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A Review

Phytonanotechnology: Recent applications and the role of Biocorona

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Phytonanotechnology is lately gaining increased interest owing to its potential to modernize agriculture for better yield and nutritional quality. Consequently, Nano-Agri products like nano-biosensors, nano-carriers, and growth augmenters are being developed and applied. However, the limited knowledge of molecular interactions taking place at nano-bio interface remains a major concern. The nanotechnological interventions for healthier crops could rather turn out tobe risky and inefficient in the absence ofclear understanding of molecular mechanisms of nano-bio interactions. Upon entry into tissues or cells, nanoparticles (NPs) adsorb biomolecules forming a biocorona which determines NP uptake, translocation, and reactivity. The composition of biocorona is dependent on the physicochemical characteristics of the NPs, their surroundings, and the interaction time. Recent nascent studies in plants showed the potential of biocorona to influence major cellular pathways or plant responses like energy synthesis, pathogenesis, stress tolerance, and leaf senescence. This mini-review aims at summarizing the recent application of phytonanotechnology, the current status of biocorona studies with an overview of research bottlenecks and future prospects.

Keywords: Agriculture, Biocorona, Nano-bio interaction, Phytonanotechnology

Introduction

To feed humans on planet earth, the global food yield needs to be increased by 70% as the rapidly growing human population is expected to cross 9 billion by the year 2050. While agriculture is already considered as the economic backbone for several developing countries, developed countries are also keen to boost their food yield owing to longer lifespans and increasing immigration. Under this “extreme performance pressure” agriculture has constantly adopted newer technologies like precision breeding, genetic engineering, and nanotechnology. There has been a tremendous growth in Phytonanotechnology lately due to its potential to address global food challenges. Many Nano-Agri products like nanobiosensors, nanocarriers, fertilizers, and other nano formulations have been developed for commercial exploitation. The overwhelming interest and NP usage have, however, raised concerns, many a time, about their potential toxicity and there have been a number of conflicting reports.

The existing methods for environmental risk assessment were questioned by a group of scientists from the UK, US, Australia, Netherlands, Sweden, and Austria[1]. The paper published in the “Journal of Agriculture and Food Chemistry” referred to the evidence suggesting that the factors affecting the environmental behavior of engineered NPs differed from those influencing the substances that do not contain NPs. Industrial stakeholders, on the other hand, have demonstrated the risk-free usage of NPs. Under these circumstances, the methods used in the risk assessment urgently need a sincere revisit. Many other issues also exist that need to be taken care of. For example, toxicity and potential long-term and short-term effects of NPs on humans are not sufficiently understood. Bioaccumulation of NPs in the ecosystems and the consequent effects are also not known. The knowledge of crosstalk among NPs, plants, soil, and the immediate environment is also important for predicting the effect of nano-bio integration. These bottlenecks lead to conflicting regulatory issues. Moreover, a wide diversity of available nanomaterials makes this task humongous. The US FDA and Environmental Protection Agency (EPA) are yet to consider nanomaterials as the new chemicals. Ironically, although manufacturers are required to demonstrate that the ingredients and food products are safe for human consumption; however, there is no regulation specifying utility of NPs.

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Therefore, extensive use of nanomaterials in agriculture amidst limited understanding of the molecular mechanisms of their action and biological interactions is certainly not an intelligent move. The existing reports reveal that the majority of toxicological studies conducted using a variety of organisms showed conflicting results even with the same NP-Organism combination[2]. The observed variations could be due to the formation of distinct NP biocorona (the accumulation of biomolecules from the surrounding medium on the NP surface). NP biocorona majorly constitutes proteins besides lipids, secondary metabolites, flavonoids, and nucleic acids. The NP biocorona is commonly referred to as protein corona (PC) due to the higher affinity of proteins toward the NP surface. Biocorona modulates the NP identity, cellular internalization, distribution, and effects[3]. After its formation, biocorona evolves spatially and temporally[4]. Initially, abundant proteins get adsorbed to NPs to form an unstable entity, followed by the accumulation of molecules with a better affinity for the particle surface. Nanoparticles show differential behavior depending upon their shape within the same plant. Likewise, species-dependent variation in NP behavior is also observed. Therefore, the analysis of biomolecular corona fingerprint is crucial to gain insights into NP-plant crosstalk and fine-tuning of NPs for their safe and sustainable use in agriculture.

