The Planta RBS, Vol.-5, Issue-2, October 2024: 1607 – 1617 // ISSN: 3048:8494
PRUTHVIRAJ *et.al.*, (Series Chapter No. – 178 // ISBN: 978-81-965701-9-4)
https://www.pgrindias.in

NANOTECHNOLOGY: SIGNIFICANCE IN PLANT PATHOLOGY

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Abstract

The term 'nano' is used as a prefix to denote one billionth, i.e., 1 nanometer means 1 billionth of a meter. Various definitions of nanotechnology has been put forward, but the current widely accepted definition of nanotechnology covers a number of fundamental aspects: 1. research and technology development at the atomic, molecular, or macromolecular levels using a length scale of approximately 1 to 100 nanometers in at least one dimension; 2. the creation and use of structures, devices and systems that have novel properties and functions because of their small size; and 3. the ability to control or manipulate matter on an atomic scale. In agriculture, new tools for molecular and cellular biology are needed that are specifically designed for separation, identification and quantification of individual genes and molecules. Nanotechnology has that potential to deliver the genes to specific sites at cellular levels and rearrange the atoms in the DNA of the same organism to get expression of desired character, thus skipping the time consuming process of transferring the gene from foreign organisms. Nanotechnology has also shown its ability in modifying the genetic constitution of the crop plants thereby helping in further improvement. Mutations both natural and induced have long since played an important role in crop improvement. Instead of using certain chemical compounds like EMS, MMS and physical mutagens like X-ray, gamma ray, etc. for conventionally induced mutation studies, nanotechnology has showed a new dimension in mutation research.

Citation: Pruthviraj, Sachin, M. L., Madankumar, M and Manjunath Madhukar Mopagar (2024), Nanotechnology: Significance in Plant Pathology, *The PLANTA Research Book Series*, *5*(2), 1607-1617. www.pgrindias.in

Introduction

Nanotechnology is an interdisciplinary field of research and development in chemistry, physics, biology and technical sciences (Roco *et al*., 1999). The development and use of nanotechnology may revolutionize the world, enabling the production of novel substances through manipulations at the atomic and molecular levels. The development of this field will facilitate correlation between the macroscopic properties and structure of a substance within plant- and animal derived biological materials (Kulzer *et al*., 2004). It is believed that the development of nanotechnology will enable scientists only to group atoms into structures with desired properties (Huang *et al*., 2001). Nanotechnology products, due to their unique physical and chemical properties, have already been widely used in information technologies, telecommunication industry, medicine, automatics industry, chemical industry, environmental protection, as well as in agriculture and food industry (Liu *et al*., 2003).

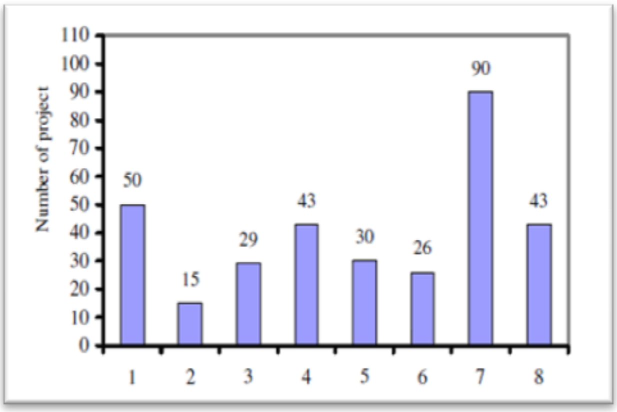

Fig-1. Number of projects in different fields of agriculture in the USA: 1-biosensors; 2-environmental protection; 3- agricultural incentives; 4-pathogen detection; 5-plant and animal production; 6-veterinary medicine; 7-biofood and 8-nano-bioindustrial products.

This is why more than 40 countries have adopted long-term national programmes of fundamental and applied research in nanotechnology. Investments into the scientific research in this field are rapidly growing and, accordingly, the number of nano structured products increases proportionally with the rising investment growth rate. The world market for nanotechnology-based products is expected to reach one billion dollars by 2015. Modern agriculture makes use of computers and global satellite systems to measure highly localized environmental conditions and precisely identify the nature and location of problems. By using centralized data, seeding, fertilizer, chemical and water use can be fine-tuned to lower production costs and potentially increase production.

Globally, many countries have identified the potential of nanotechnology in the agricultural sector and are investing significant funds into nanotechnological research. For example, most funds in the USA are being invested in applied research.

