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# Introductory Chapter: Atlas of Ultrastructure Interaction Proteome between Barley Yellow Dwarf Virus and Gold Nanoparticles

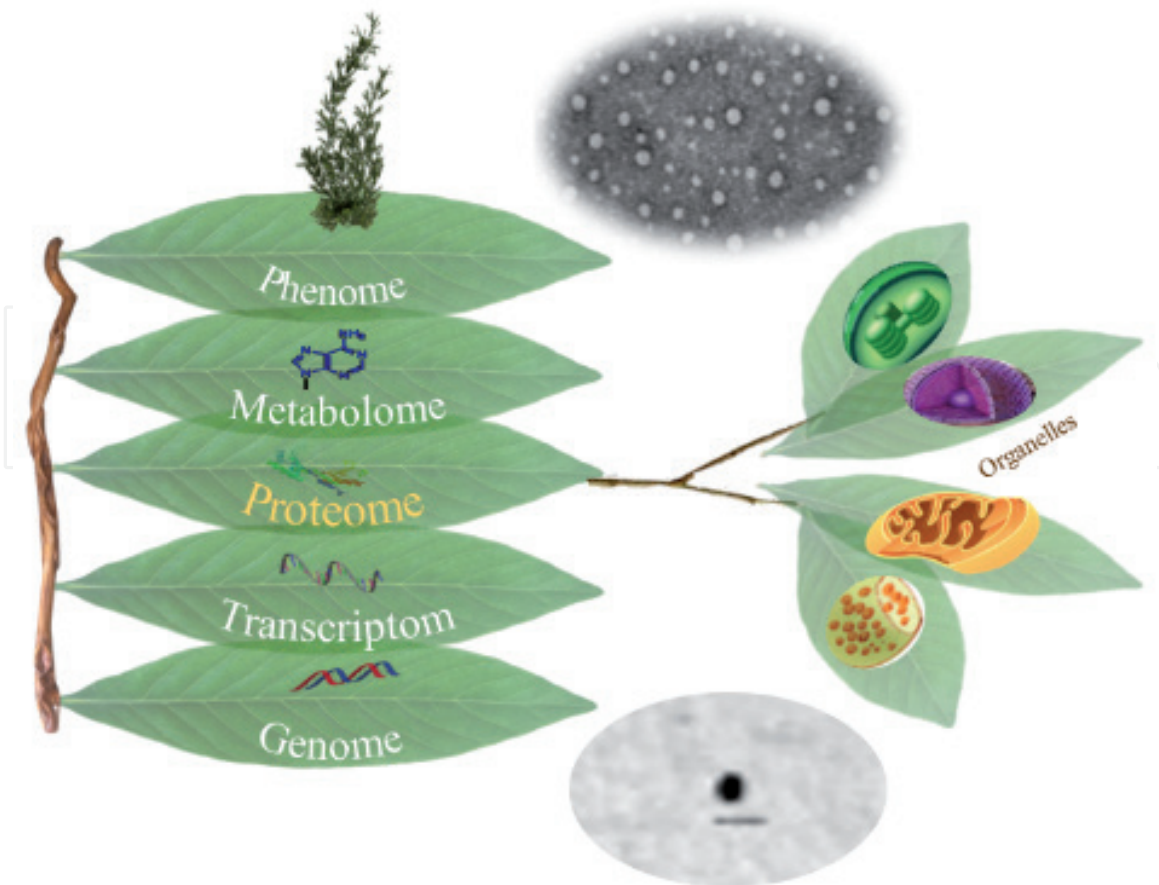
Noorah Abdulaziz Othman Alkubaisi  
and Nagwa Mohammed Amin Aref