The methods of biocorona synthesis and their characterization are very well-reviewed[5]. Biocorona is synthesized by allowing the interaction of NPs with biomolecules under controlled conditions. The biocorona thus synthesized are isolated by either centrifugation or column chromatography. NP-biomolecule complexes can be analyzed by various tools and techniques like SDS-PAGE analysis, electron microscopy, mass spectrometry (MS), UV-Visible spectroscopy, DLS (dynamic light scattering), CD (circular dichroism), FTIR (Fourier-transform infrared spectroscopy), among others[6-8]. In this review, we summarize the current knowledge of plant biocorona research, its limitations, and future prospects.

Applications of phytonanotechnology

Nanoparticles owing to their nanoscale size and tunable properties offer many potential applications for sustainable agriculture (Fig. 1). From the Nano-Agri products like biosensors and nanocarriers to the acceleration of adaption of plants towards changing climate, NPs hold a bright future. Nano-fertilizers are gaining popularity as alternatives to chemical fertilizers. Other nanocomposites have also emerged useful for the genetic engineering of plants and defense against insects, weeds, and fungi. Here, we discuss some of the major applications of nanotechnology in plant sciences (Table 1).

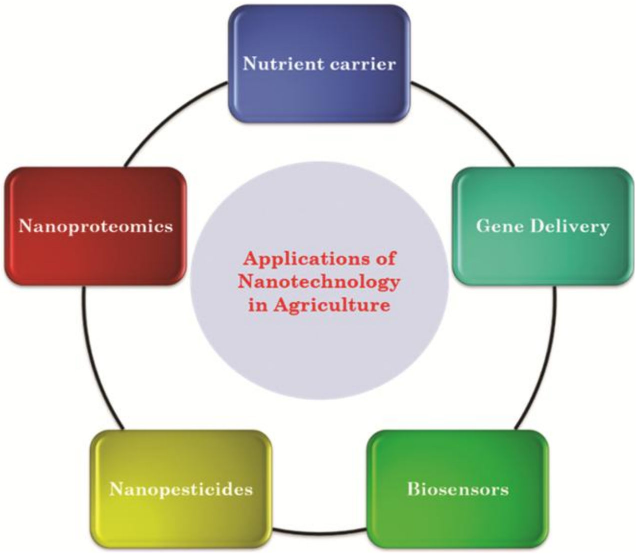

Fig. 1 — Common applications of nanotechnology in agriculture

Nanoscale carriers for plant nutrient deficiency mitigation

Phytonanotechnology offers solutions for the growing concern of nutrient deficiency in edible crops that significantly affects human health. Inspired by the targeted delivery of drugs in the animal and human systems, plant scientists started using NPs for such utility in plants. The conventional methods of agrochemical application lack controlled and consistent release of active compounds. Volatilization of the active compounds, soil quality deterioration, and eutrophication of water bodies are some other challenges. Nanoscale carriers have been helpful in the alleviation of these obstacles. Therefore, different types of NPs composed of plant-derived molecules are used for the delivery of macronutrients (N, P, and K) and essential metal nutrients like Fe, Zn, Cu, Mn, and Cobalt. For example, a group of researchers from Israel led by Prof. Schroeder loaded the nutrients to NPs that are designed to be stable inside plants. These nanocomposites could penetrate the cells. They made use of 100-nanometer size liposomes containing lipids derived from soy (*Glycine max*) plants to deliver and release Fe and Mg across tomato leaves to treat the acute nutrient deficiency that was otherwise