History

The concept of manipulating molecules and atoms has been postulated in 1860s, but the first experiments with actual observations and measurements in the nano-meter range have not been done until the early 1900s. In 1959, physicist Richard Feynman foretold the benefits that can be realized by being able to manipulate materials at the atomic and molecular scale and described a conceptual framework to achieve it (Feynman, 1959). The term 'nanotechnology' was first used by Norio Taniguchi in 1974 in describing the significant improvement in dimensional accuracy (in the order of 1 nanometer) for manufacturing processes at that time (Taniguchi, 1974). In 1980s, development in this field was greatly enhanced with advances in electron microscopy, which allowed scientists to 'see' and manipulate individual atoms and molecules. One defining moment in nanotechnology happened in 1989 when Donald Eigler used a scanning tunnelling microscope (STM) to produce the now widely-popular image of individual xenon atoms arranged to spell out the letters 'IBM' (Eigler and Schweizer, 1990). Another milestone is the discovery of a new form of carbon by Harold Kroto, Richard Smalley and Robert Curl. Formed out of 60 carbon atoms and shaped like a ball, it was named Buckminsterfullerene (or often called 'buckyball') and earned the three scientists Nobel Prize in Chemistry (Kroto, 1985). Since then, various advances were made in atomic and molecular manipulation, which gave rise to different nano-scale materials and devices with multitude of applications. An overview of the scale of materials produced with nanotechnology (Figure 1), relative to nano-scale things that occur in

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nature, is available from the website of the U.S. National Nanotechnology Initiative (www.nano.gov).

Indian Scenario

In India the nanotechnology is still at infancy and it requires sustained effort of experts in Applied Mathematics, Electronics, Drafting, Embedded systems, hardware and software, Agriculture, Genetics, Artificial Intelligence, Computer Graphics and Animation, Chaos theory, number theory etc to catch up with the leaders.

The Government has recognized the importance of research and development in nanotechnology and accordingly investments have been made to engage with and harness benefits from such emerging technologies.

Experts including this technology's advocate Dr Kalam have felt that nanotechnology poses great opportunity to enhance national agricultural productivity and reduce production costs in the face of reduced arable land, water, and workforce as well as increased population and climatic variation. Even Mr. Pawar the minister of Agriculture and food industries in his inaugural address at ASSOCHAM's "Fifth Knowledge Millennium Summit B2B on Bio and Nanotechnology" expounded on certain bio-nanotechnology interventions that could help alleviate the problem of food security. A sub-group set up by the Planning Commission headed by ICAR director-general has stated in its report that nanotechnology such as, nano-sensors and nano-based smart delivery systems could help ensure efficient utilization of natural resources like water, nutrients and chemicals in agriculture. Nano-barcodes and nano-processing could also help monitor the quality of agricultural produce.