## 1. Introduction

Interaction between Barley yellow dwarf virus, BYDV-PAV, and gold nanoparticles AuNPs application revealed great effect whether in *vitro* or *Vivo*. The significant effect of virus particles occurred inside the plant cell due to the existence of AuNPs treatment. It was clear that using tiny AuNPs 3.151 to 31.67 nm had a potential agent to ruined virus particles inside the infected cells. AuNPs cause damage to the virus-like particles (VLPs) of the barley yellow dwarf virus-PAV. Where they observed puffed and deteriorated VLPs decorated with AuNPs, as well as destroyed and vanished particles, using Transmission Electron Microscopy TEM. Generally, the plant cell contained different organelles that exhibited ultrastructure changes in Nucleus, Chloroplast, Plant cell wall, Mitochondria, cytoplasmic matrix, and viable cellular composition of the infected cell with AuNPs. TEM is a powerful tool in elucidating plant cells' fine details at the nanoscale. The present Atlas describes each organelle's structure of plant cells revealed by TEM in healthy, infected, and treated with AuNPs in **Figure 1**.

The purpose of this work is expressed via TEM, which is a very accurate tool for judging the AuNPs behavior inside the plant infected cell. Recent remarkable innovations in KSU. Platforms [1, 2] provide crucial resources to promote research in AuNPs applications and applied plant species as *Hordeum vulgare* (Barley) due to lacking knowledge in the field of virus pathogenicity at the level of ultrastructure. A combinatorial approach using the integration of virus with AuNPs in our proteome platform is now an effective strategy for clarifying molecular systems integral to improving plant productivity of the frequency and importance of ultrastructure abnormalities in Barley crop development caused by BYDV-PAV. It was finding out the critical feedback of using AuNPs applications on plant cells by examining the virus's behavior conjugated with AuNPs by ultra-structure in TEM on the diseased plants.

The method of inhibiting a plant virus using AuNPs is a method of inducing plant resistance against viral disease caused by BYDV by introducing a thoracically adequate amount of polydispersed AuNPs system integrated with the virus particles wherein virus particles were dissolved and melted in **Figure 2**. The application of



**Figure 1.**

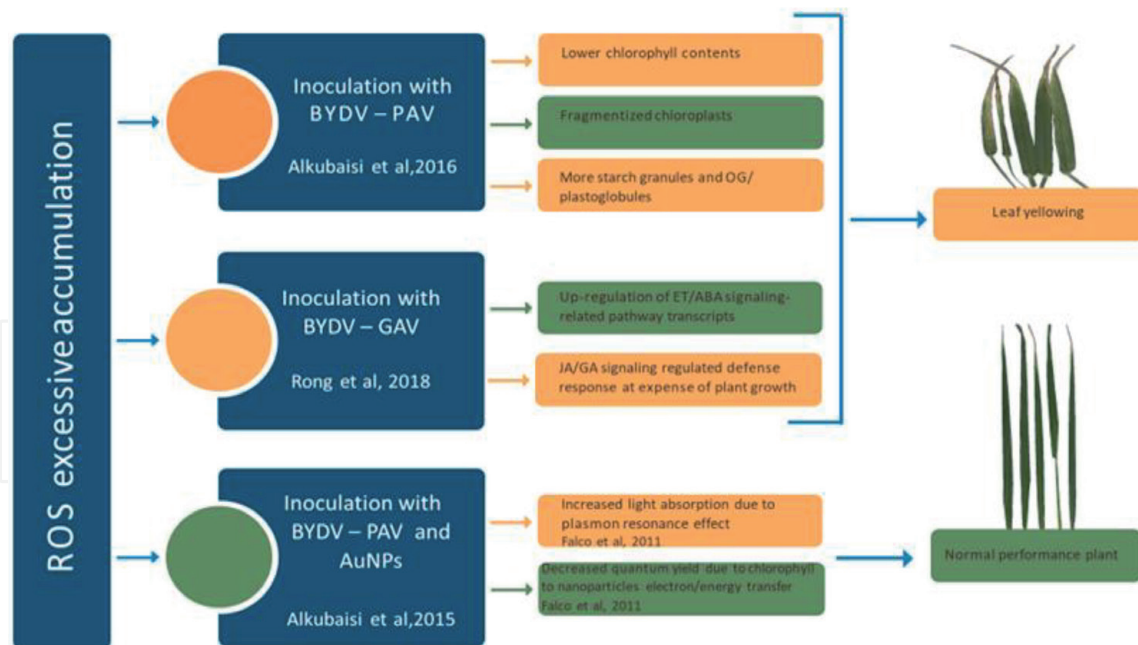
*The plant cell contained different organelles that exhibited ultrastructure changes in nucleus, chloroplast, plant cell wall, and mitochondria that describes each of them by TEM in healthy, infected, and treated with (AuNPs) as interaction preteome of BYDV- PAV.*