Table 1 — Major applications of phytonanotechnology and associated pros and cons

S. No.	Application	Limitations of conventional approaches	Nano-product/ technology examples	Benefits	Limitations of Nano-based approaches
1.	Nutrient Delivery	➢ Uncontrolled and inconsistent release of active compounds. ➢ Volatilization of active compounds. ➢ Soil qualitydeterioration. ➢ Eutrophication of water bodies.	➢ Geolife Nano Mg ➢ Tropical Nano Phos ➢ Nano urea: IFFCO	➢ Slow release ➢ In some cases, increase abiotic stress tolerance ➢ Increased nutrient use efficiency	➢ Presents health safety issues ➢ Phytotoxicity ➢ Very high reactivity is a concern
2.	Genetic Engineering	➢ Species limitation ➢ Poor transfection specificity ➢ Poor target specificity	➢ CRISPR/Cas9 Nano-Delivery	➢ No species limitation ➢ Target specificity	➢ Formation of biocorona and disintegration of original cargo
3.	Nano Biosensors	➢ Molecular assays (nucleic acid and antibody-based) for the detection of plant diseases are laborious and complex.	➢ Plasmonic AuNPs for detection of fungi	➢ Early sensing of infections and stress conditions. ➢ Real time sensing	➢ Current challenges reside in the identification, production and purification of enzymes specific to each target
4.	Nanopesticides	➢ Similar to that of conventional fertilizers	➢ Polymer stabilized bifenthrin nanoparticles	➢ Low concentration is required ➢ Site specific	➢ Similar to that of conventional fertilizers
5.	Nanoproteomics	➢ Inability to capture very low abundant stress/disease markers	➢ Magnetic Fe_3O_4 NPs surface functionalized by multivalent ligand molecules making particles prone to bind to the phosphate groups for phosphoproteins enrichment	➢ Identification of very abundant markers ➢ Easily combined with conventional Mass spectrometry	➢ High sensitivity of biocorona towards all reaction parameters

untreatable by conventional practices[9]. Interestingly, approximately 1/3rd of these particles could penetrate the leaves and translocate to the other leaves and roots. Finally, liposomes were internalized by the cells, wherein they released their payload.

Indian Farmers Fertilizer Cooperative Limited (IFFCO), India`s leading fertilizer company, developed nano urea, a source of nitrogen, in association with Nano Biotechnology Research Centre (NBRC) Kalol, Gujarat, India, through proprietary patented technology. Foliar application of Nano Urea in liquid form has been reported to effectively fulfill the need for nitrogen for better quantity and the quality of crops during field trials. The government of Sri Lanka has recently decided to procure IFFCO`s nano urea as part of its organic strategy, especially for maize and paddy plantations. Although the use of nanomaterials in plants raises some toxicity issues, biogenic NPs may be a better option.

NP mediated genetic engineering in plants

Genetic editing of plants allows sustainable efforts towards meeting the needs of the growing human population and natural product synthesis for the pharma industry. Genetic engineering adopts new methods for passive transport of coding genetic elements into a wide range of crops. Gene vectors play an important part in genetic engineering. Nano-based non-viral vectors for gene delivery offer several advantages over viral vectors. Some of these benefits areno species limitation, tunable properties, target specificity, larger payload, improved transfection efficiency, and low cytotoxicity. These vectors can be constructed using inorganic NPs, liposomes, carbon nanotubes, protein/peptide NPs, and nanosized polymers. Therefore, NP-mediated gene delivery in the plant system has become the current trend and mesoporous silica NPs were recently used for gene delivery into suspended tobacco cells[10]. Ultrasonic treatment was used to increase the gene transfer events, however, a high level of sonoporation had adverse effects on cells. Under optimized conditions, the NP-mediated ultrasonic method of gene transfer is considered economical, straightforward, and safer. Nanoparticle mediated biotransformation has been combined with CRISPR (clustered regularly interspaced short palindromic repeats) technology to discourage transgene silencing, improve transformation efficiency, and productivity. Researchers tried multiple ways to combine the CRISPR–Cas9 system with nanocarriers like exosomes or liposomes and achieved an effective strategy for transferring the CRISPR–Cas9 system[11].

Nanobiosensors

Besides the rapidly growing human population, global food demand faces another challenge in the form of climate change. Plant pathogen-induced damage varies from 12% to 80% in major crops with an annual loss of around 50 thousand crores in India[12]. Tools for the early determination of plant diseases are essential for sustainable agriculture. Molecular assays (nucleic acid and antibody based) for the detection of plant diseases are laborious and complex. Fortunately, nano-inspired biosensors are being developed to deal with such challenges by early sensing of plant infections, pathogens, and other stress conditions simply and more effectively. Metal-based NPs, polymers, and carbon allotropes offer better reproducibility, faster detection, stable immobilization of receptor molecules, and efficient/enhanced signaling, among others. The specificity of the sensors is increased by targeting molecular interactions or recognition elements like nucleic acids, enzymes, and antibodies. Some methods also utilize the optical properties of NPs. For example, platinum NPs functionalized with IgG were used to detect bacterial cues from the soil and roots of the carrot plant[13]. Similarly, AuNPs based nano biosensor was developed for colorimetric sensing of Tomato yellow leaf curl virus[14]. The infection could be detected by visual color changes in AuNPs suspension besides detection of change in the LSPR (local surface plasma resonance) peak of AuNPs post their interaction with viral cues.