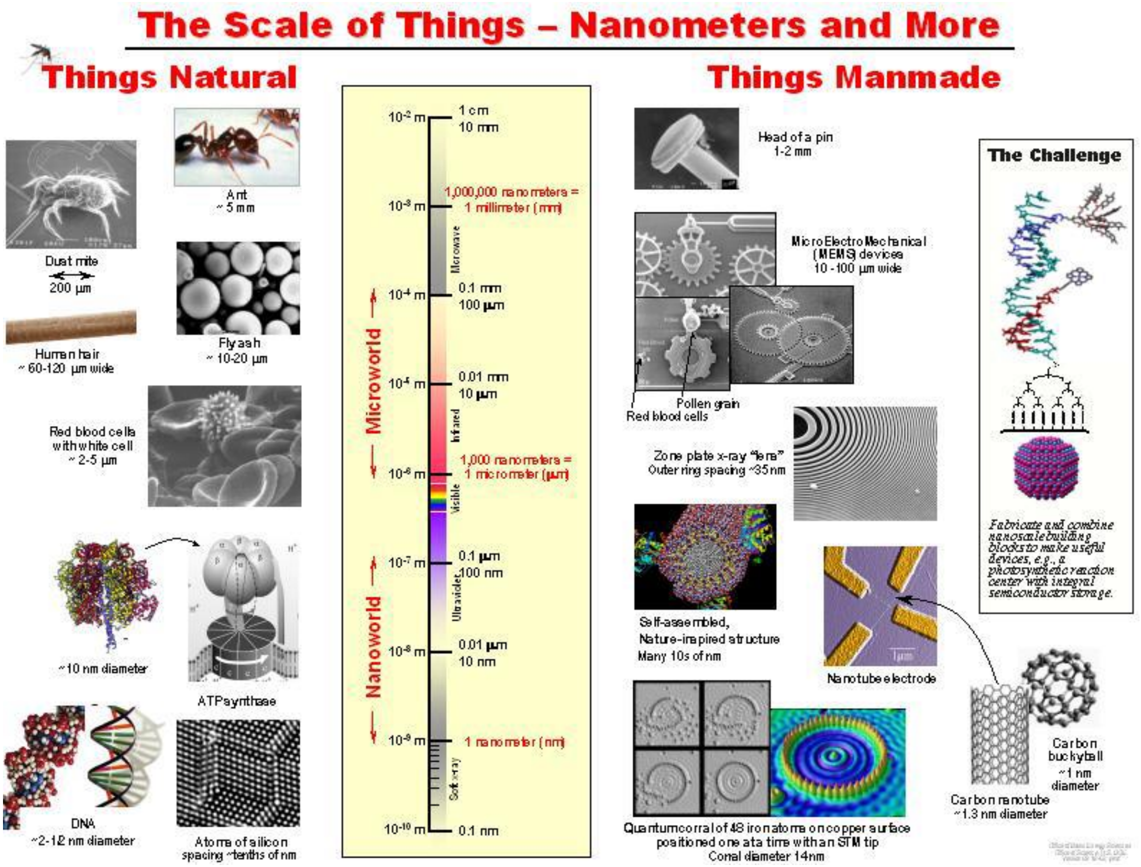

Fig-2. Natural and man made nano-scale materials

The report has called for the creation of a National Institute of Nanotechnology in Agriculture (NINA) under National Agricultural Research System. Further, the vast infrastructure of universities and research institutes in agriculture in India could be spruced up to engage with nanotechnology. Also, creation of nanotechnology centres in these organizations drawing expertise from different areas of agriculture could provide a platform to the scientific community to explore this technology better. Since, nanotechnology is interdisciplinary in nature and possesses application potential in several areas of agriculture, its successful usage would require a greater degree of interaction and collaboration among the disciplines and actors. In this regard, the sub-group has proposed for the establishment of a national consortium on nanotechnology R&D. The consortium would include, besides the proposed NINA, several other institutes active in nanotechnology R&D (IIT, IISc, NPL, BHU, agricultural universities, Jamia Hamdard University, and apex scientific and research bodies).

What is Nanotechnology?

The term 'nano' is used as a prefix to denote one billionth, i.e., 1 nanometer means 1 billionth of a meter. Various definitions of nanotechnology has been put forward, but the current widely accepted definition of nanotechnology covers a number of fundamental aspects: 1. research and technology development at the atomic, molecular, or macromolecular levels using a length scale of approximately 1 to 100 nanometers in at least one dimension; 2. the creation and use of structures, devices and systems that have novel properties and functions because of their small size; and 3. the ability to control or manipulate matter on an atomic scale (EPA, 2007). An essential aspect of nanotechnology is the nanometer scale of dimensions involved. At this scale, the classical rules of physics and chemistry ordinarily valid at larger, macro scales no longer apply; instead nanoscale materials exhibit unique quantum properties that may be radically different from the properties of the same material at macro-scale. For instance, the characteristics and properties of a typical material observed at macro-scale such as colour, strength, electrical conductivity, thermal properties, and reactivity, can be significantly different at the nano-scale (Anonymous, 2003). Additionally, as structured materials are created in smaller dimensions, the ratio of the exposed surface to the volume of the material increases rapidly, thereby making the material more reactive. These scale-related phenomena open up the range of possibilities which enable nano-scale researchers to come up with novel materials and processes with unique functions and properties that were previously not available at larger scales.

Nanoparticles (NP)

NP's are considered substances that are less than 100 nm in size in more than one dimension.Particles in the nano-sized range have been present on earth for millions of years and have been used by mankind for thousands of years. Soot for instance, as part of the Black Carbon continuum, is a product of the incomplete combustion of fossil fuels and vegetation; it has a particle size in the nano-meter micrometer range and therefore falls partially within the ''nanoparticle'' domain. Recently, however, nanoparticles have attracted a lot of attention because of our increasing ability to synthesize and manipulate such materials.

