Chapter

Nanophytovirology: An Emerging Field for Disease Management

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Abstract

Nanotechnology positions as a new armament in our collection against the increasing challenges in disease management and plant/human health. The application of nanotechnology in plant/human disease administration, diagnosis, and genetic transformations is still in its early stages. Apart from the scope of this chapter, there is also a mounting collection of new tools and techniques where nanoparticles are employed as delivery vehicles for genetic material in plants. Due to their nanoscale dimensions, nanoparticles may knockout virus particles and thus may open a novel arena of virus control in plants/humans. Our aim is to enlighten and enthuse researchers about the swiftly expanding prospects of nanotechnology in plant pathology i.e., "nanophytovirology."

Keywords: nanoparticles, plant pathology, human pathology, virology, disease diagnosis, disease management, plant protection, case study

1. Introduction

Food security has always been the principal apprehension for mankind [1]. Food losses because of crop infections by pathogens like bacteria, fungus and viruses are known as obstinate issues in agriculture since centuries around the globe [2]. Even countries, societies and their administrations have been facing this problem a long time. Quarantine strategies employed for crops and ornamental plants a requite effective in preventing harmful diseases and arthropod pest epidemics from being imported and getting spread in the purchasing country [3]. Plants are infected by a number bacterial, fungal and virus species [4–8]. Viruses are considered as the minutest known microbes to the mankind and yet they reason for the most significant losses in agriculture sector [9], thus putting the plants under stress [10]. Same holds true for humans as well. Many a time, the finest recognized treatment for viruses is the innate immunological resistance system of host; else, the initial prevention of viral infection is the only substitute [11]. Consequently, diagnosing host for viruses at earliest is the prime approach toward controlling and eliminating harmful virus [12, 13]. The starter of a novel class of nanoscale particles with numerous exceptional properties and functions has flashed a series of innovative applications [14]. Engineered nano-materials (nanoparticles) range from 1 to 100 nm in size [15]. Engineered nanoparticles can be synthesized to precise dimensions and intended in numerous composite arrays, making their function and efficacy applicable in many fields. Suitable sensors and good delivery systems might help infighting viruses and other crop pathogens.

Nanoparticles might employ an important integrity in future plant and human disease management that might range from disease diagnosis to disease treatment [16]. In recent past several nanoparticles has been synthesized across the globe by eminent scientists in various forms [17, 18]. Like quantum dots, metalloids, metallic oxides, nonmetals, carbon nanomaterials [19], dendrimers, liposomes [20], Virus-based nanoparticles (VNPs) are few examples of this category [21, 22]. Nanoparticles greatest advantage lies in their small size, greater surface area and strong reactivity: such efficient activity favors for vast application in plant and human pathology [23]. Nanoparticles can be synthesized either by chemical route or by green synthesis method taking in account the top down or bottom up approach, whichever better feasible. This can be further categorized into chemical, reduction, microemulsion, colloidal, sonochemical, electrochemical, microwave, solvothermal and microbial synthesis of nanoparticles [24]. The present study focuses and centric towards the above said aspects of nanoparticles vs. plant virology ("nanophytovirology"), thus summarizing the available scattered literature at one place for the common audience.

2. Pre-era of "nanophytovirology"

Earlier several methods have been given by pioneers for virus detection in the host plant (crops, ornamental plants, weeds) [25]. Therefore, techniques for recognition and detection of viruses, equally in crops and carrier vectors, participate for a decisive role in virus disease management. All of them are listed as: electron microscopy [26], symptoms determination [27, 28], biotest [29, 30], mechanical transmission [31], seed transmission [32], serological techniques [enzyme-linked immunosorbent assay, phage display, tissue blot immunoassay (TIBA), lateral flow devices, immunocapture transmission electron microscopy (ICTEM)] [33, 34], restriction fragment length polymorphism (RFLP) [35], thermostable amplification based methods [PCR and reverse transcription-polymerase chain reaction (RT-PCR), multiplex PCR/RT-PCR, immunocapture PCR (IC-PCR), immuno-precipitation PCR (IP-PCR), nested PCR, multiplex nested PCR, real time PCR (qPCR), multiplex real time PCR, Co-operational PCR (Co-PCR)] [36–39], isothermal amplification based methods [helicase dependent amplification (HDA), recombinase polymerase amplification (RPA), nucleic acid sequence base amplification (NASBA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA)] [40, 41], nucleic acid sequence hybridization techniques [in situ hybridization, microarray, lateral flow microarrays] [42, 43], next generation sequencing (NGS), recombinant DNA approach [44]. Accessibility of the few above-mentioned diagnostic methods endow with a superior elasticity, increased sensitivity, and specificity for quick judgment of virus diseases. The accurate and reliable detection of the associated virus pathogens therefore forms the first line of defense in management of these diseases.