Nanopesticides

The nano pesticides commonly applied in the form of emulsions or capsules are emerging technological advancements that offer improved efficiency, better stability, enhanced solubility, controlled release, targeted delivery, faster decomposition, and in some cases protection against premature degradation. Moreover, these pesticides are required in relatively very low amounts making them cheaper and easy to carry for farmers with lesser accumulation in foods.

However, there are some rising concerns around the use of nanopesticides. For example, nanopesticides have been shown to enter the cells and interact with chloroplasts obstructing their function[15]. Both carbon-based, as well as titanium-based nanopesticides, were found to be cytotoxic to plants by perforation of cellulose membranes. Therefore, adequate research is required for safe application of nanopesticides in agriculture.

NP Biocorona

Nanomaterials affect plant species both negatively and positively depending upon particle and plant species-based variables besides the mode of NP application. The same kind of NPs with distinct shapes behave differentially within similar plant species. For example, rod-shaped cerium oxide NPs were more reactive than cubic cerium oxide NPs in cucumber[16]. Likewise, plant species-dependent variation in NP reactivity and stability was also observed. For instance, identical cerium oxide NPs showed different degrees of translocation in corn, cabbage, wheat, and soybean[17].The literature has many such reports showing variable behavior of NPs in plants. This type of dichotomy can only be understood through the understanding of molecular interactions or the study of biocorona formed around NPs. At present, the study of plant biocorona is in its infancy. Nevertheless, the early insights from a few studies have provided exciting results that showcase the ability of biocorona to influence major cellular pathways or plant responses like energy synthesis, pathogenesis, stress tolerance, and leaf senescence, among others (Fig. 2 and Table 2).

Despite a higher number of secondary metabolites in the xylem, NP biocorona consisted of more proteins. Biocorona proteins usually undergo a change in their secondary structure leading to increased β-sheet content. With the ability to concentrate biomolecules on their surface, NPs are being employed for the nano harvesting of metabolites. The rhizosphere, the earliest site of NP-bio interaction during route passage, involves the scavenging of flavonoids affecting pathogenesis and NP uptake. On the other hand, the Phyto-enzymes exuded from roots could degrade carbon-based NPsin the rhizosphere. Manganese NPs breached seeds, formed PC, and improved the seedling salt stress response in the *Capsicum annuum*.

Temporal evolution of biocorona

Biocorona temporally evolves from a soft biocorona (poorly attached biomolecules) to hard