Properties of Nanoparticles

Intrinsic Properties of Nanoparticles: The fate, behaviour and therefore the ecotoxicology of nanoparticles will be closely related to their intrinsic properties. There are several aspects to nanoparticles which can easily be dismissed in error due to their apparent ability to behave more like molecules than larger colloidal suspensions.

Particle Mobility: The process of diffusion is controlled by several factors: gravitational forces, buoyancy and Brownian motion. These factors can all be taken into account using Einstein's law of diffusion:

$$Df = kT$$

where D = diffusion coefficient, f = the frictional coefficient for the particle, k = Boltzmann constant and T = temperature.

The frictional coefficient of a nanoparticle may be derived from Stokes law:

f =6πήa, where ή = the viscosity of the medium and a = particle radius.

This means that the diffusion coefficient is inversely proportional to the radius of the particle and the average displacement of a single particle in time t will be proportional to the inverse square root of the particle radius. Figure 4 shows a plot of the average distances moved after 20 time units for particles of different sizes. This shows that the distance moved by a particle of 50 nm will be an order of magnitude less than that of a particle, or molecule, 0.5 nm is diameter.

Production of Nanoparticles

Top-down vs. bottom-up Approach

There are two fundamental approaches to nanotechnology, pertaining mostly to the manner in which the novel nanomaterials are created (EPA, 2007). 'Top-down' approach involves creating nano-scale materials by physically or chemically breaking down larger materials using micro and nano-lithography and etching techniques. Currently, this is the well-established but more expensive process of creating nanomaterials. 'Bottom-up' approach applies to assembling nanomaterials atom-by-atom or molecule-by molecule, also known as molecular nanotechnology described by Eric Drexler in 1980s. Using forces of nature for 'self-assembly' or by individually manipulating atoms or molecules (i.e., using the probe tip of a STM), materials with desired structure and form can be 'grown' in an organized manner. Typically, this approach allows the incorporation of specific features into the final form and is considered less expensive since only the required amount of the basic element is used (unlike in top-down approach where in a large amount of material taken out of the base material is discarded).

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including7: (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) Drug release profile desired; and (f) Antigenicity of the final product.

Nanoparticles have been prepared most frequency by three methods:

(1) Physical methods; (2) Chemical methods; and (3) Biological methods

1. Physical Methods

Generally solid precursors are used which are directly converted to nano-size by mechanical or vaporization techniques.

a) Mechanical methods: this is generally done using high energy ball mills. By mechanical grinding one can attain particles in the nano scale but it has some limitations such as i)it gives a mixture of different sized and shaped nano-particles, and ii) the distribution of size and shape are uncontrolled. However, this technique can suitably be used in agriculture for making non-soluble mineral sources more useful as sources of various plant nutrient and amendments in soil.

b) Vaporization methods: following are the several techniques under this category

i) Inert gas condensation
ii) Pulse laser ablation
iii) Spark discharge generation
iv) Ion sputtering

2. Chemical Methods

The liquid and vapour precursors are allowed to undergo chemical reaction for achieving the supersaturation that is required to induce homogeneous nucleation of particles

Different Chemical Methods used for Preparation of Nanoparticles are:

a) Simple chemical reaction
b) Microemulsion method
c) Sol-gel method
d) Precipitation method
e) Chemical vapour synthesis
f) Spray pyrolysis
g) Laser pyrolysis/ photothermal synthesis
h) Thermal plasma synthesis
i) Flame synthesis
j) Flame spray pyrolysis

3. Biological Methods

Higher plants and a number of microbes are employed for the synthesis of nano-particles. The development of reliable and ecofriendly biological processes for the production of nano-particles is still a challenging task in present scenario of nanotechnology research.

