosensors, Fishery and Aquaculture, Pest Management, Crop Improvement.

Applications of nanotechnology in crop protection

Nanoparticles can serve as 'magic bullets', it contains the coated or protective layered pesticide, fertilizer and other agrochemicals that enable effective penetration through cuticles and tissues, allowing slow and constant release of the active substances for protection against pests and pathogens, early detection of plant disease and pollutants including pesticide residues by using nanosensors (Ghormade *et al.*, 2011) [2], nano magnets are used for removal of soil contaminants.

Different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver have been developed. Silver nanoparticles (Nano-Ag) have proved to be most effective as they exhibit potent antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms (Guo *et al*., 2003) [4]. Nowadays they are found in clothing, food containers, wound dressings, ointments etc. and also used in food packaging and food processing material (US Food and Drug Administration). Application of AgNPS in soil and as seed/seedling coatings may not only control the phytopathogen, but also stimulate plant growth by several known and unknown mechanisms. The antifungal activity of silver nanoparticles was evaluated against sclerotium-forming phytopathogens, *R. solani, S. sclerotiorum* and *S. minor*, during a study conducted by Min *et.al*. (2009) [9], who demonstrated that the nanoparticles strongly inhibited the fungal growth and sclerotial germination growth.

Agrilife research scientist utilizes drone to detect wheat disease progression

Dr. Charlie Rush, a Texas A&M Agrilife Research plant pathologist in Amarillo, with the help of Ian Johnson used a helicopter drone to track disease progression across wheat fields and to make better irrigation decisions. Mite-vectored virus diseases (transmitted by the wheat curl mite) are predominant. Drone uses visible spectrum -focus on the yellow band of light and captures the typical symptoms of wheat streak mosaic.

Conclusion and future perspectives

As status and importance of various diseases have changed over years, more sophisticated technologies have to be carried out in the future. Society, consumers and growers will only be able to continue to benefit from plant pathology if the discipline can evolve appropriate disease management schemes that can respond to the significant changes in agricultural practices in India; the ultimate goal being to produce more and safer food in sustainable agricultural systems that conserves natural resources and the environment. Information technology, communication and the integration of conventional and new technologies are essential and must be integrated by the modern practitioners of plant pathology into effective disease management schemes that can be implemented at the farm level. So, at present, we, the plant pathologists, have to be more proactive in our approach and identify new and novel techniques to counter the threat posed by plant pathogens.

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