Likewise, number of methods has been devised in viral disease management [45–48]. It is conceivable that destruction/killing arthropod vectors, either via biological control or with traditional methods, helpful in reducing the viral populations. *Scymnus offmanni*, *Coccinella septempunctata*, *Propylaea japonica*, *Euseius cutalis*, etc. are some natural predators of whiteflies. Chemiecological technique employs honeydews excreta of whitefly, which work as a kairomone to attract natural prey, i.e., *Encarsia formosa*. Such approach has been successfully used in the Mediterranean regions against whiteflies vector. Even plant age is also crucial in vector population controlling. Field trial has been successful where young plants were covered with plastic bag (yellow polyethylene film) or grown under green house until maturing phase, limits the contact with arthropods vectors. Similarly masking the crop with living ground covers of perennial peanuts, cinquillo and coriander plants condensed the impact of incoming whitefly adults. An old age practice helps in diminishing the virus populations by destroying the weeds growing in the near vicinity of the crop fields, or even sowing the seeds a little later when the vector populations flourishes. Both help in viral disease management [49].

Further the use of insecticides against various arthropod vectors is also helpful to a certain extent. Few of them are Neonicotinoids, Buprofezin, Thiamethoxam 70 WS, Imidacloprid 600 FS, Imidacloprid 70WS and Carbosulfan 25 DS, Triazophos, Ethion, Imidacloprid, Acephate 95 SG, Spirotetramat, Diafenthiuron, Nitroguanidines, Thiamethoxam, Ryanodine and Pymetrozine, quite lethal against whiteflies [50]. Two remarkable technology has come up as one the best solution against plant viruses, i.e., interference RNA (RNAi) and clustered regularly interspaced short palindromic repeats (CRISPR). RNAi-mediated virus resistance was reported against potato virus Y (PVY) in transgenic tobacco plants, against African cassava mosaic virus (ACMV), croton yellow vein mosaic virus (CYVMV) and many more [51–53]. RNAi is usually associated with methylation of nuclear DNA corresponding to the transcribed region of the target RNA despite transcription levels of the transgene remains unaffected [54]. CRISPR-Cas (CRISPR Associated Systems) is an adaptive immune system in many archaea and bacteria that cleaves foreign DNA based on sequence complementarity. Virus based guide RNA (gRNA) delivery system for CRISPR/Cas9 mediated plant genome editing cause mutations in target genome locations and resulted in transgenic plants showing resistance against viruses [55].

3. Epoch of "nanophytovirology"

"Nanophytovirology" is a front-line science which customs nanotechnology in diagnosis, detection and management of plant viral diseases and their pathogens especially arthropods at an initial phase, helping in plant protection from the epidemic diseases [56]. Among the various plant diseases, the diseases caused by viruses are the most difficult to manage [57], as one must stop the spread of the disease by the vectors. Nanotechnological-based disease diagnosis and management for virus infecting crop plants is attaining magnitude with the increased spread of viruses and threats of their epidemics [58]. Therefore, there is a demand for an improved management of viruses employed by a series of strategies [59, 60]; in-fact such practices relied on the ecology of the virus. Many approaches have been used to decrease crop losses due to viruses, only a few are effective in their management. Even understanding the plant mediated interactions between viruses and their carrier vector is quite important to tackle epidemiology of viral diseases [59, 60]. Developments in nanofabrication and nanotechnology endow a crucial part in plant viral disease detection, simplicity in handling and are cost-effective as compared to other plant viral diagnostic methods.