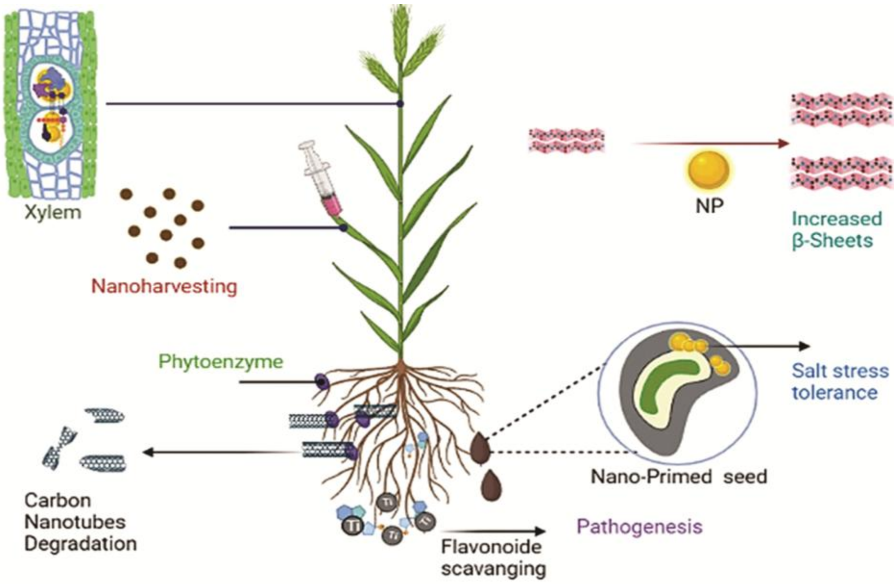

Fig. 2 — Schematic illustration of behavior of NP biocorona in plant systems

Table 2 — Biocorona studies conducted in plants and major conclusions

S. No.	System	NP type	Major conclusions	Reference	Journal
1.	*Brassica juncea* leaf protein isolates	Au	➢ Protein biocorona evolves temporally from an unstable entity to stable entity. ➢ The study showed potential ability of AuNPs to influence energy synthesis and leaf senescence in *Brassica juncea* NP protein binding is a selective process.	Prakash & Deswal 2020	Plant Physiology and Biochemistry
2.	Plant proteins	Superparamagnetic FeO	➢ NP increased the protein fibrillation through *β*-Sheet assembly.	Li *et al*. 2019	Scientific Reports
3.	Glutenin, gliadin, zein, and soy protein	TiO_2	➢ NPs undergo altered size and surface charge. ➢ Proteins undergo altered secondary structure.	Bing *et al.* 2021	Food Hydrocolloids
4.	*Capsicum annuum* seeds	Mn	➢ Nano priming of seeds followed protein biocorona formation leading to improved salt tolerance. ➢ NP bound proteins displayed altered secondary structure.	Ye *et al*. 2021	ACS Sustainable Chemistry and Engineering.
5.	*Arabidopsisthaliana* leaves	TiO_2	➢ TiO_2 NP biocorona are enriched for flavonoids and lipids ➢ Metabolite classes compete with each other for binding the NP surface	Kurepa *et al.* 2020	Journal of Nanobiotechnology
6.	Xylem sap of Pumpkin plant	CuO	➢ The biocorona was composed primarily of proteins, despite the higher abundance of carbohydrates in xylem fluid. ➢ The protein–CuO NP interactions were quasi-irreversible, while carbohydrate–CuO interactions were reversible.	Borgatta *et al.* 2021	Environmental Science: Nano

biocorona (tightly attached biomolecules)[18,19]. Initially, high abundant molecules are adsorbed in more numbers to form an unstable entity (soft biocorona) followed by adsorption of biomolecules having higher affinity (hard biocorona), a process commonly known as biocorona hardening or 'Vroman effect'. We also observed similar kind of temporal shift in the composition of protein corona (PC) in *Brassica juncea*[20]. AuNPs-leaf protein coronae were obtained from 2 h to 36 h keeping temperature and buffer pH at 25°C and 7.6 respectively, to match the *in vivo* conditions. By SDS-PAGE analysis, we could observe two distinct regimes (up to 8 h and from 16 h to 36 h) characterized by the different polypeptide profiles. Regime I(8 polypeptides) could be easily visualized by silver staining, while regime II (30 polypeptides) showed prominent background on the SDS-PAGE gel probably due to non-protein components like flavonoids and lipids. Therefore, a complete metabolomic analysis should be done to comprehend the biocorona. Nevertheless, timely evolved PCs were also scanned by a Spectrophotometer. A colloidal suspension of AuNPs is expected to give λ_{max} at around 530 nM, however, it may deviate due to the formation of biocorona. Optical scanning also showed similar regimes. Thus, the temporal evolution of biocorona from soft biocorona to hard biocorona appears to be a universal phenomenon. However, insome places in literature, the inner layer of proteins around the NPs has been termed hard corona while the upper layers of proteins are regarded as soft biocorona.