nanotechnology in agriculture, even at its global level, is at its nascent stage. The most crucial moment of plant virus entry considering is inducing viral infection crossing the cell/wall barriers. In our study, we knocked out and interfered with the virus as bio Nanoparticles with other metal AuNPs on the plant; [3] we selected the most severe BYDV isolates for Nano application on the plants [4].

The studied BYDV is the type member of the luteovirus group [5]. The Latin "luteo" name means yellow [6] and describes the most typical infected plants by luteoviruses. BYDV considered a model for dealing with the "yellowing" virus diseases, as reported [7]. Most infections appear as necrosis in the phloem, which leads to external symptoms such as stunning and leaf chlorosis [5]. The exact symptoms were reported [8] that might cup inward, tender, and show more stiffness than usual. BYDV is spread by aphids and induces the most widely and most distributed and most destructive virus disease globally.

## 2. Gold nanoparticles have adual positive effect

The prospect antiviral characteristic of Metal nanoparticles (MeNPs) in nano-agriculture drive them as a potential factor for commanding these histological agents. It is essential to detect the dosage of NPs, the application intervals, their effect as a biostimulant. The clarification of the mechanisms of action, are not fully understood [9]. Application of AuNPs in the presence of virus infection encourages the plants to produce Reactive Oxygen Species (ROS) continually in structures such as chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum (ER),



**Figure 2.** Methodology, pathological alternation of the plant cellular components, and the dual beneficial effect of AuNPs on the plant performance through the following treatments in the electron micrographs; I. healthy cells from barley leaves, II. Infected cells from by BYDV- PAV from barley leaves, III. Pretreated leaves of barley with AuNPs and infected by BYDV-PAV.

and plasma membranes [10]. These resemble components of the defensive system that have been classified according to their catalytic activity, molecular weight, the compartment where they act, and level of defense or mechanism of action [11]. The positive effect of AuNPs, therefore, needs further study to explore the physiological and molecular mechanisms. However, due to the tiny size, reactivity, and efficient penetration ability, metal nanoparticles could reach many intracellular and extracellular plant sites. That may trigger a set of physiological processes such as senescence affecting plant growth, crop yield, and ecological productivity [12]. Nanoparticles (NPs) have unique physicochemical properties, i.e., high surface area, high reactivity, tunable pore size, and particle morphology. The appropriate elucidation of the physiological, biochemical, and molecular mechanisms of nanoparticles in plants leads to better plant growth and development [13]. The chemical reactions, especially reduction–oxidation reactions, were catalyzed by Ag, Au, Fe, and Co. The released nano ions may alter proteins while entering into cells. Mechanical effects rely on the size of nanoparticles [14]. For example, the cell wall damage can be caused by the high concentration adsorption of hydrophobic nanoparticle retention and may cause clog pores, which can interfere with water uptake [15]. The ability to pass through the cell wall might not be a prerequisite for causing oxidative stress and toxicity. Some researchers suggest that despite nano-materials' inability to pass through plants' cell walls, they can cause oxidative stress and eventually lead to chromosome condensation [16].