Earlier fluorescent dyes were used straight away for staining the viruses, now nanoparticles and quantum dots (QDs) have been developed which helps in carrying the detection tags (dyes or anti-viral antibodies) and are quite efficient in identifying the viruses, which are also helpful as labeling and imaging agents. Such fluorescent tags are easily detected in flow cytometry enabled devices. In yet another instance nano-biosensor was developed against plasmodiophoromycete *Polymyxa betae* which is responsible for the carrier of beet necrotic yellow vein virus (BNYVV) and caused the deadly disease rhizomania in sugarcane plants. The authors used specific antibodies against conjugated with Cadmium-Telluride

QDs against the glutathione-S-transferase protein's (GST). The developed nano-biosensor showed enough fluorescence resonance energy transfer (FRET) to detect the plasmodiophoromycete *Polymyxa betae*. Cadmium-Telluride QDs conjugated to antibodies were also developed against citrus tristeza virus (CTV) using the similar approach [61].

Surface plasmon resonance (SPR) is also an optical based technique which employs diagnosis of viruses by change in refractive index on a metal surface. In this gold nanoparticles are conjugated with anti-viral antibodies adsorbed on a glass substrate and are sensed by SPR. Quartz-crystal microbalance (QCM) is a well-known, commercially accessible mass sensor technique generally employed in quantitative measurement of the thickness of thin films. The principal is that the exterior part of the quartz-crystal device (microchip: dimensions in nanometer) is typically coated with anti-viral antibodies against the targeted plant viruses. Now when a virus is encountered on the quartz-crystal surface there results an increase in the mass, thereby resonant frequency decreases, the change in the frequency before and after of the chip is measured subsequently. SPR and QCM based nano-biosensor has been manufactured successfully for the detection of orchid viruses, tobacco mosaic virus (TMV), cymbidium mosaic virus (CymMV), odontoglossum ringspot virus (ORSV), etc. [62].

Microcantilevers are in the micrometer range, but their tip end is in the nano-meter scale and is widely used for various biosensing applications. Microcantilevers works in two different modes, i.e., straining and resonating mode, both are helpful in identifying viruses. Resonating mode is like QCM, whereas straining mode relies on the changes in electrical resistance whenever a virus particle bound to the surface. The major limitation is the low performance of device in the liquid medium, hence the sample need drying before application. For virus particle detection nanowire employed transistors have been devised. An immuno-biosensor was developed for the detection of Plum pox virus in plum (*Prunus domestica*) and tobacco (*Nicotiana benthamiana*) leaves sap, where gold electrodes were modified with 1,6-hexanedithiol, gold nanoparticles, anti-PPV IgG polyclonal antibody and BSA. Nanowires can be engaged against the target virus, and when encountering a charged virus capsid, a depletion or gain of charge in the nanowires is thus recorded as a simple conductance change. In a similar instance a lithographically patterned nanowire electrodeposition (LPNE) technique was used to develop a label-free chemiresistive sensors based on a polypyrrole (PPy) nanoribbon conjugated with anti-viral antibodies against cucumber mosaic virus (CMV) [63].

Nanotechnology benefits agriculture sectors and diminish environmental pollution. This is carried out by manufacturing of pesticides and chemical fertilizers using nanoparticles and nano-capsules and has the capability to control or delayed the delivery and absorption of pesticides and chemical fertilizers with lower dose. "Nano-5" is a marketed product pesticide to control several plant viruses. It was found effective at dilution of 1:500 against Mosaic, ringspot, transitory yellowing, tristeza virus, exocortis viroid by spraying "Nano-5" onto the surface of leaves and apply to the roots once every 3 days. It was reported that chitosan nanoparticles have the ability to induce resistance in host crops against few viruses, for example mosaic virus of alfalfa, snuff, peanut, potato, and cucumber were targeted. Similarly, gold nanoparticles showed antiviral effects against Bean mild mosaic virus in beans, barley yellow mosaic virus in barley and tobacco necrosis virus in tobacco plants. Peoples also claimed silver nanoparticles application made the host plant resistance against sun-hemp rosette virus (infecting bean plants) and bean yellow mosaic virus (causing disease in faba bean crops) [64].