NP mediated molecular scavenging and structural shifts

The molecular basis of NP-mediated effects involves their direct interaction with biomolecules, thereby, modulating the respective pathways or interactions through scavenging of biomolecules and/or structural changes within them. For instance, the interaction between proteins and NPs involved an assembly of β-sheets revealed in a study that utilized magnetic iron oxide NPs and plant protein fibrils[21].Iron oxide NPs helped with accelerating the fibrillation probably due to hydrogen bonding, ionic interactions, and surface energy transfer between NPs and proteins. In another study, proteins like glutenin, gliadin, zein, and soy were found to have a modified secondary structure post interaction with TiO_2 NPs as revealed by infrared spectroscopy, fluorescence quenching, and circular dichroism[22]. During FTIR analysis, the change in protein structureis detected through amide I (1700cm^{-1}-1600 cm^{-1}) and the amide II (1550 cm^{-1}-1530 cm^{-1}) bands. Bing and Xiao, 2021 observed a red shift in the peak of amide I and amide II for all the tested proteins. The similar red shift of peaks of amides was previously reported for human serum albumin absorbed on the carbon nanodots[23]. Further, the far-UV CD spectra of glutenin showed an increase in the β-sheet content of the protein. Similarly, Ye and colleagues also reported an altered protein structure when *Capsicum annuum* seeds were treated with low concentrations of manganese nanoparticles (MnNPs)[24]**.** Therefore, NP-dependent alteration of protein structure seems to be a general occurrence, although more research is required to establish such relationships. Increased β-sheet composition is associated with increased protein stability. Notably, the biocorona enzymes like MDH (Malate dehydrogenase) and FBA (Fructose-bisphosphate aldolase) were spectrophotometrically active[20].

Besides, over 10% of proteins of the hard biocorona obtained by the interaction of AuNPs with *Brassica juncea* leaf isolates were proteases that made us speculate that the AuNP mediated increase in leaf number reported in *Brassica juncea* could be a result of scavenging of proteases leading to delayed senescence[20,25].NP's ability to scavenge biomolecules has been utilized for nano-harvesting of natural products/metabolites from plant cells, a process termed "nanoharvesting", coined by Smalle and associates[26]. The group observed that 20 nM wide phosphorylated anatase TiO_2NPs could enter the plant cells and accumulate molecules having catechol groups (flavonoids) *in situ*, and exit plant cells. This type of metabolite harvesting does not require the usage of organic solvents. In addition, different types of NPs can be surface functionalized to harvest a variety of metabolites of interest from plant cells.

Biocorona alters the NP size and surface charge

The biocorona formation follows the physicochemical changes in the NP properties like size, charge, surface chemistry, and hydrophobicity. For example, we observed modified zeta potential and increased AuNP protein complex size following the interaction of AuNPs with *Brassica juncea* leaf proteins[20]. Interestingly, the scatter plot of adsorbed protein content versus time formed a sigmoidal curve. Hence, the PC evolution had "three" distinct phases. Phase I included a steep rise in biocorona protein concentration from 2 to 8 h indicating rapid adsorption of proteins on the pristine NPs surfaces. This followed a competition among proteins for NP surface leading to the formation of a plateau from 8 h to 24 h (Phase2). Again, an abrupt rise in accumulated protein concentration was observed after 24 h suggesting protein-protein interaction (Phase 3). Consequently, the adsorbed proteins led to a consistent increase in NP size as revealed byDynamic Light Scattering (DLS) sizing. The final size of the complex was measured to be 253.56 nm, approximately 10 times higher than the original particle size (24.74 nm). Similar to the complex size, the interaction of AuNPs with the *Brassica juncea* leaf proteins also changed the zeta potential of interacting AuNPs. High zeta potential of nanomaterials is desirable so that they are stable inside the tissue and do not aggregate. Aggregation ofnanomaterials triggers oxidative stress in crops by choking the membrane channels, thereby, causing nutrient and water deficiency[27,28]. The zeta potentials AuNP protein complex dramatically fell to -21 mV from −38.5 mV at 2 h interactions likely due tothe accumulation of positively charged proteins. However, the zeta potential of the AuNP-protein complex then underwent a consistent increase from -21 mV to −50 mV at 36 h. Similarly, the surface zeta-potential of the negatively charged TiO_2NPs (−18.7 mV) changed to positive when interacted with cationic proteins[29].A change of zeta potential from +7.3 mV to −5.5 mV was recently reported for chitin nano whiskers post formation of PC[30]. These reports support that the NPs lose their original surface characteristics upon the formation of biocorona.

Biocorona modulates energy synthesis and stress response in plants

Plant response to stress conditions involves morphological, physiological, and metabolic shifts. There are reports of NP-mediated increased seed germination, seedling growth, photosynthesis, and nitrogen metabolism. Besides, the activities of stress related enzymes and chlorophyll amount arealso affected.