Mode of Action of Nanoparticles

Studies disclosed that nanoparticles carrying features like high surface area, charge, ability to deliver a large number of antibiotics or other substance, size and shape makes them ideal antimicrobial weapon (Bhosale et al., 2018; Fabiyi et al., 2020; Yien et al., 2012). These features saliently or latently may contribute to its antimicrobial efficacy. The size of nanoparticles are proven features that confers antimicrobial potency. on the substance. Besides the innate antimicrobial potential of NPs, the vast varieties of analyses on NPs suggested that the large surface area of the NPs are imperative for microbial attachment and rapid penetration into the cell. The inhibitory mechanism that is followed by the nanoparticles is not correctly assembled and fully explained. However, evidence shows that induced oxidative stressare thoroughly followed, models. Moreover, microbial cell wall penetration, the generation of reactive oxygen species (ROS), damaged DNA and proteins, loss of cellular integrity are the underlying mechanisms that cause inhibition for bacteria, fungi, and likewise viruses. Cell penetration is often the initial step in the stages involved in some microbial cell inhibition process before other mechanisms are adopted. Adsorption or diffusion of NPs at the cell surface is the main penetration mechanism. Adsorption can be attained through the binding of NPs with the negative charged functional groups of proteins resulting in protein destruction and cell death (Padmavathy and Vijayaraghavan, 2011). While evidence has reported ROS formation into pathogenic cells through the diffusion process (Zhang et al., 2013). Besides, microbial destruction and inactivation due to an interaction between various surface-exposed groups of microbes and nanoparticles may seem to be a possible mechanism (Ray et al., 2007; Rogers et al., 2008). From the overall discussion, some characteristics of nanoparticles are explored which can be very beneficial to future researchers. The antimicrobial activity of nanoparticles has been studied against a wide range of microorganisms including bacteria, fungi, viruses from time to time.

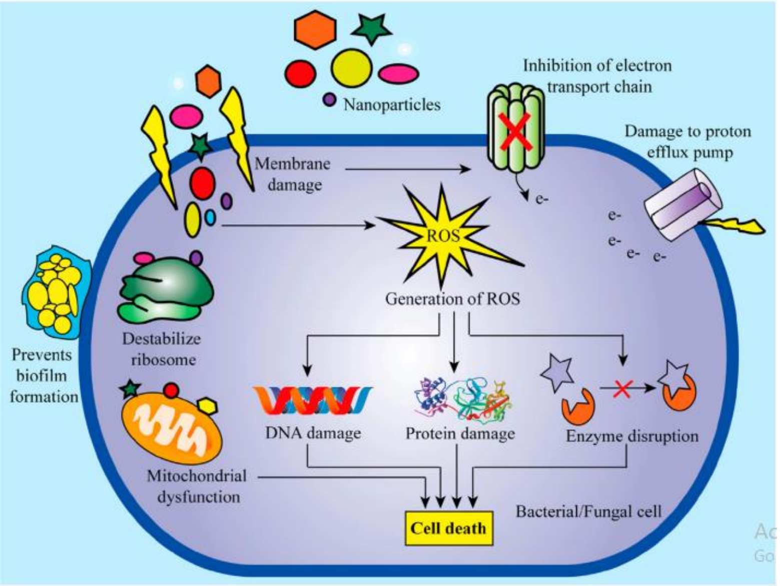

Fig-3. Schematic represents antimicrobial (bacteria and/or fungi) mechanisms of various nanoparticles. The antimicrobial (bacteria and/or fungi) activity of NPs has been attributed to their direct interaction with the bacterial and fungal cell wall/membrane and prevention of biofilm formation. In addition, NPs display potent antibacterial/anti-fungal effects through the generation of innate along with adaptive host immune responses, production of toxic reactive oxygen species (ROS), and stimulation of intracellular effects (e.g., enzyme disruption, DNA damage, and protein damage). (Sharmin et al., 2020)

Overview of Nanotechnology Applications

Various nanomaterials, techniques, and processes arising from nanotechnology can have significant benefits in diverse fields. Some examples of the current and potential applications of nanotechnology are as follows.

Applications of Nanotechnology in Pests and Plant Diseases Management

Today use of chemicals such as pesticides, fungicides and herbicides is the fastest and cheapest way to control pests and diseases. Also biological control methods are very expensive currently. Uncontrolled use of pesticides has caused many problems such as: adverse effects on human health, adverse effects on pollinating insects and domestic animals, and entering this material into the soil and water and its direct and indirect effect on ecosystems. Intelligent use of chemicals on the nano scale can be a suitable solution for this problem. These materials are used into the part of plant that was attacked by disease or pest. Also these carriers in nano scale has self-regulation, this means that the medication on the required amount only be delivered into plant tissue. Nanotechnology helps to agricultural sciences and reduce environmental pollution by production pesticides and chemical fertilizers by using the nano particles and nano capsules with the ability to control or delayed delivery, absorption and more effective and environmentally friendly; and production of nano-crystals to increase the efficiency of pesticides for application of pesticides with lower dose. Nano particles for delivery of active ingredients or drug molecules will be at its helm in near future for therapy of all pathological sufferings of plants. There are myriad of nano materials including polymeric nano particles, iron oxide nano particles and gold nano particles which can be easily synthesized and exploited as pesticide or drug delivery piggybacks. The pharmacokinetic parameters of these nano particles may be altered according to size, shape, and surface functionalization. They can also be used to alter the kinetic profiles of drug release, leading to more sustained release of drugs with a reduced requirement for frequent dosing (Sharon *et al*., 2010). Diseases are one of the major factors limiting crop productivity. The problem with the disease management lies with the detection of the exact stage of prevention. Most of the times pesticides are applied as a precautionary manner leading to the residual toxicity and environmental hazards and on the other hand application of pesticides after the appearance of disease leads to some amount of crop losses. Among the different diseases, the viral diseases are the most difficult to control, as one has to stop the spread of the disease by the vectors. But, once it starts showing its symptoms, pesticide application would not be of much use. Therefore, detection of exact stage such as stage of viral DNA replication or the production of initial viral protein is the key to the success of control of diseases particularly viral diseases.