Similarly, CuO nanoparticles can also cause oxidative damage to plant DNA and can be detected in plant cells [17]. Particles with anoxic surface often form a layer of OH– groups at the surface; these negatively charged groups attract positively charged side groups of proteins [14]. Surface effects have engaged a great deal of attention in the field of nanotoxicology.

The positive, neutral, and negative charge of Au nanoparticles are resemble hydroponic exposure to Rice plants. The distribution of the bioaccumulated Au nanoparticles due to the negative surface charge of the nanoparticles, which is more toxic in above-ground organs [18]. The small particle modulation of 15 nm or 25 nm AuNPs



was transported to the shoot in poplar. At the same time, larger particles (50 nm) could hold their size *in vivo*. AuNPs were located within the roots in a large amount than in leaves. AuNPs were detected in different tissues, phloem complex, xylem, cell wall, plastids, mitochondria, and more abundantly in the plasmodesmata [12]. Negatively charged, anionic carboxylate AuNPs conferred protection to the model lipid membrane against the extreme pH (=12) via shielding effects, whereas positively charged, cationic amino-AuNPs could penetrate and disrupt the model membrane [19].

Nanotoxicity is based on empirical data by an exact predictive model, which is explained by the interaction between surface charge and particle size that affects AgNPs toxicity in both the prokaryotic and eukaryotic model organisms [20]. The interaction between particle size and potential surface charge influencing ENM phytotoxicity has not received much attention. Therefore, the potential effect surface charge density remains to be tested in plants. ORF3 encodes a major coat protein (CP) of 22 kDa [21]. The coat protein plays a crucial role in maintaining a high level of accumulation of genomic RNA, though unnecessary for PAV replication [22]. ORF4 is entirely nested within ORF3 and codes for a 17 kDa nonstructural protein required for BYDV-PAV to spread systemically in plants [23]. The expression of ORF 4 is associated with a unique regulatory mechanism of the ribosome leaky scanning mechanism [24]. The ORF4 translation product is similar to that of the homology of ORF4 in potato leafroll virus (PLRV), which has biochemical properties specific to known movement proteins, including the ability to be phosphorylated, binding nonspecifically to nucleic acids [25, 26] and localization to the plasmodesmata [27]. PAV ORF5 is fused to CP as a readthrough domain and encodes a 50 kDa protein expressed as a 72 kDa fusion protein via a readthrough suppression of the ORF3 stop codon [23, 28–30]. A frameshift mutation within ORF6 was reported to be incompatible with BYDV-PAV RNA replication in protoplasts [31]. In [32], it is found that the RNA sequence encoding or flanking ORF6, rather than the protein product of ORF6, is required for PAV replication in oat protoplasts [33].

The viral infection starts with virus replication in the infected cell initially and spread to neighboring cells through plasmodesmata which are considered intercellular conduit connecting cell walls. This process is called cell-to-cell (short-distance) movement, facilitated by viral movement protein (MP). The following phase is termed (long-distance) movement which viruses could enter the vascular tissue, dispense, and flood into non-infected tissues, helped by the phloem stream [34]. It is presumed that the cell-to-cell movement is an active function, requiring specific interaction between the virus and plasmodesmata, whereas systemic viral spread through the vascular tissue is a passive process, driven by the flow of photoassimilates [35]. The discovery that a 30 kDa movement protein (MP) encoded by the Tobacco mosaic virus (TMV) was required for viral cell-to-cell movement [36, 37] exploring the trafficking mechanisms of a more comprehensive viral array opened a new path. Trafficking viral protein and RNA by viral MP into the phloem and their inter-organ regulation of plant development were rarely studied for some viruses [38] and [39]. The viral nucleic acid conjugate with the MP, which could transport it through plasmodesmata. The first viral MP of the Tobacco Mosaic Virus (TMV) was discovered that had 30 kDa protein (P30) and was able to bind single-stranded nucleic acid [40], mediated by two independently active domains of the MP [41]. The P30-TMV RNA complex measures a diameter of 1.5–3.5 nm [41] and [42] and may interact with the cytoskeletal elements to facilitate the transport of the P30- TMV RNA complex from cytoplasm to plasmodesmata [43] and [44]. The diameter is even smaller than a protein-free, folded TMV RNA, allowing easy access through dilated plasmodesmata [45, 46]. The MP could bind nonspecifically to single-stranded RNA and DNA *in vitro* [25] and associate with plasmodesmata in host plants [47]. ORF 4 proteins in luteo-viruses may provide a clue for assisting virus cell-to-cell spread in host plants [48] as