RNAi technique were also employed in coupling with nanotechnology, a remarkable study was carried out to show resistance against cucumber mosaic virus (CMV) and pepper mild mottle virus (PMMoV). In this wonderful approach dsRNA was loaded onto LDH (Layered double hydroxides) nanoparticles, called BioClay and were sprayed on the challenged plants. Plants showed resistance against the above-mentioned viruses for 20 days as compared to controlled ones [65]. It is clear from the above discussion that "nanophytovirology" represent an attractive advancement, owing to their potential advantages for the plant disease management against deadly crop viruses.

4. Challenges adjoining "nanophytovirology"

The application of nanoparticles, particularly in plant disease management, need specific structural and physicochemical features, and any slight variations to their planned properties can hinder the function and performance of designed nanoparticle conjugate [66]. Hence, numerous factors related to nanoparticle synthesis are quite important for the development of an effective virus detection assay. Further, the methods employed in making/synthesizing nanoparticles seem easy and quick for large scale production but getting the final product in uniformity (shape and size) remains challenging. Nanoparticles itself cannot detect the viruses solely and thus need additional biomolecule specific in sensing the pathogen. In comparison to nanoparticles, biomolecules are quite delicate to severe chemical and physical alterations (high temperature, high salt concentrations, reducing agents) which might can harmfully affect their reactivity and specificity [67]. Therefore, such procedures demand for proper optimization steps aimed at in detecting viruses.

But when employing/using nanoparticles and its conjugates for the application in virus disease management, their biosafety and toxicity on human health and environment is yet another a major challenge. For example, nano-pesticides might get inhaled by the workers during treatment process. Similarly, other nano-composites might get deposit on the leaves or flowers, can affect animals, birds, honey bees, etc. They may clog the stomatal pores and might hinder the penetration of pollen grains on stigma. If get inside the plant system, nanoparticle might affect plant metabolism and can cause similar effect on humans as well. Cellular toxicity can be induced by nanoparticles (NPs) that lead to toxic side effects such as enhanced ROS generation, disruption of redox homeostasis, lipid peroxidation, impaired mitochondrial function, and membrane damage. Due to their long persistence and greater reactivity nano-pesticides may contaminate water and soil system [68]. Regardless of these developments in nanotechnology, there are some unsolved problems concerning the detection of many plant viruses due to their low titer in the plants, their uneven distribution, the existence of latent infection and lack of validated sampling protocols.

5. Conclusion

Virologists need complete knowledge about viral infection and of effects on host plants so that correct control procedures can be implemented [69]. Specificity of viruses varies greatly [70]. Some of them can colonize different species and some are specific or interact to specific cell machinery. There is more focus on reduction of crop loss by controlling pathogen movement from infected plants to healthy plants rather than treating the infected plants [71]. The work on the development of nanoparticles done by the pioneers in the field is particularly significant and beneficial for the humans and to the agriculture sector which supports the lives of growing population [72, 73]. Nanoparticles affect the pathogens in a similar way as the chemical pesticides do at a very low concentration [74]. Nanomaterials have been used as carrier of active ingredients of pesticides, host defense inducing chemicals, etc. [75, 76], to target the viral pathogens. These nano-based diagnostic kits not only increase the speed of detection but also increase the power of the detection. Thus, finding nanotechnology-based solutions, will enable researchers to explore better management practices against viruses in a better way for the plants which are constantly challenged in the natural conditions.

Conflict of interest

The authors have no conflict of action to declare.

Author contributions

A.M. and R.K.G. drafted the manusxxcript. A.M. and R.K.G. contributed to acquisition of literature data. All authors read and approved the final version of the manuscript.

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