Nano-based viral diagnostics, including multiplexed diagnostic kit development, have taken momentum in order to detect the exact strain of virus and stage of application of some therapeutic to stop the disease. Detection and utilization of biomarkers that accurately indicate disease stages is also a new area of research. Measuring differential protein production in both healthy and diseased states leads to the identification of the development of several proteins during the infection cycle. These nano-based diagnostic kits not only increase the speed of detection but also increase the power of the detection (Prasanna, 2007). In the future, nano scale devices with novel properties could be used to make agricultural systems “smart”. For example, devices could be used to identify plant health issues before these become visible to the farmer. Such devices may be capable of responding to different situations by taking appropriate remedial action. If not, they will alert the farmer to the problem. In this way, smart devices will act as both a preventive and an early warning system. Such devices could be used to deliver chemicals in a controlled and targeted manner in the same way as nano medicine has implications for drug delivery in humans. Nano medicine developments are now beginning to allow us to treat different diseases such as cancer in animals with high precision, and targeted delivery (to specific tissues and organs) has become highly successful (Joseph and Morrison, 2006).

Crop Improvement

In agriculture, new tools for molecular and cellular biology are needed that are specifically designed for separation, identification and quantification of individual genes and molecules (Warad and Dutta, 2005). Nanotechnology has that potential to deliver the genes to specific sites at cellular levels and rearrange the atoms in the DNA of the same organism to get expression of desired character, thus skipping the time consuming process of transferring the gene from foreign organisms.

Nanotechnology has also shown its ability in modifying the genetic constitution of the crop plants thereby helping in further improvement. Mutations both natural and induced have long since played an important role in crop improvement. Instead of using certain chemical compounds like EMS, MMS and physical mutagens like X-ray, gamma ray, etc. for conventionally induced mutation studies, nanotechnology has showed a new dimension in mutation research. In Thailand, Chiang Mai University's Nuclear Physics Laboratory has come up with a new white-grained rice variety from a traditional purple coloured rice variety called Khao Kam through nanotechnology. Using nanotechnology, the scientists changed the colour of the leaves and stems of Khao Kam from purple to green and the grain became whitish (ETC, 2004). The research involves drilling a nano-sized hole through the wall and membrane of a rice cell in order to insert a nitrogen atom, using a particle beam and the nitrogen atom is shot through the hole to stimulate rearrangement of the rice's DNA. This newly derived organism through the change at the atomic level is designated as 'Atomically Modified Organism (AMO).

Merits and Demerits of Nanotechnology

Merits

- Label free detection sensors are currently the most promising technologies suitable for Use in POC settings
- Biocare SPR sensor surfaces are most widely used and are commercially available
- QCM Immunosensors offers new insights in detection of plant pathogenic viruses up to 1ng within 1hr.

Demerits

- Electrochemical detection having low sensitivity, high cost and complicated.
- Nanoparticles may cause inflammation, tissue damage or growth of tumors and harm the body’s immune system
- In SPR nucleotide extraction is needed thereby making it less attractive for POC analysis

Conclusion

Antibody-based sensors offer the potential of plant pathogen detection and rapid analysis. Biosensors released within hours or even minutes, rather than several days. Adoption of nanotechnology-based diagnostics for the diagnosis of infectious plant diseases in diverse settings throughout the globe, preventing epidemics and safeguarding human economic wellness. Application of nanotechnology in plant pathology at the global level is at its nascent stage, and its success is based on its ultimate acceptance by the researchers.Plant Pathology is the essential core of development of nanotechnology especially when it is to be focused not only to the needs of farm but also the non-farm sector.

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