there is a high similarity of amino acid sequence between ORF 4 protein encoded by luteoviruses and PLRV MP [49, 50]. BYDV-PAV MP may also help transport the viral genome into the nucleus as the MP is present in the cytoplasm and the nucleus [51]. After entry into the cytoplasm, protein synthesis is initiated [52] and enhances replication and transcription efficiency; viruses use the strategy of compartmentalization in specific intracellular components [53]. Its replication is almost totally restricted within the plant phloem tissue [54, 55], i.e., phloem parenchyma cells, companion cells, and sieve tubes. The restricted site of infection in phloem tissue is an essential feature of the *Luteoviridae* [56].

The systemic spread is suspected to be associated with vascular transport of virions due to the discovery of BYDV particles in vasculature samples [57, 58]. The critical role of MP was emphasized by the association between the long-distance movement of some viruses and viral gene expression. For example, geminiviruses coded two proteins responsible for long-distance transportation and viral DNA with a single-stranded genome in and out of the nuclei [59, 60]. However, studies of the function of putative luteoviral MPs remain limited [61]. A 17 kDa protein encoded by ORF 4 is required for BYDV-PAV to spread systemically in plants [62, 63]. The replication of the plant virus genome occurs in host cells [64]. The genome of viruses must be transported into the nucleus by mechanisms requiring viral MP [65].

Three stages of infection were proposed by [57, 33]. At the first stage, densely staining material appeared in plasmodesmata, and an amorphous substance and viral RNA containing filaments appeared in the host cytoplasm, Figures 5 (I)-(M) in chapter 3. In the second stage, filaments became visible in the nuclear pores. During this stage, the nuclear outline became distorted and massive clumping of heterochromatin occurs, Figures (5) and (6) in chapter 1. In the nucleus, viral particles were seen at the last stage after the disintegration of the nuclear membrane. The virus could only infect phloem parenchyma, sieve elements, and companion cells, while it could not be seen in the mestome sheath or the xylem.

The characterization of VLPs was defined by their diameter, their circular outlines, and high electron opacity. Viral particles were detected within areas containing filamentous material Figures (2) and (3) in chapter 4.

**Our study concludes** that Nanoscience leads to developing a range of inexpensive nanotech applications for enhanced plant growth. The included data proved an efficient means to control virus infection in a fashion way to reduce collateral damage. AuNPs have a dual positive effect on controlling the plant viral disease and enhance strong plant growth performance.

Transmission electron microscope	TEM
Gold nanoparticles	AuNPs
King Saud University	KSU
Barley Yellow Dwarf Virus	BYDV-PAV
Reactive Oxygen Species	ROS
Endoplasmic reticulum	ER
Silver	Ag
Gold	Au
Iron	Fe
Cobalt	Co
Copper oxide	CuO
Hydroxide	OH

NanoMeter	nm
Silver nanoparticles	AgNPs
Engineered nanomaterial	ENM
Metal nanoparticles	MeNPs
Open Reading Frame	ORF3
Coat protein	CP
kilodalton	kDa
Ribonucleic Acid	RNA
Potato leafroll Polerovirus	PLRV
Movement protein	MP
Tobacco mosaic virus	TMV
Deoxyribonucleic Acid	DNA

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