The biocorona studies in plants also revealed PC-mediated modulation of plant energy yielding pathways and plant stress responses. For example, our findings showed that AuNPs could alter the *Brassica juncea* yield as approximately 30% of the biocorona proteins associated with stable PC were part of energy-yielding processes. Similarly, data from Ye and colleagues suggested a linkage between the PC and salt stress response in *Capsicum annuum* seeds[31]. The group observed enhanced root elongation besides decreased negative effects of salt during seed germination. Manganese NPs breached the seed coats forming NP PC. It was hypothesized that the formation of NP-protein complexes played a crucial role in salt tolerance. However, direct priming of seeds with NPs assumes that the NP surface is available for new interactions without any resistance and the NP uptake/entry is solely dependent on the properties of pristine NPs, however, the transformation of these NPs before they enter the plant, *i.e.*, in the rhizosphere must not be ignored. The root exudates and microorganisms in the rhizosphere might interact and alter the NPs affecting their stability and bioavailability. And once we put these possibilities in to consideration, the resultant NP PC might be significantly different from what is observed by the direct priming of seeds with uncoated NPs. Notably, poor uptake of nanomaterials through roots has been reported when applied through the soil in comparison to the uptake under an aqueous medium. Thus, it is evident that the complexity of the rhizosphere leads to altered NP properties. For example, the work by Kurepa and the group showed that flavonoids have a very good affinity for TiO_2 NPs. Focusing on the biomolecules other than proteins, Kurepa and colleagues utilized *Arabidopsis* 4 mutant lines enriched in lipids. Further, Lipid capped TiO_2 NPs were reacted with leaves to harvest nano-metabolite complexes[26]. The biocorona around TiO_2 NPs showed enrichment of flavonoids and lipids. The group suggested that the TiO_2 NPs could affect biological functions through altered lipid signaling and flavonoid content. Since flavonoids attract some nitrogen-fixing bacteria and regulate rhizobial nod genes expression[32], it reflects the potential of NPs to interfere with flavonoid dependent processes like parthenogenesis and allelopathic interactions like rhizobia legume symbiosis in the rhizosphere[26,33]. For instance, the nodulation process and arbuscular mycorrhizal symbiosis were reduced in plant roots exposed to TiO_2 NPs[34]. Besides, there are reports of the oxidative degradation of engineered carbon-based nanomaterials like carbon nanotubes or fullerene by the phyto-enzymes like peroxidases in the rhizosphere[35]. As a result, the size, shape and surface property of these NPs might change making these less cytotoxic to humans[36].

Biocorona formation is a selective process

Biocoronaaffects the distribution of NPs within living systems. NPs mainly enter through the leaf or roots. Long-distance transport happens through the xylem and phloem. Borgatta *et al*. 2021 examined the interaction of CuONPs with xylem fluid of pumpkin plant[37]. Copper oxide NPs are considered effective micronutrient source against fungal diseases. Xylem contains more carbohydrates than the proteins. Interestingly, the biocorona was dominated by proteins and not carbohydrates. Interestingly, the most abundant biocorona proteins were not the most abundant proteins in the xylem fluid. Further, using *in situ* attenuated total reflectance FTIR, they showed that the interaction between carbohydrates and NPs was reversible while the interaction between proteins and NPs was irreversible. Similarly, we earlier reported that the Myrosinase enzyme which was abundantly present in the *Brassica juncea* leaf nuclear fraction was not a part of AuNP- leaf nuclear protein complexes[20].

Nanoparticle assisted proteome mining

Since, the advent of protein mass spectrometry, proteomics underwent technological upgrades for almost three decades though, it is still limited by the inability to identify very low abundant proteins. The problem has been partially solved with the abundant protein depletion protocols. Nano-based proteomics, commonly known as nano proteomics offers deeper mining of proteomes or PTM (Post translational modification) enrichment without using labor-intensive protocols[38,39]. Nano proteomics involves the modulation of NP biocorona to enrich low-abundant proteins. For example, Prof. Ying Ge's group and associates developed Fe_3O_4 NPs with magnetic properties and surface functionalized by multivalent ligand molecules creating a specific affinity of these NPs towards the phosphate groups for phosphoproteins enrichment[38].

Nano proteomics also enables the discovery of low abundant biomarkers of diseases and stress conditions. The composition of PC is highly sensitive to fluctuations in the health conditions of humans or plants. These molecular signatures are identified by comparative analysis of PC synthesized under stressed *vs* control conditions. For example, multiple biomarkers associated with triple-negative breast cancer (TNBC) were detected by the screening of serum obtained from patients. Metal NPs were used for the concentration of proteins for SDS-PAGE and LC-MS/MS analysis[40].

Major limitations in the study of plant biocorona

The study of biocorona in plants is in its infancy and faces multiple challenges including the extreme sensitivity of biocorona towards both NP and plant-based factors (Fig. 3). The unavailability of any *in vivo* model plant system is also a major hurdle, therefore, only *in vitro* or *in situ* biocorona studies have been conducted so far. Currently, the available protocols for the recovery of NPs from plant tissue are either destructive and harsh or are unable to provide desirable yield for biocorona characterization. Hence, the development of an appropriate protocol for *in vivo* biocorona analysis would be a significant breakthrough in phytonanotechnology.

Biocorona analysis often requires biophysical instruments like Zetasizer Nano that are generally not available in plant biology laboratories or facilities. Besides these technical issues, the biocorona analysis in plants also struggles due to a lack of awareness and a meagre workforce as only a few groups are currently working in this area. As a result, phytonanotechnologists are yet to harvest the benefits of biocorona analysis. It is important to mention here that the biocorona may be easily employed for the discovery of low abundant and novel stress associated markers that are very difficult to capture otherwise.

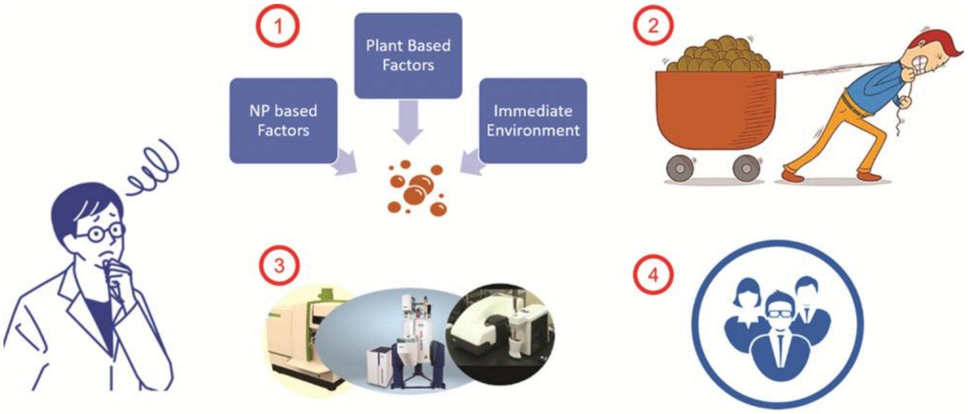

Fig. 3 — (1-4) Major bottlenecks in plant biocorona research. (1) Biocorona is highly sensitive to various plant based, particle based and microenvironment-based factors. (2) A protocol for recovery of analyzable NP-biomolecule complexes from plants is not developed yet. (3) Common NP research instruments like FTIR, NMR and Zetananosizer are generally not available in plant research labs or facilities. (4) There are only a few groups working on biocorona in plants

Conclusion

NP biocorona in plants consists of biomolecules, majorly proteins. The characteristics of the biocorona are governed by NP-based factors like size, shape, and surface chemistry. Further, the NP proteins interaction alters the NP-complex size, surface functionalization, and zeta potential. On the other hand, proteins exhibit altered secondary structures. Biocorona influences the cellular pathways by altering protein structure and/or scavenging biomolecules. More such studies are required to establish the observations and enable the sustainable utility of NPs in agriculture.

Despite multiple hurdles, attempts to characterize the mechanisms of NP interaction with biological systems including plants are slowly picking up. Biocoronas formed on the surface of NPs are the true identities of NPs entering the plants. A high sensitivity of biocorona towards multiple factors makes its prediction difficult. Hence, intensive studies with perfectly controlled designs are required to gather data to decode the interaction mechanism. The apprehension of the attributes of biocorona and the resulting biological responses are of great importance for designing the engineered nanomaterials (ENMs) as per the need. The effect of NPs can be ameliorated by revising the initial surface chemistry of NPs by taking the advantage of biocorona data. During *in vivo* administration, NPs interact with a wide range of molecules, such as proteins, lipids, enzymes, and carbohydrates. Therefore, future efforts could be on the development of an *in vivo* system to understand the behavior of NPs within the plants.

Conflict of interest

All authors declare no conflict of interest.

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